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RECENT ADVANCES IN CHEMOTHERAPY

By

G. M. FINDLAY

O.B.E., M.D., D.Sc.

Wellcome Bureau of Scientific Research, London

WITH A FOREWORD BY

C. M. WENYON

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Director-in-Chief of the Wellcome Bureau of Scientific Research, London

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FOREWORD

THE work of Ehrlich and those that immediately followed him opened up new fields of inquiry in which the old empiricism was replaced by definite attempts to correlate the specific action of drugs on parasites with chemical constitution. An ever-increasing number of investigators have studied the action of drugs on parasites, and by varying the constitution have found that the action is thereby increased, diminished or not altered at all. The literature of the subject, which is of the greatest importance as regards improvements in therapeutics, is now so extensive, that without some adequate guide it is difficult, if not impossible, for an individual to grasp its significance. With the exception of "The Principles and Practice of Chemotherapy," by J. A. Kolmer, a work which deals more especially with the treatment of syphilis, and scarcely at all with many other important branches of chemotherapy, there is no work available in the English language which describes the present position of the subject from the point of view of medicine. On this account Dr. G. M. Findlay's survey of the recent advances should prove of great service to the many workers in this field.

The greater part of the work is necessarily devoted to a study of the action of chemical agents on diseases due to helminths, protozoa and spirochætes, for as yet little progress has been made in the chemotherapeutic treatment of bacterial diseases, and still less in those of virus infections. The reason for this is to be found in the fact that helminths, protozoa and spirochætes can readily be detected by direct examination of, for instance, a blood film or fæces. Their presence before and their absence after the administration of a drug is relatively easy to determine, whereas the detection of bacteria and ultra-microscopic viruses requires more elaborate cultural or inoculative tests. Furthermore,

protozoal infections, such as those due to trypanosomes in experimental animals, run fairly regular and rapid courses, during which the parasites are easily seen in the blood. It is in these cases a relatively simple and rapid procedure to determine whether the infection is influenced in any way by chemotherapeutic agents. On this account a large proportion of the published works deal with protozoal infections, particularly those due to trypanosomes in experimental animals. Quite apart from the reasons just mentioned, the study of chemotherapy has developed along the lines of testing drugs on parasites of an animal nature, for it has been found that they are actually more vulnerable to drugs introduced into the body of the host than bacteria and allied organisms. It might be true to state that the more highly organised a parasite is, the more probable it is that it will be influenced by chemotherapeutic agents.

C. M. WENYON

PREFACE

RECENT advances in such a subject as chemotherapy are by no means easily defined. Countless drugs have been introduced for the specific treatment of parasitic diseases, but it is only after constant trials that the value of any compound can be accurately assessed and by this time its introduction can hardly be described as recent. Nevertheless, when such a period as the last ten years is surveyed as a whole, it is apparent that certain definite advances have been made in chemotherapeutic treatment, while in addition to the use of new compounds, a better understanding has been gained of the mode of action of well-established medicaments.

An attempt has therefore been made to describe the more important advances in chemotherapy which have occurred during the post-war period, special emphasis being laid on the fact that the focus of attention has tended to shift from a study of the direct interaction of drugs with infecting organisms to an investigation of the part played by the tissues in chemotherapeutic treatment.

This book could not have been written without the invaluable assistance, which I most gratefully acknowledge, of Dr. C. M. Wenyon, Director-in-Chief of the Wellcome Bureau of Scientific Research, and of Dr. T. A. Henry, Director of the Wellcome Chemical Research Laboratories.

My sincere thanks are also due to Mr. W. H. Gray, Dr. J. W. Trevan, and my colleagues Major H. C. Brown and Dr. J. C. Broom for their kindness in reading proofs and for their constant aid and advice. The help of Miss I. M. Bellis, Librarian to the Wellcome Bureau of Scientific Research, has also been of the utmost value. I am indebted to Colonel R. B. Lloyd, I.M.S., and the Editor of the *Indian Journal of Medical Research* for permission to reproduce the graphs illustrating the chapter on kala azar, and to the Wellcome Historical Museum for the use of the portraits of Ehrlich, Pelletier, Caventou and Magendie. I desire also to express my indebtedness to Dr. G. M. Dyson's "Chemistry of Chemotherapy." Finally, I must express to my publishers my appreciation of their courtesy and ever-ready help.

G. M. FINDLAY

CONTENTS

CHAP.		PAGE
	FOREWORD	v
	PREFACE	vii
I.	INTRODUCTION	1
II.	CHEMOTHERAPY OF HELMINTHIC INFECTIONS	9
	ANKYLOSTOMIASIS	15
	SCHISTOSOMIASIS	55
III.	CHEMOTHERAPY OF AMŒBIASIS	77
IV.	CHEMOTHERAPY OF MALARIA	106
	BLACKWATER FEVER	154
V.	CHEMOTHERAPY OF LEISHMANIASIS	169
	KALA AZAR	169
	CUTANEOUS AND MUCO-CUTANEOUS LEISHMANIASIS	216
VI.	CHEMOTHERAPY OF TRYPANOSOMIASIS	221
VII.	CHEMOTHERAPY OF PIROPLASMOSIS	308
	OROYA FEVER	311
VIII.	CHEMOTHERAPY OF SYPHILIS	312
IX.	CHEMOTHERAPY OF YAWS	407
X.	CHEMOTHERAPY OF RELAPSING FEVER	418
	SPIROCHÆTAL JAUNDICE	423
XI.	CHEMOTHERAPY OF MYCOTIC DISEASES	426
XII.	CHEMOTHERAPY OF LEPROSY	430
XIII.	CHEMOTHERAPY OF TUBERCULOSIS	466
XIV.	CHEMOTHERAPY OF GRANULOMA INGUINALE	476
XV.	CHEMOTHERAPY OF ACUTE BACTERIAL INFECTIONS	480
XVI.	CHEMOTHERAPY OF VIRUS DISEASES	514
XVII.	CHEMOTHERAPY OF CANCER	515
	INDEX	526

RECENT ADVANCES IN CHEMOTHERAPY

CHAPTER I

INTRODUCTION

THE word "chemotherapy" literally means the treatment of disease by chemicals. It should, therefore, include all forms of chemical treatment, whether the object is to eradicate the cause of the disease or merely to alleviate its symptoms. As originally defined by Ehrlich, and as now generally understood, however, chemotherapy has a somewhat narrower connotation and implies the treatment of parasitic diseases by chemicals with the object of destroying the specific parasites of the disease. Wright (1927), in fact, would prefer to call this specific anti-parasitic chemotherapy "Pharmacotherapy," since it implies treatment by chemical substances other than those naturally occurring in the body or produced in the body during the process of immunisation.

Although the idea of driving out the cause of disease by drugs is by no means new, and is to be found in the medical prescriptions of the ancient Egyptians, yet the great contribution of Ehrlich (1854–1915) to medicine lies in his recognition of the fact that there are chemicals which are specifically parasiticidal *in vivo*, such remedies as quinine in malaria, ascaridole in hookworm disease and emetine in amœbic dysentery constituting clues which the chemist can use as starting points in researches designed to find the ideal curative drug.

The foundation of chemotherapy as a science is, in fact, due almost entirely to the work of Ehrlich. Struck by th fact that certain vital dyes are able to stain specifically certain cellular

elements, Ehrlich conceived the idea that chemical substances might be produced which would unite with and destroy the parasitic agents of disease without in any way injuring the cells of the body. This theory of the action of chemotherapeutic agents formed an essential part of Ehrlich's side-chain theory. No chemical could hope to have a parasiticidal action unless it was fixed by the parasites—" corpora non agunt nisi fixata." Ideally, any chemical substance which is fixed by the parasites or is parasitotropic should not be fixed by the tissue cells and should not, therefore, be organotropic. In practice, however, every substance which is toxic to parasites is also more or less toxic to the body tissues, the object of chemotherapeutic research being to produce substances which are tolerated by the tissues in large doses, but are fatal to the parasites in small doses. The ratio

$$\frac{\text{Maximum tolerated dose}}{\text{Minimum curative dose}} = \text{Chemotherapeutic index.}$$

The greater the chemotherapeutic index of any chemical compound the greater therefore is its efficiency.

Ehrlich's theory of the action of chemotherapeutic agents thus implied that there is a direct action by the chemical on the protoplasm of the parasites. More extensive observations have shown that unfortunately chemotherapeutic action is not as simple as this. Emetine, certain anthelminthics and possibly Bayer 205 may perhaps have a direct action on some parasitic entamœbæ, on certain worms and certain trypanosomes respectively, but, with the majority of remedies, there is no evidence of any direct action on the parasites. The chemicals must therefore act indirectly through their reactions with the body tissues.

The mechanism of parasiticidal activity may therefore call into play any one or more of the following factors :—

(i.) A direct chemical interaction between the compound, as administered or after transformation in the body, and some protoplasmic constituent of the parasite, resulting in the death or injury of the latter by interference with its vital processes.

(ii.) A physical or physico-chemical interaction with the protoplasmic colloids of parasites, involving precipitation, coagulation,

changes in electrical charge, etc., sufficient to destroy or injure the parasites.

(iii.) The production of new compounds from the tissues as a result of the action of the chemotherapeutic agent on tissue-constituents, these new compounds being capable of chemical or physico-chemical interaction with the protoplasmic constituents of invading parasites.

(iv.) The production of antibodies by the release of antigenic substances from the parasites.

Even when a chemical compound has been found which is capable of destroying the parasites by one or more of these means it does not follow that a successful chemotherapeutic agent has been evolved, for, apart from any toxic action on the tissues of the parasite's host, other factors may militate against efficient chemotherapeutic action. In the first place, the drug may be so readily changed in the body or may be so rapidly excreted that it does not remain in a parasiticidal concentration for a sufficiently long period of time. In the second place, the parasites may be situated in a tissue where they cannot be reached by a parasiticidal concentration of the drug. Finally, either as the result of selection or of mutation, the parasites may become resistant to the drug.

The selection of a suitable chemotherapeutic agent is thus a matter beset with very considerable difficulties.

The experimental production of diseases in suitable laboratory animals has, however, provided one means of overcoming certain of the difficulties encountered. The chemotherapeutic utility of the arsphenamines in spirochætal infections, of Bayer 205 in trypanosomiasis and of plasmoquin in malaria could hardly have been determined had it not been for the use of laboratory animals infected with spirochætes, trypanosomes and plasmodia. Although the fact that a particular drug has a chemotherapeutic action in experimental infections does not necessarily imply that it will have a similar action in the same infection in man, nevertheless, broadly speaking, it may be said that any drug which is devoid of action in experimental animals will also be devoid of action in man.

In order to ensure that chemotherapeutic results in animal infections are of value, the following precautions must be taken :—

(i.) A standard strain of animals must be bred under standard environmental conditions as regards light, diet, temperature.

(ii.) The use of animals not differing greatly from the normal weight of their species and at an age which is comparatively uniform.

(iii.) The use of standardised methods of obtaining the required pathological condition.

(iv.) The use of a sufficiently large number of animals for each experiment in order to reduce any uncertainty concerning the results.

(v.) The use of a standard infection as regards the virulence and number of the organisms injected.

(vi.) Comparison of the therapeutic action of the unknown compound with that of a substance of known activity, preferably belonging to the same chemical series, the two drugs being tested simultaneously in two groups of animals, preferably litter mates, injected with suspensions of the same organism.

In perhaps no other department of science is there psychologically so great a will to success as in chemotherapy. Throughout the literature of pharmaceutical chemistry, more especially that of certain continental countries, compounds are hailed, by their discoverers at least, as the final solution of the problem in hand, substances which to judge by the efficacy and freedom from toxicity claimed for them should be of immense service to humanity, yet which in the light of more critical examination prove to be only of mediocre activity if not distinctly harmful.

Some of the pitfalls which befall the laboratory worker in determining chemotherapeutic efficiency have already been indicated. It now remains to deal with the precautions which must be taken by the clinician in order to determine the chemotherapeutic efficiency of any drug :—

(i.) The cases treated should represent an average sample of the population commonly affected by the particular disease.

(ii.) A sufficiently large number of cases should be investigated to ensure against errors of random sampling.

(iii.) The cases treated should be living under standard environmental conditions to which they have been accustomed before the special treatment is begun.

(iv.) No other treatment should be given at the same time as the special treatment.

(v.) The patients should remain on the treatment for a sufficient length of time to ensure the maximal therapeutic effect.

(vi.) The patients should remain under observation for sufficiently long to ensure that the infection has been entirely eradicated and is not temporarily in abeyance.

(vii.) During the period of observation there should be no risk of reinfection.

(viii.) A sufficiently large number of controls must be placed under conditions absolutely similar to those of the treated cases except in regard to the special treatment.

It is realised that these conditions represent an ideal which can be only rarely, if ever, attained, nevertheless the closer the approximation to these conditions the greater will be the statistical value of the results obtained by clinicians.

This is not the place to enter into any discussion of the theory of statistics, nor of the methods employed in assessing the significance of results. For any detailed account of these matters reference should be made to the text-book by Yule (1927), or, in the particular sphere of medical statistics, to that of Raymond Pearl (1923), while for dealing with small groups of animals Fisher's " Statistical Methods for Research Workers " (1928) is invaluable, as it gives methods not found in other works. Nevertheless the matter is of such importance that little solid progress can be made in chemotherapeutic research until both the laboratory worker and the clinician realise the errors into which lack of elementary statistical knowledge may lead them.

Topley and Wilson (1929) have recently discussed certain of these errors in connection with work on immunity and infection. Their remarks may be summarised, even in a book of this kind, with reference to their bearing also on these same statistical problems in chemotherapeutic research.

The majority of chemotherapeutic problems are of the following

nature: In order to test the influence of a particular drug in a given infection, m animals are treated and n are left as controls. It is noted that p per cent. of the m animals die and q per cent. of the n controls. What reliance can be placed on an observed average value? Is the difference between two observed values sufficiently great to be significant?

If an experiment, in which the fate of m test animals and n normal controls is involved, is repeated a large number of times, there is not observed the same difference between the mortalities in the test and control series every time. What is required is some measure of the degree of variability which will enable us to calculate the reliability of the mean or the significance of the difference. The measure of variability adopted is known as the Standard Deviation. The ratio of a particular difference to its standard deviation enables one to calculate, on certain assumptions, how often a difference as great as, or greater than this, should be encountered, if the two samples under comparison were really identical in all relevant characters and the difference actually observed were due solely to chance in the statistical sense. Thus if the observed difference is equal to about two-thirds of its own standard deviation it is as likely as not that in any particular experiment there will be observed a difference between the test animals and controls at least as great as this, even though the experimental procedure has been without influence on the result. If the difference is one and a half times as great as its standard deviation the odds against its being due to statistical chance, that is to errors of random sampling, become about 5 to 1. When the ratio increases to 2 to 1, the odds increase to about 22 to 1. With a ratio of 3 to 1 the odds are about 400 to 1, and with a ratio of 4 to 1 they become about 16,000 to 1.

Thus by comparing the observed difference with its standard deviation it is possible to calculate the probability that it is a real, and not an accidental difference, *i.e.*, that it is significant in the statistical sense.

To enable this test to be applied to cases recorded in chemotherapeutic literature, the relevant formula may be given, without proof.

If of n animals x die and y survive under one set of conditions

and of n^1 others x^1 die and y^1 survive under other conditions, then the mean chance of death for both cases together is $\frac{x + x^1}{n + n^1}$, which is usually expressed p_o, and the mean chance of survival is $1 - p_o$, which is expressed as q_o. The difference in the number of deaths is $x - x^1$, and the standard deviation of this difference is equal to $\sqrt{p_o q_o (n + n^1)}$. If proportions of deaths are used instead of actual numbers the difference becomes $\frac{x}{n} - \frac{x^1}{n^1}$ with a standard deviation of $\sqrt{p_o q_o \left(\frac{1}{n} + \frac{1}{n^1}\right)}$, and if these proportional percentage values are multiplied by 100, percentage values are obtained.

To give a concrete example, suppose forty animals are given a particular disease, twenty are left untreated and twenty are given a chemotherapeutic drug. If all the control animals die, the ratios between the observed difference and its standard deviation for sixteen, twelve or eight deaths among the treated animals are 2·1, 3·16 and 4·14 respectively, so that when all the controls die at least eight of the treated animals must survive for the result to attain the degree of significance represented by odds of 400 to 1. If, however, five of the controls live, at least fifteen of the treated animals must survive for this degree of significance to be attained, while if half the controls live, not more than one of the treated animals must die.

Although it is always desirable to apply this simple test for statistical significance to any observed difference in mortality it must not be supposed that it is a mechanical device which can be applied blindly or which yields infallible results. The method employed in calculating the standard deviation is based on certain assumptions which may not always be justified (Greenwood (1913): this paper gives methods for estimating the significance of differences for small groups and for small values of p_o). All that the statistical test can do is to say that a particular difference is not likely to be due to chance, as defined for the particular case of random sampling. It cannot detect the intervention of chance in the wider sense.

The application of simple statistical methods to chemotherapeutic results, whether obtained as the result of research or of clinical experience, would lead however to much saving both of time and energy.

To apply to chemotherapy the words of Topley and Wilson (1929) in regard to the study of infection and resistance: "One of the most valuable gifts that an investigator in this field can cultivate is a healthy scepticism, especially if he learns to apply it to his own results and conclusions as well as to those of others."

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CHAPTER II

THE CHEMOTHERAPY OF HELMINTHIC INFECTIONS

THE most primitive chemotherapy of which there is record was undoubtedly concerned with the finding of some remedy for the intestinal worms which so frequently parasitize man and his domestic animals. The passage in the fæces of large round worms and tapeworms could not fail to attract notice at a very early stage of man's history, and, though the relationship between cause and effect is difficult to determine, it is probable that in the course of time the evacuation of these worms came to be associated with the eating of certain substances. Since vegetables form a large part of the food supply of primitive man, it is not surprising that the earliest known anthelminthics were also of vegetable origin. It is, in fact, only within recent years that organic compounds, other than those of plant origin, have come into use in anthelminthic treatment. Among the many hundreds of vegetable substances which were formerly used as anthelminthics, some, such as vermouth (wormwood), are now put to different uses; others, such as male fern, which was esteemed by Theophrastus (370–285 B.C.), Pliny (A.D. 23–79) and Galen (A.D. 130–201) still retain their old prestige.

Hall (1928) divides the study of anthelminthics into three periods. The first of these was characterised by the purest empiricism when any drug which would remove some worms was recommended as an anthelminthic, without consideration of the number of worms which remained behind. This first epoch of empirical medication, with its constantly growing number of unstudied drugs, came to an end with the recognition of the economic importance of hookworm disease. In 1880 male fern was proposed for the treatment of hookworm disease by Perroncito, and thymol by Bozzolo in 1881, while chenopodium

was reported on unfavourably by Baumler (1881). Perroncito's recommendation of male fern was based on tests on hookworm larvæ *in vitro*, Bozzolo's proposal of thymol on clinical experience, while Baumler's dissatisfaction with the use of oil of chenopodium in ankylostomiasis was due to the unsatisfactory treatment of a single case, following which more than thirty years had to elapse before Schüffner and Verwoort (1913) reported favourably on its use.

Meanwhile Bentley (1904) had found β-naphthol satisfactory. Treatment of ankylostomiasis by male fern passed out of fashion as a result of the clinical successes obtained with thymol, while in time chenopodium came to have preference over both thymol and β-naphthol, although these drugs are still used for the removal of human hookworms. This second period of critical empiricism was characterised by a growing tendency to test the absolute and relative efficacy of the various anthelminthics used for the removal of hookworms. The evacuation of worms and the clinical improvement were naturally the first criteria used in formulating judgments on the drugs. However, the worms removed told nothing about those which remained, and clinical improvement could not be accurately measured. The examination of fæces for worm eggs before and after treatment gave information of some value, but there are many pitfalls in this procedure, more especially in unskilled hands. Attempts to correlate the egg-count with the number of worms present, by various methods, gave additional but only approximate information. Further evidence, however, was obtained by using a more or less standard treatment following a test treatment, the worms removed on both occasions being carefully counted. This method, which can easily be applied to man, has given much information as to the value of anthelminthics, but it lacks that exactness which is necessary if research into the subject of anthelminthic medication is to be placed on a scientific basis.

The third epoch dates from the year 1915, when, in the United States, Hall and his colleagues introduced a critical method for testing anthelminthics. This was not, however, the first time that critical testing had been attempted, since Grassi and Calandruccio (1884) and Perroncito (1880) had already devised

a method of critical testing in their investigations on liver fluke disease, and had established by post-mortem examinations of sheep that male fern can kill liver flukes. Both as a practical proposition and as a scientific procedure, however, their work remained neglected for more than a quarter of a century.

The method of critical testing which Hall adopted in studying anthelminthics was to administer known doses of drugs to animals of various species, to collect all the worms from the fæces for at least four days, sometimes for weeks or months, to kill the animals, and at the autopsy collect, identify and count all the worms present. This method gives exact information as to the number of worms originally present, the number removed, and the number left after treatment. It also enables new drugs to be tested which it would be inadvisable to administer to human beings without preliminary tests on animals. Experience has shown that many of the experimental findings are applicable in human medicine with but little modification.

By using the method of critical testing Hall and his co-workers have been enabled to introduce two drugs of the greatest importance in both human and veterinary medicine—carbon tetrachloride and tetrachlorethylene. Carbon tetrachloride was proposed as an anthelminthic in 1921. Since then it has been employed in some millions of cases of human hookworm disease, and in combination with oil of chenopodium or with the active constituent of the latter—ascaridole—it is now the drug most commonly used in cases of combined hookworm and ascaris infection. It is rapidly becoming the standard drug for the removal of hookworms and ascaris from dogs and cats, and is of value for stomach worms and small trichostrongyles in sheep and for ascaris, strongyles and bots in horses. Recently also it has been found to be of great value in the destruction of liver flukes in sheep, for which purpose it has to a large extent replaced male fern and the other drugs previously used. Tetrachlorethylene, which was introduced in 1925, though no more effective than carbon tetrachloride in human infection, is probably more efficacious and less toxic in removing hookworms and ascarids from dogs and foxes.

A revolution has also occurred in the treatment of schistosomiasis, for with the introduction of tartar emetic and emetine it is now possible to cure the greater number of infections due to schistosomes both in man and animals. The use of gentian violet in the treatment of experimental clonorchiasis is also of promise, although insufficient time has yet elapsed to determine the value of the drug in human cases of this disease.

Side by side with these curative experiments a large amount of work has been carried out, largely by Caius and Mhaskar in India, in an attempt to correlate anthelminthic action with molecular constitution. The results which have been achieved are by no means conclusive.

Bound up with the correlation of chemical composition and anthelminthic action is the question of the actual mode of action of anthelminthics, of which nothing is definitely known. This is a subject which might well be studied more thoroughly by experiments on parasitic worms *in vitro*, though it should be said that toxicity tests on earthworms *in vitro* tell nothing about the value of anthelminthics for parasitic worms, an apparently self-evident fact which seems to have been forgotten or overlooked by a number of workers.

Anthelminthic therapy still has many problems to solve. A vast number of drugs has been proposed for the treatment of dracontiasis, though, as Fairley (1924) points out, even if some drugs cause the death of the worm they do not cause its absorption, so that surgical measures are still indicated. Fairley and Liston (1924) in India failed to confirm the good results obtained by Macfie (1920) in Africa, using 1-gr. doses of tartar emetic intravenously every other day up to six doses. Filariasis is unaffected by any known drug, as shown by Anderson (1924), though Chopra and Rao (1929) have recently claimed successful results with tryparsamide in clearing up the chyluria, while Dalal (1927) states that injections of neoarsphenamine are effective in reducing the fever. Hydatid cysts yield only to surgical interference, though recent *in vitro* tests by Ross (1927) suggest that brood capsules and scolices may be killed by acriflavine diluted 1 in 10,000 in eight hours, and 1 in 100,000 in seventeen hours.

Finally, though the treatments are many, there is no known cure for oxyuris infection.

In human medicine the anthelminthic drugs which are at present regarded as of value in the treatment of the common intestinal worms are :—

(1) Oil of chenopodium and its active constituent ascaridole.
(2) Carbon tetrachloride.
(3) Tetrachlorethylene.
(4) Santonin.
(5) The latex of *Ficus laurifolia.*
(6) β-naphthol.
(7) Thymol.
(8) Tanret's pelletierine.
(9) Male fern.

While of use in veterinary medicine are :

(10) Carbon bisulphide.
(11) Copper sulphate.
(12) Kamala.
(13) Nicotine.
(14) Areca nut.

Finally for worms outside the digestive tract in man there are :—

(15) Tartar emetic and sodium antimonyl tartrate.
(16) Emetine.
(17) Gentian violet (?).

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ANKYLOSTOMIASIS

HALOGEN DERIVATIVES OF CARBON

(A.) Carbon tetrachloride

Owing to the fact that in certain tropical and subtropical countries a large percentage of the population is infected with hookworms, the discovery of some anthelminthic having a high degree of toxicity to hookworms and a low degree of toxicity to man is a matter of the greatest importance. The ideal anthelminthic is, of course, one which is capable either directly, or indirectly, as a result of its interaction with the tissues, of destroying all the infecting parasites without in any way injuring the host. Such an ideal anthelminthic has not yet been discovered, although a gradual approximation to the ideal is being attained. While thymol, β-naphthol, chloroform, and Manson's mixture of chloroform and eucalyptol still have their uses in the treatment of individual cases of ankylostomiasis, their employment for the treatment of mass infections has been largely superseded by oil of chenopodium and carbon tetrachloride.

Caius and Mhaskar (1920) found that chloroform was by no means inefficient in destroying hookworms; they suggested, however, that some related compound with a higher halogen content might prove even more efficient, since the anthelminthic value of chloroform appeared to be correlated with its chlorine content. For substantially the same reasons Hall (1921[1]) was led to test the anthelminthic power of carbon tetrachloride (CCl_4) against hookworms in dogs. The results were remarkable, for with a dose of 0·3 c.cm. per kilogram of body weight, all the hookworms were expelled, a finding which had never before been obtained with any previously tested drugs, such as thymol, chenopodium, chloroform and combinations of chloroform and chenopodium. In order to test its toxicity to man, Hall himself took a dose of 3 c.cm. of carbon tetrachloride by mouth without any dire results. When doses of carbon tetrachloride, even up to 1·5 c.cm. per kilogram of body weight, were given to dogs there were no macroscopic changes in the liver, though necrosis of the

liver in dogs was commonly produced by doses of chloroform of 0·3 c.cm. per kilogram of body weight. Hall (1921[2]) therefore felt justified in recommending carbon tetrachloride as an anthelminthic against hookworm in man. Successful results were quickly reported from various parts of the tropics. Thus, Caius and Mhaskar (1923) in India tested the effects of gradually increasing doses of carbon tetrachloride, the drug being shaken up with an ounce of gum acacia and dispensed in a single dose, to be followed, in one test, by a purgative. The total number of hookworms harboured by each individual was determined by the subsequent administration of 60 gr. of thymol.

Percentage of hookworms removed by one test treatment of Carbon tetrachloride (Caius and Mhaskar, 1923)

Test treatment.	Number of cases treated.		Hookworms removed with one test treatment.			Percentage of hookworms removed with one test treatment.			Percentage of cases cured with one test treatment.
			A. duodenale.	*N. americanus.*	*A. duodenale and N. americanus.*	*A. duodenale.*	*N. americanus.*	*A. duodenale and N. americanus.*	
1 c.cm. (with purge)	22	Test treatment .	3	437	440	11·5	90·8	86·7	33 3
		Subsequent treatment	23	44	67				
		Total hookworms .	26	481	507				
2 c.cm. (with purge)	21	Test treatment .	113	1,023	1,136	72·4	89·6	87·4	42·8
		Subsequent treatment	43	118	161				
		Total hookworms .	156	1,141	1,297				
3 c.cm. (with purge)	20	Test treatment .	81	1,519	1,600	76·4	96 3	95·7	45·0
		Subsequent treatment	25	58	83				
		Total hookworms .	106	1,577	1,683				
4 c.cm. (with purge)	22	Test treatment .	134	1,117	1,251	93·0	98·1	97·6	77·2
		Subsequent treatment	10	21	31				
		Total hookworms .	144	1,138	1,282				
5 c.cm. (with purge)	24	Test treatment .	85	1,055	1,140	96·5	99·9	99·7	83·3
		Subsequent treatment	2	2	4				
		Total hookworms .	87	1,057	1,144				
5 c.cm. (no purge)	52	Test treatment .	74	1,219	1,293	94·8	99·0	98·7	67·3
		Subsequent treatment	4	12	16				
		Total hookworms .	78	1,231	1,309				

Carbon tetrachloride was thus shown to be highly effective against hookworms, though it had but little effect on ascaris or oxyuris in man. While these encouraging results were being obtained, Smillie and Pessôa (1922) found that in certain individuals toxic symptoms, with hæmaturia, developed after the administration of carbon tetrachloride. Meyer and Pessôa (1923) and Davis (1924) therefore examined the microscopical lesions produced by carbon tetrachloride in dogs. In the liver there was noted at the periphery of the lobules fatty infiltration, in the centre of the lobules necrosis of the liver cells and round-celled infiltration. In the kidneys the microscopic changes consisted of fatty changes in the epithelial cells lining the convoluted tubules and loops of Henle and occasionally congestion in the glomeruli. Similar changes were described in man by Docherty and Burgess (1922), who, in Ceylon, treated three condemned criminals with carbon tetrachloride, two receiving 5 c.cm. by mouth, the third 8 c.cm. in two doses. At the subsequent post-mortem all the prisoners were free from ancylostoma and ascaris, but in all cases the liver showed necrosis and fatty change. These findings necessitated a thorough investigation of the pharmacology and toxicity of carbon tetrachloride.

When given by mouth carbon tetrachloride passes for the most part through the stomach unchanged. Absorption probably takes place throughout the whole length of the large and small intestines and is apparently complete in from twenty-four to thirty hours, though the rate of absorption may be increased by the addition of alcohol or fatty substances. Wells (1925) found that a high percentage of the absorbed drug was rapidly excreted in the expired air. Degenerative changes in the liver were produced by whatever route the drug was given, and were noticeable as early as twelve hours after administration. Repair began in from three to four days, but was not usually completed until after the lapse of five weeks. Lamson and Wing (1926) found that, as might be expected, repeated administration of carbon tetrachloride to dogs produced early cirrhotic changes in the liver; the blood fibrin was also reduced by carbon tetrachloride administration. Lamson and McLean (1923) investigated the

liver function following the administration of carbon tetrachloride. When a dose of 2 c.cm. per kilogram of body weight was given to dogs the liver function as tested both by the phenol tetrachlorophenol phthalein and lævulose tests was not deranged, but with doses of 4 c.cm. per kilogram there was definite impairment with a return to normal within ninety-six hours. In human cases of carbon tetrachloride poisoning Straub (1925) has found that the serum gives both a direct as well as an indirect positive van den Bergh reaction.

A curious fact, however, was noted by Lamson, Gardner, Gustafson, Maire, McLean and Wells (1923) when investigating the toxicity of carbon tetrachloride for dogs, for it was found that dogs which had received a well-balanced diet showed no signs of intoxication after very large doses. Minot (1927) next reported that if dogs were kept on a diet of lean meat without bones, even very small doses of the drug caused a severe and usually fatal poisoning. When 3 or 4 c.cm. per kilogram of body weight were given to dogs thus fed the following events took place. During the first twelve to twenty hours no symptoms other than slight nausea and malaise were noted, though diarrhœa occasionally occurred. Then the dogs became definitely ill, with fibrillary tremors in most of the skeletal muscles. Visible twitching of the paws and lips soon followed and gradually became more marked. Occasionally there were violent general convulsions, especially after some external stimulus. At this stage a foul blood-stained diarrhœa was seen with vomiting of black material containing blood. Death sometimes occurred during coma, but more commonly the animals gradually grew progressively weaker without losing consciousness.

The occurrence of cases of acute intoxication only after a radical reduction in dietary calcium as well as the similarity of the convulsive symptoms to the tetany associated with calcium deficiency suggested the treatment of the poisoned animals with calcium salts. The administration of calcium chloride intravenously or of Collip's parathyroid extract, did, in fact, bring about a rapid cure in the intoxicated animals. Chemical investigations on dogs intoxicated by carbon tetrachloride by

Minot and Cutler (1928) showed that there is a definite retention of guanidine in the blood, bilirubinemia and hypoglycemia. There appears, in fact, to be some correlation between the high guanidine content of the blood and the symptoms of carbon tetrachloride poisoning, for if guanidine hydrochloride is administered to a normal dog so as to produce a high concentration in the blood, symptoms of nervous irritability, gastro-intestinal irritation, hypoglycemia and death occur. When calcium is given to dogs poisoned either by guanidine or by carbon tetrachloride, the nervous symptoms are relieved and the blood-sugar level is restored to normal. There are, however, at least two antagonistic actions between calcium and guanidine, because if the blood sugar is raised by dextrose administration the nervous hyperexcitability persists until calcium also is given. At least three factors therefore seem to contribute to the acute need for calcium in carbon tetrachloride poisoning, (i.) a deficient calcium intake, (ii.) the necessity for increased calcium to combat the effects of guanidine, and (iii.) the reduction of ionised calcium in the blood by combination with retained bile pigments.

In addition to calcium salts and parathyroid extract, the administration of ammonium chloride or hydrochloric acid also counteracts the toxic effect of carbon tetrachloride by increasing the ionised calcium of the blood.

Lamson, Minot and Robbins (1928) regard calcium deficiency as a possible cause of many of the fatal results recorded as following the administration of carbon tetrachloride. They therefore recommend that children especially should be given milk or calcium lactate for some days before receiving the carbon tetrachloride. Other predisposing causes of death after carbon tetrachloride administration are chronic alcoholism, the presence of undigested fat in the intestine and mechanical obstruction by ascaris, which is stimulated to great activity by the drug. It is also essential to employ perfectly pure carbon tetrachloride. Thus Khalil (1926[1]) tested one consignment of carbon tetrachloride on a dog of 10 kgm. and a monkey of 3 kgm. body weight, the dog receiving 160 c.cm. and the monkey 20 c.cm. without any ill effects, but when a second consignment was similarly

tested the monkey died next day, the lumen of the small intestine being filled with bloodstained mucus. It is believed that the toxic effects of this sample of carbon tetrachloride were due to sulphur compounds which distil below 70° C., for by discarding the first portion of the distillate the toxicity of the carbon tetrachloride was destroyed. Although carbon bisulphide can be added to carbon tetrachloride without increasing the toxicity of the latter for laboratory animals, it seems that if the carbon bisulphide is removed, the unknown sulphur compounds are also eliminated. As a simple test for the presence of carbon bisulphide, Perkins (1924) recommends the following :—10 c.cm. of the sample of carbon tetrachloride are boiled and agitated for a few minutes with 3 c.cm. of an alkaline solution of potassium plumbite and 1 c.cm. of absolute alcohol. The mixture is then allowed to stand. A darkening of the upper layer signifies the presence of carbon bisulphide. The alkaline plumbite reagent is prepared by dissolving 0·5 gm. of lead acetate in 20 c.cm. of water and then adding 20 gm. of pure caustic potash. The test will detect 5 parts of carbon bisulphide in a million.

A pure sample of carbon tetrachloride should also pass the following tests :—

(i.) When 10 c.cm. of the sample are shaken with 20 c.cm. of distilled water for five minutes and time is allowed for the complete separation of the liquids, the wash water employed—

(*a*) Should be neutral to litmus.

(*b*) Should show not more than a slight opalescence when treated with 4 drops of a solution of silver nitrate (5 gm. silver nitrate dissolved in 100 c.cm. distilled water).

(*c*) Should not give any colour with 1 c.cm. of a solution of cadmium iodide (5 gm. cadmium iodide dissolved in 100 c.cm. distilled water) and 2 drops of mucilage of starch.

(ii.) Twenty-five c.cm. of the sample should leave no residue when evaporated on a water bath.

(iii.) After shaking 20 c.cm. of the sample with 10 c.cm. of pure sulphuric acid for fifteen minutes, both the acid and the carbon tetrachloride should be nearly colourless.

(iv.) A small well-cleaned silver coin should show no darkening when immersed in the sample for ten minutes.

(v.) No positive reaction should be obtained with Schiff's reagent.

(vi.) The benzidine test should be negative.

Up to the present carbon tetrachloride has been used in some millions of cases, with a very low fatality rate and in the mass treatment of hookworm infections it has given results far superior to those previously obtained with oil of chenopodium alone. In Fiji, for instance, Lambert (1928) recorded the treatment of three million cases, with the result that three years after the last treatment clinical cases of hookworm infection were still rare. If precautions are taken regarding the purity of the carbon tetrachloride used and the calcium deficiency of the patient's diet is remedied there seems no reason why the percentage of fatalities should not be still further reduced. Bais (1924), also, in the Dutch East Indies, reported that despite ten years' use of oil of chenopodium, the disease was still rife, yet after two mass treatments with carbon tetrachloride in one and a half years, the health of the population was immensely improved, the average worm count dropping to thirteen.

Soper (1924) has suggested that in the routine treatment of hookworm, oil of chenopodium should be combined with carbon tetrachloride. As Smillie and Pessôa (1925) point out, carbon tetrachloride in diminished doses, when used in the treatment of hookworm disease, has a selective action on female hookworms. Ascaridole, the active principle of chenopodium, in diminished doses has a selective action on male hookworms, and is very efficient in removing ascaris. A mixture of the two drugs does not increase their toxicity towards the host, since ascaridole affects mainly the central nervous system, while carbon tetrachloride affects chiefly the liver. Two c.cm., consisting of 4 parts of carbon tetrachloride and 1 part of ascaridole, would seem to be an efficient mixture, especially when *Ancylostoma duodenale* and not *Necator americanus* is the predominant parasite. Against the latter carbon tetrachloride alone is highly effective. The exact way in which carbon tetrachloride acts upon parasitic worms does not appear to

have been investigated, though, judging from its similarity to chloroform, it seems not improbable that carbon tetrachloride interferes with certain of the cell enzymes. Even small doses of carbon tetrachloride inhibit the egg-laying capacity of the hookworms, though according to Sweet (1924) the depressing effect, as judged by egg-counts, passes off within five days. Manalang (1927) has found that when hookworms obtained at autopsy are left in clean water at room temperature (25° to 30° C.) for twenty-four hours and are then crushed between two slides, there are released, either motile free swimming larvæ, or, at least, coiled larvæ moving within the shells. After the administration of carbon tetrachloride, however, no active larvæ are produced, either free swimming or motile within the shell. Instead, the ova are swollen, the shells being filled with fine granulations, while in others the shell can hardly be distinguished. Fat globules are frequently seen within the ova. Ova expelled after treatment with thymol or oil of chenopodium develop normally.

Although carbon tetrachloride is probably the most effective drug known for the expulsion of hookworms of the genus *Necator*, it is markedly less effective for *Ancylostoma* infections, not more than 30 to 40 per cent. of cases being completely cured. On *Ascaris*, carbon tetrachloride has some action, though less marked than that of santonin. *Oxyuris* is also expelled in large numbers, but *Trichuris* and *Strongyloides* are unaffected.

Tænia and *Hymenolepis* are not destroyed in the dog, but in man carbon tetrachloride is of value in removing both *T. solium* and *T. saginata*, for Daubney and Carman (1928) found that in the case of boys with fairly light infections of *T. saginata*, carbon tetrachloride has an efficacy of approximately 97 per cent. Barlow (1927) has recently employed carbon tetrachloride in the treatment of fasciolopsiasis in China, while Khalil (1926[2]) and Cawston (1928) have both used it as adjuvant to antimony in the treatment of schistosomiasis.

Carbon tetrachloride has been very extensively used in veterinary practice. While dogs are comparatively resistant to the toxic effects of the drug, foxes, especially young ones, are less resistant; rabbits are very susceptible. Chandler and Chopra

(1925) found that cats show toxic symptoms after doses of 0·25 c.cm. per kilogram of body weight, a result which may possibly be correlated with lack of calcium in the diet.

Montgomerie (1926), as the result of experiments with sheep, came to the conclusion that while these animals were not very susceptible to the toxic effects of carbon tetrachloride, they were readily cured of liver flukes by a dose of 1 c.cm. for a sheep weighing 140 lb. Only mature flukes, however, were destroyed. Montgomerie (1928), therefore, experimentally infected sheep with flukes. He found that when the period between infestation and the dose of 1 c.cm. of carbon tetrachloride was eight weeks, or less, few, if any, parasites were killed, while if the time interval was increased to ten weeks, nearly all the flukes were destroyed. Ten c.cm. killed some of the parasites after four weeks' infestation, and almost all five to eight weeks after infestation. According to Hall and Shillinger (1923) carbon tetrachloride is effective in every case of stomach worms of sheep. Chickens and turkeys have a very high tolerance, and would seem to be almost immune to the toxic effects of the drug which in doses of from 2 to 5 c.cm. per kilogram of body weight is usually effective against *Ascaridia* and *Heterakis*.

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(B.) Tetrachlorethylene

In a search for other halogen derivatives of carbon which should be less toxic than carbon tetrachloride, but equally, if not more, effective against hookworms, Hall and Shillinger (1924) tested the anthelminthic effects of ethylene dichloride ($C_2H_4Cl_2$). This substance was less efficient than either chloroform or carbon tetrachloride.

Hexachloroethane or carbon trichloride (C_2Cl_6) was also quite ineffective as an anthelminthic (Hall and Cram, 1925) against hookworms in dogs, although quite recently Hilz and Scheuble (1928) have employed carbon trichloride against liver flukes in sheep and cattle with satisfactory results, the drug being specially effective when injected intraperitoneally.

Tetrachlorethylene, more correctly tetrachloroethylene, (C_2Cl_4), however, was found by Hall and Shillinger (1925 [1, 2]) to be quite as efficient as carbon tetrachloride in removing hookworms from dogs in doses of from 0·2 to 0·3 c.cm. per kilogram of body weight. Lambert (1925), who tested the drug on man, found that the mild symptoms of intoxication were similar to those produced by carbon tetrachloride, over which it had no advantages in taste or colour. Soper (1926) reached similar conclusions. Carbon tetrachloride, either alone or in combination with oil of chenopodium, was more efficient against *Necator americanus* than was tetrachlorethylene. When given alone, the superiority of carbon tetrachloride for *Ancylostoma duodenale* was not apparent, but when combined with oil of chenopodium it was undoubtedly more efficient than tetrachlorethylene. Schapiro and Stoll (1927) found that doses of 3 c.cm. reduced the count of hookworm eggs, on an average, 93 per cent. in fourteen patients examined. In India Maplestone and Mukerji (1929) found tetrachlorethylene less efficient than carbon tetrachloride against hookworms. The toxic effects of tetrachlorethylene were investigated by Schlingman and Gruhzit (1927), who recorded necrotic changes in the liver as with carbon tetrachloride. Chickens were least and horses most susceptible to the drug. Montgomerie (1926) was

unable to cure sheep infected with the common liver fluke by means of doses of 2 c.cm. Hanson (1927), on the other hand, found that tetrachlorethylene was less toxic than carbon tetrachloride to foxes, and was as effective as carbon tetrachloride in the removal of hookworms ; it was more effective in the removal of ascaris, while there was also less danger of intoxication from inhalation.

In one respect, however, tetrachlorethylene is inferior to carbon tetrachloride, namely, in its relative instability, for even at room temperatures the drug may decompose, giving rise to phosgene, hydrochloric acid and chlorine. If, however, it is kept from the air in well-stoppered amber bottles it does not break down on standing. Lamson, Robbins and Ward (1929) have recently investigated the pharmacology and toxicity of tetrachlorethylene. In normal dogs receiving as much as 10 c.cm. per kilogram of body weight, or fifty times the therapeutic dose, there is no absorption from the intestines, but if fat or alcohol is present in the intestine a certain amount of the drug is absorbed. Mice and rabbits apparently absorb the drug rapidly, and pass into a state of narcosis when given amounts of 4 c.cm. per kilogram of body weight. In cats and puppies the tendency to absorb tetrachlorethylene is less than in mice and rabbits, but greater than in dogs.

Tetrachlorethylene failed to produce necrotic changes either in the kidneys or liver, while functional tests for these organs were not in any way abnormal, nor was there any fall in blood sugar or change in blood guanidine even when the dogs were kept on a diet low in calcium.

In view of these findings the possibility of toxic effects on the host appears to be much less with tetrachlorethylene than with carbon tetrachloride.

It was originally suggested by Caius and Mhaskar that the anthelminthic action of the halogen derivatives is correlated with their chlorine content. If such were the case, however, carbon trichloride or hexachloroethane should be even more potent as an anthelminthic than carbon tetrachloride, as although the proportion of chlorine is 3 : 1 instead of 4 : 1, the molecule actually contains six chlorine atoms as compared with four.

Others have believed that the decisive factor in connection with the anthelminthic action of these compounds is the solubility. Thus Kudicke and Borchardt (1926), using strongyloides larvæ as test objects, found that the toxic effects of certain hydrocarbon compounds of chlorine varied with the solvent. When the compounds were dissolved in pancreatic juice the toxic effects paralleled those obtained with watery solutions, but in bile, a better solvent for these compounds, there was a definite lowering of toxicity.

The solubilities of the compounds in question are :—

Ethylene dichloride	$C_2H_4Cl_2$	1 part in 120 parts of water.
Chloroform	$CHCl_3$	1 part in 161 parts of water.
Carbon tetrachloride	CCl_4	1 part in 1,250 parts of water.
Tetrachloroethylene	C_2Cl_4	1 part in 10,000 parts of water.
Hexachloroethane	C_2Cl_6	1 part in over 10,000 parts of water.

It is thus obvious that it not possible to correlate the anthelminthic and toxic properties of these halogenated aliphatic hydrocarbons with either their structural formulæ or their solubilities.

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OIL OF CHENOPODIUM

Before the introduction of carbon tetrachloride, oil of chenopodium was probably the most widely used drug in the treatment of hookworm infection. Even at the present time the use of a mixture of carbon tetrachloride and oil of chenopodium is regarded as preferable by many authorities, more especially when both ascaris and hookworms are to be treated. Others, such as Clayton Lane (1928), regard the adoption of carbon tetrachloride as definitely retrogressive, and would restrict themselves to thymol and oil of chenopodium in the treatment of ankylostomiasis.

Oil of chenopodium is obtained principally from *Chenopodium anthelminticum*, or American wormseed, commonly known as "Jerusalem oak," or "Mexican tea." In India there are at least seven species of *Chenopodium*, but only two of these, *C. ambrosioides* and *C. botrys*. yield an oil with anthelminthic properties. A variety of *C. ambrosioides* is cultivated in Java and yields a good quality of oil. Infusions of the leaves and seeds of chenopodium have long been employed as a household remedy for worms throughout the entire western hemisphere; in fact, there is some evidence to show that infusions of "apazote" were used by the Aztecs centuries before the Spanish conquest.

The essential oil distilled from the seeds was first introduced into therapeutics as an ascaricide, but was not used to any considerable extent in the treatment of ankylostomiasis until Schüffner and Verwoort (1913) tested it for this purpose on the rubber estates of eastern Sumatra with results which were far superior to those previously obtained with thymol. During the next few years oil of chenopodium remained the standard treatment for hookworm infections. Between 1916 and 1921, in Brazil alone, more than a million treatments were carried out by the International Health Board in co-operation with the Brazilian Government. The pharmacological action of oil of chenopodium has been known for some years. In the mouth the oil has a sharp burning taste, which stimulates the flow of saliva and reflexly that of the gastric juice. On passing into the stomach there is a feeling of warmth and slight irritation accompanied by increased vascu-

larity of the gastric mucosa. There would seem to be little or no absorption from the stomach, but absorption from the duodenum is rapid, and if the drug is introduced directly into it characteristic effects immediately result. The peristaltic action of the intestines is decreased, a fact which probably accounts for the constipating effect of the drug in man. Intravenous injection of 0·02 c.cm. of the oil per kilogram of body-weight causes a fall in blood pressure and a depressed action of the heart. The respirations are also slowed, probably owing to a direct action on the respiratory centre of the medulla. Neurotoxic symptoms are very marked in patients suffering from the poisonous effects of the drug, for after transitory stimulation there is much depression, ending in coma and death. The oil is partly excreted by the lungs, while the terpenes and other bodies are principally eliminated through the kidneys.

Little was known as to the effects of large doses of oil of chenopodium until reports began to appear of untoward symptoms following its administration for ankylostomiasis. Hall (1918) suggested that the injury to the patient probably depends on the rate of absorption of the drug, two factors requiring consideration in this connection—the immediate local effect on the gastro-intestinal tract and the more remote irritant and toxic effects on the circulatory, respiratory, excretory and nervous systems.

Salant and Bengis (1917) drew attention to the irritant effects of oil of chenopodium, more particularly on herbivora such as rabbits. The elimination of fat-soluble dyes by the kidneys was partially inhibited as the result of poisoning by oil of chenopodium, while when rabbits, fed exclusively on oats, were given small doses of the oil dissolved in cocoanut oil, by mouth, albumin, and hyaline and granular casts promptly appeared in the urine. A diet consisting of carrots, with a high carbohydrate content, protected against this toxic effect of oil of chenopodium. Molloy (1923) found that in man also the nutritional condition exerts considerable influence on the toxic effects of the drug. Among more than 200,000 cases treated in Nicaragua three children in extreme stages of inanition succumbed to minimum doses of the drug, while a well-nourished American marine survived a dose of 160 minims given in error. A high

carbohydrate diet given for some time before treatment considerably decreased the number of cases of poisoning. Biesin (1929), who has recently analysed the literature, finds that a total of forty-one cases of poisoning and thirty deaths have been recorded.

It has long been known that samples of oil of chenopodium vary in their anthelminthic efficiency. Attempts have, therefore, been made to obtain some stable anthelminthic product from oil of chenopodium.

Schimmel and Company (1908) and Nelson (1911) both separated from oil of chenopodium a considerable portion with a specific boiling point, to which they gave the name ascaridole. Although it has been generally assumed that the value of the oil as an anthelminthic is due to its ascaridole content, Hall and Hamilton (1918) believed that the greatest anthelminthic efficacy resides in the lighter fraction of the oil, the efficacy suffering a diminution as the heavier fractions are used. In view of this confusion, Henry and Paget (1921) undertook a re-examination of the constituents of chenopodium oil. Minute quantities of the lower fatty acids, chiefly butyric acid, and less than 0·5 per cent. of methyl salicylate were present. The remainder of the oil consisted of at least 60 per cent. of ascaridole, with about 5 per cent. of the corresponding glycol and 30 to 40 per cent. of a mixture of hydrocarbons made up approximately of cymene 15 per cent., α-terpinene 5 per cent., and a new lævorotatory terpene 10 per cent., which Henry and Paget (1925) subsequently identified as in all probability $\Delta^{2:8(9)}$ *p*-menthadiene. Small traces of a substance *sym*-dimethylethylene oxide C_4H_8O were also isolated. No evidence was obtained of the presence of sylvestrene, limonene, phellandrene, safrole or camphor, all of which have been suggested or recorded as constituents of the oil.

Caius and Mhaskar (1919) also brought forward evidence to support the suggestion that the anthelminthic action of oil of chenopodium is due to the ascaridole, for on treating the oil with ferrous sulphate the ascaridole is converted into the corresponding glycol and the anthelminthic efficacy of the oil then disappears. Smillie and Pessôa (1924), however, tested the anthelminthic action of the various fractions isolated by Henry and Paget on the

destruction of hookworms in dogs. The fæces were carefully examined after the administration of the substance to be tested, and ten days later the animals were killed and examined for worms. The results were as follows :—

Fraction I A is a mixture of terpenes and has a boiling point of 85° C. at 15 mm. pressure. It forms about 35 per cent. of the total oil, but has no ascaridole. No toxic symptoms followed and no worms were expelled.

Fraction III.—Ascaridole. A dose of 0·05 c.cm. per kilogram of body weight caused no intoxication and removed all the hookworms. A dose of 0·08 c.cm. per kilogram of body weight caused slight intoxication and removed all the worms. The toxæmia was similar to that caused by the entire chenopodium oil.

Fraction IV.—Ascaridole glycol. No worms were expelled. A dose of 1 c.cm. per kilogram of body weight killed the dog.

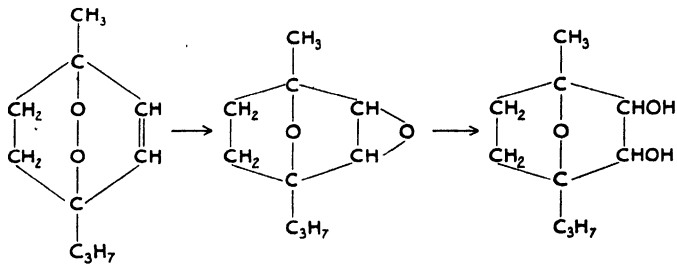
Fraction V.—Ascaridole glycol anhydride. No worms were expelled and there was no toxicity in doses of 1·5 c.cm. per kilogram of body weight.

Methyl salicylate in doses of 0·5 c.cm. per kilogram of body weight produced slight intoxication and removed one out of eight hookworms.

These experiments were followed up by experiments on human cases. The drug to be administered was given on an empty stomach and was followed by a purge two hours later. The fæces were carefully examined for worms, and ten days later a standard dose of oil of chenopodium was given, the number of worms expelled by the standard treatment being also carefully noted.

Drug.	Number of cases.	Average dose in c.cm.	Percentage of worms expelled by drug.
Methyl salicylate	2	2·0	0·9
Fraction I A (terpenes)	5	0·5—2·0	1·5
Fraction III (ascaridole)	17	1·07	94·7
Fraction IV (ascaridole glycol)	15	1·26	0·3
Fraction V (ascaridole glycol anhydride)	16	1·20	1·6
Stock oil of chenopodium	26	1·80	90·3

From these experiments it will be seen that the anthelminthic action of oil of chenopodium is bound up with the structure of ascaridole, even the slight intramolecular change to ascaridole glycol anhydride causing loss of anthelminthic power, while hydration of the anhydride to ascaridole glycol does not restore it. On hydration ascaridole glycol anhydride yields a mixture of α- and β-ascaridole glycols, which in turn can be converted into thymol and carvacrol, which show anthelminthic properties. Starting with an active compound (ascaridole), it is thus possible to pass through three inactive compounds and end up with an active substance, thymol.



In order to avoid the possibility of adulteration of oil of chenopodium, Paget (1926) devised a method of titrating the ascaridole against titanous chloride, 1 gm. of ascaridole being reduced by 1·2770 gm. of titanous chloride.

Considerable discussion has arisen as to the best method of administering oil of chenopodium. Earlier observers recommended 1·5 c.cm. divided into two doses an hour apart. Smillie (1922), however, prefers to give 2 c.cm. of oil of chenopodium, preceded by a purge the evening before treatment and followed two hours after treatment by a saline purge.

Clayton Lane (1928) states that 0·8 c.cm. oil of chenopodium is quite sufficient when the ascaridole content of the oil is 90 per cent. He points out that some of the fatalities with oil of chenopodium have been due to the fact that 48 minims have been given instead of the 48 drops (1·2 c.cm.) measured by the international drop counter, which Schüffner originally recommended.

The after-purge is of considerable importance, since it removes the drug from the gut and thus decreases the risk of poisoning to the host. It also removes the dead and dying worms and any toxins which the defunct worms may be producing. Clayton Lane recommends a dose of 0·5 c.cm. of ascaridole. Smillie and Pessôa (1924), on the other hand, give 1 c.cm. on an empty stomach, followed half an hour later by a saline purge which successfully eliminates the risk of toxic absorption.

As with so many other anthelminthics, the exact action of ascaridole on parasitic worms is unknown. It is suggested, however, that the drug penetrates the cuticle and causes first stimulation and later paralysis of the musculature, for Bliss (1925) has shown that ascaris placed in solutions containing oil of chenopodium in dilutions of 1 in 5,000 to 1 in 10,000 exhibits tonic muscular contractions followed later by paralysis.

The indications against the administration of this drug are malnutrition, gastro-enteritis, gastric stasis and constipation. It also appears to be dangerous during pregnancy, although carbon tetrachloride is not contraindicated.

Recently, prior to the administration of carbon tetrachloride or oil of chenopodium for the removal of hookworms, the use of liver extract has been recommended, as by this means the anæmia, so characteristic of ankylostomiasis, with all its attendant weakness, is counteracted, and the patient is far more able to withstand the toxic effects of the anthelminthic drug.

In comparing oil of chenopodium with carbon tetrachloride, it may be said that while carbon tetrachloride is the more effective against the New World hookworm, *Necator americanus*, oil of chenopodium is slightly more efficient, against the Old World hookworm, *Ancylostoma duodenale*. Chenopodium is far superior to santonin in removing ascaris both from man and from all animals on which it has been tested. Carbon tetrachloride is almost as useful as chenopodium for the ascaris of dogs and cats, and both carbon tetrachloride and carbon bisulphide are more effective in removing ascaris from horses.

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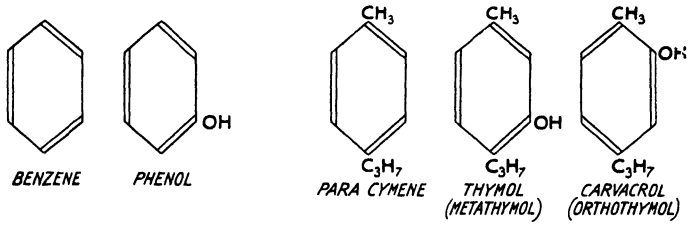
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THYMOL, β-NAPHTHOL AND THE RELATIONSHIP BETWEEN CHEMICAL CONSTITUTION AND ANTHELMINTHIC ACTION

Although thymol and β-naphthol were employed for many years in the treatment of hookworm disease, their use has now been largely discontinued in favour of carbon tetrachloride and oil of chenopodium. Considerable interest has, however, been aroused by the possible relationship between chemical constitution and anthelminthic action exhibited by these substances and compounds closely related to them. It is not improbable that the simple mono-, di-, and tri-hydric phenols are all capable of yielding anthelminthics of practical value in proportion as their solubility and toxicity are modified by the nuclear substitution of either alkyl or acyl groups. Phenol itself has been used as a remedy for thread worms, but is, of course, too irritant for general use, a finding which also applies to its methyl, ethyl, propyl and butyl homologues. Thymol (methyl*iso*propyl phenol), which is solid at ordinary temperatures and sparingly soluble in water, is also possessed of anthelminthic properties. Clayton Lane (1928), in fact, still regards it as the best anthelminthic for hookworms, since after doses of 60 grains no deaths have been attributable to its use. Thymol is one of the hydroxyl derivatives of *p*-cymene, another being carvacrol. The anthelminthic properties of other thymol isomerides have not been investigated.

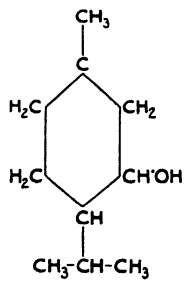


Caius and Mhaskar (1919) have shown that from 94 to 99 per cent. of the hookworms are removed by thymol in doses of from 30 to 60 grains, *Ancylostoma duodenale* being more resistant than *Necator americanus*. Carvacrol, which is fluid at ordinary tem-

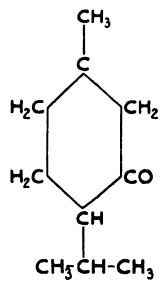
peratures, has been recommended as a substitute for thymol; it is, however, a somewhat weaker anthelminthic, for Caius and Mhaskar (1923) found that it would only remove 38·8 per cent. of *Ancylostoma duodenale* and 88·3 per cent. of *Necator americanus* in doses of 60 minims, thus confirming the International Health Board, who in 1919 reported unfavourably on its efficiency, as owing to its being more liquid it was in practice more irritant than thymol. With a dose of 60 minims there were well-marked toxic symptoms, salivation, retching, burning pain in the abdomen, and giddiness, all of which were absent with the corresponding dose of thymol. Carvacrol, as well as being a more feeble anthelminthic, is, therefore, more toxic to man than thymol.

Attempts have also been made to lower the toxicity of alkylphenols by converting them into non-irritant esters, such as carbonates, carbamates and N-dimethylcarbamates. One of these, thymol carbonate, frequently known as thymotal, is stated to have given good results in the outbreak of hookworm disease which occurred during the boring of the St. Gothard Tunnel, but more recent trials by Caius and Mhaskar (1923) have shown that it is quite useless in ankylostomiasis. Another of these compounds, *p*-benzylphenyl carbamate, has been recommended as a remedy for oxyurids.

While esterification of the phenolic hydroxyl group leads to the formation of non-anthelminthic, non-toxic compounds, such as thymol carbonate, hydrogenation of the benzene nucleus, as in the reduction of thymol to menthol, lowers very considerably the



MENTHOL

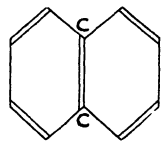


MENTHONE

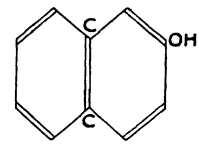
toxicity to the hookworms, but increases the toxicity to the host, for Caius and Mhaskar (1923) found that doses of 24 gr. removed only 20·9 per cent. of hookworms.

Conversion of the secondary alcohol group of menthol into a carbonyl group produces menthone, which is possessed of no anthelminthic action.

The observations on thymol and its derivatives suggest that anthelminthic action may be related to the introduction of an hydroxyl group into the benzene nucleus, for just as insertion of an hydroxyl group in *p*-cymene yields thymol, so the introduction of the same group in naphthalene yields β-naphthol.



NAPHTHALENE



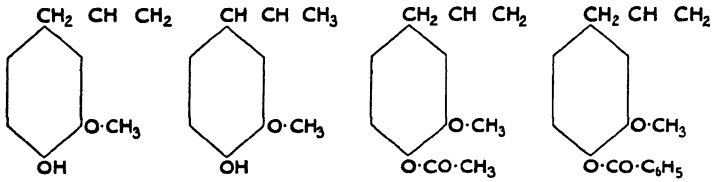
BETANAPHTHOL

Similarly the esterification of thymol with the formation of thymol carbonate destroys the anthelminthic power, and the formation of benzo-naphthol (benzoyl-naphthol) is also attended with complete loss of anthelminthic activity.

Although these esters are broken up in the alimentary tract by hydrolysis forming the original hydroxyl compound, they do not seem to attain sufficient concentration to be effective as anthelminthics. On the other hand, the phenolic hydroxyl group alone does not confer anthelminthic properties, since Pessôa and da Palma (1926) have shown that *iso*amylphenol and *iso*butylphenol have very slight anthelminthic properties, despite the presence of a phenolic group similar to that of thymol and β-naphthol.

Anthelminthic activity is also exhibited by the propenyl phenols. Eugenol, which is an *iso*propenyl phenol and occurs in oil of cloves, has strong anthelminthic properties, for Caius and Mhaskar (1922) found that in doses of 90 minims it was capable of expelling 70 per cent. of hookworms. *iso*Eugenol, which is an isomer of eugenol, is as potent an anthelminthic as eugenol itself. The introduction of an acetyl group into eugenol reduces its anthelminthic pro-

perties, while the introduction of a benzoyl group does away with them completely. In both cases the phenolic hydroxyl group is covered.



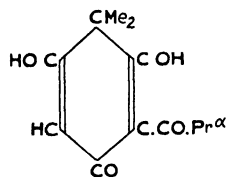
EUGENOL *ISO EUGENOL* *ACETYLEUGENOL* *BENZOYLEUGENOL*

Certain other results obtained by Caius and Mhaskar (1921) seem to show that anthelminthic action depends upon whether the phenolic hydroxyl is covered or free. Thus salicylic acid and its esters with acetic acid (aspirin), with phenol (salol), and β-naphthol (betol) have no anthelminthic power, although methyl salicylate, which liberates salicylic acid and methyl alcohol in the intestines, is said to exert some action in the unhydrolysed state. Possibly the methyl alkyl intensifies the anthelminthic action of the phenolic hydroxyl, or the methyl salicylate being more stable is less rapidly absorbed from the intestine. It seems doubtful, however, whether any anthelminthic tests yet devised are sufficiently delicate to distinguish between feeble anthelminthics such as these.

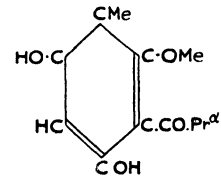
Comparatively little recent work has been carried out on male fern, the classical remedy for cestodes, or on kamala and kousso, though Hall and Shillinger (1926) have found that kamala is probably the best known remedy for tapeworms in poultry. Various attempts have, however, been made to prepare synthetic compounds analogous to those found in male fern which should have a high anthelminthic value.

Since β-naphthol and thymol are both phenols, work has been chiefly devoted to a study of the phenols, following a suggestion made by Fränkel that the active constituents of a number of well-known anthelminthics, such as filicic acid, derived from *Filix mas*, and rottlerin, derived from kamala, contain butyric or *iso*butyric acid residues with or without phloroglucinol. Karrer (1919) prepared an extensive series of condensation products of aliphatic

acids with phloroglucinol and resorcinol analogous to filicic acid and aspidinol, which may be regarded as the nuclear units on which the complex and more active constituents of *Filix mas* are built up.

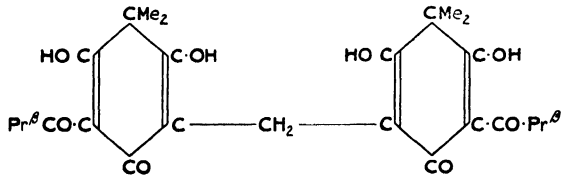


FILICIC ACID

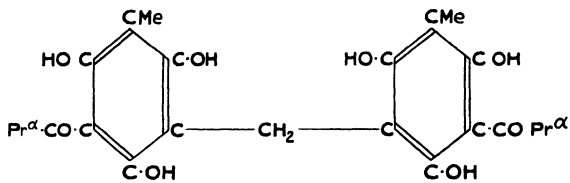


ASPIDINOL

Of the synthetic compounds prepared, the *iso*hexoyl derivatives of either resorcinol or phloroglucinol appeared the most promising, while the *iso*acyl residue conferred greater activity than the normal acyl residue, phloro*iso*butyrophenone being twice as active as phlorobutyrophenone. The naturally-occurring fern compounds, however, become more active with increasing complexity, albaspidin being more active than filicic acid and less active than filixic acid, which probably contains three rings.



ALBASPIDIN



2.4.6:2':4':6'-HEXAHYDROXY-5 5'-DIBUTYRO-3:3':-DIMETHYLDIPHENYL-METHANE

The synthetic compounds on the contrary become less active with increasing complexity: thus methylphlorobutyrophenone condenses with formaldehyde in presence of alkali to form

2 : 4 : 6 : 2′ : 4′ : 6′- hexahydroxy-5 : 5′ -dibutyro-3 : 3′-dimethyl-diphenylmethane, which should have an activity similar to that of albaspidin, but is in fact less active than the starting material.

It will thus be seen that in the majority of cases the relation between chemical constitution and anthelminthic action is not very clear. It seems, however, that, in addition to chemical constitution, physical properties such as solubility and volatility must be taken into account in determining the activity of a particular compound. Slight changes in chemical constitution may completely alter the physical properties on which the anthelminthic activity of a compound entirely depends.

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SANTONIN

Although santonin was only discovered by Kehler and Alms in 1829, the anthelminthic value of the plant from which santonin is obtained, *Artemisia maritima* Linn., was well known to the Greeks and Romans, by whom a knowledge of its properties was transmitted to the Arabian physicians of the Middle Ages.

Recent experiments in man on the anthelminthic action of santonin have in part confirmed the reputation which it has so long enjoyed, for the observations of Caius and Mhaskar (1923) and Chopra and Chandler (1924) showed a high percentage of cures in human ascaris infections.

Critical tests on animals, however, have not been so successful. Hall and Foster (1918) reported that santonin in single doses of from 1 to 13 gr. removed only about 24 per cent. of ascaris from dogs, while Mote (1924) found that the drug affected only from 0 to 24 per cent. of ascaris in swine. Shillinger (1927) obtained the following results in dogs. Santonin, without purgation, and in doses varying from 1 to 16 gr., removed 7 per cent. of whipworms but no hookworms, ascaris or tapeworms. In doses of from 6 to 12 gr. administered simultaneously with 5 to 10 gr. of calomel, 4 per cent. of hookworms, and 88 per cent. of whipworms were removed, but neither ascaris nor tapeworms. In doses of from 1 to 6 gr., followed by 1 ounce of castor oil administered fifteen hours later, santonin removed 25 per cent. of ascaris, but had no effect on hookworms, tapeworms or whipworms. In swine the minimal effective dose was 15 gr., while in horses santonin proved entirely ineffective.

The exact action of santonin on round worms is at present but little understood. Caius and Mhaskar (1923) found that the majority of the worms expelled were injured, but not killed. Previous experiments by Trendelenburg (1916) had shown that when ganglion-free strips of ascaris muscle are suspended in Ringer's solution the addition of santonin in dilutions of 1 in 5,000 to 1 in 10,000 causes intense muscular contractions. These muscular contractions may be sufficiently violent to detach the

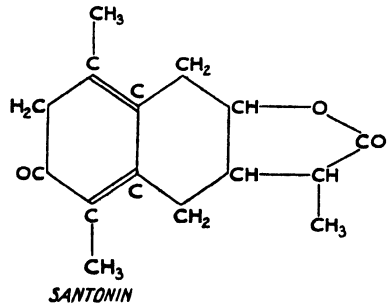
worms from the intestinal wall. Numerous attempts have also been made to determine with what peculiarity in the molecular structure of santonin anthelminthic activity is associated. Lautenschlager (1921) believed that the anthelminthic action of santonin is due to the association of the lactone group with a carbon ring structure for such an action was produced, not only by lactone groups in association with dimethylnaphthalene, as in santonin, but also by phthalide and its dimethoxy derivative, meconin. The stimulating action on ascaris muscle could be increased by replacing one of the methylene hydrogen atoms by an alkyl group or diminished by similar substitution of an aromatic group.

Oshika (1921[1 & 2]), however, believed that the lactone structure was not the essential group in determining the anthelminthic action of santonin, for while repeating and confirming Trendelenburg's experiments on the divergent action of santonin and its sodium salts, he suggested that the lack of anthelminthic action in the acids derived from santonin might be due to the acid character of the compounds rather than to the absence of the lactone structure, the action of the esters being similar to that of santonin itself. The question of the correlation between vermicidal action and chemical structure was thoroughly investigated by Caius and Mhaskar (1923), who studied the effect of santonin and various santonin derivatives, not on the musculature of worms *in vitro*, but on the number of worms removed and the number of human cases clinically cured, equal doses of the drugs being administered under similar conditions. The " test treatment " consisted of a single 5-gr. dose of santonin, or of a santonin derivative, at night and a purge of Epsom salts early next morning. The number of worms expelled during the next three days was recorded. The total number of round worms harboured by each person was then determined by a " standard treatment " with 5 gr. of santonin at night and a purge of Epsom salts next morning. A microscopic examination of the fæces for round worm ova was made on the fourth, fifth and sixth days, and if ova were absent a further examination was made after an interval of three days. The absence of round worm ova in the fæces on the tenth, eleventh

The Anthelminthic Action of Santonin and its Derivatives (*Caius and Mhaskar*, 1923).

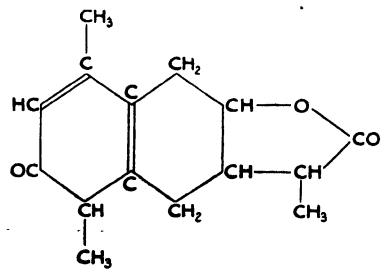
Drug.	Dosage.	Number of cases treated.	Roundworms removed.		Total worms removed.	Percentage of worms removed with one test treatment.	Number of cases cured by one test treatment.	Percentage cured by one test treatment.
			With test treatment.	With subsequent standard treatment.				
Santonin . . .	5 grains	20	27	4	31	87·3	16	80·0
Chromosantonin .	,,	6	3	8	11	27·2	3	50·0
Desmotroposantonin .	,,	8	1	21	22	4·5	—	—
Santonone . .	,,	6	—	9	9	—	—	—
Santoninic acid . .	,,	26	33	4	37	89·2	19	73·1
Santonic acid . .	,,	19	26	4	30	86·6	16	84·2
Santonous acid . .	,,	18	19	1	20	95·0	12	66·6
Photosantonic acid .	,,	11	46	3	49	93·8	9	81·8

and twelfth days after treatment was considered to be evidence of " cure." It is perhaps somewhat unfortunate that the number of patients treated with each compound was small. Chemically, santonin is now believed to be the inner anhydride or lactone of santonic acid, a derivative of naphthalene.

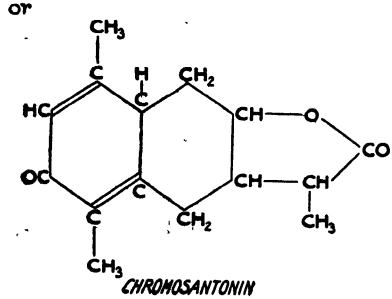


SANTONIN

On exposure to light, especially direct sunlight, santonin acquires a yellow colour. Yellow or chromosantonin is a modification of ordinary santonin due to the wandering of an atom of hydrogen, and its formula is believed to be either

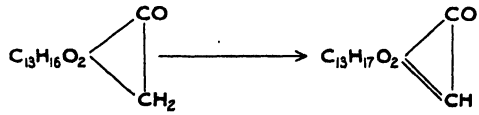


or



CHROMOSANTONIN

Thus, the lactone structure of santonin is preserved in its isomer, chromosantonin, but through the migration of an atom of hydrogen from the methylene radical adjacent to the carbonyl, the group $-CO-CH^2$ becomes $-CO-CH=$



SANTONIN *CHROMOSANTONIN*

This migration of the atom of hydrogen is attended with marked changes in the properties of santonin, and results in the formation of a compound which, though still toxic to ascaris, is much less toxic to the parasites than santonin.

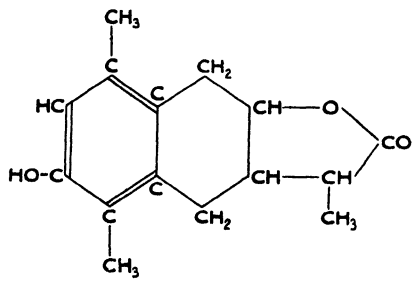
The urine of patients treated with yellow santonin still gives the crimson colour after the addition of alkalies, as does santonin itself.

By dissolving santonin in hydrochloric acid and heating at 60° C. for six hours an isomer of santonin, desmotroposantonin is formed. Its behaviour to reagents shows that the ketonic character of santonin has disappeared, to be replaced by phenolic properties, the change being accounted for by the transformation of the group $-CO-CH_2$ into $-COH=CH$.



SANTONIN *DESMOTROPOSANTONIN*

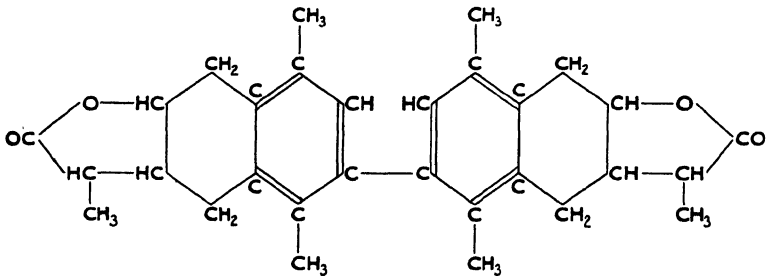
Thus the structure of desmotroposantonin is



DESMOTROPOSANTONIN

Although desmotroposantonin retains the lactone group of santonin, it has no anthelminthic value, nor does it give the colour reaction with alkalies.

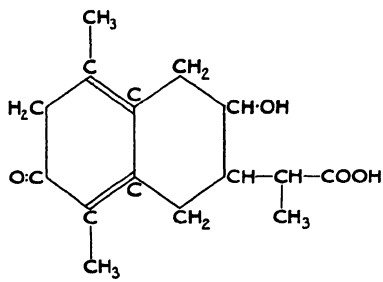
Santonone may be looked upon as resulting from the union of two molecules of santonin in which the $-CO-CH_2-$ group has been changed to $=C=CH-$. Santonone thus contains two lactone groups in its molecule, and has the structural formula :



SANTONONE

Santonone has no action as an anthelminthic, nor does it give the colour reaction with alkalies.

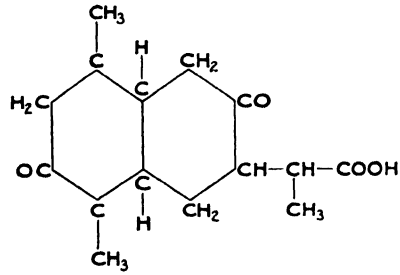
Santoninic acid is prepared by boiling santonin with sodium hydroxide solution, adding an excess of hydrochloric acid and extracting the water-insoluble santoninic acid with ether. Santonin is thus the anhydride of santoninic acid, which is represented by the formula



SANTONINIC ACID

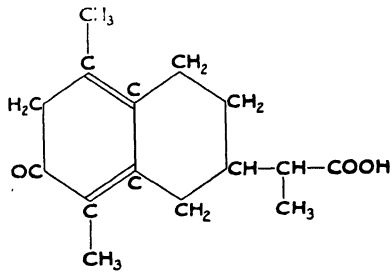
Santoninic acid does not contain a lactone structure, but it does retain the group $-CO-CH_2-$. It is as good an anthelminthic as santonin, and it gives the colour reaction with alkalies.

Santonic acid is much more stable than its isomer santoninic acid, and cannot be converted into santonin by removal of the elements of water. Its constitution is



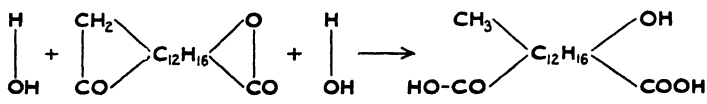
SANTONIC ACID

Santonic acid is thus not a lactone, though it retains the — CO—CH_2 — group. It is as good an anthelminthic as santonin, and gives the characteristic colour reaction with alkalies. Santonous acid has the properties of a phenol, while both its behaviour with alkalies and its anthelminthic action on round worms suggest the constitution



SANTONOUS ACID

Photosantonic acid is derived from santonin by fixation of two molecules of water.



SANTONIN *PHOTOSANTONIC ACID*

Thus in this compound the naphthalene nucleus of santonin has been ruptured, but there is no weakening in the anthelminthic power, nor is the colour reaction with alkalies modified. In addition, there is some evidence to show that while santonin and the other anthelminthic compounds previously mentioned act merely as narcotics to the worms, photosantonic acid is a true vermicide in that it acts as a direct poison to ascaris, which is invariably expelled dead.

The anthelminthic properties of santonin and its derivatives are thus correlated, according to Caius and Mhaskar, not with the lactone structure, for the lactones desmotroposantonin and santonone have no action on the worm, but with the group $-CO-CH_2-$, for chromosantonin, which contains the group $-CO-CH-$, is inferior as an anthelminthic to santonin, while desmotroposantonin with the group $-COH=CH-$ and santonone with the group $=C=CH-$ have no anthelminthic value.

On the other hand, photosantonic acid which has lost the $-CO-CH_2-$ group is an active anthelminthic. If Caius and Mhaskar's conclusions are correct, substances such as pulegone, menthone and camphor should also show some activity against ascaris, while it is difficult to explain Lautenschlager's findings as to the activity of phthalide and meconin.

There seems, however, to be some correlation between the anthelminthic properties and the reaction with alkalies, for both desmotroposantonin and santonone fail to give the characteristic crimson colour. It is thus obvious that further work is required to determine what peculiarity in the molecular structure of santonin is responsible for its anthelminthic action.

The exact manner in which santonin acts on intestinal worms is not only of scientific interest, but of considerable practical importance. The most successful manner of attacking the problem would seem to be by a comparative study of the chemical constitution of the drug and the changes which it undergoes when absorbed by the animal organism.

Absorption of santonin begins a few minutes after its introduction into the stomach, and lasts for some hours. Absorption occurs, however, chiefly in the duodenum, possibly after the

formation of alkali santoninates, for the upper part of the jejunum will be found free from the drug. As, however, santonin is found in the lower part of the intestine even when the duodenum is ligatured, or the drug administered subcutaneously, it must be concluded that there is a partial re-excretion into the lumen of the bowel.

The Japanese workers, Kohgame and Asada (1927), claim in fact to have obtained experimental data to prove that the santonin absorbed from the intestine passes to the liver, where it combines with bile to form a compound which is then highly toxic to ascaris, santonin itself having no action on the worms. According to these workers, bile is slightly toxic to ascaris. Santonin given by subcutaneous, intramuscular or intravenous injection is also toxic to round worms, for out of forty cases treated in this way with " santonin-sodium," Morinaka and Ishikawa (1926) succeeded in curing thirty-five.

The santonin absorbed from the intestine passes into the urine, in part unaltered, in part oxidised. Two or three hours after the administration of a relatively small dose the urine assumes a yellow colour and leaves a yellow stain on drying, while the addition of alkali gives the characteristic crimson colour. The formation of this yellow substance has recently been reinvestigated by Marshall (1927), for it is in some way related to the " yellow vision," which is such a striking symptom of santonin intoxication. The coloured substance is present in the blood plasma, and also in small quantities in the lumen of the bowel, where it is probably formed, at least in part.

In the disorder of colour vision which even moderate doses of santonin may produce, objects at first appear of a bluish colour. This aberration is, however, of short duration, and may even pass unnoticed; it is followed by a longer period of yellow vision, during which brightly-illuminated objects have a yellow tinge, blue seem green, and violet are indistinct. These symptoms pass off in the course of a few hours, a second stage of violet vision occasionally intervening before complete recovery. According to Marshall (1927), the xanthopsia is not due to any discoloration of the media of the eye, but is peripheral in origin, and is due to

stimulation followed by depression of the violet-perceiving mechanism of the eye, a condition similar, but for the absence of "dazzle," to the exhaustion of violet vision produced by violet light.

Apart from personal idiosyncrasy, santonin appears to be a safe remedy when used therapeutically, while owing to the fact that it does not produce gastro-enteritis it can be given repeatedly. Infants, per unit of body weight, are, however, considerably more sensitive to santonin than adults. In one dog which received a little over a dram of santonin in the course of three months, Hall (1920) found that the hair had come out on the ventral surface of the neck and abdomen, around the eyes, and in the axillary and inguinal regions.

On purely chemical grounds, as Gray (1926) points out, santoninoxime should be much less toxic than the other derivatives, or even than santonin itself; its therapeutic action, however, is not at present sufficiently known, and further investigation of this derivative is required before its use can be recommended. Careful tests on man are also needed before the anthelminthic value of even santonin itself can be definitely ascertained.

Recently von Oettingen and Garcia (1929) have studied the anthelminthic action of β-angelicalactone and the dilactone of acetonediacetic acid in ascaris infections of cats. The dilactone was the more efficient compound, and in seven out of ten experiments in cats all the parasitic roundworms were removed, a single dose of 0·3 gm. per kilogram of body weight being sometimes sufficient. The M.L.D. of β-angelicalactone was 0·7–1·25 gm. per kilogram of body weight in the cat, that of the dilactone considerably greater for 2·6 gm. per kilogram of body weight produced only a transient slight depression.

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THE TREATMENT OF WHIPWORMS
(*Trichuris trichiura*)

The latex of *Ficus laurifolia* appears to be of some use as an anthelminthic against whipworms. The active anthelminthic principle is at present unknown, though it probably resides in the solid portion of the latex, as the expressed juice has no value as an anthelminthic. A patented method [British patent No. 243,325, of 27.10.25], has, in fact, described the preparation of an anthelminthic from this material. In its natural state, the latex is a perfect emulsion of rubber and resinous substances in a slightly sweet vegetable juice. It also contains an albumin and a substance of fixed composition yielding ammonia. It seems probable that the active principle is associated with the ammonia-yielding substance. Unfortunately the latex readily ferments, frothing freely and giving off carbon dioxide. Under ordinary conditions it will thus only keep for four or five days, though it can be preserved in the ice-box for as long as thirty-five days. According to Hall (1928), a method of preserving it for shipment has now been developed, but data are still lacking as to the anthelminthic value of the preserved product. Although this drug always brought away whipworms, some were always left after one treatment with a dose of 30 c.cm., or three such doses on three successive days. Montoya (1922) finds that the drug may give rise to toxic symptoms. Colic, nausea and vomiting may occur, or occasionally muscular cramps, delirium, syncope, urticaria, rectal and vesical spasms, and partial suppression of urine. Hall and Shillinger (1926) report that mercurochrome-220 given to dogs in capsules each containing 1·5 gm. removes from 88 to 100 per cent. of the whipworms present.

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SCHISTOSOMIASIS

The number of persons suffering from schistosomiasis is probably second only to the number of those infected wtih hookworms, for schistosomiasis is found not only throughout Africa, but in parts of China, in the Philippines, Formosa, and in one of the Japanese islands, as well as in British Guiana and the West Indies. In Egypt alone it is estimated that between four and five millions of the population are infected.

In attempts to cure schistosomiasis a large number of drugs has been used, ranging from the prescription to be found in the Ebers papyrus to synthetic chemicals such as arsphenamine; time alone appeared to be the only remedy, when the introduction of tartar emetic and emetine so completely revolutionised treatment that a rapid cure is now certain in all except the most chronic forms of the disease.

As an adjuvant to tartar emetic, the use of carbon tetrachloride has recently been suggested both by Khalil (1926) and by Cawston (1928), who conclude that though the gradual destruction of schistosomes by intravenous antimony is the method of choice, yet the immediate appearance of signs of degeneration of the adult worms after one or more doses of carbon tetrachloride raises the hope that massive doses, given at sufficiently long intervals to enable the system to recover completely from the effects of each dose, may be equally effective in producing a cure in certain cases of the disease. Injections of mercurochrome-220 have also been tried in treatment, but so far without success.

COMPOUNDS OF ANTIMONY

McDonagh (1915), in his treatise on venereal diseases, was the first to recommend the use of antimony in the treatment of vesical bilharziasis. His patients, however, do not appear to have been observed for long enough to establish a definite cure. In the meantime Christopherson (1918), while injecting a patient with antimony for kala-azar, noticed an improvement in

the symptoms of urinary bilharziasis, and thus independently rediscovered the curative action of tartar emetic, successfully treating a series of cases of infection due to *Schistosomum hæmatobium* at the Khartoum Civil Hospital. Christopherson advocated a full course of 1·95 to 2·0 gm. (30 gr.), given intravenously over a period of thirty days, the injections being made on alternate days, beginning with an initial dose of 0·03 gm., and increasing to a maximum of 0·13 gm. The drug was considered to exert a lethal action on both schistosomes and living ova, and not only to produce a clinical cure, but to eradicate the carrier. As a result of the publication of Christopherson's paper, McDonagh (1918) directed attention to the fact that in 1912 he had successfully treated twenty-three cases of bilharziasis, using not only tartar emetic, but also colloidal antimony and antiluetin, his results with the two latter drugs being even better than with tartar emetic itself. Wiley (1918) also reported that in 1916 he had cured a case of vesical bilharziasis with antimony at the Australian Dermatological Hospital in Cairo. Many successful cases were recorded during the year 1919 by Low, Taylor, Innes, Low and Newham, Cawston and Fairley. Innes suggested that a total course of 0·9 gm. was quite adequate. Baujean (1921) reported cures in nine cases of intestinal bilharziasis (*Schistosomum mansoni*), while Carrasquillo (1922) and Martins (1923) also had successful results from tartar emetic therapy in this type of infection.

Since 1920, numberless cases have been treated by tartar emetic. Cawston, from 1919 onwards, and Sharp (1924) have recorded successful experiences in South Africa, while Lasbrey and Coleman (1921 and 1924) and Day (1921 and 1924), have analysed the results obtained in several thousand cases in Egypt. Day (1921) regarded a course of 0·78 gm. (13 gr.) as the smallest curative amount, though temporary improvement followed 0·48 or 0·54 gm. given during the first week. Usually 1·56 gm. or more were needed to produce a cure. Lasbrey and Coleman (1924) treated 4,655 patients in five years. They recommended an intensive course consisting of daily injections for six days, followed by six more on alternate days, making a total of 1·75 gm.

The initial dose was 0·06 gm., increasing to a maximum of 0·15 gm.

Clinical relapses following treatment have been recorded by a number of observers, but they have almost always been due to an inadequate course of treatment involving less than the standard 1·9 gm. Phease (1923) found that 20 per cent. of cases relapsed after two years where an average of 0·7 gm. had been given, while Spence (1925) reported that 5 per cent. had viable ova after 1·3 gm. After a full course given two and a half years previously, Smith (1924) found 3·5 per cent. of cases still infected, while Christopherson (1923) estimated that 79 per cent. of unselected cases were cured by a full course of treatment. In some few cases the worms appear to be very resistant even to large doses of tartar emetic.

Khalil (1926) found that of 1,680 cases of schistosomiasis treated during the last five months of 1924 with twelve injections of tartar emetic, 1,523, or 90·6 per cent., were cured. In a further communication, Khalil (1928) states that among 284,934 cases treated with tartar emetic during twelve months there were only six fatal cases.

Owing to the toxicity of potassium tartar emetic, various attempts have been made to find less toxic preparations of antimony. Fargher and Gray (1921) found that quinine antimonyl tartrate was five times less toxic to mice than tartar emetic. Sodium antimonyl tartrate $2(NaSbO.C_4H_4O_6).H_2O$ is preferred by many in the treatment of bilharziasis owing to its lesser toxicity and greater solubility: though it was formerly more difficult to obtain in a chemically-pure form, a scale preparation is now available for intravenous injection. Cawston (1924), however, states that the potassium salt is less painful and more efficient than the sodium salt. Day (1924) has in a few cases employed a lithium tartar emetic which, according to Fargher and Gray, is two and a half times less toxic than potassium antimony tartrate, and one and three-quarter times less toxic than sodium antimony tartrate. Of the pentavalent organic compounds which have proved of such importance in the treatment of leishmaniasis, the majority, such as urea stibamine, neostam or stibamine gluco-

side, neostibosan (von Heyden 693) and amino-stiburea have not been extensively used in the treatment of helminthic infections.

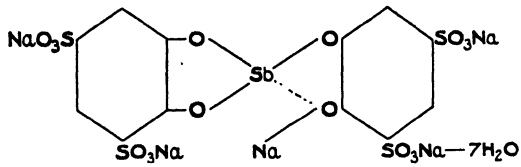
Walravens (1927), however, reports six cases successfully cured with stibosan (von Heyden 471). The course of injections consisted of one of 0·2 gm. and eight of 0·3 gm. given every other day. Goldie (1929) treated two cases with stibenyl, but the injections were followed by toxic reactions. Three cases treated with stibosan, on the other hand, were cured by twelve to fifteen injections of from 0·1 to 0·4 gm.

An organic trivalent antimony compound of a somewhat different character, antimosan (von Heyden 661), has been introduced by the von Heyden Company. It is stated by Uhlenhuth, Kuhn and Schmidt (1924) to be a pyrocatechol derivative, and though its exact nature is not disclosed, it is probably an antimony compound of an alkali pyrocatechol-sulphonate or -carboxylate in which the antimony is attached to the oxygen atoms of the two phenolic residues. Specht (1926) has cured two cases with antimosan, which is said to have had none of the unpleasant after-effects of tartar emetic. Tootell (1927) also treated five cases due to infection with *S. hœmatobium*. Orenstein (1928) has used antimosan with success in two cases of bilharziasis in South Africa, while Cawston (1929) also records its use in one case. Although less toxic than tartar emetic, ova were still found in the urine after thirty-six days. It can be given subcutaneously and intramuscularly as well as intravenously. Of four cases treated by Walravens (1927) two had relapses after having received 45 c.cm. and 40 c.cm. respectively, while the others remained negative after 43 c.cm. and 32 c.cm. Goldie (1929) also used antimosan in nineteen cases, eight of the cases showing symptoms of intolerance. Doses of 40 to 50 c.cm. were insufficient to bring about a cure, but 60 to 90 c.cm. ensured a successful result.

Khalil (1926) has used a compound Bayer SB 212, which is a complex salt of an organic antimonyl acid containing 25 per cent. of organic antimony. The minimal lethal dose for dogs is 10 c.cm. (0·005 gm. antimony) per kilogram of body weight. The drug can be given intramuscularly, and causes much less reaction than tartar emetic, while the amount of antimony which can be adminis-

tered in a given time is considerably greater. The site of injection must be frequently changed, however, to avoid œdema ; another disadvantage of the drug in its present form is its bulk.

Quite recently another trivalent antimony compound has been prepared by Professor Hans Schmidt, and has been used in Egypt by Khalil, Nazmi, Peter, el Din and el Betash (1929). The compound, which has been named "fuadin," is a trivalent antimony compound of pyrocatechin sodium disulphonate, the formula being described as :—



In a 7 per cent. solution this compound remains stable for as long as six months, even at the temperature of an Egyptian summer. Pharmacological experiments show that the antimony is largely excreted by the kidneys, only a small portion passing into the intestine. The injections can be given intramuscularly in the gluteal region, there being little or no reaction at the site of injection, while, as shown by X-ray examination, the antimony is very rapidly absorbed. With the exception of vomiting in some 2½ per cent. of cases, there are no toxic sequelæ beyond an occasional slowing of the pulse. The following course of injections is recommended :—

First day.	1·5 c.cm.	7 per cent. solution of fuadin.
Second day.	3·5 c.cm.	7 ,, ,, ,,
Third day.	5·0 c.cm.	7 ,, ,, ,,

The same dose is given on alternate days till the fifteenth day, to a total of 40 c.cm. The maximum dose of 5·0 c.cm. contains 42·5 mgm. of antimony. On the fifth to the seventh day after the fifth injection, the eggs in the urine become granular, and by the end of the course, if not before, they are all dead. So far among 200 cases there have been no fatalities.

Shattuck and Willis (1928) have employed both antimony

sodium thioglycollate and antimony thioglycollamide, compounds which have been used with success in the treatment of granuloma inguinale. Antimony sodium thioglycollate was given in doses of from 0·01 to 0·03 gm., six injections being given in twelve days with a total amount of 0·17 gm. The eggs in the urine began to degenerate on the sixth day of treatment. At the present time the value in the treatment of bilharziasis of antimony compounds other than tartar emetic cannot be fully estimated until a more extensive trial of the less toxic antimony salts has been carried out.

While tartar emetic is preferably given intravenously, both Wilson (1922) and Scott (1922) report satisfactory results with large doses given *per rectum*. Scott also injected the drug directly into the bladder. From the enormous doses which were given, it does not appear probable that much of the drug was absorbed.

Tartar emetic has also been used in the treatment of infections due to *S. japonicum*. Cawston (1921) and Christopherson (1921) both recorded the cure of one case, and in the same year Sanders and Priston treated three cases with good results. Tyau (1922) used it with success in China, while Tootell (1924) reported the treatment of twenty-four cases. Thirteen were cured after 1·25 gm. had been injected, seven improved, and four, who would not persist, showed no change. Libby (1924) found that in chronic cases with hepatic changes and splenomegaly, the progress of the disease was unmodified. As most of the cases in China belong to this category, Libby expressed doubts as to the efficacy of tartar emetic in the Far East. Ova disappeared from the stools after from 0·26 to 0·65 gm. and at least 1·3 gm. were considered necessary for effective results. Faust and Meleney (1924) advocated an intensive course of treatment in which the patient's limit of tolerance was quickly reached, a total of 1·5 to 2·0 gm. being given in eighteen to twenty days, provided the disease was not too advanced. Meleney, Faust and Wassell (1925) advise that tartar emetic should be given during the first and second stages of infestation, but only very cautiously when there is cirrhosis and ascites. Eggs ceased to appear in routine fæcal smears after an average of twenty-one days with an average

administration of 0·9 gm., but miracidia could still be hatched from the fæces up till the thirty-sixth day, or until an average of 1·8 gm. had been given.

The curative effects of tartar emetic have also been studied in experimental bilharziasis in animals. Nishi (1923), who investigated the effects of the sodium salt of tartar emetic on dogs infected with *S. japonicum*, found that injections, given shortly after exposure to cercariæ, killed the schistosomulæ and prevented the development of an attack. The therapeutic dosage was estimated at between 1 and 5 mgm. per kilogram of body weight. Faust and Meleney (1924) state that they too were able to cure dogs infected with *S. japonicum*, but give no details as to treatment.

Fairley (1924) found that in goats experimentally infected with *S. spindalis*, tartar emetic was less satisfactory than emetine hydrochloride, while urea stibamine was quite useless. For goats, using a 1 per cent. solution of potassium antimony tartrate, the minimal lethal dose proved to be 16 mgm. per kilogram of body weight. In a series of twenty-two animals treated with tartar emetic on alternate days, or at slightly longer intervals, clinical cures were obtained in thirteen, while in a subsequent series, where the injections were given at closer intervals, six out of seven animals were cured. At the autopsy of the nineteen animals, schistosomes were demonstrated to have survived treatment in ten instances, though even in these there had been a considerable reduction in the parasitic level, the sex ratio showing that the female worms were particularly affected.

Christopherson (1924) has described the technique for administering tartar emetic intravenously. The injections are best given some two and a half hours after a light meal. The most suitable vein is one in front of the elbow, the vein being rendered prominent by means of a tourniquet round the arm. Great care must be taken that no tartar emetic is injected into the tissues surrounding the vein, as necrosis is liable to occur. During the course of the injection the face and neck become temporarily flushed, there is a feeling of constriction round the chest, colic in the abdomen, a metallic taste in the mouth, and finally a hard, dry cough. After

the injection, the patient should rest for at least half an hour. After the fourth or fifth injection, rheumatic pains are common at night in the joints and muscles, especially of the shoulder. The symptoms of acute antimony poisoning, which may sometimes occur, are vomiting, giddiness, a marked rise or fall of temperature, diarrhœa, reduction in the amount of urine, cramp in the muscles of the calf, and delirium. Very rarely there are symptoms of collapse. In chronic antimony poisoning there is great muscular weakness, loss of weight, anæmia, cracked and ulcerated tongue, and diarrhœa. Christopherson and Gloyne (1926) have drawn attention to the fact that in certain cases there is developed a condition of hypersusceptibility to antimony after some few injections have been given. This condition, which may resemble shock, may be due to an alteration of protein metabolism, or possibly to the destructive action of antimony on the liver cells liberating histamine or histamine-like substances. It is well known that antimony salts are capable of producing very definite fatty degeneration and necrosis of the liver parenchyma. Debility, myocarditis and renal disease are usually regarded as contraindications to the administration of tartar emetic, though according to Nogue and Boulay (1923) patients with nephritis and œdema stand the drug well.

As a result of the first injection of tartar emetic there is usually increased hæmaturia, but after the second or third injection the blood-clots disappear and the smokiness of the urine decreases. Ova, however, disappear more slowly, and though quite degenerate, may still occasionally be found in the urine months or even years after the successful cure of the disease. The early improvement in the patient's subjective symptoms is very striking, and is accompanied by an increase in the body weight and disappearance of anæmia.

In order to determine whether a patient has been definitely cured, four tests have been suggested :—

(I.) The complement-fixation test.
(II.) The disappearance of eosinophilia.
(III.) França's ovimetric test.
(IV.) The microscopic appearance of the ova.

(I.) In patients treated with 2·0 gm. (30 gr.) of tartar emetic

even a year previously, definite positive serological reactions may still be obtained, despite the absence of clinical evidence of the disease. Fairley (1926) suggests that such serological findings in man indicate the survival of a limited number of schistosomes which are exclusively or mainly of the male sex, the females, if present, being too scanty to deposit living ova in demonstrable numbers or to produce vesical symptoms.

(II.) Eosinophilia may be due to many other causes than bilharzia.

(III.) The ovimetric method devised by França (1923) rests upon the assumption, at present unproved, that female bilharzial worms, devitalised by antimony, lay very small eggs.

(IV.) Microscopic examination of the ova in the urine or fæces probably provides the best evidence of the progress of treatment.

According to Day (1921), by the end of the first week there should be an appreciable reduction in the number of ova passed. By the ninth day the ova hatch out in water more slowly, while the miracidia are less active and die more rapidly. By the end of the second week a considerable proportion of the ova cease to hatch. The shells of these ova are distended as usual by imbibition of water, but the enclosed miracidia show little or no movement. Small immature ova are common, the contents appearing brown and granular instead of clear.

During the third week the few miracidia that hatch out are usually globular, move more slowly, and soon die in distorted shapes. Most of the ova no longer absorb water, while the delicate and refractile structure of the contained miracidium becomes obscure and granular. Death of the embryo is followed by a deepened opacity, the contents of the shell appearing brown or black by transmitted light. This change occurs first in the cells and granules around the embryo, and later in the degenerated miracidium itself.

By the end of the third or during the fourth week all the ova are dead, most of them being black. These blackened ova are often massed together by mucus. Occasionally some hundreds of ova may still be found in the urine, but in the majority of cases the ova are few and far between.

These changes in the ova naturally lead up to the question whether tartar emetic is lethal both to worms and to ova in the tissues. The question is not entirely an academic one, for if the worms were killed, but the ova still remained viable, the patient would continue to be a disseminator of the disease.

Christopherson (1919) believed that tartar emetic not only kills the adult worms, but sterilises the ova which are deposited in the tissues of the bladder and rectum. Day (1921) supports this view, and believes that the primary action of tartar emetic is on the ova. Fairley (1919), on the other hand, is of opinion that the primary action of the drug is on the adult schistosomes, the evidence that the miracidia are directly killed in the host's tissues resting on a less secure foundation. Experimental observations on monkeys suggested that normally mature ova must either reach the exterior rapidly or perish *in situ* as the result of cellular-humoral and tissue reactions. Hence once the female worms ceased depositing eggs in the tissues, living ova would shortly afterwards cease to appear in the excreta. Hodson (1921) also supported this view, and estimated that the normal time taken for a crop of eggs to pass from the worm to the urine is from five to seven days. This is also the period at which improvement takes place during tartar emetic treatment. Khalil (1922) found that, as Christopherson and Newlove (1919) had shown, tartar emetic in a dilution of 1 in 46 invariably prevents hatching of the schistosome eggs, but this result would appear to be due to its effect on the osmotic pressure of the fluid rather than to any direct toxic effect of tartar emetic on the eggs. Miracidia were not affected by concentrations of tartar emetic such as could possibly be obtained within the body during treatment.

Fairley (1924) found that female worms, present in goats infected with *S. spindalis*, showed after treatment with tartar emetic fewer ova than worms from control animals, while the ova present in the tissues usually contained motile, fully developed miracidia. *In vitro* experiments were also carried out by Fairley (1926) on the effects of tartar emetic on bilharzia cercariæ. A dilution of tartar emetic 1 in 200 was not lethal to the cercariæ when suspended in water for three hours, but a dilution of tartar emetic

1 in 160,000 caused death when the cercariæ were suspended in goat serum. This striking difference may be due to the fact that tartar emetic forms a highly toxic compound with some substance present in the serum, but it is more probably correlated with the *p*H at which the tartar emetic is acting. Although the evidence is far from conclusive, it seems probable that the primary action of the tartar emetic is on the bilharzia worms, the female worms being especially sensitive to the toxic action of the drug.

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EMETINE *

The curative effects of emetine in schistosomiasis were first described by Hutcheson (1913), who successfully treated five cases of bilharzial dysentery due to *Schistosomum japonicum*. About the same time Tsykalas (1913) in Egypt recorded a case of urinary schistosomiasis treated by emetine. As a secret remedy emetine seems to have been used by the Greek population of Egypt for some years before Diamantis (1916) recommended its general use in urinary schistosomiasis, with a record of success in thirty cases. The routine which he advocated was to give the drug intravenously, beginning with 0·06 gm. on the first day, 0·09 gm. on the second day, and thereafter 0·10 or 0·12 gm. on the third, fifth, seventh, ninth and twelfth days, so that a total of 0·65 to 0·75 gm. was administered. In cases of very severe hæmaturia two further injections of 0·12 gm. were recommended at intervals of three days. Mayer (1918) reported that in 1914 he had successfully treated a case of mixed infection, while Erian (1919) recorded fifty recoveries, injections of 2·7 to 3 gr. being given every four or five days. Bonne (1919) found that in twenty-two cases of infection with *S. mansoni* five daily injections of 1 gr. each were sufficient to cause the disappearance of ova from the stools, though they reappeared after an interval of about two months; it was uncertain, however, whether such effects were due to the death of the parasites or to the fact that the worms wandered away from the bowel wall as a result of the treatment.

A very large number of clinical observations have now been made on the treatment by emetine of infections due to *S. hæmatobium* and *S. mansoni*. All observers agree that cures can be brought about in a certain percentage of cases, though the efficiency of emetine is less than that of tartar emetic. Thus Khalil (1926) found that 32·2 per cent. of cases were cured by intravenous injections of emetine hydrochloride, in contradistinction to 87 per cent. of cures following injections of tartar emetic. In Egypt, where amœbic dysentery is a not infrequent complication of

* The chemical constitution is discussed in relation to Amœbiasis.

bilharziasis, emetine is of considerable use, since it improves both conditions at the same time. Since emetine can be given intramuscularly, its use is indicated in obese persons where intravenous injections are difficult, while its employment is preferable in cases suffering from advanced renal or hepatic disease or in individuals with an intolerance to antimony.

Although emetine injections are not associated with the immediate symptoms of distress which follow intravenous antimony injections, nevertheless, Young and Tudhope (1926) have found that in experimental animals there is a definite cumulative effect. The cardiac weakness would seem to be due to the direct action of emetine on the heart muscle, while the neuritis which may follow intravenous medication is not a true inflammation of the nerves, but a Wallerian degeneration of the myelin sheaths. This degeneration begins peripherally, usually in the motor fibres, and is associated with chromatolytic changes in the motor cells of the anterior horn of the cord. Cawston (1922) recommends that, as there is difficulty in determining the onset of cardiac depression in adults, for whom large doses are required, emetine should only be given to children and young persons who are able to tolerate a dose of from 12 to 15 gr. within a period of not more than twenty-four days. As a result of intramuscular injections there is apt to be much local tenderness, which can be minimised by dissolving the emetine in a 1 per cent. solution of carbolic acid and by the application of warm sea-water to the sites of injection. Although, following injections of emetine, there is usually a cessation of hæmaturia, Sharp (1924) has found that in children viable ova may still be present in the urine, even in the absence of clinical symptoms. It is, therefore, advisable to allow at least six months to elapse before pronouncing a definite cure. Gordon (1926) has reported the results of treating *S. hæmatobium* in fourteen West African children, thirteen of whom received 0·5 gr. daily for fifteen injections. Living ova completely disappeared within a period of seven to nineteen days after injection. Striking results were also recorded with the oral administration of emetine periodide in fourteen other severely infected children, 1 gr. being given thrice daily for fifteen days. Viable ova had disappeared

from the urine of all but two cases within twenty-one days of starting treatment. Cawston (1928), however, believes that the amount of emetine periodide which must be given orally to produce a cure is so large that symptoms of cardiac depression are bound to arise. In one case treated orally with emetine periodide for eight days living ova were still present in the urine.

Emetine has also been used in the treatment of infections due to *S. japonicum*, successful cures having been reported by Cawston (1921). Kawamura, Kasama and Tanaka (1924) found that emetine hydrochloride gave promising results as a specific anthelminthic in animals experimentally infected with *S. japonicum*, and confirmed their opinion of its value in thirteen human cases. Even in very severe cases the clinical symptoms improved rapidly, and the fæces ceased to show eggs after from eighteen to twenty-one injections. These results were confirmed by Kawamura, Ohmori and Tanaka (1924) in another series of cases.

Just as the mode of action of tartar emetic is uncertain, so the means by which emetine cures bilharzial infection is still unknown. Tsykalas (1921) believed that emetine acted directly on the worms and on the embryos enclosed within the egg. Direct cystoscopic examination of the bladder wall during the course of treatment showed that the bilharzial granulations were converted into patches of a dirty yellow colour, an appearance which Bonnet (1921) believes to be due to the dead ova beneath the submucosa. Fairley (1924), in India, has studied the effect of emetine injections on the infection produced by *S. spindalis* in goats. Goats not exceeding 25 lb. in weight were given 1 c.cm. of a 1 per cent. solution of emetine hydrochloride intravenously, the injections being given daily for ten to fifteen days. In two animals which were autopsied 111 and 125 days after the beginning of treatment no worms were found, while in two others examined thirteen days after the initial injection all the worms were dead and in various stages of degeneration, while microscopically the defunct worms were being actively phagocytosed by leucocytes. Direct microscopical examination of the bowel wall showed that there were no ova in the animals which were killed 111 and 125 days after treatment, while dead ova were present in the others in small

numbers. In the animals dying shortly after the beginning of treatment the complement-fixation test was positive, but in the two animals which were killed after a long interval the test gave negative results. Microscopically the livers of these animals showed periportal fibrosis with minimal round-celled infiltration, the last remaining traces of schistosome infection. In a further series of experiments on goats infected with *S. spindalis*, Fairley (1926) found that intravenous injections of emetine hydrochloride resulted in the clinical cure of eight out of eight animals. At the autopsy in six goats not a single schistosome survived. Of four infected animals treated by subcutaneous injections clinical cure resulted in three cases, the course consisting of ten to fifteen daily injections, varying from 0·7 to 1 mgm. per kilogram of body weight. Emetine hydrochloride thus proved quite as specific an anthelminthic as tartar emetic in curing *S. spindalis* infection in the goat, but its cumulative effects developed more rapidly and its toxicity was definitely greater than that of tartar emetic. One curious fact brought to light by these experiments was that, in treatment with emetine, as with tartar emetic, the female worms died first, while even some time before their death their reproductive functions were inhibited.

In order to test whether emetine has any direct lethal action on bilharzia cercariæ *in vitro*, cercariæ of *S. spindalis* were placed in water, saline and serum at 37° C., to which had been added emetine hydrochloride to make dilutions varying from 1 in 100 to 1 in 160,000. Three lots of sera were used, normal goat serum, goat serum containing bilharzia antibody and the same serum heated for twenty minutes at 55·5° C. to destroy complement. While the cercariæ survived for eighty minutes in saline and water containing emetine hydrochloride 1 in 100, all were dead in the sera containing emetine hydrochloride 1 in 10,000. The greater lethal effect of emetine in serum was noted in normal serum, in serum containing bilharzia antibody or in the same serum bereft of complement. The suggestion, therefore, was made that the increased toxic effect of emetine in serum might be due to the formation of some more toxic compound of emetine and a constituent of normal serum. Unfortunately for this theory, it is

known that many alkaloids, such as quinine and emetine, only display their full parasiticidal properties when present as the free base, that is to say, when the *p*H of the medium in which they are present is in the neighbourhood of 7·0. As will be shown in discussing the action of emetine on *Endamœba histolytica*, a slightly increased acidity of the medium causes a definite reduction in the amœbicidal power of the emetine. The buffering action of serum probably, therefore, accounts for the enhanced cercarial killing power exhibited by emetine hydrochloride when dissolved in serum.

Although further evidence is required, it thus seems probable that the action of emetine on the schistosomes is direct.

Emetine has also been tested for its curative effects in other helminthic infections. In infections due to *Clonorchis* Faust and Ke-fang (1926) believed that emetine was entirely ineffective.

In pulmonary distomiasis due to *Paragonimus westermanni*, Kikuiko and Imamura (1918) and Murashima (1922) reported cases successfully treated by intravenous injections. Martin (1927), in reporting two cases, found that an initial treatment by mercurochrome followed by emetine eventually produced a cure.

Emetine has also been recommended in guinea-worm infections (dracontiasis), Tournier (1922) claiming to have treated successfully seventeen cases by emetine given both orally and intravenously. These results require confirmation. Emetine preparations have also been used against filarial infections, especially *F. bancrofti*, but with unsuccessful results.

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CLONORCHIASIS

Infection with *Clonorchis sinensis* Cobbold is by no means rare in China and Japan. In certain parts of Central Japan, in fact, from 56 to 67 per cent. of the population are said to be infected. The worms live within the bile ducts, which become thickened and dilated, while the surrounding liver tissue undergoes atrophy.

Carbon tetrachloride, emetine, tartar emetic and arsphenamine have all been used in the treatment of the disease, and though with the last two drugs ova may disappear from the stools for a time, they almost always tend to recur. Methylene blue is said to have been long used in Japan, while the effect of various aniline dyes upon Clonorchis *in vitro* has been tested by Hasui (1917). The living flukes are susceptible to methyl violet, crystal violet and Nile blue sulphate. Methyl violet given intravenously to dogs was found to stain the liver intensely and to be excreted in the bile, but doses of methyl violet which were toxic to but half the number of worms were fatal to the dog. Nile blue sulphate was even more toxic to the host than methyl violet. In view of the fact that mercurochrome-220 (hydroxymercuric dibromofluoresceine) and gentian violet have been shown by Churchman (1925) to be excreted by the bile in considerable quantities, Faust, Khaw, Ke-Fang and Chao-Yung-An (1927) investigated the effects of these drugs on experimental clonorchiasis of the cat, an animal which is the common natural reservoir of *Clonorchis sinensis* throughout the Orient. The certified gentian violet used is defined by Conn (1925) as " either penta-methyl or hexamethyl pararosaniline or else a mixture of methylated pararosanilines, composed primarily of the two compounds just named." The most conspicuous effect of the oral administration of both mercurochrome and gentian violet in doses of 50 mgm. per kilogram of body weight was a stimulation of egg production as determined by the number of ova in the fæces. This excessive production consisted for the most part of immature, imperfectly formed non-viable ova, and was followed by a sudden fall in the number of eggs produced. The first hyper-production was not

due to death of the worms and disintegration of their uteri, for mercurochrome in doses lethal to the host had no clonorchicidal value ; the worms were alive, though their uteri were empty.

Gentian violet, on the other hand, was found to be definitely toxic to the worms, for after a dose of 50 mgm. per kilogram of body weight the average daily egg-count fell rapidly. When smaller doses of gentian violet such as 16 mgm. per kilogram were given, the average daily egg-count fell only after several administrations of the dye.

It is possible, therefore, that the action of gentian violet is cumulative. Autopsies on the cats treated with gentian violet showed a worm reduction of from 84 to 96 per cent. With an oral dose of 50 mgm. or more per kilogram of body weight there were symptoms of intoxication, characterised by vomiting and loss of weight. If this dose were repeated death ensued. In light infections amounts up to 35 mgm. per kilogram were administered without toxic effects, while even in heavy infections with much liver damage doses of from 15 to 17 mgm. per kilogram were given daily with apparent immunity.

These striking results do not yet appear to have been extended to man. Olivier and Kandou (1927), however, report that as the result of 20 c.cm. of a 1 per cent. solution of gentian violet given intravenously, followed three days later by a further 30 c.cm., Clonorchis ova disappeared from the fæces of a Chinaman who clinically, at any rate, was cured. This appears to have been an early case : in more advanced cases, with much fibrous alteration of the hepatic tissue, the parasites might not be so easily reached.

The use of aniline dyes as anthelminthics has as yet been scarcely attempted. Hall (1928), however, has recently found that mercurochrome (hydroxymercuric dibromofluoresceine) will kill whipworms in dogs, the dead worms coming away stained, whereas other species of worms are not killed and take up little or none of the stain. Kudicke (1925) has also carried out *in vitro* experiments with a number of dyes on *Cysticercus tenuicollis*, and on the filariform larvæ of *Strongyloides stercoralis* placed in glasses so that the action of the dyes can be watched. Among other dyes, malachite green, brilliant green, crystal violet, fuchsine,

rhodamin B, congo-red, trypan red, trypan blue, and thionin blue were investigated.

Both acid and alkaline dyes penetrated the cell wall, while as a general rule those which diffused badly penetrated less thoroughly than those which diffused rapidly. Practically all the dyes showed slight toxic action on the cysticercus and on the larvæ. No experiments were carried out *in vivo*.

Further experiments in regard to the action of dyes on parasitic worms should provide results of considerable interest.

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CHAPTER III

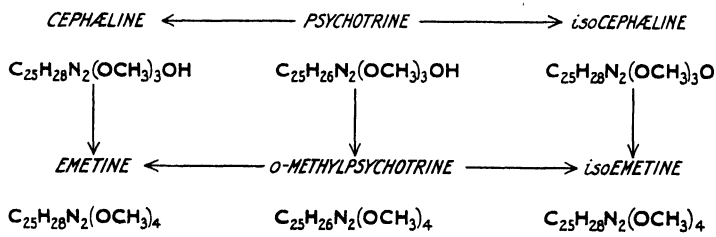
THE CHEMOTHERAPY OF AMŒBIASIS

THE results obtained during the war in the treatment of amœbic dysentery with emetine showed very definitely that this drug failed in a large percentage of cases to eradicate the parasitic amœbæ from the tissues. Numerous attempts have therefore been made to find other substances which would replace emetine, but in one form or another emetine still retains its pre-eminence in the chemotherapeutic treatment of amœbiasis.

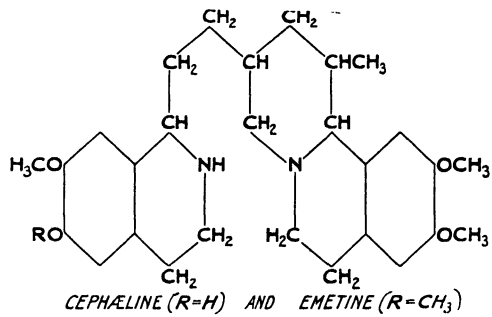
EMETINE

Emetine is one of the alkaloids obtained from various species of Psychotria. The roots of *Psychotria ipecacuanha* constitute the Brazilian ipecacuanha of commerce, and probably also that obtained from Johore, in Malaya. A second commercial variety known as Carthagena ipecacuanha is believed to be derived from *P. acuminata*, which is collected in Colombia. The medicinal qualities of ipecacuanha were apparently known before the Spanish Conquest to the natives of Brazil, whence it was introduced to Europe by Guillaume le Pois (Guilielmus Piso : 1658), who in laudatory terms described the root as " Salutifera et celeber radix "—" Benedicta illa radix—præstans remedium—qua nullum præstantius aut tutius." Helvetius successfully used the drug in the treatment of Louis XIV. The alkaloid emetine was first isolated by Pelletier and Magendie (1817), but was only obtained in a pure state by Paul and Cownley (1894), who separated from commercial emetine the phenolic base cephæline, and later obtained a third alkaloid, psychotrine. To these Pyman (1917) added emetamine and O-methylpsychotrine, psychotrine on treatment with methylsulphate in presence of sodium amyloxide being

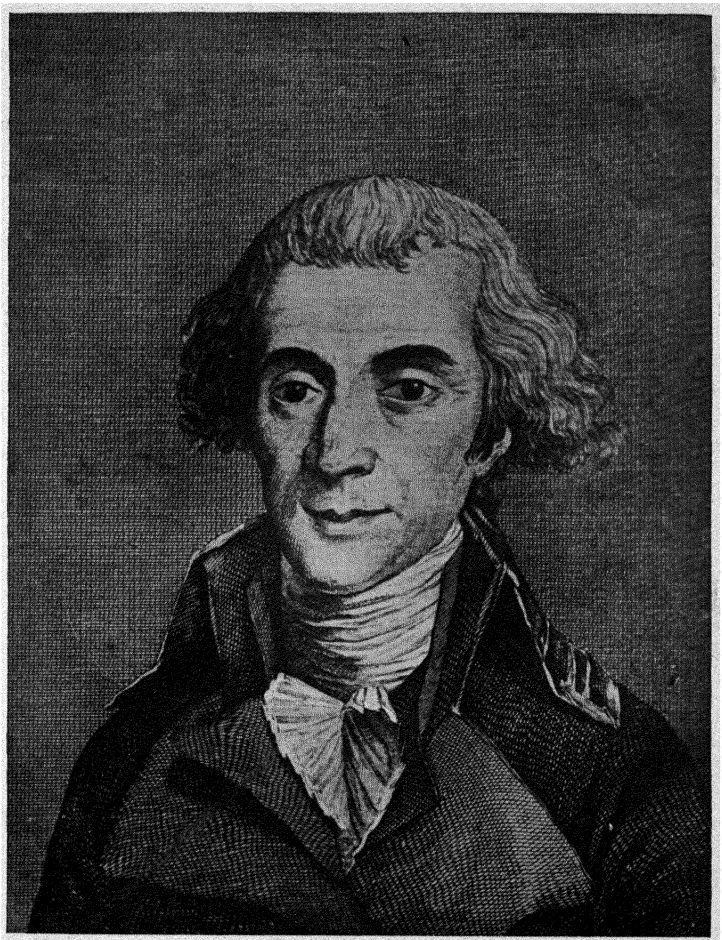
converted into O-methylpsychotrine. Carr and Pyman (1914) also showed that on reduction psychotrine yields a mixture of cephæline and *iso*cephæline. These are probably stereoisomerides, and on methylation with sodium methyl sulphate in presence of sodium amyl oxide yield respectively emetine and *iso*emetine, these also being produced by the reduction of O-methylpsychotrine. *iso*Emetine appears to be one of the reduction products of emetamine. These interconversions are thus mainly brought about by the addition of hydrogen or by methylation of hydroxyl groups. The inter-relationships they imply may be graphically represented thus :



The structural formulæ of the ipecacuanha alkaloids has recently been elucidated by Brindley and Pyman (1927).

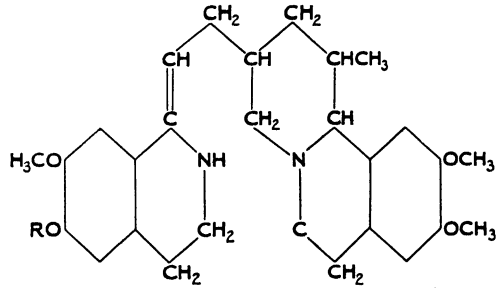


As emetine, on oxidation with potassium permanganate, yields a simple *iso*quinoline derivative, the ipecacuanha alkaloids may be placed in the *iso*quinoline group.

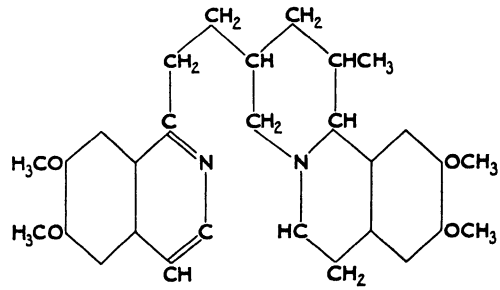


P. J. PELLETIER (1788–1842), who first isolated the alkaloids emetine and quinine.

[*To face p.* 78.



O-METHYLPSYCHOTRINE (R=CH3) AND PSYCHOTRINE (R=H)



EMETAMINE

The Pharmacology of Emetine

The introduction by Rogers (1912) of emetine in place of the total alkaloids of ipecacuanha marked a notable advance in the treatment of amœbiasis. Certain of the other alkaloids of ipecacuanha have been found to have little or no curative action in amœbic dysentery, methylpsychotrine in particular having been shown by Jepps and Meakins (1917) to have no action on infections due to *Endamœba histolytica*. The pharmacology of emetine has, therefore, naturally been more extensively studied than that of the other ipecacuanha alkaloids.

Arrillaga and Guglielmetti (1921) found that the conductivity of the heart muscle was affected by emetine, a result confirmed by Chopra and Ghosh (1922), who showed that injections of emetine caused a weakening of the heart's action and a fall in blood pres-

sure, death resulting from either auricular or ventricular fibrillation. There was no action on the musculature of the uterus.

Emetine when directly applied to a mucous surface such as the tongue has, according to Chopra, Gupta and Pillai (1927), an irritant action; there is a flow of saliva. The activity of the starch-digesting ferment is, however, decreased. Peptic digestion, on the other hand, is stimulated by dilutions higher than 1 in 2,000. When given intravenously, emetine is excreted, at least in part, by the intestinal mucosa, and the tone and movement of the gut are stimulated, the effect being greater as one passes from the stomach distally down the colon. The stimulation is due to a direct action of the alkaloid on the musculature of the tract, the nervous mechanism being unaffected. In addition, there is an increased vascularity of the gut, the volume and automatic movements of the spleen also being augmented. This pooling of blood in the splanchnic vessels, the area affected by *E. histolytica*, may possibly assist the parasiticidal action of the emetine. While in cats emetine is apparently excreted by the kidneys, in man it would seem, at least in the first place, to be excreted by the intestines. Mattei (1920) and others have shown, however, that even in man, emetine is ultimately excreted in the urine, but the excretion is slow, continuing for days or even weeks.

The toxic effects of prolonged emetine administration were recognised during the early days of the war, when large doses of emetine hydrochloride were occasionally administered to bacillary as well as to amœbic dysentery patients, symptoms of low blood pressure with the "emetine pulse," cardiac irregularity, neuritis, desquamation of the skin, striation and atrophy of the nails, and mental depression being by no means rare.

Dale (1915), who was the first to study the cumulative effects of emetine, found that in rabbits a profound diarrhœa set in, while in cats there occurred a lethargy deepening into coma. In man, cases of neuritis and cardiac derangement were not infrequently encountered. Young and Tudhope (1926), who recount a personal experience, noted that after 18 gr. of emetine in the course of twelve days, there were weakness of the arms and legs, ankle drop and some muscular wasting, extensors and flexors being equally

affected. There was no pain, either subjectively or objectively, and no tenderness on squeezing the muscles. The knee-jerks, however, were sluggish, and the mechanism of accommodation in the eye was easily fatigued. The only sensory symptom noted was a loss of taste for tobacco and food. After the cessation of the emetine injections, the palsy lasted for about one month. In rabbits and guinea-pigs the symptoms produced were weakness of the hind limbs, wasting and diarrhœa, the effects being more acute with larger doses. Histologically, there were noticed granular degeneration and cloudy swelling of the heart muscle and hæmorrhages in the interstitial tissue. The lungs were congested, while the liver showed, in addition, areas of necrosis. The kidneys were hyperæmic, with swelling and desquamation of the renal epithelium and occasional areas of hæmorrhage and necrosis. The suprarenals also exhibited hæmorrhages in the medulla. The changes in the nervous system were of much interest, for the nerves showed Wallerian degeneration at their peripheral extremities, while in the spinal cord there was definite chromatolysis of the anterior horn cells. There was no evidence of any inflammatory change in the nervous system.

Similar findings have been recorded by Chopra, Ghosh and Premanker De (1924). Rabbits injected with 1 mgm. per kilogram of body weight exhibited toxic symptoms after the tenth injection, though even after the sixth or seventh injection the mucosa of the stomach and intestine was found to be injected and hæmorrhagic. The liver and heart muscle were always degenerate, the cardiac fibres being much shrunken. In the fatal cases recorded by Soca (1922) and Bais (1921) there were symptoms of neuritis and cardiac derangement. Bais's patient, a Javanese woman, had only received 0·1080 mgm. in eighteen injections over a period of six weeks, but at the autopsy there was evidence of considerable degeneration of the cardiac musculature. The petechial hæmorrhages which form so constant a feature of the histological picture in chronic emetine poisoning would seem to be due to a direct toxic action on the capillary endothelium, for with intramuscular injections of emetine hydrochloride, Acton and Chopra (1924) found no necrosis or hyperæmia, and only slight sub-

cutaneous and intramuscular œdema, but petechial hæmorrhages were common both at the site of inoculation and extending for some distance into the surrounding muscles.

Emetine hydrochloride

It is now generally agreed that when emetine hydrochloride is used hypodermically not more than twelve daily injections of 1 gr. should be given, in order to avoid the risk of toxic symptoms. After an interval of some weeks, during which the greater part of the emetine is eliminated, a similar course may be repeated. At the present time, however, it is more usual to combine the injection of emetine hydrochloride with the oral administration either of ipecacuanha or more commonly of some other form of emetine. Some workers, such as Vaccarezza (1921), believe that the failure of emetine introduced subcutaneously or intramuscularly is due to the employment of too small a dose or to the cessation of treatment on the disappearance of symptoms. Vaccarezza, therefore, gives 0·15 gm. daily for not more than four days, or 0·10 gm. for not more than seven days.

Cawston (1922) suggests that the pain commonly caused by emetine injections can be avoided by dissolving the alkaloid in 1 per cent. carbolic acid immediately before the injection.

Sellards and Leiva (1923), having found that rectal injections of 10 mgm. of emetine per kilogram of body weight, diluted 1 in 1,000, were more efficacious in curing cats of amœbic dysentery than hypodermic injections, Willmore (1923) supplemented oral administration of emetine by medication *per rectum*. Emetine base 1 grain is mixed with ether (6 drachms) and olive oil (8 oz.), and after a preliminary washout, injected slowly into the bowel, the patient being retained in a position as much "inverted" as possible. The injections of "emetol," as the mixture with olive oil is called, are given on alternate days, six to twelve completing a course, which should be controlled by sigmoidoscopic examination. Unfortunately, with this form of lavage it is only possible to reach the distal third of the colon, so that lesions in the ascending or transverse colon are unaffected.

Manson-Bahr (1925) finds that emetine injections *per rectum* in man are extremely irritating and painful. When given in a dilution of 1 in 2,000 (2½ gr. of emetine in 12 oz. of water) they produce intense intestinal spasms, which may last as long as six hours. In two chronic cases treated in this manner an acute attack was precipitated. In a third case, when given twice weekly for three weeks on end, a favourable result was obtained.

Intravenous injections of emetine have been recommended by Petzetakis (1924), who gives in chronic or moderately severe cases gradually-increasing doses, beginning with 0·03 to 0·05 gm. on the first day, and reaching a maximum of 0·1 gm. on the fourth day, a total of from 0·35 to 0·45 gm. of emetine in a space of ten days. In serious cases slightly larger doses are given up to a maximum of 0·6 gm. in the same period. The results, it is claimed, are rapid and striking, and much superior to those obtained by the ordinary subcutaneous or intramuscular route. As may be surmised, and as Bensaude, Cain and Rachet (1924) have shown, the intravenous injection of emetine is liable to be followed by nausea and vomiting within from fifteen to sixty minutes of the injection, while delayed toxic symptoms due to a cumulative action are by no means rare.

Emetine bismuthous iodide

The double iodide of emetine and mercury was first employed in the treatment of dysentery in India by Tull Walsh (1891), but was entirely neglected until DuMez (1915) reintroduced emetine bismuthous iodide in the treatment of amœbic dysentery. The compound is a brick-red powder containing approximately 30 per cent. of emetine.

It was originally employed under the impression that, being insoluble both in water and hydrochloric acid, it would pass into the intestine and remain in contact with the ulcerated mucosa.

Martindale (1923[2]), however, points out that in contact with hydrochloric acid in physiological concentration, the compound is slowly decomposed with the formation of bismuth oxychloride. The great drawback to the use of this compound is the intense

nausea and vomiting to which it so frequently gives rise. Attempts which have been made to counteract this action have in some cases resulted in the non-absorption of the drug. Thus Low and Dobell (1916) have shown that the drug passes through the intestine unabsorbed if compressed into a hard tablet or if coated with paraffin, vaseline, resin, keratin or stearin. Dobell, Gettings, Jepps and Stephens (1918) have shown that stearin and salol-coated pills are not so efficacious as gelatine capsules containing the powder. Jepps (1921) found that over 45 per cent. of her cases relapsed after treatment with salol-coated pills: an excellent method of administration, however, was to give 3 gr. of the salt dissolved in half an ounce of liquid paraffin. If dropped into a glass containing two to three ounces of water, the dose was easily taken. Given by this method, only 10 out of 75 cases had unpleasant sequelæ, while only 12·7 per cent. relapsed after a course of 36 gr. Rennie (1922) concluded that the best form of administering emetine bismuthous iodide was as a loose powder in gelatine capsules. Three of these, each containing 1 gr. of the salt, are given on an empty stomach last thing at night, 10 minims of tincture of opium being given half an hour previously if the patient is intolerant. The patient should be in bed, the diet light and easily assimilated, but not necessarily restricted to milk. Vomiting delayed as long as four hours after ingestion does not necessarily indicate non-absorption, but rather that the drug is having a therapeutic effect. Diarrhœa occurring during treatment must be similarly regarded, three or four dark fluid motions being passed daily. Most patients tend to lose weight during the course, but quickly regain it afterwards, though restriction in diet for a month after treatment is advisable. Willmore (1923), in treating the chronic cases at the Ministry of Pensions Hospital, gave 60 gr. of emetine bismuthous iodide in twenty days, 10 gr. of emetine being injected during the same period. Of 358 cases so treated, 28 per cent. were cured after the first course; 20 per cent. after a second course, and 5 per cent. after a third course. One hundred per cent. of those receiving more than three courses relapsed.

It is thus obvious that though emetine bismuthous iodide is of very considerable value in the treatment of chronic cases of

amœbic dysentery, nevertheless, it is by no means a specific cure, the most intractable cases being those which pass from one acute relapse into another, encystment of *E. histolytica* failing to occur at all.

Emetine periodide

Emetine periodide ($C_{29}H_{40}N_2O_4$6I), which was prepared by Martindale (1923[1]), is a dark purple crystalline powder containing about 38·7 per cent. emetine and 61·3 per cent. iodine. It is insoluble in water and ether, but readily soluble in acetone, and to a less extent in alcohol and chloroform. In contrast to emetine bismuthous iodide, it liberates practically no emetine in the presence of acids, but in a 2 per cent. solution of sodium bicarbonate emetine is liberated. Willmore (1923), who gives it in capsules in doses of 2 gr. three times a day, together with 5-gr. tablets of ox bile to increase the absorption, believes that it is equal if not superior to emetine bismuthous iodide, and in addition emetine periodide is less toxic since it is not followed by nausea and vomiting, nor by cardiac or muscular weakness, depression or desquamation of the skin. It may be given as an intensive fifteen days' course of 90 gr., and this may be repeated after an interval of ten days. Of 91 cases treated by emetine periodide, 43, or 47 per cent., were apparently cured by a first course, while of 37 receiving a second course, 14, or 38 per cent., were presumably cured.

Gordon (1923) has not formed an equally high opinion of the drug, for out of 16 cases, 8 relapsed within one month, while its administration in gelatine capsules was unsatisfactory, as they frequently passed through the gut without dissolving. Manson-Bahr (1925) believes that the periodide is more easily tolerated by the patient than the bismuthous iodide, but is less efficacious in eradicating chronic infections. Of 12 cases treated by Manson-Bahr and Sayers (1927), 5 had to be treated subsequently with emetine bismuth iodide to obtain a permanent cure.

Auremetine

Auremetine, which was introduced by Willmore and Martindale

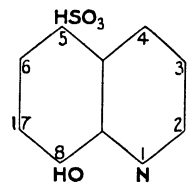
(1926), is a combination of the periodides of emetine and auramine (tetramethyldiamino-diphenyl-ketoniminehydrochloride), with the approximate composition of:

Emetine	28 per cent.
Auramine	16 per cent.
Iodine	56 per cent.

It is a dark maroon powder, insoluble in water, but slowly split up in the intestine with the liberation of emetine and auramine by which the stools are stained a distinct orange colour. It inhibits the growth of free living amœbæ. It can be given by mouth, its use being unattended by vomiting, nausea, abdominal pain or purging. Hypodermically, it is also less depressing than emetine, and it is not therefore necessary to keep the patient in bed on this account alone. Auremetine is given in 1-gr. doses in gelatine capsules four times daily after food on alternate days for seven days, and then daily to a total of 40 to 60 gr. Willmore and Martindale prefer to combine it with stovarsol.

IODOHYDROXYQUINOLINESULPHONIC ACID

Yatren, loretin, quinoxyl ($C_9H_6O_4SNI$) is 7-iodo-8-hydroxyquinoline-5-sulphonic acid mixed with roughly 20 per cent. of sodium bicarbonate. It contains about 28 per cent. of iodine, and its structural formula is said to be:



It is a pale yellow powder which easily absorbs moisture, and must therefore be kept dry, as otherwise the sulphonic acid combines with the sodium bicarbonate to form iodine oxyquinoline sulphate of sodium with the liberation of carbon dioxide. This change also occurs when it is stirred in water at 80° C., so that sterilisation is not advisable. At ordinary temperatures it is soluble to the extent

of 4 per cent. Yatren, which for some time had been used as an intestinal disinfectant, has during the past eight years been largely employed in the treatment of amœbic dysentery. Although it can be injected either intravenously or intramuscularly, it is now usually administered either orally or *per rectum*. When given *per rectum* it is readily absorbed, while to a large extent it is excreted in the urine, where after five or six hours its presence can be detected by the use of iron perchloride, with which it gives a green coloration. Its employment is unaccompanied by any unpleasant symptoms, though if too strong a solution is used for intestinal lavage tenesmus may occur.

When given orally in amœbic dysentery, yatren is made up either into pills containing 0·25 gm. (4 gr.), or in cachets containing 0·5 gm. (8 gr.), a total dosage of 1 gm. per day being administered. As an enema, 3 to 5 gm. are added to 200 c.cm. of water at 80° C. The total amount of liquid given should not exceed 1 litre, and is run into the bowel at body temperature by means of a funnel and rubber tubing. After a little practice the patient is able to retain it for several hours.

Mühlens and Menk, in 1921, reported the cure by yatren of eight resistant cases of amœbic dysentery, two of which had undergone cæcostomy and appendicostomy without success. For intramuscular injection they recommended injections of 10 c.cm. of a 5 per cent. solution. Treatment given orally and *per rectum* was continued for from 8 to 14 days, being controlled by sigmoidoscopic examination of the bowel and microscopical examination of the stools; after a week's interval treatment was repeated for from three to seven days, followed by a similar interval and treatment again for from three to five days. Rest in bed and careful dieting were essential to complete success.

Since then many observers have reported cures with yatren. Birt (1923) reports on 28 cases treated in Shanghai, of which 16 were believed to be cured. He used enemata of 100 c.cm. containing 10 gm. of yatren, with the result that some of his patients had considerable pain. Bax (1924) gave 3 gm. doses daily in cachets for ten consecutive days, the patients remaining in bed on a milk diet; a second course was given after a week's interval.

A painless diarrhœa ensued from the absorption of the drug, but a permanent cure appears to have been produced in 16 cases, while 2 failed to respond. De Langen (1923) also treated 36 cases with satisfactory results. Menk (1922) found that a few cases of chronic amœbic colitis were entirely uninfluenced by yatren, while 2 cases of apparent cure subsequently developed liver abscess, which is said to have been cured solely by the administration of yatren. Dalmeyer (1926) also claims to have cured a liver abscess by yatren without operation. Lichtenstein (1923), however, only succeeded in really curing one out of four cases of intestinal amœbiasis, while Gordon (1923) also met with failure, and Boyers (1925) never completely succeeded in eradicating *E. histolytica* with yatren.

The majority of workers now combine yatren with emetine therapy, the combination, according to Acton and Knowles (1924) and Manson-Bahr and Sayers (1927), giving excellent results. Relapsing cases especially react favourably to emetine bismuth iodide by mouth and yatren *per rectum*. Twenty-two cases were given 3 gr. of the salt every night, while every morning they received 200 c.cm. of a 2½ per cent. solution of yatren per rectum. Cure resulted in every case, the changes in the mucosa of the lower part of the intestine being carefully watched with the sigmoidoscope. By this treatment lesions both in the cæcum, transverse colon and descending colon are reached, the former being inaccessible to the effects of yatren administered *per rectum*.

Yatren appears to have little or no direct action on *Endamœba histolytica*. Kofoid and Wagener (1925), who investigated the effects of various drugs on *E. histolytica* grown on egg-Locke-blood medium, found that yatren was only lethal after twenty-four hours in a dilution of 1 in 1,250, while " yatren casein " was lethal only in 1 in 250. York and Adams (1926) also found that yatren in a 5 per cent. solution had no action on cysts of *E. histolytica in vitro*. Vogel (1927) found that yatren 1 in 100 killed the amœbæ in a few hours in culture, while 1 in 5,000 inhibited multiplication and caused the cultures to die out; 1 in 10,000 had no effect. Dilutions of 1 in 1,000, however, required more than fifteen hours to destroy entirely the amœbæ present in a culture, for if sub-

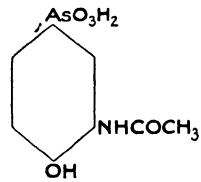
cultured into yatren free medium in a shorter time, the amœbæ regained their powers of multiplication.

Wagner (1928) found that a dilution of 1 in 50 was required to kill cultures of *E. histolytica* in twenty-four hours, dilutions of 1 in 100 merely injuring the amœbæ.

Boyers, Kofoid and Swezy (1925) have, in combination with emetine and other drugs, employed an organic iodine compound, iodo-oxy-benzenepyridine sulphonate.

ACETYLAMINOHYDROXYPHENYLARSINIC ACID

3-acetylamino-4-hydroxyphenylarsinic acid (*stovarsol,* spirocid, kharophen, orarsan*), which has the following graphic formula,



is a white powder practically insoluble in water and with an arsenic content of 27·2 per cent. It was originally studied by Ehrlich and Hata (1911) and reinvestigated by Fourneau and his colleagues (1921 and 1923). It has been used in the treatment of syphilis, malaria and yaws, as well as in amœbiasis. Marchoux (1923), who first employed stovarsol in dysentery, found that three chronic carriers who were quite resistant to emetine were rapidly cured when treated with stovarsol in tablet form, 0·25 gm. being given twice daily. Seven other chronic cases were also treated, only one relapsing subsequently. The tablets were given with food, the course of treatment lasting in most cases for six weeks. Chronic cases sometimes took two tablets the first day, three the second and four the third, fourth, sixth, eighth, tenth and twelfth days, though in most cases after the eighth day it was possible to reduce the number, three tablets being

* The prefix "stov" appears to be a play on the word "furnace," the English translation of Fourneau's name.

given every other day for one week and two tablets every other day for the following week; a single tablet was then given every day for three weeks. In addition to its action in chronic amœbic dysentery, there was definite improvement in the patients' general condition, the appetite was regained, colic disappeared and sleep returned. Nogue and Leger (1923) find that in negroes doses somewhat larger than those recommended by Marchoux are necessary, twenty-nine cachets being given in a period of thirteen days with a break on the fourth, eighth and twelfth days. Although the clinical symptoms rapidly disappeared, relapses occurred and had to be treated with emetine. Rubenthaler and Jausion (1924) also advocate three to four tablets daily from the beginning of treatment, no toxic symptoms being observed by these workers, though others have described dermatitis and jaundice. Manson-Bahr (1925) has reported two cases of relapsing amœbic dysentery which had proved particularly resistant to emetine bismuth-iodide. One was cured by daily doses of 0·75 gm. stovarsol, the other by 0·5 gm. daily. Johns and Jamison (1925) treated forty-six patients with daily doses of from 0·5 to 1·0 gm. Twenty-one remained free from relapses for an average period of more than three months. Stovarsol appeared to be of special value when emetine was contra-indicated, owing to cardiac lesions, in pregnancy and in small children.

Petzetakis (1925) also recommends its use in infants. Up to one year of age 0·05 to 0·08 gm. can be given daily, from one to two years, 0·08 to 0·10 gm., and from two to five years, 0·10 to 0·25 gm. Some cases of amœbic dysentery were found to be quite resistant to the action of stovarsol, however, while a high percentage relapsed. An attempt was made by Petzetakis to determine whether stovarsol has any prophylactic action against amœbic infection. Two tablets of stovarsol were taken daily for three days by a volunteer, and on the fourth day two 1 c.cm. capsules containing dysenteric stools with numerous amœbæ and cysts were given, followed on the same day by eight tablets of stovarsol. During the next five months there were no symptoms of dysentery and no evidence of *Endamœba histolytica* in the stools. Unfortunately, it is impossible to judge of the value of this experiment, as only about 10 per cent.

of human beings can be experimentally infected with *Endamœba histolytica.*

Newman and Davies (1926), who treated alternate cases with emetine hydrochloride injections and stovarsol by mouth, found that the clinical symptoms disappeared in from four to five days with stovarsol, as against eight days with emetine : the period in hospital was about the same, but 33·3 per cent. of cases treated with stovarsol relapsed, as against 11 per cent. treated with emetine.

The majority of workers now prefer to combine stovarsol with emetine. Thus Willmore and Martindale (1926) give 4 gr. of stovarsol three times a day on alternate days to auremetine for a period of seven days, emetine in olive oil being given *per rectum* on the stovarsol days. Seibert (1927) also finds that stovarsol by mouth and emetine hydrochloride hypodermically give better results than emetine bismuth iodide. Only one of 300 cases treated with arsenic developed a slight rash, and this disappeared in from one to three days after discontinuing treatment. Other observers, however, have noted severe skin rashes, which have sometimes simulated measles.

" Spirocid," which appears to be chemically identical with stovarsol, has been used in Germany, while some, such as Ravaut (1926), claim to have obtained good results in chronic cases with arsphenamine, 0·6 gm. being given in the first twenty-four hours, with 0·2 to 0·3 gm. on succeeding days. Françon and Hutinel (1924) combine neoarsphenamine and emetine. Arsphenamine and neoarsphenamine have been used in America, Kofoid and Wagener (1925) having shown that neoarsphenamine is lethal to cultures of *E. histolytica* within twenty-four hours in a dilution of 1 in 420,000. Garin and Lépine (1924) have found acetylarsan in association with emetine of considerable service.

TREPARSOL

3-formylamino-4-hydroxyphenylarsinic acid, containing 28·75 per cent.of arsenic, has been employed by Flandin (1925), who gives 0·25 gm. twice daily. In association with emetine, this compound

is said to have some action on infections due to Giardia and Trichomonas. Treparsol seems, however, to have been but little used in the treatment of dysentery. Vialard and Dargeluy (1925) gave a total of 5·25 gm. to each of five chronic cases with successful results, there being a complete absence of toxic sequelæ. Both treparsol and stovarsol are decomposed in the intestine and excreted in the urine.

RIVANOL

Rivanol, which is 2-ethoxy-6 : 9-diamino acridine, was originally prepared by Morgenroth as an antiseptic, and is more fully dealt with in discussing the chemotherapy of acute bacterial infections. It is a yellow dye-stuff, soluble to the extent of 1 part in 15 parts of water. Its use for intestinal lavage in dysentery was originally suggested by Urchs (1926), who claimed that its effect was mainly on the secondary infections of the specific ulcers; 500 to 800 c.cm. of a rivanol solution (1 in 2,000) in water at body temperature were run into the rectum, the patient retaining the solution as long as possible. Peter (1927), however, who used it in a dilution of 1 in 10,000 in rectal clysmas three to four times a day for three or four days, found that not only were the dysenteric symptoms cleared up, but that both the vegetative and encysted forms of *Endamœba histolytica* rapidly disappeared. According to Wagner (1928), dilutions of rivanol 1 in 1,000 kill all the *E. histolytica* in cultures in from twenty to twenty-four hours, while emetine under similar conditions acts only in a dilution of 1 in 100. Experiments with cats infected with *E. histolytica* did not prove that rivanol had any very definitely curative action when given *per rectum*. When solutions stronger than 1 in 10,000 were given *per rectum* to man, Peter (1927) found that spasmodic contraction of the colon occurred. Schaumann (1928), who studied the pharmacology of the drug, found that in cats rivanol produced spasmodic contractions of the smooth muscle of the gut very similar to those caused by papaverine.

In addition to rectal administration Peter (1928) has given rivanol by mouth both in cases of amœbic dysentery and of non-

specific mucous colitis. After a large dose of Carlsbad salts 0·05 gm. of rivanol is given three times a day for from four to eight days, to a total of from 0·9 to 1·7 gm. Thirty-three cases of amœbic dysentery so treated were all eventually found to be free from infection. Up to the present these striking results lack confirmation. Weitzmann (1929), in fact, in a short note has failed to find any curative action following the administration of rivanol in doses of 0·05 gm. for six days, while van den Branden (1929) only cured three cases, three others remaining totally unaffected.

KURCHI

The bark and seeds of *Holarrhena antidysenterica*, a small deciduous tree belonging to the Apocynaceæ, and found throughout India and Burma, have long been valued as a remedy for dysentery in the form of an infusion of kurchi, the Bengali name for the plant. An alkaloid, conessine ($C_{12}H_{20}N$), was obtained by Haines (1858) from the bark. This substance when injected subcutaneously produces necrosis, but can be given orally or intravenously without any local reaction. Brown (1922) tested the action of conessine on free living amœbæ, and found that not only had the drug a marked amœbicidal action, but that even in a dilution of 1 in 1,000,000 it was capable of inhibiting the growth of amœbæ in culture. The action of conessine in this respect was equal to that of emetine. These results were confirmed by Chopra, Gupta, David and Ghosh (1927), who found that conessine has a specific action on *E. histolytica* obtained from the stools of infected kittens, for it kills the entamœbæ in mucus flakes in dilutions of 1 in 280,000 in eight minutes in the presence of an alkali, and in eighteen minutes in the absence of alkali, thus confirming the results obtained by Henry and Brown (1923) with free-living protozoa. Emetine was found to kill *E. histolytica* in dilutions of 1 in 200,000 in the presence of alkali, but to have no effect in the absence of alkali. Pharmacologically it was found that when given intravenously there was a very marked and persistent fall of blood pressure. In addition to the alkaloid

conessine, Pyman (1919) isolated holarrhenine from kurchi, while Ghosh and Ghosh (1928) have recently found two other alkaloids—kurchine and kurchicine—present in the bark.

Decoctions of kurchi bark have been used by a number of investigators in the treatment of amœbic dysentery. Though the endamœbæ are not entirely eradicated, the clinical symptoms are very rapidly ameliorated by its administration. Tabloids of kurchi bark extract in doses of 20 to 30 gr. a day have a similar effect. In a recent investigation Knowles and his colleagues (1928) found that the simple administration of kurchi by the mouth cured seven out of sixteen cases, and was preferable to the hypodermic injection of both conessine hydrochloride and the hydrochlorides of the total alkaloids of kurchi bark. Drake-Brockman (1926) also speaks well of kurchi as a useful adjuvant in the treatment of chronic amœbic dysentery. Acton and Chopra (1929) have reached the conclusion that the results of treatment with total alkaloids of kurchi bark are far superior to those obtained with emetine. The kurchi alkaloids are much less toxic than emetine, and appear to be without depressant, irritant or emetic effects. Intramuscular injections of 2 gr. of the total alkaloids caused transient hyperæmia and œdema, but this was not visible to the eye unless the dose was injected subcutaneously, the swelling passing off in from twenty-four to forty-eight hours. In cases of acute amœbic dysentery injection was the best method of administration. Kurchi bismuthous iodide could be given orally in 10 gr. doses twice a day for ten days without any deleterious effects. In chronic amœbic colitis 4 gr. of kurchi bismuthous iodide given orally twice a day for ten days cured twelve out of eighteen cases, compared with one out of every two with emetine bismuthous iodide. Intramuscular and oral administration of the total alkaloids of kurchi bark was found to have a curative action on non-suppurative amœbic hepatitis. Its action on liver abscess has still to be investigated.

BISMUTH SUBNITRATE

Bismuth subnitrate was introduced about 1908 by Decks in the treatment of amœbic dysentery in Panama, the results being

analysed in a paper published in 1914. The drug is administered in heroic doses, 180 gr. being given in effervescent water every three hours, night and day in severe cases, while in very chronic cases it is as well to continue one or two daily doses for at least a month after convalescence has been established. James and Deeks (1925) strongly advocate combined treatment with emetine and bismuth subnitrate, for James (1913) believes that bismuth acts indirectly on the amœbæ, either by neutralising some product essential to the existence of the amœbæ or by altering the bacterial flora, which in part constitutes the food of the amœbæ. Occasional untoward effects, such as cyanosis and forcible action of the heart, are said to be due to the use of impure bismuth.

Acton and Knowles (1924) also combined emetine hydrochloride given subcutaneously with 2 drachms of bismuth carbonate every four hours, as by this means the alkalinity of the gut is slightly increased. Knowles, Napier and Das Gupta (1923) have noted that in amœbic dysentery the *p*H of the contents of the gut is on the average 6·22. As recovery takes place the reaction approaches neutrality. Since emetine has a higher amœbicidal action in an alkaline medium, it is thought to be of great importance to render the gut as alkaline as possible.

A large number of vegetable extracts have been employed from time to time in the treatment of amœbic dysentery, the most satisfactory being *Castela Nicholsoni*, which according to Sellards and Leiva (1923) possesses an active principle, probably a glucoside. Other observers have failed to find any curative action from the administration of this bark.

From what has been described it is obvious that none of the compounds described can alone be relied upon to produce a cure in every case of amœbic dysentery. Combinations of various drugs have, therefore, been tested, but further work on the possibilities of combined treatment is required before any finality can be attained. It is perhaps unfortunate that there is a growing tendency to introduce on somewhat inadequate grounds still more preparations in the therapy of amœbic dysentery before the true efficacy of those at present in existence has been determined.

AMŒBIC HEPATITIS AND HEPATIC ABSCESS

There is now almost general agreement that in hepatic amœbiasis emetine exerts a more striking and lasting curative action than in intestinal amœbiasis. In fact, many are of the opinion that operative measures are no longer necessary, since, provided the collection of pus in the liver is not too extensive, emetine alone will bring about its absorption.

Hodson (1922) believes that the amœbæ in the liver are killed by the emetine, and that the absorption of the inert pus is brought about much in the same way as in hepatic gummata. Lenoble and Jegat (1922) have shown that pus can actually be absorbed in this manner, having verified the presence of the pus by aspiration, and then watched its absorption under emetine therapy. Manson-Bahr (1925) also recorded a case where after no less than three operations for the evacuation of liver pus, a fourth abscess developed, the pronounced physical signs and symptoms of which were resolved in a comparatively short time by the administration of emetine and emetine bismuthous iodide. Fry (1924) has similarly described two cases in which large abscesses were certainly present, though all signs and symptoms had disappeared after the administration of 12 gr. of emetine.

Escomel (1922), in fact, believes that if no improvement in the symptoms follows 3 gr. of emetine given intravenously, then the diagnosis of amœbic abscess is at fault.

There is considerable evidence to show that with the introduction of emetine as a routine therapeutic measure the number of cases of liver abscess following amœbic dysentery has decreased; thus Ralli and Panayotatou (1923) state that in Alexandria before the introduction of emetine there were from twenty to forty operations a year for liver abscess, but afterwards only from three to five. Ludlow (1926), in the East, also finds that no case of liver abscess has developed, either in a Korean or in a foreigner efficiently treated with emetine.

Some authors have claimed that yatren alone will cure amœbic abscess, but others, such as van Steenis (1927), prefer to combine

yatren with emetine therapy, as there is thus less chance of relapse occurring.

The open operation for liver abscess has now been largely abandoned in favour of aspiration through Potain's aspirator, a method originally suggested by Rogers (1922). Emetine, however, is indicated both before and after the operation, and is regarded by Thurston (1924) as quite as important as the actual aspiration. This combined therapy materially reduces the period during which the patient must remain under treatment, and has the advantage of being remarkably safe. Thus Rogers (1922) tabulated 111 cases treated by this method with only sixteen deaths, or almost exactly one-fourth of the number occurring in the days before emetine therapy, while Chatterji (1922), in a further series of 186 cases, had a mortality of only 1·6 per cent.

Primary amœbic abscess of the lung, of which several cases have now been recorded, also reacts very rapidly to the exhibition of emetine.

THE ACTION OF EMETINE ON *ENDAMŒBA HISTOLYTICA*

While it is obvious that emetine has a specifically lethal action on *Endamœba histolytica*, the method by which it acts still remains uncertain, the alternatives being a direct action on the amœba and the formation from the tissues of some amœbicidal complex. Evidence for the latter view does not exist, but certain facts recently brought forward strongly suggest that emetine has a direct parasiticidal action on the protoplasm of the parasitic amœbæ. Prior to the successful cultivation of *E. histolytica* by Boeck and Drbohlav (1925), the only methods of approaching the problem were by a study of the lethal action of emetine on endamœbæ obtained from the fæces and intestinal mucosa of men or cats suffering from dysentery, or by an examination of the effects of adding emetine to cultures of free living amœbæ.

The first experiments on the amœbicidal action of emetine were carried out by Vedder (1911), who found that free living amœbæ

in broth cultures were easily killed by emetine. Shortly afterwards Rogers (1912) tested the effect of emetine hydrochloride on *E. histolytica* in dysenteric stools. It was found that on placing a piece of mucus containing active amœbæ in normal saline solutions of emetine the parasites were immediately killed and materially altered in their microscopical appearances by a 1 in 10,000 dilution, while after a few minutes they were rendered inactive and apparently killed by as weak a dilution as 1 in 100,000.

It was, therefore, concluded, possibly on somewhat slender evidence, that emetine had a direct action on *E. histolytica*.

A few years later Pyman and Wenyon (1917) attacked the question by incorporating various alkaloids in the medium used for the growth of free-living amœbæ. The salts of emetine, cephæline, N-methylemetine, and N-methylcephæline were found to be equally inhibitory.

In the same year Dale and Dobell studied the effects of the ipecacuanha alkaloids on *E. histolytica* obtained from scrapings of the intestinal mucosa of dysenteric kittens. The amœbæ were suspended in physiological saline at 37° C. The conditions of the experiments were necessarily somewhat artificial, since the amœbæ suspended in saline alone only remained active for from three to four hours. The toxicity of the alkaloids in ascending order was found to be as follows :—

Demethoxy-emetine > Methyl-psychotrine = N-methyl-emetine > Ethyl-cephæline > Emetine = Cephæline > Psychotrine.

It was thus obvious that though emetine did exhibit a power of killing *E. histolytica* suspended in saline, yet the concentration required to kill the amœbæ within the period of their survival *in vitro* was in all cases far beyond the concentration which could be produced in the circulation of a patient without killing him.

The only conclusion which could be legitimately drawn was that emetine and the amœbæ were not the only factors to be considered in the cure of amœbic dysentery. The missing factor must be supplied by the host as the result of the interaction of emetine and the host's tissues. The value, however, of this conclusion is

rendered doubtful, as will be discussed later, by the ease with which emetine is adsorbed by tissue colloids.

With the successful cultivation of *E. histolytica* more refined methods of investigating the action of emetine became possible.

Kofoid and Wagener (1925) added various substances to cultures of *E. histolytica*, the culture medium consisting of coagulated egg slants covered with 10 c.cm. of Locke's solution, containing 0·5 per cent. of defibrinated rabbit's blood. The cultures were examined after twenty-four and forty-eight hours, the lowest lethal dilutions being :—

Emetine hydrochloride . . .	1 in 10,000
Acetylarsan	1 in 10,000
Stovarsol	1 in 95,142
Neoarsphenamine	1 in 142,000
Arsphenamine	1 in 100,000
Sulpharsphenamine . . .	1 in 3,330

It is noticeable that even in the control tubes there appears to have been a reduction in the number of amœbæ present at the end of forty-eight hours, while the authors themselves suggest that there are many factors making for error in the figures obtained. Sautet (1927) also obtained results which are somewhat anomalous, for at the end of twenty-four hours the highest lethal dilution of emetine was found to be 1 in 500 to 1 in 1,000, whereas cephæline killed in 1 in 75,000.

In the meantime the whole question was reinvestigated by Dobell and Laidlaw (1926), who used a medium consisting of solid inspissated horse serum and liquid white of egg diluted with Ringer's solution—a medium which at the time was the simplest available. The results obtained showed that the full specific action of emetine on susceptible amœbæ is only disclosed if the alkaloid is allowed to act for a sufficient length of time, *i.e.*, at least two days. In addition, it was pointed out that though the original concentration of the alkaloid in the fluid part of the medium was recorded, an unknown amount of the added alkaloid would pass into the solid portion of the medium, so that owing to this adsorption the lethal concentrations given were

relative rather than absolute. Brown (1922) had already pointed out that the lethal action of emetine on free-living amœbæ was greatly reduced if the emetine was in contact with intestinal mucus. It was found, however, that under the experimental conditions described, both emetine and cephæline were specific poisons for *E. histolytica*, and were at least fifty times more poisonous than *iso*emetine, psychotrine, methylpsychotrine, demethoxyemetine and noremetine. Emetine was about ten times as poisonous as stovarsol and about fifty times as poisonous as quinine. *E. coli*, *E. gingivalis* and *Endolimax nana* were comparatively insensitive to the action of emetine in cultures, *E. coli* being able to withstand a concentration of the alkaloid at least 100 times greater than that which is lethal to *E. histolytica*. In a later paper, Laidlaw, Dobell and Bishop (1928) studied the effects of emetine on *E. histolytica* grown in an entirely fluid medium, for when media such as egg-serum or inspissated horse serum-egg Ringer are used, an erroneous idea of the absolute toxicity of emetine for *E. histolytica* is obtained, owing to the fact that the distribution of emetine varies between the solid and liquid portion of a medium with the nature of the solid, the reaction of the system and the time.

With a simple liquid medium it was found that a dilution of emetine hydrochloride 1 in 5,000,000 is lethal for *E. histolytica in vitro* within four days, provided the reaction of the medium is not more acid than *p*H 6·4. With greater acidity the potency of emetine *in vitro* is much reduced. Incidentally no evidence was obtained of the amœbæ becoming resistant to emetine. These results compare with those obtained by Henry and Brown (1923), who found that the lethal effect of emetine on free-living protozoa was greatly reduced in an acid medium. They also fall into line with the observations of Knowles, Napier, and Das Gupta (1923) on the changes in the reaction of the intestinal contents during amœbic dysentery. To ensure the full potency of emetine it must, therefore, be allowed to act both *in vivo* and *in vitro* in an alkaline medium—a medium which, as Deschiens (1927) points out, is itself unfavourable to the growth of *E. histolytica*.

The evidence which is at present available is thus in favour of

the view that the therapeutic efficacy of emetine in human amœbic dysentery is probably due to a direct toxic action of the alkaloid upon *E. histolytica*, while on other intestinal protozoa the action is much less marked, since Bishop (1929) has found that emetine only kills *E. coli* growing *in vitro* in a liquid medium in dilutions between 1 : 300,000 and 1 : 600,000 with a *p*H varying from 6·8 to 7·2. Dobell and Bishop (1929) have likewise shown that in macaques, emetine bismuthous iodide given *per os* eradicates infections due to *E. histolytica*, but leaves untouched those due to *E. coli*, *Endolimax nana*, *Enteromonas* (= *Tricercomonas*) and *Giardia*. Since emetine thus affects the various intestinal protozoa of these monkeys, as it does the comparable species in man, it is obviously possible to employ macaques in chemotherapeutic experiments.

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CHAPTER IV

THE CHEMOTHERAPY OF MALARIA

DURING the past ten years a very large amount of work has been carried out on the chemotherapy of malaria, with the result that though the alkaloids of cinchona still retain their pre-eminence in treatment much has been learnt in regard to their true values and limitations.

The discovery also that the parasites of bird malaria react to drugs in a manner closely akin to that of the human plasmodia has enabled synthetic remedies to be studied under laboratory conditions, so that in plasmoquin there has been evolved a compound which, though it does not displace the cinchona alkaloids, is nevertheless of the greatest value in the treatment of malaria. It is perhaps not too much to hope that during the next few years there will be evolved synthetic compounds which will not merely hold the parasites of malaria in check, but will suffice to eradicate them from the tissues.

CINCHONA

As is well known, in or about the year 1639, Juan del Vego, physician attendant to the Countess Anna del Chinchon, wife of the Governor of Peru, introduced " the Peruvian bark " into Spain for the purpose of treating the ague on his mistress's estates.

In 1655 the bark was sold in England under the name of " Jesuits' powder," and was prescribed by Brady in Cambridge in 1658, and some two years later by Willis in London. The genus *Cinchona* was established by Linnæus in 1742, the tree now known as *Cinchona officinalis* being described by him in 1753.

It is still uncertain whether the antipyretic properties of cinchona bark were known to the South American natives before

the Spanish Conquest, though it was generally believed at the commencement of the eighteenth century that such was the case, as is shown by Gray, quoting Arrot, who travelled in South America in 1737. Humboldt, however, was informed at Loxa in 1807 that the natives regarded cinchona bark as a dangerous drug, while the cascarilleros, who collected it for export, were convinced that the only purpose for which it could be employed was the dyeing of cloth. The derivation of the word quinine is also uncertain. While some would derive it from the name of the aforesaid Countess Anna, others would refer it to the word "kinia," which in the old Peruvian language means bark. The duplication, as in many primitive languages, merely signifies something of special importance as " tse-tse " or " beri-beri." In 1820 Pelletier and Caventou, in Paris, isolated from the bark two alkaloids which they named quinine and cinchonine. Cinchonidine and quinidine were isolated thirty-two years later.

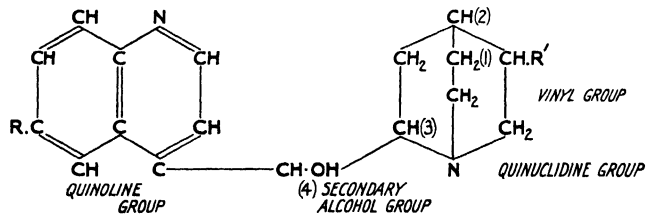
The cinchona alkaloids occur in the various species of two rubiaceous genera, *Cinchona* and *Remijia*, which are indigenous to the eastern slopes of the Andes between the latitudes 10° N. and 20° S., the average height at which the trees flourish being from 5,000 to 8,000 feet above sea-level.

About 1860, attempts were made to introduce cinchona trees in India, Ceylon, Jamaica, Australia, and Java, but at first success was only attained in India and Ceylon. Of late years, however, cultivation has declined in these countries, and has so enormously increased in Java that the latter is now the most important cinchona district in the world, a position which is chiefly the result of the long-continued chemical and botanical investigations carried out under the auspices of the Dutch East Indian Government.

The Alkaloids of Cinchona

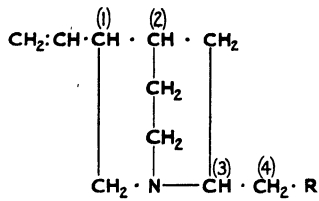
The four chief crystallisable alkaloids derived from cinchona bark are quinine, quinidine, cinchonine and cinchonidine, but in addition to these, over twenty other alkaloids have been isolated from various species of cinchona and cuprea. The majority of these alkaloids, the non-crystallisable and amorphous alkaloids,

are sometimes described collectively as quinoidine. The four alkaloids, quinine, quinidine, cinchonine and cinchonidine, form two pairs of isomerides of which each member of the first pair differs from each member of the second by the residue of a methoxyl group—CH_2O. In addition, the members of each pair yield for the most part the same products under the action of various reagents and the products furnished by the two pairs form parallel series differing constantly by the residue of a methoxyl group—CH_2O. Rabe has assigned the following general formula to this group of alkaloids.



In quinine and quinidine R = OCH_3. R′ = $CH : CH_2$.
In cinchonine and cinchonidine R = H. R′ = $CH : CH_2$.
In cupreine R = OH. R′ = $CH : CH_2$.
In the hydro-bases R′ becomes $CH_2.CH_3$.
In the alkylcupreines R becomes OAlK (homologues of quinine).
In the alkylhydrocupreines and alkylhydrocupreidines R becomes OAlK, and R′ becomes $CH_2.CH_3$ (homologues of dihydroquinine and dihydroquinidine).

The carbon atoms numbered (1), (2), (3) and (4) in the general formula are asymmetric. Since cinchoninone and quininone in which the asymmetry of carbon atom (4) has been destroyed by the conversion of the secondary alcohol group (CHOH) into a carboxyl group (CO) are both dextrorotatory and both yield β-vinyl-α′-quinuclidineoxime of the same optical activity, it follows that cinchonine, cinchonidine, quinine and quinidine must be optically identical as regards carbon atoms (1) and (2), that the distribution in space is the same about these atoms, and in all four cases is dextrorotatory in total effect.



DEOXY-BASES

The deoxy-bases, however, obtained from cinchonine and quinidine and from cinchonidine and quinine are structurally identical, but differ in optical properties, the first pair being dextro- and the second pair lævorotatory. These deoxy-bases have the same general formula where R is C_9H_6N for cinchonine and cinchonidine, and $C_9H_5(OMe)N$ for quinine and quinidine, and carbon atom (4) is no longer asymmetric. It follows that the difference in optical activity in these bases, and therefore in the four alkaloids from which they are derived, depends on the arrangement of groups round carbon atom (3), and is different in sign in the two pairs.

It is thus possible to divide the cinchona alkaloids into two series, a dextrorotatory and a lævorotatory :—

Chemical name.	Natural alkaloid.	Corresponding hydroalkaloid.
Cinchonine Series—Dextrorotatory Alkaloids		
Cinchonine.	Cinchonine.	Hydrocinchonine.
Hydroxycinchonine.	(Cupreidine—unknown)	Hydrocupreidine.
Methoxycinchonine.	Quinidine.	Hydroquinidine.
Cinchonidine Series—Lævorotatory Isomerides		
Cinchonidine	Cinchonidine.	Hydrocinchonidine.
Hydroxycinchonidine.	Cupreine.	Hydrocupreine.
Methoxycinchonidine.	Quinine.	Hydroquinine.

Acton (1922) believes that the pharmacological properties of the two groups also show a divergence, which is dependent on three factors in the structure of the complex alkaloidal molecule :—

(I.) The grouping of the quinuclidine system round the asymmetric atom (3). The dextrorotatory alkaloids are more powerful in their effects than the lævorotatory alkaloids of the cinchonidine series, as shown by their toxicity to mice and to *Paramecium*, by their inhibitory action on enzymes, and by their effects on blood pressure and uterine muscle. The cinchonidine series act more powerfully as local anæsthetics.

(II.) The vinyl group ($CH = CH_2$) in the quinuclidine system. The natural alkaloids are rather more toxic to *Paramecium* than the hydroalkaloids. The latter are more toxic to mice, inhibit enzyme action, and cause a greater fall of blood pressure and greater uterine contraction than the natural alkaloids.

(III.) The group R in the quinoline ring. The higher members of both series of the hydroalkaloids are more toxic to mice, *Paramecium*, bacteria and leucocytes, and are more powerful local anæsthetics. Their action on the inhibition of enzymes, on blood pressure and uterine muscle is reduced.

Considerable interest in the past few years has been aroused by the modified cinchona alkaloids, for with hydrogenation of the vinyl group and replacement of the methoxyl group of quinine and quinidine by higher alkyloxy groups, the following compounds are produced. When the alkyloxy group is

- $-OCH_3$ there is formed Methylhydrocupreine.
- $-OC_2H_5$ there is formed Ethylhydrocupreine (Optochin).
- $-OCH(CH_3)_2$ there is formed *iso*Propylhydrocupreine.
- $-OCH_2—CH(CH_3)_2$ there is formed *iso*Butylhydrocupreine.
- $-OCH_2—CH_2—CH(CH_3)_2$ there is formed *iso*Amylhydrocupreine (Eukupin).
- $-O(CH_2)_5—CH(CH_3)_2$ there is formed *iso*Octylhydrocupreine (Vuzin).

Optochin is highly toxic to the pneumococcus, vuzin to *Corynbacterium diphtheriæ*, while both eukupin and vuzin have been used as dressings for septic wounds and are said to be powerful anæsthetics. Although Izar and Nicosia (1914) have recommended the use of optochin in malaria, it is actually much less effective and far more toxic than quinine. The same is true of

eukupin and vuzin. These compounds are discussed in relation to the chemotherapy of acute bacterial infections.

The Relative Efficiency of the Crystallisable Cinchona Alkaloids in the Treatment of Malaria

During the war the alleged failure of quinine to rid the blood of malarial parasites, and to prevent the occurrence of relapses led to many investigations on the relative curative value of the alkaloids, quinine, quinidine, cinchonine and cinchonidine. The curative properties of cinchona febrifuge were also reinvestigated.

Cinchona febrifuge was first prepared in India in 1874 at the instance of Dr. J. E. de Vrij, who suggested the manufacture of a powder containing all the cinchona alkaloids derived from the red bark of *Cinchona succirubra*, in which quinine constitutes about one-third of the alkaloids present. This powder was called quinetum (kinetum) or febrifuge. It was made by exhausting the powdered red bark with water acidulated with hydrochloric acid, precipitating the liquor with caustic soda and drying the crude deposit. It is clear, therefore, that the name " febrifuge " was originally employed to describe a mixture of the total alkaloids extracted from cinchona bark. Now quinine, the first of the cinchona alkaloids to be isolated, was commonly believed to be the only one of pharmacological value. As a result, the idea became widely current that the curative value of cinchona bark is entirely dependent on its content of quinine. There thus arose a demand for a bark containing a higher percentage of quinine than that found in *C. succirubra*, with the result that the cultivation of yellow bark from *C. calisaya*, *C. ledgeriana* and their hybrids, was encouraged, since in these species the proportion of quinine to the other alkaloids is about 70 per cent. Now as Gage (1925) points out, *C. calisaya* and *C. ledgeriana* require a very special climate and special soil, and though they can be raised with care in the Eastern Himalayas, provided the season be not too dry, the ideal conditions are only found in their natural home in Bolivia and in Java. Undoubtedly also the Dutch spent much more time and money in research than the British in India. It

thus came about that Java became possessed of a virtual world monopoly, not of cinchona bark, but of quinine. About 1903 the scarcity of *C. succirubra* in India led to an alteration in the process of manufacture of cinchona febrifuge, which now consisted of the alkaloids which remained after the extraction of the quinine from yellow bark, some quinine being added to make it more or less similar in composition to the original febrifuge. Cowan (1929) has recently discussed the whole question of the cultivation of cinchona in the British Empire.

Composition of Cinchona Febrifuge from Different Sources (modified from Howard (1925))

	1	2	3	4	5	6	7	8	9
	Dr. Vrij's analyses of Java febrifuge (1876).	Macgilchrist's formula. (1915).	Composition calculated from Hooper's bark analyses.	Indian Goot Tablets. (1913.)	Febrifuge from I. G. Madras factory (1923).	I. G. Febrifuge 1922, quoted by Gage (1925).	B.K.E. make analysed for Dr. Dale. 12.2.24.	B.F.K. make from India. 7.3.22.	League of Nations Malaria Commission.
Quinine	2·9–22·2	7·40	24·0	2·7	8·0	10·5	5·8	11·9	15·0
Cinchonidine	24·0–60·4	5·84	28·0	3·4	21·0	7·0	12·2	9·2	35·0
Cinchonine	18·0–54·0	16·58	15·0	12·3	21·0	23·0	20·0	15·3	25·0
Quinidine	2·8– 5·4	23·83	8·0	12·5	4·5	16·0	8·7	4·8	5·0
Amorphous alkaloid	0·4–21·0	29·12	25·0	54·9	30·0	33·0	41·3	45·4	20·0
Ash, moisture	—	16·12	—	14·1	—	10·5	3·7	—	—
		98·89				100·0			100·0

It is obvious, as pointed out by Howard (1925), that considerable differences are liable to arise in the composition of cinchona febrifuge. Recently, however, under the name of " malarene," a standard cinchona febrifuge is said to have been prepared by the Director of Agriculture (Cinchona), Madras. The form of quinetum approved by the Malaria Commission of the League of Nations (1927) consists almost entirely of alkaloid bases.

In attempting to assess the value of the various preparations of cinchona alkaloids two types of investigation have been carried out. In the first, the clinical symptoms and the disappearance of

parasites from the blood have been studied. This type of investigation, therefore, merely gives an indication of the value of any preparation in producing a clinical as distinguished from a radical cure. In the second type of investigation, the clinical symptoms and the disappearance of parasites from the blood have also been studied, but in addition the freedom from relapse, as determined by frequent blood examinations, over some two months, has been noted. A somewhat longer period of observation would perhaps be more satisfactory, but at any rate this second type of investigation, which gives some evidence of a radical cure, is obviously much more difficult to carry out satisfactorily, since it implies not only strict observation, but also the avoidance of any possibility of reinfection. An ample supply of patients, military discipline and a suitable climate can alone supply the necessary conditions.

Quinoidine (combined amorphous alkaloids of cinchona bark).—While Waters (1916) and Prain (1924) found it to be as effective in malaria as quinine, Row (1919), Acton (1920) and Fletcher (1923) found it useless in removing parasites, and far too poisonous for general use in malaria, as it frequently caused vomiting, diarrhœa and collapse. In doses of 5 gr. twice a day the temperature was controlled, but the parasites remained in the blood, except in one out of ten cases. It seems possible that the drug used by Waters and Prain differed from that used by the other investigators. Possibly the modern methods employed in extracting alkaloids from the bark with hot mineral oil render the non-crystallisable residue less efficient and more toxic than when the extraction is carried out with acidulated water.

Cinchona Febrifuge.—During the war, partly owing to the alleged failure of quinine to control malaria and partly owing to the quinine shortage, attempts were again made to determine whether cinchona febrifuge is of value in treating malaria. Acton, Curjel and Dewey (1920) studied their cases from the point of view of relapse, the possibility of reinfection being slight, and found that of 110 patients with benign tertian malaria 48 per cent. relapsed, whether the daily dose of 21 gr. of cinchona febrifuge was given for ten or twenty-one days. Quinine, however, given

to benign tertian cases, was followed by from 60 to 75 per cent. of relapses, while it was more toxic and less pleasant to take than the febrifuge. Later Acton and Knowles (1924) found that cinchona febrifuge given with alkali was more effective than quinine. Fletcher (1925), who carried out careful tests with cinchona febrifuge in Kuala Lumpur, showed that while doses of 5 gr. twice a day were sufficient to clear the peripheral blood of asexual malarial parasites, the same amount of febrifuge was insufficient. When the febrifuge was given in doses of 10 gr. twice daily the results were equal to those with quinine. Doses of 20 gr. twice daily were too toxic, as they caused vomiting and diarrhœa. Ciuca and his colleagues (1925) also made similar comparative tests, and found that quinetum, containing all the alkaloids but only 15 per cent. of quinine and 5 per cent. of quinidine, was as effective as pure quinine hydrochloride in clearing the blood of asexual malarial parasites, although crescents were unaffected.

Very similar conclusions were reached by the Malaria Commission of the League of Nations (1927), who found that quinetum was of practically equal value to quinine in producing clinical cures, all the available evidence strongly suggesting that a standard preparation consisting of a mixture of the principal alkaloids and purified only so far as to be free from the more toxic constituents, would serve for the treatment of malaria quite as well as quinine.

Finally, Sinton and Bird (1929), in their carefully controlled investigations at Kasauli, in which there was no possibility of reinfection, found that cinchona febrifuge gave an average of 73·1 per cent. of relapses in simple tertian infections, which, while slightly higher than that for quinine, cinchonine and cinchonidine, was superior to that obtained with quinidine. Sinton (1925) had previously found that in malignant tertian infections 50 per cent. relapsed after 30 gr. of quinine daily for seven days.

All these investigations, therefore, go to show that cinchona febrifuge, provided its composition is standardised, is but little inferior to quinine, both in producing clinical and radical cures in

malaria, while from the economic standpoint its use is much to be preferred.

Quinine.—Dogmatic faith in the curative value of quinine was somewhat shaken during the years of war by the numerous reports received from all parts of the world of the failure of the drug to control malaria. Numerous inquiries were, therefore, carried out to determine the real value of quinine in the treatment of the various forms of malaria. The first of these investigations, by Stephens, Yorke, Blacklock, Macfie and O'Farrell (1919), in Liverpool, where the possibility of reinfection after treatment was absolutely excluded, showed that in whatever way quinine was given it failed to control the malarial parasites or to prevent relapses. From the table it will be seen that of sixty patients treated with prolonged courses of quinine by mouth nine relapsed.

Daily dose of quinine in grains.	Number of weeks quinine given.	Number of cases treated.	Number of relapses.	Percentage of relapses.
20	14	5	1	20
30	5	14	4	28·6
(30	3)	22	2	9·9
(45	1)			
45	3	19	2	10·5

The effect of various short courses of quinine in the treatment of simple tertian malaria is shown in the table on page 116 (Yorke and Macfie, 1924).

The patients treated with 90 gr. of quinine sulphate daily on each of two consecutive days were divided into two series at different seasons of the year. Of 76 cases treated in this way from July to September, 29, or 38 per cent., relapsed, while of 89 treated between January and April, 83, or 93 per cent., relapsed. Anderson (1922), in Macedonia, also found that cases treated during the winter months showed a much greater tendency to relapse than during the summer months. It is possible that, as the Liverpool workers concluded, season has a considerable influence on the efficacy of the quinine treatment of malaria,

though other correlations occur to the mind, such as, from the soldiers' point of view, the greater attraction of hospital during the winter months.

The results obtained at Liverpool and in Macedonia were in contrast to those of Rennie, Acton, Curjel and Dewey (1920). Working at Dagshai, in India, where reinfection was also carefully excluded, it was found that 25 to 30 per cent. of cases of simple tertian malaria were cured after a three to eight weeks' course of quinine, during which from 1,050 to 4,200 grains of quinine were given to each case. In cases of malignant tertian

Daily dose in grains and salt of quinine used.	Method of administration.	Number of consecutive days quinine given.	Number of cases treated.	Number of relapses.	Percentage of relapses.
5 sulphate	Oral.	2	12	11	91·6
5 hydrochloride	,,	2	18	18	100
10 sulphate	,,	2	10	10	100
15 sulphate	,,	2	14	14	100
15 hydrochloride	Intramuscular	2	20	19	95
15 base	,,	1–2	38	31	81·6
30 sulphate	Oral.	2	14	14	100
45 sulphate	,,	2	12	9–12	75–100
60 sulphate	,,	2	12	7	58
90 sulphate (1st series)	,,	2	76	29	38·1
90 sulphate (2nd series)	,,	2	89	84–86	94–97
120 sulphate	,,	2	15	9	60
30 bihydrochloride	Intramuscular	12	30	26	86·6

infection, however, Acton, Curjel and Dewey (1920) obtained a cure rate of over 90 per cent. of cases. Quinine, therefore, appeared to be more efficient in the treatment of malignant than of simple tertian malaria, a result which was confirmed by Sinton (1925), who succeeded in curing 80 per cent. of infections due to *P. falciparum* with a daily dose of 30 gr. of quinine and alkali given for seven days.

Acton suggests that the efficacy of quinine is due to the fact that the malignant tertian parasites sporulate in the mesenteric vessels, where quinine is present in greatest concentration, while the simple tertian and quartan parasites are found in the peripheral blood-stream where the concentration of quinine is lower.

The fact is therefore emphasised that there is not one malarial fever, but three malarial fevers, due to three distinct species of plasmodium, which react quite differently to drugs. In support of this view, Mayne (1920), Wenyon (1921), Walch and Walch-Sorgdrager (1921) and Mühlens and Kirschbaum (1921) have all produced good evidence to show that quinine is more potent against the schizonts of *P. falciparum* than against those of *P. vivax*, whereas for the gametocytes the condition is reversed. The reaction of quartan malaria to quinine would seem to differ also from that of the other two species, for Leslie (1923) has found that in this form quinine gives most unsatisfactory results.

In addition to the type of parasite involved, the chronicity or acuteness of the infection is also of importance in determining the reaction to quinine.

Thus in cases of simple tertian infections Sinton and Bird (1929) found that quinine cured 76 per cent. of primary infections, while 24 per cent. relapsed, but in chronic cases there were cured only 32 per cent., with a relapse rate of 68 per cent. These results go to show that chronic infections with simple tertian malaria are more difficult to eradicate than primary infections, an opinion which was widely held by many workers during the war (Seguin, 1921 and 1922). The results of treatment of benign tertian malaria induced by the inoculation of infected blood for the cure of mental disease also favour this view, since the majority of such infections completely recover after therapeutic doses of quinine given for less than one week. Thus York and Macfie (1924) treated sixty-one patients for three days with 30 gr. daily and obtained only 1·6 per cent. of relapses. Yorke (1925), in a later paper, stated that he had observed over 100 similarly treated patients with a relapse rate of about 2 per cent. Rudolf (1927) collected the results of treatment of 455 cases in which the relapse rate was only 2·2 per cent. Of seventy-one patients treated by this worker with a total of 200 gr. of quinine in seventeen days, one only was recorded as relapsing, while of seven patients who received only a single dose of 20 gr. all relapsed. Since similarly low figures have been reported by other workers, it seems evident that malaria

following the inoculation of infected blood is easily cured by small quantities of quinine given for a very short time.

When malaria is therapeutically induced by the bites of infected mosquitoes the results of quinine treatment are not so good. Yorke and Macfie (1924) found that of thirty-one patients receiving 30 gr. daily for three days, four, or 13 per cent., relapsed, while in a later series of thirty-seven cases similarly treated, Yorke (1925) had 57 per cent. relapses. Rudolf (1927) gives 61 per cent. as the relapse rate with this method of infection, James (1926) 25 per cent. after a treatment of 30 gr. of quinine daily for five days. Nicol (1927) obtained 50 per cent. of relapses, whether 30 gr. was given for five days or 15 gr. for ten days. The relapse rate of 24 per cent. obtained by Sinton and Bird (1929) thus compares very closely with that obtained in primary infections induced by the bites of mosquitoes, although it bears no relation to the 90 to 95 per cent. of cures which Bass (1921 and 1922) obtained, using the standard treatment adopted by the National Malaria Committee of the United States of America.

The reason why primary infections of simple tertian malaria are more readily cured than chronic infections is at present unknown. One reason which has been put forward is that the malarial parasites acquire a resistance to quinine.

Quinine Resistance

The evidence for quinine-resistant malarial parasites rests largely on analogy with what is known in regard to certain other parasitic protozoa.

MacGilchrist (1915) did not observe any instance of quinine-resistance, or even of relative quinine-resistance, among 149 patients treated in India, and the same conclusion was reached by Acton, Curjel and Dewey (1920), who found that when relapses occurred after quinine had been stopped and it was necessary to repeat it the parasites had not become quinine-resistant owing to the prolonged administration of the drug, for they disappeared from the peripheral blood in as short a time at the fifth relapse as at the first. James (1913), who has discussed the question of relapse in patients

undergoing treatment with quinine, states that the following hypotheses have been put forward to account for such relapses: (i.) Failure of quinine due to a new species of parasite, (ii.) non-absorption of quinine on account of gastro-intestinal disease, (iii.) failure to swallow the quinine prescribed, (iv.) habituation of parasites to quinine (quinine-resistance).

Although the profound difference between ordinary endemic malaria and malaria in epidemic form had not been widely recognised before the war, yet the examination of countless blood films in the war showed that, as Wenyon (1921) pointed out, the parasites corresponded in every way with those studied in other parts of the world.

The failure of many cases of concurrent dysentery and malaria to react to quinine was regarded as due to non-absorption of the drug as the result of the intestinal inflammation, although no attempts seem to have been made to determine the amount of quinine excreted by the fæces in these cases. Fletcher (1923), who carefully investigated this question, found that in spite of dysentery the drug was readily absorbed and quickly appeared in the urine.

The true explanation of quinine resistance would seem to be the failure of the patient to swallow the quinine. Fletcher (1923), who investigated forty-four so-called quinine resistant cases at Kuala Lumpur and a number of similar cases among soldiers in England, reached the conclusion that when the quinine was actually swallowed and retained without vomiting, the parasites disappeared rapidly from the blood.

Quinine-resistant parasites do, however, occur, although they are extremely rare. Fletcher mentions one possible case, a Tamil woman, in whom malignant malarial parasites persisted in the blood for six months although she was given quinine during the whole of that period. These parasites, which did not cause fever or appear to affect her health in any way, finally disappeared from the blood under the influence of cinchona febrifuge given in doses of 20 gr. for twelve days. Unfortunately absolute proof of quinine resistance would only have been attained if the parasites had been transmitted to another person and then found to be resistant to

quinine. Et. and Ed. Sergent (1921), who have investigated the effects of quinine on bird malaria, find that 0·7 mgm. of quinine given every day for a month readily controls the parasites, and also exercises a powerful influence on the infecting strain, so that when the infection is passed on from treated to untreated birds the latter suffer from the disease in a milder form, the normal virulence being regained only after passage through other untreated birds. In one among 966 canaries infected with malaria the influence of quinine was of quite another kind. This canary had been infected for a period of nine months, during the whole of which time it had been given daily doses of quinine and had shown no symptoms. Suddenly parasites appeared in the blood and persisted in spite of further treatment. Blood of this bird was virulent for other birds, the resistance to quinine being retained in full during its passage through two other canaries and in a lessened degree through a third.

Though the occurrence of quinine-resistant parasites cannot, therefore, be denied, such an explanation of the failure of any particular case to react to quinine, should not be invoked until all other simpler explanations have been disproved.

Cinchonine, Cinchonidine and Quinidine

In 1854 Pepper wrote on the possibilities of replacing the salts of quinine by those of cinchonine in the treatment of intermittent fevers, and in 1866 the Madras Cinchona Commission was appointed to investigate the curative effects of the four principal alkaloids of cinchona—quinine, cinchonine, cinchonidine and quinidine. Since the parasites of malaria had not then been discovered, the effect of the drugs was judged solely by their action on the clinical symptoms of the disease. The conclusion reached was that all four alkaloids were of equal value in the treatment of malaria. The first scientific investigation of the relative value of these alkaloids with regard to the presence or absence of malarial parasites in the blood was carried out by MacGilchrist (1915), who found that, judging by the minimum therapeutic dose, quinine, cinchonine and quinidine sulphates were, for all practical purposes, of equal

value in the treatment of simple tertian infection, though if there was any difference at all, cinchonine was slightly more powerful than quinine, quinidine slightly less powerful. Cinchonidine seemed to act rather more powerfully in malignant tertian infections.

Acton, Curjel and Dewey (1920) believed that quinine was a specific for *P. falciparum*, but that quinidine acted much more powerfully on *P. vivax* infection, as judged by its action in preventing relapses, for of sixty-two patients treated with daily doses of 21 gr. for twenty-one days, only 37 per cent. relapsed; cinchonine, on the other hand, in doses of 20 gr. for twenty-one days, gave a relapse rate of 57 per cent. Special Report No. 96 of the Medical Research Council (1925) failed, however, to confirm the specific action of quinidine in simple tertian infections.

The only other investigation carried out to determine the value of the four alkaloids in preventing relapse is that by Sinton and Bird (1929), who investigated chronic infections due to *P. vivax*, in which all chance of reinfection during the observation period of two months was excluded.

The Relative Effect of the Cinchona Alkaloids in Preventing Relapse in Chronic Benign Tertian Malaria. (*Sinton and Bird*, 1929.)

Alkaloid.	Total patients.	Number lost sight of.	Number not relapsing.	Number of relapses.	Percentage of relapses.				
					Observed.	Possible maximum.	Observed minimum.	Average.	Deviation from average.
Quinine . .	667	66	184	417	69·4	72·4	62·5	68·0	− 3·2
Quinidine . .	208	14	30	164	84·5	85·6	78·8	83·0	+ 11·8
Cinchonine . .	72	3	22	47	68·1	69·4	65·3	67·6	− 3·6
Cinchonidine .	107	24	23	60	72·3	78·5	56·0	68·7	− 2·5
Cinchona febrifuge	110	25	19	66	77·6	82·7	60·0	73·1	+ 1·9
Total . .	1,164	132	278	754	73·0	76·1	64·7	71·2	

The Effect of the Cinchona Alkaloids in Preventing Relapses

From the table it will be seen that the most active alkaloid in preventing relapse was cinchonine, the least powerful quinidine.

Numerous observations have been made on the comparative action of the alkaloids in producing a clinical cure. Thus Silvestri (1921) believed that cinchonine was quite as effective as quinine. Bini (1921) also recommended cinchonine. Fletcher (1925) found that in doses of 0·1 gr. per kilogram of body weight, cinchonine was less effective than quinine in reducing the fever and in removing malarial parasites from the peripheral blood. In doses of 0·1 gr. per pound of body weight it was as effective as quinine. Cinchonine was not more toxic than quinine. Quinidine was as good as, or even slightly better than, quinine bisulphate in its immediate effects.

Ascoli (1926) reported results very similar to those of Fletcher, though it was considered that cinchonine of Dutch origin was less effective, an increase in the dose of one-third to one-half being required to render it equal to quinine. Quinidine, however, was more effective in malignant tertian infections, 0·5 to 0·66 gm. being equal to 1·0 gm. of quinine. Filipella (1923) came to the conclusion that cinchonine was as effective as quinine against all types of parasites. Cordes (1924) found 0·5 gm. cinchonine hydrochloride per day for three days less efficient than 0·2 gm. of quinine hydrochloride in the treatment of malignant tertian malaria. Kligler, Shapiro and Weitzman (1924) also found quinidine to be quite effective in treating both adults and children suffering from simple tertian infection. As judged by the return of fever, there was a relapse rate of 26·6 per cent., while with quinine the relapse rate was 28·5 per cent.

In treating cases of chronic malaria in Algeria, the Sergents and Catanei (1925) found that the effects of cinchonidine and cinchonine in removing the malarial parasites from the peripheral blood and in reducing the hypertrophy of the spleen were very similar to those of quinine, but that cinchonidine was more powerful than cinchonine in reducing splenomegaly.

Ciuca, Irimesco, Manoliu, Alexa and Constantinesco (1925), in comparing the effects of cinchonine and quinine, found that cinchonine was slightly less efficient in removing the schizonts of simple tertian infection from the blood, and much less efficient in dealing with gametocytes. Baqueé, Céard, Dekester and Melnotte

(1925) regard cinchonine as being as effective as quinine, in doses of 1 gm. per diem.

In bird malaria, the results with cinchonine, cinchonidine and quinidine closely parallel those obtained in man.

Giemsa, Weiss and Tropp (1926) found that quinidine was equal to quinine, while cinchonine was less efficient, thus confirming the results which Giemsa and Werner (1914) had obtained in human malaria.

Cinchonine according to the brothers Sergent and Catanei (1924) was a third less efficient than quinine, cinchonidine was quite ineffective in non-toxic doses, while quinidine was too toxic for use.

In summarising these results on the relative efficiency of quinine, cinchonine, cinchonidine, quinidine and cinchona febrifuge or quinetum, the Malaria Commission of the League of Nations (1927) stated that in doses of 1 gm. daily, quinine, quinidine and quinetum are equally efficient in producing a clinical cure in malaria, although the depressant action of quinidine on the heart muscle must not be lost sight of. Cinchonine in doses of 1·5 gm. equals the efficiency of the other three alkaloids. These results show that in cases of quinine idiosyncrasy the other alkaloids of cinchona can be administered with excellent results in malaria. Thus Fletcher and Travers (1923) report the case of a lady in whom quinine dihydrochloride had given rise to a dermatitis; cinchonine base, however, in a daily dose of 16 gr. a day, was well tolerated. Dawson and Garbade (1930) report a case which was sensitive to the dextrorotatory but not to the laevo-rotatory cinchona alkaloids.

The Antimalarial Action of some Derivatives of Quinine and Cupreine

Various attempts have been made to determine the relative values of a number of derivatives of cinchona and cupræa alkaloids in malaria.

Quinine base, according to Fletcher (1923), removed the parasites from the peripheral blood within three days in three benign tertian, three mixed tertian and four malignant tertian infections. In this connection the observation of Henry and Brown (1923) that the toxicity of the base to *Paramecium* is greater than that of the salts is of interest.

Quinine tannate, though tasteless and expensive, is apparently useless in producing a clinical cure in malaria.

Euquinine (quinine-ethylcarbonate), which is tasteless but rather bulky, has been shown by Fletcher (1923) to be effective in removing parasites from the peripheral blood. Biginelli (1928) has recently pointed out that one method of avoiding the bitterness of quinine is to replace the hydrogen of the hydroxyl group of the alkaloid by the residue (R) of either an acid such as acetic, benzoic or salicylic, or by the half ester of a dibasic acid, such as ethyl hydrogen carbonate. This change can be represented as—

$$C_{10}H_8ON—CH.OH—C_9H_{14}N - C_9H_{14}N \longrightarrow C_{10}H_8ON - CH.OR - C_9H_{14}N.$$

Euquinine thus depends for its therapeutic efficiency on the facility with which it undergoes hydrolysis to quinine in the body.

Experiments *in vitro* show that it is only partially hydrolysed under acid and not at all under alkaline conditions. It is, therefore, definitely inferior to quinine itself.

There is some uncertainty in regard to the action of the hydroalkaloids in malaria. Giemsa and Werner (1914) found that hydroquinidine and hydrocinchonine were not more effective in human malaria than the corresponding alkaloids. MacGilchrist (1915), on the other hand, found hydroquinine hydrochloride even more effective than quinine sulphate.

In bird malaria, Giemsa, Weise and Tropp (1926) found that hydroquinine was almost as effective as quinine itself. Boyd (1926) found that hydroquinine chloracetdiethylamide and hydroquinine 4-chloracetylamino-guaiacol also had some action in delaying the appearance of the parasites in the blood.

Giemsa and Werner (1914) found that cupreine given as the sulphate was, in doses of 1 gm., a good substitute for quinine in the treatment of human malaria, though it was both difficult and expensive to procure, and in fact is now unobtainable as Cuprea bark is no longer collected.

These observers obtained the most striking results with quinethyline and quinpropyline, which in doses of 0·3 to 0·4 gm. caused a rapid disappearance of parasites within a few days.

Giemsa, Weise and Tropp (1926) found that these compounds and quinamyline had a similar action in bird malaria.

The Action of Cinchona and Allied Alkaloids in Bird Malaria (Giemsa, Weise and Tropp, 1926)

Preparation.	Birds received on four consecutive days c.cm.	Per cent strength of solution.	= mg. substance.	Number of days on which parasites appeared later than in controls.	Action in human malaria.
Cupreine . .	0·25–0·3	0·5	1·25–1·5	0–4	0 or +
Quinine . .	0·25–0·3	1	2·5–3·0	12–13	++
Quinidine . .	0·25–0·3	0·5	1·25–1·5	12	++
Cinchonine . .	0·25–0·3	1	2·5–3·0	0–5	+
Quinethyline .	0·1	1	1	8–40	+++
Quinpropyline .	0·15–0·2	1	1·5–2·0	4–10	+ or ++
Quinamyline .	0·15–0·2	1	1·5–2·0	7–11	unknown
Hydrocupreine .	0·15–0·2	1	1·5–2·0	10–14	unknown
Hydroquinine .	0·25–0·3	1	2·5–3·0	11	++ or+++
Ethylhydrocupreine (Optochin).	0·25–0·3	0·5	1·25–1·5	6–11	+ or ++
Hydrochlorquinine	0·25	1	2·5	9–11	unknown
Hydroiodoquinine .	0·15	1	1·5	8–10	,,
Quinine dibromide.	0·20–0·25	1	2–3	5–12	,,
Dehydroquinine .	0·25–0·3	0·25	0·62–0·75	5–7	,,
Quitenine . .	0·25–0·3	2	5·0–6·0	0	0
Ethylquitenine .	0·2	1	2	5–12	+ or ++
Quinine chloride .	0·15	1	1·5	0	unknown
Desoxyquinine .	0·20	0·25	0·5	0	,,
Quininine . .	0·30	1	3	0	,,
Acetylhydroquinine	0·10–0·20	1	1–2	0	,,
Quinicine . .	0·15	0·5	0·75	0	0
Quininone . .	0·25–0·30	1	2·5–3·0	0	unknown
5-aminohydroquinine	0·25–0·30	1	2·5–3·0	11–12	++ or +++
5-chlorhydroquinine	0·15–0·20	1	1·5–3·0	11	unknown
5-bromohydroquinine	0·10–0·15	1	1·0–1·5	9–10	,,

0 = no action.
\+ = action less than that of quinine.
++ = action equal to ,, ,,
+++ = action greater than ,, ,,

Optochin, ethylhydrocupreine, was used in the treatment of human malaria by Izar and Nicosia (1914), but disappointing

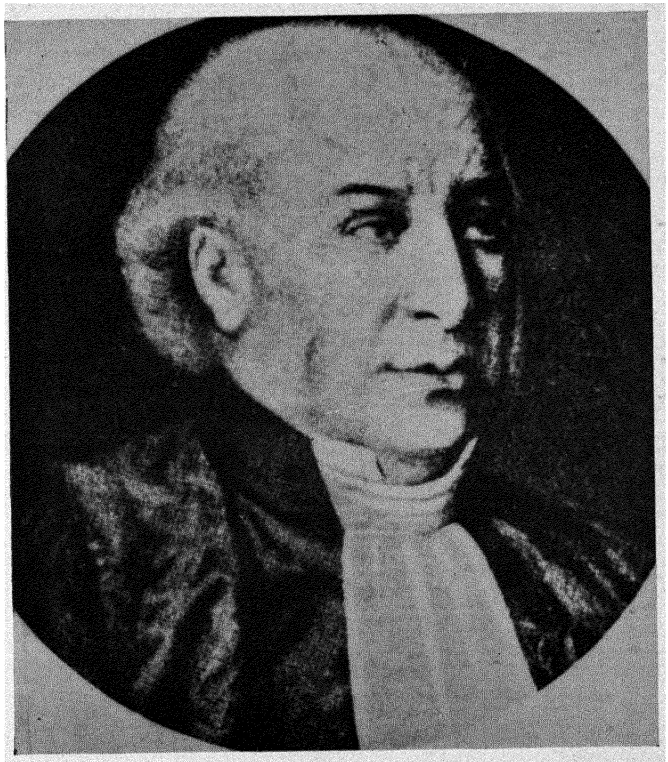
results were obtained both by MacGilchrist (1915), and by Morgenroth, Abraham and Schnitzer (1926).

The Method of Administration of Quinine

The most suitable time, route, medium and interval of administration have all been the subject of much disagreement.

Despite dogmatic statements as to the most susceptible stage in the life-history of the malarial parasite, there is little or no scientific evidence by which the question may be decided. By some there is a widely held belief that quinine is effective only during sporulation, that is to say, against the extra corpuscular stages. Others just as firmly hold the dogma that quinine administration must be started when schizonts are very young, as growth then ceases and degeneration ensues, for if started when the schizonts are half grown, some are killed and degenerate, others carry on to sporulation. If quinine is begun during sporulation, this is not checked.

Oral Administration.—By reason of the millions of persons involved, the oral route must of necessity be ordinarily employed. Its efficacy has been amply proved, though the vast majority of cases are of course unpublished. One reason for the apparent failure of oral administration has been indicated by Fletcher (1925): the quinine is never swallowed, or if swallowed, is promptly vomited. Another suggested reason is its administration in tablet form. It has, however, been amply demonstrated that properly made tablets of quinine hydrochloride produce at least as much urinary excretion of the base as does the same quantity of quinine in liquid form (Seidelin, 1922). There has been a tendency to blame sugar coating as preventing absorption, but sugar coating cannot but rapidly dissolve in any aqueous fluid. The fault really lies in the tablet's menstruum, which, as Blanchard (1922) has shown, may resist solubility for months and defy the attacks of a hammer. It is as useless to expect absorption by solution of the quinine which lies in the centre of an impervious lump of concrete as it is unreasonable to condemn the sugar-coated rapidly disintegrating tablets of proved efficacy. There is



J. B. CAVENTOU (1795–1877), who with P. J. PELLETIER first isolated the alkaloid quinine.

[*To face p.* 126.

considerable evidence to show that the less soluble salts of quinine are absorbed from the gastro-intestinal canal at least as energetically as the more soluble. Thus MacGilchrist (1911), finding the maximum elimination of quinine, taken fasting, to be three to six hours, and taken with food six to twelve hours, concludes that quinine, in whatever form it is given, must before absorption be precipitated as an alkaloid by the alkaline intestinal juice.

The use of alkalies in association with quinine has been recommended by Acton (1921), who found that the toxicity of quinine to *Paramecium caudatum* was increased when the medium was alkaline. Sinton and Lal (1924) subsequently pointed out that in malarial subjects larger doses of alkalies were required to render the urine alkaline than in normal persons. Sinton (1922), after the usual purge of 3 gr. of calomel and an ounce of magnesium sulphate, gave three doses of an alkaline mixture containing sod. bicarb. gr. 60, sod. citrat. gr. 40, aq. ad. 1 oz. at two hourly intervals, followed by an ounce of a second mixture containing quinine sulphate gr. 10, acid. sulph. dil. 10 minims, mag. sulph. gr. 60, aq. ad. 1 oz. in the morning. Subsequently an ounce of each mixture was given in the same order with thirty minutes' interval three times a day for four days and twice a day for two days, thus making a total of 180 gr. of quinine in seven days. During the succeeding eight weeks, the relapse rate was only half that occurring with the same doses of quinine given in dilute sulphuric acid. Sinton (1925) subsequently treated 800 cases of malignant tertian malaria with 30 gr. of quinine daily for seven days with the addition of alkali, and obtained 80 per cent. of cures, as contrasted with 50 per cent. in the cases receiving the same dose of quinine, cinchona febrifuge or febrifuge and alkali. The advantages of the alkali would seem to be due, at least in part, to the increased rate of absorption through the intestinal wall.

Standard Treatments.—Although from what has been previously said, it is obviously impossible to lay down any hard and fast rules for the oral administration of quinine in malaria, certain standard treatments are at present favoured. Thus Ross (1921) recommends a ten-weeks' course entailing the administration of 15 gr. of quinine sulphate or hydrochloride daily for two weeks, and

thereafter 10 gr. daily on six days a week for eight weeks—a total of 690 gr. The great majority of the 24,000 cases with which the Ministry of Pensions had to deal were cured by this plan.

The Standard Treatment of the United States National Malaria Commission, advocated by Bass (1921), consisted of 30 gr. (2 gm.) daily of quinine sulphate so long as clinical symptoms continued. Thirty grains in doses of 10 gr. each were continued for four days longer, and thereafter 10 gr. daily for eight weeks, this course being held to disinfect 90 per cent. of the cases met with in the Southern United States.

In Palestine this treatment was found insufficient to cure chronic cases, and, owing to the attendant difficulties and expense, it was not recommended for universal application.

Mayne and Carter (1919) recommend an easily remembered routine :—

40 gr.	of	quinine	sulphate	for	5	days	= 200 gr.
20	,,	,,	,,	,,	10	,,	= 200 ,,
10	,,	,,	,,	,,	20	,,	= 200 ,,
5	,,	,,	,,	,,	40	,,	= 200 ,,

Hehir (1927) gives the following plan for a three-months' course :—

First Week.—(*a*) In benign tertian, 30 gr. on the day of the next expected attack, and on alternate days until 120 gr. have been taken during the week.

(*b*) In quartan, 30 gr. as before, and on every third day until 90 gr. have been taken.

(*c*) In malignant tertian, as for benign tertian.

(*d*) In double benign and double malignant tertian, 30 gr. daily for three days, and then 20 gr. daily for another three days, none on the seventh day.

Thereafter the course is the same for all types.

Second Week.—15 gr. daily.

Third and Fourth Weeks.—10 gr. daily, with 20 gr. every seventh day.

Fifth to Eighth Weeks.—10 gr. daily.

Ninth and Tenth Weeks.—5 gr. daily, with 10 gr. on two consecutive days each week.

Eleventh and Twelfth Weeks.—5 gr. daily, with 10 gr. once a week.

Panama Canal Zone Standard Course of 1925.—The patient is given a preliminary purge of 2 to 3 gr. of calomel followed by a dose of Epsom salts; 15 gr. of quinine are given three times a day and continued for a week or until the temperature has been normal for five or six days. Then 10 gr. are given three times a day for ten to twelve days.

Pratt-Johnson and Gilchrist (1921), in South Africa, advise the following treatment: 10 gr. of quinine three times a day for three weeks, 10 gr. twice a day for one month, and once a day for two months.

Rectal Administration.—Fletcher (1924) states that the absorption of quinine by the rectum, as judged by the amount excreted in the urine, is poor, irregular and unreliable, and as judged by its effect on parasites in the blood quite unreliable, for in eleven out of sixteen patients on whom it was tried for a week parasites were still present: under oral quinine the parasites promptly vanished. In many of the patients subjected to rectal injections mucus and membranous shreds were passed, thus indicating the irritant action of the drug on the mucous membrane.

Intravenous Injection.—Although there is no evidence that sterilisation of the blood is more readily obtained with quinine given intravenously than with oral quinine, yet where rapid access of the drug to the blood is imperative, as in coma or the antecedent hyperparasitism, this method of injection may be employed, as also in those rare instances where for some reason quinine cannot be given by mouth. The quinine should be injected slowly to avoid any fall in blood pressure, and strict aseptic precautions should, of course, be taken. If, as Clayton Lane (1924) points out, intravenous injections of tartar emetic can be accomplished by Indian sub-assistant surgeons at the rate of three or four cases a minute, the operation cannot be regarded as one beyond the technical skill of a medical man with Western training. Thus Cantlie and Moubarak (1924), in an epidemic of malaria in the Soudan, gave 2,484 intravenous injections, with slight acceleration of the pulse and fainting only on two occasions. Although the average length

of time in hospital was diminished, 42·5 per cent. of the cases relapsed. Stradomsky (1924) reported a relapse rate of 77 per cent. among 1,703 cases each of which had received from six to ten injections. It was found, however, that if an intravenous injection was given on the fever-free day of benign tertian malaria, the paroxysm due for the following day was aborted in 85 per cent. of cases, as against 64 per cent. with oral administration. Thrombosis of the veins not infrequently prevented the continued use of this method.

While quinine dihydrochloride is the salt most generally used for intravenous injection, Rogers (1917) found that in rabbits the dihydrobromide was somewhat less toxic, and employed it with success in grave cases of algid malaria. McCarrison and Cornwall (1918) also found that in doses of 15 gr. the dihydrobromide was less toxic to the respiratory centre than the hydrochloride; if a fall in blood pressure occurs, adrenalin should be given at the same time. Knowles (1917), who believes that the intravenous use of quinine is much less dangerous than is generally supposed, gives 7·5 to 10 gr. of the dihydrobromide in 15 to 20 c.cm. of distilled water. Incidentally Crawford (1922) has found that while distilled water dissolves 1 part of neutral hydrochloride in 35 parts, Ringer's solution dissolves 1 part in 110 parts.

In cases of cerebral malaria, intravenous injection is the method of choice. Thus Cordes (1928) reports that of fourteen cases of cerebral malaria eleven were saved by the intravenous injection of 0·5 gm. of quinine in 10 c.cm. of water. In such cases puncture of the cisterna magna at the base of the brain and the withdrawal of 50 c.cm. of cerebro-spinal fluid is probably preferable to lumbar puncture. Nogue (1922) also recommends the use of intravenous injections of quinine in children. In one case of malignant tertian fever with cerebral symptoms, 40 per cent. of the erythrocytes were parasitised, yet the child was cured by the injection of 1 gm. daily for three days.

Intramuscular Injection.—The intramuscular injection of quinine salts is followed in many cases by such severe consequences that this means of injection should be used only when oral or intravenous administration is quite impossible. All observers have

found that the injection of dilutions as weak as 1 in 150 causes extensive necrosis of muscle, nerve and artery, while a considerable part of the quinine is deposited in the necrotic area. Mariani (1903) found 60 per cent. of quinine dihydrochloride still present in rabbit muscle seventeen hours after the injection. McLay (1922) has also found that the parasites in the blood disappear more rapidly when quinine is administered orally than when it is injected intramuscularly. Apart, therefore, from the risks of tetanus, cases of which have been recorded by Borel and Maire (1923) and by Acton and Chopra (1924), and of such injuries as muscular paralysis due to involvement of one of the large nerve trunks, the intramuscular method of giving quinine is definitely inefficient.

Rogers (1918), however, found that cinchonine dihydrochloride, which is more soluble than the corresponding quinine salt, was absorbed more rapidly from the subcutaneous or muscular tissues than quinine, for cinchonism could be induced within half an hour by the injection of doses which have no effect when quinine is given in the same manner. Comparative experiments made with quinine and cinchonine injected intragluteally in rabbits indicated also complete absorption of the cinchonine. Silvestri (1923) has confirmed these results, intragluteal injections of 200 mgm. of the dihydrochlorides being given to rabbits. The animals were killed at intervals after the injections, and estimations were made of the alkaloids present at the site of injection and in the viscera.

The results were as follows :—

Hours after injection.	Recovered from site of injection in mgm.		Recovered from viscera in mgm.	
	Quinine.	Cinchonine.	Quinine.	Cinchonine.
12	80	40	30	88
24	65	30	26	65
72	28	10	8	2

Cinchonine thus entered the circulation more rapidly than quinine after intramuscular injection, while after oral administra-

tion Tanret's reaction in the urine during the first twenty-four hours gave much more turbidity after cinchonine than after quinine. There was also complete absence of local necrosis at the site of intramuscular injection. Acton and Chopra (1924) also studied the effects of intragluteal injections of quinine, cinchonine, quinidine and cinchonidine dihydrochlorides in rabbits. They found that with cinchonine and cinchonidine the amount of base precipitated at the site of injection was less than with quinine and quinidine. On the third day after injection, however, there was œdema, necrosis and hæmorrhage present in all cases, there being little difference between the local reactions due to the four alkaloids.

In view of these findings it is, therefore, safer to avoid entirely the intramuscular injection of any alkaloid of cinchona.

Quinine in Pregnancy

Pharmacologically quinine salts are found to produce contraction of the uterine muscle, and as a result many have believed that the abortions which are not uncommon in persons suffering from malaria are due to the action of quinine. There is now, however, general agreement that premature termination of pregnancy is much more liable to occur as the result of malaria than from the administration of quinine. Martone (1923) found that with oral quinine in small and divided doses there was no risk of abortion, although with larger doses there was some risk. Kadaner (1928) in the Congo found 0·3 to 0·5 gm. of quinine daily essential for the prevention of abortion in patients suffering from malaria.

The Prophylactic Use of Quinine

In the eradication of malaria from an endemic focus there are two essential methods of attack—the destruction of the malaria-bearing mosquito and the elimination of the human carrier of the sexual forms of malarial plasmodia. During the war many failures were reported from the prophylactic use of quinine, due in many instances to the fact that in the army there was wide-

spread evasion of the taking of quinine. On the other hand, in highly infected areas such as parts of tropical Africa, the daily use of small doses of quinine during the malarial season is regarded as of the greatest value in preventing the dangerous forms of malarial infection, even though milder attacks may not be entirely prevented.

The true action of quinine as a preventative of malaria has been elicited by a study of the therapeutic inoculation of malaria for mental disease. Thus Yorke and Macfie (1924) have shown that quinine taken before or for a very few days after being bitten by mosquitoes infected with benign tertian parasites does not prevent the attack occurring after the usual ten days' incubation period. If, however, quinine is continued for more than ten days after infection the malarial attack is prevented. In other words, the prophylactic use of quinine is due to its action in so reducing the number of malaria parasites that their activities are insufficient to produce any clinical symptoms. Quinine has no action on the development of the sporozoites injected by the mosquitoes, so that it is not a true prophylactic. As the Malaria Commission of the League of Nations (1927) points out, quinine taken over a sufficient period of time and in appropriate doses can often prevent the appearance of symptoms, thus enabling the organism to rid itself of the parasites. There is, therefore, no advantage whatever in giving quinine as a prophylactic to people going to malarial countries before their arrival, while in countries with comparatively low incidence, where the malaria is mainly or entirely of the mild benign tertian type, the continued use of quinine as a preventive of serious malarial attacks will find little place in prophylaxis against the disease. In countries, on the other hand, where a large part of the indigenous population is infected with malignant tertian parasites, and there are present foci from which Europeans are almost bound to be infected, it is probable that 5 or 10 gr. of quinine will still be taken daily as a means of warding off the severe attacks of malignant malaria. Various reports from tropical Africa continue to show the value of prophylactic doses of quinine. In the Belgian Congo, Seidelin (1924) found that 15 gr. of quinine twice a week rendered the infections mild and few,

only 0·8 per cent. of working days being lost. Van den Branden and van Hoof (1923) reported that in Leopoldsville those who had taken quinine once a week for a year had considerably less infection at the end than at the beginning of the period. In Liberia 1 gm. of quinine on Sunday and 1 gm. in the middle of the week has been advised, cases of blackwater fever being almost unknown with this *régime*. In Algiers the brothers Sergent, Parrot, Foley and Catanei (1925) conclude that though not an absolute preventitive against attack, nevertheless daily doses of quinine render the attacks milder and minimise the danger of becoming a carrier. Where malaria is thus systematically treated the number of cases infected has decreased, and eight villages have been rendered healthy.

In the Dutch East Indies, Hendriks (1924) lays much stress on the quininisation of the population, especially when applied to schools. In the Near East similar results have been obtained, Gill (1916), in Arabia, noted that 10 gr. every other day was more effective than 5 gr. every day, while in Palestine, Kligler (1923) reported that the regular administration of 30 gr. daily for five days, followed by 10 gr. daily until the end of the malarial season, reduced the loss of working days to less than one-sixth of that of those not taking the drug. Even after this prolonged quininisation, however, one-fourth of the cases had parasites in the blood within four days of ceasing quinine, thus showing that the infection was merely masked, not eradicated.

In Italy, Switzerland and Corsica extensive quinine prophylaxis has assisted in reducing the incidence of malaria in many districts. It must, however, be emphasised that quinine prophylaxis, unless associated with the destruction of anopheline mosquitoes, is useless in rendering a district entirely free from malarial infection.

THE PHARMACOLOGY OF THE CINCHONA ALKALOIDS IN RELATION TO CHEMOTHERAPY

The evidence derived from a study of the pharmacology of the cinchona alkaloids throws but little light on their mode of action in curing malaria. The greatest attention has naturally been

given to quinine. As with the majority of alkaloids, quinine passes through the stomach and is absorbed largely from the small intestine. Acton and Chopra (1925) have shown that the administration of alkalies before quinine increases its rate of diffusion through the mucous membrane of the intestine; diffusion is thus dependent on physical laws, since it increases when there is an excess of OH ions present at the cell membrane and decreases when there is an excess of H ions. By a previous administration of alkali the concentration of quinine in the mesenteric vessels can be greatly increased. Estimations of the amount of quinine in the blood by Hatcher and Weiss (1926) show that within five minutes of the intravenous injection of quinine only about 5 per cent. still remains in the blood. A large part of the quinine which has thus disappeared is taken up by the capillary endothelium, since it can be regained by perfusion. Similar results were obtained by Weiss and Hatcher (1927) with quinidine.

Morgenroth (1918) estimated the quinine concentration of the blood as 1 in 20,000 a few minutes after intravenous injection, while after oral administration of therapeutic doses it was only 1 in 150,000. Ramsden, Lipkin and Whitley (1918) found that quinine was present in the blood in a strength of 1 in 100,000 twenty-seven hours after its administration, while Hatcher and Gold (1927) made estimations on twelve specimens of blood averaging about 0·25 per cent. of the total blood, drawn from patients at intervals of from thirty minutes to two days after the oral or intramuscular administration of quinine. Quinine could not be detected in a single specimen of blood despite the fact that the patients had taken a total of 14·5 gm., though a trace was recovered from the combined extract of all the specimens. Numerous attempts have been made to determine whether quinine is distributed evenly between the red corpuscles and the plasma. Baldoni (1912) found that the red blood corpuscles contained more. Morgenroth (1918) and Schilling and Boecker (1919) also found that when quinine was added to defibrinated blood the red corpuscles contained more quinine than the serum. On the other hand, Hartmann and Zila (1918), and also Ramsden, Lipkin and Whitley (1918) showed that after intraperitoneal injection of

quinine in guinea-pigs the ratio of quinine in serum to that in corpuscles was as 8 to 1. Blood from a man during blackwater fever with quinine present in whole blood in 1 part in 140,000 gave a ratio of 2·2 to 1, while even when quinine in physiological saline was added to defibrinated calf's blood the ratio of concentration of quinine in serum to that in corpuscles was on the average 3 : 2. Acton and King (1921) concluded that the distribution between corpuscles and serum was approximately equal, a finding confirmed by Gibbs (1928), while Pantschenkow and Kirstner (1928) and Binet and Fabre (1929) again find a greater concentration in the red cells.

This question is of some practical importance, for it has been thought that since the malarial parasite is intracorpuscular no drug could be of use as a chemotherapeutic agent unless it readily penetrated the red blood cells. Shaw (1928) has, therefore, investigated the absorption by human and fowl red blood cells of a large number of chemical compounds. The relationship between the chemotherapeutic action of the drugs in bird malaria and the partition coefficient, by which is meant the concentration of quinine in the corpuscles over the concentration in the surrounding medium, has been studied by Hegner, Shaw and Manwell (1928).

A certain correlation seems to hold between the partition coefficient and the action on bird malaria.

Compound.	Partition coefficient.		Action on bird malaria.
	Man.	Chicken.	
Quinine	4·6	5·0	+
Quitenine . . .	0·2	0·3	—
Cinchotenine ethylester .	2·6	1·9	+
Quinidine . . .	4·6	4·4	+
Cinchonine . . .	—	4·3	+
Cinchonidine . . .	3·5	3·8	+
Optochin . . .	4·9	—	(+)
Optochin chloroacetamide .	—	0·2	—

It is obvious that in view of the contradictory results as to the partition of quinine the question requires further investigation.

After absorption in the body two fates await quinine. It may be excreted unchanged by the kidneys, or it may be stored in the tissues. The amount of quinine thus excreted seems to vary with the individual. Hartmann and Zila (1918) hold the percentage to vary from 15 to 35, though it is more generally placed between 23 and 65. According to Ramsden, Lipkin and Whitley (1918) it lies between 7 and 11 per cent. when 90 gr. has been administered for two days. Quinine may be detected in the urine within fifteen minutes of its exhibition by mouth, and it continues to be excreted in some quantity during the next twenty-four hours and in smaller amounts up to about seventy-two hours. At first the ratio of concentration of quinine $\frac{\text{urine}}{\text{blood}}$ is very high, ratios from 20 to 40 being common, and in one case Ramsden (1920) found the figure not less than 375 if reckoned for whole blood and 250 if reckoned for plasma. The quinine which is not excreted by the kidneys is somewhat unevenly distributed in the various tissues. The suprarenals, the spleen and the kidneys accumulate quinine at much higher concentrations than the blood, while the amount in the lymphatic glands is said to be less than in the blood. Lipkin (1919) has found that certain organs, such as the liver, kidney, striated muscle and intestinal wall have the power of destroying quinine when incubated with it *in vitro*. The suprarenals, spleen and bone marrow lack this power. The quinine-destroying agent can be extracted from the liver, is thermolabile, being inactivated at 100° C., and acts best in a neutral medium. It does not act in the absence of oxygen. In this connection it is interesting to note that Cornwall (1919) has found that quinine given intramuscularly or intravenously to rabbits over a period of nine months causes degenerative changes in the cellular elements of the adrenals and kidneys.

One of the substances formed from quinine by the action of liver is quitenine. This involves a change in the vinyl group ($CH = CH_2$) of quinine, which is converted into the COOH group.

Quitenine, however, is without action in human malaria, as shown by Stephens and his colleagues (1919), while both Giemsa, Weise and Tropp (1926), and Hegner, Shaw and Manwell (1928),

find that it has no action in controlling bird malaria. There is thus no evidence that quinine forms any antimalarial compounds on incubation with the tissues.

THE MODE OF ACTION OF QUININE

Very little light is thus thrown on the antimalarial action of quinine from a study of its pharmacology. Evidence of a direct action on the plasmodia is difficult to reconcile with the rapid excretion, even though Bass (1922) has shown that quinine dihydrochloride in a concentration of 1 in 4,000 when incubated for five hours with the schizonts of *P. falciparum* may produce some degeneration in the parasites, and possibly inhibit further development. Kirschbaum (1923), however, incubated together for five to twenty-four hours equal portions of citrated blood containing *P. vivax* and a 1 in 5,000 solution of quinine. The morphology of the parasites compared favourably with that of parasites which had been kept in glucose without quinine for a similar length of time. In addition, the quinine-treated blood produced typical malaria when inoculated into man.

These experiments suggest that the action of quinine is indirect. The effective therapeutic agent may be a metabolite resulting from the breakdown of quinine in the tissue, though of the existence of such a metabolite there is no evidence. Yorke and Macfie (1924), on the other hand, have suggested that the essential factor in the production of cure in malaria is the capacity of the host to produce immune-body, in response to the antigen formation resulting from the destruction of a considerable number of parasites by a medicament. Quinine, therefore, may destroy some, but by no means all, the parasites in the body, but the antigen thus liberated would stimulate the production of more antibody which, if formed in sufficient amount, would completely sterilize the tissues. If, for any reason, there is an insufficient production of antibody, sterilization does not take place and a relapse will occur. In some cases the parasites, though reacting to quinine, may become resistant to immune body, with the result that constant relapses occur. James (1929) believes that quinine continued

for too long after an attack may interfere with the formation of immune body. The evidence in favour of the formation of immune body is at present somewhat poor, though the fact that antibodies cannot be demonstrated in the blood does not of necessity imply the absence of a high degree of tissue immunity. The therapeutic inoculation of blood containing malaria parasites for the treatment of general paralysis of the insane has shown that malaria so produced is either easily cured by a short course of quinine, one single dose of 5 gr. being apparently as efficacious as 30 gr., or in some cases cures itself without the administration of any medicament (James, Nicol and Shute, 1927). Such cured cases are extremely difficult to reinfect. In addition, experiments have shown that there are certain individuals who cannot be infected with malaria by any known means. Such individuals, therefore, appear to be immune, though whether this immunity is acquired as the result of previous attacks of the disease or is "natural," is at present unknown. Various other suggestions have been made in regard to the action of quinine; thus Kingsbury (1925) and also Ross (1927) have brought forward evidence to suggest that in malignant tertian infections the parasite-containing corpuscles are lysed by the action of quinine with the formation of bilirubin, the amount of which in the blood is thus temporarily increased, falling to normal as the parasites disappear from the peripheral circulation. Morgenroth (1918) believes that malaria parasites are unable to enter red blood corpuscles which have been treated with quinine, while Bass (1921) suggests that quinine renders the cells permeable to the lytic action of the blood serum. These views appear to be hypothetical, although in the present state of our knowledge it is not possible to advance beyond the stage of hypothesis.

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ARSENIC COMPOUNDS

Stovarsol

Arsenic has probably been used in the treatment of the acute fevers from very remote times. With the discovery of the curative action of the organic compounds of arsenic in trypanosomiasis, various organic arsenic derivatives were tested in the treatment of malaria. Although atoxyl and neoarsphenamine have occasionally been found to have some effect in removing benign tertian parasites from the blood, stovarsol is the only organic arsenic compound that has been at all generally used in the treatment of malaria.

Stovarsol was first investigated by Ehrlich and Hata (1911), and was studied by Fourneau and his colleagues in 1921 and 1923 as No. 190 of his series of organic compounds. Stovarsol—$C_6H_3(OH)(NH.COCH_3)AsO_3H_2$—3-acetylamino-4-hydroxyphenylarsinic acid, contains 27·2 per cent. of arsenic. Baermann (1923) seems to have been the first worker to use this drug in malaria. He reported unsatisfactory results with it in the prophylaxis of tertian malaria. Valenti and Tomaselli (1924) found, however, that in a heavy quartan infection which had resisted quinine, stovarsol given by mouth caused the disappearance of parasites from the peripheral circulation and diminution in the size of the spleen. In a single case of simple tertian infection this treatment, carried out for five days, produced similar results. The patient with quartan fever, however, had a paroxysm of fever after the beginning of treatment, though he afterwards remained free during an observation period of one month. Marchoux and Cohen (1925) tested the sodium salt of stovarsol by the intravenous injection of 1 gm. in 10 c.cm. of distilled water on seven patients who had been artificially infected with *Plasmodium vivax* for the treatment of general paralysis of the insane and on two naturally infected cases. In every case the peripheral blood became free from parasites within twenty-four hours. Marchoux (1925) found that this single injection stopped relapses for two months in two-thirds of his cases, though all had previously relapsed when treated

with quinine. The drug appeared to have a definite action on the older pigmented parasites, the older schizonts and the gametocytes, in contradistinction to quinine, which attacks the younger forms. The pigmented parasites first disappeared from the blood, usually in from six to eight hours. Stovarsol was then tried in patients suffering from malaria in Algiers. Stovarsol itself was given orally, while its sodium salt was given intravenously, intramuscularly and subcutaneously as well as by mouth. The results confirmed the previous experiences; though malignant tertian and quartan parasites were unaffected, the simple tertian parasites were removed from the peripheral blood-stream by a single dose of 1 gm. for adults and 0·37 gm. for children. After even a single injection of but 0·75 gm. of stovarsol two-thirds of the cases failed to relapse within two months.

Marchoux's findings have since been repeated by Foley, Catanei, Brouard and Leblanc (1925), who continued work in Algiers. Pontano (1925) and Valenti and Tomaselli (1924) all failed in quartan and malignant tertian infections. Sinton and Eate (1926) found that the oral administration of 1 gm. of stovarsol for three consecutive days with or without quinine did not prevent relapses occurring in the majority of cases, though *P. vivax* disappeared from the blood in about 98 per cent. of the patients within forty-eight hours of the beginning of treatment. Mazza, Cossio and Aybar Albarracin (1926), on the other hand, found that one injection of 1 gm. of sodium stovarsol was sufficient to prevent relapse in seventeen patients with simple tertian infections, while a like result was obtained in thirteen cases by Gravot (1926). Ciuca and Alexa (1926), however, found that the majority of their cases relapsed within ten days of the cessation of treatment, while Vialatte (1926) also reported that three injections of 1 to 1·25 gm. intravenously at intervals of six to eight days failed to prevent relapses. Sinton (1927) found that of twenty-five patients suffering from benign tertian infections treated with intravenous injections of sodium stovarsol in varying amounts up to a total of 4gm. in five days twenty-three, or 92 per cent., relapsed during the next two weeks, although in a large series of cases treated with various cinchona alkaloids the relapse rate was only

73·1 per cent. The average duration of parasites in the blood was 19·8 hours.

Guérin, Borel and Advier (1927) treated ten cases of *P. vivax* infection, five cases due to *P. falciparum*, three due to *P. malariæ*, and one mixed infection of *P. vivax* and *P. falciparum*. A single dose of 1 gm. was given to each case. *P. vivax* in every instance disappeared from the blood, but the other parasites were unaffected, and in every case of simple tertian infection a relapse occurred within two months. Similar results were obtained by Freiman (1927) in Cyprus, and by van Nitzen (1927). The claim that a clinical cure can be produced by intravenous injections of sodium stovarsol thus appears to have been confirmed by a number of observers, though optimistic reports of freedom from relapse have not been substantiated, nor, according to Tardres (1926) and Sinton (1927), has stovarsol so definite an action as quinine in reducing splenomegaly.

On the other hand, a large number of observers have noted that stovarsol has a curious action on the temperature, severe rigors occurring in a high percentage of cases within eighteen hours of the commencement of injections. A similar rise in temperature in a malarial subject treated with neoarsphenamine has been noted. Various toxic phenomena have also occurred as the result of stovarsol or sodium stovarsol injections. Ciuca and Alexa (1928) report symptoms of acute nephritis with albumin, blood and casts in the urine, while, in addition, Vialatte (1926) mentions colic, diarrhœa, erythema, urticaria, ocular troubles, tachycardia, giddiness, collapse and glycosuria. Sinton (1927) was untroubled by these sequelæ, either because a magnesium sulphate purge helped to eliminate the arsenic, or because the liver and kidneys were protected by the glucose and alkali which his patients also received.

Stovarsol has been tested for its effects on bird malaria by Giemsa, Weise and Tropp (1926).

Hegner, Shaw and Manwell (1928) were unable to detect any action of the drug on *Plasmodium cathemerium* infections in canaries.

The action of stovarsol on *Plasmodium vivax* is of some interest.

Marchoux (1925) followed the changes in the parasites by taking hourly slides. The action appeared to be progressive, the first parasites to disappear from the peripheral blood-stream being the large pigmented forms, which could no longer be found six to eight hours after the administration of the drug. The younger forms were more resistant, a reversal of the findings with quinine. Stovarsol did not produce a fragmentation of the parasite, but a condensation of the protoplasm, and more especially of the chromatin. Later the protoplasm lost its power of staining and gradually melted away. The nucleus at this period was still dense, well coloured and swollen, surrounded by a faint outline of cytoplasm. In the end the nucleus also melted away, leaving the red cell intact, since in many cases there could be seen red blood corpuscles free from parasites, but filled with Schüffner's dots. Ciuca and Alexa (1926) failed to find any special susceptibility on the part of the large pigmented forms and gametocytes. Sinton (1927), however, found that these stages were definitely the first to degenerate, though curious changes also occurred in the smaller forms of the parasite. One hour after injection the size of the vacuole had increased, the protoplasm had thinned, and the chromatin dot stained more deeply. By the fourth to sixth hour the protoplasm of the ring forms had become thinned, and in many cases had broken up into fine connected strands, sometimes with a ragged cobweb-like appearance. The vacuole, if present, was very large and the chromatin was deeply stained. Many accolé forms were present, and double or even treble infections of the same cell were by no means rare, though such had not been found before the injection of stovarsol. These young unpigmented forms were still present in the peripheral blood many hours after all large pigmented forms had vanished, though their numbers had much decreased. The action of the drug seemed either to prevent the normal increase in size of these young forms or to destroy them when they had reached a certain developmental stage.

No satisfactory explanation has yet been forthcoming of the rigors so commonly produced by the injection of stovarsol. It is possible, however, that they are due to the liberation of toxin from the rapid destruction of parasites. Now Yorke and Macfie

(1924), in discussing the mechanism of the radical cure of malaria by quinine, have suggested that quinine given to patients whose blood contains numerous parasites destroys either directly or indirectly many, but not all the parasites, thus setting free a considerable quantity of soluble antigen. This antigen provokes the formation of immune body, which if present in sufficient amount then kills the remaining parasites with complete sterilisation of the tissues. Theoretically, therefore, stovarsol should be of great value in the radical cure of malaria, though practically it unfortunately fails completely to prevent relapses.

Quinine Stovarsol

Since stovarsol and sodium stovarsol have absolutely no effect on quartan and malignant infections, numerous attempts have been made to prepare derivatives of stovarsol which should have this action. Stovarsol urethane was tested by Marchoux (1925), but was found to be far too toxic. Fourneau, however, prepared a new salt of the drug, quinine stovarsol, which contains about equal amounts of quinine and stovarsol. This compound was tested by Boyé (1926) in six cases of quartan infection, with the result that the clinical manifestations were controlled more easily than with an equal amount of quinine hydrochloride. Marchoux and Quilici (1926) treated a case of malignant tertian malaria, 0·25 gm. being given twice daily. The temperature had fallen to normal by the third day. Raynal (1927) also gave 0·5 gm. to a case of malignant tertian infection twice a day for ten days. Five days' rest was then allowed, after which another course was given, this routine being continued for two months. Parasites disappeared from the blood and splenomegaly decreased, but unfortunately the subsequent history of the patient was not obtained. Legendre and Cienfuegos (1927) used quinine stovarsol in the treatment of one simple tertian and four quartan cases. The clinical symptoms were successfully controlled.

Quinine stovarsol was given by van Nitzen (1927) in doses of 1·5 gm. daily to twenty-three adults, and a sixth to a third of that dose to eight children. Crescents disappeared in from three to

thirty-six days in adults, and in from five to twenty-one days in children ; on the schizonts the drug had much less effect, for they persisted in the blood after 30 to 43 gm. had been taken.

Sinton, Bird and Eate (1928) have also used quinine stovarsol in the treatment of twenty-three cases of simple tertian infection. The observed minimum relapse rate was 69·5 per cent., with a possible maximum of 73·9 per cent., the corresponding figures for controls treated with various cinchona alkaloids being 57·4 per cent. and 61·1 per cent. The results are thus better than those following the intravenous injection of sodium stovarsol, but very similar to those obtained with quinine and stovarsol given separately. Although the clinical course of the disease was controlled, rigors developed in nearly half the cases within twenty-four hours of the beginning of treatment.

Quinine Troposan

Quinine troposan, which has been used by Sinton, Bird and Orr (1928), is the quinine salt of 5-acetylamino-2-hydroxyphenylarsinic acid. Its chemotherapeutic index for *Trypanosoma equiperdum* infections in mice is said to be 1 : 100, and it is dispensed in tablets of 0·25 gm. each containing 50 per cent. of quinine and 40 per cent. of troposan. Nineteen cases of simple tertian infection received six tablets for twenty-eight days, while thirty-eight cases received nine tablets for fourteen days. In the first series ten, or 55 per cent., relapsed, in the second fourteen, or 42 per cent., as compared with no relapses in cases treated with plasmoquine and quinine. Quinine troposan acted quite as rapidly as quinine alone in overcoming the fever, though the splenic enlargement was less reduced than by other drugs. There were, however, no toxic symptoms, and the drug was well tolerated. Prophylactic measures must be taken against arsenic poisoning.

Dimethylarsinate of Quinine

Puyal, Navarro-Martin and Alvarez-Cienfuegos (1928) have employed dimethylarsinate of quinine, $C_{20}H_{24}N_2O_3.OHAsO(CH_3)_2$,

which they have termed " caco-quin." The maximum tolerated dose for rabbits is 0·18 gm. per kilogram of body weight intravenously, and 0·066 gm. intramuscularly. Three patients with simple tertian infections were treated intravenously with doses varying from 0·5 to 2 c.cm. of a 5 per cent. solution of the drug. The parasites rapidly disappeared from the peripheral circulation, and no relapses occurred within two months of the injections.

Bromide of mesothorium and quinine have also been recommended by Tanon and Jamot (1926).

Methylene Blue

Methylene blue, although it cannot be compared with the alkaloids of cinchona, has occasionally been used with success, either alone or in association with other drugs. Thus Pitschugin (1925) found that 0·01 gm. for every year of age three times daily for a week cured benign tertian infections. Vorobiew (1927) reports that the injection of 0·2 gm. of methylene blue solution intravenously followed by neoarsphenamine four hours later, the injection being repeated once in most cases, reduced the relapse rate to 19 per cent. Couto (1926) believes that methylene blue is as efficient as quinine in the treatment of simple tertian infections, 0·10 to 0·20 gm. being given with the food during a meal; in malignant tertian infections, 0·05 gm. of methylene blue dissolved in 5 c.cm. of distilled water was injected intravenously two to five times a day, but without any great success.

Other Drugs used in Malaria

Various other drugs have from time to time been used in the treatment of malaria, but have failed to fulfil the expectations of their originators. Mercury in the form of the perchloride, mercurophen, mercurosal and mercurochrome 220 have been recommended, but have all failed to display any curative action, either in human or in bird malaria. Iodine, Peracrina 303, which is said to be a compound of specific albuminates and trypaflavine, *Vitex peduncularis* and smalarina have also been tested without success.

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BLACKWATER FEVER

The ætiology of blackwater fever is still unknown, although there is general agreement that malignant tertian malaria is almost always associated as a predisposing cause. Most authorities also believe that the dissolution of the red corpuscles occurs in the peripheral circulation, and not in the kidneys alone.

From the chemotherapeutic standpoint the main interest lies in the possible association between quinine and the onset of the hæmoglobinuria. Although it is now recognised that the proper use of quinine is probably the most essential factor in the prevention of blackwater fever, yet it is an undoubted fact that once a person is predisposed to blackwater fever by repeated attacks of neglected malaria full doses of quinine are often an exciting cause in precipitating the hæmoglobinuria. The administration of this drug may also produce a recurrence of the symptoms after a few days' intermission.

While *in vitro* quinine has long been known to produce hæmolysis, the occurrence of hæmolysis *in vivo* in non-malarial subjects is of extreme rarity. Cases do, however, occur, such as that recorded by Brahmachari and Sen (1925), in which a non-malarial patient invariably developed hæmoglobinuria after taking 5 gr. of quinine by mouth. There was no increased vulnerability of the red cells to the hæmolytic action of quinine *in vitro* and no evidence of any hæmolysin in the peripheral blood. Kligler (1923) has suggested that the hæmolytic action of quinine may be increased by the presence of bile in the blood, while Kessler (1925) has found that *in vitro* lecithin also increases the hæmolytic action of quinine, but serum rich in lipoids, even from a case of diabetic coma, produced no increase in quinine hæmolysis.

Ghiron (1927) believes that there is a hæmolysin present in the serum of cases of blackwater fever which can only be demonstrated when red cells from a case of malaria and quinine are added to the blackwater fever serum. Nierenstein (1919) claims to have isolated a derivate of quinine, hæmoquinic acid, from the urine of cases of blackwater fever.

Until the introduction of plasmoquin it was, therefore, a moot point in what way to remove from the blood the malarial parasites when present in association with blackwater fever. It is generally agreed that when malarial parasites are present in the blood the case should be treated with quinine, and also when parasites reappear after an attack. This reappearance was found by Thomson (1924) to occur in from five to fourteen days after the urine had cleared, though post-mortem examination of fatal cases showed that parasites might be found in the internal organs even when they were absent from the peripheral blood-stream. As pointed out in discussing plasmoquin, this compound has been given with apparent success in a small number of cases of blackwater fever, though until a much larger series of cases has been treated its value cannot be accurately gauged, since recovery normally occurs in from 75 to 80 per cent. of cases. Trabadoros (1928) has recently claimed excellent results with plasmoquin compound in three cases.

The use of alkalies, either by mouth or intravenously, to render the urine alkaline has been strongly recommended by many authorities.

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PLASMOQUIN

The first announcement concerning plasmoquin was made in September, 1926. In accordance with a policy which is a compliment to the initiative and enterprise of the fine chemical industries of other countries, but which is much to be deplored in the interests of the progress of chemotherapy, the structure of the drug has not been wholly disclosed, although, according to Hörlein (1926), the director of the pharmaceutical scientific department of the I. G. Farbenindustrie, the compound is N-diethyl-amino-*iso*pentyl-8-amino-6-methoxyquinoline, thus differing from quinine principally in the absence of the quinuclidine ring with two intermediate CH_2 radicals.

Some confusion appears to have been created by calling this compound " beprochin," " plasmochin," and later, in English-speaking countries, " plasmoquin " or " plasmoquine."

The first biological experiments with the new compound were carried out by Roehl (1926) on the parasites of bird malaria. Some difficulty was at first encountered in determining the tolerated dose of plasmoquin for canaries, as when given intramuscularly the delicate birds often died as a result of the intense local reaction, while when mixed with the food it was impossible to be certain how much of the drug had been absorbed. Later a special pipette was devised by means of which a definite amount of plasmoquin could be deposited in the crop. By this means it was found that when doses of 1 c.cm. for every 20 gm. of body weight were given to canaries, the highest dilution of plasmoquin tolerated was 1 : 1,500, the lowest curative dilution, 1 in 50,000, thus giving a chemotherapeutic index of 1 : 33 as compared with 1 : 4 for quinine. These results were confirmed in bird malaria by Hegner and Manwell (1927), who found that daily doses of 1·5 mgm. given orally were effective in preventing the appearance of parasites in the blood, but were not easily tolerated. Heavy doses of 1 mgm. given daily for the five days following inoculation prevented acute infections developing, though a few parasites appeared in the blood-stream. The parasites may thus be controlled, but cannot be eradicated from the tissues. Mazza (1928)

has also found that *Hæmoproteus* may be temporally removed from the peripheral blood-stream of thrushes, falcons and *Brachyspiza*, one of the Fringillidæ, while Godoy and Lacorte (1928) find that in the pigeon plasmoquin removes the gametes of *Hæmoproteus* from the peripheral blood-stream, though they return again after a few days. Plasmoquin also possesses a destructive action on the sporozoites, but not on the schizonts.

Sioli (1926) was the first to use plasmoquin in the treatment of human malaria, forty cases of general paralysis therapeutically infected with simple tertian malaria being successfully cured. A daily dose of 0·15 gm. was considered to be the maximum, for with 0·25 gm. toxic symptoms occurred. In one case after a total dosage of 0·6 gm. taken over eight days there was pain in the region of the liver and some cyanosis, though without methæmoglobinæmia. Recovery was rapid, but the cyanosis did not completely disappear for nearly three weeks. Mühlens (1927) and Mühlens and Fischer (1927) employed plasmoquin in the treatment of 172 cases of naturally acquired human malaria. They had all received previous hospital treatment in Hamburg, as they were either chronic or acute relapsing cases. In benign tertian and quartan infections daily doses of from 0·05 to 0·1 gm., with in a few instances 0·15 gm., were effective in causing the fever to fall after the second or third day, while parasites disappeared from the peripheral blood-stream in from five to seven days. Although relapses were less frequent than after quinine treatment, such symptoms as cyanosis of the fingers, toes, lips and face were not uncommon, while spasmodic gastralgia occurred occasionally, especially when the drug had been given on an empty stomach or in large individual doses. On the other hand, none of the usual after-effects of quinine was experienced, plasmoquin also having a further advantage in being almost tasteless. In tertian and quartan infections plasmoquin was quite as effective as quinine in destroying both the sexual and asexual forms of the parasites, but in the treatment of subtertian infection plasmoquin was less efficient than quinine, for although crescents were more readily removed from the peripheral blood-stream relapses were more frequent than with quinine,

owing to the fact that the ring forms of the subtertian parasite were but little affected.

To prevent relapse quinine was, therefore, added, the combination being known as " Plasmoquin compound," or more simply as " Plasmoquin Co." The largest amount of plasmoquin given to any one case was 3·25 gm. in the course of sixty-eight days. In one instance Mühlens gave a daily dose of 0·18 gm. without any severe symptoms of intoxication developing. Children, and even babies, were found to be very tolerant to the drug.

The following results were obtained by Mühlens: after the administration of pure plasmoquin there were no relapses among four cases of quartan infection, three relapses among forty benign tertian infections, and thirty-four relapses among forty-nine subtertian infections. When quinine was combined with plasmoquin to form plasmoquin co. there were no failures among the simple tertian infections and only four relapses in subtertian cases.

As some uncertainty prevailed as to the strength of the plasmoquin co. tablets, Mühlens (1927) stated that each tablet contained 0·01 gm. of plasmoquin and 0·125 gm. of quinine bisulphate. In simple tertian and quartan infection he gave one tablet of plasmoquin three times a day, and in subtertian infection six plasmoquin co. tablets a day.

Two cases of blackwater fever were successfully treated with plasmoquin by Mühlens.

In Spain, Roehl (1927) cured three cases of simple tertian fever with pure plasmoquin, though in three subtertian cases schizonts continued in the peripheral blood-stream. In Italy, Schulemann and Memmi (1927) report having treated over 100 cases. The course of treatment consisted of (i.) 0·02 gm. plasmoquin three times a day for a week; (ii.) four days' interval; (iii.) 0·02 gm. three times a day for three days followed by another interval of four days; treatment for three days, followed by an interval of four days, was then continued for six weeks. Of twenty-four cases of simple tertian infection only one relapsed, while of four quartan infections none relapsed. The malignant tertian infections were uncontrolled. Sixty-three cases of this type of infection were, therefore, treated thrice daily with 0·02 gm. plasmoquin and 0·25

gm. quinine, with the result that no parasites were found after the eighth day of administration. Thirteen cases, however, relapsed during the course. Toxic effects occasionally occurred. Slight cyanosis of the lips and cardiac arrhythmia were common, while in three cases the cyanosis was of an alarming nature. A relative lymphocytosis was also not uncommon, lymphocytes in some cases amounting to 50 per cent. of the white cells in the blood. Gastralgia was another sequela, though it was rare when plasmoquin was given on an empty stomach. Vomiting was never encountered after plasmoquin, and only twice after plasmoquin compound, as the result of an idiosyncrasy to quinine, since it ceased when plasmoquin alone was administered.

One curious fact noted was that changing from plasmoquin to quinine or *vice versâ* frequently resulted in fever or in the reappearance of parasites in the peripheral blood-stream. Schulemann and Memmi also found degeneration of the adult parasites under plasmoquin treatment in simple tertian infection, the degenerate forms showing a uniform dark blue protoplasm with small drop-shaped portions partly cut off.

Radojičič (1927), in Jugo-Slavia, treated forty-nine cases of malaria with pure plasmoquin, daily doses of 0·06 to 0·08 gm. being given. Djokič and Stambuk (1927) also treated 102 cases with plasmoquin, doses of from 0·08 to 0·14 gm. being given daily. In simple tertian infections both sexual and asexual forms disappeared from the blood within five days of the commencement of treatment, the temperature fell, splenomegaly was reduced, and the general condition rapidly improved. The results were equally satisfactory in quartan infections. Plasmoquin appeared to be as effective in relapses as in primary attacks. The results in subtertian infections, however, were unsatisfactory, small rings remaining in the blood-stream, though crescents invariably disappeared. In many cases of subtertian infection the administration of plasmoquin was followed by the sudden appearance of schizonts in the blood, but since crescents were absent these cases ceased to be carriers of the infection. Polychroniades (1927) treated 188 cases in Salonika. Four simple tertian and two quartan infections were successfully treated by 0·02 gm. of plasmoquin three times a day,

while of thirty-eight subtertian cases similarly treated nineteen relapsed, nine cases continuously showing the presence of small rings in the blood. Eight of these cases showed cyanosis, eight gastralgia. Three quartan cases and 139 subtertian cases were given plasmoquin compound—0·02 gm. plasmoquin and 0·25 gm. quinine—three times daily. In all cases gametocytes had disappeared by the eighth day of treatment, while in all the malignant cases schizonts had vanished by the tenth day, only to reappear subsequently, however, in fifteen cases. Twenty-two cases suffered from abdominal pain and two from cyanosis.

Polychroniades also reported three cases of blackwater fever cured by plasmoquin. The first case had hæmoglobinuria and fever, with simple tertian rings in the blood. Three days after treatment with 0·06 gm. plasmoquin daily, parasites had disappeared from the peripheral blood and hæmoglobin from the urine, while by the sixth day the temperature was normal. On the fourteenth day, however, malarial parasites again appeared in the blood, though unaccompanied by fever or by hæmoglobinuria. As the result of the administration of 0·06 gm. of plasmoquin the parasites disappeared again, only to return on the twenty-second day. One gram of quinine dihydrochloride was then given by mouth, with the result that the urine again contained blood for twenty-four hours. After an interval of a day plasmoquin compound was given for three days, and after five days' interval for a further three days. Both parasites and hæmoglobinuria vanished. Later quinine was again tried in increasing doses and was tolerated up to 0·75 gm. The second case had fever and hæmoglobinuria only. Under treatment the blood disappeared after twenty-four hours, while the temperature was normal by the fifteenth day. Simple tertian rings were found in the peripheral blood on the twenty-first day without any recurrence of fever. After administration of plasmoquin compound the parasites disappeared without recurrence of the blackwater symptoms. The third case was very similar.

Sliwensky (1927), in Burgas, Bulgaria, treated 225 cases of malaria in hospital and fifty-nine cases as out-patients. The usual good results were reported in simple tertian and quartan

cases. Of eight quartan infections none relapsed, while among twenty-six simple tertian cases there were but two relapsing cases, one of which had received only 0·08 gm. for five days, an obviously insufficient dose. Among 125 malignant tertian infections receiving 0·03 gm. plasmoquin and 0·375 gm. quinine sulphate twice a day for from five to twelve days, thirty-eight relapses took place. Crescents had as a rule vanished from the peripheral blood-stream after eight days' medication, while cyanosis and gastralgia were only occasionally encountered. Two cases of quinine idiosyncrasy, with epistaxis, and one case of blackwater fever tolerated plasmoquin and were successfully cured by Sliwensky (1927). The patient with blackwater fever, a female forty years old, had had a subtertian infection treated with quinine for about two months. After a dose of 0·4 gm. of quinine a very severe attack of hæmoglobinuria occurred, with jaundice, vomiting and coma. Under symptomatic treatment and plasmoquin the patient gradually improved, a tolerance for quinine being eventually established.

Manaloff-Sliven (1927) also recorded ten cases from Bulgaria. Quartan infections, which had been treated with quinine for some considerable time without freeing the peripheral blood-stream from parasites, became negative on the fourth day of plasmoquin medication.

Baerman and Smits (1927) reported one death following treatment with plasmoquin, the chief pathological change noted at the autopsy being degeneration of the liver.

The physicians of the United Fruit Company, in their Annual Reports for 1926, 1927 and 1928, have had a very extensive experience of treatment with plasmoquin and plasmoquin compound. The inefficiency of pure plasmoquin in controlling subtertian infections was confirmed, while occasionally cases of simple tertian and quartan infection failed to react. There was one fatal case in a negro, the main histological finding being early central necrosis of the liver. Slight jaundice, cyanosis and abdominal pain were by no means rare. Barber (1928) finds that comparatively small doses of plasmoquin render crescents non-infective to anopheline mosquitoes, a discovery of considerable importance, since Mühlens

and Kirschbaum (1924) showed that a patient receiving quinine still remains infective to mosquitoes. Barber, Komp and Newman (1929) find that a single dose of plasmoquin renders crescent-containing blood non-infective for three days. Brosius (1928) believes that in simple tertian and quartan infections plasmoquin and quinine are more effective in destroying the asexual forms than quinine alone.

Manson-Bahr (1927, 1928[1], 1928[2]) also finds that plasmoquin compound is very effective. For simple tertian and quartan infections two tablets of plasmoquin co. are given three times a day (0·06 gm. plasmoquin and 0·75 gm. quinine daily) for seven days. Five such courses are necessary, with intervals of four days between each, the whole treatment thus occupying fifty-one days. Of eighteen cases of simple tertian infection thus treated only two relapsed within six months. The parasites vanished from the peripheral blood-stream after an average dose of 0·135 gm. plasmoquin and 1·687 gm. quinine. The drug was well taken; though tasteless, it was found when chewed to have a curious anæsthetic action on the tongue and pharynx. One case showed slight cyanosis and one dizziness, while occasional abdominal discomfort was not uncommon. A drachm of glucose given daily, either in powder or liquid form, was sufficient to prevent these sequelæ. In one case of quartan infection which had failed for four months to react to quinine, no further relapses occurred after plasmoquin co. treatment. In the subtertian cases vomiting and pyrexia occurred in some, and had to be controlled with intramuscular injections of quinine. With plasmoquin alone some cases of subtertian infection developed methæmoglobinuria and icterus, the attacks resembling a mild blackwater fever. Definite signs of degeneration were noted in the crescents within thirty-six hours of the commencement of plasmoquin medication, while twenty-four hours later the crescents were entirely disintegrated.

Eiselberg (1927) also had a severe case of methæmoglobinæmia and methæmoglobinuria, which improved only after a blood transfusion had been performed, while Low (1928) has noted cyanosis and vomiting after the administration of plasmoquinin.

In Malaya, Fletcher and Kanagarayer (1927) treated ninety-

seven cases with plasmoquin. The usual good effects were obtained in simple tertian and quartan fevers, but the frequency of toxic symptoms, sometimes of an alarming nature, and the large number of relapses rendered mass treatment so impossible that they consider that plasmoquin should only be used under hospital conditions.

Cordes (1928) reports on seventy-two cases of subtertian malaria in which alternate cases received plasmoquin and quinine respectively. The only advantage of plasmoquin appeared to be that the crescents disappeared more frequently from the peripheral blood-stream; on the other hand, four patients became seriously ill, two eventually dying. In India, Sinton and Bird (1928) also record somewhat unsatisfactory results with pure plasmoquin, the effect on the temperature and on splenomegaly being less than with quinine, while the relapse rate was 30 per cent. In addition, in twenty-two out of twenty-nine patients the course of treatment had to be interrupted owing to the development of toxic symptoms, four patients failed to complete the course and two became dangerously ill. When plasmoquin was combined with quinine the relapse rate was reduced to 20 per cent., a better result than that obtained with quinine alone.

M'Hutchison and Duff (1928), in Malaya, also found that with three tablets of plasmoquin compound and 30 gr. of quinine daily there was very rapid improvement in the clinical symptoms and reduction in splenomegaly.

In the Philippines, Hasselmann and Hasselmann-Kahlert (1928) also used plasmoquin and plasmoquin compound. The results were not quite so striking as those described by many of the German workers. In double infections there was frequently noted a curious provocative effect of plasmoquin. In cases where in the beginning only simple tertian parasites were found, after the administration of pure plasmoquin subtertian schizonts, and even crescents, appeared in the peripheral blood. On the other hand, in cases of what at first appeared to be pure subtertian infections, simple tertian parasites would appear after treatment with plasmoquin compound. These observers also met with a few cases which were quite resistant to the action of plasmoquin.

Ruge (1928) has compared the length of time during which schizonts and gametocytes remain in the blood during treatment with quinine and plasmoquin.

Comparatively little work has been carried out on the pharmacology of plasmoquin. Eichholtz (1927), however, found that in cats doses of 2·5 to 5 mgm. per kilogram of body weight caused methæmoglobin formation, while given intravenously to rabbits,

The Effects of Treatment on the Survival of Sexual and Asexual Malaria Parasites in the Peripheral Blood following Treatment (Ruge, 1928)

Treatment.	Number of days schizonts remained in blood.	Number of days gametocytes remained in blood.
Simple tertian infections—primary.		
Quinine hydrochloride (134) .	1·8	2·5
Plasmoquin (26) . . .	2·7	3·0
Simple tertian infections—relapse.		
Quinine hydrochloride (427) .	2·4	2·6
Plasmoquin (23) . . .	2·5	3·2
Subtertian infections.		
Quinine hydrochloride (275) .	2·8	13·9
Plasmoquin (30) . . .	3·2	4·0
Plasmoquin co. (35) . .	15·8	11·4
Quartan infections.		
Quinine hydrochloride (47) .	2·7	3·7
Plasmoquin	2·0	4·0

cats and dogs there resulted cardiac inco-ordination characterised by duplication or suppression of systoles. The cardiac arrhythmia could be controlled by the injection of quinine or adrenalin. The toxicity of plasmoquin, according to Le Heux and de Lind van Wyngaarden (1927), varies with different species of animals. Thus for cats the M.L.D. was 5 mgm. per kilogram of body weight whether given intravenously or subcutaneously, while orally the M.L.D. was 7·5 mgm. per kilogram of body weight. Rabbits

succumbed to 3·5 mgm. per kilogram of body weight when the drug was given intravenously, but tolerated up to 20 mgm. injected subcutaneously, or 225 mgm. if given by mouth. Cats recovered more quickly than rabbits from sublethal doses. Death occurred with symptoms of dyspnœa, asphyxia, brachycardia and arrhythmia, with methæmoglobinæmia. Plasmoquin was found to have the power of forming methæmoglobin *in vitro* from the blood of sheep, pig, ox, horse, cat, rabbit, dog and man. It seems probable that the action of plasmoquin in malaria is at least partly due to this capacity for forming methæmoglobin, with consequent lysis of the red cells.

Orachowatz (1928), who has studied the effects of plasmoquin on the blood in man, finds that a third of the oxygen capacity of the blood may be lost as a result of the conversion of hæmoglobin to methæmoglobin. Cordes (1928) has noticed that a leucocytosis sometimes follows the administration of plasmoquin, many young undifferentiated leucocytes being present in the blood. Karamchandani (1928), therefore, prefers plasmoquin to quinine when malaria is complicated by pneumonia, since quinine is apt to cause a leucopenia.

Plasmoquin is, at least partially, excreted in the urine, where its presence can be detected according to Schulemann, Schoenhoefer and Wingler (1927) by the following test :—

Two or three hundred cubic centimetres of urine are mixed with 20 c.cm. of 50 per cent. caustic potash solution to liberate plasmoquin base, which is then extracted by agitating with ether three times, using 30 c.cm. each time. The total ethereal extract (90 c.cm.) is filtered, washed twice with 10 c.cm. of water containing 2 drops of normal soda solution and the plasmoquin extracted from it by thorough shaking with 6 c.cm. of a 2 per cent. acetic acid solution. The latter is separated, warmed on the water bath to remove dissolved ether, and is then ready for the application of the colour reaction ; 3 c.cm. of acetic acid are added, and about 0·5 gm. of chloranil and the mixture, contained in a test-tube of 1·6 c.cm. diameter, is heated for one and a half minutes in a free flame. If more than 1 part of plasmoquin in 50,000 is present a blue or blue-green colour appears. The solution is then cooled,

left for some minutes to allow excess of chloranil to crystallise out, filtered, and to the filtrate 1 to 1·5 c.cm. of ether is added. The ether remains in solution until the acetic acid is partially neutralised by the addition of a few drops of 50 per cent. caustic potash solution, when it separates as an intensely blue upper layer. Applied in this fashion, 100 c.cm. of urine containing 1 in 2,000,000 of plasmoquin gives an ethereal layer of a bluish green colour, which is not interfered with by the presence of quinine in the urine.

It will thus be seen that owing to its toxic effects the use of pure plasmoquin has in large part been abandoned. Its employment, however, would appear to be indicated in cases of quinine idiosyncrasy, in pregnancy where there is risk of abortion from the use of quinine, and possibly in malaria in children, by whom it is well tolerated. Schiassi and Merighi (1928), in fact, state that an infant of from one to six months can tolerate as much as 0·005 gm. twice daily. The combination with quinine as plasmoquin compound, however, is at least equal, if not superior, to quinine alone. Its action in rendering gametocytes non-infective to mosquitoes is also of the greatest importance, for quinine by itself appears to have but little effect on sexual forms. With plasmoquin compound there is thus a possibility of sterilising the carrier. The anti-malarial effects of plasmoquin were first demonstrated by experiments on bird malaria. By means of this experimental procedure the activities of all synthetic products can be so easily determined as to hold out the reasonable hope that plasmoquin is but the first of a destined series of still more efficient anti-malarial compounds.

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CHAPTER V

THE CHEMOTHERAPY OF LEISHMANIASIS

THE use of antimony in the treatment of leishmaniasis constitutes one of the most important chemotherapeutic advances of the present century, for before the introduction of antimony the death rate from kala azar in India was almost 90 per cent. Two periods may be distinguished in the treatment of leishmanial infections, an earlier period during which trivalent antimony compounds of the tartar emetic type were exclusively used, and a later one characterised by the employment of pentavalent antimony derivatives.

The use of antimony in medicine is by no means new, tartar emetic, first described in 1631 by Adrian de Mynsicht, having long been used in the treatment of pulmonary diseases. The introduction of antimony into tropical medicine, however, was due to the discovery by Plimmer and Thomson (1908) that the injection of tartar emetic or the analogous sodium salt into rats and mice suffering from trypanosomiasis caused a rapid disappearance of trypanosomes from the peripheral blood-stream.

KALA AZAR

The credit for bringing to the notice of the medical world the specific action of antimony in leishmanial infections is due to Gaspar Vianna (1913), who employed tartar emetic with success in the treatment of South American cutaneous leishmaniasis. Confirmation of these results was quickly forthcoming. In Italy Di Cristina and Caronia (1915) found that the drug was highly efficacious in the treatment of infantile kala azar, while Rogers (1915) was the first to employ the drug in India, to be followed shortly afterwards by Muir (1915). Of the thirteen cases treated by Rogers eight were either cured or improved, while four either did not

change or became worse, and one died. Of Muir's eight cases one died of phthisis, the others all rapidly improved. Rogers and Hume (1916) and Rogers (1916–17) gave details of the treatment of thirty-five Europeans, six of whom were children. Of these twenty-six were reported as cured, eight were improved, while one died of phthisis. Rogers (1917) also described the treatment of a series of thirteen natives of India. Eight were said to have been cured, three much improved, and two to have shown no change. Rogers noted that the treatment had to be continued for some time after complete recovery had apparently taken place.

The use of tartar emetic in the treatment of kala azar then became general throughout India. Muir (1916) gave an account of the treatment of a series of about 100 cases, and in 1917 of another series of 143 cases, all in natives. At Shillong, Knowles (1920) treated eighty-six cases, of which forty-six were discharged as cured; of the twenty-three deaths in this series many were due to the influenza pandemic which was then raging.

Mackie (1915), Brahmachari (1915), Ghosh (1916) and Jackson (1916) all recorded successful results in properly diagnosed cases of Indian kala azar, while Low (1919) reported the treatment of a case which had apparently contracted the disease in Mesopotamia. In Assam treatment with tartar emetic was undertaken on a large scale, out-patient treatment having to be adopted in many cases owing to the lack of hospital accommodation. According to Kundu (1920) the improvement was so rapid that many of the out-patients ceased to attend after a few injections, whereas in order to effect a permanent cure prolonged treatment is essential.

Occasionally, however, toxic effects followed the use of potassium tartar emetic. Rogers (1918), therefore, completely gave up the use of the potassium in favour of the corresponding sodium salt—sodium antimonyl tartrate—which he believed to be less toxic. This salt had already been used with success by Brahmachari (1915) and by Ghosh (1916). Dodds-Price (1920) and Elwes (1920), although obtaining good results with tartar emetic, also preferred to use the sodium salt.

Successful results from the use of tartar emetic were reported also from other areas where leishmaniasis is endemic. In Italy, where, as already mentioned, the treatment of kala azar by tartar emetic had originated, Di Cristina and Caronia (1915) described ten cases varying in age from fifteen months to six years. Two of the cases which were in the final stages of the disease died soon after treatment had begun, while a third died of acute nephritis. The remaining seven cases were either cured or were reported as progressing so favourably that a cure was ensured. Jemma (1916), in Naples, cured twenty-one out of twenty-six cases of infantile leishmaniasis, while Spagnolio (1916) also recorded four successful cases treated by tartar emetic.

The most extensive series of Italian cases consisted of 110, which were treated at Jemma's clinic in Naples between October, 1918, and July, 1920. An account of this series was given by Javarone (1920) and Foti and Javarone (1921). Before the introduction of tartar emetic almost all patients died, but of the sixty-three cases treated by tartar emetic, forty-nine recovered, while only fourteen died.

In Khartoum, Christopherson (1917) treated one case of mucocutaneous leishmaniasis with success, and three cases of kala azar, two of which died during treatment.

The use of potassium or sodium antimonyl tartrate has thus revolutionised the treatment of kala azar wherever it occurs.

At first, in addition to the intravenous route, attempts were made to give the drug by mouth or *per rectum*. Injections were also given intraperitoneally or intramuscularly. In the latter case the local reaction is very marked, and may end in abscess formation, while subcutaneous injection almost always leads to cellulitis.

Various small differences in the mode of administration of tartar emetic have from time to time been suggested. Napier (1927[2]), as the result of his unrivalled experience in the treatment of kala azar, finds that there is little to choose between the sodium and potassium tartar emetic provided that the salt is absolutely pure. The sodium salt can be readily obtained in scale form, as can a triple salt, potassium sodium antimony tartrate. The

strength of the solution is also of but little importance. One per cent. or 2 per cent. solutions are most frequently used ; 5 per cent. solutions are apt to produce cough and retching in a certain number of patients. It is also important to use a freshly-prepared solution, as moulds are very apt to grow and produce toxins. Solutions can, however, be kept for some weeks if 0·25 per cent. of carbolic acid is added at the time of preparation. The solution may be made either in distilled water or in tap water, with or without the addition of 0·85 per cent. sodium chloride.

The dosage naturally varies according to the age and general condition of the patient. Assuming that a 2 per cent. solution is used, Napier recommends for the average adult patient a primary dose of 2 c.cm. (0·04 gm. of the salt) ; doses should be increased on each occasion by 1 c.cm. up to a maximum of 5 c.cm. (0·1 gm.) ; subsequently 5 c.cm. is given on each occasion. For more debilitated patients the initial dose should be 0·5 c.cm., increased by 0·5 c.cm. up to a maximum of 5 c.cm.

For infants of three years it is advisable to begin with 0·5 c.cm., making 2 c.cm. the maximum. For children of twelve the maximum is 3·5 c.cm., the doses for intermediate ages being in proportion ; though there is a relation between the age of the patient and the dose, it will be noticed that the dose per 100 lb. of body weight is proportionately larger in the case of children than in the case of adults.

The injections are given on alternate days throughout the course of treatment. Although it is permissible to extend the interval by one day in certain cases, the injections must never be given less frequently than twice a week.

It is of the utmost importance when treatment is once begun to continue it uninterruptedly. It may be necessary to discontinue the injections when there appear symptoms which are directly due to the effects of the salt that is being given, but when the common complications of the disease itself arise, the treatment is best continued. Slight diarrhœa, œdema of the feet, the presence of a trace of albumin in the urine, severe bleeding from the gums, are indications that specific treatment must be begun immediately, or, having been begun, be actively continued. Where there is

severe dysentery, however, it is advisable to treat this condition first, postponing the specific treatment until the patient's condition has improved.

The length of time during which treatment must be continued is still somewhat uncertain. Rogers (1917), using a 1 per cent. solution, recommended an initial dose of 5 c.cm., a maximum dose of 10 c.cm. for a European, and 7 c.cm. for an Indian. When given every third day or twice a week the injections were continued for two to three months. Muir (1918) stated that a four months' course of thrice weekly injections should be given in every case, involving the administration of a total of 5 gm. of tartar emetic. Knowles (1920) suggested that for an adult a total of 2 gm. was sufficient to ensure a cure, but since some of his patients subsequently relapsed it is doubtful whether this amount can be regarded as sufficient.

Napier (1927[2]) stated that the routine adopted in the Carmichael Hospital for Tropical Diseases, Calcutta, was to give a course of thirty injections to each patient. A spleen or liver puncture was then done, and two or three N.N.N. tubes were inoculated with the material obtained. If no flagellates developed in the culture tubes, and the patient's general condition and blood count were satisfactory, he was discharged as cured; otherwise he was given a further course of ten to fifteen injections and the spleen puncture repeated. In general practice it may be said that in any but a case which is definitely resistant to the action of the antimony tartrates, the maximum total dose necessary to effect a cure is 4 gm. per 100 lb. body weight of the patient. This maximum course may be modified if the clinical condition of the patient improves, the factors to be taken into account being (i.) the general condition, (ii.) the fever, (iii.) the size of the liver and spleen, (iv.) the blood.

General Condition.—In the majority of cases the patient improves after a very short course of treatment. The hair ceases to fall out and begins to regain its normal lustre, the appetite is improved, and there is a greater sense of well-being. Quite frequently there is a loss of body weight for the first week or two, no increase taking place until the last weeks of treatment. In

women the menses do not usually begin again until after the treatment has ended.

The Fever.—The average time for the disappearance of the fever is about twelve weeks. There are some cases in which the temperature falls after about the third injection and does not rise again, but there are also a few cases in which the fever does not subside for more than a month. It is not, however, a signal for despair, even if the temperature remains above the normal after as many as forty injections. A few cases show a slight reactionary rise of temperature following each injection, while in cases running an afebrile or almost afebrile course the commencement of injections of antimony tartrate frequently causes the reappearance of a remittent or intermittent type of pyrexia.

The Spleen and Liver.—The behaviour of the spleen is by no means consistent: in some cases the spleen diminishes rapidly in size from the beginning, and disappears under the costal arch before the patient leaves hospital; in others there is very little decrease in the size of the organ. The behaviour of the spleen is naturally dependent on its consistence. In a long-standing case, in which the spleen is hard and fibrous, diminution in size beyond a certain point is naturally impossible, while if kala azar has occurred in a chronic malarial subject with a previously enlarged spleen, specific treatment for kala azar can hardly be expected to produce much improvement.

Generally speaking, the diminution in size of the liver is less noticeable than that of the spleen.

The Blood.—The blood picture improves rapidly under treatment. By the time the patient has completed his course the white blood count has returned to normal, and not infrequently a moderate leucocytosis is present. In the differential count the most noticeable increase is in the eosinophils. The red blood count also shows a distinct improvement.

In infantile kala azar, as Mallardi (1923) has shown, the effect of the injections of antimony tartrate may be estimated by an examination of blood films. As is well known, the blood, in addition to leucopenia, lymphocytosis and oligochromæmia, shows the presence of immature blood cells of various types, not

only normoblasts and other cells of the red corpuscle series, but also myelocytes. In certain cases the latter may be so numerous as to simulate a leukæmia. During the course of treatment the blood generally resumes its normal characters, and in cases which are responding to antimony the gradual disappearance of cells of the immature type can be easily traced. In certain cases, however, the blood picture does not improve, while cells of the immature type become more numerous : there is then but little to be gained from continuing the injections of antimony tartrate.

Napier (1927[2]) has proposed that the full course of 4 gm. per 100 lb. body weight should be modified to thirty injections (2·88 gm. in an adult), provided that—

(I.) The body weight has increased ;

(II.) The spleen is reduced to the level of the costal arch, or, where it was very large, by at least 4 inches ;

(III.) The white blood count is above 6,000 per c.mm. ;

(IV.) The temperature is normal by the seventh injection.

In cases where the temperature falls to normal after the seventh, but before the tenth, injection, thirty-five injections should be given. In cases where the temperature falls to normal after the tenth, but before the sixteenth injection, forty injections should be given.

Following a full course of injections of tartar emetic, Napier and Halder (1927) believe that an 80 per cent. cure rate can be obtained.

As in the case of bilharziasis, so in leishmaniasis treated by tartar emetic, complications are by no means uncommon. The most frequent is severe coughing immediately after the injection, associated sometimes with vomiting. Pneumonia is also by no means rare, and appears to be directly due to the injection of antimony tartrates, since it is rarely seen as a sequela in patients treated with pentavalent antimony compounds. Joint and muscle pains are also very common complications, though fortunately they seldom occur except towards the end of a course of treatment. They usually come on some four or five hours after the injection, and last for anything up to twelve hours. A less common complication is an acute arthritis, affecting particularly

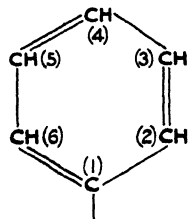
the wrist, knee and ankle joints. This arthritis generally subsides in about ten days, the condition of the patient being much improved as a result of the attack.

Very marked slowing of the heart is met with in some cases towards the end of a course of injections; it is an indication that treatment should be suspended. An irritating papular eruption also occasionally occurs, and usually does not disappear until the injections have been discontinued. Severe headaches may follow the injections, while rigors, fainting or even cessation of breathing have also been recorded.

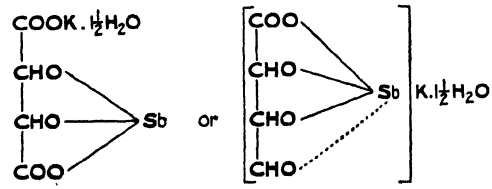
These toxic sequelæ represent a serious disadvantage in the use of the antimony tartrates. The length of time necessary for a complete cure and the fact that the salts can only be administered intravenously have also militated against their extended use. Numerous attempts have therefore been made to find other antimony compounds which, while less toxic to the host, are yet more rapid than the antimony tartrates in curing leishmanial infections. Colloidal antimony suphide, colloidal antimony, lithium antimonyl tartrate, strontium, ethyl, quinine, cinchonine, narcotine, antimonyl tartrates, antimony trioxide dissolved in glycerine or in tartaric acid have all been used from time to time in the treatment of kala azar, but with results for the most part inferior to those obtained with potassium or sodium antimonyl tartrate.

Antimosan has also been employed both by Mühlens (1926) and Struthers (1927[2]). Though rather less toxic than tartar emetic, its action is almost as slow in producing a cure in kala azar.

In the pentavalent antimony compounds, however, there have



PHENYLSTIBINIC ACID



POTASSIUM ANTIMONYL TARTRATE

been discovered substances which fulfil the requirements of reduced toxicity to the host and increased toxicity to the parasite. The preparation of organic antimony compounds was first made possible in 1911, following the discovery of the method of diazo-synthesis by Schmidt (1920). In this process, by the interaction of diazotised aniline and antimony trioxide in the presence of alkali, there is formed phenyl stibinic acid.

From an examination of the structural formulæ it will be seen that the difference between the phenyl stibinic acid series and the "emetic" series lies in the fact that in the former the therapeutically-active element antimony is directly united to the carbon, while in the emetic series it is joined to carbon through oxygen.

In the first series a large number of compounds can be formed by substitution of one or more hydrogen atoms in positions 2 to 6 in the benzene hexagon by other atoms or groups such as the amino (NH_2), hydroxyl (OH) or chlorine (Cl) groups. In all these pentavalent antimony compounds the characteristic antimony reactions, such as the red precipitate of antimony sulphide, are obscured.

The pentavalent antimony compounds which have so far been prepared can be classed under three headings :—

I. Salts of *para*-aminophenylstibinic acid.
   - (*a*) Sodium *para*-aminophenylstibinate—stibamine ;
   - (*b*) Diethylamine *para*-aminophenylstibinate—von Heyden 693, von Heyden 693 B, or neostibosan.

II. Derivatives obtained by substitutions in the amino-group of *para*-aminophenylstibinic acid :
   - (*a*) Sodium *para*-acetylaminophenylstibinate—stibenyl.
   - (*b*) Ammonium *para*-carbamylaminophenylstibinate (?) —urea stibamine.
   - (*c*) Urea and glucose combined with *para*-aminophenylstibinate—amino-stiburea.
   - (*d*) Nitrogen glucoside of sodium *para*-aminophenylstibinate—neostam.
   - (*e*) Urea and *para*-aminophenylstibinic acid—urea-stibol.

III. Derivatives obtained by substitutions in the benzene nucleus of *para*-amino-phenylstibinic or *para*-acetylaminophenylstibinic acid.

(*a*) Sodium *meta*-chlor-*para*-acetylaminophenylstibinate stibosan—von Heyden 471.

The first of these compounds to be tested was sodium *para*-aminophenylstibinate ($NH_2 . C_6H_4 . SbO(OH)(ONa)$) (**stibamine**), which is the antimony analogue of atoxyl. This compound, when examined by Uhlenhuth, Mulzer and Hügel (1913), and later by Brahmachari (1922), proved too unstable in solution and too uncertain in its action to be used therapeutically.

Sodium para-acetylaminophenylstibinate (**stibacetin,** later known as **stibenyl**) ($CH_3 . CO . NH . C_6H_4SbO (OH)(ONa)$) was, however, found to be much more stable and less toxic.

Fargher and Gray (1921) found that the M.L.D. for mice was 133 mgm. per kilogram of body weight, compared with minimal lethal doses for sodium and potassium antimony tartrate of 25 mgm. and 16 mgm. respectively. In fowl spirochætosis, and rabbit syphilis also, stibenyl was found to have a more favourable chemotherapeutic index than tartar emetic.

Brahmachari (1922) also worked out the relative toxicity of this and other antimony compounds, but he used guinea-pigs and gave the injections intramuscularly: by this method the results obtained are apt to be irregular. Stibenyl was first used in the treatment of leishmaniasis by Caronia (1916).

In the meantime Brahmachari (1922) had introduced a compound which he termed **urea-stibamine.** This substance was at first stated to be urea combined with *para*-aminophenylstibinic acid, with the empirical formula $C_7H_{12}O_4N_3Sb$. Further analyses by Brahmachari and Das (1924) showed that this formula was correct except that the material contained in addition a molecule of water. Study of the reactions of the substance showed, however, that it was not a simple salt of the two components as originally supposed, and it was suggested that a more correct formula would be$NH_2.CO.NH.C_6H_4.SbO(OH)(OHN_4)$ammonium-*para*-carbamylaminophenylstibinate. The work of Ghosh, Chopra and Chatterjee (1928) has thrown very considerable doubt on the validity of this

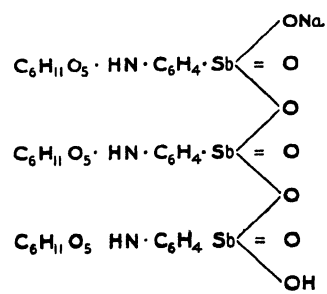
latter formula, for they found that most of the urea could be washed out in the cold with a mixture of absolute alcohol and ether. Estimations of the nitrogen by combustion also showed very wide variation in different samples of urea stibamine, while the antimony content of different samples varied from 20 to 43 per cent. This compound undergoes a chemical change if kept in contact with the air for any length of time.

Napier (1927[2]) finds that the lethal dose for mice is 250 mgm. per kilogram of body weight, while the tolerated dose is 175 mgm. per kilogram of body weight.

Two other compounds somewhat on the same lines as Brahmachari's urea stibamine have also been introduced, **amino-stiburea,** which was produced by the Union Drug Company of Calcutta, and **urea-stibol,** which was introduced by Chopra, J. C. Gupta, Mullick and A. K. D. Gupta (1928). Amino-stiburea is said to be the ammonium salt of *para*-aminophenylstibinic acid combined with urea and glucose $C_6H_{11}O_5$-NH.CO.NH.C_6H_4.SbO(OH)(ONH_4). The toxic dose for mice is given by Napier (1927[2]) as 250 mgm. per kilogram of body weight, while the tolerated dose is 175 mgm. per kilogram of body weight.

" Urea-stibol " is claimed to be a salt of urea and *para*-aminophenylstibinic acid. It is said to be toxic to mice after intravenous injection in doses of from 220 to 230 mgm. per kilogram of body weight.

Another direct derivative of *sodium para-aminophenylstibinate* has been prepared by Gray (1925), who has converted it into a stable N-glucoside, which, like other stibinic acids, has a condensed molecule.



It has been used therapeutically under the name of **neostam.** Napier (1927[2]) finds that the lethal dose for the mouse is 500 mgm. per kilogram of body weight, while the tolerated dose is 300 mgm. per kilogram of body weight.

Uhlenhuth, Kuhn and Schmidt (1925) also described another derivative of *para*-aminophenylstibinic acid, diethylamine-*para*-aminophenylstibinate, which has been employed clinically under the name of **von Heyden 693.** The lethal dose for mice is given as 800 mgm. per kilogram of body weight, the tolerated dose as 650 mgm. per kilogram of body weight. Napier (1927[1]), however, found the M.L.D. for mice to be 350 mgm. per kilogram of body weight. This salt, when used clinically, was found to produce vomiting in certain cases. A slightly different method of preparation was therefore employed which resulted in the formation of a form of the diethylamine salt of *para*-aminophenylstibinic acid with greatly reduced toxic properties. This compound is known as **neostibosan.**

One other pentavalent antimony derivative requires notice, **stibosan** or **von Heyden 471.** This compound was introduced by von Heyden & Co. in 1923, and is formed by substitution of a chlorine atom in the benzene nucleus of *para*-acetylaminophenylstibinic acid, with the formation of sodium *meta*-chlor-*para*-acetylaminophenylstibinate $(CH_3 . CO . NH)(Cl)C_6H_3 . SbO(OH)(ONa)$. The lethal dose for mice was found by Napier (1927[2]) to be 275 mgm. per kilogram of body weight, the tolerated dose 200 mgm. per kilogram of body weight. This compound may be kept in an ill-stoppered bottle for as long as two years at tropical room temperature without undergoing any change.

In addition to the pentavalent antimony compounds which have actually been used in the treatment of kala azar, a very large number have been tested for their toxicity to mice and their trypanocidal action. A very small change in the constitution of the molecule produces a very considerable change in the properties. Sodium *para*-acetylaminophenylstibinate, for instance, has trypanocidal action, and an M.L.D. for mice of 12 mgm. Sodium *para*-chlorphenylstibinate, on the other hand, has no trypanocidal action, and an M.L.D. for mice of 1 mgm.

Pentavalent Antimony Compounds

Compound.	Formula.	Percentage content of antimony.	Minimum lethal dose for mice in mgm. per kilogm. of body weight.	Maximum tolerated dose for mice in mgm. per kilogm. of body weight.	Reference to first clinical report.
Sodium *para*-aminophenylstibinate "Stibamine."	$NH_2.C_6H_4.SbO(OH)ONa$ . .	40–42·1	—	—	—
Sodium *para*-acetylaminophenylstibinate "Stibenyl."	$CH_3.CO.NH.C_6H_4.SbO(OH)ONa$ . .	33	133	—	Caronia, G. (1916).
Ammonium *para*-carbamineaminophenylstibinate "Urea-stibamine."	$NH_2.CO.NH.C_6H_4.SbO(OH)ONH_4$ . .	20–43	250	175	Brahmachari, U.N. (1922).
Glucose and urea with *para*-aminophenylstibinic acid "Aminostiburea."	$C_6H_{11}O_5.NH.CO.NH.C_6H_4.SbO(OH)(ONH_4)$	24·8	250	175	Napier, L. E. (1925).
Urea with *para*-aminophenylstibinic acid "Urea stibol."	—	—	220–230	—	Chopra, R.N., Gupta, J.C., Mullick, R. N., and Gupta, A. K. D. (1928).
N-glucoside of sodium *para*-aminophenylstibinate. "Neostam."	$(C_6H_{11}O_5.NH.C_6H_4.SbO)_2O_2(OH)(ONa)$.	30	500	300	Napier, L. E. (1924).
Diethylamine *para*-aminophenylstibinate "von Heyden 693."	$NH_2.C_6H_4.SbO_3H.NH(C_2H_5)_2$ . . .	40	350	250	Napier, L. E. (1925).
Sodium *meta*-chlor-*para*-acetylaminophenylstibinate "Stibosan" ("von Heyden 471").	$(CH_3.CO.NH)Cl.C_6H_3.SbO(OH)(ONa)$.	31	275	200	Napier, L. E. (1923[a]).

It is now possible to test the pentavalent antimony compounds therapeutically on the striped Chinese hamster (*Cricetulus griseus* M.Edw.), or on the European hamster (*Cricetus cricetus L.*), which recent researches have shown to be easily infected with leishmania. A few chemotherapeutic experiments have been carried out by Smyly (1926), who found that tryparsamide, sodium potassium tartrobismuthate and sodium antimony tartrate were all ineffective in curing hamsters, though there occurred a reduction in the size of the spleen roughly proportional to the amount of the drug administered. More recently, Roehl (1929) has shown that the hamster is of extreme value in determining the chemotherapeutic index of antimony preparations. Antimosan has a chemotherapeutic index of 1 : 5, stibosan 1 : 5 to 1 : 7, and neostibosan 1 : 50.

The Clinical Application of Pentavalent Antimony Compounds

The first of the pentavalent antimony compounds to be tested clinically was stibenyl, which was employed in Italy by Caronia (1916) in the treatment of four cases of infantile kala azar. The injections were given every other day into the gluteal muscles. For children under two years of age, an initial dose of 0·03 gm. was given, increased to 0·1 gm. In older children the doses ranged from 0·05 to 0·15 gm. Of the four cases, a boy aged three and a half years had completely recovered after three months' treatment, during which he had received 3·6 gm. of the drug: a girl aged twenty-three months was cured by nine injections in the course of sixteen days, and another aged twelve months by six injections in ten days: the fourth case, a boy of seventeen months, improved considerably, but died from an abscess in the lumbar region, which was present before treatment began.

The treatment of a single case, with favourable result, was also reported by Khàrina-Marinucci (1916). Since then favourable results have been obtained by Spagnolio (1920), and by Foti and Javarone (1921), who used stibenyl in ten cases, of which eight were treated by intramuscular injections and two intravenously. For intramuscular injections they began with 0·03 gm. increased to 0·1 gm. The time necessary for treatment was considerably longer than with intravenous injections, though seven out of the

ten cases were eventually cured. Other reports from Mediterranean countries have for the most part proved favourable to stibenyl. In Spain, Catala (1924), Lozano (1924), Moragas y Garcia (1925), and Martinez (1927) have all found that cures result from intramuscular injections. In only one instance were there anaphylactic-like symptoms, though some completely resistant cases were encountered. In France, Klippel and Monier-Vinard (1922), Renault, Monier-Vinard and Gendron (1922), Levy (1924) and Giraud and Massot (1927) have all used stibenyl either on autochthonous cases or on cases imported from Morocco. Artamanoff (1926), Smorodintsev (1926), and Korchitz (1926) reported cures from the Russian endemic areas. In England Manson-Bahr (1920) treated two cases of Indian kala azar. One case was cured by intravenous injections of stibenyl; in the other a dose of 0·4 gm. produced nausea and vomiting, the patient eventually dying from amœbic dysentery, while leishmania, proved by culture to be living, were still present in the organs at the autopsy.

Despite these favourable reports from Europe, stibenyl, in India, has proved quite ineffective. Mackie (1921), and Napier (1922), who reported the results in ten cases, both found that toxic symptoms were extremely common, possibly owing to the fact that the compound was unsuitable for export to the tropics.

In the meantime, Brahmachari (1922) produced urea-stibamine, and recorded the treatment of eight cases. Shortt and Sen (1923), working at Shillong, confirmed these results, and reported very favourably on the compound. Greig and Kundu (1925) also reported the treatment of fifty-one patients, some of whom had received previous treatment. They gave an average dose of 2·12 gm., which was equivalent to 2·4 gm. per 100 lb. of body weight. 0·25 gm. was given as a maximum dose, though in one instance a total of 10 gm. of the preparation was given to a very resistant case without producing any improvement. Another series was reported by Foster (1924), the patients being members of a tea-garden coolie force. Proof of cure was by spleen puncture only. Four deaths occurred in a series of sixty-seven cases, while the average dose was about 2·0 gm. Napier (1924) had unsuccessful results in a few cases, but later (1927[2]) he reported that he

had treated forty-eight patients with only three deaths. The mean total dose of the series was 2·08 gm., the relative dose 2·7 gm., and the mean of the number of injections given in each case was 11·8. Thirty-six of these patients are known to have remained free from the disease for at least six months. Mitra (1925) reported the treatment of sixteen cases, while Chatterjee (1926) had thirteen recoveries out of eighteen cases, while five died. Napier (1929[1]) records a series of seventy patients treated with this compound; of fifty whose subsequent history was known only one relapsed. In view of the results recently obtained by Ghosh, Chopra and Chatterjee (1928), it seems essential that some method of standardising urea-stibamine should be introduced.

Stibamine glucoside—Neostam.—Comparatively few reports have been published on the use of this compound. Napier, in 1925, recorded the results in ten cases. There was one death, the other nine being discharged cured on the strength of a negative liver puncture. Seven patients are known to have remained free from the disease. The mean total dose was 2·58 gm., or 4·21 gm. per 100 lb. of body weight, and the mean number of injections was 13·8. It seems possible that an unnecessarily high total dose was given in these cases, for Greig and Kundu (1925) subsequently reported the cure of two patients with doses of 1·85 gm. and 2·05 gm. respectively.

Struthers (1927[1]) treated eighteen cases, of which three died, while the others were cured. The initial dose was 0·05 gm., increased at each injection by 0·05 gm., till a maximum of 0·2 gm. was reached. The total dose given varied between 2 and 3 gm., the average duration of treatment being about one month. Low (1927) also cured five cases with this preparation, one, a child, receiving intramuscular injections of from 0·25 to 0·75 gm. till a total of 2 gm. was given.

In a more recent paper, Napier (1929[2]) describes the treatment of fifty-seven consecutive cases with stibamine glucoside. Of forty-four patients whose subsequent history could be traced, thirty-five remained in good health, while nine relapsed. Of the patients whose subsequent history is known, thirty-nine had had no further treatment; of this group the mean actual total dose

was 2·19 gm., the relative total dose 3·06 gm., and the relapse rate 15·4 per cent. Of sixteen patients who received a relative dose of more than 3 gm. (mean 4·32 gm.), only one relapsed.

Amino-stiburea.—Napier (1928[2]) describes the treatment of fifty-two cases. There were two deaths in the series, one from an intercurrent pneumococcal meningitis, and two very resistant cases which failed entirely to respond. The mean total dose of the series was 2·4 gm., which is equivalent to a dose of 3·35 gm. per 100 lb. weight of patient, and the mean of the number of injections was 12·06, the average number of days under treatment being 28·9. Of the forty-eight patients who were discharged cured, thirty-five were in good health six months later.

Hodgson, Sen and Das (1928) also describe the results of treatment of eighteen cases, all of whom were discharged after a negative spleen puncture. The minimum dose which produced a cure was 1·5 gm., the average 2·99 gm.

Urea-stibol.—Chopra, Gupta, J. C., Mullick and Gupta, A. K. D. (1928), report the treatment of fifteen cases with this compound.

The dosage was the same as with urea stibamine: success was obtained in patients who, in addition to kala azar, were suffering from bronchitis, diarrhœa or cancrum oris.

Stibosan—von Heyden 471.—Napier (1926[2]) described the treatment of 104 cases with this compound. The death rate of this series was 10·6 per cent.: of the survivors, two did not respond to any form of antimony, and both eventually died. Of the patients discharged as cured, seventy-seven were traced for six months after their discharge: seventy had remained well and seven had relapsed. The mean total dose was 2·78 gm. The mean number of injections was 13·3, the injections being given thrice weekly, with a maximum dose of 0·3 gm. Calculated with reference to the body weight of the patient, the dose per 100 lb. body weight was 4·00± 0·97 gm. Of the previously untreated patients who received a relative dose (*i.e.*, dose per 100 lb. of body weight)

of between	2	and	3	gm.	.	.	.	18·2	per cent. relapsed.
,,	,,		3	,,	4	,,	.	.	.	9·2	,,	,,
,,	,,		4	,,	5	,,	.	.	.	5·0	,,	,,
,,	more	than		5	,,	.	.	.	0	,,	,,

Greig and Kundu (1925), in a much smaller series of cases, gave a mean dose of 2·0 gm. The mean relative dose necessary to effect a cure was 2·73 gm., but the cases were not followed up. Struthers (1927[2]) treated seven cases, with one death. The initial dose was 0·05 gm., increased to 0·2 gm., the mean dose being 2·4 gm.

Požariski (1927) also records the cure of a girl of two and a half years, who received fifteen injections of stibosan, following treatment with stibenyl.

Von Heyden 693.—This compound has been reported on by Napier (1927[1]), who treated sixty-one cases, fifty of whom have remained well for a period of not less than six months. Two cases failed to respond to treatment, and died about one year after discharge. The mean total dose was 2·19 gm., which represents 3·35 gm. per 100 lb. of body weight. The mean total number of injections was 10·85.

With the exception of vomiting, there were no disagreeable symptoms or sequelæ.

In order to avoid the vomiting **von Heyden 693b** or **neostibosan** was prepared. Twenty-six patients were treated, 0·3 gm. of neostibosan, dissolved in distilled water so as to make a 25 per cent. solution, being injected intramuscularly, the solution being isotonic with the blood serum. Eight daily injections were given, the total amount of the compound administered being 2·3 gm. There was an entire absence of nausea or vomiting, and no local reaction at the site of injection, the patients being able to tolerate the maximum dose from the first. Napier and Mullick (1929) found that six months after the termination of the course only three patients had relapsed. Corrochano (1928), who has treated 100 cases of leishmaniasis in Spain with tartar emetic, antimosan, Bayer 211 and 212, stibenyl and stibosan, also records remarkable results in two cases treated with neostibosan.

The initial dose of urea-stibamine, aminostiburea and neostam advocated by the manufacturers is 0·05 gm., increased by 0·05 gm. at each injection up to 0·2 gm. Napier (1927[2]), however, gives 0·1 gm. as an initial dose, 0·2 gm. as a second dose, and 0·25 gm. for each subsequent dose. With stibosan and von Heyden 693 an initial dose of 0·2 gm. can be given, with subsequent doses of

0·8 gm. In the case of neostibosan the maximum dose of 0·8 gm. may be given even at the first injection.

Children, although tolerating proportionately larger doses than adults, receive smaller initial doses. Thus an infant from eighteen months to three years will tolerate a dose of 0·1 gm., children of six years 0·15 gm., children of ten years 0·2 gm., and children of fourteen years, 0·25 gm. Debilitated patients require a smaller initial dose, which must be increased with great caution.

The Course of the Disease under Treatment

The Fever.—After about the fifth injection the patient is usually free from fever, even when, as in the case of neostibosan, the injections are given on consecutive days. In some cases the fever disappears after the first or second injection, although in others a low fever persists until some time after the injections have ceased. A reactionary rise of temperature may occur after each injection, though a sudden sharp rise in a patient who has not previously shown any reaction is indicative that too large a dose has been given.

Body Weight.—As in treatment with the antimony tartrates, there is first a decrease, later a rapid increase in weight.

Spleen and Liver.—In the majority of cases the spleen decreases rapidly in size, and is beneath the costal margin by the end of treatment. In some few cases the decrease is slower, but the decrease continues for some time after treatment has terminated. The liver, on the other hand, rarely decreases until after the injections have been discontinued.

General Condition.—The general condition improves almost immediately, the improvement being noticeable some days before the fall in temperature.

The Length of the Course of Treatment.—The length of the course of treatment is, as with the antimony tartrates, a problem of some difficulty. Napier (1927[2]) recommends that a thorough treatment should be given first, and then if a relapse occurs another more severe treatment. For stibosan, Napier (1926[2]) recommends a course amounting to 3·5 gm. per 100 lb. body weight. With this course, a 9 per cent. rate of relapse should be expected.

In the urea-stibamine and aminostiburea series the mean relative dose given by Napier was 2·7 gm., the relapse rate being very low. If the relative dose be 3 gm., this means, with a maximum dose of 0·2 gm., twelve or fifteen injections per patient. In the von Heyden 693 series the mean total dose was 2·19 gm., and the relative dose 3·35 gm., entailing a total of ten injections. For neostibosan, Napier and Mullick (1929) recommend 2·3 gm. per 100 lb. body weight, given in eight injections, though possibly the number of injections might be increased to ten with advantage.

The Evidence of Cure.—Apart from the improvement of the clinical symptoms, the best evidence of cure by the pentavalent antimony compounds is an entire absence of symptoms for a period of at least six months after the termination of treatment. The results of spleen and liver puncture are, as Napier and Greig and Kundu (1925) have pointed out, apt to be fallacious, for the process of cure apparently continues for some time after the last injection has been given. Thus a patient who gives a positive liver or spleen puncture some few days, or even a month, after the cessation of treatment, may fail to show any further signs of the disease, while another puncture carried out some weeks later will be negative. The formol leucogel (aldehyde) reaction introduced by Napier (1921) may also be employed, for it becomes negative when complete serological cure has occurred. This test consists of adding one drop of 40 per cent. formaldehyde to 1 c.cm. of serum. The mixture is then well shaken and allowed to stand at room temperature. If the blood is from a well-established case of kala azar of from four to five months' duration, the serum immediately becomes viscid, and within a minute or two will have "set," so that the tube can be inverted. Within three to twenty minutes the whole of the serum will have become absolutely solid and opaque, like serum coagulated by heat. If the serum happens to be hæmoglobin-stained, the coagulated serum has a pink tinge, which changes to a chocolate brown after twenty-four hours. Unfortunately, this test does not become definite until the infection has lasted for some time—so that its absence in early cases cannot be taken as an absolute indication of cure.

Two other tests may also be used for the control of treatment

of kala azar. In 1927, Chopra, Gupta and David found that when a solution of urea-stibamine is added to blood serum from a patient with kala azar, flocculation occurs at the junction of the two fluids. The test is best carried out with a 4 per cent. solution of urea-stibamine and serum diluted 1 in 10. Napier (1928[1]), who has critically examined this test, finds that it is positive in 64·6 per cent., doubtful in 13·4 per cent., and negative in 21·9 per cent. Fabris (1928) also finds that the serum test is positive when carried out with stibosan, in place of urea-stibamine, and with the sera of cases of infantile kala azar. Tartar emetic does not give the test. During treatment with urea-stibamine the test becomes uncertain after about the twelfth injection, which roughly corresponds to the number of injections necessary to produce a cure.

A second test has recently been proposed by Lloyd and Paul (1928). In 1924 Ray found that in kala azar there was a relative increase in the euglobulin content of the serum. Lloyd and Paul confirmed this finding. In normal serum there is on an average 0·16 gm. of euglobulin per 100 c.cm. of serum, or approximately 5 per cent. of the total globulin, while in a well-established case of kala azar there is from 1·5 to 2·5 gm. per 100 c.cm. of serum, or from 40 to 50 per cent. of the total globulin. The study of a full protein graph of a kala azar case under treatment with von Heyden 693 shows :—

(1) A first stage in which the total globulin and pseudoglobulin show an enormous and very rapid fall, while the albumin shows an equally sudden rise. During this change the euglobulin undergoes very little change. As a result of its rapid fall the pseudoglobulin graph soon cuts the euglobulin graph.

(2) After its initial fall the pseudoglobulin begins to rise to intersect the euglobulin curve a second time. This intersection corresponds to the point at which the globulin/albumin ratio first becomes normal. From this point onwards the pseudoglobulin curve steadily climbs almost to touch the total globulin graph, the euglobulin graph falling by an equal gradient to the normal 0·16 gm.

(3) The form of these curves is very characteristic, and is shown with minor variations in every case examined.

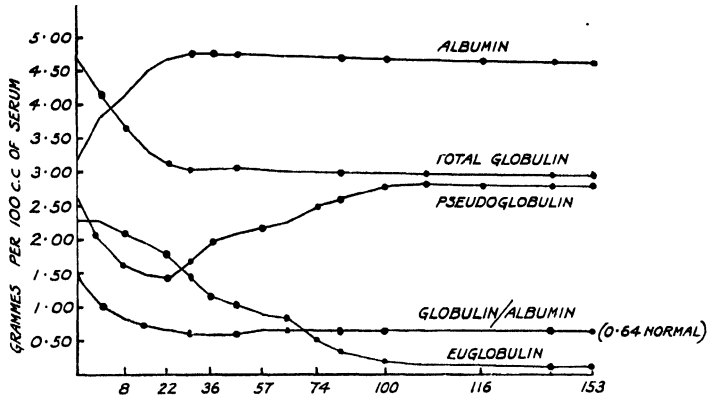
The typical features of the graph are so regular in their appearance that they may be used as a serological standard for the treatment of kala azar, just as the Wassermann reaction is used in the control of the treatment of syphilis.

In a later paper, Lloyd, Napier and Paul (1929) have applied the method of protein graphs to an estimation of the cure of kala azar when treated by different methods and by different compounds of antimony. Graphical records were made of the behaviour of the serum protein fractions resulting from the following different methods of treatment :—

(I.) Concentrated intravenous courses of neostibosan 693 B.
(II.) Alternate daily intravenous injections of neostibosan 693 B.
(III.) Intramuscular injections of neostibosan 693 B.
(IV.) Interrupted intravenous courses of neostibosan 693 B.
  (*a*) one injection of neostibosan 693 B.
  (*b*) two injections ,, ,,
  (*c*) four ,, ,, ,,
(V.) Urea-stibamine.
(VI.) Sodium antimony tartrate.

The treatment of resistant and atypical cases was also followed by means of a study of the serum proteins. All the protein fraction estimations were made by the refractometric method described by Robertson (1924).

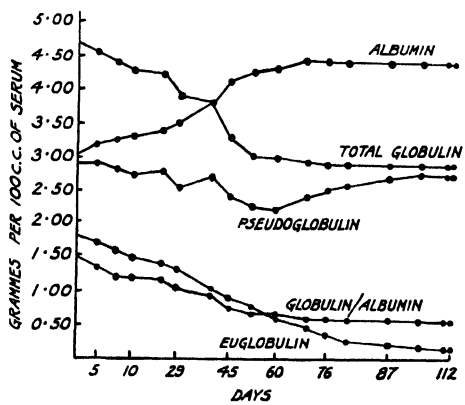
A concentrated course of 693 B, producing the standard graph, consisted of eight daily intravenous injections, the first dose being 0·2 gm., the subsequent doses 0·3 gm., thus making a total of 2·3 gm. The graph produced by such a concentrated course was of an absolutely fixed type. In Graph 1 the initial changes, termed the first stage, comprise rapid crossing of the albumin and total globulin graphs and double intersection of the pseudoglobulin and euglobulin graphs, the point of second intersection corresponding with the point at which the albumin and total globulin values first stabilise themselves. The second stage is characterised by a steady ascent of the pseudoglobulin, and a steady descent, by an equal gradient, of the euglobulin, the latter finally falling to the normal figure of approximately 0·16 gm. per



GRAPH 1.

100 c.cm. of serum. During the second stage the albumin and total globulin values remain absolutely steady. The first stage occupies about one month, the second stage about three months. During the first stage the euglobulin falls very gradually, while in the second stage the fall is more rapid. In the case illustrated in Graph 1, the temperature had fallen to normal by the ninth day, the general response to the treatment being good.

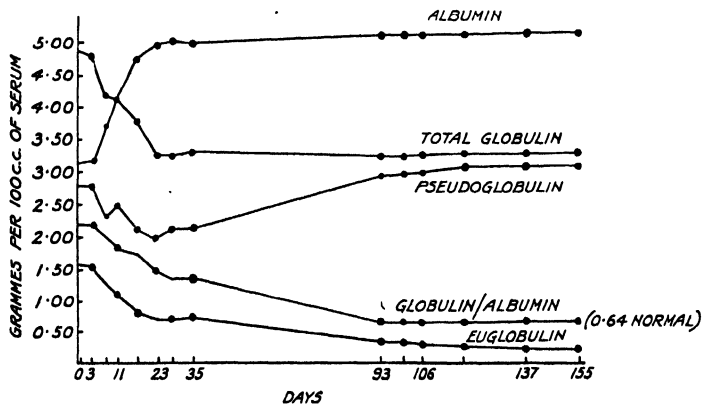
Treatment by eight injections of 693 B on alternate days.—The first injection was 0·2 gm., all subsequent injections being



GRAPH 2.

0·8 gm., with a total dosage of 2·3 gm. Graph 2 shows the record of a case treated on these lines. The sudden fall in the pseudoglobulin in the initial stage of treatment is not seen, while the fall in the globulin and rise in the albumin are slower, so that intersection takes place later. Also, the onset of the second stage is much slower than in the standard graph, being entered on only about the fifty-second day, a period approximately twice as long as in the standard graph. Since the initial sudden fall of the pseudoglobulin was absent, the pseudoglobulin graph does not intersect the euglobulin curve, but the onset of the second stage is seen as the point where the pseudoglobulin and euglobulin curves diverge. Eight alternate daily injections of 693 B thus produce a much slower protein response than the concentrated course of the same total dosage. The temperature of the patient became normal on the sixteenth day. The spleen culture was positive on the twenty-eighth day, but negative on the fifty-sixth day.

Treatment by intramuscular injections of 693 B.—(Concentrated course of nine daily injections.) It will be seen in Graph 3 that, in spite of daily injections, there was no protein response for three days, after which the usual changes occurred, producing the

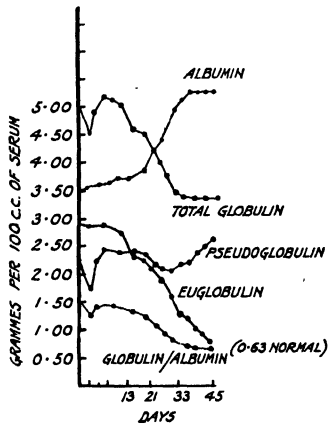


GRAPH 3.

typical graph. In this case formalin gave only a clear gel on the hundredth day, and by the 187th day the patient was serologically

cured, with the protein fractions normal and no gel with formalin. It is clear from this graph that intramuscular treatment of this type of case with high total globulin and euglobulin figures is effective in the usual dosage.

Treatment by interrupted intravenous courses of 693 B.—(*a*) *The effect of one injection of* 693 *B followed after an interval by the usual concentrated course.*—Graph 4 shows a typical case with high total globulin and euglobulin values. A single injection of 693 B caused the tremendous drop in the pseudoglobulin so con-

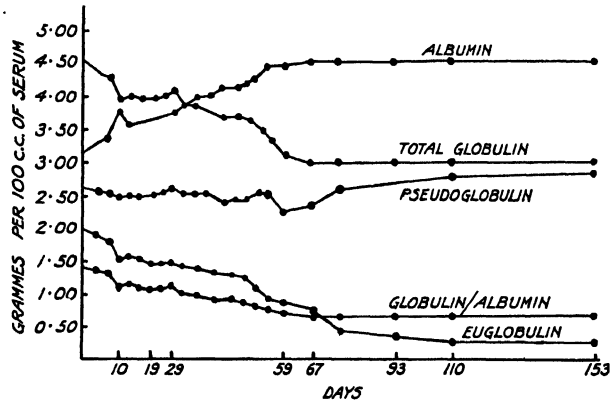


GRAPH 4.

stantly met with in treatment by concentrated courses of 693 B, the euglobulin and albumin being but little changed. Later it will be seen the proteins relapsed, until seven days after the single injection the globulin was actually higher than before. A full concentrated course of 693 B, however, begun on the thirteenth day, produced the usual response.

(*b*) *The effect of two injections of* 693 *B.*—Graph 5 is of considerable interest. Two injections, each of 0·3 gm., produced the usual fall in total globulin and rise in the albumin. Practically no change occurred in the pseudoglobulin, most of the fall being in the euglobulin. The protein progress was normal up to the tenth day, after which there was a slight tendency to relapse. The

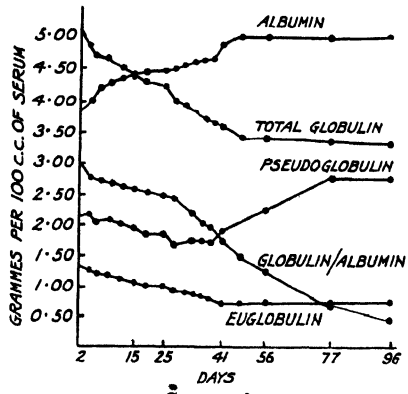
patient was watched very closely, and as he was doing well no further treatment was carried out. On the twenty-sixth day there was a definite turn downwards of the total globulin, and from this point the graph progressed normally to reach serological cure on the 110th day, *i.e.*, within the usual period required for serological cure with concentrated courses of treatment. The onset of the second stage was delayed to about the fifty-ninth day.



GRAPH 5.

This graph indicates that the patient's resistance was just able to effect a cure with the aid of two doses of 693 B. It also illustrates the very curious but nevertheless constant feature that no matter whether the treatment given is much or little, provided that it is sufficient for cure, the period required to reach serological cure is always about the same (four months), even though with very low dosage, such as was given in this case, the second stage is delayed till the fifty-ninth day. The body appears to possess a curious power of compensating for slowness in the first stage by increased speed in the second stage.

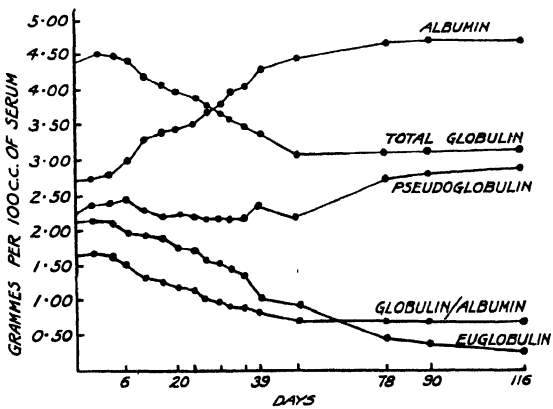
(*c*) *Treatment by four doses of* 693 *B.*—Graph 6 shows that four doses of neostibosan were amply sufficient to cure the patient. After a slight hesitation on the twenty-fifth day the total globulin curve definitely turned downwards, and the patient entered the second stage on the forty-first day, and with the sharp upward



GRAPH 6.

turn of the pseudoglobulin, from then onwards the progress was excellent. In this case, before treatment, the euglobulin was higher than the pseudoglobulin, the euglobulin reaching the exceptionally high figure of very nearly 3 gm. per 100 c.cm. of serum. This, however, does not offer any obstacle to cure even by a half course of total dosage 1·1 gm.

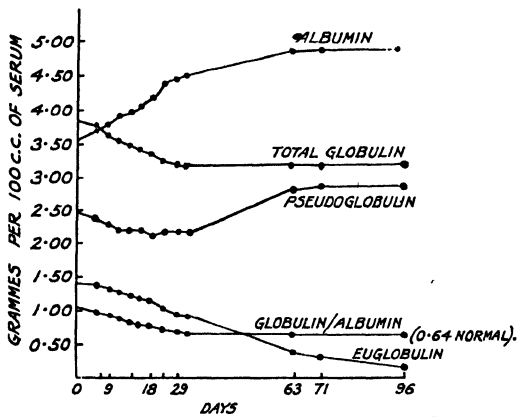
Treatment by urea stibamine.—Graph 7 is very similar to Graph 2, which shows the effect of alternate daily injections of.



GRAPH 7.

693 B, thus indicating, as might be expected, that the general form of the graph is independent of the use of any particular compound of antimony. The treatment here consisted of ten alternate daily injections, a first injection of 0·2 gm., and nine subsequent injections of 0·25 gm., *i.e.*, a total dosage of 2·45 gm. The second stage was reached on the fifty-third day, the serological cure being nearly effected by the 116th day. There was, however, no sign in this case of the sudden drop in the pseudoglobulin.

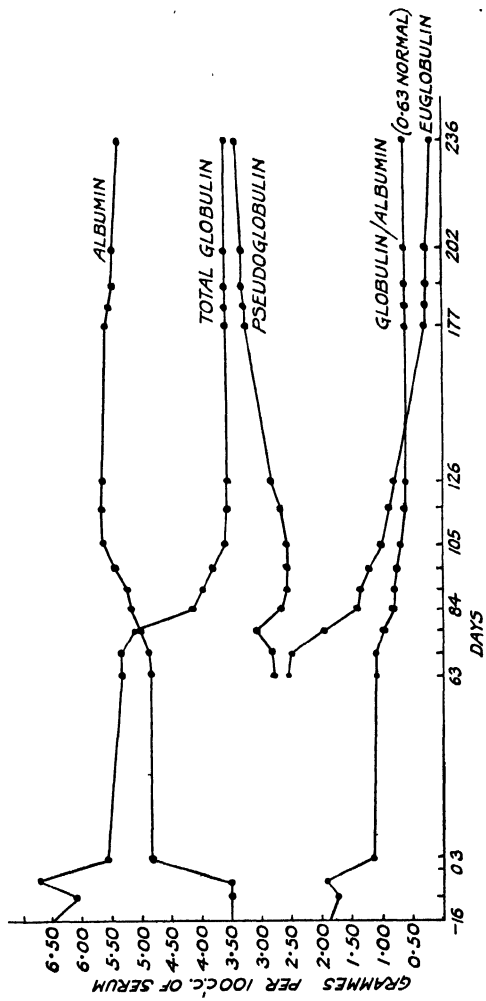
Treatment by sodium antimony tartrate.—Graph 8 is of very similar type to the previous graph. After receiving sixteen injections—that is, only half the usual course—the patient insisted on



GRAPH 8.

leaving hospital. The second stage was reached on the thirty-first day, while serological cure was complete by the ninety-sixth day.

Treatment of "resistant" cases.—As there is no clinical means of determining in advance whether any given untreated case of kala azar will or will not require more than the usual number of injections, the only sense in which the term "resistant" can be used in kala azar is in connection with a case in which previous treatment has failed to effect a cure. Graph 9 is of considerable interest. The patient was gravely ill on admission, and had previously received two courses of sodium antimony tartrate and two

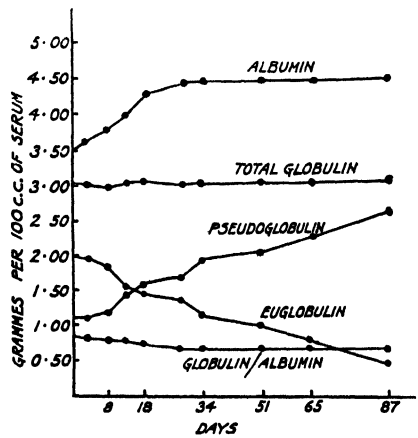


GRAPH 9.

courses of pentavalent antimony. After a concentrated course of 693 B, with dosage rather larger than usual, he absconded and reappeared on the sixty-third day, much improved. While out of

hospital he had had no treatment. When he returned his serum proteins were again graphed, and as his euglobulin was still high he was obviously far from cured. He was observed for a time, and without any further treatment the typical protein response commenced, the albumin and total globulin curves crossing in the usual manner, the euglobulin falling and the second stage beginning about the 105th day. After this the upward movement of the pseudoglobulin began, associated with the corresponding fall in the euglobulin. Serological cure began on the 236th day, after nearly eight months, double the time required to produce a cure in the ordinary case. The practical value of the graph in this case was that it showed that the patient was being slowly cured, although he had had no treatment for months. In view of the past history, further courses of treatment would certainly have been given had there been no graph. This further treatment would certainly have been unnecessary, and quite possibly harmful. From the analogy of other diseases, it is quite conceivable that the avoidance of over-treatment may be of very great importance, for the defensive processes of the body, being greatly weakened by grave degrees of infection, may be altogether broken down by excessive amounts of a highly toxic compound.

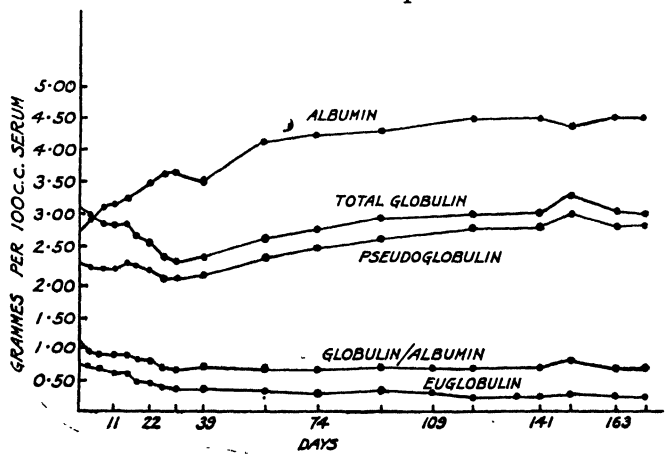
Treatment of Atypical Cases.—Graph 10 is of a case of kala azar



GRAPH 10.

with the albumin higher than the globulin, associated also with a very high euglobulin and a very low pseudoglobulin. There is also the low total protein figure of 6·5 gm. per 100 c.cm. of serum. This patient also showed an incomplete formol leucogel reaction, a gel of some degree of opacity forming in ten minutes, but remaining unchanged up to twenty-four hours. The duration of the case was only about three months, which is too early for the development of a fully-positive formalin reaction. The case, which had had no previous treatment, gave a fairly satisfactory clinical response, though there was no gain in weight, and the leucocyte count was only 6,000 per c.cm. at the time of discharge. In this case there was no question of the crossing of the globulin and albumin curves, but the albumin, which was much below the normal, increased under treatment, whereas the globulin, which was 3 gm. per 100 c.cm. of serum before treatment, continued at about the same level.

Graph 11 shows a case of about one year's duration with the "enteric" type of onset, giving a weak formol leucogel test. There was again a low total protein value of 5·84 gm. per 100 c.cm. of serum. The patient, who was given four doses of 693 B, in order to see if a half course would produce a cure, did not do well. After the first course the temperature did not settle to



GRAPH 11.

normal, and indications of cancrum oris developed. A second full course was therefore given, after which the temperature fell to normal within six days. An examination of the graph shows that about the twenty-ninth day there is a tendency to relapse in the globulin/albumin ratio. This is the point at which signs of cancrum oris appeared. The large increase in the total protein produced by treatment is noticeable. The patient was discharged from hospital, but was readmitted two months later with occasional fever and an enlarging spleen, in which parasites could still be demonstrated. Reference to the graph shows that after the second course of treatment the euglobulin relapsed somewhat, eventually falling again almost to normal by the 121st day. By the 141st day there was again evidence of a rising euglobulin, and the next observation taken on the 150th day, after a third course of treatment had been commenced, showed a very definite relapse in all the protein fractions. The last course of treatment has apparently brought about a cure.

It would appear from this case that the association of a long history with low total proteins and a weak or absent formol leucogel reaction is not of good omen.

In examining the graphs of cases treated with 693 B it will be seen that the second stage begins about the fourth week. This is also the period at which spleen and liver punctures are usually negative. Thus :

> of NNN cultures taken from spleen or liver puncture material from the eighteenth to twenty-fourth day, eleven out of twenty-nine (or 38 per cent.) were positive.
>
> of NNN cultures taken from spleen or liver puncture material from the twenty-fourth to thirtieth day, three out of thirteen (or 23 per cent.) were positive.

After the thirtieth day, parasites were absent. These figures suggest that when the protein graph reaches this particular stage conditions are such that the parasites are unable to exist as a general systemic infection.

Although the general form of the graphs is not dependent upon the use of any one compound of antimony, or upon any special method of administration, yet the standard graph produced by

concentrated courses of 693 B does exhibit one additional factor, namely, the sudden fall in the pseudoglobulin in the initial stages of treatment. The reason why this sharp dip in the pseudoglobulin occurs only with concentrated courses of 693 B is by no means clear. It tends to be absent in " resistant " cases. Since, however, with concentrated courses of 693 B the fall in the total globulin, of which the pseudoglobulin fall is a part, is more rapid, and the second stage is more quickly entered than with any other form of treatment, it seems probable that the pseudoglobulin fall is part of the process of cure rather than a toxic phenomenon due to the extreme potency of 693 B, notwithstanding the fact that, when the case eventually reaches serological cure, the pseudoglobulin is higher than before treatment. As in a well-established case of kala azar the pseudoglobulin is usually somewhat below the figure for normal serum, it is curious that the first effect of treatment should be to reduce it still further.

It seems probable that the general form of the graphs, so far from being peculiar to any particular method of antimony treatment, is not special to kala azar, for cases of secondary syphilis treated with organic arsenicals exhibit somewhat similar curves, although they do not show the heavy pseudoglobulin fall in the initial stages of treatment. It may be anticipated that similar graphs will be met with in other diseases in which the globulin is higher than the albumin.

Two very striking features of the standard graphs are their extraordinary concordance with one another, and their remarkable freedom from minor irregularities opposed to their general direction. These are important practical points, which render the protein graph a more accurate index of the condition of the disease than any clinical observation. Variations from the standard graph have in every case been in agreement with deviations from the normal clinical progress. While the point of serological cure is accurately shown by the extinction of the formol leucogel reaction, this test cannot be used as an indication of the progress under treatment. It seems probable also that the protein graphs may be used for estimating the relative value of different treatments.

Cases of particular interest from the point of view of the mechanism of cure are those associated with a low total protein figure. These cases tend to be associated with a weak or absent formol leucogel reaction, and to give a subnormal response to courses of treatment which are ordinarily adequate. Now in India kala azar is almost always associated with malaria, and for many years was looked upon as a severe form of this disease. It seems possible that a malarial attack, or an attack of some other disease, such as typhoid, in which the protein content of the serum is low, is an essential predisposing factor, and that once the parasite of kala azar has invaded the system a vicious circle is established, whereby the albumin is maintained at a low level, and the euglobulin increases until the cycle is broken by the antimony treatment. In malaria, the characteristic protein graph exhibits a low total protein, the albumin being greatly, and the globulin slightly, reduced. Under quinine treatment, both curves rapidly rise to normal, and parasites disappear from the peripheral blood. It is very seldom indeed that during a kala azar attack malarial parasites are found in the peripheral blood, but once the antimony treatment has taken effect, typical malarial attacks are common. It would seem that both parasites exist under conditions associated with a low total protein content of the serum. Kala azar, however, produces an increase in the globulin factor, a reaction which may be evidence of an immunity response. The high globulin content is unfavourable to the malarial parasite, which therefore disappears from the peripheral circulation, only to reappear, in many instances, when the globulin content falls under antimony treatment.

Incidentally, the beneficial effects of malaria in syphilis may be due to the fact that the low albumin content of the serum in malaria counteracts the high globulin content of the cerebrospinal fluid in syphilis.

It is suggested that if the high globulin content of a serum in kala azar represents an immunity response, such cases should react rapidly to treatment, while the low total protein type of case with a weak or absent formol leucogel reaction represents the opposite condition which should show a worse response.

From the accompanying table it is seen that of a total of 255 cases, 18 relapsed, of which 13 were associated with a weak or absent formol leucogel reaction. Of those treated with stibosan,

Table showing the Association between the Number of Relapsing Cases and the Strength of the Formol Leucogel (Aldehyde) Reaction prior to treatment, as compared with the normal Relapse Rate.

Group.	Course of treatment.	Number of cases and number of relapses in each group.	Formol leucogel complete or nearly so.	Formol leucogel weak or absent.
I. (100 cases)	471 (stibosan) normal relapse rate, 10 per cent.	Number of cases . .	59	41
		Number of cases relapsing	3	4
II. (55 cases)	Stibamine glucoside, normal relapse rate, 13 per cent.	Number of cases . .	34	21
		Number of cases relapsing	2	5
III. (100 cases)	693 B (neostibosan), 4 per cent.	Number of cases . .	65	35
		Number of cases relapsing	0	4

the weak leucogel class show 4 relapses out of 41, or approximately 10 per cent., as against a figure for the strong leucogel class of 3 in 59, or approximately 5 per cent. Of those treated with stibamine glucoside, the weak leucogel class shows a relapse rate of 5 in 21, or approximately 25 per cent., as against a figure for the strong leucogel class of 2 in 34, or approximately 6 per cent. Of the group treated with 693 B, the weak leucogel class shows a relapse rate of 4 in 35, or approximately 11 per cent., as against no relapses in 65 cases with strong leucogel reaction.

The connection between the tendency to relapse after treatment and a weak formol leucogel reaction before treatment is thus very marked. It must, of course, be borne in mind that the weak formol leucogel class includes not only those cases in which the assumed process of immunisation has failed to occur, but also early cases of under five months' duration, in which it has not yet had time to develop. Although the term "immunisation" must be

used in this connection only in the most general sense, since there is no known protein antigen which takes five months to evoke the corresponding antibody, yet it seems highly probable that the clue to the action of antimony in kala azar will be found in its correlation with or stimulation of immune bodies. This view finds support in the fact that cases of moderate duration tend to react to treatment more readily than those with a short history. Waiting for some time may even improve the prognosis. The well-established case, with its high total globulin and euglobulin values, responds so rapidly to treatment that it suggests that the characteristic protein changes are of the nature of an immunity response. The disease having begun to cure itself, the addition of antimony merely serves to tilt the balance, as it were, in the patient's favour.

The question of these protein graphs has been considered at some length not only because of their great practical importance in kala azar, but also on account of their bearing on the mechanism of cure both in this and in other diseases.

COMPLICATIONS ASSOCIATED WITH TREATMENT BY THE PENTAVALENT ANTIMONY COMPOUNDS

While lung complications are common after treatment with the tartar emetic compounds, they are exceedingly rare when the pentavalent antimony derivatives are employed.

Vomiting, however, is one of the commonest complications. It usually comes on some twenty minutes after the injection, and may be preceded by giddiness and nausea. With stibosan, vomiting is rare except when the injections are pushed beyond the usual maximum dose; but with urea-stibamine, aminostiburea and von Heyden 693, vomiting occurs in about 10 per cent. of cases. In these the dose has usually to be kept down to about half the maximum, though the tendency to vomit can be frequently overcome by very cautiously increasing the dose. Vomiting after the injection of neostam occurs frequently according to Napier (1929[2]), and constitutes a serious drawback to the use of this compound, but Low (1927), Struthers (1927[1]) and Chesterman (private communication) experienced no difficulty of this kind.

Neostibosan, on the other hand, appears to be entirely free from this drawback.

Diarrhœa.—Occasionally the vomiting is associated with diarrhœa, although a true dysentery does not occur.

Hepatitis.—In a few instances during treatment with stibosan and urea stibamine, symptoms suggestive of acute hepatitis have developed. The liver becomes much enlarged, and the patient complains of severe pain over the liver. Jaundice is present, and the fever, if absent, recurs. If the treatment is immediately discontinued the symptoms usually subside.

Anaphylactic-like Syndrome.—This group of symptoms generally occurs quite suddenly after the sixth or seventh injection, when the patient has been receiving a maximum dose for the last few injections. Within a few minutes of giving the injection the patient's face becomes puffy, and an urticarial rash comes out all over the body; the voice becomes husky, and there is difficulty in breathing. The patient, in the most severe cases, becomes collapsed, the pulse being imperceptible at the wrist, or the collapse may be accompanied by violent diarrhœa and vomiting, cyanosis, stertorous breathing, and unconsciousness lasting for some minutes. The symptoms usually disappear rapidly, but in a few cases the swelling of the face lasts for twenty-four hours. In two cases a severe shivering attack, lasting two hours, was the main feature. Napier (1926[1]) has found that the condition was commonest with urea-stibamine and aminostibura, rare with stibosan.

Although the symptoms are alarming, no death has been reported. As further administration of the smallest dose may lead to a recurrence of these symptoms, it is best to abandon treatment altogether with the particular compound, recommencing it with minute doses of some other compound.

THE CHOICE OF A PENTAVALENT ANTIMONY DERIVATIVE

With the evidence which is at present available, it is somewhat difficult to compare the various pentavalent antimony compounds with a view to determining the most suitable for general use.

Apart from the cost of manufacture and the stability of the compounds under all conditions of temperature, the factors which have to be taken into account are (I.) the presence of toxic sequelæ, (II.) the relapse rate, (III.) the time necessary for a course of treatment, (IV.) the ease of administration.

While the anaphylactic-like condition is by no means uncommon with aminostiburea and urea-stibamine, it is rare with stibosan, and has not been observed with von Heyden 693 or neostibosan. Jaundice, also a common sequelæ, is apparently rare with von Heyden 693, while vomiting, which is so frequent a complication, is entirely absent in cases treated with neostibosan.

In so far as relapses are concerned, Napier found that of sixty-one patients treated with von Heyden 693, none relapsed, while of twenty-six patients treated with neostibosan, three relapsed. With stibosan, Napier obtained a 9 per cent. relapse rate after a mean relative dose of 4·01 gm., whereas with a mean relative dose of 2·7 gm. of urea-stibamine the relapse rate was only 2·8 per cent. While stibosan is the most stable compound, aminostiburea is the most powerful, and is especially useful in the treatment of relapses.

As regards ease of administration, neostibosan would appear to be superior to all the other pentavalent antimony compounds, for of these only 0·1 gm. can be given intramuscularly, as a maximum, while 0·3 gm. of neostibosan can be injected without pain and without any local reaction. If the results already obtained by Napier and Mullick (1928 and 1929) are confirmed, neostibosan would appear to be the most useful drug yet produced for the treatment of kala azar.

THE ADVANTAGES OF THE PENTAVALENT AS COMPARED WITH THE TRIVALENT ANTIMONY COMPOUNDS

The advantages which are claimed for the pentavalent antimony compounds as compared with the trivalent derivatives are as follows :—

The pentavalent compounds are much less toxic, and can therefore be administered in larger doses.

The total amount of antimony necessary to effect a cure can be administered in a much smaller number of doses. About ten to twelve doses of the pentavalent compounds, as against thirty doses of sodium antimony tartrate, can be looked upon as the average number necessary for an ordinary case. This reduction in the number of injections means that the time required for treatment is reduced from two months to three weeks, or in the case of neostibosan to eight or ten days, so that a far higher percentage of the dispensary class of patient is able to complete a full course of treatment.

Resistant cases which show little, if any, improvement with the tartrates rapidly improve when given relatively large doses of pentavalent antimony compounds.

Disagreeable symptoms, such as cough and severe joint pains, which are frequently associated with the tartrate treatment, do not occur when the pentavalent antimony derivatives are used.

The death rate amongst kala azar patients under treatment has been very markedly reduced since the introduction of the pentavalent antimony compounds, pneumonia, one of the most frequent causes of death during treatment, having been almost entirely eliminated. Before the introduction of antimony in the treatment of kala azar in India it is probable that the death rate was almost 90 per cent. With the use of the antimony tartrates, Muir (1918) reported a 12 per cent. death rate in a series of 150 cases ; Knowles (1920) a 25·6 per cent. death rate among eighty-six cases. Napier (1924), in a series of 139 cases treated in 1922 with sodium potassium tartrates, had 14·4 per cent. of deaths, but (1926[1]), in a small series of thirty-five cases treated with sodium antimony tartrate, the death rate was 22·8 per cent.

These figures are in marked contrast to the 4·2 per cent. death rate obtained by Napier (1926[2]) in a series of 167 cases treated during 1925 with six different pentavalent antimony compounds.

The disadvantages of the pentavalent compounds are few. Jaundice is perhaps rather more common in patients treated with these compounds, as is the anaphylactic-like group of symptoms. These symptoms, according to Chopra (1927), are associated with extreme changes in the pulmonary blood pressure,

together with contraction of the bronchial musculature. All the organic antimony compounds on intravenous injection cause a rise of pressure in the pulmonary artery and in the inferior vena cava, while at the same time the systemic blood pressure falls, due partly to dilatation of the splanchnic vessels, partly to lessened output of the left ventricle. Unless, however, very large doses are given, the fall is rapidly compensated.

The only serious disadvantage of the pentavalent antimony compounds is their high cost, a matter of some moment when, as in the case of Assam, Bengal, and China, some hundreds of thousands of kala azar patients require treatment.

METHOD OF ACTION OF ANTIMONY COMPOUNDS IN KALA AZAR

The means by which antimony destroys the parasites of leishmaniasis in the body is at present unknown.

In vitro, as Noguchi (1924) has shown, tartar emetic has very little action on cultures of leishmania, the highest dilution which is lethal being 1 in 100: nor is the lethal action of antimony increased by bringing it in contact with fresh animal tissues, or by first injecting it intravenously into rabbits. Arsphenamine and neoarsphenamine, on the other hand, while having no curative action in kala azar, are ten times more lethal than antimony to leishmania *in vitro*. Other evidence also makes it doubtful whether *in vivo* the action of antimony is directly parasiticidal. The largest single dose of any antimony compound that can be given does not amount to one hundred-thousandth part of the body weight, and since antimony is fairly evenly distributed throughout the body, except perhaps in the kidney, its concentration is not likely to be greater at any one time than 1 in 10,000. In addition, according to Brahmachari (1922), pentavalent antimony compounds are excreted even more rapidly than trivalent antimony derivatives, for whereas 30 to 40 per cent. of urea-stibamine ($Sb^{(v)}$) is excreted within twenty-four hours of injection, only 6 per cent. of antimony tartrate ($Sb^{(III)}$) is excreted in the same time. Even trivalent antimony is rapidly eliminated, for Mallardi (1921) found that in cases

of infantile kala azar treated with either tartar emetic or stibacetin, the whole of the antimony had been excreted by the end of the third day. Only in very debilitated patients was the excretion still continued till the end of the eighth day.

In this connection it is of interest that Boyd and Roy (1929) have developed a colour test, by means of which the excretion in the urine of pentavalent antimony compounds, such as von Heyden 693 or urea-stibamine, can be studied.

To 0·5 c.cm. of urine containing a known quantity of von Heyden 693 is added one drop of dilute hydrochloric acid, followed by 1·5 c.cm. of water, the mixture being cooled on ice. After about ten minutes one drop of a 1 per cent. solution of sodium nitrate is added, mixed, and the test tube again put back in ice for about a minute. Then 1 c.cm. of a 1 per cent. solution of α-naphthol, in 20 per cent. caustic soda, is added and mixed. A red colour develops in about five minutes, the depth of the colour being proportional to the concentration of the antimony compound in the solution. This solution forms the standard, which is compared in a colorimeter with a known amount of the patient's urine similarly treated.

There remains, of course, the possibility that very small quantities of antimony may either combine with certain of the body tissues to form parasiticidal compounds, or liberate from the tissues immune bodies, which then destroy the parasites. In mice infected with dourine, Uhlenhuth, Kuhn and Schmidt (1925) have pointed out that tartar emetic rapidly damages the trypanosomes, since they disappear from the peripheral blood-stream in two to three hours after the injection. With the pentavalent compounds, on the other hand, there is an interval of from one to two days before the trypanosomes disappear. Whether this latent period is due to a gradual reduction to a trivalent compound is unknown. In human kala azar also there would seem to be a latent period before the parasites are destroyed, for while a spleen puncture performed shortly after a course of pentavalent antimony compounds may be positive, a further spleen puncture performed some weeks later may be entirely negative, although in the interval no further antimony has been given. This suggests that antimony in some

way stimulates certain of the body functions, whereby the environment becomes unsuitable for the development of leishmania, this reaction continuing even after the antimony has been excreted.

In this connection, the experiments of Chopra and Das (1927) are of interest. Pentavalent antimony derivatives, when injected intravenously, cause enlargement of the liver and spleen, the rhythmic contractions of which are strongly stimulated, while tartar emetic produces a much smaller reaction.

If blood films are examined within twenty minutes of an intravenous injection of 0·05 to 0·1 gm. of urea-stibamine, Leishman-Donovan bodies will be found free in the peripheral circulation, possibly because the congestion and rhythmic movements of the organs have ruptured certain of the endothelial cells. If, however, the action of antimony were merely stimulatory, it would be easier to imagine that better results would follow repeated small stimuli spread over a long period rather than a sudden violent stimulation lasting for a short time. Yet clinical experience has shown that the most satisfactory results are obtained by concentrating the treatment to the point just short of producing immediate symptoms of over-dosage.

Further work on the parasitotropic action of antimony must be awaited before the curative action of the pentavalent antimony compounds is fully understood.

The results which have been described show that the use of antimony in kala azar has reduced the death rate from 90 per cent. to under 5 per cent. In addition, as Shortt, Craighead and Swaminath (1928) point out, the use of antimony in the mass treatment of kala azar has had a remarkable effect in limiting the epidemic ravages of the disease. It seems probable that had it not been for the widespread sterilization of the blood due to treatment, the outbreak in Assam during the years 1917–1927 would have been even more disastrous than that of the years 1891–1901, when vast areas of country became entirely waste, for even when antimony fails to cure completely the individual patient he is prevented, at least for a time, from spreading infection. Even, therefore, if antimony did not curtail the duration of the epidemic, it at any rate limited it in area and in numbers.

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CUTANEOUS AND MUCO-CUTANEOUS LEISHMANIASIS

Under this heading are included oriental sore, muco-cutaneous leishmaniasis, seen more especially in South America, and the dermal leishmaniasis, first described in India by Brahmachari (1922).

The intravenous injection of antimony, in some form or another, is, according to Napier (1927), the only treatment that has any effect on the lesions in this last condition. Cases in which there is no previous history of kala azar clear up rapidly, especially after the injection of stibosan, when the nodules usually disappear after about ten injections, there being little or no tendency to recurrence after treatment is discontinued. Those cases, however, which have been previously treated for kala azar with antimony sometimes prove resistant, one case having received over 5 gm. of stibosan with little or no improvement.

ORIENTAL SORE

After the introduction of tartar emetic in South American leishmaniasis by Gaspar Vianna, the antimony tartrates were also employed in the treatment of oriental sore, though the records of its use are not as numerous as might be expected.

Scott (1917) was the first observer to publish an account of the treatment of oriental sore by this method, success being obtained with sixty-three cases of frontier sore in India. Unfortunately, leishmania were found only in four of twenty-seven cases examined microscopically. Sinton (1917), however, cured six diagnosed cases in a period of from eight to thirty-seven days, the scarring left after treatment being very slight.

Christopherson (1917) obtained a good result in a severe case in the Sudan which had persisted for four years, while Greig (1917) treated eighteen cases from Mesopotamia, with recovery in from sixteen to fifty-two days. It must, however, be remembered that oriental sore is a comparatively benign disease which almost invariably cures itself, generally in from six to eighteen months; in addition there are no serious consequences, so that the intra-



F. MAGENDIE (1783–1855), who with P. J. PELLETIER first isolated the alkaloid emetine.

[*To face p.* 216.

venous injection of tartar emetic has been regarded by many as a somewhat drastic procedure. Karamchandani (1927), after injecting 5 c.cm. of a 1 per cent. solution of tartar emetic, had one fatal case among 300.

The method of injecting tartar emetic in cases of oriental sore does not vary from that used in the treatment of kala azar. Karamchandani gives a first injection of 5 c.cm., followed by 7·5 c.cm. and 10 c.cm. of a 1 per cent. solution.

Pentavalent antimony compounds have also been employed in the treatment of oriental sore, though only to a limited extent. Thus Plessier (1922) cured one case by injections of stibenyl: four months, however, elapsed before cure took place, a total of 8 gm. of the drug being injected in doses of from 0·2 to 0·6 gm. Snijders (1927) has also employed stibenyl, while Talaat (1928) has used both antimosan and stibosan. Owen (1928) also reports a case of oriental sore of seventeen years' duration, which, though it resisted intravenous injections of tartar emetic, was eventually cured by injections of stibosan and stibamine glucoside. Dostrowsky (1929), who has carefully compared the length of time required for healing under different methods of treatment, suggests that X-rays and antimosan are the two most efficient methods. Various local methods of treatment have been employed from time to time, but none can be looked upon as specific. The use of a 2 per cent. solution of tartar emetic ointment, X-rays and ionisation have all been used with occasional success.

In 1920 Photinos described a method which he had found efficacious in twenty-three cases in Crete. A solution of emetine hydrochloride was injected around and below the lesion both subcutaneously and intracutaneously. For a small sore the total amount of the drug required was 0·01 gm., for large sores 0·05 gm. One sitting was alone necessary, healing taking place in from fifteen to thirty days.

More recently, Sinderson (1925), in Iraq, records 147 sores, only one of which failed to disappear after treatment with a 2 per cent. solution of emetine hydrochloride.

Castellani (1928) has recommended the injection of phosphorated oil, while more recently Varma (1927) has used injections of

berberine sulphate with success. This method of treatment has also been adopted by Karamchandani (1927), who uses 0·25 grain dissolved in 1·5 c.c. of distilled water. Healing took place in five cases in an average of fourteen days, as compared with eighteen days in the tartar emetic treated series.

More recently, Das Gupta and Dikshit (1929) have studied the toxicity of berberine sulphate on cultures of *Leishmania tropica* and *Leishmania donovani*. Berberine sulphate, even in dilutions of 1 in 80,000, was able to inhibit the growth of the organisms, while the highest toxic dilution of quinine was 1 in 1,000. Emetine 1 in 1,000 had no action, while stibosan did not kill *Leishmania tropica*, even in dilutions of 1 in 100. The action of berberine sulphate on leishmania was also observed directly under the microscope; in dilutions of 1 in 200 the protozoa were immediately killed, though in dilutions of 1 in 20 stibosan the organisms were quite active after three and a quarter hours. Following injections of berberine sulphate into the muscles of rabbits, the only histological changes found were dilatation of the vessels, with some swelling of the capillary endothelium.

Devi (1929) also found that of eighteen sores on twelve patients, six healed completely after one injection, five after two injections, and five after three injections. One case only failed to show any improvement, and in this case the original diagnosis was doubtful.

From the number of treatments which have been used, it is obvious that the highly specific action of antimony in visceral leishmaniasis is not so apparent in oriental sore.

MUCO-CUTANEOUS LEISHMANIASIS

For reasons which are at present unknown, the muco-cutaneous lesions of South America are also more resistant to antimony than infections due to *Leishmania donovani*. This is especially true of the mucous lesions, which yield far less readily than those on the skin. Escomel (1917) states that he has successfully treated several cases with injections of Martindale's oxide of antimony solution, 6 to 8 c.cm. of which can be given once a week, or 1 to 2 c.cm. every two days.

Lindemberg (1926) has used the trioxide of antimony (Sb_2O_3) as a local application, with results superior to those of tartar emetic.

The pentavalent antimony compounds have been but little used in South American leishmaniasis, though Wilson and Shrewsbury (1926) succeeded in curing a case of espundia with stibosan following tartar emetic injections. Lindemberg (1926) has also applied stibenyl and stibosan in the form of an ointment with good results.

Curettage and the local application of an 80 per cent. lactic acid solution has been found superior to antimony injections by de Rezende (1925). Stovarsol, Bayer 205, antimosan and iodobismuthate of quinine have all been employed with varying success, while recently Pupo (1926[1&2]) claims success with "eparseno," which is amino-arseno-phenol of Pomaret, corresponding to dioxy-diamido-arsenobenzene, Ehrlich's 592. Injections of 0·125 to 0·25 gm. can be given intramuscularly at intervals of from two to three days.

A specific treatment for muco-cutaneous leishmaniasis has yet to be found.

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CHAPTER VI

THE CHEMOTHERAPY OF TRYPANOSOMIASIS

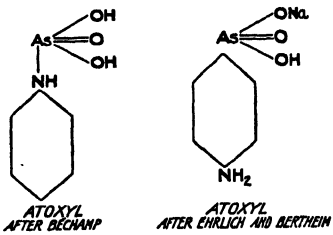
BEFORE the war the only drugs extensively used in the treatment of trypanosomiasis were atoxyl and tartar emetic. During the last ten years, however, there have been definite advances in the chemotherapy of trypanosome infections, for new pentavalent arsenicals have been introduced such as Tryparsamide and Fourneau 270, while in Germanin—Bayer 205, and Moranyl—Fourneau 309, a drug has been produced of a type entirely new to chemotherapy. Unfortunately the high hopes which followed the introduction of this substance have not been entirely fulfilled, and there is thus need for still further improvement in the chemotherapeutic treatment of trypanosomiasis. In sleeping sickness compounds are required which can be given with success by mouth: infections due to *Trypanosoma rhodesiense* are far less amenable to treatment than those due to *T. gambiense*, and in veterinary medicine tartar emetic, in default of anything more efficient and despite the risks attendant on its use, is still largely employed. Finally, in the trypanosomiasis of the New World there is as yet no drug which has the least curative action.

THE TRYPANOCIDAL ACTION OF COMPOUNDS OF ARSENIC

Trypanosome infections were first treated with preparations of arsenic by Bruce (1895) and Lingard (1899), who employed them in nagana and surra. Some few years later, Laveran and Mesnil (1902) used inorganic arsenic in an attempt to cure trypanosomiasis in laboratory animals. Ehrlich and Shiga (1904) also studied the effects of various substances, including atoxyl, on trypanosomes *in vitro*, but found that organic pentavalent arsenicals were quite inert. In 1905, however, Thomas showed that *in vivo* atoxyl

was capable of definite curative action in laboratory animals infected with a number of different species of trypanosomes. These experiments led to a reinvestigation of the chemistry of atoxyl, which was found to be, not the anilide of arsinic acid, as was thought by its discoverer, Béchamp (1863), but *para*-aminophenylarsinic acid.

As the result of its success in the trypanosome infections of laboratory animals, atoxyl was employed by Koch (1907), in Africa, in the treatment of sleeping sickness on a large scale. The preliminary reports inspired great hopes, but later it was found that



relapses frequently occurred, while optic atrophy was by no means an uncommon sequela.

The trypanocidal activity of atoxyl *in vivo* and its failure *in vitro* were explained by Ehrlich as due to its reduction in the body to arsenic in the trivalent state. Since *in vitro* the trivalent organic arsenicals were more actively trypanocidal than the pentavalent derivatives, and were devoid of toxic action on the nervous system, Ehrlich took up the study of the trivalent organic arsenicals. It was found that reduction of the pentavalent phenylglycine-*para*-arsinic acid with sodium hyposulphite resulted in a trivalent compound, *para*-arsenophenylglycine. This substance, in which the two arsenic atoms are linked by a double bond and each is coupled to the benzene nucleus by a single linkage, represents the arsenobenzene type of organic arsenical, and is therefore the precursor of the arsenobenzene compounds which are discussed in relation to the chemotherapy of syphilis.

Owing to the interest aroused by the arsenobenzenes, attention was for a time diverted from the pentavalent organic arsenicals

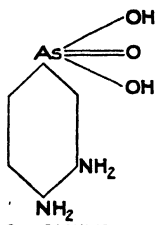
and various modifications of the atoxyl molecule were attempted, among other compounds produced being arsacetin, the sodium salt of *para*-acetylaminophenylarsinic acid ($CH_3CO.NH.C_6H_4.AsO_3HNa$).

Browning (1908) found that in mice the maximum tolerated dose of this compound by subcutaneous injection was 3·0 gm. per kilogram of body weight: mice infected with *T. brucei* or *T. equiperdum* could be cured by the subcutaneous injection of from 1·2 to 2·0 gm. per kilogram of body weight. Similar trypanocidal properties were noted in the *p*-hydroxybenzylidene derivative of atoxyl.

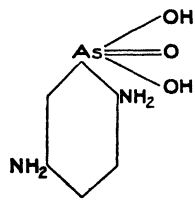
Since the war the study of the pentavalent organic arsenicals has been actively pursued. At the Rockefeller Institute, New York, the investigations of Jacobs and Heidelberger resulted in the discovery of tryparsamide, while the work of Fourneau and his collaborators at the Pasteur Institute, Paris, not only produced derivatives such as Fourneau 270 and stovarsol, but has thrown light on the relationship between chemical constitution and trypanocidal action. King and his colleagues (1924–1928) have also made important contributions to this question.

Fourneau and his collaborators (1923, 1925, 1926) studied more especially the phenylarsinic acids and the effect on toxicity and trypanocidal activity of introducing various radicals into the molecule. When an amino group is introduced in the *para*-position in phenylarsinic acid, the toxicity diminishes slightly and the therapeutic activity is increased, yielding atoxyl. The *ortho*-aminophenylarsinic acid, however, is very toxic and is quite inactive therapeutically, and the *meta*-aminophenylarsinic acid has only a slight trypanocidal action. If a second amino-group is introduced into *para*-aminophenylarsinic acid in the *ortho*-position relative to the first group and in the *meta*-position relative to the arsenic a compound 3 : 4-diaminophenylarsinic acid (209) is obtained of low toxicity, but of irregular trypanocidal action, owing to its rapid elimination. In the case of the diamino-derivatives, the position of the groups is thus also of great importance; 2 : 5-diaminophenylarsinic acid and 2 : 4-diaminophenylarsinic acid are about five times as toxic as 3 : 4-diaminophenyl-

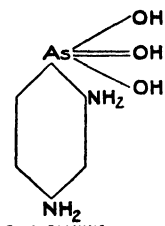
arsinic acid ; 2 : 5-diamino-acid has no trypanocidal action, but 2 : 4-diamino-acid, with an amino-group in the *para* position, has a chemotherapeutic index of 1 : 2·5.



3:4-DIAMINO-PHENYLARSINIC ACID



2:5-DIAMINO-PHENYLARSINIC ACID



2:4-DIAMINO-PHENYLARSINIC ACID

The introduction of a third amino-group does not confer any trypanocidal action, 3 : 4 : 5-triaminophenylarsinic acid being rather more toxic than 3 : 4-diaminophenylarsinic acid. The maximum tolerated dose of the aminophenylarsinic acids in grams per 20 gm. of body weight of mouse is :

Phenylarsinic acid	0·003
Aminophenylarsinic acid . . .	0·0035
Diaminophenylarsinic acid. . .	0·020–0·10
Triaminophenylarsinic acid . .	0·020

If in place of an amino, a hydroxyl group is introduced into the *para* position of phenylarsinic acid, a compound is obtained which is found to have a chemotherapeutic index of between 1 : 2 and 1 : 3.

A hydroxyl group introduced in the *meta*-position of phenylarsinic acid causes a slight loss in therapeutic activity, while the same group in the *ortho*-position produces a totally inert compound.

The therapeutic importance of the position of the hydroxyl group is also seen by the introduction of this group in the *para*-position in *meta*-aminophenylarsinic acid (Fourneau 240). The resulting compound (Fourneau 189), *meta*-amino-*para*-hydroxyphenylarsinic acid, is at least ten times as toxic as 240 and five times more active therapeutically, and has, according to Navarro-Martin

(1922), a chemotherapeutic index of between 1 : 2 and 1 : 3 in laboratory animals infected with *T. rhodesiense* and *T. brucei*. Unfortunately trials of Fourneau 189 in sleeping sickness by Blanchard and Lefrou (1922) have given unsatisfactory results.

On the other hand, the addition of an amino-group in the *meta*-position of *para*-oxyphenylarsinic acid causes only a very slightly enhanced chemotherapeutic action. In other words, the introduction of a hydroxyl group into an amino compound produces greater therapeutic action than the introduction of an amino group into a hydroxyl compound.

The effect of variation in the position of the amino and hydroxy groups on the chemotherapeutic index of the ten phenylarsinic acids prepared by Fourneau and his colleagues is shown in the following table :—

Arsinic acid.	Number.	Chemotherapeutic index.
4-amino-2-hydroxyphenyl . .	269	1 : 8
4-amino-3-hydroxyphenyl . .	248	1 : 2
5-amino-3-hydroxyphenyl . .	455	1 : 3
6-amino-2-hydroxyphenyl . .	459	1 : 1
2-amino-3-hydroxyphenyl . .	218	1 : 2·2
2-amino-4-hydroxyphenyl . .	258	1 : 1
2-amino-5-hydroxyphenyl . .	242	1 : 2·5
3-amino-4-hydroxyphenyl . .	189	1 : 5
3-amino-6-hydroxyphenyl . .	224	1 : 1
3-amino-2-hydroxyphenyl . .	416	1 : 1

The effects of introducing a methyl group into the molecule of the phenylarsinic acids have also been investigated. As a general rule, this change leads to a reduction in the chemotherapeutic index, the exception being 3 : 4-diamino-phenylarsinic acid. The reason for this exception may be that the diamino-phenylarsinic acids are very rapidly excreted, the introduction of a methyl group delaying this excretion.

As a general rule also the introduction of a chlorine atom increases the toxicity of the phenylarsinic acids, except, as shown in the table, in the case of 3-amino-6-chlorophenylarsinic acid

(425) and 4-glycineamide-2-chlorophenylarsinic acid (429). Haythornthwaite (1929) finds that the introduction of bromine into the nucleus of the simple phenylarsinic acids also raises the chemotherapeutic index.

The influence of acylation of the amino-group is of considerable interest. In certain cases, as in the formyl-derivative of 4-amino-2-hydroxyphenylarsinic acid, the compound is more active when given by mouth than when injected. In the case of the compounds derived from 3-amino-4-hydroxyphenylarsinic acid it is noticeable that acetylation or the formation of a urethane or of any amide group is associated with a complete disappearance of trypanocidal action. On the other hand, the dimethylglycinamino-(415) and the acetyllactylamino-derivatives (192) both have an increased chemotherapeutic action. This increased action is probably due to a greater diffusibility of the compounds.

Because a particular modification in structure appears to augment the therapeutic activity of one particular compound, it does not follow that the same modification will of necessity increase the activity of another compound.

Thus, 4-amino-2-hydroxyphenylarsinic acid (269) [$C_6H_3(NH_2)$-(OH) (AsO_3H_2) = 4 : 2 : 1] has a chemotherapeutic index of 1 : 8, while tryparsamide [$C_6H_4(NHCH_2.CONH_3)$ (AsO_3H_2) = 4 : 1] has a chemotherapeutic index of 1 : 3. The addition of a phenol group in the *ortho*-position in tryparsamide might be expected to produce a substance of increased activity. As a matter of fact, 4-phenyl glycineamide-2-hydroxyphenylarsinic acid (439) [$C_6H_3(NHCH_2.CONH_2)$ (OH) (AsO_3H_2) = 4 : 2 : 1] has a chemotherapeutic index of only 1 : 2.

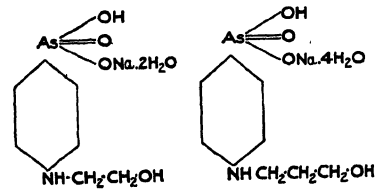
A study of the table (pp. 232–3) shows that when the amino-group is in the *para*-position acylation of the amino-group causes a reduction in the chemotherapeutic index. The therapeutic activity of compounds derived from 4-amino-2-hydroxyphenylarsinic acid is especially noticeable.

These results indicate that the action of any particular compound cannot yet be predicted from a study of its chemical structure, and that only certain very broad rules can be laid down for the determination of trypanocidal activity. Unfortunately

the fact that a compound has a curative action in experimental trypanosomiasis does not necessarily imply that it will have the same action in sleeping sickness. If, however, a compound does not display any curative activity in the experimental trypanosome infections of laboratory animals it is safe to assume that it will be useless in the more chronic trypanosomiasis of man.

Stimulated by the results obtained with tryparsamide and Fourneau 270, other workers have essayed the production of pentavalent arsenical derivatives. One aim, as yet unattained, is the formation of some compound which is actively trypanocidal when given by mouth, another is the discovery of substances which will penetrate the central nervous system even more readily than tryparsamide.

One attempt to produce compounds which will readily pass into the central nervous system has been made by Stratman-Thomas and Loevenhart (1928[1, 2]), who have prepared the monosodium salts of *p*-*β*-hydroxyethylamino-phenylarsinic acid (Etharsanol) and *p*-*γ*-hydroxypropylamino-phenylarsinic acid (Proparsanol).



ETHARSANOL *PROPARSANOL*

It will be noted that in both compounds the side-chain is in the position *para* to the arsinic acid group. Etharsanol differs from tryparsamide in having a primary alcohol group at the termination of the side-chain, while tryparsamide has an acid amide group. This change in structure yields a compound which is approximately twice as toxic as tryparsamide. Proparsanol differs from etharsanol in having an extra methylene group in the side-chain, though this difference in structure produces no change either in toxicity or type of action. The arsenic content of etharsanol is 20·32 per cent. and that of proparsanol 20·68 per cent. It was found that etharsanol produces no effect in trypanosome infections unless

given in doses approaching the lethal. Owing, however, to its very rapid excretion, it can be given repeatedly in large doses without producing deleterious effects. Thus, while 0·35 gm. per kilogram of body weight, given intravenously, is the maximum tolerated dose for a rabbit, three doses of 0·25 gm. may be given on alternate days without toxic effects. The maximum tolerated dose, the minimum curative dose and the chemotherapeutic index of etharsanol are shown in the following table :—

Animal.	Mode of administration.	Maximum tolerated dose in gm. per kilogm.	Minimum curative dose in gm. per kilogm.	Chemotherapeutic index.
Mouse	Intraperitoneal	0·50	—	—
Rat	Intravenous	1·25	0·06	1 : 21
	Subcutaneous	0·90	0·10	1 : 9
	Intramuscular	1·00	0·10	1 : 10
	Intraperitoneal	0·90	0·10	1 : 9
Guinea pig	Subcutaneous	0·15	—	—
	Intramuscular	0·15	—	—
Rabbit	Intravenous	0·25	0·05	1 : 5
	Intramuscular	0·40	0·10	1 : 4
	Oral	0·35	—	—

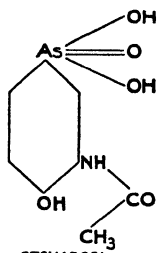
Experiments on rats infected with *T. brucei*, *T. equiperdum*, *T. equinum*, *T. lewisi* and *T. rhodesiense* and on rabbits infected with *T. brucei*, *T. equiperdum* and *T. rhodesiense* showed that etharsanol is an effective drug in the treatment of experimental trypanosomiasis. Rabbits could even be cured when, as shown by lethargy and paralysis, the central nervous system was affected, and on analysis, the brain substance was found to contain large amounts of arsenic. Intramuscular injections were unattended by any local reaction. Up to the present etharsanol has only been used in the treatment of a very few human cases of sleeping sickness, but amblyopia has not been seen, possibly owing to the rapid excretion of the drug, for in man 75 per cent. was excreted in five hours, and 91 per cent. within four and half days. The results of further clinical trials will be awaited with considerable interest.

The curative action of various derivatives of arsinic acid on mice infected with *T. equiperdum* has also been investigated by Ewins and Everett (1927), the compounds being given either by mouth or by injection, and the curative effect being judged by the sterilization of the peripheral blood-stream maintained for at least seventy-two hours. The results are shown in the table:

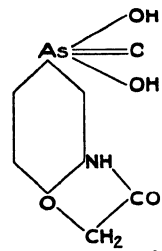
The Effect of Arsinic Acid Derivatives given by Mouth on T. equiperdum *Infection in Mice (Ewins and Everett* (1927))

Compound.	Maximum tolerated dose in mgm. per gm. of body weight.	Minimum curative dose in mgm. per gm. of body weight.	Chemotherapeutic Index.
3-acetylamino-4-hydroxyphenylarsinic acid (stovarsol)	4·5	0·4	1 : 11
4-acetylamino-2-hydroxyphenylarsinic acid	10·0	0·1	1 : 100
5-acetylamino-2-hydroxyphenylarsinic acid	10·0	0·1	1 : 100
3-acetylamino-4-methoxyphenylarsinic acid (O-methyl stovarsol)	2·0	0·8	1 : 2·5
N-phenylglycineamide-4-arsinic acid (tryparsamide)	14·0	0·6	1 : 23
3-hydroxy-1 : 4-benzisoxazine-6-arsinic acid	3·0	0·12	1 : 25
3-hydroxy-1 : 4-benzisoxazine-8-amino-6 arsinic acid	14·0	> 7	1 : < 2·0
3-hydroxy-1 : 4-benzisoxazine-8-acetylamino-6 arsinic acid	16·0	0·2	1 : 80
3-hydroxy-1 : 4-benzisoxazine-8-glycinamido-6-arsinic acid	16·0	> 8·0	1 : < 2·0
3-glycinamido-4-methoxyphenylarsinic acid	12·0	> 5·0	1 : < 2·4
Benzoxazolone-4-arsinic acid	1·0	0·1	1 : 10
Benzoxazolone 6-acetylamino-4-arsinic acid	14·0	0·6	1 : 23
3-acetylamino-4-hydroxy-5-aminophenylarsinic acid	7·0	> 5·0	1 : < 1·4
3 : 5-diacetylamino-4-hydroxyphenylarsinic acid	10·0	> 8·0	1 : < 1·2
2-hydroxy-5-diglycineamidephenylarsinic acid	12·0	> 8·0	1 : < 1·5

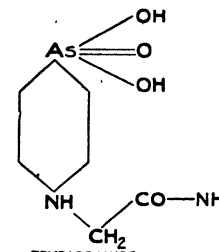
It will be noted that the second and third compounds are isomers of stovarsol. The results from the oral administration of stovarsol were superior to those obtained by Levaditi, Nicolau and Galloway (1926) in the prophylaxis of nagana infections in



STOVARSOL



3-HYDROXY 1:4-BENZISOXAZINE 6 ARSINIC ACID



TRYPARSAMIDE

rabbits. The substitution of a methoxy- for the hydroxy-group of stovarsol did not endow the compound with a permanently curative action such as has been described by Hewitt, King and Murch (1926) in the case of certain other methoxy-compounds. The trypanocidal action of the benzisoxazinearsinic acids was also investigated. They are heterocylic compounds having some

Curative Action of Benzisoxazinearsinic acids in T. equiperdum *Infections in Mice.*

Compound.	Method of administration.	Maximum tolerated dose in mgm. per gm. of body weight.	Minimum curative dose in mgm. per gm. of body weight.	Chemo-therapeutic index.
3-hydroxy-1 : 4-benzisoxazine-6-arsinic acid.	Intravenous inj.	0·2	0·15	1 : 1·3
	Subcutaneous inj.	0·2	0·15	1 : 1·3
	Per os . .	3·0	0·12	1 : 25·0
3-hydroxy-1 : 4-benzisoxazine-8-acetylamino-6-arsinic acid.	Intravenous inj.	1·2	0·3	1 : 4·0
	Subcutaneous inj.	1·2	0·3	1 : 4·0
	Per os . .	16·0	0·2	1 : 80
3-hydroxy-1 : 4-benzisoxazine-8-glycinamido-6-arsinic acid.	Intravenous inj.	1·5	>1·0	1 : <1·5
	Subcutaneous inj.	1·5	>1·0	1 : <1·5
	Per os . .	16·0	>8·0	1 : <2
3-hydroxy-1 : 4-benzisoxazine-8-amino-6-arsinic acid.	Intravenous inj.	0·7	>0·5	1 : 1·4
	Subcutaneous inj.	0·7	>0·5	1 : <1·4
	Per os . .	14·0	>7·0	1 : 2·0

The Effect of Introducing a Methyl Group into the Constitution of the Phenylarsinic Acids on Chemotherapeutic Action (*Fourneau, M. and Mme. Tréfouel and Mme. de Lestrange-Trévise* 1926)

Acid.	No.	Formula.	Maximum tolerated dose in gm. per 20 gm. of mouse.	Curative dose in gm. per 20 gm. of mouse.	Chemotherapeutic index.
3-amino-4-hydroxyphenylarsinic acid	189	$[C_6H_3(NH_2)(OH)(AsO_3H_2) = 3:4:1]$	0·035	0·007	1 : 5
3-amino-4-hydroxy-5-methylphenylarsinic acid	299	$[C_6H_2(NH_2)(OH)(CH_3)(AsO_3H_2) = 3:4:5:1]$	0·005	0·005	1 : 1
3-amino-4-hydroxy-6-methylphenylarsinic acid	300	$[C_6H_2(NH_2)(OH)(CH_3)(AsO_3H_2) = 3:4:6:1]$	0·020	0·008	1 : 2·5
4-acetylamino-2-hydroxyphenylarsinic acid	270	$[C_6H_3(NHCOCH_3)(OH)(AsO_3H_2) = 4:2:1]$	0·020	0·001	1 : 20
4-acetylamino-2-hydroxy-5-methylphenylarsinic acid	423	$[C_6H_2(NHCOCH_3)(OH)(CH_3).(AsO_3H_2) = 4:2:5:1]$	Subcutaneous 0·020 *Per os* —	0·007 0·025	1 : 3 —
2-amino-4-acetylaminophenylarsinic acid	272	$[C_6H_3(NH_2)(NHCOCH_3)(AsO_3H_2) = 2:4:1]$	0·040	0·009	1 : 4.
2-amino-4-acetylamino-5-methylphenylarsinic acid	420	$[C_6H_2(NH_2)(NHCOCH_3)(CH_3)(AsO_3H_2) = 2:4:5:1]$	Subcutaneous 0·025 *Per os* relapse after	0·009 on tenth day 0·030 gm.	1 : 3
3 : 4-diaminophenylarsinic acid	209	$[C_6H_3(NH_2)(AsO_3H_2) = 3:4:1]$	0·060	irregular	—
3 : 4-diamino-6-methylphenylarsinic acid.	304	$[C_6H_2(NH_2)(CH_3)(AsO_3H_2) = 3:4:6:1]$	0·060	0·030	1 : 2

Influence on Chemotherapeutic Action of Neutralization of the Amino-Group in Phenylarsinic Acids

Acid.	No.	Formula.	Maximum tolerated dose in gm. per 20 gm. of mouse.	Curative dose in gm. per 20 gm. of mouse.	Chemotherapeutic index.
3-amino-4-hydroxyphenylarsinic acid	189	$[C_6H_3(NH_2)(OH)(AsO_3H_2) = 3:4:1]$	0·035	0·007	1 : 5
3-acetylamino-4-hydroxyphenylarsinic acid	190	$[C_6H_3(NHCOCH_3)(OH)(AsO_3H_2) = 3:4:1]$	—	slight action	0
3-methylcarbamino-4-hydroxyphenylarsinic acid (Urethanes.)	349	$[C_6H_3(NHCO_2CH_3)(OH)(AsO_3H_2) = 3:4:1]$	0·008	slight action	0
3-ethylcarbamino-4-hydroxyphenylarsinic acid (Urethanes.)	284	$[C_6H_3(NHCO_2C_2H_5)(OH)(AsO_3H_2) = 3:4:1]$	—	no action	0
3-dimethylglycylamino-4-hydroxyphenylarsinic acid	415	$[C_6H_3(NH.CO.CH_2N(CH_3)^2)(OH)(AsO_3H_2) = 3:4:1]$	0·050	0·035	1 : 1·4
3-acetyl-lactylamino-4-hydroxyphenylarsinic acid	192	$[C_6H_3(NH.CO.CH(O.CO.CH_3).CH)(OH)(AsO_3H_2) = 4:1:3]$	Subcutaneous 0·030 *Per os* 0·160	0·010 0·020	1 : 3 1 : 8
3-ethoxyacetylamino-4-hydroxyphenylarsinic acid	421	$[C_6H_3(NHCOCH_2OC_2H_5)(OH)(AsO_3H_2) = 3:4:1]$	0·120	slight action	0
3-glycineamine-4-hydroxyphenylarsinic acid	350	$[C_6H_3(NHCH_2.CO.O)(AsO_3H_2) = 3\text{–}4:1]$	0·040	slight action	0
3 : 4-diaminophenylarsinic acid	209	$[C_6H_3(NH_2)(AsO_3H_2) = 3\text{–}4:1]$	0·060	irregular	0
3 : 4-benzimideazol-phenylarsinic acid	318	$[C_6H_3(N{=}CH.NH)(AsO_3H_2) = 3\text{–}4:1]$	0·015	0·015	1 : 1
3 : 4-methylbenzimideazol-phenylarsinic acid	317	$[C_6H_3(N{=}C(CH_3)NH)(AsO_3H_2) = 3\text{–}4:1]$	0·020	0·015	1 : 1·3
4-amino-2-hydroxyphenylarsinic acid	269	$[C_6H_3(NH_2)(OH)(AsO_3H_2) = 4:2:1]$	0·015	0·002	1 : 7·5

4-acetylamino-2-hydroxyphenylarsinic acid	270	$[C_6H_3(NHCOCH_3)(OH)(AsO_3H_2) = 4:2:1]$.	0·020	0·001	1 : 20
4-ethylcarbamino-2-hydroxyphenylarsinic acid	418	$[C_6H_3(NHCO_2C_2H_5)(OH)(AsO_3H_2) = 4:2:1]$.	Subcutaneous 0·003 *Per os* 0·030	0·002 0·010	1 : 1·5 1 : 3
4-formylamino-2-hydroxyphenylarsinic acid	417	$[C_6H_3(NHCOH)(OH)(AsO_3H_2) = 4:2:1]$ . .	Subcutaneous 0·015 *Per os* 0·100	0·003 0·006	1 : 5 1 : 16
4-propionylamino-2-hydroxyphenylarsinic acid	438	$[C_6H_3(NHCOCH_2CH_3)(OH)(AsO_3H_2) = 4:2:1]$.	0·015	0·001	1 : 15
4-acetyllactylamino-2-hydroxyphenylarsinic acid	437	$[C_6H_3(NHCO.CH.CH_3)(OH)(AsO_3H_2) = 4:2:1]$ (OCOCH$_3$)	0·025	0·003	1 : 8
4-phenylglycineamide-2-hydroxyphenylarsinic acid	439	$[C_6H_3(NHCH_2CONH_2)(OH)(AsO_3H_2) = 4:2:1]$.	0·020	0·010	1 : 2
2 : 4-diaminophenylarsinic acid	273	$[C_6H_3(NH_2)(AsO_3H_2) = 2\text{–}4:1]$ . . .	0·022	0·009	1 : 2·5
2-amino-4-acetylaminophenylarsinic acid	272	$[C_6H_3(NH_2)(NHCOCH_3)(AsO_3H_2) = 4:1]$ . .	0·040	0·009	1 : 4
2 : 4-diacetylaminophenylarsinic acid	396	$[C_6H_3(NHCOCH_3)(AsO_3H_2) = 2\text{–}4:1]$ . .	0·030	0·018	1 : 1·6
2 : 4-dihydroxyphenylarsinic acid	259	$[C_6H_3(OH)(AsO_3H_2) = 2\text{–}4:1]$ . . .	0·022	0·009	1 : 2·5
5-amino-2 : 4-dihydroxyphenylarsinic acid	458	$[C_6H_2(NH_2)(OH)(AsO_3H_2) = 2\text{–}4:1]$ . .	0·003	very slight action.	0
5-acetylamino-2 : 4-dihydroxyphenylarsinic acid	456	$[C_6H_2(NHCOCH_3)(OH)(AsO_3H_2) = 5:2\text{–}4:1]$.	0·048	feeble action	0

Influence on Chemotherapeutic Action of Neutralization of the Amino-Group in Phenylarsinic Acids—contd.

Acid.	No.	Formula.	Maximum tolerated dose in gm. per 20 gm. of mouse.	Curative dose in gm. per 20 gm. of mouse.	Chemo-therapeutic index.
5-formylamino-2 : 4-dihydroxyphenylarsinic acid	461	$[C_6H_2(NH.COH)(OH)(AsO_3H_2) = 5:2\text{–}4:1]$	0·050	very feeble action.	0
5-propionylamino-2 : 4-dihydroxyphenylarsinic acid	462	$[C_6H_2(NHCOCH_2CH_3)(OH)(AsO_3H_2) = 5:2\text{–}4:1]$	0·035	no action	0
3 : 5-diaminophenylarsinic acid	447	$[C_6H_3(NH_2)(AsO_3H_2) = 3\text{–}5:1]$	0·018	0·010	1 : 1·8
3 : 5-diacetyldiaminophenylarsinic acid	444	$[6_6H_3(NHCOCH_3)(AsO_3H_2) = 3\text{–}5:1]$	0·050	0·030	1 : 1·6

The Effect on Chemotherapeutic Action of Introducing Chlorine into the Constitution of the Phenylarsinic Acids

Acid.	No.	Formula.	Maximum tolerated dose in gm. per 20 gm. of mouse.	Curative dose in gm. per 20 gm. of mouse.	Chemo-therapeutic index.
4-amino-phenylarsinic acid (atoxyl)	—	$[C_6H_4(NH_2)(AsO_3H_2) = 4:2]$	0·0033	0·0033	1 : 1
4-amino-2-chlorophenylarsinic acid	431	$[C_6H_3(NH_2)(Cl)(AsO_3H_2) = 4:2:1]$	0·003	slight action	0
4-amino-3-chlorophenylarsinic acid	464	$[C_6H_3(NH_2)(Cl)(AsO_3H_2) = 4:3:1]$	0·001	„ „	0

4-acetylamino-phenylarsinic acid (arsacetin) . .	—	$[C_6H_4(NHCOCH_3)(AsO_3H_2) = 4:1]$ . . .	0·033	0·033	1 : 1
4-acetylamino-2-chlorophenylarsinic acid .	422	$[C_6H_3(NH.COCH_3)(Cl)(AsO_3H_2) = 4:2:1]$.	0·017	0·013	1 : 1
4-acetylamino-3-chlorophenylarsinic acid .	450	$[C_6H_3(NH.COCH_3)(Cl)(AsO_3H_2) = 4:3:1]$.	0·002	slight action	0
4-phenylglycineamide-arsinic acid (tryparsamide)	—	$[C_6H_4(NHCH_2.CONH_2)(AsO_3H_2) = 4:1]$ . .	0·040	0·013	1 : 3
4-glycineamide-2-chlorophenylarsinic acid .	429	$[C_6H_3(NH.CH_2.CONH_2)(Cl)(AsO_3H_2) = 4:2:1]$	0·050	0·010	1 : 5
4-glycineamide-3-chlorophenylarsinic acid .	443	$[C_6H_3(NH.CH_2.CONH_2)(Cl)(AsO_3H_2) = 4:3:1]$	0·012	0·008	1 : 1·5
3-amino-phenylarsinic acid .	240	$[C_6H_4(NH_2)(AsO_3H_2) = 3:1]$	0·0045	slight action	0
3-amino-6-chlorophenylarsinic acid .	425	$[C_6H_3(NH_2)(Cl)(AsO_3H_2) = 3:6:1]$ . .	0·006	0·006	1 : 1
3-amino-4-chlorophenylarsinic acid .	441	$[C_6H_3(NH_2)(Cl)(AsO_3H_2) = 3:4:1]$ . .	0·0005	slight action	0
3-acetylamino-phenylarsinic acid	241	$[C_6H_4(NH.COCH_3)(AsO_3H_2) = 3:1]$ . .	0·010	0·005	1 : 2
3-acetylamino-6-chlorophenylarsinic acid .	426	$[C_6H_3(NH.COCH_3)(Cl)(AsO_3H_2) = 3:6:1]$.	0·010	slight action	0
3-acetylamino-4-chlorophenylarsinic acid .	445	$[C_6H_3(NH.COCH_3)(Cl)(AsO_3H_2) = 3:4:1]$.	0·003	very slight action.	0
3-amino-4-hydroxyphenylarsinic acid	189	$[C_6H_3(NH_2)(OH)(AsO_3H_2) = 3:4:1]$ . .	0·035	0·007	1 : 5
3-amino-4-hydroxy-5-bromo-phenylarsinic acid . .	469	$[C_6H_3(NH_2)(OH)(Br)(AsO_3H_2) = 3:4:5:1]$.	0·010	0·010	1 : 1
3-acetylamino-4-hydroxy-5-bromo-phenylarsinic acid.	470	$[C_6H_3(NHCOCH_3)(OH)(Br)(AsO_3H_2) = 3:4:5:1]$	0·004	no action	0
3-amino-4-hydroxy-5-chlorophenylarsinic acid .	467	$[C_6H_3(NH_2)(OH)(Cl)(AsO_3H_2) = 3:4:5:1]$.	0·008	irregular action.	0
3-acetylamino-4-hydroxy-5-chlorophenylarsinic acid .	468	$[C_6H_3(NHCOCH_3)(OH)(Cl)(AsO_3H_2) = 3:4:5:1]$.	0·020	„ „	0

resemblance on the one hand to stovarsol, on the other to tryparsamide.

From the table it will be seen that the minimum curative doses of certain of the benzisoxazinearsinic acids are the same whether the compound is injected or given by mouth, thus differing from numerous other arsinic acids where, in general, a much larger dose is required *per os* than by injection to produce a curative effect.

Chesterman and Todd (1927) have tested 3-hydroxy 1 : 4-benzisoxazine-6-arsinic acid (Cyclosan) in human trypanosomiasis. It caused diarrhœa and vomiting when given by mouth and produced no amelioration in the symptoms. Stovarsol by mouth was also ineffective.

King and his co-workers (1924–1928) have prepared and tested the trypanocidal action of a large number of derivatives of the phenylarsinic acids. Among the arylamides of *p*-aminophenylarsinic acid the three isomeric monoaminobenzoyl-derivatives were found to have slight curative action. Glyoxaline-4 (or 5)-carboxyl-*p*-aminophenylarsinic acid and its amino-derivative were also therapeutically active. Amphoteric *s*-carbamido-arylarsinic acids and the *s*-carbamides and arylamides of naphthylamine di- and tri-sulphonic acids were either inactive or only very slightly active, as were derivatives of β-aminoethyl and γ-aminopropylarsinic acids.

Barber (1929) has found that the introduction of an unsubstituted phenyl group in the 4-amino group of 3 : 4-diaminophenylarsinic acid results in a marked increase in toxicity, despite the lower arsenic content and somewhat greater trypanocidal action.

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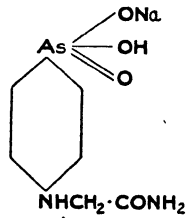
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SODIUM-N-PHENYLGLYCINEAMIDE-*p*-ARSINATE. TRYPARSAMIDE

At the present time, probably the most widely used pentavalent arsenic compound is tryparsamide, which was first described by Jacobs and Heidelberger (1919).



Tryparsamide is the sodium salt of N-phenylglycineamide-*p*-arsinic acid and is a colourless, crystalline salt, extremely soluble in water and forming neutral solutions which are comparatively stable, so that a 10 per cent. solution may be boiled without appreciable cleavage of ammonia or arsenic. It contains 24·57 per cent. of arsenic. Pearce and Brown (1919[1],[2],[3]) described the chief biological effects of the compound. The minimum lethal dose is as follows :—

Minimum Lethal Dose of Tryparsamide in Animals in Grammes per Kilogram of Body Weight

Animal.	Method of administration.		
	Subcutaneous.	Intravenous.	Intraperitoneal.
Mouse . .	2·75	2·0	2·0–2·25
Rat . .	1·0	—	0·75
Rabbit . .	1·10	0·75–0·90	1·10
Guinea-pig .	1·50	—	1·50

The symptoms of intoxication were those commonly associated with the pentavalent compounds of arsenic—nervous and nutritional. The nervous symptoms consisted of tremors with incoordination of movement, clonic spasms, weakness or prostration. In mice a curious jerking of the head was not uncommon, while circular " waltzing " movements were also seen. The nutritional symptoms were loss of appetite, emaciation and occasional diarrhœa.

The pathological changes seen in the early stages of intoxication of small laboratory animals consisted in moderate vascular dilatation and congestion, with a few scattered petechial hæmorrhages, occasional effusions into the serous cavities and widespread cellular degenerations or even necrosis in some organs. In the kidney the glomerular tufts were swollen and the covering epithelium degenerated, while degeneration and necrosis were also seen in the cells lining the ascending loops of Henle and the convoluted tubules. Hæmorrhagic foci were found in the adrenals, heart muscle and central nervous system. The administration of the drug in therapeutic amounts was not followed by manifestations of organic or functional injury. The minimum therapeutic dose for mice infected with *T. brucei* was found to be 0·2 gm. per kilogram of body weight, given subcutaneously or intravenously, and 0·275 gm. given intraperitoneally within twenty-four hours after infection, thus giving a chemotherapeutic index of 1 : 8, while for rats infected with *T. evansi* the index was 1 : 3. Infections due to *T. equiperdum* and *T. evansi* in mice were more resistant to the action of tryparsamide. In rabbits infected with *T. brucei*, tryparsamide was found to have a curative range of from 0·2 to 0·35 gm. per kilogram of body weight when given intravenously, or from one-third to one-half the minimum lethal dose. Later observations (1921) showed that in rabbits, rats and mice, infections due to *T. gambiense* were more readily treated than those due to *T. rhodesiense*, for whereas cures could be obtained in this class of infection by the administration of a single large dose of the drug amounting to approximately two-thirds of the maximum tolerated dose, in infections due to *T. gambiense* the curative dose was from one-ninth to one-fifth of this dose.

As a result of these experiments, Miss Pearce was sent by the Rockefeller Institute to the Belgian Congo, in May, 1920, and conducted a long series of most careful observations on human trypanosomiasis at the Government Laboratory and Hôpital de la Reine in Léopoldville : her report, based on observations on seventy-seven cases, was published in December, 1921.

The cases due to infection with *T. gambiense* represented all grades of infection and included previously untreated patients, as well as several who had been already treated with one or more drugs. Preliminary investigations were made to determine how soon the gland juice became negative after a single dose of tryparsamide. In thirty-three patients gland punctures were invariably negative within twenty-four hours of a dose varying from 0·3 to 7 gm. (4·7 to 112 mgm. per kilogram of body weight) ; similarly in thirty-five cases the blood was negative within twenty-one hours after the administration of 1 to 5 gm. of the drug. The general conclusion was that from 1 to 5 gm. of tryparsamide brings about peripheral sterilization in from six to twelve hours, when the drug is given either intravenously or intramuscularly.

The duration of peripheral sterilization obtained by a single dose of the drug was then determined ; twenty-one patients, either in the first stage of the disease, or early in the second stage, as indicated by a slight excess of cells in the spinal fluid, were given 0·5 to 5 gm. of the drug ; of these, twelve relapsed within from seventeen to fifty-eight days, and nine did not relapse within an observed period of from 40 to 111 days. In fourteen of the cases with normal or slightly abnormal spinal fluids, lumbar puncture was made some five weeks after the administration of the drug, and in every instance but one, in which the result was uncertain because of blood contamination, the second cell count continued to be normal or was decreased towards the normal. The general physical improvement of the patients was prompt and satisfactory, the subjective symptoms disappeared, and within a few days the pulse rate and temperature became normal.

The effect of repeated doses of tryparsamide in early and in advanced cases was next investigated. Ten patients were injected intravenously at weekly intervals with from four to nine

injections, varying from 20 to 111 mgm. per kilogram of body weight; four were placed on a fortnightly schedule. A second group of thirteen early cases received from 1 to 5 gm. at weekly or fortnightly intervals. Trypanosomes were found in the lymph juice before treatment in all cases. In both groups the preliminary results were excellent, the blood and gland juice became permanently negative, there was general clinical improvement, and in those cases in which the spinal fluid was originally slightly changed before treatment it tended to become normal again.

Finally twenty-eight patients in the second stage of the disease were treated; of these ten had received previous treatment.

As in the early cases, there was immediate peripheral sterilization of the blood and improvement in the physical state. In three cases premature termination of the treatment was followed by clinical exacerbation and death. The nervous and mental symptoms present in many instances were greatly improved or completely eliminated, except in two very advanced cases. There was also a marked and rapid diminution in the number of cells in the spinal fluid. In general, three or four weekly doses of from 3 to 5 gm. caused decreases of from 57 to 98 per cent. in the cell content of the cerebrospinal fluid in an average of five weeks. The use of bi-weekly doses of 2 gm. had no advantage over weekly doses of 3 or 4 gm. The only untoward symptom was dimness of vision in nine advanced cases, five of whom had received previous arsenical treatment. There was no instance of any visual disturbance either in the early cases or among those in the first stages of the cerebrospinal period of the disease. In the majority the visual disturbance was purely transitory.

The later history of some of the cases treated by Miss Pearce is given by van den Branden and van Hoof (1923). Twenty of the early cases were followed, and at least three more relapsed after from seventeen days to thirteen months. All those who relapsed were again treated and were apparently cured, the observation period varying from six months to two years and seven months.

Of the thirty-five advanced cases observed six months to a year after treatment, three had died, sixteen had a normal spinal fluid and were apparently cured, in three the spinal lympho-

cytosis had increased, in two it had remained stationary, and in the remaining eleven it had diminished, but had not become entirely normal.

Chesterman (1922 and 1924) published a series of observations on the therapeutic action of tryparsamide, the work being carried out in the Belgian Congo between August, 1921, and September, 1922. Thirty-seven cases showing definite symptoms of nervous involvement were given a course of eight weekly injections of 3 gm. of tryparsamide. Of twenty-four cases which had received no previous treatment, fourteen were unaffected, three died, one was untraced, and nine were cured. Ten cases which had previously received treatment with arsenic or tartar emetic yielded seven relapses, with four deaths. Thus of the thirty-seven cases treated, fifteen remained well without relapse for an average period of two years after a single course of treatment with tryparsamide, though one subsequently died (1925) of sleeping sickness, thus reducing the percentage alive two and a half years after treatment to 37·8.

Letonturier, de Marqueissac and Jamot (1924) also tested the action of tryparsamide on fourteen cases of sleeping sickness in the Cameroons. Only two cases had relapsed a few months after cessation of treatment, while all cases in the second stage which received from 14·5 to 21·5 gm. were greatly benefited, the cell content of the cerebrospinal fluid being greatly reduced.

Van den Branden (1925 and 1926), who has had extensive experience in the Belgian Congo, believes that in order to obtain real and permanent results in chronic trypanosomiasis, it is necessary to give from 70 to 80 gm. of tryparsamide; failures, when they occur, are thought to be due either to the coexistence of neuro-syphilis or to a previous course of arsenical treatment which has rendered the trypanosomes arsenic-resistant.

Tryparsamide has now been extensively used in the treatment of sleeping sickness, and of those who have employed it the majority are agreed that it produces immediate peripheral sterilization of the blood in infections due to *T. gambiense*, not only in early cases, but also in those with pronounced nervous symptoms.

In infections due to *T. rhodesiense*, on the other hand, tryparsamide, according to Keevil (1926), fails to sterilize either the peripheral blood or the spinal fluid and produces but little immediate physical improvement. Similar results have been obtained by Maclean (1926) and by Corson (1928) in Tanganyika Territory, for in previously untreated cases of infection due to *T. rhodesiense* it failed to cause trypanosomes to disappear from the peripheral circulation, despite the administration of a considerable number of doses. Lauterburg (1929) also failed with *T. rhodesiense* infections, though even with the severest cases of *T. gambiense* infection a 60 per cent. cure rate could be obtained. In early infections due to *T. gambiense*, the peripheral sterilization of the blood may last so long as to suggest a permanent cure.

The curative effects of tryparsamide in the second stage of infections due to *T. gambiense* first described by Miss Pearce have been amply confirmed by later observers. Thus Kellersberger (1926) found that of 100 cases treated with tryparsamide, many of them in the second stage and *in extremis*, only one died, the improvement in even the most hopeless cases greatly impressing the natives.

Laigret (1926) obtained remarkable recoveries of cases with pronounced nervous symptoms in the late stages of the disease, and even in some instances in the terminal stages when death was only a matter of a few weeks. In the first stage of the disease the results were equal to those obtained with atoxyl, for in 100 first stage patients sterilization of the blood continued for at least a year, while of thirty cases well advanced in the second stage, twenty-nine showed a persistent sterilization both of the blood and cerebrospinal fluid, and in twenty the latter even returned to normal. It is noteworthy that since the introduction of tryparsamide the mortality in the hospital at Brazzaville in the Belgian Congo has fallen to about one-fourth of its former rate.

Van den Branden and seven of his colleagues (1927) have published the results of their extensive experience with tryparsamide or with an equally efficient Belgian product having the same formula and sold under the trade name of " Tryponarsyl." In patients in the first stage of infection due to *T. gambiense*,

a total of from 20 to 40 gm. sufficed to produce a cure, but in chronic cases in fair condition from 50 to 100 gm. were necessary in doses of 3 gm. Children were given doses of from 0·5 to 2 gm. No serious toxic symptoms occurred, and visual disturbances cleared up completely on cessation of treatment. Relapses or incomplete cures were almost always due to insufficient dosage. The conclusion drawn by the Belgian workers was that tryparsamide is by far the best drug in all stages of sleeping sickness and gives rapid, constant and lasting results, provided that the dosage is sufficient. Since the time required to produce a cure is comparatively short, the use of tryparsamide is, in the end, cheaper than that of atoxyl, while the results obtained have gained the entire confidence of the native population.

In advanced cases of *T. gambiense* infection, Jamot and Vernon (1927) also obtained 77·5 per cent. of results, which could be classified as excellent or at least favourable. It was noted, however, that the cytological changes did not always coincide with the clinical results, for the lymphocytosis in the cerebrospinal fluid might persist and the general condition be greatly improved, or *vice versâ*. The treatment in this series of 100 cases consisted of ten weekly injections, each of about 4 cgm. per kilogram of body weight, the maximum dose for an adult rarely exceeding 3 gm. The value of tryparsamide in the treatment of the second stage of sleeping sickness is possibly correlated as pointed out by Voegtlin, Smith, Dyer and Thompson (1923), with the ease with which it penetrates into the cerebrospinal fluid. Fordyce, Rosen and Myers (1924), however, failed to find any significant difference in the arsenic content of the nervous tissues following the intravenous administration of tryparsamide and neoarsphenamine.

Although tryparsamide is preferably given intravenously, it can also be inoculated intramuscularly (King, 1926). When given subcutaneously it usually produces abscess formation. Chesterman and Todd (1927) treated cases orally with tryparsamide, which can be tolerated up to 0·15 gm. per kilogram of body weight. Progress, though definite, was, however, very slow, and slight diarrhœa and vomiting were not uncommon. Van Hoof (1928) found that though one early case was appa-

rently cured, oral administration was ineffective when the cerebrospinal fluid was impaired,

The toxic symptom most commonly arising from the administration of tryparsamide is amaurosis, which may go on to complete blindness. Occasionally vomiting, slowing of the pulse and loss of consciousness occur immediately after an injection, reactions very similar to those seen with the arsenobenzenes. The onset of these toxic sequelæ may, in part, be correlated with the rate of excretion, for as Young and Muehlberger (1924) point out, in three out of four persons, 88 to 95 per cent. is excreted within twenty-four hours, but in the fourth there is a much slower rate of excretion.

Tryparsamide in the Treatment of Trypanosomiasis in Animals

Tryparsamide has not been extensively used in the treatment of trypanosomiasis in domestic animals. Smillie (1923), who tested the efficacy of the drug upon a number of horses affected with mal de caderas in Brazil, concluded that single doses of tryparsamide of from 5·0 to 8·0 gm. given intravenously to horses and mules produced a definite reduction in the number of parasites in the circulating blood. In addition, there was prompt cessation of fever. The administration of two doses of 8·0 gm. separated by an interval of three weeks was highly effective in maintaining peripheral sterilization, there being no toxic sequela or other evidence of constitutional injury. When the disease has progressed, tryparsamide offers only a problematical measure of success. More recently Hornby (1925) came to the conclusion that when given to cattle infected either naturally or artificially with *T. congolense* tryparsamide has no beneficial effect, but on the contrary appears to make the blood extraordinarily favourable for the multiplication of the parasites. Edwards (1926), in India, found that in cattle infected with *T. evansi*, the intravenous injection of the maximum therapeutic dose did not bring about a complete sterilization of the system, since the trypanosomes reappeared intermittently in the blood-stream, though their numbers were so limited that they no longer endangered the life

of the animal as they did before their activity was checked by the administration of tryparsamide. Tryparsamide is also of limited value in the treatment of equine surra, whether judged by the permanence of peripheral sterilization or by the extent to which it is capable of arresting the active progress of the disease towards cerebrospinal involvement. Its prophylactic action is negligible.

The Prophylactic Action of Tryparsamide in Sleeping Sickness

Up to the present, tryparsamide has not been extensively used in the prophylaxis of sleeping sickness. Experience of its use on a large scale is required in order to determine how far its systematic use in all infected persons in any one area will reduce the number of new infections in that area. Levaditi, Nicolau and Galloway (1926) have, however, found that, orally, tryparsamide exerts a most efficient prophylactic action against nagana infections in rabbits, the prophylactic dose being well within the maximum tolerated by the animals. The prophylaxis of sleeping sickness by the oral administration of drugs may, therefore, be within the bounds of possibility.

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SODIUM *P*-AMINOPHENYLARSINATE (ATOXYL, SOAMIN, SODIUM ARSANILATE)

Little need now be said in regard to the use of atoxyl in the treatment of sleeping sickness, though the drug is still used, more especially in French Equatorial Africa.

Although a considerable proportion of first stage cases may be cured by repeated injections, there is nevertheless considerable risk of causing optic atrophy unless very great care is taken with the dosage. In cases where there is involvement of the cerebrospinal fluid the drug is quite valueless. Ouzilleau and Lefrou (1923) found that the best results were obtained with doses of from 0·015 to 0·02 gm. per kilogram of body weight or 1 to 1·25 gm. for an adult, the doses being given at intervals of from ten to sixteen days, a series of six injections constituting a course. In the second stage, moderate doses diminished the meningeal reaction and produced some slight clinical amelioration, but the effects were of short duration, and at the end of four or five months the patients had completely relapsed. A series of injections was, therefore, necessary to prevent the recurrence of lymphocytosis in the cerebrospinal fluid. By this means, forty out of fifty-two

patients were kept alive for from nine to twelve months. Jamot (1926) finds that treatment of the population with atoxyl on a large scale fails to eradicate the disease, for during the last ten years some 50,000 natives in the Cameroons have been injected with atoxyl, at intervals varying from ten days to two months. Although in some areas the number of cases has decreased, the disease has by no means disappeared. This is due to the fact that patients at the second stage, on whom atoxyl has only a temporary effect, represent more than half the total number of cases, and when to these are added those at the first stage who are refractory to atoxyl, nearly two-thirds of the total number of cases are insensitive to the action of atoxyl. Boyé (1927) believes that a course of six injections at intervals of ten days represents the best method of using the drug. The introduction of tryparsamide and Bayer 205 has, however, largely removed the necessity for employing atoxyl.

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SODIUM-4-ACETYLAMINO-2-HYDROXYPHENYLARSINATE (FOURNEAU 270)

Fourneau 270 or the sodium salt of 4-acetylamino-2-hydroxyphenylarsinic acid ($C_6H_3NHCOCH_3)(OH)AsO_3H_2$) was found by Fourneau, Navarro-Martin, M. and Mme. Tréfouel (1923) to have a definitely curative action in experimental nagana infections in mice, its chemotherapeutic index being 1 : 20.

It is a white powder readily soluble in water and can be injected subcutaneously, the injections being painless and the absorption rapid. No abscess or local irritation occurs when solution is complete, but if some of the drug remains undissolved a mild inflammation may ensue.

The action of this compound in sleeping sickness was first investigated by Ledentu and Daude (1926). Preliminary observations indicated that in the first stage the dose of 0·001 gm. per kilogram of body weight led to a disappearance of trypanosomes from the glands in forty-eight hours, sterilization lasting eleven days ; with doses of 0·005 gm. per kilogram of body weight the trypanosomes disappeared in less than twenty-one hours and reappeared, not in the glands, but in the peripheral blood between the fourth and fifteenth days. Doses gradually increasing from 1·5 cgm. to 5 or 6 cgm. per kilogram of body weight were given to fifteen patients in the first stage, the injections being made at intervals of a week. Two patients thus treated died, probably as a result of the injections, two relapsed and eleven remained sterile during an observation period of from three to six months. In patients at the second stage, the results were comparable with those obtained by the use of tryparsamide, and even when advanced symptoms such as somnolence, tremor and incoordination were present, the results were remarkable. Of nineteen advanced cases, four died, one relapsed, one became blind, one had amblyopia, seven were ameliorated and five, as judged by the cerebrospinal fluid, were cured. The later history of some of these patients is given by Ledentu and Vaucel (1927). Of four early cases, three remained sterile for more than a year, a fourth relapsed or possibly had been reinfected, while of seventeen cases

in the second stage, eight relapsed and nine were cured. As the result of treatment of these and other cases, the conclusion was reached that the drug must be given in doses of from 0·02 gm. to 0·05 gm. per kilogram of body weight during the first stage. In the second stage vomiting and wasting render smaller doses necessary, 0·015 gm. rising gradually to 0·04 gm. per kilogram being within the margin of safety. The toxic sequelæ are similar to those associated with tryparsamide, and occur in about the same proportion of cases. Van den Branden (1927) also has used 270 in fourteen advanced cases of sleeping sickness. One patient died during treatment, but in all the others the lymphocytosis of the cerebrospinal fluid either decreased or returned to normal.

Levaditi, Nicolau and Galloway (1926) have tested the prophylactic action of 270 when given by mouth to rabbits subsequently infected with nagana. Both 270 and tryparsamide were found to have a prophylactic action, the latter drug being somewhat the more efficient. Stovarsol and sodium stovarsol—the sodium salt of acetyloxyaminophenylarsinic acid—were ineffective. Incidentally, stovarsol has been used without success in trypanosomiasis by van den Branden (1925), while Bacchelli (1927) has also unsuccessfully tested sodium stovarsol in experimental trypanosome infections.

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4-AMINO-2-HYDROXYPHENYLARSINIC ACID AND 4-FORMYLAMINO-2-HYDROXYPHENYLARSINIC ACID (FOURNEAU 269 AND 417)

In an attempt to discover arsenical compounds which might be administered by mouth, van den Branden (1928) tested two of the many arsinic acid derivatives prepared by Fourneau, Navarro-Martin, M. and Mme. Tréfouel (1923).

4-amino-2-hydroxyphenylarsinic acid (269) [$C_6H_3(OH)(NH_2)(AsO_3H_2) = 4 : 2 : 1$] is the parent substance of 270, which is the sodium salt of the acetyl derivative of the same acid. According to Fourneau and his colleagues, 0·014 gm. is well tolerated, but 0·015 gm. produces nervous symptoms in mice. The curative dose for nagana infections in mice is 0·002 gm., while even with 0·001 gm. the trypanosomes disappear from the peripheral blood-stream for about a week. The chemotherapeutic index is thus 1 : 7·5.

Five cases of trypanosomiasis were treated with 269. One advanced case died with symptoms of sleeping sickness after having received 4 gm. of the drug, another was only observed for two months after the same dosage. His blood remained sterile during the whole period. One early case was apparently cured by 13 gm., while in two others, at the beginning of the early stage, the cerebrospinal fluids returned to normal after 28 gm. and 42 gm. respectively, given in courses of 4 gm., 1 gm. being given daily, with intervals of three to six days between the courses.

4-formylamino-2-hydroxyphenylarsinic acid (417) [$C_6H_3(NHCOH)(OH)(AsO_3H_2)] = 4 : 2 : 1$] is the formyl derivative of No. 269, and is stated to have a chemotherapeutic index of 1 : 16 when given orally and 1 : 5 when given subcutaneously.

Four cases were treated by van den Branden with this preparation. In one chronic case the blood was rendered sterile, though the cerebrospinal fluid did not return to normal; in another case at the beginning of the second stage, the blood became sterile and the cerebrospinal fluid returned to normal after a dose of 12 gm. Of two patients in the early stages, one relapsed again

and again even after 20 gm., the blood of the other patient was sterilized after a total dose of 12 gm.

So far as these observations go, 269 appears to be superior to 417. Further investigations on the curative effects of 269 will be awaited with interest.

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HYDROXY-ACETYLAMINOPHENYLARSINATE OF DIETHYLAMINE (ACETYLARSAN)

Acetylarsan, which is prepared by the Usines du Rhône, France, is said to be the diethylamine salt of 3-acetylamino-4-hydroxy-phenylarsinic acid. It can be injected both intramuscularly and subcutaneously, and has been used by van den Branden (1927) in the treatment of chronic trypanosomiasis. The treatment consisted of the administration of 12 gm. of acetylarsan in sixteen injections of 0·75 gm. each, given at intervals of a week. Although the general condition improved, and the lymphocytosis of the cerebrospinal fluid decreased, the results were inferior to those obtained with tryparsamide. Ledentu and Vaucél (1927) have also employed acetylarsan in treating twenty-two patients in the first stage and seven in the second. They conclude that in the first stage the drug is inferior to atoxyl, while in the second stage it is inferior both to tryparsamide and Fourneau 270. Vomiting and diarrhœa frequently followed the injections, and in only two cases in the first stage did the blood remain sterile for as long as seventy days.

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BR. 68

This organic arsenical differs from those previously described in that the arsenic is not linked to a benzene nucleus. Binz and Räth (1927) have recently succeeded in introducing arsenic into heterocyclic ring systems with cyclically bound nitrogen (pyridin-quinoline), and have produced a number of new compounds of this type.

Giemsa and Mayeda (1927) have studied the effect of one of these compounds, BR. 68, the formula of which is not disclosed, on mice infected with *T. brucei*. BR. 68 is a yellowish-brown powder, readily soluble in water and forming a neutral solution which can be immediately injected.

The toxicity for mice per gram of body weight on subcutaneous injection, compared with atoxyl and tryparsamide, is as follows :—

	Maximum tolerated dose.	Minimum lethal dose.
BR. 68 . . .	0·2 mgm.	0·25 mgm.
Tryparsamide . .	0·62 ,,	0·70 ,,
Atoxyl . . .	0·2 ,,	0·25 ,,

When the therapeutic action of the three substances was tested on mice infected with the nagana strain " Prowazek " and an arsenic resistant strain " 30," it was found that BR. 68 was distinctly more efficacious than the other two drugs, for the chemotherapeutic index was :—

	" Prowazek " strain.	Strain 30.
BR. 68 . . .	1 : 25	1 : 5–6·6
Tryparsamide . .	1 : 2	0 : 1
Atoxyl . . .	1 : 1	0 : 1

Corson (1928) has used the drug intravenously in two relapsed cases, in doses of from 0·15 to 0·8 gm. The clinical effects and action

on the blood were good, but in the doses used it did not appear to influence the infection of the nervous system. Febrile reactions were common, and on one occasion immediate vomiting occurred. Coghlan (1929) has found the drug useless in infections due to *T. rhodesiense*.

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BR. 34

The sodium salt of this compound produced by Binz has been employed by Kleine (1928), who found that with as small a dose as 0·2 gm. intravenously the blood remained free from parasites for from eight to nine days. In view of this result the further testing of the drug seems indicated.

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BR. 34 A

An arsenical pyridine preparation, insoluble in water, has also been prepared by Binz and Räth, and has been used prophylactically and curatively by Collier and Krause (1929) in infections due to *T. brucei* in mice. The subcutaneous injection of 1 c.cm. of a 1 in 2,000 concentration in olive oil 30 minutes after the inoculation of trypanosomes prevented infection. The chemotherapeutic ratio was 1.20.

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HOECHST 2754

This compound, which is said to be closely related chemically to tryparsamide, contains 27·2 per cent. of arsenic. It can be given intravenously in doses of 2 gm., according to van den Branden, Clévers and Moreels (1927), who found that it had a definite action in cases at the beginning of the second stage, but it is apt to produce symptoms of nephritis. In two patients the lymphocytosis of the cerebrospinal fluid returned to normal after one series of ten injections of 2 gm. given every five or six days, while in a third the lymphocytosis decreased markedly. Mules infected with *T. congolense* were not cured by the drug. Kleine (1928) gave the drug by mouth with results which were discouraging, though its tonic effect was definite.

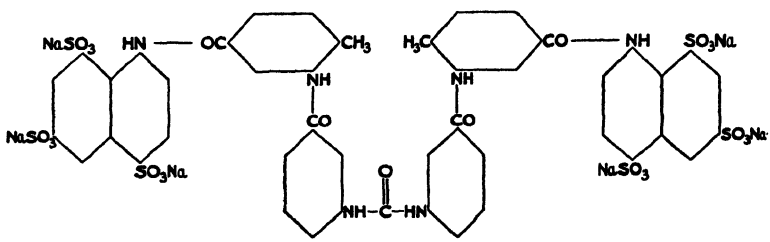
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VAN DEN BRANDEN, F., Mlle. CLÉVERS and M. MOREELS, 1927, Essai de traitement de la trypanosomiase humaine et des infections animales à *T. congolense* par le " 2754 " Hoechst. *Bull. Soc. Path. exot.*, XX., 734.

BAYER 205 (GERMANIN) AND FOURNEAU 309 (MORANYL)

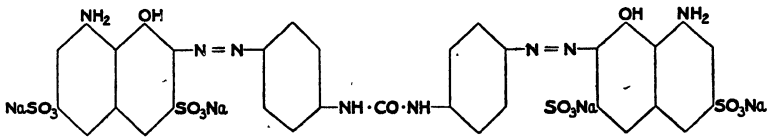
Towards the end of 1920 it became known that a new synthetic remedy for trypanosomiasis had been produced in Germany. The composition of this drug—Bayer 205 or Germanin—was not at first disclosed, though as the result of the researches of Fourneau and his colleagues (1923–1924) a compound has been synthesised which, it is now agreed, is identical in composition with the German product. The constitution of Bayer 205 is given by Schlossberger (1928) as :



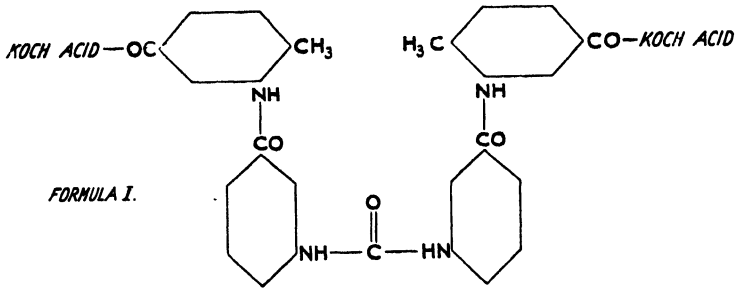
BAYER 205

a structural formula identical with that arrived at by Fourneau (1924).

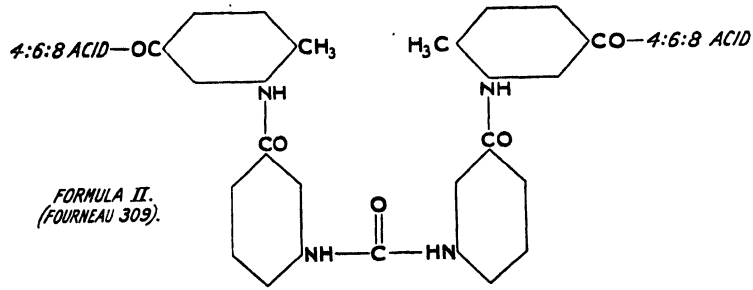
The introduction of non-metallic compounds of this type into chemotherapy was due to the discovery in 1906 that the dyestuff afridol violet is possessed of some trypanocidal action. Although not in itself an efficient trypanocide, it served to direct attention to the chemotherapeutic possibilities of this type of molecule.



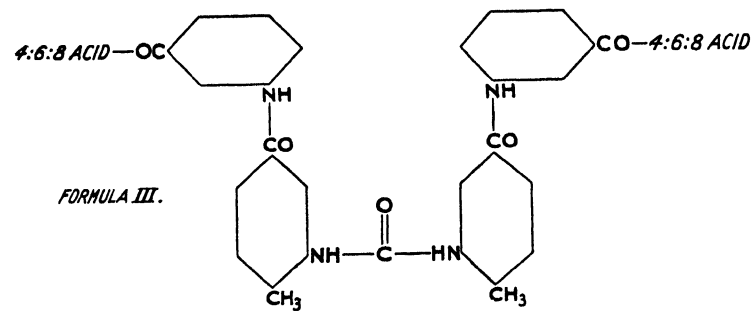
From the year 1912 onwards there appeared in Germany a number of patents dealing with ureas of the aminonaphthalenesulphonic acid type. Acting on this suggestion, Fourneau prepared a long series of urea compounds with the three acids—1-amino-8-naphthol-3 : 6-disulphonic acid (H acid), 1-aminonaphthalene-4 : 6 : 8-trisulphonic acid (4 : 6 : 8 acid) and 1-aminonaphthalene-3 : 6 : 8-trisulphonic acid (Koch acid). After producing compounds of H and Koch acids, it was found that compounds containing the *meta*-aminobenzoyl nuclei were more effective than those with the *para*-aminobenzoyl residue. Further research showed that partial replacement of the aminobenzoyl residues by aminotolyl gave a compound the trypanocidal activity of which was distinctly encouraging, since it had a therapeutic index of 1 : 12 (Formula I). At this stage it was found that naphthylamine-4 : 6 : 8-trisulphonic acid was more conducive to the development of trypanocidal action than the Koch acid previously used; a new series of compounds entirely analogous to those obtained with Koch acid was therefore prepared, with the result that the 4 : 6 : 8 acid compound (Formula II) analogous to Formula I was found to have a high chemotherapeutic index, which, together with the fact that 4 : 6 : 8 acid, *m*-amino-benzoic acid and *m*-amino-*p*-methylbenzoic acid were obtained by the acid hydrolysis of Bayer 205, goes far to show that it is identical with the German compound (Kolle and Bauer (1926)).



FORMULA I.



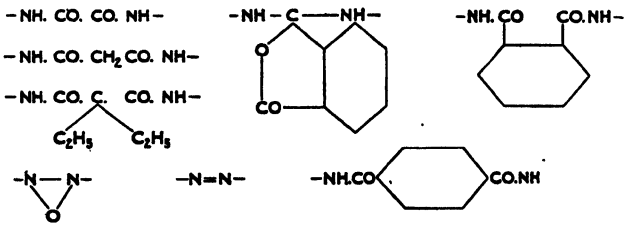
FORMULA II.
(FOURNEAU 309).



FORMULA III.

It is of interest to note that as far as is at present known the slightest deviation from the molecular arrangement of Formula II is attended with a diminution in the trypanocidal activity of the compound. Thus the compound represented by Formula III differs only from Fourneau 309 in that the *m*-aminobenzoyl and the *m*-amino-*p*-tolyl residues have exchanged positions, but it is entirely without action on trypanosomes.

Similarly the central substituted urea residue seems an essential part of the molecule for the development of trypanocidal activity. Conversion to the thiourea lowers the activity considerably, and condensation of the unphosgenated amine with oxalyl, malonyl, diethylmalonyl, phthalyl, *iso*phthalyl and terephthalyl chlorides, or the production of an azo or azoxy analogue, as shown, leads in every case to the formation of a trypanocidally inactive compound.



The methyl group of the *m*-amino-*p*-tolyl residue would also appear to play some important part, since its replacement by higher homologous alkyl groups, by methoxyl or halogens has been found to lead invariably to the production of inactive or slightly active compounds. Finally the function of the sulphonic acid groups cannot be entirely physical in relation to the solubility in aqueous media of otherwise insoluble substances, but must be bound up with physiological action since, as Fourneau and others have demonstrated, replacement of naphthylamine-4 : 6 : 8-trisulphonic acid by other naphthylamine sulphonic acids yields compounds which have only slight or no trypanocidal properties.

Bayer 205 is a fine white flocculent powder, very easily soluble in water, the solution having a faint pink colour. The solution can be sterilized. It has a slightly bitter taste, and when given by mouth it tends to repeat itself for some hours. The drug was originally sent for trial to Haendel and Joetten (1920) and to Mayer and Zeiss (1920), who found that in small doses it cured mice, rats, guinea-pigs and rabbits infected with *Trypanosoma brucei*, *T. equiperdum*, *T. equinum*, *T. gambiense* and *T. rhodesiense*. A dose of 0·0006 gm. or about 0·0024 gm. per kilogram of body weight was found to cure mice, which became immune to reinoculation for a period of about three months. Administered

prophylactically, Bayer 205 was found to prevent infection. The drug was then tested by Pfeiler (1920) in 200 cases of dourine infection in horses. The results, which were most satisfactory, were confirmed by Miessner and Berge (1921) and Ellinger (1920). Wenyon (1921) also found that mice infected with *T. equiperdum* could be cured by 0·005 gm. per kilogram of body weight. The ratio between the curative and the maximum tolerated dose was as 1 : 60, but while antimony and arsenic caused a very rapid disappearance of trypanosomes from the blood-stream, with Bayer 205 the organisms did not disappear for some forty-eight hours. Mayer (1921) found that Bayer 205 could be given by mouth, for 50 gm. given orally to a goat during a period of eleven days completely cured infection due to *T. rhodesiense*. The intrathecal injection of the compound also appeared possible, for 0·7 gm. given to a dog by this route caused no untoward symptoms. Brumpt and Lavier (1922) also tested the effects of Bayer 205 on protozoal infections in small animals. *T. cruzi* was unaffected, but *T. venezuelense* (=*T. evansi*) rapidly disappeared from the peripheral blood-stream. Infections due to *Babesia mutans* or *B. bigemina* were unaffected.

The first human cases of trypanosomiasis to be treated with Bayer 205 were recorded by Mühlens and Menk (1921). One of these, a German from Fernando Po, with infection due to *T. gambiense*, received intravenously two doses each consisting of 0·2 gm. of the drug at an interval of seven days. The trypanosomes, so far from disappearing, increased in numbers in the peripheral blood, a result due, as subsequent experience has shown, to the fact that the doses given were too small.

The second case, infected with *T. rhodesiense*, was a patient under Yorke (1921) and had proved refractory to tartar emetic, atoxyl, and other forms of treatment. After 2·5 gm. of Bayer 205 had been given intravenously, the injections were stopped owing to the development of albuminuria and slight hæmaturia. The patient, whose blood had become negative within a few hours of the first injection, rapidly improved and was alive four years later.

Mayer and Menk (1922) recorded a third case, a Belgian from the Congo, who had suffered for two years with *T. gambiense*

infection. Three injections of 1 gm. each given on three successive days caused the trypanosomes to disappear from the peripheral circulation.

In view of the successful treatment of these cases, Kleine and Fischer were sent out to Northern Rhodesia and the Congo to test the therapeutic value of the drug on a large scale. Preliminary experiments recorded in 1922 showed that 0·25 gm. of Bayer 205, given orally on two consecutive days to monkeys, caused *Trypanosoma brucei* and *T. rhodesiense* to disappear from the blood, but a relapse occurred within three weeks unless the drug was given on four consecutive days. Infections due to *T. bovis* (=*T. vivax*) and *T. capræ* were unaffected. In Rhodesia thirty-five cases of *T. rhodesiense* infection were treated, about half of which were in the second stage. Routine treatment consisted of 1 gm. injected subcutaneously on the first, tenth and eighteenth days respectively; those with trypanosomes in the cerebrospinal fluid and those which relapsed with parasites in the blood received two further injections. The immediate results were excellent, but in 1925 Kinghorn found that of the original thirty-five cases, thirty had died from sleeping sickness, though the five which survived were apparently free from trypanosomiasis.

In the Congo, Kleine and Fischer treated about 150 patients, most of whom were in the first or second stage of the disease, with about 10 per cent. in the third stage. Routine treatment consisted of 1 gm. of the drug intravenously on the first, third, and fifteenth days. In all cases in the first and second stages improvement was obvious, and of ninety-six patients whose blood was examined at least every week for five months after the third injection, two only—both children—relapsed. In seven cases in the third stage five injections of Bayer 205 produced no improvement. Later accounts of these cases treated at Kiambi in the Tanganyika, Moero district, were given by Fontana (1924) and Walravens (1925). Of ninety-five cases, twenty-seven (28·4 per cent.) were dead, and of the forty-four which were re-examined, twenty-five had a normal spinal fluid and negative blood, five appeared to be sterile, twelve were in the second stage and one had a doubtful condition of the cerebrospinal fluid.

Strada and Lopes (1927) also reported the ultimate results of the treatment of forty-three advanced cases treated with Bayer 205 in 1923 to 1925. Only two remained well; eleven had died, eleven were worse, and the rest still showed signs of the disease.

These results do not fulfil the first hopes which were entertained of the curative value of the drug. Nevertheless, in early cases, many of the results have been extremely successful. Low and Manson-Bahr (1922 and 1923), for instance, record a series of nine cases in Europeans, one case being infected with *T. rhodesiense* and eight with *T. gambiense*. The injections each consisted of 1 gm. in a 10 per cent. solution intravenously, although occasionally 2 gm. in a 20 per cent. solution were given; they were given at intervals of a week, ten injections as a rule being sufficient. One case of infection due to *T. gambiense* and the case infected with *T. rhodesiense* died, but in the others the symptoms rapidly improved, the return of the finer co-ordinated movements and the psychical characteristics being especially noteworthy. The continued progress of these seven cases is recorded by Low (1923), who also reports the successful treatment of four other cases of sleeping sickness.

The treatment of these cases, however, emphasizes the fact that the administration of Bayer 205 is not without danger. In certain cases an erythematous rash appeared, commencing on the forearms and spreading to the rest of the body; the distribution of the eruption, which had central raised papules, was rather patchy (it finally disappeared with desquamation); pruritus was constantly present. Purulent conjunctivitis and stomatitis have also been recorded. A more serious effect of the drug, however, is the presence in the urine after the second or third injection of albumin and casts and in some cases blood; anuria has also been reported. As a rule, the symptoms of urinary irritation subsided after the termination of the treatment, but in a few cases they persisted. Duncan and Manson-Bahr (1923), who studied the effects of Bayer 205 on the tissues of healthy mice, found that in the kidneys there was very extensive degeneration and exfoliation of the epithelium of the convoluted and other secreting tubules of the cortex, though no fatty change was noted. The straight

tubules and excretory ducts appeared unaffected. Some of the tubules contained hyaline casts, while here and there were necrotic foci. The blood vessels were much engorged, minute hæmorrhages being common in the cortex. The blood vessels of the cortex also showed a very marked degree of perivascular round-celled infiltration. In the liver and lungs there was much vascular engorgement with, in the former, fatty change and focal necrosis. Minute hæmorrhages were present in the lungs, cerebrum and cerebellum.

These pathological findings indicate that Bayer 205 must be administered with considerable caution, more especially as fatal cases of acute nephritis have followed its use in man. Thus Stones (1924) records a fatal case where acute nephritis and anuria followed the administration of only 3·5 gm. of the drug in four doses spread over a period of six weeks. Chesterman (1924) also had two deaths from acute nephritis after only five and ten weekly doses each of 1 gm. On the other hand, the majority of cases are able to tolerate ten or twelve weekly injections of 1 gm. each, with only slight albuminuria.

Bayer 205 has now been extensively used in various parts of Africa in the treatment of sleeping sickness, and a sufficient time has elapsed to allow a correct evaluation of the results obtained. While early cases, more especially those due to *T. gambiense* and a proportion of those due to *T. rhodesiense*, respond to the action of the drug with a peripheral sterilization of the blood so prolonged as to suggest permanent cure, cases of chronic trypanosomiasis with altered cerebrospinal fluid show as a rule a slight, but only transient, improvement. In a few cases the cerebrospinal fluid improves several months after the cessation of treatment or the condition remains stationary. In the greater number the disease continues to progress slowly to a fatal issue.

Thus van den Branden and van Hoof (1923) found that of thirteen patients in the second stage treated by intravenous injections of from 3·4 to 17·5 gm. six died, six remained negative, but in bad condition, and one relapsed with trypanosomes in the blood. Combined intravenous and intrathecal administration was both useless and dangerous, since the intrathecal injection of a dose of

0·05 gm. was liable to produce symptoms, while 0·3 gm. was often fatal. Saunders (1928) found that even 20 c.cm. of a 0·1 per cent. solution of Bayer introduced intrathecally, produced alarming symptoms. In the early stages the conclusion was reached by van den Branden and van Hoof that the curative and clinical results were no better than those obtained with atoxyl, for while of nineteen first stage cases treated with Bayer 205 twelve remained sterile for from five to sixteen months, one died and five others were known to have relapsed. In a later communication van den Branden (1926) records the later history of seven patients who had been treated three years previously with 3·5 gm. of Bayer 205. All these patients could be regarded as definitely cured. Two of the patients had relapsed with trypanosomes in the blood some weeks after the end of treatment, and after showing parasites irregularly for some months had finally lost their trypanosomes without further treatment. Letonturier, de Marqueissac and Jamot (1924) tested Bayer 205 on thirty-five cases from the Cameroons. The total dose for an adult was 3 gm. in two or three injections at three to seven days' intervals. Of the thirteen patients in whom the cerebrospinal fluid was normal nine were apparently cured, while four relapsed. Of the twenty-two patients in whom the cerebrospinal fluid was more or less altered, eleven died, two were in bad condition and nine were well, though there was no appreciable modification in the meningeal reactions. The results obtained by Chesterman (1924) in seventeen cases in the second stage were also disappointing, for after from two to six doses of 1 gm. at weekly intervals two died of intercurrent bilharzial dysentery, two from acute nephritis, two who during previous treatment with atoxyl had suffered from amblyopia became completely blind, eight returned in a worse condition than before, one remained stationary, and only two cases were definitely improved. Examination of the cerebrospinal fluid in eleven cases showed that excess of albumin and a considerable lymphocytosis remained four months or more after treatment. Hanington (1924) recorded the treatment of cases at Sherifuri, Northern Nigeria, doses of 1 gm. being given on the first, third, fifth, twelfth, and nineteenth days. Here again the results in

early cases were satisfactory, as were those obtained by Maclean (1926), who treated twenty-seven cases due to infection with *T. rhodesiense*. Fourteen survived for a year or more, eight of them, of which seven were very early cases, being in good health. Dye (1926) also treated early cases of infection due to *T. gambiense* with Bayer 205, doses of 1 gm. being given on the first, third and fifth days, followed by 1 gm. at intervals of five to seven days, until a total of 6 to 7 gm. had been given; the results were excellent, since sterilization of the peripheral blood was rapidly obtained when the drug was given in doses of 1 to 1·2 gm. over a period of four to five weeks. Kellersberger (1926), in the Belgian Congo, likewise found that Bayer 205 cures almost 100 per cent. of cases in which the cerebrospinal fluid is normal and the infection is restricted to the glands and blood-stream, the earlier the infection the more efficacious being the drug. In cases in which the central nervous system is definitely attacked, however, there is only temporary benefit, followed later by relapse, while in contrast to the arsenicals the drug has no influence on the cell content of the cerebrospinal fluid.

The question of the intervals between the doses is apparently one of considerable importance. Fischer (1927) records a case of resistance to Bayer 205 where relapses continued to occur, possibly because the weekly intervals between the injections allowed the trypanosomes to become resistant.

Mayer (1928) also believes that it is important to give as large a dose as possible in the shortest possible time; thus either 1·5 gm. can be given on the first, second and fourth days or 2 gm. doses on alternate days. If a total of 4 or 5 gm., given as quickly as possible, is without action, it is useless to repeat the course of treatment during the following week or month.

The results with moranyl or Fourneau 309 are very similar to those obtained with Bayer 205. Laigret and Blanchard (1925) first tested the drug on guinea-pigs infected with *T. pecaudi* (= *T. brucei*) and *T. gambiense*, and found that it exerted a trypanocidal action exactly comparable with that of Bayer 205. A dose of 0·05 gm. per kilogram of body weight sufficed to cure guinea-pigs if given within a month of infection. In the treat-

ment of human trypanosomiasis peripheral sterilization was easily obtained but was of short duration, and in the majority of cases caused albuminuria. Keevil (1926) used this drug in the treatment of nine early cases of human trypanosomiasis. A total of 3 to 5 gm. was given in from ten to fifteen days, with results which were equal to those obtained with Bayer 205. The albuminuria cleared up satisfactorily after the cessation of treatment. In a later communication Keevil (1928) states that three cases examined two years later were in excellent health.

It may, therefore, be concluded that moranyl is as efficient as Bayer 205 in the treatment of early cases of sleeping sickness, its action on infections due to *T. gambiense* being less, however, than on *T. rhodesiense* infections. Cases in the later stages are largely uninfluenced.

Bayer 205 and Fourneau 309 in the Treatment of Trypanosomiasis in Animals

Bayer 205 or Naganol, as it has been termed when employed in veterinary medicine, has now been extensively used in the treatment of trypanosome infections of domestic animals. Pfeiler (1920) and Miessner and Berge (1921) found the drug efficacious in artificially induced dourine of horses, but uncertain when employed in natural infections, since parasites tended to reappear after having been absent from the peripheral blood for as long as two months. In a case of surra Rodenwaldt and Douwes (1922) used Bayer 205 unsuccessfully, but Herzog and Lavier (1923) cured a case of debab in a camel, while both Balozet, Lavier and Velu (1923), and Pataki (1923) obtained cures in dourine. According to Migone and Osuna (1922), Bayer 205 is of value in the treatment of mal de Caderas, and in surra, Baermann (1922), Bubberman, Douwes and van Bergen (1925), Bakker (1925) and Berg (1925) all report successes. Van Saceghem (1924) obtained sterilization of the peripheral blood in cattle infected with *Trypanosoma vivax* and *T. congolense*, but relapses invariably took place after about a week. Using Fourneau 309 van Saceghem (1925) obtained even more rapid sterilization of the blood than

with Bayer 205, but here again relapses invariably occurred. Edwards (1926) finds that Bayer 205 far surpasses all other medicaments in the treatment of surra in horses. A suitable therapeutic dose for intravenous administration was found to be 5 gm. in 10 per cent. aqueous solution, per 1,000 lbs. of body weight. Bayer 205 is only very slowly excreted in horses and remains in the circulation in sufficient concentration to exert a definite trypanocidal action for about two months, a point of considerable importance since relapses are thus guarded against. The lack of diffusibility of the drug operates adversely, however, in one important respect, in that, possibly owing to the large size of the molecule, it is unable to penetrate into the subarachnoid space in a concentration great enough to exert trypanocidal action; hence, although prolonged sterilization of the circulatory system may be effected by the administration of a single intravenous dose, in well-established cases the trypanosomes may multiply in the safe retreat afforded by the cerebrospinal canal, and the animal may ultimately succumb from surra affecting only the central nervous system. This possibility may be counteracted by the introduction of Bayer 205 intrathecally at the same time as intravenously in a dosage arithmetically equivalent to that introduced intravenously, having regard to the relative weights of the body and the central nervous system. The intrathecal dose thus applied is 20 c.cm. of a 0·1 per cent. solution per 1,000 lbs. body weight, injected through the occipito-atlantal space. The therapeutic dose of Bayer 205 can be safely introduced into the circulating blood when large numbers of trypanosomes are present in it, for since complete destruction of the parasites takes a considerable period of time, the alarming symptoms set up by some other drugs as the result of rapid destruction of the parasites are not seen. Symptoms of drug intoxication, manifested notably in the form of locomotor disturbances, closely similating those of laminitis, and urticaria, may appear following intravenous injection, but these symptoms disappear after a few days. Occasionally when the combined intravenous-intrathecal treatment is repeated after a month's interval, the symptoms of drug intoxication are very pronounced.

There appears to be no advantage in combining " Bayer 205 " with other trypanocidal agents.

The occurrence of toxic symptoms in horses treated with Bayer 205 has been recorded by a number of other workers besides Edwards. In a horse affected with mal de Caderas and treated with Bayer 205, Migone and Osuna (1922) noted swelling of the hind-quarters, pharynx and testicles, and on the fourth day the appearance of eczema round the anus, on the nasal mucous membrane and parts of the genitalia. These symptoms disappeared, however, in the course of eight to ten days. Pataki (1923) found that swellings and even necrosis of the skin occurred in horses affected with dourine, and in several cases there was evidence of inflammation of the coronets. Baermann (1922) in the course of his experiments on the treatment of equine surra with Bayer 205 found that too large doses caused podo-dermatitis and albuminuria accompanied by wasting and anæmia. Rodenwaldt and Douwes (1922) noted ulcerous erosions on the mucous membrane of the mouth, lips, tongue and rectum, together with dermatitis on the limbs and a hypersensitiveness of the skin. The emaciation and general symptoms of intoxication not infrequently progressed to a fatal issue.

The doses tolerated by various animals vary considerably with the species. While cattle are less tolerant than man, they are far more tolerant than horses. It is thus extremely difficult, if not impossible, to make deductions in regard to dosage in large animals from the results obtained from experiments on small laboratory animals. As Ruppert (1923) points out, the lethal dose for rabbits is about 0·5 to 0·6 gm. per kilogram of body weight, but for horses 0·02 gm. per kilogram of body weight, so that the drug is twenty-five times as toxic for horses as it is for rabbits.

It is not surprising, therefore, to find that the therapeutic dosage of Bayer 205 employed in the treatment of various forms of trypanosomiasis in horses has been subject to considerable variation. Schmidt and de Oliveira (1924) gave doses of 2 to 3 gm. to horses and mules infected with mal de Caderas, a total of 7 to 9 gm. being found sufficient to effect a cure. Pataki (1923), on

the other hand, obtained what would appear to be encouraging results in dourine by means of Bayer 205 administered in doses of only 0·01 gm. per kilogram of body weight. In the treatment of infection due to *Trypanosoma venezuelense*, Tejera (1924) found that while a single injection of 4 gm. was inadequate a second dose of 3 gm. administered after an interval of ten days was effective. Numerous attempts have also been made to cure bovine trypanosomiasis. Van den Branden (1926) treated a number of zebus and native Dahomey cattle with three doses of Bayer 205, ranging from 1·5 to 2·0 gm. with two or three days' interval between the injections. While infections due to *T. congolense* were cured, those due to *T. dimorphon* (=*T. congolense ?*) were entirely unaffected. Ledentu and Daude (1926) found that though the peripheral blood of cattle could be sterilized by an injection of 10 gm., yet relapses invariably occurred within six days.

In camel trypanosomiasis in the Sudan, Bayer 205 acts almost as a specific, according to Knowles (1927), who finds that 10 gm. in a 10 per cent. solution given intravenously is almost invariably successful in producing a cure. Fifty-six out of sixty animals so treated became negative to the formol gel test. Bayer 205 (naganol) in association with tartar emetic also gives good results, and is considerably cheaper as a method of routine treatment.

The Prophylactic Action of Bayer 205 and Fourneau 309 in Trypanosomiasis of Man and Animals

Up to the present time no satisfactory and cheap method of prophylaxis of sleeping sickness has been discovered. All such measures as the clearing of bush, the evacuation of heavily-infected areas and the controlled movements of natives merely serve to decrease the number of infected cases at a high administrative cost. Owing to the fact that small animals cured of trypanosomiasis by Bayer 205 remain immune to fresh infection for a considerable period, as a result of the continued presence of a trypanocidal substance in the peripheral blood-stream, hopes were held out that this drug might be effective not only as a curative

but as a prophylactic agent in human trypanosomiasis. It must, however, be remembered that if in infected districts only suspected natives receive treatment, the source of infection would not necessarily die out, since there is a possibility that man may not be the only reservoir of the virus which has to be taken into account.

The prophylactic use of Bayer 205 and Fourneau 309 has now been investigated in many areas in Africa. One of the earliest experiments of this nature was carried out by van den Branden (1926) who, prophylactically, gave three injections of Bayer 205 to the 256 inhabitants of a native chiefdom. The results strongly suggested that if they are to of value prophylactic inoculations of Bayer 205 must be repeated at least every nineteen months.

In a later communication (1927) van den Branden records the results obtained with prophylactic injections made in 1925. The infected cases were treated in the ordinary manner, the healthy adults being given two injections of the drug, each of 1 gm., the young people two injections of 0·5 gm., the children two of 0·25 gm. and the babies two of 0·1 gm., each at intervals of two to three weeks. Examinations made six, twelve and eighteen months after the prophylactic injections revealed the presence of only three fresh cases, whereas previously seventeen fresh cases had been found. Fourche (1927) was enabled to examine in the same manner, and after the same interval two areas which were equally infected at the primary examination but which had been subjected to two different methods of treatment; in one of them not only the definitely infected were treated but also the suspects, while in the other only the certainly infected were given treatment. In the first district in which suspects also were treated, nineteen new cases were discovered among 1,889 persons examined, while in the second district the number examined was 6,532 and 345 new cases were found. It would, therefore, seem that the risk of infection is, at any rate, decreased by widespread treatment of all active and suspected cases.

Bossert (1927) also carried out a carefully controlled test in French Equatorial Africa with Fourneau 309. In the selected area all the infected cases were set aside and treated with atoxyl; those free from infection were then divided into two equal groups

as regards numbers, age and sex. The first group served as a control, the second was injected with Fourneau 309. In all, 500 persons were injected with doses varying from 0·02 gm. to 0·04 gm. per kilogram. Seven months later, among 375 of the injected there were no fresh cases, but among 313 of the 503 controls eight new cases had developed. Fourche and Ricklin (1928) also studied the percentage of fresh infections in a healthy Bayerised and non-Bayerised population, the sick and suspect having been treated with atoxyl or tryparsamide. The index of contamination among the Bayerised and non-Bayerised healthy population was as follows :—

Healthy.	Delay in re-examination in months.	Index of contamination percentage.
Bayerised (nearly all 2 injections) .	7	0·00
Bayerised—2 injections . . .	10	0·22
Bayerised—1 injection . . .	10	0·54
Non-Bayerised	10	1·00

The general conclusion may, therefore, be drawn that although treatment of the sick and suspect cases with arsenicals is in part responsible for the low infection rate found at the second examination, nevertheless, prophylactic treatment with Bayer 205 has a very definite influence.

These observations, therefore, justify the International Sleeping Sickness Commission (1928) in stating that moranyl (Bayer 205 or Fourneau 309) affords protection for a long period provided that the drug is employed for the protection of healthy individuals at the same time as the sterilization of the blood of patients is being carried out by the judicious use of atoxyl or other arsenical remedies.

In domestic animals Bayer 205 has been employed prophylactically in a variety of trypanosome infections. For prophylaxis against dourine in horses Pfeiler (1922) recommends 6 gm. during the covering season in two doses of 8 gm., administered at an interval of eight days. Infection with *T. venezuelense* is inhibited,

according to Tejera (1924), by a dose of 2 gm. repeated every six months. The results obtained by Kleine (1923) and Berg (1925) in cattle were disappointing, for even 10 gm. for a bullock of 5 cwt. in repeated doses failed to prevent infection by trypanosomes. Against surra, Baermann (1922) found that protection was obtained for about thirty to forty days by injecting 2 to 6 gm. of Bayer 205. Edwards (1926) also obtained complete protection against surra for a period extending to twenty-two days by 7·5 gm. per 1,000 lbs. of body weight, given intravenously.

Launoy, Nicolle and Prieur (1929[1] & [2]) have recently shown that the length of time for which a mouse is protected by Fourneau 309 varies directly with the amount of the drug injected, as shown in the table.

Table showing the Relationship between the Dose of Fourneau 309 and the Length of Time for which the Mouse is Protected against Trypanosome Infection

Dose in gm. (intravenous) for a mouse of 20 gm. body weight.	*T. brucei.*		*T. evansi.*		*T. equiperdum.*	
	Minimal prevention in days.	Maximal prevention in days.	Minimal prevention in days.	Maximal prevention in days.	Minimal prevention in days.	Maximal prevention in days.
0·00005 .	0	3	2	3	1	4
0·00010 .	3	5	5	3	8	10
0·00020 .	3	10	15	26	15	25
0·00030 .	10	16	15	31	20	25
0·00040 .	10	25	33	40	33	40
0·00050 .	25	40	35	40	33	40
0·00060 .	31	41	35	41	40	40

The Pharmacology of Bayer 205 and Fourneau 309

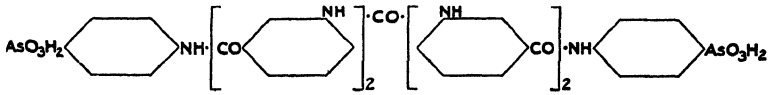
Comparatively little is known of the pharmacology of Bayer 205. When given by mouth it is slowly absorbed from the alimentary tract, for infections may be cured not only by the intravenous administration of Bayer 205, but, as Levaditi and Klarenbeck (1927) have shown, by oral ingestion, as in rabbits 0·1 gm. per kilogram

of body weight confers protection against infection with nagana for as long as eighty-seven days. The presence of a trypanocidal substance in the blood following the injection of Bayer 205 was first demonstrated by Mayer and Zeiss (1920), who believed that the drug was bound up with the serum proteins. This fact was confirmed by Wenyon (1922), who showed that 0·25 c.cm. of human cerebrospinal fluid, removed one week after the intravenous injection of 1 gm. of Bayer 205, was capable of freeing the peripheral blood of mice from *T. rhodesiense*, although relapse invariably occurred.

It is of course obvious that the trypanocidal substance present in the blood may be either Bayer 205 itself, a compound of Bayer 205 and some constituent of the blood or some substance having no chemical relationship to Bayer 205, but produced by its action on the tissues. Similar possibilities apply to the trypanocidal substance which Mayer and Menk (1922) noted in the milk of a goat injected with Bayer 205. A trypanocidal substance can also be detected in the urine, for Thomson and Robertson (1922) found that 1 c.cm. of human urine obtained twenty-eight days after the last dose of a 14 gm. course of Bayer 205 caused a temporary disappearance of *T. rhodesiense* from the blood of a rat.

Collier (1925) has shown that in certain strengths Bayer 205 lessens or actually abolishes the coagulation of blood serum by heat, possibly because a compound is formed between the drug and the serum. One per cent. of Bayer 205 was found to have this effect, whereas 0·5 per cent. was without effect.

The success of Bayer 205 as a trypanocidal agent has inspired the preparation of a number of complex ureas containing arsenic, in the hope that such complex substances would remain and exert a trypanocidal action for a prolonged period in the body. The final products of the following type were devoid of trypanocidal



action, but some of the intermediate amides containing a free amino group were found by King and Murch (1924) to cause a

temporary disappearance of trypanosomes from the peripheral blood-stream.

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THE TREATMENT OF SLEEPING SICKNESS BY PREPARATIONS OF ANTIMONY

The discovery having been made by Broden and Rodhain (1906), that antimony can be injected intravenously, this metal was first

introduced in the treatment of trypanosomiasis by Plimmer and Thompson (1908), who found that the blood of laboratory animals infected with *T. brucei* or *T. evansi* was sterilized by injections of potassium or sodium antimonyl tartrate. These two preparations are the most commonly used inorganic derivatives of antimony, though Masters (1918) claims to have obtained excellent results from the injection of antimony oxide suspended in equal parts of glycerine and water.

Various organic preparations of antimony, either in the trivalent or pentavalent form (*cf.* " Chemotherapy of Leishmaniasis and Schistosomiasis "), have been tried in the treatment of sleeping sickness. Both van den Branden (1926[1, 2]) and Kleine (1928) failed to obtain any success with antimosan or Sb 212, and though stibosan was found to be trypanocidal *in vivo*, its action was much less rapid than that of tartar emetic. Recently, Dunning and Macht (1928) have prepared a number of azo-dyes containing antimony, but though results have been obtained in laboratory animals, experiments on sleeping sickness do not yet appear to have been made.

With the introduction of Bayer 205 and the pentavalent arsenicals, the use of antimony has decreased except in the treatment of those cases which are resistant to the action of arsenic.

The Treatment of Trypanosomiasis in Animals by Antimony

Although in sleeping sickness the use of antimony has now been almost entirely discarded, nevertheless, in veterinary medicine it still finds a place in the treatment of trypanosomiasis.

Hornby (1919), who summarizes the earlier work relating to the use of tartar emetic in animal trypanosomiasis, believes that antimony constitutes a most valuable drug in the treatment of *T. congolense* infections in cattle, although the results are not good enough to allow of relaxation in the search for a better remedy.

Tartar emetic is especially useful in the treatment of animals used for transport purposes in flybelts. In fact, it appears to be worth while to inject mules in a flybelt with 25 c.cm. of a 4 per cent. solution of tartar emetic every five days, for, though this treatment does not prevent infection, it checks fever and con-

serves energy. Thus Hornby and Burns (1926), in an attempt to keep oxen alive in a flybelt in Tanganyika Territory, where there was constant exposure to infection with *T. congolense* and *T. vivax*, gave fortnightly injections of tartar emetic with the result that the greater number of the animals remained alive. No advantage, however, was derived from combining Bayer 205 with tartar emetic.

Et. and Ed. Sergent, Donatien and Lhéritier (1921), who carried out experiments on the treatment of debab in camels with tartar emetic, found that while a camel weighing 300 kg. could tolerate doses of 1 gm., 1·5 gm. was liable to cause death. One gramme caused a disappearance of trypanosomes from the blood and a lowering of the temperature for eight days. Weekly injections of 1 gm. of tartar emetic produced apparent cure and allowed the female camels to pass through pregnancy without fear of abortion.

Curson (1922) found that satisfactory results were obtained in nagana by five intravenous injections of tartar emetic. The drug was given as a 5 per cent. solution in normal sterile saline, the dose being 1·5 gm. for adult cattle, horses, donkeys and mules, and 0·1 to 0·125 gm. for dogs weighing 25 lbs.

In 1917, and again in 1919–1920, Cross tested the efficacy of intravenous injections of tartar emetic upon camels naturally infected with surra. The conclusion reached was that a course of ten injections, each of 60 c.cm. of a 1 per cent. solution of tartar emetic given intravenously to full-grown camels, was insufficient to effect a cure, as was one large dose of 600 c.cm. of a 1 per cent. solution. Ten to twelve injections of tartar emetic, however, given on alternate days commencing with 150 c.cm. of a 1 per cent. solution and increasing to 300 c.cm., gave considerable promise of success. Later, Cross and Patel (1922) recommended intravenous injections of a 1 per cent. solution given on alternate days as follows :—

1st day	50 c.cm.
3rd day	100 ,,
5th and 7th day	150 ,,
9th to 31st day	175 ,,
33rd and 35th day . .	200 ,,

Leckie (1925), who has also adopted the method of a gradual increase in the dose of tartar emetic, begins the treatment of surra in the camel with 20 c.cm. of a 0·5 per cent. solution, reaching a dose of 200 c.cm. of a 1 per cent. solution on the forty-third day, the total amount of tartar emetic administered being about 30 gm. Careful watch must be kept for symptoms of poisoning, such as rise of temperature to 100° F., cessation of rumination, loss of appetite, orange or blood-tinged urine, yellow discoloration of the mucous membranes, lachrymation, dermatitis, constipation, paraplegia, muscule tremors and disorganization of the eye movements. The post-mortem findings in animals dying under treatment are fatty degeneration of the liver, kidneys and heart. Magnesium sulphate in doses of 2 to 4 lbs. has a curative effect in cases of poisoning. A few experiments have been carried out with sodium in place of potassium antimonyl tartrate. The sodium salt is said to be less depressing and can be given in larger doses, while its effect in clearing the blood from trypanosomes is equal to that of the potassium salt.

In an attempt to detect the early symptoms of antimony poisoning, Hornby (1928) has employed the van den Bergh test in cattle, as by this means early necrosis of the liver is revealed, since it was found that when a series of medicinal doses of tartar emetic is given, the injection which causes fatal necrosis is generally preceded by one which occasions only slight necrosis but, nevertheless, provokes a van den Bergh reaction.

Edwards (1926) treated buffaloes and horses infected either experimentally or naturally with *T. evansi*. For buffaloes during the septicæmic stage 5 c.cm. of an M/10 solution per 100 lbs. of body weight were found to produce a rapid "cure." It would seem that in cattle repression of virulent septicæmic invasions is never followed by complete sterilization, since the trypanosomes reappear intermittently in the blood-stream. Each return, however, is overcome with increasing success by the host's own defensive powers, until the septicæmia becomes less and less intense and finally ends. Experiments with horses indicate that after intravenous injection of a full therapeutic dose the tartar emetic remains in trypanocidal concentration in the blood-stream for

about twenty-four hours; hence the therapeutic dose must be repeated either daily or, at any rate, on alternate days. When, however, full therapeutic doses are given to horses with a trypanosome septicæmia sudden death may occur either from occlusion of the blood capillaries or from liberation of a toxic substance by the dead trypanosomes. It is, therefore, preferable to begin the treatment of horses infected with *T. evansi* with very small doses, gradually increasing until the full therapeutic dose is tolerated. Prophylactically, as may be supposed, tartar emetic is of little use.

Curson (1926) finds that in Zululand systematic treatment of trypanosomiasis in cattle by tartar emetic has caused a marked fall in the death rate in infections due to *T. brucei* or *T. congolense*. During 1920 approximately 33 per cent. of the cattle population died of nagana, and in 1921 25 per cent., over 90 per cent. of the infected animals eventually succumbing. In 1922, after the issue of tartar emetic to farmers, 5 per cent. of the total cattle population died, and of the infected only 20 per cent. Drug-resistant trypanosomes were produced if the doses given were too small.

In Uganda, Richardson (1928) finds that *T. brucei* is usually non-pathogenic to cattle, while *T. vivax* has a negligible pathogenicity, although virulent strains do exist. Infections due to *T. vivax* are uncontrolled by tartar emetic, but those due to *T. congolense* are for the most part cured, although a few animals remain chronically infected. Schwetz (1929), in the Belgian Congo has, however, found just the reverse; *T. vivax* infections are rapidly cured, *T. congolense* infections uncontrolled. The question obviously requires further investigation.

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THE TREATMENT OF TRYPANOSOMIASIS WITH BISMUTH

In view of the successful results obtained with bismuth in the treatment of spirochætal infections, it is not surprising that this metal should have been tested in trypanosomiasis.

The results, however, have not been encouraging. Van Saceghem (1925) has given intravenous injections of colloidal bismuth to cattle suffering from infections due to *T. vivax*, *T. congolense* and *T. brucei*, but only in the last did the blood become sterile. A basic oxyaminophenylarsinate of bismuth has been

found by Nicolau, Doskocil and Galloway (1925) to have a slight action in experimental nagana in the rabbit, though less than in fowl spirochætosis.

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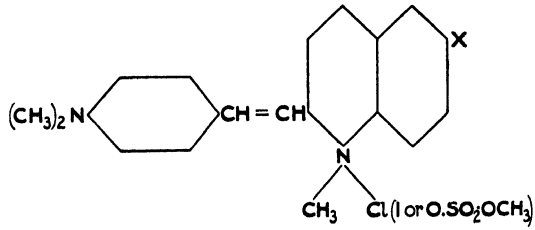
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ANIL- AND STYRYL- QUINOLINE DERIVATIVES IN THE TREATMENT OF TRYPANOSOMIASIS

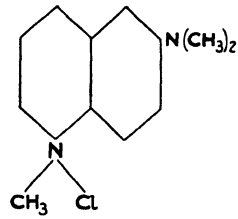
Derivatives of anil- and styryl-quinolines have been introduced in the treatment of trypanosomiasis by Browning, Cohen, Ellingworth and Gulbransen (1926 and 1929). The existence of trypanocidal activity in these compounds appears to depend upon the presence in each of the nuclei of basic groups, or at any rate of acylamino groups. The most marked trypanocidal action was exerted by substances which contain a free basic group in one nucleus and an acylamino group in the other, especially in the styryl series. The anil quinolines in general had but little trypanocidal effect, although they mostly possess powerful bactericidal action. Some trypanocidal effect was shown, however, by 2-(*p*-aminoanil) 6-dimethylaminoquinoline methochloride, 2-(*p*-dimethylaminoanil) 6-aminoquinoline methochloride and 2-(*p*-aminoanil) 6-aminoquinoline methochloride. Certain acylated derivatives of the latter substances also showed some action, as did also the 7-acetylamino-derivatives.

The styryl quinolines were, as a rule, much more effective than the anils as trypanocidal agents, the most effective trypanocides amongst the styryl compounds having, however, very weak antiseptic action on bacteria. The dimethylaminostyryl derivatives examined had the general formula :—

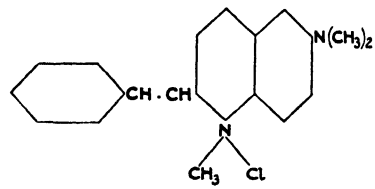


Where x was a tertiary amino-group ($x = N(CH_3)_2$) the substance was inactive in the tolerated dose. When x was NH_2 or when the amino-group was acylated, the resulting substances had some action. The most active was the acetylamino-derivative, the chemotherapeutic index being between 1 : 20 and 1 : 30. Introduction of halogen into the acyl group diminished the trypanocidal action. As the acetylamino-derivative was the most efficient of the dimethylamino series, the tertiary basic group was replaced by a primary one. The substance 2-(*p*-aminostyryl)-6-acetylamino-quinoline methochloride was found to have a chemotherapeutic index which on occasions was as high as 1 : 50. This compound, the corresponding methosulphate, the propionylamino- and the 7-acetylamino-derivative were all active, but their trypanocidal activity against various strains of *T. brucei* in mice showed considerable variation. The conditions for effective trypanocidal action in this group of compounds appear to be (*a*) an acylamino group, which may be situated either in the benzene or the quinoline nucleus, (*b*) a basic group, either primary or tertiary, situated in the other nucleus. The action of an acetyl group frequently decreases the toxicity to the host and results in increased trypanocidal activity, a point of considerable interest in view of the effect of acetylation on the activity of aminophenylarsinic acid. In order to throw light on the influence of the constituent parts of the molecule in determining trypanocidal action in such a compound as 2-(*p*-acetylamino styryl)-6-dimethylaminoquinoline methochloride, the trypanocidal action of the following substances was tested :—

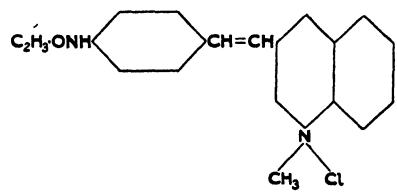
(i.) Dimethylaminoquinaldine methochloride.



(ii.) 2-(*p*-acetylamino styryl)quinoline methochloride.



(iii.) 2-styryl-6-dimethylaminoquinoline methochloride.



The last two substances, having no basic group in the quinoline nucleus and no acetyl amino or amino group in the benzene nucleus respectively, were inert and relatively toxic. The first substance, though poisonous, possessed a slight trypanocidal action.

It is therefore evident that the free basic group, the acetylamino group and the styryl linkage all play a definite part in the trypanocidal action of 2-(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride. Both this compound (No. 90) and 2-(*p*-amino styryl)-6-acetylaminoquinoline methochloride (No. 8), which were the two most active substances prepared, had no prophylactic action against infection with *T. brucei* in mice and were not absorbed when taken by mouth. *In vitro* there was little or no trypanocidal action.

These two compounds were also used successfully in the treatment of *T. brucei* infections in rabbits, five out of eight rabbits being cured after receiving 0·02 gm. per kilogram of body weight subcutaneously. Little is known of the pharmacology of these substances, though No. 90 at any rate appears to be excreted in the urine. The results of experiments with these compounds in sleeping sickness and the trypanosomiases of domestic animals will be awaited with interest.

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DRUG RESISTANCE

It has been known for many years that races of trypanosomes may be readily obtained which are resistant to the action of drugs or sera.

Although much work has been carried out on the subject of drug resistance, more especially that produced by arsenic, it has not yet been definitely determined whether the change is due to a direct action on the protoplasm of individual trypanosomes, or whether it is due to a process of selection whereby naturally resistant trypanosomes alone survive.

The observation of Werbitzki (1910), Kudicke (1911), and Laveran and Roudsky (1911-12) that when rats infected with *T. brucei* or other trypanosomes are treated with oxazine or acridine dyes, there appears in the blood of the animals a certain number of trypanosomes which have no parabasal body (= kineto nucleus) suggests that the mutation is brought about by a direct action of the drug on the protoplasm of the trypanosome. The question then arises as to whether it is the parabasal body which is directly attacked by the drug as suggested by the observations of Kudicke (1911) on *T. lewisi* and by Laveran and Roudsky (1912) on *T.*

duttoni, or whether the drug exerts its influence on the dividing forms so that at division a parabasal containing and an aparabasal individual are produced. The occurrence of aparabasal trypanosomes in races which have not been subject to the action of drugs, as shown by Wenyon (1928) in the case of *T. equinum*, at any rate appears to be due to some irregularity in division.

Earlier experiments, summarised by Laveran and Mesnil (1912) and by Dobell (1912) had shown that in addition to arsenic resistant strains, strains could be easily produced which were resistant to various dyes. Moreover, it was found that resistance was not absolutely specific, for certain arsenic free substances, such as pyronine and acridine, were able to produce strains which had some resistance to atoxyl. Dyes of the *para*-quinoid type, such as *para*-rosaniline, had no such action.

A similar lack of specificity has been recorded by Leupold (1924) and Fourneau (1925) in connection with a strain of *T. brucei* resistant to Bayer 205, for it was found that the Bayer 205 resistant strain was also partially resistant to arsacetin and trypaflavine, though not to neoarsphenamine nor tartar emetic.

Curative Dose

	Arsacetin.	Trypaflavine.	Neoarsphenamine.	Tartar emetic.
T. brucei (normal)	1/800	1/8000	1/2000	1/6000
T. brucei (resistant to Bayer 205) . .	1/400	1/4000	1/2000	1/6000

Conversely it was found that a strain resistant to trypan blue was also resistant to Bayer 205. In the same way Browning, Cohen, Ellingworth and Gulbransen (1929) found that a strain of *T. brucei* rendered resistant to 2-(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride was also resistant to arsacetin, trypaflavine and tryparosan, though not to trypan blue or Bayer 205. Another strain resistant to 2-(*p*-acetyl-amino-

styryl)-6-dimethylaminoquinoline methochloride was, however, still susceptible to tryparosan.

This lack of specificity is somewhat difficult to reconcile with the theory of specific chemoreceptors, as is the fact, first noted by Mesnil and Brimont (1908), that the arsenic resistance of a particular strain of trypanosome passaged in the mouse is lost if the strain is passaged in the rabbit or dog, but is regained when returned to the mouse. This fact suggests that the host's tissues play some part in the development of the resistance.

In this connection an interesting phenomenon has been brought to light by Browning and Gulbransen (1922). It was found that when mice were fed with parafuchsine and were subsequently inoculated with a strain of trypanosome resistant to parafuchsine, the therapeutic action of injections of trypaflavine was greatly reduced. This phenomenon was termed "therapeutic interference." Its existence was confirmed by Schnitzer (1926), who has shown that in order to demonstrate interference it is not necessary to employ trypanosomes which are resistant to parafuchsine. Schnitzer and Rosenberg (1926 and 1927) found that a suitable subtherapeutic dose of parafuchsine prevented the action of various other chemotherapeutic agents, such as trypaflavine, arsacetin or arsphenamine. The interference effect was manifested when the dose of parafuchsine was injected along with or preferably a certain time, such as four hours, before. With a strain of trypanosome resistant to parafuchsine much larger doses of parafuchsine were needed to inhibit the action of arsacetine than were effective for trypaflavine, and still larger doses were required to inhibit the action of arsphenamine. With the normal strain of trypanosomes, the optimum dose of parafuchsine required to produce interference was the same for each of the three other trypanocidal agents. With excessive doses of parafuchsine in animals inoculated with the normal strain, interference sometimes failed to occur, possibly as the result of cumulative effects. The curative action of tartar emetic was found by Schnitzer and Silberstein (1927[1]) to be interfered with by parafuchsine in a proportion of animals. If instead of parafuchsine other dyes were given, interference would also occur. Thus Schnitzer and Silber-

stein (1927[2]) found that pyoktanin (a mixture of penta- and hexamethyl-triaminotriphenylmethane hydrochloride), the analogue of ethylviolet, interfered with the therapeutic action of trypaflavine, arsacetin, arsphenamine and tartar emetic with both parafuchsine-resistant and normal strains ; in its action pyoktanin closely resembled parafuchsine. With tryparosan it failed to produce interference, while an analogue of Döbner's violet, brilliant green (tetraethyldiaminotriphenylmethane sulphate), showed only a trace of interfering action. Browning and Gulbransen (1927) found that interference with the therapeutic action of arsacetin is produced by ethylviolet (penta- and hexamethyl triaminotriphenylmethane hydrochloride), a dye related to parafuchsine. Tryparosan (a chlorinated parafuchsine) and Döbner's violet (diaminotriphenylmethane hydrochloride), on the other hand, had only an uncertain interfering effect, while trypan blue (one molecule tetrazotised toluidine plus two molecules of the sodium salt of aminonaphthol disulphonic acid 1 : 8 : 3 : 6) had some interfering action. A small dose of parafuchsine interfered with the trypanocidal action of the related substance tryparosan, but did not interfere with a subsequent therapeutic dose of itself. This fact supports the suggestion that chemotherapeutic interference is due to the direct influence of the interfering agent on the parasites and not to an indirect influence exercised through the tissues of the host. In favour of this direct action is the observation of Schnitzer and Rosenberg (1926) that the administration of parafuchsine along with one of the other chemotherapeutic agents does not diminish the toxicity of the latter for the mammalian host. In addition, according to Schnitzer (1926), very minute doses of parafuchsine may produce the interference phenomenon, *e.g.*, 0·5 c.cm. parafuchsine 1 : 50,000 inhibits the action of a large dose of trypaflavine in 50 per cent. of animals infected with the parafuchsine resistant strain.

Although the evidence is thus in favour of a direct action of the interfering agent on the trypanosomes, it is somewhat difficult to explain the phenomenon on the theory of chemoreceptors if these are regarded as more or less constant structures qualitatively fixed in their constitution, though possibly capable of quantitative

variation. As shown by Browning (1908), parafuchsine, ethyl-violet and trypan blue are drugs which do not lead to the development of strains resistant to arsacetin or *vice versâ*. Now when the substance whose effect is interfered with and that which produces the interference do not lead to mutually resistant strains, that is to say, presumably, do not unite with the same receptors (*e.g.*, parafuchsine in the first instance and arsacetin in the second), it cannot be concluded that the latter agent fails to exert trypanocidal action on account of its corresponding receptor having already combined with the interfering substance. To meet this difficulty, Browning and Gulbransen (1927) suggest that the receptor apparatus of the trypanosomes is capable of very rapid modification, a conception which is not opposed to Ehrlich's views as shown by his work with Roehl and Gulbransen (1909) on the rapid development of serum resistant strains *in vitro*.

However, while the experimental results of chemotherapeutic interference are not incompatible with the receptor theory, they do not at present permit of any simple explanation. In this connection the results obtained by Silberstein (1928) are of interest, for they would appear to demonstrate a close relationship between chemo- and immune receptors, since when there is incomplete interference there may be early relapse or intermittent infection, the relapse or intermittent strains being different from the original strain of trypanosome.

It is obvious that at present no satisfactory explanation of drug resistance can be given. It is, however, inevitable that the drug resistance which can be acquired by trypanosomes, spirochætes and bacteria, either *in vivo* or *in vitro*, should be compared with the acquired tolerance towards drugs, such as alcohol, exhibited by man. In the case of so-called arsenic habituation, there is, according to Schwartze (1922) and Schwartze and Munch (1926), very little unequivocal evidence in favour of any increased tolerance. Gunn (1923), in fact, is very sceptical as to the possibility of any organism acquiring by habituation a destructive action which it did not originally possess or increasing a pre-existent destructive power. On the other hand, Weller (1927) has brought forward very suggestive evidence that guinea-pigs can acquire a tolerance for lead.

Possibly a new method of attack on the problems of drug resistance has been opened up by the observations of Wilson (1922) that tissue cells in cultures may actually acquire an increased tolerance to copper sulphate and sodium arsenite when grown in weak solutions of these poisons.

In attempting to analyse the mechanism of drug resistance it is obvious that two possible factors may play a part:—

(i.) The acquisition of a newly acquired character which is hereditarily transmissible.

(ii.) The selection by means of the drug of individuals which already possess a resisting power to the drug, this innate resisting power being transmissible by heredity.

One difficulty encountered in studying drug resistance is to be found in the fact that all members of a strain are not identical, but possess certain characteristics in relative amounts. It is, therefore, always possible to argue that a modification of the original strain has arisen as a result of the selection of a small number of survivors whose original capacity was above that of the other members of the strain.

Nevertheless there is certain evidence in favour of the acquisition of a new character which is hereditarily transmissible. Freund (1925), for instance, in his work on *T. brucei*, found that certain trypanosomes of a given strain (Nagana-Prowazek) exposed in mice to ineffective doses of " Bayer 205 " had lost their capacity to form relapse strains when exposed to antimony. But Bayer-resistant trypanosomes formed relapse strains and the strain removed from a mouse insufficiently treated with the drug, though unable, after twenty-four hours, to form a relapse strain when treated with antimony in the passage mouse, would do so if removed after forty-eight hours. Freund calls this loss of recidival capacity the antimutative action of the drug, which seems an unsuitable description. The so-called antimutative action of Bayer 205 as a general principle is called in question by Kritschewski and Kaganova (1929), but this does not invalidate the facts described by Freund, which afford a good instance of modification of the trypanosomes as distinct from selection because, whatever the relative proportions, all the types of origin present

in the second sample must be present in the first even if in a different state. A similar observation has been made by Robertson (1929) in the case of *Bodo* exposed to acriflavine, where it was found that a culture in a concentration of the drug bordering upon the lethal was not viable in the early days of development, but became so at a later date even though the actual numbers taken were higher at the non-viable attempt than later.

Robertson (1929) has also described drug hypersensitiveness of cultures of *Bodo* to acriflavine in which no selection process of the killing-off type would account for the result. These experiments are in agreement with certain observations by Duke (1927), who describes a state in trypanosomiasis where the host is becoming more and more resistant and the trypanosomes still survive in the blood, but when tsetse flies are fed on the host the trypanosomes fail to develop, having in fact become less transmissible. An analogous condition has also been produced in artificial trypanosomiasis in mice where strains treated with potassium hexatantalate, while uninfluenced in their evolution in the treated host, become untransmissible by means of the syringe into further mice (Morgenroth and Rosenthal, 1911).

In the same way that trypanosomes may become resistant to drugs, so also they may slowly lose this character. The loss of resistance has, however, received relatively little detailed study, and there is possibly an undue impression of the solidity of this character. Morgenroth and Rosenthal (1911), however, admit in a general statement the gradual loss of arsenic resistance in certain strains. Browning (1908) notes a highly atoxyl resistant strain which lost this character in the eighty-ninth passage. Mesnil and Blanchard (1916) have similarly demonstrated the slow appearance of sensitiveness to human serum in trypanosome strains of human origin which had been passaged in mice. Robertson (1929) also gives an account of the slow loss of resistance to acriflavine on the part of *Bodo*.

The evidence at present available, therefore, suggests that the acquisition of drug resistance in trypanosomes is brought about by the interaction of selective inheritance and an actual modification of the qualities of the organism by evolution in the drug.

The modification is not apparently in the nature of a mutation, but rather a piling up of gradual changes in a particular direction, the modification being similarly lost by a series of gradual changes in the reverse direction.

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THE ACTION OF DRUGS IN TRYPANOSOMIASIS

In considering the means by which trypanosomes are destroyed by drugs in the body, two distinct problems arise:

(i.) The mechanism by which drugs inert *in vitro* become trypanocidally active *in vivo*.

(ii.) The point of attack on the trypanosome of the active trypanocidal compounds.

The Trypanocidal Action of Arsenic and Antimony

Since the arsinic acids are relatively inactive in the destruction of trypanosomes *in vitro*, but active *in vivo*, while the arsenoxides are active *in vitro*, it was thought by Ehrlich that the tissues have the power of reducing the pentavalent arsinic acids to the trivalent arsenoxide stage, which then destroys the trypanosomes.

$$R.AsO_3H_2 \longrightarrow R.AsO$$

This theory, which is now generally accepted, was extended by Voegtlin and Smith (1920[1, 2]), to explain the trypanocidal action of the pentavalent antimony compounds. Since the time required *in vivo* for pentavalent arsenic and antimony derivatives to exert trypanocidal activity is longer than for trivalent compounds, it is assumed that the longer latent period is due to the reduction of a sufficient amount of the drug to the trivalent form. Since also arsenic in the arsenoxide form acts much more rapidly than the arsphenamine derivatives, it is suggested that these compounds are oxidised in the body to the oxide.

$$R.As = As.R \longrightarrow R.As = O$$

Thus if the minimal effective dose of a drug belonging to the arsenoxide group is injected into a mouse, it is found that the number of trypanosomes at once decreases until all the parasites have been killed; this may require from ten to sixty minutes, depending on the amount of the drug injected. On the other hand, the injection of twice or even several times a minimal effective dose of the arsphenamine derivatives and the pentavalent arsenicals does not produce a material change in the trypanosome count in the peripheral blood until from one to six hours have elapsed.

This conception is strengthened by the fact that the toxicity of a slightly alkaline aqueous solution of arsphenamine or neo-arsphenamine is increased when kept in contact with air, probably

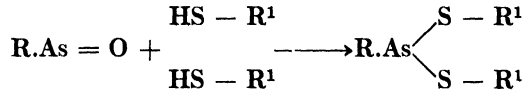
owing to oxidation to *meta*-amino-*para*-hydroxyphenylarsenoxide, since on treating arsphenamine in alkaline solution with hydrogen peroxide, Ehrlich and Bertheim (1912) produced *meta*-amino-*para*-hydroxyphenylarsinic acid. In addition, an aqueous solution of the sodium salt of arsphenamine which has been incubated at 37° C. for three hours exerts a much greater trypanocidal activity than freshly prepared solutions, and has no latent period in its trypanocidal action. Since reduction of the pentavalent arsenicals to the arsenious oxide stage would require a longer time than oxidation of the arsphenamines to the same stage, a longer latent period for trypanocidal action would be necessary for the arsinic acids than for the arsphenamines. This has been found to be the case by Voegtlin and Smith, the latent period for the arsphenamines being from one to three hours, for the pentavalent arsinic acids from twelve to eighteen hours.

Although pentavalent arsenic derivatives are not directly trypanocidal *in vitro*, the work of Levaditi and Yamanouchi (1908) and Levaditi (1909) showed that when atoxyl was incubated at 37° C. with an emulsion of liver, kidney, brain or muscle, trypanocidal properties were produced in the mixture, due to the production of a hypothetical substance which Levaditi has termed "trypanotoxyl." It is obvious that this trypanotoxyl may be either a new compound formed between atoxyl and the tissues, or a simple chemical change in the atoxyl whereby it is rendered trypanocidal *in vivo*. In a more recent communication Levaditi, Anderson and Manin (1928) have suggested that the constituent in the tissues which converts atoxyl into a trypanocidal substance is reduced glutathione. It was found that a mixture of glutathione in saline and atoxyl kept at 37° C. for three hours became possessed of trypanocidal properties. In addition there exists a certain relationship between the glutathione content of the organs and their capacity for transforming atoxyl into trypanotoxyl. But this relationship is neither absolute nor constant, for there is, for example, a greater difference in the glutathione content of liver and striated muscle than there is between their powers of activation. It is therefore argued that there must be a second factor operating in addition to glutathione, since when atoxyl and

issues are incubated together, some glutathione always remains n the tissues, and even after extracting the tissues with trichlor- icetic acid, which removes glutathione, they retain the power of forming trypanotoxyl. Furthermore, blood which is poor in glutathione activates atoxyl. The activating substance in blood is absent from serum, but is present in hæmoglobin, which is equally potent after desiccation, resolution in saline and repre- cipitation by alcohol.

Despite the fact that there is thus general agreement that pentavalent arsenicals must undergo some change before they become trypanocidal *in vivo*, there is little or no agreement as to the mechanism by which this change is produced or the exact compound which results from the change, although the bulk of the evidence suggests that a reduction takes place to the arsenoxide stage.

Even less agreement exists as to the mechanism by which the arsenoxide attacks the trypanosomes. Voegtlin, Dyer and Leonard (1923) have suggested the interesting hypothesis that, having arrived at the oxide stage, the arsenic reacts with the reduced glutathione present in the tissues and the trypanosomes. In other words the —SH group forms the chemoreceptor for arsenic.



In support of this view are the following facts :—

(i.) By the nitroprusside reaction it is possible to show that trypanosomes contain an —SH group.

(ii.) If sodium thioglycollate is injected simultaneously with a minimum fatal dose of 4-hydroxyphenylarsenious oxide, a delay is noticed in the death of the animal.

(iii.) Feeding with glutaminic acid and cysteine offers protection against a minimum fatal dose of 4-hydroxyphenylarsenious oxide administered three hours later.

(iv.) The injection of compounds containing an —SH group simultaneously with a trypanocidal drug slows the rate of dis-

appearance of trypanosomes from an infected animal. Substances which act in this way include reduced glutathione, thioglycollic, thiolactic and thiosalicylic acids and cysteine.

The analogy between the chemotherapeutic interference previously described with parafuchsine and the action of the —SH group will be obvious.

Much criticism has been directed against the hypothesis that the —SH group in trypanosomes represents the receptor for arsenic, on the ground that the most obvious deduction from the experimental data is that the decrease in activity of the drug when an excess of sulfhydryl molecules is present, is due to the removal of much of the drug from active service. No deduction concerning the nature of trypanocidal activity is therefore logically justified. Brown and Kolmer (1929), in addition, have failed to establish any relationship between the reduced glutathione content of animal organs and the varying liability of various species to arsenic intoxication. Thiosulphates have long been known to have a curative action in dermatitis due to arsphenamine, yet neither human nor animal skin contains glutathione, so that the cure cannot be caused through any sulfhydryl molecules. Furthermore, small doses of arsphenamine or neoarsphenamine do not lower the glutathione content of the organs *in vivo*. It is, in fact, quite impossible to correlate the action of arsenicals with any direct action on glutathione.

Although there is thus but little agreement as to the mode of action of the pentavalent arsenicals and the arsphenamines, it seems almost certain that some arsenic compound is formed in the tissues which is slowly liberated in a trypanocidal form for a considerable period. This was the view held by Ehrlich and Bertheim (1912) as to the trypanocidal action of dyes, and it is supported in the case of the arsenicals by the following observations. Voegtlin, Dyer and Miller (1924) have shown that resistance to arsenic can be acquired by trypanosomes in rats which have been treated with arsphenamine prior to infection. This may be due to the persistence in the body of small quantities of arsenic or to the formation of a trypanocidal compound from the tissues and arsphenamine. Possibly the spleen acts as a depôt for this

deposit of arsenic, for Kritschewski (1927[1], [2]) has shown that in mice splenectomy destroys the sterilizing action of various trypanocidal drugs; blockade of the reticulo-endothelial system on the other hand does not. These results have been confirmed by Rubinstein (1928) in mice, and Lisgunova (1928) in rats, while Kritschewski himself (1928) has further shown that loss of the spleen is compensated for if, in the splenectomised animal, the drug is injected in combination with agar, so as to form a depôt from which slow absorption may occur.

Cytological observations on trypanosomes subjected to drugs have failed to advance knowledge as to the action of these drugs. The results originally obtained by Werbitzki (1910) on the correlation between the loss of the parabasal body or kinetonucleus have already been discussed. This question has, however, been still further complicated by the work of Leupold (1925), who has found that arsenic-resistant trypanosomes cannot be made "ablepharoplastic" by trypaflavine. Trypan blue resistant trypanosomes have a similar property, though strains resistant to tartar emetic and Bayer 205 are just as susceptible to the action of trypaflavine as are normal strains. It is possible, therefore, that the parabasal body is so changed by arsenicals or trypan blue as to be no longer susceptible to the action of trypaflavine. However, a trypanosome strain resistant to tryparosan and aparabasal is nevertheless destroyed by trypaflavine in the same way as a normal strain. Moreover, it is possible to make the tryparosan-resistant aparabasal strain resistant to arsacetin, with the result that the doubly resistant strain is now found to be resistant to trypaflavine. No explanation of this phenomenon can as yet be given.

Saito (1927) has found that parafuchsine and trypan red first stimulate the trypanosomes and then paralyse them. Later the trypanosomes become rounded and granules and vacuoles appear in the cytoplasm. Trypaflavine especially causes the appearance of very numerous granules in *T. gambiense*.

Tartar emetic seemed to act especially on the nucleus. Leckie, (1925), however, found that the first action of tartar emetic was to interfere with the staining reaction of the cytoplasm,

later the centrosome and flagellum disappeared, and finally the nucleus disintegrated.

It has long been known that when an animal is cured of a trypanosome infection it remains immune for some time to a fresh infection. In the case of Bayer 205 this " immunity " is most probably due to the retention of the drug in the body, but in the case of the pentavalent arsenicals which are rapidly excreted there would seem to be a true liberation of immune bodies, for a prophylactic injection of a pentavalent arsenical does not necessarily protect against subsequent infection, and in addition, the immunity only holds for the homologous strain. Thus Browning (1927) found that the immunity response following treatment by the same agent may vary in degree with different strains of *T. brucei*, for after curing mice heavily infected with the " Prowazek " strain, complete failure of the second inoculation to develop occurred even after an interval of twenty-two days, but when the second inoculation was made with the " Ferox " strain (Ehrlich), complete immunity was not obtained. In addition, it is by no means rare to find that with certain chemotherapeutic agents the administration of a dose at any early stage of infection may at first appear to be without effect, since the trypanosomes continue to multiply and increase in numbers in the blood for some days. Then they disappear suddenly and permanently, and the tissues are sterilized. This phenomenon may also occur when a prophylactic injection of a drug of the aminostyryl-quinoline type is given even as long as thirteen days before the injection of trypanosomes. The only possible explanation seems to be that under the influence of the chemotherapeutic agent a proportion of the trypanosomes are killed, thereby inducing an immunity reaction which kills the remainder. This might account for the observations of Schnitzer and Silberstein (1928) that when mice which are doubly infected with a normal and a drug-resistant strain of trypanosome are treated with the drug, against which one strain is resistant, it is found that the drug-resistant forms disappear with the normal forms from the blood, though only when the normal and the drug-resistant strains are immunologically identical. An immunological response must also be at least a

partial explanation of the following facts observed by Browning and Gulbransen (1928). When mice are infected with *T. brucei*, cure may eventually be brought about after a number of relapses, each of which is treated with the same dose of 2-*p*-acetylaminostyryl-6-dimethylaminoquinoline methochloride. From the irregular intervals occurring between the relapses, and the fact that prophylactic inoculations of the drug fail to prevent attacks it is obvious that mere accumulation of the drug in the tissues could not alone account for the cure. The factors involved in the explanation of the phenomenon would seem to comprise, on the part of the host, the capacity of the tissues to co-operate with the therapeutic agent by immunity mechanisms and, on the part of the parasite, accommodation to the antibodies produced (serum resistance) as well as alterations in susceptibility directly or indirectly to the drug.

In relation to the combined action of immune bodies and chemotherapeutic agents, Brown and Broom (1929) have made certain interesting experiments which are described more fully in discussing the chemotherapy of leptospiral diseases. In brief, however, it was found that a negatively charged colloid was more toxic to trypanosomes in presence of a specific immune serum and complement than in presence of normal serum, the suggestion being that the immune serum reduces the negative charge of the protozoa which, therefore, attract the negatively charged colloid.

If little is known in regard to the trypanocidal action of the dyes, arsenic and antimony, even less is known of the mechanism by which Bayer 205 proves lethal to trypanosomes. It is, however, certain that after the injection of Bayer 205 into an animal the blood possesses trypanocidal powers for some days after the injection.

Various observers, including Mayer and Zeiss (1920), Dervish and Laigret (1923) and Sei (1923), working with mice, Shintake (1923), in mice and rats, and Saito (1927), *in vitro*, have described in trypanosomes subjected to Bayer 205 an increase in the number of divisional forms, some having as many as four nuclei. Moschkowski (1927), however, in guinea-pigs and mice, was unable to detect any definite increase in the percentage of divisional

forms. Dervish and Laigret (1928) noted especially the granulation of trypanosomes subjected to the action of Bayer 205.

The work of Kligler and Weitzman (1925) shows that Bayer 205 kills *T. evansi in vitro* in a dilution of 1 in 1,600, thus showing a fairly close correspondence with the effective dose *in vivo*. The conclusion is therefore drawn that the therapeutic acitivity of Bayer 205 is due to a direct action on the trypanosomes.

Roehl (1926) believes that the sulpho-acid groups in Bayer 205 react with certain of the basic substances in the cell nucleus to form insoluble salts, with the result that metabolism is altered and division becomes impossible, so that the enfeebled parasites are readily destroyed by the tissues.

The factors involved in drug therapy in trypanosomiasis may therefore be summarised as follows :—

(i.) The circulating drug, either in the form introduced, or after some simple chemical change in the body, acts directly on the trypanosomes, thereby destroying them :—Kligler and Weitzman (1925), Moschkowski (1927), for Bayer 205, while Papamarku (1927) gives interesting data on the effective doses *in vivo* and *in vitro* of some other drugs.

(ii.) The drug produces either enfeebled or otherwise modified trypanosomes ; the effect of potassium hexatantalate in rendering the strain non-transmissible is an example of this mode of action (Morgenroth and Rosenthal (1911)).

(iii.) The drug produces antigens from the bodies of the trypanosomes.

(iv.) The action of the spleen and reticulo-endothelial system :

(*a*) In producing a natural resistance to trypanosomes apart from any drug. This is shown by the high resistance in all the relapsing trypanosome diseases in nature. It seems that protozoan infections especially, held in check in normal animals, break into acute manifestations in splenectomised individuals, a typical example being bartonella infection in rats. This natural action of the spleen is at its minimum in the artificial *T. brucei* infections in mice as is shown by Jungeblut (1927).

(*b*) In pouring out immune body in response to the trypanosome

antigens liberated by the drug. (Kikuth and Regendanz, 1929.)

(*c*) In clearing up cell-detritus.

(*d*) In serving as depôts for the concentration of the drug. (Voegtlin, 1925.)

(*e*) In changing the drug into a more efficient trypanocide. (Kotake, Masai and Mori (1922), Voegtlin, Dyer and Miller (1924), Jungeblut (1927), Feldt and Schott (1927) and Kritschewski (1927[1, 2]).

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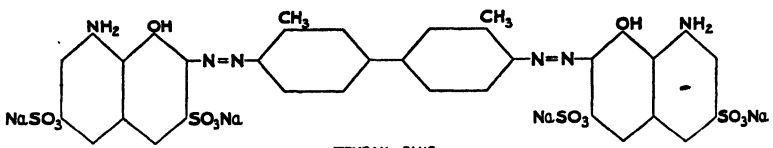
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CHAPTER VII

CHEMOTHERAPY OF PIROPLASMOSIS

THE sub-order *Piroplasmoidea* comprises the two protozoal families *Babesiidæ* and *Theileriidæ*. The first family contains a large number of species infecting cattle, sheep, goats, horses and dogs; the second has as its most important member *Theileria parva*, the agent of East Coast fever.

Though many substances, including the various arsenic derivatives and Bayer 205, have been employed in the treatment of piroplasmosis, the only drug which has anything like a specific action is trypan blue, which was first used by Mesnil and Nicolle (1906) in the treatment of experimental trypanosomiasis. Its trypanocidal action, however, is slight. Trypan blue is the sodium salt of tolidinedisazo-diamino-8-naphthol-3 : 6-disulphonic acid.



TRYPAN BLUE

In 1909, Nuttall and Hadwen found that a dose of 5 to 10 c.cm. of a 1 or 1·5 per cent. solution of trypan blue had the effect of causing *Babesia canis* to degenerate and eventually disappear from the blood of dogs. In this way the serious symptoms resulting from heavy infections could be averted, but, though the animals recovered clinically, the blood remained infective sometimes for many years. Meyer (1912), and others, confirmed the curative action of trypan blue in canine piroplasmosis, though trypan red was without action on the protozoa. Trypan blue has a curative action also on *B. bigemina*, *B. caballi* and *B. motasi*. Thus Stirling (1927) finds that a dose of 40 c.cm. cures 95 per cent. of cases in

cattle. On the other piroplasmata of cattle and horses, and on the parasite of East Coast fever, trypan blue has no action whatsoever. It thus appears to be a specific cure for the larger but not for the smaller piroplasmata.

In infections with *B. equi*, methylene blue has some curative effect, but unfortunately de Kock (1918) found that it is liable to produce anæmia in horses. Toxic effects also may follow the intravenous inoculation of trypan blue, according to Donatien and Lestoquard (1927), a rise of temperature, muscular tremors and difficulty in breathing being the most important symptoms. It seems probable that these symptoms are due to a liberation of toxins from the dead and dying parasites. Recently various silver preparations have been used, more especially in Russia, in the treatment of infections due to *T. bovis* by Yakimoff (1927), who has found ichtargan (7·1 per cent. mortality), arrhena (9·1 per cent. mortality), and protargol (13·0 per cent. mortality) the most satisfactory.

In Palestine, where theileriasis is due either to organisms belonging to the group of *T. dispar* or *T. annulata*, atoxyl with antimosan or stibosan yields the most satisfactory results, according to Freund (1929), provided treatment is begun sufficiently early. Atoxyl should be given subcutaneously in 7 per cent. solution, while antimosan is best injected either intravenously or intramuscularly. The combination of drugs is superior to atoxyl alone, as antimosan reduces the fever and improves the general condition of the oxen.

In East Coast fever, among a large number of other substances which have proved without avail are tartar emetic, picric acid and brilliant green. Earlier work on the chemotherapy of this condition is summarised by Velu (1922).

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CHEMOTHERAPY OF OROYA FEVER

Noguchi (1928), during his investigations on *Bartonella bacilliformis*, the alleged causal agent of Oroya fever, studied the effects of various drugs on the parasites *in vitro* and on the development of the lesions experimentally produced in *Macacus rhesus*. In the *in vitro* experiments, the substances to be tested were added directly to the culture media, the cultures being incubated at 25° C. for a period of thirteen days. Neutroflavine inhibited growth in 1 in 10,000,000 dilution, formalin was almost as effective, and neoarsphenamine, novasurol and mercuric chloride were effective up to 1 in 100,000. Mercurochrome and tartar emetic required concentrations of at least 1 in 10,000 to prevent growth. Curative experiments were made on monkeys, in which the cherry-red verrucous lesions on the abdominal skin and eyebrows had reached maximal size, and had persisted in that state for some days. Cures were brought about by two injections of 0·05 c.cm. arsphenamine, by a mixture consisting of 1 c.cm. of 1 per cent. bismuth lactate, 1 c.cm. of 1 per cent. neutroflavine, and 1 c.cm. of 1 per cent. urotropine injected intravenously, or 1 c.cm. of moogrol (the esters of chaulmoogra oil). These substances, however, did not act prophylactically. Arsphenamine had already been recommended in the treatment of Oroya fever by Arce (1918).

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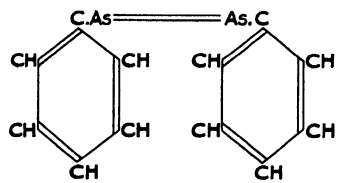
CHAPTER VIII

THE CHEMOTHERAPY OF SYPHILIS

THE therapeutic use of the arsenobenzene derivatives constitutes the greatest achievement yet made, not only in the treatment of syphilis, but in the whole of chemotherapy; nevertheless, since the war, still further important advances have been made in treating syphilis and other spirochætal diseases.

The introduction of bismuth has very largely displaced the use of mercury, and as an adjunct to the arsenobenzene derivatives bismuth is now recognised as a drug of considerable value.

Unfortunately much confusion has arisen in regard to the nomenclature of the arsenobenzene derivatives. The term "arsenobenzene" is frequently misused in medical literature. It should be strictly applied only to the nuclear substance represented by formula I,



FORMULA I. ARSENOBENZENE

which is itself of no therapeutic value, and is of medical interest only as the parent from which salvarsan and the allied trivalent organic arsenic compounds are derived. "Salvarsan" and the derivative names "neosalvarsan," "silver salvarsan," etc., are the subject of proprietary rights, and for that reason the names invented for these substances in the United States—arsphenamine, neoarsphenamine, silver arsphenamine—are used in this work. Unfortunately the only official guide to names for this series

of substances so far provided in this country is the misuse of the name arsenobenzene already referred to in the Therapeutic Substances Act, 1925.

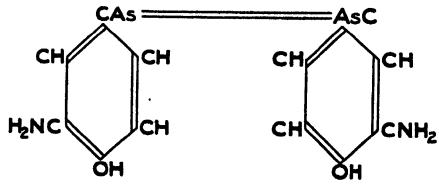
The necessity for standardization of the arsphenamines, both in regard to toxicity and therapeutic activity, is fully recognised and, with increasing experience, the frequency of toxic reactions has materially decreased. The use of pentavalent arsenicals has also been introduced, although, as far as can be judged at present, they are not as efficient as the arsphenamines.

Many problems still, however, remain. There is no clear explanation of the toxic reactions following injections of the arsphenamines. The mode of action of the arsphenamines and of bismuth is unknown, and finally, there is still uncertainty as to whether modern methods of treatment really eradicate syphilitic infection, or whether they merely render the disease latent. Further research alone can solve these problems.

THE ARSENOBENZENE DERIVATIVES

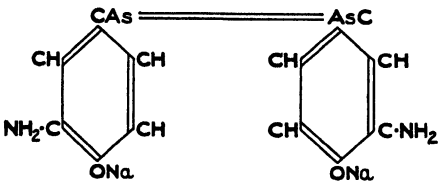
The discovery of the spirochæticidal power of the arsphenamines was the result of Ehrlich's belief that pentavalent arsenicals, such as atoxyl, are reduced in the body to the trivalent form, which is actively parasiticidal. Various substances obtained by reduction of the arsinic acids were, therefore, studied, among them the trivalent *d*-arsenophenylglycine, which is formed on reduction of phenyl-glycine-*p*-arsinic acid: *p*-arsenophenylglycine contains two arsenic atoms linked together by a double bond and each linked to the benzene nucleus by a single bond. It is, therefore, the precursor of arsphenamine, salvarsan or "606."

Simple amino- or hydroxy-derivatives of arsenobenzene were found to be highly toxic, but the simultaneous introduction of amino- and hydroxy-groups into the benzene nuclei produced a much less toxic compound which constituted the arsphenamine base.



ARSPHENAMINE BASE

The di-hydrochloride of 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene is the form that usually appears on the market. As this salt is acid when dissolved in water, it requires neutralisation before use, with the formation of the monosodium salt. With a slight excess of alkali, the disodium salt is produced, this being much less toxic than either the base or the monosodium salt.



3:3′-DIAMINO-4:4′-DIHYDROXYARSENOBENZENE

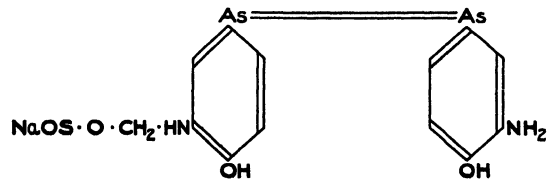
Stabilarsan, which consists of arsphenamine base combined with glucose, on the theory that one molecule of arsphenamine base combines with two molecules of glucose to form a stable compound, has also been much employed in recent years.

There is, in fact, no chemical compound which has been more intensively studied, both biologically and chemically, than arsphenamine (salvarsan, arsenobenzol, arsenobillon, kharsivan). As a result, various modifications in the molecular structure of arsphenamine have been made with a view to increasing its therapeutic efficiency and at the same time decreasing its toxicity.

Thus it was found that the introduction of two additional amino groups into arsenobenzene, as in 3 : 3′ : 5 : 5′-tetra-amino-4 : 4′-dihydroxyarsenobenzene, increased the toxicity of the parent substance 39 per cent., while the therapeutic dose remained the same. Arsalyt, 3 : 3′ : 5 : 5′-tetra-amino—4 : 4′-dimethyl-aminoarsenobenzene has also failed therapeutically. The intro-

duction of various fatty acid groups into both amino-radicals of arsphenamine lowered both the toxicity and the therapeutic effect. According to Bertheim (1912), the introduction of methyl groups into the amino-radicals increases the toxicity of arsenobenzene. Thus 3 : 3′-dimethyldiamino-and 3 : 3′-tetramethyldiamino-4 : 4′-dihydroxyarsenobenzenes are about ten times as toxic as the parent substance, while the 3 : 3′-hexamethyldiamino-4 : 4′-dihydroxyarsenobenzene is three to five times as toxic. The introduction of iodine into the benezene ring also decreases the therapeutic effect.

The most efficient derivative of arsenobenzene, however, is neoarsphenamine, or neosalvarsan (novarsenobenzol, novarsenobillon, neo-kharsivan, " 914 "), which is sodium 3 : 3′-diamino-4 : 4 -dihydroxyarsenobenzene-N-methylene-sulphinate, being derived from arsphenamine by the introduction of a methylene-sulphinic group into one of the amino groups.

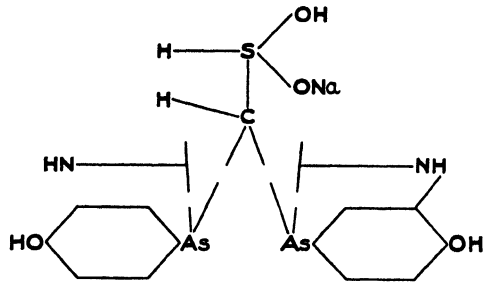


NEOARSPHENAMINE

Salkin (1929) has recently brought forward evidence to suggest that neoarsphenamine differs considerably in constitution from arsphenamine.

While the arsenic in neoarsphenamine requires but two iodine atoms for oxidation, the arsenic in arsphenamine requires four iodine atoms for its oxidation. As in both cases the arsenic is oxidised to the pentavalent form, it is concluded that in arsphenamine the two arsenic atoms are linked by a double bond, while in neoarsphenamine the arsenic atoms are either bridged by a group which allows a normal oxidation to occur, or are not linked at all. Absence of the free amino groups may be assumed from the fact that on precipitating neoarsphenamine with hydrochloric acid, no hydrochloride of the amine is formed, the precipitate

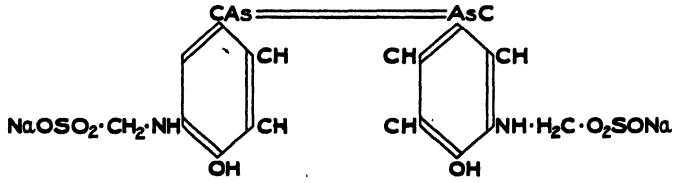
containing no chlorides. The new structural formula suggested is :—



Since neoarsphenamine contains on the average 19 per cent. of arsenic, as compared with 30 per cent. in arsphenamine, the curative activity of the former is less, the ratio of therapeutic activity, according to Ehrlich and Hata (1911), being such that 0·6 gm. of arsphenamine is equivalent to 0·9 gm. of neoarsphenamine, as judged by experiments on fowl spirochætosis and mice infected with relapsing fever.

The advantage of neoarsphenamine, apart from its decreased toxicity, is that it does not require neutralization. On the other hand, its keeping powers are not so great, it oxidises readily on shaking the solution, and its toxicity is apt to vary in different samples.

Sulpharsphenamine (sulpharsenobenzene, sulpharsenol, kharsulphan, myosalvarsan).



SULPHARSPHENAMINE

The arsenic content is from 20·7 to 23·6 per cent. and the sulphur from 8·7 to 12·8 per cent., the atomic ratio of arsenic to sulphur ranging from 1 : 0·91 to 1 : 1·45.

Silver Sodium Arsphenamine (silver salvarsan).—In 1915

Ehrlich and Karrer published details of complex salts of arsenobenzene and the heavy metals characterised by deep colours and great stability. The only examples of these metal-arsenobenzenes to be used therapeutically to any extent are the silver sodium arsphenamine and silver neoarsphenamine. Myers (1922) regards silver sodium arsphenamine as the sodium salt of the monosilver 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene $[(NH_2(NaO)C_6H_3As_2)_2Ag_2O]$. Raiziss and Gavron (1922), however, suggest that silver sodium arsphenamine is a mixture of sodium arsphenamine and colloidal silver, while Gray (1923) has shown that in silver sodium arsphenamine the silver is present as oxide and probably to some extent as metal, both being protected by the colloidal sodium arsphenamine or its hydrolytic product, " arsphenamine base."

Recently compounds of bismuth and arsphenamine have been introduced into therapeutics.

Some importance also attaches to a compound introduced by Albert (1924) under the name of " Albert 102," which, though its use in syphilis and trypanosomiasis has given disappointing results, is of interest chemotherapeutically from two points of view. In the first place, it represents the principle of stabilizing an easily oxidised product by the introduction of the hydrazone, phenylhydrazone or semicarbazone of a ketone group as a side chain, and in the second place it points to the fact that neither the nuclear amino-group nor the nuclear hydroxy-group are absolutely essential for the development of therapeutic activity in the arsphenamine type of molecule.

THE PHARMACOLOGY OF THE ARSENOBENZENE DERIVATIVES

Although the usual method of administering the arsphenamines is by intravenous injection, various other routes have from time to time been used.

The rate of absorption after intramuscular injection depends primarily on the degree of injury produced, neoarsphenamine, which is less irritating than arsphenamine, being absorbed much

more rapidly. Thus Swift (1913) found that in rabbits injected intramuscularly with arsphenamine, some 30 to 40 per cent. of the arsenic was absorbed during the first week, about 50 per cent. by the end of the second week, while at the end of six weeks about 80 per cent. had disappeared from the muscles. In the case of neoarsphenamine, 80 per cent. of the arsenic had been absorbed during the first week, and 90 per cent. by the end of the second, while only about 5 per cent. remained in the tissues at the end of six weeks.

According to Kolmer and Schamberg (1912), arsphenamine can be absorbed when given by mouth, but after rectal administration, at least in children, Noeggerath and Reichle (1923) found only minute traces in the urine. Evidence as to the degree of absorption can be obtained, as Kolmer (1926) has shown, by injecting rats intraperitoneally with *Trypanosoma equiperdum*, and treating them twenty-four hours later with the drug.

Drug.	Minimal curative doses per kilogram of body weight in rats infected with T. *equiperdum* by			
	Intravenous injection.	Intramuscular injection.	Oral administration.	Rectal administration.
Arsphenamine .	0·004	0·004	0·028	0·045
Neoarsphenamine	0·020	0·012	more than 0·030	more than 0·060
Sulpharsphenamine	0·008	0·010	0·040	0·060

The minimal curative dose of the three different drugs administered by intramuscular injection was practically the same as by intravenous injection, and some six to ten times less than by oral or rectal administration. Voegtlin and Smith (1921) also reached the conclusion that the minimal curative doses are practically the same by intramuscular and intravenous injection.

On intravenous injection of the arsphenamines, practically none of the arsenic is taken up by the blood corpuscles, according to the experiments of Myers (1919), and Clausen and Jeans (1922): the arsenic, however, disappears very rapidly from the blood-

stream, and in from one to three hours after injection only a trace can be found in the circulation.

Jiminez de Asua and Kuhn (1928) have shown that after intravenous injections of neoarsphenamine the reticulo-endothelial cells of the spleen, liver and lymph glands become swollen, and by appropriate methods of silver impregnation the actual granules of neoarsphenamine can be demonstrated within the cells. After very large injections the granules of neoarsphenamine also appear in the capillary endothelium of the intestines and salivary glands. In splenectomised animals, according to Jiminez de Asua, Kuhn and Torino (1928), the elimination of arsenic is slower than in normal animals, thus suggesting that the reticulo-endothelial system is occupied with the conversion of the arsphenamine into active germicidal substances.

Numerous estimations have been made of the distribution of arsenic in the various organs after intravenous administration. According to Bulmer (1923), there is at first a high concentration of arsenic in the liver, followed by a rapid reduction, probably as the result of elimination in the bile. The lungs also contain a large amount of arsenic, the high content being maintained for several days. The same is true for the long bones. Underhill and Dimick (1928) found that, as the result of repeated injections of neoarsphenamine, the quantity of arsenic in the different organs and tissues of dogs bore no direct relationship to the quantity of neoarsphenamine introduced, nor to the number of injections given. The individual variation in the concentration of arsenic in the tissues indicated an individual difference in the capacity to absorb and excrete the drug. In the case of arsenious oxide poisoning, the liver was pre-eminently the storage place, then the kidney, spleen, cord and brain, the heart and muscles. The arsenic content of the spleen was equal to that of the brain. With neoarsphenamine, on the other hand, the kidneys contained the most arsenic, then the spleen, liver, thyroid, adrenals, heart, muscle, reproductive organs and brain. The subordinate position of the liver is explained by the rapid elimination through the bile, which has a high arsenic content.

A question of very considerable importance in connection with

the arsphenamines is their penetration into the central nervous system. After intravenous injection, the drugs naturally circulate in the blood through the brain and spinal cord, but the point of special interest in relation to the treatment of neurosyphilis is their penetration to the cerebrospinal fluid.

McIntosh and Fildes (1916) were unable to detect arsenic in the brain after the intravenous injection of arsphenamine, while more recently Rudolf and Bulmer (1923) obtained similar results. Voegtlin, Smith, Dyer and Thompson (1923), however, have shown that appreciable quantities of arsenic can be found in the brains of rabbits after the intravenous injection of arsphenamine, neoarsphenamine, sulpharsphenamine and pentavalent organic arsenicals. The average arsenic content of the cerebrospinal fluid of eight normal rabbits fed for several weeks on oats and kale was 0·69 micromgm. per gram of fluid, with a maximum of 1·55 and a minimum of 0·5 micromgm. After the intravenous injection of the disodium salt of arsphenamine in doses of 0·04 gm. per kilogram of body weight, the maximum increase of arsenic in the spinal fluid was found to occur about four hours later, and amounted to 40 micromgm. at a time when the blood arsenic was 15 and the brain arsenic 2 micromgm. The brain tissue of normal control rabbits contained an average of 0·02 micromgm. of arsenic. After the intravenous injection of neoarsphenamine in doses of 0·064 gm. per kilogram, the arsenic content of the blood, brain and spinal fluid four hours later was about 3·0, 0·2 and 30 micromgm. respectively. Although with sulpharsphenamine the amount of arsenic found in the brain and cerebrospinal fluid was less than after injections of arsphenamine or neoarsphenamine, yet sulpharsphenamine proved more efficient in destroying trypanosomes placed in the subarachnoid space.

Fordyce, Rosen and Myers (1923), in an extensive investigation of the arsenic content of the cerebrospinal fluid of syphilitics injected intravenously with arsphenamine, found arsenic in the cerebrospinal fluid of more than 80 per cent. of cases, at least during some period of their treatment, while Cornwall and Myers (1923), in a study of the cerebrospinal fluids from 151 cases of neurosyphilis, treated with intravenous injections of 0·1 to 0·2 gm.

of silver arsphenamine, claimed that arsenic was to be found in quantitative amounts in 78 per cent. of cases after two hours, and in 68 per cent. at the end of seventy-two hours. Siengalewicz (1924) found that neoarsphenamine increased the permeability of the choroid plexus to trypan blue. After the intravenous injection of arsphenamine, as previously noted, there is a rapid disappearance of arsenic from the peripheral blood-stream. In fact, immediately after injection, according to Fordyce, Rosen and Myers (1923), as much as 60·0 per cent. of the arsenic injected has disappeared from the blood. During the next twenty-four hours the arsenic content decreases, to rise again during the ensuing twenty-four hours, finally decreasing again after some days. Weiss and Raiziss (1922) found that by far the greatest amount of arsenic was eliminated in the urine during the first three days after the injection of 0·6 gm. of arsphenamine, the excretion thereafter falling to the normal level about the fourteenth day. Neoarsphenamine is more rapidly eliminated by the kidneys than arsphenamine.

In addition to the urine, a considerable quantity of arsenic is eliminated by the fæces. Clausen and Jeans (1922), for example, found five times more arsenic in the fæces than in the urine of children injected intravenously with 0·01 gm. of arsphenamine per kilogram of body weight. Underhill and Davis (1922) also found that the percentage of arsenic in the fæces was greater than in the urine, but while arsenic appeared in the urine a few hours after an intravenous injection, it was not found in the fæces till the third or fourth day. It seems probable that the greater part of the arsenic occurring in the fæces is due to excretion in the bile; some, however, may be directly excreted by the intestinal mucosa. Arsenic is also eliminated in the perspiration, saliva and milk (Fordyce, Rosen and Myers (1924)), while if there is any analogy with what occurs in acute arsenic poisoning as shown by Willcox (1922), and Althausen and Gunther (1929), a considerable portion of the arsenic of arsphenamine must also be eliminated through the hair.

It has, however, been found experimentally in rats, that the rate of excretion of arsenic after intravenous injection shows

very wide individual variation, and this seems to be one of the important factors regulating toxicity and therapeutic activity. Damage to the kidneys produces considerable variation in the rate of excretion, but this is not the only factor, although the rate of excretion of neoarsphenamine, which is more irritant to the kidneys than arsphenamine, is always more irregular than in the case of arsphenamine. The trivalent arsenoxides show the slowest rate of excretion, while the pentavalent arsenicals, with the exception of arsinic acid, are eliminated most rapidly, the arseno-compounds occupying an intermediate position. The ratio between the urinary and fæcal excretion of arsenic in these different compounds also shows great variations in otherwise normal rats. There is every reason to believe that similar variations exist among human beings, probably even to a greater degree, while the rate of elimination by kidneys and bile may be still further altered as the result of syphilis, previous medication or disease.

Although, after prolonged administration of arsphenamine, large amounts of arsenic may be stored in the organs of the body, it appears doubtful whether this stored arsenic is therapeutically active, or even capable of being rendered so after reabsorption into the blood. In fact, Wechselmann, Lockemann and Ulrich (1923) state that clinical observations indicate that stored arsenic is therapeutically inactive, the maximum spirochæticidal activity occurring during the comparatively short period in which the arsenic is present in the blood in large amounts.

This view is in agreement with the observations of Voegtlin, Dyer and Miller (1922), that ligation of both ureters increases the toxicity and trypanocidal activity of those arsenical compounds, such as neoarsphenamine or atoxyl, which normally show a rapid rate of urinary excretion. Complete obstruction of the bile-duct enhances the parasiticidal action of both arsphenamine and neoarsphenamine, although the toxicity of these compounds is scarcely increased, but the therapeutic activity of atoxyl is unaffected, probably owing to its rapid excretion by the kidneys.

In therapeutic doses, arsphenamine, given in alkaline solution,

has little or no effect on the blood pressure, the chemical constitution of the blood, or the number and character of the blood corpuscles.

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TOXIC EFFECTS RESULTING FROM TREATMENT WITH THE ARSENOBENZENE DERIVATIVES

Shortly after the introduction of arsphenamine in the treatment of syphilis, accounts began to appear of toxic reactions and deaths following the administration of the drug. In Germany, more especially, reports of fatalities became so frequent that the Government was forced to make two official enquiries, in 1914 and 1917, concerning the value of salvarsan in treatment, and the injurious effects which were alleged to follow its use. In this country the whole question of the toxic effects following employment of the arsenobenzene derivatives was investigated by a Committee of the Medical Research Council, who published their report in 1922. During the past few years, with greater knowledge of correct methods of administration, the number of cases exhibiting toxic sequelæ appears to have decreased, although in this country no exact figures are available. The figures given by the Salvarsan Committee of the Medical Research Council (1922) show that among 479,912 cases of syphilis, with a total of 2,019,935 injections, there were 1,575 cases with complications due to arsphenamine, that is to say 0·33 per cent., while the deaths numbered 175. Phelps (1929), in reviewing the reactions following the administration of arsenic for syphilis in the United States Navy, states that among 191,778 injections there were eleven fatal cases and 167 cases of reactions (0·087 per cent,), the classification of which is shown in the table :—

Reactions following Arsenic Treatment of Syphilis in the United States Navy. (*Phelps* (1929))

Drug.	Total No. of doses administered.	Mild reactions.	Severe reactions.	Fatal reactions.	Ratio all reactions to doses.	Ratio fatal reactions to doses.
Arsphenamine.	17,767	15	10	1	1 to 683	1 to 17,769
Neoarsphenamine .	167,442	110	31	10	1 to 1,109	1 to 16,744
Sulpharsphenamine .	2,081	0	0	0	—	—
Tryparsamide .	4,488	1	0	0	1 to 4,448	—
Total .	191,778	126	41	11	1 to 1,077	1 to 17,434

De Souza Coelho (1926) noted 117 cases of reaction among 3,460 injections given in Rio de Janeiro.

So various have been the ill effects following the administration of the arsenobenzene derivatives that there is no absolutely satisfactory method of classification. The following list is given by the Salvarsan Committee of the Medical Research Council (1922):

(I.) Immediate reactions, *e.g.*, diarrhœa, vomiting, pyrexia, headache, and also the vasomotor or so-called "anaphylactoid effects"—"crises nitroïdes."

(II.) Effects involving the nervous system—"encephalitis hæmorrhagica."

(III.) Effects involving the liver. Clinically these may be grouped as :—

(*a*) "Early jaundice"—coming on a few days after an injection. This is usually mild and evanescent, but may occasionally be more severe and persistent.

(*b*) "Late jaundice"—occurring not earlier than several weeks after the end of a course of treatment. This is usually more severe and prolonged than the early jaundice.

(*c*) Acute yellow atrophy of the liver—often supervening on "late jaundice."

(IV.) Exfoliative dermatitis and slighter skin reactions. The former may be complicated by broncho-pneumonia or septicæmia and end fatally.

(V.) Various rare lesions—acute hæmorrhagic nephritis, ulcerative enteritis, aplastic anæmia.

(VI.) Complications now regarded as relapses of the syphilis, or as due to spirochætal toxins, suddenly liberated by the action of the drug (Herxheimer reaction). These include affections of the nervous system, such as deafness, cranial nerve palsies, and other forms of neuro-recurrence.

In addition there are the accidents due to faulty injection, such as perivascular infiltration and necrosis at the site of injection, thrombosis or phlebitis.

Very few accurate figures have been published to show the relative frequency of these various forms of reaction. This is due

to the fact that the standards adopted in reporting reactions vary enormously.

Some observers include in their reports such ill effects as a slight rise in temperature, headache and mild vomiting, while others consider these too trivial to mention. In the case of skin affections, some include minor forms, such as urticaria and herpes labialis, others recognise only severe conditions, such as exfoliative dermatitis. Even in the case of the more striking reactions, such as dermatitis and acute yellow atrophy, there is the difficulty that these may be delayed so long a time after the last injection of the arsphenamine compound that the connection may go unappreciated. Jaundice and dermatitis are, however, the more commonly reported sequelæ, cerebral symptoms being comparatively rare except in the first German enquiry of 1914.

Immediate Reactions

Very slight but transient symptoms often occur during the intravenous injection of the drug, more frequently with arsphenamine, less frequently with neoarsphenamine or sulpharsphenamine. There is a slight metallic taste, a feeling of fullness in the head, and flushing of the face. Faintness, nausea and perspiration may be due to apprehension on the part of the patient. The more severe immediate reactions are divided by Kolmer (1926) into two types :—

(*a*) Acute physico-hæmoclastic crises.

(*b*) Vasomotor reactions.

The acute physico-hæmoclastic crisis, now rarely seen, was usually associated with the intravenous injection of an insufficiently neutralised solution of arsphenamine. During the actual injection the expression of the patient's face became drawn, hiccough or short spasmodic cough developed, and there was severe pain in the chest, with a sense of constriction and suffocation. The pulse became rapid, feeble, and later irregular. Coma generally followed, and death occurred either within a few minutes or after one or two hours. In less severe cases bronchopneumonia and pulmonary embolism developed after an interval. The cause of this acute crisis is unknown, though various explanations have been offered.

Toxic Effects After Arsphenamine Administration (Medical Research Council Special Report No. 66.)

Sources.	Total cases of syphilis.	Number of injections.	Jaundice.	Dermatitis and other skin lesions.	Cerebral symptoms.	Other complications.	Total complications.	Total deaths.	Ratio of complications to		Ratio of deaths to	
									Total No. of cases.	Total No. of injections.	Total No. of cases.	Total No. of injections.
German inquiry of 1914	74,018	288,942	8	—	60	574	642†	56*	—	—	1 in 1,322	1 in 5,141
German inquiry of 1917	265,158	1,268,946	—	—	—	—	—	70*	—	—	1 in 3,788	1 in 10,984
Leredde's table (France)												
1910 . . .	—	—	—	—	—	—	—	—	—	—	—	1 in 3,000
1911 . . .	—	—	—	—	—	—	—	—	—	—	—	1 in 8,700
1912 . . .	—	—	—	—	—	—	—	—	—	—	—	1 in 18,000
1913 . . .	—	—	—	—	—	—	—	—	—	—	—	1 in 54,000
British Mil. Hospitals :												
A . . .	4,887	39,036	13	96	1	16	126	3	1 in 39	1 in 310	1 in 1,629	1 in 13,000
B . . .	18,500	125,000	62	188	—	—	250	10	1 in 74	1 in 500	1 in 1,850	1 in 12,500
C . . .	9,758	—	—	—	—	—	—	26	—	—	1 in 375	—
Four British military hospitals combined table . . .	29,946	—	187	279	14	—	480	—	1 in 62	—	—	—
Civil centres in England in 1921 . .	77,645	298,011	38	27	2	10	77	10	1 in 1,000	1 in 3,870	1 in 7,765	1 in 29,800

* Some deaths should be deducted as having no connection with the treatment.
† Some of these complications would now be considered as not connected with arsphenamine treatment.

It has been suggested that arsphenamine is capable of coagulating the blood proteins, but of this there is no evidence.

Table Showing Number and Nature of Complications after Administration of Various Forms of "606" or "914" in Four Military Hospitals
(Medical Research Council Special Report No. 66)

	Arsphenamine.	Neoarsphenamine.	Galyl.	Luargol.	Two or more drugs	Total.
Total cases of syphilis summarised . .	12,177	6,027	676	26	11,140	29,946
Total cases without complications .	11,918	5,849	673	25	11,001	29,466
Total cases with complications . .	259	78	3	1	139	480
Jaundice . .	103	21	—	—	63	187
Dermatitis .	96	30	1	1	54	182
Erythema .	6	7	1	—	2	16
Urticaria . .	44	16	1	—	20	81
Cerebral symptoms, convulsions . .	10	4	—	—	—	14

In 1920, Kolmer and Yagle found that, *in vitro*, arsphenamine was lytic to washed red cells. In the presence of serum or other protective colloids, however, this hæmolytic action was not in evidence.

There seems, on the other hand, to be a possibility that agglutination of the red cells may occur *in vivo* as the result of the injection of acid solutions of arsphenamine, for in many of the earlier cases of fatal arsphenamine poisoning the presence of multiple emboli was noted, more especially in the lungs. Kolmer and Lucke (1921) also found, experimentally, that thromboses were present in the lungs and other organs of rabbits and rats injected intravenously with arsphenamine, though these changes did not occur to the same extent after neoarsphenamine injections; similar results were obtained by Oliver and Yamada (1922).

Intravascular agglutination is prevented, however, by injecting the arsphenamine in association with a protective colloid such as gelatine (Oliver, Yamada and Kolos (1923)).

Of more importance than the physico-hæmoclastic crisis is the acute vasoparetic reaction, the "crise nitritoïde" of French

writers, who have likened the symptoms to those produced by the inhalation of amyl nitrite. The commonest symptom-complex, occurring immediately after or during an injection, is as follows: flushing of the face, dilated pupils, slightly increased pulse rate, and some dimness of vision. If stomatitis is present the patient may complain of pain in the gums and teeth. In more severe cases these symptoms are intensified: the lips and tongue may become swollen; there is a feeling of constriction in the throat and upper part of the chest, with dyspnœa; tingling in the extremities; the conjunctivæ are congested, and lachrymation is always in evidence. The patient may fall if standing when the reaction comes on. Still more severe cases show urticaria, either limited to portions of the body, or spreading over the entire trunk and limbs. Very rarely the patient becomes partially unconscious and remains so for some hours. These symptoms usually occur in isolated cases, and often a patient is similarly affected after each injection. When a number of patients injected on any one day are affected, the fault probably lies with the qualities of the drug as prepared for injection or with the speed of the injection. Thus silver arsphenamine, unless injected slowly and in dilute solution, almost always causes vasomotor symptoms.

At times the face becomes pale, the pulse feeble, and vomiting may ensue. Another type of early reaction is characterised by rigors and headache. It is much commoner after the first than after subsequent injections. Occasionally severe vomiting, often accompanied by diarrhœa, follows a few hours after the injection, while herpes labialis not infrequently appears in such patients a day or two after the main reaction has passed off.

Although the symptoms above described may be alarming at the time, they are rarely, if ever, fatal. They may occur after the administration of any of the arsphenamines, even with the best technique, but they are most commonly provoked by improperly prepared solutions or faulty samples of the drug.

Keidell and Kemp (1924) find that cases of cardiac syphilis, with aortic insufficiency and myocarditis, are especially liable to develop alarming symptoms of sudden syncope after injections of

arsphenamine. The symptoms comprise cold sweats, gasping respiration, greenish pallor, bradycardia and low blood pressure, and are apparently associated with acute cardiac damage.

Various theories have been suggested to account for these immediate vasomotor responses to the injection of arsphenamine.

Earlier observers, finding that the injection of adrenaline relieved the symptoms, believed that insufficiency of the adrenal glands might be the cause. Observations have shown, however, that in rabbits, at any rate, arsphenamine and neoarsphenamine in therapeutic doses do not affect the adrenalin content of the suprarenals. Danysz (1916 and 1917), on the other hand, has brought forward evidence in favour of the view that arsphenamine may be precipitated in the blood vessels. It was found that all the products (mono- and di- sodium salts) of arsphenamine gave precipitates in the presence of chlorides, oxalates, carbonates, sulphates, and especially the alkaline phosphates. These precipitates, if formed rapidly in solution, may render the product extremely toxic to experimental animals. When rabbits were injected with a solution of arsphenamine, rendered opaque in less than ten minutes by adding 0·8 per cent. sodium chloride, immediate convulsions followed, resulting either in death or recovery, according to the dose employed. Widal, Abrami and Iancovesco (1920) also believe that the reactions after arsphenamine are due to flocculation or precipitation in the body fluids.

Attempts to confirm Danysz's experiments in carnivora have, however, failed, while Schamberg, Raiziss, Weiss and Kolmer and Tokuda (1920 and 1921) have found that *in vitro* concentrations of inorganic salts equivalent to those found in the blood do not precipitate arsphenamine in alkaline solutions. At present, therefore, the hypothesis of Danysz is not supported by adequate experiments, either *in vivo* or *in vitro*.

On the other hand, as was first pointed out by Kolle, Schlossberger, and Leupold (1920), the symptoms show a close resemblance to those seen in anaphylactic shock, although there is ample evidence that the immediate reaction may be produced by a first injection of arsphenamine. Unfortunately the mechanism of anaphylactic shock is as yet unexplained. In anaphylaxis, how-

ever, as Lewis (1927) has pointed out, there is evidence of the liberation of histamine or of a histamine-like substance. Many of the symptoms of the immediate reaction due to arsphenamine would also seem to be due to the liberation of histamine or a histamine-like substance.

In addition to adrenaline—1 c.cm. of a 1 in 10,000 solution, injections of pituitrine and atropine sulphate, $\frac{1}{50}$ grain, have been given for the relief of the vasomotor symptoms. Power (1927) recommends administering a minute dose of the arsenical preparation thirty minutes before giving the larger therapeutic dose.

Stokes and McIntyre (1928) have found ephedrine especially useful in guarding against the vasomotor paresis, possibly, as they suggest, because the fall in blood pressure which may occur after injections of arsphenamine is prevented.

The relationship between the acute arsphenamine reaction and the changes of an anaphylactic nature brought about by the injection of various colloids has recently received considerable attention in France.

Girard and Peyre (1926) found that by injecting animals with cæsium eosinate they could prevent the symptoms of shock resulting from anaphylaxis or from the injection of various shock-producing colloids.

The explanation of this phenomenon was found in a modification of the colloidal character of the plasma. When a drop of blood serum from an animal injected with cæsium eosinate was examined under the ultramicroscope it was found that the colloidal particles were more widely dispersed than in a normal serum. This dispersion was due to the fact that coloured colloidal particles adsorbed a number of molecules of water, as a result of which their precipitation by colloids was prevented.

Gougerot, Peyre and Bertillon (1929), believing in the anaphylactic nature of the immediate arsphenamine reactions, have therefore injected patients who suffered from reactions with 10 to 15 c.cm. of a 6 per cent. solution of cæsium eosinate at the same time as the neoarsphenamine. The reactions in twenty-one cases so treated were entirely abolished. The technique is of the simplest,

the neoarsphenamine being dissolved in from 5 to 20 c.cm. of a 6 per cent. aqueous solution of cæsium eosinate in place of distilled water. The sole difficulty at present is the cost of the cæsium eosinate and the danger of impurities which render it toxic.

So-called Hæmorrhagic Encephalitis.—In certain cases the cerebral and meningeal congestion characteristic of the vasoparetic reaction produces extremely dangerous symptoms, coming on from two to five days after the injection of arsphenamine preparations. The onset is usually sudden, the first symptoms, according to Fraser and Duncan (1923) and others, being severe headache, fever, œdema of the face, vomiting and dyspnœa. Thereafter the patient has an epileptiform convulsion with clonic spasms, followed by unconsciousness. The deep reflexes are lost, a positive Babinski sign, squint and continued convulsions indicate the involvement of the central nervous system. Coma gradually deepens, there is incontinence of urine and fæces, and death frequently occurs within forty-eight hours of the onset of symptoms.

Such cases have been met with after the use of various arsphenamine preparations, and so frequently have they been recorded that it would seem that they constitute about one-half of the so-called "salvarsan deaths." It must, however, be remembered that owing to the striking character of the symptoms, anyone seeing them for the first time would wish to report them.

Hæmorrhagic encephalitis, it is generally agreed, most commonly occurs during the secondary stage of syphilis, though it must be remembered that the majority of syphilitics are in the secondary stage when they first come under treatment. While a few cases have been described after a single injection, the majority have occurred after the second injection.

Examination of the brain for arsenic in these cases has given such variable results as to afford little help in the solution of the problem. In some analyses no arsenic has been found, in others it has been present in abundance.

The best account of the pathology of the condition is given by von Marschalkó and Veszprémi (1912), whose description was founded on a single but very typical case. Microscopically hæmorrhages were observed in different parts of the brain, some-

times punctiform, sometimes larger. The latter were regarded as being due to aggregations of smaller hæmorrhages. Most of the lesions were in the pons varolii, they were less abundant in the corpus callosum, temporal lobes and lenticular nuclei. There were no signs of softening or inflammation. The microscopic changes may be summarised as follows :—

(i.) Numerous small hæmorrhages : no evidence of softening or breaking down of the brain tissue.

(ii.) Capillaries were occasionally filled with hyaline thrombi. Some larger vessels contained red blood corpuscles so aggregated together as to be no longer individually recognisable. Other vessels exhibited typical parietal thrombosis, not completely obliterating the lumen, but still allowing a partial blood flow through the centre. The thrombus consisted partly of blood platelets, partly of these combined with fibrin, and contained leucocytes in varying numbers.

(iii.) The lungs, spleen, kidneys, liver and stomach were all hyperæmic, but the blood vessels were free from thrombi.

Lissauer (1917), who also described the pathological changes in hæmorrhagic encephalitis, emphasised the presence of thrombi in the cerebral capillaries which were surrounded by small zones of necrotic brain tissue. The presence of these small thrombi is apparently evidence of damage to the capillary endothelium by arsphenamine, either directly or indirectly, for von Marschalkó and Veszprémi (1912) found that when rabbits were injected intravenously with 0·11 to 0·12 gm. of arsphenamine per kilogram of body weight, death occurred in from two to two and a half days. In these animals symptoms identical with those of encephalitis hæmorrhagica occurring in man were seen, and the animals died after repeated convulsions. Moreover, the brain showed similar changes, consisting of punctiform hæmorrhages and hyaline thromboses in the capillaries without any inflammatory change. Although hyaline thrombosis of capillaries in organs other than the brain has not been observed in cases treated with arsphenamine, there is evidence pointing to damage of capillary endothelium elsewhere in the body. Jersild (1918), for instance, in a fatal case of encephalitis hæmorrhagica, reported the presence of

multiple small hæmorrhages in the liver, spleen, kidneys and lungs in addition to the characteristic extravasations in the brain. Stühmer (1919) has described three cases of very considerable interest in relation to their bearing on the pathology of hæmorrhagic encephalitis, for in all there was œdema of the brain with, in two cases, acute internal hydrocephalus, but no hæmorrhage. Cases with symptoms suggesting encephalitis hæmorrhagica, but ending in recovery, have occasionally been recorded. Thus Klewitz (1918) reported one case in which all the typical symptoms, headache, convulsions, coma, unequal pupils, incontinence of urine, followed soon after the sixth injection of arsphenamine. The fluid obtained by lumbar puncture was clear and under high pressure (150–160 mm. of water). Three days later the cerebrospinal fluid contained red cells and free hæmoglobin. The patient made a complete recovery.

Hamson (1916) has recorded somewhat similar cases. Beyond venesection, the withdrawal of some 15 c.cm. of cerebrospinal fluid, and the intramuscular injection of adrenaline as soon after the development of symptoms as possible, little can be done in the way of treatment.

Various theories regarding the ætiology have been put forward. Dudley (1920), for instance, believes that the most important cause is anoxæmia. There seems, however, to be little doubt that the ætiology of encephalitis hæmorrhagica is the same as that of the acute vasoparetic reaction following on the injection of arsphenamine.

Dermatitis and Allied Reactions

Skin reactions have been reported after the use of every arsphenamine compound, although on the whole the " neo " preparations seem to be less toxic. The severity and importance of the skin reactions vary enormously, but in Great Britain and America, where encephalitis hæmorrhagica is rare, exfoliative dermatitis is perhaps the commonest fatal complication of treatment by arsphenamine preparations. There are a large variety of skin reactions, and their different characters depend chiefly on the

sensitiveness of the patient to the drug, the amount of arsenic introduced into the body, and the degree of reaction in the skin. They are all, however, manifestations of one and the same kind of reaction, each phase of which shows its special clinical characters. A patient may pass through the various stages very rapidly, so that an initial urticaria or a discrete erythema becomes in twenty-four to forty-eight hours a confluent erythema, a day or two later appears vesicular or bullous, and subsequently passes into a generalised, exfoliating dermatitis. On the other hand, all the various phases of the eruption may be seen on different parts of the body at the same time. Unfortunately, it is not possible to foretell if a given patient is likely to be unduly sensitive to the arsenical drug.

The very multiformity of the lesions forbids any definite classification. Kolmer (1926), however, suggests that the reactions may be classified as follows :—

- Mild and early skin lesions.
  - Simple erythema.
  - Urticaria.
  - Herpes.
    - simplex.
    - zoster.
  - Exacerbation of syphilitic rashes—(Jarisch-Herxheimer reaction).
  - Pruritus.
- Severe and late skin lesions.
  - Dermatitis.
    - Scarlatiniform erythema with desquamation.
    - Erythema multiforme
      - Papular.
      - Vesicular.
    - Lichen planoid.
    - Acute exfoliative.
  - Purpura.
    - Simplex.
    - Rheumatica.
    - Hæmorrhagica.
- Chronic and recurrent skin lesions.
  - "Fixed" arsenical rashes.
  - Pigmentation:
    - Arsenical melanosis.
    - Argyria.
  - Arsenical hyperkeratoses
    - Palmar.
    - Plantar.

While the incidence of the milder skin reactions cannot be accurately assessed, it seems probable that with the severer cases

the incidence is 1 in every 500 to 1,000 injections. Sulpharsphenamine has produced a somewhat higher incidence.

Harrison (1916) reported the occurrence of 124 cases of dermatitis in the course of 80,000 injections of arsphenamine to 10,000 patients. Of these skin reactions twenty-six were severe, twenty-four moderately severe, and seventy-four mild or fleeting. There were eight deaths among the severe cases. Parnell and Fildes (1919) recorded thirty-eight cases of dermatitis among 1,240 men who had received 6,588 doses of neoarsphenamine. Only one case was very severe, and no fatality ensued. Moore and Keidel (1921), in reporting the skin reactions seen at the Johns Hopkins Hospital, Baltimore, during the period 1914–1920, met with twenty-one cases in patients to whom approximately 47,000 injections of arsphenamine drugs had been given. Two non-syphilitics, treated with arsphenamine, also developed dermatitis. Skin reactions were found to be three times commoner in the white than in the negro races. Among the cases of exfoliative dermatitis five died, giving a death rate of 27·7 per cent. Stokes and Cathcart (1923) reported thirty-eight skin reactions of various types in the course of approximately 44,000 injections of arsphenamine at the Mayo clinic. Of these thirty-three were studied in detail, and nine were classified as severe, twelve as moderate, and twelve as mild dermatitis. There were two deaths in the group of nine severe cases.

Sulpharsphenamine, according to Belding (1924), may give an incidence of skin reactions as high as 16 per cent.

Parnell and Fildes (1919) find that 48 per cent. of the skin reactions occurred after the third dose of the drug, while Brown (1922) also noted that the greatest number occurred after the third injection.

Forms of Eruption.—(i.) *Erythema.* As already mentioned in connection with the acute vasoparetic reaction, an erythematous rash is sometimes observed during or immediately after an injection of one of the arsphenamines. Sometimes this rash results in an urticaria, but more commonly it disappears together with the other symptoms. The most usual form of skin reaction is an erythema, which varies greatly in intensity from a very mild redness

to a deep red with a slightly purple tint. The eruption blanches upon pressure, and appears first on the flexures of the limbs and trunk. In very acute cases there is a petechial or hæmorrhagic element, but generally the erythema is either morbilliform, scarlatiniform, erysipelatoid, or less commonly of a blotchy, indeterminate character. The morbilliform type is the most frequent, while the individual spots either remain fairly discrete, or become confluent as in confluent measles. When this occurs there is usually some suffusion of the conjunctivæ and œdema of the eyelids. The general appearance may closely simulate a copaiba rash.

The degree of infiltration of the skin varies considerably, but in acute cases is always present to a certain degree. Sometimes there is a small discrete papular rash, though a large urticarial type is more frequent. The papular element rarely presents the true wheal-like character of urticaria. When confined to the backs of the hands, forearms and dorsal surfaces of the feet, the eruption might pass for erythema multiforme. Sometimes the papules are entirely follicular, resembling lichen ruber pilaris, and several writers, such as Smith (1925), have described cases in which the eruption closely resembles lichen planus. Sometimes the papular eruption is accompanied by a pemphigoid eruption of large vesicles and blebs, which involves particularly the hands and feet, as in the cases recorded by Nicholas, Massia and Dupasquier (1921). Liebkind (1921) has described a nodular eruption on the face and extremities persisting for three and a half months, and leaving some pigmentation. Itching is in some cases a prominent symptom.

Labial herpes is especially liable to occur, or in other cases large areas of the body may be covered by herpetic vesicles.

Occasionally the erythema leaves a considerable degree of pigmentation behind, as in the cases of melanodermia recorded by Heller (1921), Klauder (1924), O'Donovan (1928), and others. Purpura hæmorrhagica may occur without any other manifestation, as in a case recorded by Hyman (1920), which ended fatally.

(ii.) *Exfoliative Dermatitis.*—The exfoliation is always preceded

by an acute erythematous dermatitis of varying degree. In the milder cases, with slight infiltration of the skin, in which the dermatitis does not pass beyond the erythematous stage, only the superficial layers are exfoliated in the form of very fine branny scales, whereas in the more severe cases the erythema becomes confluent over large areas and much deeper in colour, sometimes with a cyanosed tint. The infiltration becomes more distinct, being most noticeable on hands, feet and ears ; vesicles, or small bullæ, may form, and rapidly rupture, and thus produce areas of acute weeping dermatitis, marked redness of the skin, exfoliation, crusting and scaling from the drying of the exudate. The whole body may be more or less uniformly affected, or the weeping and exfoliation may be confined to certain parts. The ears, scalp, armpits, groins and the folds of skin are all commonly affected. Œdema of the eyelids, conjunctivitis, and photophobia are frequent. The skin on the palms and the soles is usually shed in large thick pieces, exfoliation taking longer in these parts than in others. In the great majority of cases the general symptoms correspond very closely to the extent and severity of the skin manifestations. Fever, headache, faucial congestion and emaciation are all present in varying degree. Secondary infection frequently takes place. The very severe cases, with universal implication of the cutaneous surface, present the appearance of an acute scaling erythrodermia, with serous crusts or raw weeping areas in places. There is a loss of hair of the scalp, eyebrows and eyelashes : the nutrition of the nails is impaired, sometimes they are completely shed, or they become thickened and distorted. The tongue becomes dry, red and furred, the mucous membrane of the mouth and pharynx congested. Stomatitis has been recorded, while Laurentier (1921) has also noted otitis media. The development of purpura, though rare, is of dangerous significance (O'Leary and Conner, 1925).

The main complications are glandular enlargements, with abscess formation in the groins and axillæ. Occasionally there may be multiple subcutaneous abscesses, or even necrosis both of skin and bones. Bronchopneumonia or œdema of the lungs may supervene, exfoliative vaginitis or diarrhœa. O'Donovan (1928)

records a case of pulmonary embolism from a thrombus in the iliac veins.

Albuminuria is inconstant, and may or may not be present even in extensive cases. When recovery occurs convalescence is protracted, the skin becomes thin and atrophic, and usually assumes a red-brown pigmented tint. Fraser (1919) and others have described during convalescence numerous sterile non-inflammatory swellings in the subcutaneous tissues.

(iii.) *Chronic Skin Lesions.*—The most interesting of these are the so-called "fixed arsenical exanthemata," described by Guttmann (1921), Stokes and Cathcart (1923), Klauder (1924), and many others. In these cases a local urticarial patch on some portion of the body tends to appear afresh after each injection of an arsphenamine preparation, with the formation of one or more sharply defined, slightly elevated smooth plaques. In some cases the plaque becomes chronically inflamed, with the production of a scaly, eczematous, lichenified patch. Very rarely hyperkeratosis may be found on the palmar or plantar surface. Kogoj (1923) has reported a case of argyria in a girl treated with silver arsphenamine.

(iv.) Raynaud's syndrome with gangrene, though exceedingly rare, has been recorded by Nicolas, Massia and Dupasquier (1921). The doses of neoarsphenamine were small, 0·15 gm., 0·15 gm., and 0·3 gm., but the syndrome was sufficiently severe to involve the extremities, nose and ears. The symptoms became progressively worse until the terminal phalanges of most of the fingers were gangrenous.

(v.) Harbitz (1927) has recorded the development of a fibrosarcoma in the subcutaneous tissues at the site of injection of arsphenamine.

The Pathology of Arsphenamine Dermatitis

In general the post-mortem findings in all fatal cases of exfoliative dermatitis are very similar, and consist of innumerable hæmorrhages throughout all the organs, more especially in the lungs, gastro-intestinal tract and kidneys. The bone-marrow.

as pointed out by Moore and Keidel (1921), may also be aplastic, a finding which is in agreement with the characteristic blood picture which consists of a leucopenia with decrease in the polymorphonuclear leucocytes, well-marked eosinophilia, and an increase in the large mononuclear cells. The histological findings in the skin have been described by Kyrle (1927).

Many theories have sought to explain the occurrence of dermatoses after arsphenamine administration.

There is now, however, general agreement that the lesions are not due to an intensification of syphilitic dermatitis (the Jarisch-Herxheimer reaction), but are the direct effects of the arsenical drugs employed. Cases of dermatitis have occurred in non-syphilitic patients, who have been treated with arsphenamine, while Sicard and Roger (1918) have also found that cases of general paralysis of the insane treated with arsphenamine eventually develop symptoms typical of exfoliative dermatitis.

Stokes (1920) believes that an exacerbation of focal bacterial infection may precipitate the occurrence of the skin lesions, and that possibly an allergic instability may result either from the continued absorption of bacterial proteins from a focus of infection or from the sudden liberation of such proteins as the result of a sudden stimulation by the arsphenamines.

The hypothesis of a natural or acquired hypersensitiveness to arsenic has also been brought in to explain the arsphenamine dermatoses. Such hypersensitiveness undoubtedly does occur, for Klauder (1922) has recorded the case of a physician who had received an intracutaneous injection of 0·1 c.cm. of either a 1 in 100 or 1 in 1,000 solution of arsphenamine. Some eight months later, whenever he inhaled minute amounts of arsphenamine or neoarsphenamine during the opening of ampoules, or allowed some of the solution to come in contact with his fingers, he developed asthmatic attacks, with an erythematous scaly eruption of the fingers, and on occasions an eczematoid eruption of the face. It is true that hypersensitiveness in this case could not be passively transferred to guinea-pigs by injection of the patient's serum, but such is generally the case in allergy due to drugs. Fuhs and Riehl (1928) reported three cases, a father, son and daughter, who were all sensitive

to arsphenamine applied to the skin. Blood serum from the girl, when injected into two syphilitic but arsphenamine-tolerant patients, rendered them both sensitive to arsphenamine. Stuart and Maynard (1920) found that intracutaneous injections of dilutions of arsphenamine and neoarsphenamine gave positive reactions in two out of three patients who had recovered from exfoliative dermatitis. Twenty-three other cases who had never shown any arsphenamine skin reaction were all negative.

Whatever the reasons for the development of arsphenamine dermatitis, the recent work of Osborne (1928) has conclusively shown that the skin of normal persons treated with arsphenamine contains much less arsenic than those suffering from dermatitis. By microchemical methods it was found that in arsphenamine dermatitis arsenic was deposited deep in the corium around the arterioles and capillaries, in the walls and lumen of the sweat and sebaceous glands and in their ducts, in the hair follicles and hair shafts. The arsenic was always extracellular. The amount of arsenic present was proportional to the severity of the dermatitis, and in patients treated with similar doses of arsphenamine, but showing no dermatitis, the arsenic was present in the same situations but in relatively small amounts. Treatment of arsphenamine dermatitis with sodium thiosulphate reduced the arsenic content of the skin. The distribution of the arsenic after injection of the trivalent arsphenamine preparations was in contrast to that of pentavalent arsenicals. Pentavalent arsenic appeared to have a special affinity for structures of ectodermal origin, such as the epidermis, sweat and sebaceous glands, hairs and hair follicles and relatively little affinity for the blood vessels in the corium. Osborne suggests that this difference in distribution explains the reason why pentavalent arsenicals produce mild dermatitis, keratoses, pigmentation, wrist drop and optic atropy, whereas the trivalent compounds cause severe dermatitis, hæmorrhagic encephalitis and purpura.

Prevention and Treatment of Dermatitis and Allied Reactions.—Prodromal symptoms of arsphenamine dermatitis are not common, though itching of the palms and sole, and numbness of the fingers, may be of importance.

In the actual treatment of the lesions the intravenous administration of sodium thiosulphate, first recommended by Ravaut (1920), appears to be efficient (Dennie and McBride, 1924). Intramine has also been employed in this connection, while thiosinamine has been used by Greenbaum (1924), and others.

Polyneuritis and other Nervous Symptoms

While numbness or itching of the palms and soles occasionally occur during the administration of the arsphenamine, the most alarming nervous symptom due to arsphenamine is undoubtedly polyneuritis. Fortunately this complication, which has been fully described by Beeson (1920), is very rare.

In the majority of cases the condition has followed at an interval after the development of a dermatitis, but in other instances no skin complication has preceded it. Duhot (1912) was the first to describe this condition in five cases which had received 1·2 to 1·5 gm. of the drug every two or three days until four or five injections had been given. After an interval the following symptoms and signs appeared—pain in the calves and soles of the feet, with subsequent development of œdema, erythema and desquamation. In more serious cases the plantar, Achilles and patellar reflexes were lost, and paralysis finally ensued.

Though neoarsphenamine has produced more cases of polyneuritis than arsphenamine, cases due to the latter have been recorded by Variot and Bouquier (1918) and others. A similar polyneuritis sometimes associated with exfoliative dermatitis was encountered in the well-known English outbreak of 1901 of chronic poisoning due to the presence of arsenic in beer.

Ulcerative Enteritis

Krüger (1920) has reported the case of a young female, treated in the ordinary way with salvarsan and mercury. An intense necrosing enteritis supervened, leading to perforation of the intestine and fatal acute peritonitis. The damage to the intestinal mucosa is evidently similar in nature to that met with in exfolia-

tive dermatitis, in which hæmorrhages into the intestinal wall are frequently found in fatal cases.

Damage to the Kidneys

Very few cases have been recorded where the administration of arsphenamine compounds has proved to be the actual cause of nephritis. A true syphilitic nephritis is well known to occur, especially at the secondary stage of the disease in cases which have remained untreated, and in addition the prolonged administration of mercury may also damage the kidneys. It is, therefore, difficult to exclude these two possible causes in estimating the renal damage done by the arsphenamines. Turnbull (1920), for instance, found severe parenchymatous degeneration of the kidneys in every one of six cases of death after arsphenamine, associated with jaundice, and in two of these cases hæmorrhages were also found in the renal tissues. Although, as Kolmer and Lucke (1921) have shown, single large doses of arsphenamine or neoarsphenamine may cause extensive vascular damage to the kidneys, repeated doses equivalent to those given to man cause only inconspicuous tissue alterations.

Aplastic Anæmia

Just as benzene poisoning is known to cause an aplastic anæmia, so the arsphenamines may also, in rare cases, produce an aplastic anæmia with degeneration of the bone marrow. Dodd and Wilkinson (1928), who report a case in a negro girl of eleven, review the history of twenty-three other recorded cases. The symptoms have developed after injections of arsphenamine, neoarsphenamine, sulpharsphenamine and silver arsphenamine, the number of injections varying from a long series to two. In many there was bleeding from the gums and mucous membranes, the red count varying from 4,800,000 to 80,000, and the hæmoglobin from 90 to 13 per cent. In all cases the leucocyte count was reduced, the polymorphonuclear leucocytes being most affected. As a result of this absence of polymorphonuclear

leucocytes, stomatitis and other secondary infections are common. Post-mortem there is almost complete aplasia of the bone marrow, and, in many cases, a bronchopneumonia with a poor cellular reaction.

Disorders of the liver following administration of arsphenamine preparations

Disorders of the liver following the administration of the arsphenamines have attracted considerable attention, though the intensity of the reaction varies from a transitory jaundice to acute yellow atrophy of the liver.

Three types of jaundice are usually distinguished :—

(i.) " Early " jaundice (Benign jaundice. Ictère immédiat. Frühikterus).

(ii.) " Late " jaundice (Severe jaundice. Ictère tardif. Spätikterus).

(iii.) Acute yellow atrophy of the liver—generally as the sequel of late jaundice.

This classification is not wholly satisfactory, inasmuch as acute yellow atrophy of the liver may occur at an early and benign jaundice at a late date.

(i.) Early (benign) jaundice usually commences within a few days, sometimes within a few hours, of the injection of an arsphenamine derivative. It may come on after a first injection, or suddenly appear after any one of a number of subsequent injections. As a rule there is little or no constitutional disturbance, though this does not necessarily imply that there has not been serious hepatic damage, for cases have been recorded, as by Stewart, Vining and Bibby (1919), where only transient jaundice occurred, but some months later the patient died suddenly and post-mortem examination showed typical yellow atrophy of the liver.

(ii.) Late jaundice. By this term is understood a condition of much more serious disorder of the liver, with intense jaundice, coming on usually several weeks, and often several months, after the termination of a course of arsphenamine injections, though occasionally severe jaundice begins early. Unless acute yellow

atrophy of the liver follows, the patients usually recover. Silbergleit and Föckler (1919) found that in non-fatal cases the onset of the disease was never so severe as in those that went on to acute yellow atrophy. In five out of eight patients who recovered, fever was never present at any stage. Nervous symptoms also were less severe. The liver was generally enlarged, but not the spleen; bile and usually a trace of albumin were present in the urine.

(iii.) Acute yellow atrophy of the liver following arsphenamine treatment. Many circumstances have combined to attract attention to acute yellow atrophy of the liver—the striking symptomatology, the bad prognosis, and the mystery which has always surrounded the causation of the disease as met with under a wide variety of conditions, quite apart from syphilis and arsphenamine.

With few exceptions, such as the Newcastle cases, reported by McDonald (1918), and some of the Cherryhinton cases referred to in the Report of the Salvarsan Committee of the Medical Research Council (1922), acute yellow atrophy occurs some weeks at least after the end of a course of treatment. The main clinical features, and the changes found post-mortem, of acute yellow atrophy following arsphenamine treatment differ in no important respect from those met with in other circumstances. The first and most obvious sign is jaundice, usually intense, but often afebrile at first, and apparently of little importance. Then, quite suddenly as a rule, the clinical picture changes to one of acute hepatic insufficiency, with fever, vomiting, rigor and delirium and other nervous symptoms. Coma and death almost always result. One curious fact in regard to acute yellow atrophy of the liver associated with arsphenamine administration is that though cases occur singly, yet several very remarkable groups of cases have been met with in recent years. At Ingolstadt, in Germany, Silbergleit and Föckler (1919) recorded thirteen cases of acute yellow atrophy of the liver within four months, all occurring in syphilitic soldiers, and all beginning several weeks after the end of a course of arsphenamine treatment. During 1917 a similar outbreak of cases of acute yellow atrophy occurred at Cherryhinton Military Hospital, Cambridge, resulting in the death of fifteen patients, while Mc-

Donald (1918) also reported five cases, all occurring in the same area within a period of two months during 1917. Herxheimer (1920) gave an account of a series of six fatal cases of acute yellow atrophy, all in soldiers treated with arsphenamine, and all coming on from three to eight weeks after the course of treatment had ended. Strathy, Smith and Hannah (1920) met with eight fatal cases of acute yellow atrophy in a Canadian military hospital in England during the war, among forty-seven patients showing symptoms of hepatic disorder. In these cases an intensive treatment was employed, and in some cases patients received repeated doses of arsphenamine after symptoms of arsenical poisoning had appeared.

Various theories have been suggested to account for the occurrence of jaundice and acute yellow atrophy in association with syphilis and arsphenamine injections.

The various possibilities are :—

(i.) Syphilitic infection is the sole cause.

(ii.) The symptoms are a form of Herxheimer reaction, *i.e.*, the syphilitic infection of the liver is intensified as the result of the administration of arsphenamine preparations.

(iii.) A latent bacterial infection is stimulated as the result of injection of arsphenamines.

(iv.) Arsphenamine acts on a previously damaged liver.

(v.) Arsphenamine is the sole cause.

If syphilis is the sole cause of jaundice and acute yellow atrophy of the liver, then there should have been little change in the incidence of these conditions with the general use of arsphenamine. It has long been known that jaundice, both mild and severe, can occur during syphilis. Gruner, in his book " Aphrodisiacus sive de lue venerea," published at Jena in 1789, refers to an observation of Paracelsus : " Icterus cum morbo gallico copulatus non curatur nisi subacuta materia venerea." With regard to the possibility of an increase of acute yellow atrophy in syphilis, very few accurate figures are available. Stokes, Ruedemann and Lemon (1920) state, however, that seventy cases of jaundice occurred in approximately 5,200 cases of syphilis observed in the Section of Dermatology and

Syphilis of the Mayo Clinic from August, 1916, to July, 1920. Sixty-four of the seventy cases occurred between August, 1918, and July, 1920, though the method of administration did not alter. Bodin (1921) has published somewhat similar figures: of 254 cases of syphilis treated with neoarsphenamine between 1912 and 1914, only 2, or 0·78 per cent., developed jaundice; among

Deaths from Acute Atrophy of the Liver in England and Wales in Persons over Fifteen.

(*From the Returns of the Registrar-General.*)

	Number of Deaths.						Total 15 and over.		
	15—45			45 and over.					
	Males	Females	Persons	Males	Females	Persons	Males	Females	Persons
1913 . .	7 (1)	20	27	7	7	14	14 (1)	27	41
1914 . .	11	29	40	13	10	23	24	39	63
1915 . .	2	18	20	11	8	19	13	26	39
1916 . .	16 (6)	23	39	3	12	15	19 (6)	35	54
1917 . .	17 (10)	16	33	9	13	22	26 (10)	29	55
1918 . .	19 (15)	20	39	11 (1)	11	22	30 (16)	31	61
1919 . .	14 (6)	25	39	6	5	11	20 (6)	30	50
1920 . .	14	39	54	9	10	19	23	49	72
1921 . .	18	32	50	9	6	15	27	38	65
1922 . .	10	39	49	6	15	21	16	54	70
1923 . .	13	43	56	3	6	9	16	49	65
1924 . .	11	64	75	6	11	17	17	75	92
1925 . .	7	44	51	5	10	15	12	54	66
1926 . .	4	44	48	14	15	29	18	59	77
1927 . .	4	47	51	12	11	23	16	58	74
1928 . .	6	37	43	9	15	24	15	52	67
Average per year for 3 years, 1913–15.	6·66	22·23	29	10·33	8·33	18·66	17	30·66	47·66
Average per year for 4 years, 1916–19.	16·5	21	37·5	7·25	10·25	17·5	23 75	31·25	55
Average per year for 9 years, 1920–28.	9·67	43·2	53	8·1	11	19·1	17·77	54·22	72

Note.—Figures in brackets refer to men belonging to Naval or Military services. It is not known whether these cases were syphilitic or had been treated with arsphenamine.

113 cases treated during 1919 and 1920, 2, or 1·77 per cent., and among 472 cases treated in 1921, 34, or 7 per cent. McDonagh (1918) stated that from 1906 to 1914 he had only met with one case of acute yellow atrophy in a syphilitic, whereas during the war he saw eight cases in patients treated with salvarsan.

The question of increased frequency of acute yellow atrophy in general is complicated by the difficulties introduced by the war

years, when, as is well known, certain munitions of war, especially trinitrotoluene, were the occasional cause of death from acute liver atrophy. With this proviso it is of interest to consider the Registrar-General's returns of icterus gravis and acute yellow atrophy.

From these statistics the probable inference can be drawn that in England and Wales there has in recent years been no increase in the incidence of acute yellow atrophy of the liver except during the war years in males of a certain age group, the age group which is most liable to be suffering from the earlier stages of syphilis; there has, however, been a considerable but unexplained rise in the female death rate for the ages fifteen to forty-five during the past nine years.

It seems possible that in certain very rare cases arsphenamine may stimulate latent spirochætes into renewed activity with the production of an active syphilitic hepatitis, in a manner analogous to the production of active lesions of the optic nerve or other parts of the central nervous system when antisyphilitic treatment is begun suddenly and vigorously in cases of chronic syphilis. Fabry and Wolff (1922) have described cases of this kind, and in the treatment of chronic latent syphilis of the liver the administration of repeated large doses of an arsphenamine preparation may produce active hepatic lesions.

Numerous writers have suggested that, though arsenic may be a predisposing factor, the agent actually causing the jaundice and acute yellow atrophy is an organism from some intercurrent infection. As the result of slight damage to the liver cells by arsphenamine, the invading organisms find there a suitable breeding ground and add to the hepatic damage.

In this connection the work of Opic (1910) may be recalled. With chloroform alone, the damage produced in the liver of dogs was very variable, but by the combined injection of living bacteria and chloroform all grades of hepatic damage from acute liver atrophy to cirrhosis could occur. Hurst and Hurst (1928) have obtained similar results with manganese chloride and *Bacillus coli* injections.

Stokes, Ruedemann and Lemon (1920) have suggested that the increase in the number of cases of jaundice in syphilitics recorded

by them in the period August, 1918, to August, 1920, may be correlated with the great prevalence of respiratory disease at the time.

McDonald (1918), in the Newcastle cases of acute yellow atrophy, obtained post-mortem copious growths of organisms of the coli-typhoid group from the lungs, while Fraenkel (1920), in cases of acute yellow atrophy unassociated with syphilis, isolated a hæmolytic *Bacterium coli* and a typical *B. paratyphosus B.*

It is obvious, however, that little reliance can be placed on bacteriological examinations made after death, and on the other hand Turnbull (1920), in an investigation of eight cases of acute yellow atrophy, failed to detect micro-organisms except as terminal invaders.

Thus although there is no direct evidence of bacterial infection in association with jaundice and acute yellow atrophy of the liver, the epidemic-like nature of certain outbreaks, such as that recorded by Todd (1921), suggests that the symptoms of an epidemic of catarrhal jaundice present in the surrounding population may coincide with or be intensified by the administration of arsphenamine.

The possibility arises that previous damage to the liver, either by the spirochæte of syphilis or by some other poison, may render the liver more susceptible to the toxic action of arsphenamine. The recent work of McJunkin (1928) shows, however, that therapeutic doses of arsphenamine do not add to the hepatic injury in a liver already involved in an extensive acute necrotic process, as administration of arsphenamine did not influence the course of chronic chloroform poisoning in the rabbit, and did not produce a change resembling acute yellow atrophy of the liver.

There remains, finally, the suggestion that arsphenamine alone can exert a definitely toxic action on the liver parenchyma. Certain evidence suggests that, quite apart from those cases in which hepatic damage is shown by the occurrence of obvious signs of jaundice, a certain degree of hepatic insufficiency results after every course of arsphenamine treatment. Thus Widal, Abrami and Iancovesco (1920), using the hæmoclastic reactio n

Widal, have found evidence of hepatic insufficiency without jaundice or other overt signs, eighteen and twenty-four days after the end of a normal course of arsphenamine treatment. Spence and Brett (1921) have also investigated liver function by the lævulose tolerance test. In every patient suffering from jaundice following injections of arsphenamine, insufficiency of the liver was demonstrated in varying degree. A deleterious effect on the liver could also be demonstrated in patients who had received only one or two injections of arsphenamine. Mackenzie Wallis (1923), who has employed the lævulose and blood lipase tests, obtained the following results : the effect of one or two injections of arsphenamine preparations was almost always slight, and little evidence of hepatic damage could be found at the end of a course of six injections. Three months later, however, evidence of hepatic insufficiency could be detected almost invariably, although no clinical signs of hepatic disorder could be discovered. Six months after the last injection, on the other hand, all evidence of liver damage had disappeared. Kartamischew (1924) believes that arsphenamine has a cumulative action on the liver.

These findings are in general agreement with the observations of Kolmer and Lucke (1921), that even therapeutic doses of arsphenamine produce slight structural changes in the liver. On the other hand, Greenbaum and Brown (1924), employing the phenol tetrachlorphthalein test, found that only 25 per cent. of patients who had received from 45 to 124 intravenous injections of arsphenamine or neoarsphenamine showed signs of liver insufficiency. MacCormac and Dodds (1923) were also unable to determine any injury to the liver as the result of arsphenamine treatment, employing a variety of tests of liver function. Schamberg and Brown (1924) suggest that the van den Bergh reaction may be of use in detecting the presence of early jaundice.

One of the most puzzling facts connected with the occurrence of acute yellow atrophy of the liver following neoarsphenamine treatment is the long latent period usually occurring between the last injection and the onset of the symptoms. O'Donovan (1918), it is of some interest to note, has recorded analogous cases of delayed trinitrotoluene poisoning coming on two months, six

months and ten months after all work with trinitrotoluene had ceased.

Foulerton (1921), who believes that the arsphenamines are the sole cause of acute yellow atrophy, has suggested the following hypothesis to account for the delayed poisoning. Arsphenamine is partially stored in the fat depôts of the body ; if for any reason these fat depôts are rapidly depleted, a large amount of arsphenamine is liberated in the body and the liver is attacked. Finally, in support of the view that the arsphenamines are primarily responsible for the liver damage are the cases recorded by Golay (1920), Pulvermacher (1917), Zimmern (1919), and others, in which arsphenamine administered to non-syphilitic patients has been followed by the occurrence of jaundice.

The prevention of liver injury during arsphenamine administration is a matter of very considerable importance. While there is no evidence to support the suggestion that the use of specially toxic batches of the drug or faulty methods of administration are responsible, it is possible that unwise pushing of the dosage, both as regards size and frequency, may be a factor of some importance in causing an outbreak. This cannot, however, by itself be regarded as a sufficient explanation of the tendency of severe liver affections following arsphenamine to occur in groups like small epidemic outbreaks. The possibility of a further local and at present unknown factor, acting with increased force upon a liver already damaged by arsphenamine, cannot therefore be lightly dismissed.

In the actual treatment of jaundice and acute yellow atrophy the intravenous injection of sodium thiosulphate, thiosinamine or contramine (intramine-diortho-diamino-thio-benzene) has been recommended. The true value of these drugs is difficult to assess, for while acute yellow atrophy of the liver is almost invariably fatal, cases of uncomplicated jaundice almost always recover, whatever treatment is given.

In order to prevent the occurrence of toxic reactions following the injection of arsphenamines, Lees (1925) recommends the following precautions :—

(i.) The health of the individual patient should be studied.

Cachexia, diabetes, nephritis and cardiac lesions must be treated as far as possible.

(ii.) The patient must be carefully prepared. The bowels should be opened and no food taken for at least three hours before the injection.

(iii.) In preparing the drug, shaking should be avoided.

(iv.) The intravenous injection must be given slowly.

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THE STANDARDIZATION OF ARSPHENAMINE PREPARATIONS

In order to ensure that the arsphenamine preparations placed on the market are lacking in undue toxicity, but are nevertheless of a definite therapeutic value, certain standardization tests have been introduced. Soon after the introduction of arsphenamine, Ehrlich drew attention to the chemically-uncontrollable variations in the toxicity of the product and the necessity for testing every batch for undue toxicity before it was issued for use. Every batch of salvarsan made in Germany has from the first been tested at the Georg-Speyer Haus in Frankfurt before its issue. A rapid change in therapeutic practice appearing in the years immediately following the war has led, however, to the practical disuse of the original "606" in favour of the more easily-administered "914," or neoarsphenamine, group. It therefore became essential to introduce satisfactory tests for neoarsphenamine. Unfortunately, the composition of the product, wherever manufactured, has never corresponded closely with the theoretical formula. Makers soon discovered that by slight modifications of the manufacturing process they could produce a substance which was so uniformly low in toxicity that it easily passed the official test, which became almost a formality. Many months of experience were required to reveal the fact that lowering of toxicity had been obtained at the cost of a serious weakening of therapeutic potency.

Tests for Therapeutic Standardization

The first tests on the therapeutic standardization of neoarsphenamine were carried out by Voegtlin and Smith (1920) on rats infected with trypanosomes. Trypanosome infections in rats and mice are preferable to fowl spirochætosis or relapsing fever, since trypanosomiasis usually assumes an acute course, resulting in the death of the animal. Thus the slightest curative effect of the drug can be ascertained. Many species of trypanosomes were used for these experiments. In 1920, however, Schamberg, Kolmer and Raiziss

suggested the use of *Trypanosoma equiperdum* in preference to other trypanosomes, as the results with this species were sharper and more decisive.

Briefly the method consists in first determining the number of trypanosomes in a cubic millimetre of the blood of a seed rat (by examining a definite volume in a counting chamber under the microscope) and preparing an inoculum of definite strength by so diluting the total volume of blood for injection that it will contain the desired number of organisms. A seed rat showing about 200,000 trypanosomes per cubic millimetre of blood is bled by decapitation directly into 5 c.cm. of saline solution containing 2 per cent. sodium citrate. About 0·5 c.cm. of this suspension of parasites is injected intraperitoneally into each rat, which will then have twenty-four hours later an infection of about 100,000 parasites per cubic millimetre of blood, and will die after two days if left untreated.

The trypanosomes are counted by the method introduced by Kolmer (1915), using an ordinary pipette for counting red cells. The tip of the tail is nipped off ; the first drop of blood appearing is discarded ; then the pipette is filled up to the 5 mark with blood and made up to the 10 mark with diluting fluid.

The drug to be tested is made up in distilled water so that the total volume is from 0·3 c.cm. to 0·9 c.cm. In the case of rats, the injection is made into the leg vein previously exposed (Voegtlin and Miller (1922)), but with mice it is preferable to use the tail vein. The number of trypanosomes per cubic millimetre of blood in the animal to be treated should be from 100,000 to 250,000, preferably 100,000 to 150,000. The choice of a uniform grade of infection is very important for accurate work, as was pointed out by Kolmer, Schamberg and Raiziss (1917).

The minimum effective dose is the dose of the drug required to bring the trypanosome count to negative within twenty-four hours, or, in other words, the dose of the drug which kills from 100,000 to 150,000 trypanosomes per cubic millimetre. Greater accuracy is obtained, according to Raiziss and Severac (1928), if the blood is examined for trypanosomes not only twenty-four and seventy-two hours after injection of the drug, but twenty-one days

later. In addition, a standard chemical compound should be used from time to time as a further check to guard against any drug resistance which may have appeared in the strain of trypanosome used for the test.

In this country, Dale, White, Burn, and their collaborators (1922) have somewhat modified the Hygienic Laboratory's test, as mice have been used in place of rats. The strain of *T. equiperdum* is propagated in rats, and mice are infected for the tests by intraperitoneal injections of 0·1 c.cm. of an emulsion of rat's blood in 1 per cent. sodium citrate solution in physiological saline, so diluted that the total number of trypanosomes injected is in the neighbourhood of seven to eight millions. Two days later mice are selected from the infected group showing 100,000 to 500,000 trypanosomes per c.mm. of blood. The counts are made in the usual manner with blood from the tail diluted with Toison's fluid, to which a little formalin has been added. The medicament is prepared in solution and administered by injection into the tail vein. Blood from the tail vein is examined twenty-four, forty-eight and seventy-two hours after the injection of the drug. The least dose which causes a complete disappearance of trypanosomes within seventy-two hours is termed the minimal curative dose.

The question naturally arises as to whether the action on trypanosomes can be taken as evidence of an equal action on the spirochæte of syphilis. The work of Dale and his collaborators (1922) has shown that a very close parallelism can be obtained.

Preparation.	Minimum curative dose for mice infected with *T. equiperdum* in mgm. per gm. of body weight.	Proportion of human cases in which spirochætes were detected 18—20 hours after injection of 0·45 mgm.
B3	0·015	0 out of 6
A2	0·02	0 „ 4
A3	0·02	1 „ 6
C3	0·02	1 „ 6
C2	0·03	3 „ 6
B2	0·05	9 „ 10

Tests of Toxicity

The question of the standardization of the toxicity of the arsphenamines was carefully investigated by the International Conference on Biological Standards which met in Geneva, at the invitation of the Health Organisation of the League of Nations, in September, 1925. It was resolved that the following remedies should be the subject of internationally-recognised standardization :—

1. Dioxydiamino-arsenobenzene dihydrochloride (arsphenamine.)
2. Its metallic derivatives (silver-arsphenamine).
3. Its sodium salt.
4. Dioxydiamino-arsenobenzene sulphoxylate of sodium (neoarsphenamine.)
5. Silver neoarsphenamine.
6. Sulpharsphenamine.

The tests at present in use in different countries for the standardization of neoarsphenamine are described by Dale (1925) in a memorandum presented to the International Conference. Tests on mice are official in three countries—Great Britain, Germany and Japan. In the United States of America the official test is carried out on rats, though the mouse may be used as an alternative animal. There are minor differences in the technique :—

(i.) The British Test

(i.) Into each of five mice is injected 0·3 mgm. of neoarsphenamine per gm. of body weight. If not more than one mouse out of the five dies in three days the batch is forthwith passed for issue. If more than two die, it is rejected.

(ii.) If two mice die on the first test, a second test is carried out on a fresh ampoule of the sample, each of five mice receiving 0·4 mgm. per gm. of body weight. If not more than two mice die on this dose, the batch is passed for issue.

(ii.) The German Test

Two simultaneous tests are carried out, and their rejections are additive. Three sets of five mice each receive from three different

ampoules of the sample 0·37 mgm. per gm. of body weight. Seventy per cent., presumably eleven out of fifteen, must survive.

Three similar sets of five mice each receive, again from three different ampoules, 0·42 mgm. per gm. of body weight. Fifty per cent. must survive, presumably eight out of the fifteen. A sample which fails on either of these tests is rejected.

(iii.) The Japanese Test

Ten mice are used, and two-thirds (presumably seven) must survive with a dosage of 0·27 mgm. per gm. of body weight.

(iv.) The American Test

Five mice are used, and 60 per cent. must survive a dose of 0·288 mgm. per gm. of body weight.

The Japanese and American tests are obviously slightly more lenient than the European ones. Though there are no exact data, Japanese mice are said to be rather more susceptible to arsphenamine than European mice.

Durham, Gaddum and Marchal (1929), as the result of extensive experiments, have proposed the following simplified procedure for testing neoarsphenamine :—

(i.) Ten mice, weighing 18 to 20 gm., are each injected with 7·6 mgm. Samples which do not cause the death of more than two mice can be passed without further testing.

(ii.) All samples giving a greater mortality in the first test than 20 per cent. are injected into a second batch of ten mice in the same dose. When the total mortality of both experiments is under 40 per cent. the sample is passed.

(iii.) Any sample which has killed more than eight, but not more than fifteen, mice in the first two tests, is injected into a further batch of ten mice. Samples which have killed not more than fifteen in all out of the total of thirty mice injected at the three stages are passed, the others rejected.

In the official method prescribed by the Hygienic Laboratory of the United States Public Health Service, white rats weighing from 100 to 150 gm. are employed. For each toxicity test of

arsphenamine not less than five rats are used, and at least 60 per cent. of the animals injected must survive at least forty-eight hours after injection of not less than 120 mgm. per kilogm. of body weight. For neoarsphenamine, the rats must survive seven days after an injection of 240 mgm. per kilogm. of body weight. The maximum tolerated dose for sulpharsphenamine, silver arsphenamine and silver neoarsphenamine must be not less than 300, 140 and 180 mgm. per kilogm. of body weight respectively.

It must be emphasised that toxicity in no way guarantees therapeutic activity, since highly toxic samples may be relatively inactive therapeutically. It is therefore essential that all arsphenamine preparations should be tested both for toxicity and therapeutic action before being placed on the market.

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PENTAVALENT ARSENIC DERIVATIVES

With the introduction of tryparsamide in the treatment of sleeping sickness, interest in the pentavalent arsenicals, which had been temporarily eclipsed by the arsenobenzene derivatives, became renewed, and in addition to tryparsamide itself, various other pentavalent arsenic derivatives, such as stovarsol and acetylarsan, were introduced for the treatment of syphilis.

TRYPARSAMIDE

The original observations of Brown and Pearce (1919) on tryparsamide showed that the initial lesions of experimental syphilis in the rabbit were healed, but that living spirochætes could still be demonstrated in the tissues. Owing, however, to the successes obtained with tryparsamide in the treatment of the nervous stages of sleeping sickness, efforts were made to determine whether tryparsamide had any similar action in neurosyphilis. Lorenz, Loevenhart, Bleckwenn and Hodges (1923) found that, in association with mercury salicylate, tryparsamide produced both clinical and serological improvement in early cases of general paresis. These results were confirmed by a number of other workers, such as Solomon and Viets (1924 and 1925), Lees (1925) and Wolfsohn and Leiva (1925). The last authors gave a course of eight weekly intravenous injections of 0·3 gm. each, the injections being repeated, if necessary, after an interval of six months. Dawson (1925), on the other hand, found that though in certain cases of paresis the downward course was arrested, the results of treatment with tryparsamide were not more remarkable than those obtained with other arsenic compounds.

Silverston (1926) has in certain cases combined tryparsamide with malaria therapy. Of eight cases of general paralysis treated with tryparsamide alone, two were discharged as recovered, one improved, one died, and four were unchanged. Of seven cases treated with tryparsamide and malaria therapy, two recovered, one improved, one died, and three remained unchanged. Burkes

(1928) treated forty-nine cases of neurosyphilis, and obtained clinical improvement, both physical and mental, in thirty-four, six were serologically improved but declined clinically, while nine were unimproved. Fong (1928) and Jaenike and Forman (1928) have also recorded extensive series of cases. The former obtained thirty-one remissions among forty-eight cases, but though there was definite physical and mental improvement, there was little change in the neurological symptoms, and no marked serological response. Jaenike and Forman treated 100 cases of paresis, each patient receiving about thirty-five injections of 3·0 gm. each. Improvement was manifested in from six weeks to two months: gain in weight being first seen, mental improvement following later. The results were best in twenty-six cases, in which the condition had lasted less than three months. Patients with maniacal symptoms showed especially rapid and complete return to normal. Keith and Le Marquand (1929) also reported on the use of tryparsamide in tabes, general paralysis and neurosyphilis. Five out of six cases of paresis were enabled to return to work, while seven early cases of tabes were much improved, trophic ulcers healing after resisting every other form of treatment. Gastric crises and lightning pains were unaffected.

There is thus general agreement that in early cases of general paresis and tabes, treatment with tryparsamide is of considerable value, better results being obtained than with the arsphenamines. The results when the parenchyma is involved are superior to those obtained when the blood vessels and meninges are affected, but, while clinical improvement is soon noticeable, it requires at least eighty injections of tryparsamide to render the serological reactions negative. It has, therefore, been suggested that the value of the drug is due, not to its direct spirochæticidal action, but to its general tonic action on the tissues, for, as Moore, Robinson and Lyman (1924) have shown, it is useless in primary and secondary syphilis.

It must, however, be remembered that a variety of toxic sequelæ have been ascribed to the use of tryparsamide. Silverston (1926) has reported vasomotor reactions following the injections. Within twenty-four hours of the injection there may be

rigor, fever, headache, diarrhœa, vomiting, conjunctivitis and much mental restlessness, with insomnia or even delirium.

Moore and Sutton (1926) believe that these immediate reactions are less liable to occur in patients who have received some previous antisyphilitic treatment.

Among the more remote effects of tryparsamide injections are shedding of the nails, albuminuria, dermatitis, and in a few cases jaundice. Epileptiform convulsions have also been described, but the most important complication is undoubtedly toxic amblyopia. The occurrence of visual disturbance after the administration of atoxyl has long been known, the action on the optic tract being regarded as due to the pentavalent condition of the arsenic atom. Young and Loevenhart (1924) have, however, shown that arsenicals with an amino group or a substituted amino group in the *para* position to the arsenic produce optic atrophy in the rabbit: organic arsenicals with the amino group or substituted amino group in the *ortho* or *meta* position to the arsenic produce, on the other hand, no optic nerve lesions.

Woods and Moore (1924), in a careful study of the visual manifestations in 241 patients, found that in 10·2 per cent. there were subjective disturbances, and in 5·5 per cent. objective ocular lesions. The untoward effects usually appeared by the fifth injection. Subjective disturbances consisted solely in a dazzling sensation, but on examination of the eye the only abnormality was an occasional slight degree of hyperæmia of the fundus. The objective symptoms began with the same dazzling sensation, to be succeeded by a gradual veiling of vision. At the same time examination of the visual fields showed concentric contraction of the form of the fields, the nasal upper and lower fields being especially affected. In severe cases this contraction continued, to reach a maximum in about three weeks, and in some cases permanent blindness ensued.

It must, however, be remembered that involvement of the optic nerve is by no means rare in neurosyphilis. Thus Cady and Alvis (1926) found that among 153 neurosyphilitics who were apparently normal before treatment, only eight, or 5·2 per cent., had any visual disturbance as the result of the tryparsamide injections.

Of twenty-seven patients who were originally abnormal, ten, or 37 per cent., became definitely worse as the result of treatment. It is therefore essential that all neurosyphilitics should be examined by an ophthalmologist before treatment with tryparsamide, as, if there is optic involvement, the results of the injections must be carefully watched.

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ACETYLARSAN

Acetylarsan, diethylamine *p*-hydroxy-*m*-acetylaminophenylarsinate, has not been extensively used in this country in the treatment of syphilis. Its advantages are that it can be given subcutaneously as well as intramuscularly, and that there is little or no local reaction. In France, numerous observations have been made on its action in early syphilis by Laurent (1923), Lacapère (1926) Poli-Garnier (1927), and others. In this country Lloyd (1928) has studied its curative action in seventeen cases of primary and nine of secondary syphilis. After the injection of acetylarsan, spirochætes disappeared from the primary lesions within twenty-four hours, sometimes within six hours. For men, a total of 11 gm. was administered in doses of from 1 to 5 c.cm., while women received 8 to 9 gm. In cases of primary syphilis with a negative Wassermann reaction before treatment, no positive reaction developed, while in seven out of nine cases with positive reactions the reaction became negative. Both primary and secondary lesions healed rapidly.

Toxic effects were met with in fourteen of eighty-eight cases treated. The most frequent phenomena were headache, malaise, and in some cases a rise in temperature a few hours after the injection. Vomiting, a slight Herxheimer reaction, transient albuminuria and jaundice occurred in a very few cases, and in two others there was a toxic erythema. No toxic effects involving the nervous system were encountered. Late jaundice and exfoliative dermatitis were not encountered by Chatelier, Mahoux and Valdigué (1927) in thirty-seven cases, nor by Poli-Garnier (1927), who treated twenty-one cases during pregnancy. Laurent (1923) has reported favourably on the toleration of acetylarsan in four cases of congenital syphilis under the age of one month.

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STOVARSOL

3-acetylamino 4-hydroxyphenylarsinic acid (stovarsol, acetarsone, spirocid), which was originally studied by Ehrlich and Hata (1911) and reinvestigated by Fourneau (1921), has already been discussed in connection with malaria and amœbic dysentery. Prior to its use in the chemotherapeutic treatment of these diseases, however, Levaditi and Navarro-Martin (1922[1]) had shown that it has a curative action in experimental syphilis of the rabbit when injected subcutaneously in doses of from 0·1 to 0·2 gm. per kilogram of body weight. In addition, Levaditi and Navarro-Martin (1922[2]) found that administration by mouth brought about the healing of primary lesions in the rabbit and monkey. Eighty cases of human syphilis were also treated with 1 gm. of stovarsol by mouth for seven days, followed by another course at an interval of a week, until 12 to 16 gm. had been given. In thirty cases of primary syphilis the chancre healed in from five to fifteen days: secondary and tertiary lesions of the skin and mucous membrane also disappeared rapidly, but unfortunately relapses occurred in a large number of cases.

Apart from its value as a prophylactic, comparatively few observations have been made on the curative action of either stovarsol or sodium stovarsol in syphilis. Its curative action in experimental rabbit syphilis has, however, been confirmed by Poole (1926), while Cregor and Gastineau (1927) believe that it is more easily tolerated by man than the arsphenamines.

On the other hand, numerous toxic manifestations have followed the use of stovarsol in syphilis. Bender (1927), for instance, has reported malaise, fever, œdema, jaundice, diarrhœa, albuminuria, coryza, bronchitis, and skin disorders, consisting of diffuse erythema, dryness and pruritus. Urticarial attacks are not uncommon, while Michael (1929) has recorded a case of exfoliative dermatitis.

Stovarsol has also been used in the treatment of general paralysis of the insane by Sézary and Barbé (1929), who since 1921 have treated 125 unselected cases. The best results were obtained in patients who suffered from either megalomania or psychical

disturbance. Of thirty-one cases in this category, seventeen were able to resume work and four others were benefited. The serological reactions were improved in 34 per cent. of all cases, but this improvement bore no relation to the clinical condition.

The treatment consisted of three series of injections separated from each other by an interval of about one month. The first injection in each series was 0·5 gm., the second 1 gm., and the remaining injections 1·5 gm., the injections being made three times a week until each patient had received approximately 20 gm. of the drug.

Calcium-stovarsol phosphate.—A new combination of calcium acetylaminohydroxyphenylarsinate with calcium glucophosphate was introduced in 1926 by Sabatay of the Pasteur Institute. Under the name of " realphene " it has been used by Levy (1929), who reports favourable results in syphilis. The principal use of the compound, however, is as a general tonic.

Bismuth-stovarsol is discussed in connection with the other preparations of bismuth.

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TRÉPARSOL

Tréparsol, *meta*-formylamino-*para*-hydroxyphenylarsinic acid, has also been used with success in the treatment of syphilis, as, according to Simon (1924), its action in producing a negative Wassermann reaction in primary and secondary syphilis is as rapid as that of neoarsphenamine itself. The fact that it can be given by mouth, and that its use is attended only by very slight toxic reactions, has recommended it to many workers on the Continent, though it has not been extensively used in this country. Mercier and Costel (1926) gave it in daily doses of from 0·75 to 1·50 gm. on four consecutive days, followed by an interval of two, three or four days. Slight diarrhœa was the only unpleasant result of the treatment. Meyer (1929) has, however, recorded a fatal case attributable to the use of this drug.

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BISMUTH

Although in 1889 Balzar had suggested the use of bismuth in the treatment of syphilis, it was not until 1916 that Robert and Sauton showed that bismuth compounds possess a curative action in fowl spirochætosis.

In 1921 Sazerac and Levaditi tested the action of sodium potassium tartrobismuthate in experimental syphilis of the rabbit, and in the spontaneous infection of the same animal due to *Spirochæta cuniculi*. It was found that 0·01 gm. per kilogram of body weight given intramuscularly in the rabbit caused disappearance of spirochætes from the primary lesions and rapid healing of the sores. Therapeutic results were also obtained with bismuth citrate, lactate and subgallate, although healing was not so rapid as with the sodium potassium tartrobismuthate. In curative experiments on man, intramuscular injections of an aqueous solution of the tartrobismuthate were found to cause intense pain, but when given as a 10 per cent. suspension in olive oil there was much less pain at the site of injection. Five cases of human syphilis, one primary, two early secondary, and two tertiary, were first treated by Sazerac and Levaditi (1921), each patient receiving a total of from 1·0 to 1·11 gm. of an oily suspension of the tartrobismuthate intramuscularly. The results were excellent, only two cases exhibiting slight toxic reactions as a result of the injections.

Fournier and Guénot (1922) then treated some 200 syphilitics with bismuth. In primary cases the chancres healed rapidly, and the development of secondary lesions was inhibited. Early secondary and tertiary lesions, such as gummata, ulcers and periostitis, disappeared, while serological studies showed that the Wassermann reaction rapidly became negative. The most satisfactory course of treatment was a series of from ten to twelve intramuscular injections given over a period of a month, each injection consisting of from 20 to 30 cgm., given as a 10 per cent. suspension in oil. In the treatment of neurosyphilis, Marie and Fourcade (1922) obtained but little benefit in general paralysis of the insane, but

where there were gummata or vascular involvement, bismuth was found to have a definitely curative action.

As a result of these preliminary observations, the use of bismuth became general in the treatment of syphilis, with the result that much fresh work has been carried out on the pharmacology and toxicity of the metal.

The Pharmacology of Bismuth

When applied to the unbroken skin in the form of daily inunctions, bismuth exerts but little curative action in syphilis, although cases of poisoning after the application of bismuth paste to wounds are not unknown. Absorption from the intestinal canal is also so slow and uncertain that this method of administration has been but little used in treatment, while intravenous injection is apt to lead to the development of acute toxic symptoms (Klauder, 1928). Intramuscular injection is therefore the most favoured mode of administration, the rate of absorption depending partly on the site of injection, partly on the particular salt employed. The rate of absorption has been carefully studied by means of X-ray examinations. Beinhauer and Jacob (1925) found that a single injection of the iodobismuthate of quinine was absorbed very rapidly, neotrépol somewhat more slowly, while potassium bismuth tartrate and bismuth salicylate gave a deep shadow after twelve days. Erdman (1929) studied the absorption after a series of injections. In one case thirty-one injections of bismuth salicylate in oil were given intramuscularly : eighty-five days after the last injection bismuth was still unabsorbed. In another case, twenty-two injections of potassium bismuth tartrate were given, and 204 days later bismuth was still visible by the X-rays. In a third case only eleven injections were given, as the patient developed blue gums : 435 days later bismuth was present at the site of injection. In yet a fourth case, after ten injections of bismuth sodium tartrate, complete absorption was evident in less than two months. The variation in the rate of absorption is also brought out by the observations of Montlaur (1929). Of the insoluble preparations, bismuth hydroxide was found encysted three years

after intramuscular injection, while the other insoluble preparations were almost completely absorbed after the lapse of four months. Water-soluble bismuth salts gave no shadow the day after injection, but fourteen days later a shadow was observed, doubtless the result of decomposition and deposition of reduced bismuth. The oil-soluble preparations are rapidly and completely absorbed, while Mulzer (1925) and Levaditi, Sanchis-Bayarri, Schoen and Manin (1928) have also observed the rapid absorption of liposoluble preparations.

By chemical analyses of the injected muscles, Wolfer (1922) found that 30 per cent. of the total amount of bismuth injected had been absorbed in thirty-four days. Lomholt (1929) also studied the amount of absorption of bismuth oxychloride, suspended either in water or in oil, by a special radio-chemical method. The rate of absorption varied greatly in different guinea-pigs, but in the majority of animals more than 50 per cent. had been absorbed in ten days. Small injections were absorbed relatively more rapidly than large injections. Histological studies of the muscles of rats excised five days after the injection of tartrobismuthates also showed varying degrees of reaction. The reaction produced by watery solutions was local and intense, actual necrosis of the muscles being by no means uncommon : oily suspensions, on the other hand, caused a more extensive reaction, in which there was less necrosis but more diffuse infiltration with polymorphonuclear leucocytes. Müller, Blass and Kratzeisen (1923), who examined the muscles of a congenitally syphilitic child injected with bismuth, also found at the site of injection an accumulation of polymorphonuclear leucocytes, proliferation of fixed connective tissue cells, and numerous foreign body giant cells.

The exact method of absorption of bismuth salts is unknown, though the phagocytic cells play at least a part, for, as Mayer and Baehr (1912) have shown, bismuth can be demonstrated within them by an appropriate micro-chemical technique.

Whatever the means of absorption, there is at least some evidence that the absorbed compound is spirochæticidal, for Kolle (1924) has found that when bismuth is injected subcutaneously into the ears of rabbits it is impossible to infect the animals with

syphilis until after the deposit of bismuth has been removed by amputation of the ear.

Absorption after intramuscular injection begins comparatively rapidly, for Leonard and Seibert (1928) found that in the dog, if the muscle is massaged, bismuth appears in the blood two hours after injection : without massage, the appearance of the metal is delayed twenty-four hours. Many observations have been made on the distribution of bismuth in the tissues. Leonard (1928), after varying doses of sodium potassium bismuthyl tartrate given under different conditions, found the highest concentration in the kidneys, less being present in the liver, spleen and lungs. Blood and bile showed a varying content. Lomholt (1929), using a radio-chemical method, found in guinea-pigs a large concentration in the kidneys and intestine and a moderate concentration in the liver and spleen. The skin contained only a small amount of bismuth, and the muscles still less. In the bone tissue the concentration was considerably higher. The total amount of bismuth found in the whole body (the site of injection excepted) varied from 4·68 to 13·11 per cent. of the amount injected after ten days and from 2·83 to 5·70 per cent. after seventeen days.

Of considerable interest in regard to bismuth therapy in neurosyphilis is the question of the presence of bismuth in the central nervous system. Demelin (1922) claimed that he found traces of bismuth in the cerebrospinal fluid after the intramuscular injection of 0·2 gm. of trépol. Jeanselme, Delalande and Terris (1924), however, tested the cerebrospinal fluids of thirty-one cases of syphilis treated by intravenous injections of bismuth, and failed to find bismuth in any. Nevertheless the definite improvement in the condition of the cerebrospinal fluid in certain neuro-syphilitics treated with bismuth seems to indicate that at least minute quantities of the metal must reach the central nervous system.

The placenta is permeable to bismuth, for Leonard and Love (1928) have shown that in pregnant animals the fœtus contains bismuth after the injection of lethal doses ; when the dose was sublethal only a very small amount of bismuth could be detected in the fœtal kidneys.

Although some bismuth may be stored in the liver and other organs, the greater part is slowly eliminated by the kidneys, liver and intestinal mucosa. Excretion also occurs to some extent by the salivary glands, tears, and buccal mucosa, while Fournier and Guénot (1922) detected traces in the sweat. After the intramuscular injection of bismuth the metal appears in the urine in from eighteen to twenty-four hours, while after intravenous injection its presence can be detected after four hours. Bismuth is still being excreted by the kidneys twenty to thirty days after intramuscular injection.

The metal is also eliminated in part by the fæces, the amount being roughly one-half of that present in the urine. A part of this fæcal bismuth is apparently due to excretion in the bile, the other to direct elimination by the intestinal mucosa, more especially by the cæcum and ascending colon.

It seems doubtful whether bismuth is excreted in the milk of lactating women.

When bismuth is injected intravenously, even in small doses, there is always a fall in blood pressure, due in part to a depressant action on the vasomotor centre, but chiefly to a direct effect on the heart. The cardiac changes recently described by Masson (1926) consist of slowing and weakening of the heart-beat, with irregularities, of which the most common is heart-block. With larger doses there is increased depth and rapidity of respiration and extreme exhaustion, with inability to move the limbs.

The action of bismuth thus seems to be exerted chiefly on the medulla and cord, and to a less extent on the heart muscle.

According to Betz (1923), certain blood changes may follow the injection of bismuth. As a rule there is a mild and temporary leucocytosis following each injection, and, especially at the beginning of a course of treatment, an increase of polymorphonuclear leucocytes and eosinophils; in the later stages an increase of lymphocytes occurs. In some cases the erythrocytes are increased in number, though in a few instances there is evidence of red cell destruction with anisocytosis, but no poikilocytosis. Punctate

basophilia may occasionally occur after repeated injections of bismuth.

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Preparations of Bismuth

Since its introduction in the treatment of syphilis a very large number of bismuth preparations have been placed on the market, consisting either of the metal itself or of one of its salts. Unfortunately there is as yet no agreement as to the most suitable type of preparation for general use, and the multiplicity of compounds of unknown value is much to be deplored. The more widely-used preparations are :—

(I.) Metallic bismuth, originally used by Sazerac and Levaditi (1922), and obtained by reduction of sodium potassium bismuthyl tartrate.

Suspensions in isotonic saline, in glucose solution, oils or fatty bases have been used.

Various preparations of colloidal bismuth have also been employed.

(II.) Organic preparations of bismuth.

(i.) Alkaline bismuthyl tartrates are usually divided into two groups : (*a*) " Neutral ". compounds, termed " bismuthyl tartrates," or " carbobismuthates," which vary in their solubility in water. They are prepared by dissolving bismuth oxy-tartrate in alkali, the alkali bismuthyl tartrate being obtained as a powder by evaporation or precipitation with alcohol.

(*b*) " Acid " preparations, frequently described as " bismuth tartrate soluble." They are obtained by treating bismuth hydro-oxide with a solution of the alkali acid tartrate, filtering and evaporating.

The " neutral " compounds used for injection are :—

Sodium bismuthyl tartrate, neutral (sodium tartrobismuthate).

Potassium bismuthyl tartrate, neutral (potassium tartrobismuthate).

Sodium potassium bismuthyl tartrate (sodium potassium tartrobismuthate).

The last compound in 10 per cent. suspension in olive oil is the tartrate usually administered ; 0·2 gm. is injected intramuscularly until 2 to 3 gm. have been given.

Pautrier (1923) employs a suspension of potassium bismuthyl tartrate in a medium containing sulphur.

(ii.) Bismuth salicylate, which is a white powder insoluble in water, alcohol and glycerine, has been employed by Jeanselme, Chevalier, Pomaret, Blamoutier and Joanon (1922).

(iii.) Bismuth subgallate (dermatol).

(iv.) Sodium bismuth thioglycollate (Gruhzit and Sultzaberger, 1927), a water-soluble preparation.

(v.) Bismuthyl gluconic acid and the sodium salt of bismuthyl saccharic acid have been used in 10 per cent. watery solution by Browning, Cohen, Gulbransen, Phillis and Snodgrass (1927).

(vi.) Bismuth camphor carboxylate, suspended in oil. One cubic centimetre corresponds to 0·05 gm. of metallic bismuth.

(vii.) Bismogenol, a compound of bismuth and hydroxybenzoic acid (Ritter and Karrenberg, 1929), and milanol, which is said to be a compound of bismuth and trichlorbutylmalonic acid, are German preparations, the compositions of which are not disclosed.

(III.) Inorganic preparations of bismuth.

(i.) Bismuth hydroxide suspended in water and glycerine.

(ii.) Bismuth hydroxide suspended in oil containing a minute trace of mesothorium bromide.

(iii.) Bismuth iodide dissolved in a solution of potassium iodide.

(iv.) Bismuth oxychloride.

(IV.) Alkaloidal iodobismuthates.

A large number of these compounds are described by Levaditi (1924), of which the most commonly used is the iodobismuthate of quinine (quinby).

Bi-quinyl is a commerical preparation of the iodobismuthate of quinine and bismuth oxychloride.

(V.) Liposoluble bismuth compounds. In place of suspensions of bismuth and its salts in olive oil, various bismuth compounds soluble in lipoids have been prepared. Thus embial (540 D Merck), the composition of which has not been disclosed, has been employed by Mulzer (1925), Dahmen (1925), Plaut (1925), and others, but is said to be but slowly absorbed after injection and to cause pain at the site of injection. Spirobismol soluble is a double iodide of quinine and bismuth associated with lecithin.

Levaditi, Sanchis-Bayarri, Schoen and Manin (1928[1]) have studied two lipoid soluble compounds, bismuth camphorcarboxylate and basic bismuth α-carboxyl thyl-β-methyl-nonoate.

The chemotherapeutic index of the latter compound is given as 1 : 33 for rabbit syphilis, and its absorption is said to be rapid. Not only does the primary lesion heal rapidly, but the spirochætal infection is totally eradicated, since the animal is again susceptible to infection with *Spirochæta pallida*. Levaditi and his colleagues (1928[2]) have also studied the effects on rabbits of tricetylamine iodobismuthate.

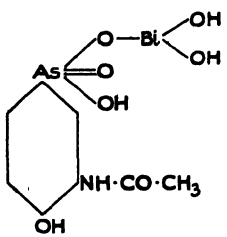
(VI.) Compounds of bismuth and organic arsenic.

(i.) Bismuth arsphenamine (bismarsen) is a bismuth derivative of arsenobenzene methylene sulphinic acid, and contains approximately 13 per cent. of arsenic and 24 per cent. of bismuth (Raiziss (1927)).

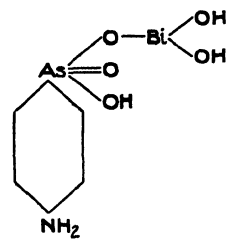
(ii.) Bismuth stovarsol (bistovol) is the basic bismuth salt of *m*-acetylamino-*p*-hydroxyphenylarsinic acid, and was first prepared by Levaditi (1925). It is used as a 10 per cent. suspension in oil, and contains 41 per cent. bismuth and 15 per cent. arsenic.

(iii.) Bismuth arsanilate contains 45·5 per cent. bismuth and 16·3 per cent. of arsenic.

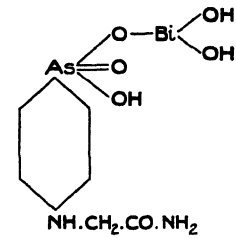
(iv.) Bismuth tryparsamide contains 40·5 per cent. bismuth and 14·5 per cent. arsenic.



BISTOVOL



BISMUTH ARSANILATE



BISMUTH TRYPARSAMIDE

Bismarsen was first used clinically by Stokes and Chambers (1927), who found that the spirochæticidal action was slow and the healing effect still slower than that of other arsenicals.

Two intramuscular injections a week of 0·2 gm. each for ten

weeks constituted a course, four courses being given with intervals of two weeks. In early syphilis the ultimate effect of this compound appeared to be equal, if not superior, to that of modern intensive treatments with other drugs, the ultimate proportion of negative serological findings being high. Toxic reactions were few, and there was a definite tonic effect. In late neurosyphilis bismarsen was not effective. O'Leary (1928) reports having given 1,145 injections of bismarsen to eighty-five patients. In cases of acute syphilis receiving thirty-two injections the results were satisfactory, but in the so-called Wassermann-fast type there was little serological improvement. Practically no pain was produced by the injections, though in one case a sterile abscess developed, and in another severe exfoliative dermatitis. Tobias (1928), who has used bismarsen in tabes dorsalis, reported some improvement in the lightning pains, but no change in the ataxia. There was no amelioration of the optic atrophy, and no effect on the spinal Wassermann reaction.

Bistovol, which was first studied experimentally by Levaditi (1925), was used by Fournier and Schwartz (1925) in the treatment of twenty patients with chancres or secondary symptoms. The spirochætes were said to have disappeared from the chancres and mucous patches in from twenty-four to forty-eight hours after a single injection. The primary or secondary lesions cicatrised rapidly, and adenitis, rashes and general symptoms disappeared with equal celerity. There was also a marked diminution or complete disappearance of the complement-fixation reaction. Anwyl-Davies (1927) was unable to confirm these results. The injections were painful, and spirochætes survived doses of even 0·6 gm. in three days. Levaditi and Fournier (1928), therefore, employed a new and stable solution of bistovol (H 13), which in rabbits infected with syphilis has a chemotherapeutic index of 1 : 35. In man this preparation could be administered intramuscularly without pain, and caused rapid healing of primary and secondary lesions. Bistovol was also given by mouth, either in solution or in solid form, and was well tolerated in doses of 2 gm. daily for a period of eight to eleven days. Even with this very considerable dosage, corresponding to 0·82 gm. of bismuth

and 0·3 gm. of arsenic per day, the only disturbance worthy of note was a slight erythema in two cases. Cases of primary and secondary syphilis in which the serological reactions were strongly positive and spirochætes were present showed rapid disappearance of the spirochætes and prompt healing of the lesions, while the serological reactions were improved.

Bismuth arsanilate was prepared by Shircore (1926), who employed it in the treatment of yaws and syphilis in Tanganyika Territory. In 538 cases of tertiary and congenital syphilis 75 per cent. showed complete healing of the lesions after six injections. Stomatitis was very rarely encountered. Levaditi (1928) used bismuth arsanilate in experimental syphilis in the rabbit. When administered intramuscularly, it was found to exert a definite curative action, although therapeutically it was not quite so active as bistovol. In man bismuth arsanilate suspended in oil can be injected intramuscularly without pain.

Bismuth tryparsamide was tested by Levaditi (1928) in natural spirochætosis of the rabbit due to *S. cuniculi*. The spirochætes disappeared on the second day after the intramuscular injection of 0·1 gm. per kilogm., and the lesions were cured on the twelfth day. Its curative action was, therefore, somewhat slower than that of bistovol and bismuth arsanilate.

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The Toxicity of Bismuth

The symptoms of chronic bismuth poisoning have been known for some years, but have excited renewed interest in view of the extended use of bismuth in the treatment of syphilis.

The first symptoms of chronic poisoning are loss of appetite with nausea, salivation, vomiting and diarrhœa. Later symptoms are stomatitis, with ulceration of the gums, tongue and buccal mucosa. Weakness, slowness and inco-ordination of movement follow and, except in a very few chronic cases, tetanic convulsions. The urine contains albumin and casts. The weakness gradually deepens into complete paralysis and death occurs. Besides the stomatitis and ulceration of the mouth, the post-mortem appearances in chronic bismuth poisoning in animals consist in congestion and necroses in the kidney and congestion and a black discoloration of the cæcum and upper part of the large intestine. The pigmentation is limited very exactly by the ileocæcal valve, and extends throughout the thickness of the bowel wall. In places the mucous membrane may be necrosed, or ulcers and hæmorrhages may be seen. The black coloration is due to a deposit of bismuth sulphide in the mucous membrane and in the capillary vessels and lymph spaces. In rabbits, according to Masson (1926), there is not infrequently distension of the right side of the heart, with fluid in the pleural and peritoneal cavities.

Histological examination of the tissues of animals suffering from bismuth poisoning has been made by Lucke and Klauder (1923), who have found that after the administration of the tartrobismuthates the most pronounced changes are present in the kidneys where the epithelium of the convoluted tubules is chiefly involved. All types of degeneration are noted, from cloudy swelling to extreme necrosis and the deposition of calcium. The glomeruli are relatively unaffected, though the glomerular capillaries frequently contain masses of agglutinated and partly hyalinized erythrocytes.

The lesions in the liver are much less conspicuous but very slight fatty infiltration and small areas of focal necrosis invaded

by a few mononuclear and polymorphonuclear leucocytes are found in most animals.

The toxic effects resulting from the administration of bismuth in the treatment of human syphilis are very similar to those occurring in animals. Although very few fatal cases of bismuth poisoning appear to have followed the intramuscular injection of bismuth compounds, yet sudden deaths, according to Magnus (1924), have followed intravenous injections; the reasons for this excessive toxicity are unknown. Munck (1927), however, records a fatal case after the administration of 5·13 gm. of bismuth during a period of eleven months. Jaundice and stomatitis were followed by delirium, collapse and death. A number of toxic phenomena have, however, been described in man following the intramuscular injection of bismuth. Of the general toxic effects, the most common are aching in the muscles and joints, lassitude and loss of appetite. In a few cases a slight rise in temperature has been noted, while after prolonged administration of bismuth there is loss of weight and asthenia. Vasomotor paresis has not been described and while jaundice, according to Hudelo and Rabut (1924), has occurred, it is extremely rare. Skin eruptions of various types have been recorded, purpura and a scarlatiniform erythema, urticaria (Galliot (1923) and Lepinay (1923)), lichenoid eruptions (Nicolas, Gaté and Lebeuf (1923)) and exfoliative dermatitis (Pinard and Marassi (1922)). Slight Herxheimer reactions have also been described, while Critchley (1926) has recorded a case of polyneuritis. The only signs of damage to the kidneys are polyuria, which has been observed in exceptional cases by Fournier and Guénot (1922), and albuminuria. Hudelo and Rabut (1924) have only observed albuminuria four times in 10,000 injections of bismuth. Galliot (1927), however, has recorded a death from hæmorrhagic nephritis following bismuth injections, while Aubertin and Destouches (1927) also saw a fatal case of stomatitis and acute nephritis as a result of the injections.

The most troublesome of the sequelæ of bismuth therapy is the development of gingivitis and stomatitis. The first symptoms are a disagreeable taste, coated tongue, malodorous breath, and a blue line along the margin of the gums. The blue line first appears

on the gingival margin of the incisor teeth of the lower jaw, both anteriorly and posteriorly, and spreads if preventive measures are not taken. It is indistinguishable from that seen in lead poisoning. Blue spots may also appear under the tongue and on the mucosa of the cheek. The appearance of a blue line is not necessarily an indication for withholding bismuth treatment, however, but rather for careful oral hygiene. Gingivitis, on the other hand, may follow the appearance of the blue line. Ordinarily this is a mild localised inflammation, but in persons with soft, spongy gums associated with tartar deposits, the gingivitis is apt to be more severe and generalised owing to the fact that fusiform bacilli and spirochætes add to the irritation and inflammation. The histology of bismuth gingivitis has been studied more especially by Azoulay (1922), who finds that the bismuth is brought to the gums by the blood capillaries, the walls of which are packed with granules of bismuth sulphide, particularly at the tips of the papillæ. The partial obliteration of the lumina of the capillaries and the inflammatory reaction around the insoluble bismuth eventually result in the formation of minute ulcers. Secondary bacterial infection then sets in.

Milian (1922) and others have recorded cases of severe enteritis and ulcerative colitis following bismuth therapy, but these complications are of extreme rarity.

The only toxic complication which may require active treatment is the stomatitis. Palazzi (1922) recommends the local application of a strong solution of methylene blue, while intravenously neoarsphenamine has an undoubted influence upon the secondary bacterial invaders. As a rule, oral hygiene and injections of sodium thiosulphate rapidly control the lesions.

Local reactions at the site of injection of bismuth compounds occasionally occur, even with the most careful technique. The common type is a hard and painful swelling with considerable infiltration, which sometimes, though rarely, undergoes suppuration. Freudenthal (1924) has, however, described a somewhat different type of reaction. Following the intragluteal administration of bismuth salicylate there were observed on three occasions general malaise, reddening and swelling of the buttock, with pain

radiating into the leg. Around the site of injection there was a bluish discoloration having a network-like appearance. Necrosis eventually occurred. Similar cases have been described by Barthélemy (1926), Gammel (1927), Martins de Castro (1929), and others. Freudenthal was able to examine one of his cases histologically, and found the cutaneous arteries blocked with needle-shaped crystals of bismuth salicylate. Nicolau (1925) found that, experimentally, in animals similar changes could occasionally be produced after the intramuscular injection of quinine bismuth iodide. Diffuse painful swelling of the muscle and a bluish-red net-like discoloration with subsequent necrosis were found to be due to emboli of the injected drug in the cutaneous arteries. When insoluble bismuth preparations were injected into the artery of the ear in rabbits necrosis invariably occurred.

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The Treatment of Syphilis by Bismuth

Although no exhaustive comparison has yet been made of the value of different bismuth preparations in the treatment of human syphilis, nevertheless sufficient time has now elapsed for a correct estimate of the relative value of bismuth as compared with other antisyphilitic remedies.

Kolmer (1926) has shown that, as determined by their curative action in rabbits experimentally infected with *S. pallida*, bismuth preparations show a somewhat lower chemotherapeutic index than the arsphenamines.

Comparison of Therapeutic Index of Bismuth and other Antisyphilitic Drugs in Rabbits.

Preparation.	Minimum curative dose in mgm. per kilogm. of body wt.	Maximum tolerated dose in mgm. per kilogm. of body wt.	Chemotherapeutic index.
Potassium sodium bismuth tartrate . .	0·050	0·200	1 : 4
Potassium iodide .	0·300	0·310	1 : 1
Tryparsamide . .	0·400	0·900	1 : 2
Sulpharsphenamine .	0·022	0·300	1 : 14
Arsephenamine . .	0·012	0·120	1 : 10
Neoarsphenamine (intravenous) . . .	0·020	0·350	1 : 17·5
Flumerin . . .	0·030	0·030	1 : 1
Mercuric salicylate (intramuscular) . .	0·005	0·005	1 : 1

At the present time it seems doubtful, therefore, whether it is justifiable to treat syphilis with bismuth alone, for though bismuth is usually efficacious in curing the clinical manifestations of the disease, its action in eradicating the syphilitic spirochætes is more uncertain than that of the arsphenamines.

Lees (1927), as the result of some 100,000 injections of bismuth given to 6,000 cases of syphilis, has reached the following conclusions: in primary syphilis, and in the mucous lesions of the secondary stage, spirochætes are not as rapidly destroyed by bismuth as by the arsphenamines. After treatment with the arsphenamines, no living spirochætes can be detected twenty-four hours later, whereas after treatment with bismuth, living spirochætes do not disappear for three or four days. Levaditi and Fournier (1928), on the other hand, state that the liposoluble bismuth preparations act quite as rapidly as the arsphenamines in destroying spirochætes. The healing effect on secondary lesions and primary sore is almost as rapid as that of the arsphenamines but in its effect on the serological reactions bismuth would seem to be definitely less efficient than arsenic preparations. The development of a positive reaction in a case of primary sero-negative syphilis treated with the arsphenamines is of great rarity, but the bismuth treatment of sero-negative cases sometimes results in a weakly positive reaction. In primary and secondary syphilitics who are sero-positive, the proportion rendered negative after one month's treatment with the arsphenamines is greater than after treatment with bismuth for a similar period. Finally, in those cases of resistant Wassermann reaction which are uninfluenced by either arsenic or mercury, bismuth also is of but little use. On the other hand, bismuth has certain advantages over mercury and arsenic, apart from the treatment of those cases which definitely fail to react to either of these latter drugs. Bismuth has an undoubted tonic action, it is not so depressing as mercury, causes less pain after intramuscular injection, and is much less likely to be followed by stomatitis and other toxic sequelæ. Old and debilitated patients seem to tolerate bismuth in appropriate doses rather better than arsenic, while bismuth can also be given for much longer periods than either mercury or arsenic.

In tertiary lesions bismuth preparations have a rapidly curative action, and tend to heal cases which are resistant to arsenic and mercury. On the other hand, Sutton (1927) and others have described cases which have been entirely resistant to bismuth. Bismuth appears to be specially effective in healing syphilitic lesions of the eye.

In neurosyphilitics bismuth has a somewhat uncertain action. Cases of general paresis are but little affected, and while in tabes gastric crises and urinary incontinence may be ameliorated, the ataxia shows scant improvement.

In congenital syphilis the action of bismuth is more marked. Müller (1922), who treated six cases, obtained a good response in all. Cajal and Spierer (1923) found that bismuth acted much more rapidly than mercury, while splenic enlargement disappeared more quickly than after treatment by arsphenamine. Genoese and Mazzacuva (1923) found that on the whole bismuth was not so rapid as arsphenamine, but was much more certain in its action. Finally, Wright (1928) reported a series of forty-seven late congenital syphilitics who had all received previous treatment with neoarsphenamine or mercurials. As the result of thirty injections of neoarsphenamine, four out of the forty-seven gave negative Wassermann reactions, but after bismuth treatment twenty-six became negative, although four subsequently relapsed. Proof of the sterilizing action of bismuth in syphilitic infections is found in the fact that reinfection after bismuth therapy has undoubtedly occurred.

Various attempts have been made to compare the action of neoarsphenamine in association with mercury and in association with bismuth. Thus Smechula (1925) treated two series of 100 cases, one with neoarsphenamine and mercury salicylate, the other with neoarsphenamine and bismuth. The first combination produced more rapid clinical and serological results, but the effects of the latter treatment were more permanent. Lees (1927) prefers bismuth to mercury in combination with the arsphenamines, but Harrison (1929), using mainly the oxychloride of bismuth suspended in glucose, has failed in a small number of cases to find it superior to mercury.

It is obvious that while the value of bismuth in the therapy of syphilis is undoubted, much remains to be learnt in regard to the relative values of the various preparations. Absorbability would seem to play a very important part in determining therapeutic activity, and absorbability in its turn depends, as von Oettingen, Todd and Sollman (1927) have pointed out, not only on the chemical constitution and physical characteristics of the compound, but upon the medium in which it is suspended.

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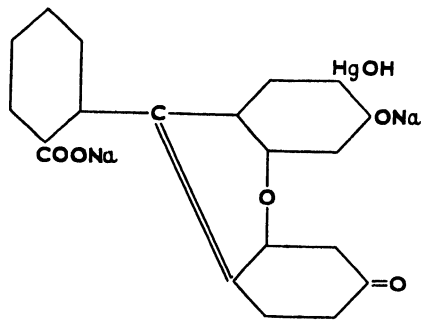
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MERCURY

While attention has been largely focussed on the curative action of arsenic and bismuth in syphilis, comparatively few fresh studies have been made on the antisyphilitic action of mercury. White, Hill, Moore and Young (1922), however, developed a new mercurial for intravenous use, and have studied its effect in human syphilis and in experimental syphilis in the rabbit. Flumerin is the disodium salt of hydroxymercuric fluorescein, and has the structural formula :



FLUMERIN

It is a reddish-brown powder, containing 33 per cent. of mercury in the non-ionised form, and is readily soluble in water. Hill and Young (1923) found that in the cure of rabbits infected with *Spirochæta pallida*, flumerin in 15 doses of 5 mgm. per kilogram produced much more rapid results than mercuric cyanide in twenty-one doses of 0·2 mgm. per kilogram of body weight, a result which is not surprising, since it is possible to introduce from six to ten times as much mercury by flumerin intravenously as by mercuric cyanide. The minimum lethal dose for rabbits was between 30 and 40 mgm. per kilogram of body weight. In the treatment of human cases Hill and Young recommended daily intravenous injections of 5 mgm. per kilogram of body weight of flumerin, together with weekly injections of neoarsphenamine. The results obtained with this drug have, however, been unsatisfactory.

2-Myristoxymercuri-3-hydroxybenzaldehyde

This is an organic preparation of mercury prepared by Henry and Sharp (1922), and dissolved in hydnocarpus oil. It is suitable for the treatment of syphilis in lepers, in whom arsenicals tend to aggravate the leprosy. Dissolved as a 0·25 per cent. solution in hydnocarpus oil, the preparation is known as "avenyl." Muir (1926), who found that hydnocarpus oil alone produced no effect on the Wassermann reaction, was able to give as much as 10 c.cm. twice weekly for fifteen doses without producing toxic symptoms of any kind. Of thirty leprous patients with positive Wassermann reactions treated with avenyl, sixteen were rendered negative, ten by one course of fifteen injections, three by two, two by three and one by four courses. In a further paper, Lloyd, Muir and Mitra (1926) state that they have never encountered unpleasant reactions or toxic effects from the use of avenyl.

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Tellurium, Vanadium and other Metals in Syphilis

As a result of the introduction of bismuth into the chemotherapy of syphilis, a number of other metals have been tested for their anti-syphilitic action.

Tellurium was first examined by Levaditi and Nicolau (1926), who found that it had a definitely curative action on syphilis in the rabbit. Further observations were made by Levaditi, Nicolau and Manin (1927), who investigated the action, not only of metallic tellurium, but of a number of organic and inorganic derivatives. Metallic tellurium was found to have a rapid curative action in rabbit syphilis, as also the trioxide and iodide and the iodotellurate of quinine. The soluble salts were much more toxic and less active therapeutically. Unfortunately when attempts were made by Fournier, Levaditi and Guénot (1927) to use tellurium in the treatment of human syphilis, it was found that, though clinically and serologically there was a definite cure, yet the results were slower and less certain than those obtained with arsenic or mercury. If larger doses of tellurium were given, signs of intoxication developed, while if the number of injections were increased there was evidence of a cumulative toxic action.

These unfortunate results have recently been confirmed by Versari (1928). Though the therapeutic effect was rapid, intense pain was produced at the site of injection, the patients invariably lost weight, and a disagreeable garlic odour was imparted to the breath.

Vanadium was first used as tetra- and hexavanadate of sodium and potassium by Proescher, Seil and Stillians (1917), who found that both in the rabbit and in man there occurred a rapid disappearance of the spirochætes, healing of the lesions, and a more or less definite improvement in the serological reactions. These results were confirmed by Fournier, Levaditi and Schwartz (1922), who successfully cured rabbits infected with syphilis with subcutaneous injections of 15 to 20 mgm. per kilogram of sodium potassium tartro-vanadate.

Platinum, as the double hyposulphite of platinum and sodium, and *gold*, as the double hyposulphite of gold and sodium, have

been shown by Levaditi, Girard and Nicolau (1925) to have some curative action in rabbit syphilis. Klauder (1924) also found that gold chloride possessed some curative action, as did thorium. Germanium, despite its close relationship to arsenic, possessed no spirochæticidal activity.

Vignati (1929) has recently attributed a spirochæticidal action to copper, for, when given in conjunction with sodium thiosulphate, there was a definite improvement in the lesions of secondary syphilitics. Levaditi (1927), however, was unable to find any curative action in copper, or in the following elements : aluminium, chromium, cobalt, nickel, selenium, niobium, molybdenum, rhodium, silver, cadmium, tin, antimony, lanthanum, neodymium, tantalum, tungsten, osmium, iridium, thallium, lead, polonium and uranium.

It is therefore obvious that there are only seven elements—arsenic, mercury, bismuth, tellurium, vanadium, platinum and gold—which have a definite action in curing syphilis. As Levaditi (1927) points out, the spirochæticidal action of these metals does not depend on the same laws as regulate the chemical properties of the elements according to Mendelejeff's classification. The reasons for the therapeutic activity of these metals is therefore still unknown.

Beinhauer and Jacob (1928) believe that in cases with a resistant Wassermann reaction, sodium thiosulphate has a definite therapeutic effect, since it rids the tissues of a saturation by the heavy metals to which the persistence of the Wassermann reaction may be due.

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CHEMOTHERAPEUTIC PROPHYLAXIS OF SYPHILIS

The prophylaxis of syphilis by chemotherapeutic means is by no means new. In a work published in 1774, de Cézan urged the value of mercuric chloride solution applied locally as a disinfectant after exposure to infection, while more recently Metchnikoff and Roux (1906), as the result of experiments both on apes and man, demonstrated that inunction with calomel cream prevented the development of syphilitic infections. Nichols and Walker (1923), working with inoculated rabbits, found that calomel proved efficacious up to eight hours after inoculation, there being no marked difference between the action of calomel in a base of lanoline and vaseline and in a base of benzoinated lard and wax.

The prophylactic action of injections of bismuth has been demonstrated experimentally in rabbits by Sazerac and Levaditi (1922), Kolle (1924), and Fournier and Schwartz (1926), the latter having shown that bismuth preserves the animal from infection for a month or more, according to the quantity and constitution of the compound injected. Tellurium has been found by Levaditi, Sanchis-Bayarri, Schoen and Manin (1928) to have a similar protective action.

The oral prophylaxis of syphilis has recently been demonstrated by Levaditi, Navarro-Martin, Fournier, Guénot and Schwartz (1922). Soluble salts of bismuth by mouth having proved unsatisfactory, attention was turned to the arsenicals, especially stovarsol. It was found that a dose per mouth of 0·1 gm. per kilogram of body weight protected a monkey from syphilitic infection for at least two, and sometimes seven, days. Two human volunteers were then inoculated with syphilis and were given, two and five hours later, 2 gm. of stovarsol by mouth. Neither developed syphilis, although monkeys inoculated with the same strain of spirochæte became infected. Nine persons who had recently been exposed to syphilitic infection were also protected by stovarsol given by mouth.

The prophylactic action of stovarsol in rabbits has been confirmed by Poole (1926), while Oppenheim and Fessler (1928) have also used it prophylactically in man. Burke (1928) believes that the prophylactic value of stovarsol is due to the pentavalency of

the arsenic atom and its consequent predilection for ectodermal structures. Administration of stovarsol causes, as it were, an "arsenical barrage" to be dropped on the structures which the syphilitic spirochæte must force in order to gain admittance to the body.

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DRUG RESISTANCE IN SYPHILIS

Although the spirochætes of relapsing fever may be rendered resistant to drugs in the same way as trypanosomes, considerable doubt exists as to the possibility of rendering *Spirochæta pallida* similarly resistant. Clinically, as is well known, cases occur which fail to react to arsenic, mercury or bismuth, but this failure does not necessarily imply that the spirochætes themselves have become drug resistant. Akatsu and Noguchi (1917), however, carried out experiments *in vitro* in which they were successful in raising to many times their original degree the resistance of *S. pallida*, *S. refringens* and *S. microdentium* to arsphenamine, neo arsphenamine and mercuric chloride. The experiments involved the cultivation of these organisms in media containing the drugs in a concentration just short of that required to suppress the growth completely. It was found that the resistance of *S. pallida* and *S. microdentium* to arsphenamine and neoarsphenamine was increased in three or four months five and a half times, while with *S. refringens* the increase was about three times. Against the action of mercuric chloride, *S. pallida* developed thirty-five times its original tolerance. The acquired drug-fastness *in vitro* gradually disappeared when the spirochætes were cultivated again in drug-free media for several generations. Although undoubtedly the spirochætes cultivated developed an increased resistance, there is some doubt whether *S. pallida* itself was cultivated. Klauder (1924), however, by treating rabbits infected with a passage strain of *S. pallida* with gradually increasing doses of arsphenamine succeeded in raising the resistance of a strain of spirochæte, for the therapeutically active dose of 0·006 gm. of arsphenamine in the control animals was raised to 0·01 gm. after six transfers. The spirochætes, however, never became absolutely resistant to the action of arsenic.

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THE ACTION OF DRUGS IN SYPHILIS

The mechanism by which the arsenobenzene derivatives and the pentavalent arsenicals destroy spirochætes appears to be very similar to that occurring in the destruction of trypanosomes. Neither the arsphenamines nor the pentavalent arsenicals seem to act directly *in vivo* on the spirochætes, but only after transformation into the arsenoxide. Voegtlin and his colleagues believe that the arsenic then reacts with the reduced glutathione of the parasites. Levaditi, on the other hand, is of opinion that both arsenic and bismuth react with certain of the tissue proteins to form toxalbumins, which are the active substances in destroying the parasites. Levaditi and Girard (1925) have found, for instance, that the amount of bismuth actually present in a syphilitic chancre is infinitesimal; they therefore suggest that the destruction of the parasites under the influence of bismuth is a lytic process where traces of the metal play the part of catalyser, the hypothetical bismoxyl being used up as soon as it is formed. An extract of a chancre in which the spirochætes have just been sterilized by bismuth is quite incapable of destroying other spirochætes. Levaditi and Howard (1929) have shown that when an extract of liver or supra-renal in saline is incubated for three hours at 37° C. with sodium potassium bismuthyl tartrate and then flocculated at 70° C. for three hours, a precipitate is obtained. When this precipitate is suspended in physiological saline and injected intramuscularly into syphilitic rabbits, cure results with amounts of metallic bismuth of the value of 0·00125 gm. per kilogram of body weight. Even with bismuth in doses of 0·000312 gm., cure eventually occurs after some delay. Levaditi, Lépine and Howard (1929) suggest that these minute quantities of metallo-proteins act synergically with the immune bodies of the tissues. In tick fever, for instance, the curative dose of arsphenamine is inversely proportional to the time which has passed since the spirochætes appeared in the blood. At the beginning of the disease, when the number of spirochætes is small, more arsphenamine is required than immediately before their disappearance, when the blood contains innumerable spirochætes. Similarly, rabbits inoculated

with *Spirochæta pallida* for 113 and 132 days were cured by much smaller doses of bismuth than one which had only been inoculated for fifty days. Brown and Broom (1929) also believe that in the destruction of leptospira by chemotherapeutic agents, immune serum in the presence of complement plays an important part. Leptospira, from their behaviour in a cataphoresis cell, are known to be negatively charged. When, however, the leptospira are acted upon by a specific immune serum containing complement, their charge is apparently reduced, with the result that a negatively-charged colloid is able to act upon them more readily.

Table showing the Lethal Action of a Negative Colloid on Leptospira in Normal and Immune Serum with Complement. (Brown and Broom, 1929.)

	10 minutes.	2 hours.
Normal serum + Cu + leptospira	Actively motile.	Actively motile.
Immune „ + „ „ .	All dead.	All dead.
Normal „ + Saline + „ .	Actively motile.	Actively motile.
Immune „ + „ + „ .	„ „	„ „

From the table it will be seen that colloidal copper is more lethal to leptospira in presence of immune than in presence of normal serum.

McDonagh (1924) also believes that all chemotherapeutic action is ultimately of an electrical nature, metals causing dispersion and non-metals condensation of the protein particles. The chief protective substance of the body is the serum protein, which exists in the form of colloidal particles, while parasites are also composed of a number of colloidal particles enclosed in a fixed area. The aim of all chemotherapy is then to increase the number of hydroxyl ions on the surface of the protective substance and decrease those on the surface of the parasite. These views, which do not lack originality, should be read in the original by those interested.

Although the usually accepted view is that the action of the

arsphenamines on spirochætes is an indirect one, there is nevertheless a certain amount of evidence to suggest that the arsphenamines have a direct toxic action on spirochætes, provided that they are allowed to act for a sufficient length of time. Thus it is generally stated that spirochætes are killed *in vitro* by arsphenamine and neoarsphenamine only when the concentration of these drugs is of the order of 1 in 7,500 to 1 in 2,000, that is to say, by concentrations which could never come into operation in the human body after therapeutic doses. In most of these experiments, however, the spirochætes were exposed to the arsenicals for a period of one hour, or at most two hours. Schamberg, Kolmer and Raiziss (1917) have found that a 1 in 128,000 solution of arsphenamine acting for two hours was sometimes sufficient to sterilize a culture of *Spirochæta pallida*. It may therefore be that solutions of the arsenicals which are inert for the first three or four hours nevertheless destroy the spirochætes after more prolonged exposure, as is the case with emetine and *Endamœba histolytica*. Nor is this possibility entirely unsupported by experiment, for Neufeld and Böcker (1914), working with *S. gallinarum*, showed that the organisms became non-motile when suspended in weak solutions (1 in 30,000 to 1 in 10,000) of arsphenamine in blood *in vitro*, but only after three or four hours' contact with these drugs. Similarly, the blood of fowls experimentally infected with these spirochætes continued to show motile forms only for three or four hours after the administration of curative doses of arsphenamine or neoarsphenamine.

It is obvious that the question whether the arsenicals cure syphilis by virtue of a direct action on the spirochæte cannot yet be regarded as settled.

Another question of great importance which has recently been revived is the power of chemotherapeutic drugs to eradicate syphilitic spirochætes from the body. The fact that reinfection may occur in patients treated with the arsphenamines or bismuth has usually been taken as evidence that there is complete eradication of spirochætes from the body. Nevertheless, Warthin (1929), as the result of extensive experience, states that he has never seen at necropsy a case of perfectly-healed syphilis. Search,

often prolonged, invariably reveals active latent lesions in aorta, heart or other organs, and this is as true of cases treated in the modern manner as it is of cases treated with mercurials. If any difference results from the two methods of treatment it would appear to be in the more frequent occurrence of chronic hepatitis in cases treated by the arsenical method. What treatment accomplishes in both cases is a rapid reduction of the average active case to a stage of latency, but there is no evidence pathologically that once infected with syphilis a patient ever becomes free from spirochætes.

Experimentally in rabbits a considerable amount of work has been carried out to determine whether syphilitic infection can be entirely eradicated. Kolle (1924) found that intensive and prolonged treatment of a rabbit when begun more than ninety days after a successful syphilitic inoculation failed to make the animal respond to reinoculation with another chancre. He therefore argued from this, in accordance with Neisser's dictum, that the original infection had not been destroyed by the treatment. Chesney and Kemp (1924 and 1925), on the other hand, treated rabbits with arsphenamine forty-one to fifty days after inoculation, and a second series 181 to 291 days after inoculation. At least forty-nine days after completion of the treatment a single popliteal gland was removed from the treated rabbits and from the untreated controls and, after emulsification, inoculated into healthy rabbits. The glands from the treated animals failed to infect even as late as 275 days after treatment, while the glands of the untreated controls infected other rabbits and were found to be still infective from 265 to 420 days after inoculation. The evidence that the treated animals had been sterilized is thus fairly strong, though there is the possibility, supported by the results of other workers that inoculation with glands and organs, such as spleen or liver in preference to popliteal glands, might have given rise to infection as a result of their greater virus content. The work of Chesney and Kemp has, however, been confirmed and extended by Uhlenhuth and Grossmann (1928), who found that not only were the organs of rabbits treated with neoarsphenamine devoid of spirochætes, but four of eleven treated rabbits became infected on

reinoculation, while seven were quite immune. This immunity, according to Breinl and Wagner (1929), increases gradually with the age of the first infection, while if Neisser's dictum that immunity to reinoculation is bound up with survival of *S. pallida* is correct there should be expected a sudden drop in the immunity with sterilization of the animal by treatment. The evidence at present available therefore suggests that in rabbits at any rate complete sterilization of syphilitic infections can be produced by chemotherapeutic agents.

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CHAPTER IX

CHEMOTHERAPY OF YAWS

ALTHOUGH yaws presents certain similarities to syphilis, there are many facts which show that the two diseases are separate pathological and clinical entities. The typical yaw, pushing up through the skin with its yellow crust, is like no eruption seen in syphilis, and is in itself sufficient to identify yaws as a disease distinct from syphilis. Yaws is also pre-eminently a disease of children, and is not transmitted by sexual intercourse, but in a manner such that white men living in close proximity to natives are only rarely attacked.

There are also chemotherapeutic differences, the most striking of which is the fact that mercurial drugs have little or no effect on *Spirochæta pertenuis*. Nevertheless, arsenic and bismuth, as in syphilis, are the substances with the most definite therapeutic action in yaws.

PREPARATIONS OF ARSENIC

Experimentally in the rabbit, Nichols (1911) was the first to show that infections due to *S. pertenuis* are much more amenable to the arsphenamines than those due to *S. pallida*. The intravenous injection of 0·0045 gm. per kilogram of body weight of arsphenamine resulted in the permanent cure of four rabbits with well-developed testicular lesions, while of five rabbits infected with *S. pallida* and treated with a similar dose three relapsed.

An abundance of clinical evidence has shown the value of the arsphenamines in yaws, with the result that potassium iodide, which was formerly much employed, is now but little used except in tertiary cases, many of which may be really syphilitic. The use of tartar emetic also has now been entirely abandoned.

While the introduction of the arsphenamines has thus revolutionised the treatment of yaws, the reported results often leave much to be desired, for the word " cure " is only too often used, when nothing more than a disappearance of the secondary eruption has been effected. Many cases thus undoubtedly pass into a latent condition. At present there is little reliable evidence, and still less agreement, as to what really constitutes an average course of treatment in various types of case, if the treatment be intended not only to cure symptoms, but to eradicate the disease.

Spittel (1922), for instance, recommends for the average male patient six injections of 0·6 to 0·75 gm. of neoarsphenamine at intervals of a week or ten days, no injection being repeated till the patient has recovered from the effects of the previous injection. Iodides are also administered, though even with this combined treatment there are many clinical relapses. Hunt and Johnson (1923), on the other hand, are content with three injections given in a period of not more than three weeks, the dose for an adult being 0·9 gm. of neoarsphenamine. Goonawardena (1923) also finds three injections sufficient. After one injection a certain number of cases cleared up, but others returned in a worse condition.

The effect of one, two or three intravenous injections of neoarsphenamine at weekly intervals has been carefully investigated by Armstrong (1925), who gave 0·6 gm. to adult males, and proportionately smaller doses to women and children. The cases were all examined eight months after the last injection.

From the table shown on p. 409 it will be seen that even with three injections there is a relapse rate of 10 per cent. within eight months. The percentage of cases remaining uncured by a small number of injections is also shown by the work of Lopez-Rizal, Gutierrez and Fernandez (1926), who among 301 cases treated with from one to seven injections of neoarsphenamine found ten relapses, while eight other cases were either unaffected or only slightly improved. Moss (1926), from the Dominican Republic, has also reported the results five years after treatment with neoarsphenamine. Of 419 cases, 195, or 46·5 per cent., were uncured, while 224, or 53·5 per cent., remained free from all symptoms. With one

Table showing Relapses in Yaws Eight Months after Treatment with Neoarsphenamine.
(Armstrong, 1925.)

	Type of Case Injected.												
	(1) Primary sore.	(2) Generalized secondary papular rash.	(3) Secondary Dermatitis.	(4) "Crab" tonas.	(5) Secondary ulceration.	(6) Gummatous ulceration.	(7) Synovitis.	(8) Dactylitis.	(9) Tibial Nodes.	(10) Slaty dermatitis.	(11) Plantar and palmar dermatitis.	(12) Hyperkeratosis.	Totals.
				Cases	receiving	three in	jections.						
Number of cases injected	6	196	110	89	5	37	1	2	3	28	54	5	536
Number of relapses .	5	32	6	8	—	2	—	—	—	—	—	—	53
Percentage of relapses	83	16 3	5·45	8·9	—	5·4	—	—	—	—	—	—	10
				Cases	receiving	two in	jections.						
Number of cases injected	1	41	30	22	2	14	1	—	—	9	13	2	135
Number of relapses .	1	13	3	1	—	2	—	—	—	—	—	—	20
Percentage of relapses .	100	41·4	10	4·5	—	14·3	—	—	—	—	—	—	14·8
				Cases	receiving	one in	jection.						
Number of cases injected	—	7	8	1	1	4	—	—	—	1	1	1	24
Number of relapses .	—	2	2	—	—	—	—	—	—	—	—	—	4
Percentage of relapses .	—	28·5	25	—	—	—	—	—	—	—	—	—	16·6

injection of 0·6 gm. neoarsphenamine, it was possible to cure just under half the cases : a second injection had no effect in increasing the percentage, but three injections raised it considerably. Navarro (1926), who investigated the Wassermann reaction in 101 cases of yaws in from three months to three years after treatment, found that eighteen continued to show a positive reaction.

It seems probable that the very striking effect produced by a single injection of neoarsphenamine carries with it some measure of disadvantage in that many native patients do not bother to return for further treatment. Even the three-injection system, which prevails not only in Samoa, but in East Africa and the Dutch East Indies, can hardly be looked upon as entirely satisfactory, for the wisdom of converting all unrelapsed cases into a possible reservoir of latent infection is open to considerable doubt. Time alone can show the percentage of children with latent infections who are destined to develop tertiary manifestations.

While the various preparations of arsphenamine have all been used in the treatment of yaws, the most striking results have been obtained with neoarsphenamine, the worst with galyl. Sulpharsphenamine, either intramuscularly or subcutaneously, has been employed by van den Branden and van Hoof (1922), and by Knowles, Chopra, Gupta and Das Gupta (1923). In adults neoarsphenamine can only be given intravenously, but in small children many workers have preferred the intramuscular method of administration. It must be remembered that toxic reactions are as liable to occur after the administration of the arsenobenzene derivatives in yaws as in syphilis : in fact, a number of observers, such as van den Zijl (1922), Hazebrock (1922), and others believe that natives are even more susceptible than Europeans, owing to the fact that in the former liver insufficiency is more frequent as the result of intercurrent protozoal or helminthic infections.

On the other hand, in certain cases of yaws, such as those recorded by Heinemann (1928), there is complete resistance to the action of neoarsphenamine.

Apart from neoarsphenamine, the most widely-used arsenical has been **3-acetylamino-8-hydroxyphenylarsinic acid** (stovarsol),

which has also found a place in the treatment of amœbiasis, malaria and syphilis. Stovarsol has the advantage over the arsphenamines that it can be given by mouth, a method involving much economy of time and energy when mass treatments are undertaken. Beurnier and Clapier (1923) recommend a dose of 1 gm. for persons of over 50 kilogm. of body weight, 0·5 gm. for those under 40 kilogm., and 0·25 gm. for those between 15 and 30 kilogm., administered in tablet form *per os* in the morning before food on alternate days for fifteen to seventeen days. If lesions have not all cleared up, treatment is recommenced with rather smaller doses after the interval of a week. The only complication encountered is diarrhœa, which soon disappears on temporarily ceasing treatment. Rouvroy (1925) also noted that diarrhœa was frequent. Tanon and Jamot (1924), in the Cameroons, found stovarsol as effective as the arsphenamines. In the case of adults they first gave 0·5 gm. to test the tolerance of the patient. If this were well borne, three tablets of 0·25 gm. were given on the second and third days, in the morning before the first meal; then after a rest of one day, four, three and two tablets respectively were administered on alternate days. During the following week one tablet was given every three days. Children from ten to fifteen years of age were given half the above doses, those from five to ten years half a tablet to begin with, and those from one to five years a quarter of a tablet. Massias (1925), in Cochin China, also obtained rapid disappearance of lesions, four tablets of 0·25 gm. being given for three days to adults. Van den Branden (1926) points out the necessity of continuing treatment until the Wassermann reaction is negative. He recommends that the total amount of the drug in grams should equal the weight of the patient in kilograms. By this means the Wassermann reaction was rendered negative in seven of eleven cases: of the four that remained positive, one, however, received 54 gm. of stovarsol. Van Nitsen (1927[1]) employed the same method of treatment with success. In the case of sodium stovarsol (1927[2]), doses of 0·5, 1·0 and 1·5 gm. were given intravenously at intervals of forty-eight hours until a total of 9·0 gm. had been given in secondary cases, and 10 to 15 gm. in tertiary cases. Bouffard

(1927) finds that relapses are extremely rare, representing only about 2 to 3 per cent. of cases treated, while Selwyn-Clarke (1926) has obtained striking results by the oral administration of stovarsol in tertiary yaws.

Chesterman and Todd (1927) gave doses of 1·5. 2·0 and 2·0 gm. by mouth on the first, fourth and seventh days to children of 15 kilogm. weight, and found that all lesions invariably disappeared ; diarrhœa was the only untoward symptom. Stovarsol thus appears to be of considerable value in the treatment of yaws in all stages of disease.

Baermann (1923) has experimented with stovarsol as a prophylactic in yaws. A European, twenty-six years of age, was inoculated from a papule rich in spirochætes taken from a Javanese suffering from yaws. 0·75 gm. of stovarsol was given by mouth on the first day, and 1·0 gm. on the succeeding days. No disease developed. Monkeys were also inoculated with yaws and given 0·25 gm. of stovarsol for four days. No disease developed in these animals, although control monkeys became infected.

Tréparsol (3-formylamino-4-hydroxyphenylarsinic acid) has been used by a few workers, such as de Almeida and de Oliviera (1926), and van Nitsen (1927[3]). The latter finds that from 4·25 to 14·75 gm. are necessary to produce a clinical cure, though the Wassermann reaction is unaffected. Beurnier and Clapier (1922) claim some success with the sodium salt of aminohydroxyphenylarsinic acid.

Acetylarsan (diethylamine - acetylaminohydroxyphenylarsinate) has been used by Boisseau (1926), who gave two subcutaneous injections at intervals of forty-eight hours of 2 cgm. per kilogram of body weight. The lesions healed rapidly, with the exception of long-standing ulcers.

More recently Chesterman and Todd (1927) have studied the effects of certain pentavalent arsenicals prepared by Ewins and Everett (1927). Hydroxy-benzisoxazine arsinic acid was found to be too toxic for use in treating yaws, while diacetylaminodihydroxyarsenobenzene was much slower than either stovarsol or the better-known arsphenamines. Aminohydroxyphenyl-dichlorarsine (halarsol) was, however, extremely active when given by subcutaneous or intramuscular injection. One to three injections

of 12 to 50 mgm. produced results in primary, secondary and tertiary yaws fully equal to those of the arsphenamines. Todd (1929) has recently confirmed these excellent results. Of forty-two cases of secondary yaws only ten relapsed. The minimum curative dose was 1 mgm. per kilogram, the minimum toxic dose 4·5 mgm. per kilogram. The only toxic results were headache and vomiting.

Incidentally the cost of production of halarsol is no greater than that of neoarsphenamine, while the dose is but one-tenth as great.

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BISMUTH

As a result of its use in syphilis, bismuth has been employed also in the treatment of yaws. Shircore (1921), in Tanganyika, called attention to the excellent results obtained with sodium potassium bismuthyl tartrate when given intramuscularly in doses of 3 grains, repeated after four days.

These results have been confirmed by Gilks (1923), in Kenya Colony, and by Howard (1923), in Uganda. The former recommends deep intramuscular injection, but others, such as Franklin (1927), find deep subcutaneous or epifascial injections much less painful. While two injections are sufficient to produce healing of the clinical lesions, it seems probable that a greater number of injections are necessary to eradicate the parasites. Irvine (1925), however, in treating 2,500 cases with two injections, had a relapse rate of under 1 per cent.

In addition to watery solutions of the bismuthyl tartrates and suspensions of the same salts in oil, bismuth subgallate (dermatol) has been used by Rebuffat (1926), 3 c.cm. of a 10 per cent. oily suspension being given intramuscularly twice weekly. The best results were obtained after ten injections, but this course was not without danger, for stomatitis, albuminuria, cachexia and death occasionally occurred. Dermatol has also been used by Mattlet (1924) and Miguens (1924). More recently, Miguens (1929) states that of a thousand cases of yaws treated with a single massive dose of dermatol, only 15 per cent. had relapsed a year and a half later. Further research can alone determine the most satisfactory bismuth salt for the treatment of yaws. Leach (1926), on the other hand, prefers to use metallic bismuth. The disadvantage of this preparation, however, is the enhanced cost as compared with the bismuthyl tartrates, which are also far cheaper for mass treatment than the arsphenamines. Van Hoorde (1927) claims excellent results with four weekly intramuscular injections of bismuth subnitrate given as a 10 per cent. suspension in oil.

Shircore (1926) has had good results with bismuth arsanilate (sobita), a compound formed by the interaction of sodium bismuthyl tartrate and atoxyl. Over 113,000 cases of yaws in the secondary or tertiary stages have been treated, and after six injections given every other day, 75 per cent. of cases were completely healed.

Parsons (1927[1, 2]) claims to have had considerable success in treating yaws with bismuto-yatren A and B. Bismuto-yatren A is said to be an aqueous solution of sodium bismuthiodohydroxy-quinoline sulphonate, containing 10 mgm. of metallic bismuth per c.cm. Bismuto-yatren B is a quinine derivative, and contains 36 mgm. of the metal per c.cm. It is an oily suspension. The weekly dose was 1 to 2 c.cm. of B given intramuscularly, and 2 to 3 c.cm. of A given either intravenously or intramuscularly. Two to seventeen injections produced a clinical cure.

Chopra, Gupta and Mullick (1928) have used an organic aromatic compound of bismuth, which is suitable for intravenous injection. This compound, "Bisnene," contains just over 50 per cent. of bismuth, and is an analogue of urea stibamine; it is said to have

the formula $NH_2.CO.NH.C_6H_4BiO.(OH)ONa$. Doses of 0·1 to 0·15 gm. have been tried in a few cases of yaws with promising results, four injections at weekly intervals being followed by disappearance of the secondary eruption.

Portois (1928) claims that the bismuth hydroxyiodogallate, "airoi," is superior to all other bismuth preparations, as the Wassermann reaction is rendered negative almost as quickly as with arsphenamine, while toxic reactions such as stomatitis only occur in about 0·5 per cent. of cases. Probably the most satisfactory preparation is metallic bismuth, owing to its sustained action and the ease of administration.

Although bismuth preparations, as a result of their cheapness, readily commend themselves for the mass treatment of yaws, it is now recognised that their true place, as in syphilis, is as an adjunct to the arsenicals. Carman (1928), for instance, has shown that after twelve injections of any of the three bismuth preparations—sodium potassium bismuthyl tartrate, sodium bismuth tartrate or precipitated metallic bismuth—a large percentage of cases are left with positive serum reactions. It is obvious, therefore, that in order to eradicate yaws, bismuth must be employed in association with arsenicals, and treatment must be continued until the serum reactions become negative.

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CHAPTER X

CHEMOTHERAPY OF RELAPSING FEVER AND OTHER SPIROCHÆTAL DESEASES

RELAPSING FEVER

THE results obtained in relapsing fever by treatment with the arsphenamines, more especially neoarsphenamine, are so striking that there has been little further attempt to search for other chemotherapeutic remedies. The injection of 0·6 gm. of neoarsphenamine is usually sufficient to cure the disease, and is attended with little or no danger provided that care is taken to administer the drug some time before the crisis, when there is no danger of collapse from toxins liberated by excessive destruction of spirochætes.

In mice infected with relapsing fever and treated with arsphenamine however, it has been found by Buschke and Kroó (1923) that, though a single injection may bring about an apparent cure, the spirochætes may still remain active in the central nervous system. Although strains of relapsing fever spirochætes differ in their resistance to arsphenamines, the continued existence of spirochætes in the brain would seem to be due to the fact that the concentration of arsenic is insufficient to cause their destruction. When, as shown by Schreus and Weisbecker (1926), the dose of arsphenamine is gradually increased the spirochætes are eventually removed even from the brain, with the result that when this has occurred, reinfection is possible.

Various other arsenicals have, however, been tested for their action in relapsing fever. Hofmann (1927) finds that Albert 102, given in doses of 0·02 gm. per kilogram of body weight, has the effect of destroying all relapsing fever spirochætes in the mouse in twenty-four hours.

Stovarsol (Chapter III.—Amœbiasis) has also been used with

success by various French workers, who cure the majority of cases with 1·5 gm. given by mouth, the spirochætes leaving the blood in from six to thirty hours. Kritschewski and Friede (1925) used stovarsol prophylactically. In the hands of de Buen (1928), however, the drug was disappointing, for of eight patients given 1 gm. by mouth, four relapsed, while only two out of eight patients injected with 0·3 gm. of arsphenamine relapsed.

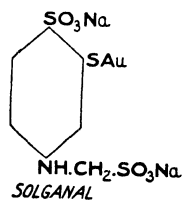
Schofield (1927), on the other hand, reports that 1·5 gm. of stovarsol given by mouth on two successive days removed all symptoms and prevented relapses. Van Nitsen (1927) has used intravenous injections of sodium stovarsol with success, 0·5 gm. being given on the first day, 1·0 gm. on the second, and 1·5 gm. on the third and succeeding days, till a total of 15 gm. has been reached.

Esquier (1928) believes that acetylarsan (Chapter VI.—Trypanosomiasis) is more effective than the arsphenamines when given subcutaneously in doses of 2 c.cm., corresponding to 0·46 gm. of the active substance.

Others find that arsalyt (Chapter VIII.—Syphilis) is of special value in relapsing fever owing to the absence of all toxic effects.

Apart from arsenic, gold salts have been used in the treatment of experimental relapsing fever. Sanocrysin, which is considered in connection with the chemotherapy of tuberculosis, has been used by Krantz (1926), who, by giving from 0·0005 to 0·001 gm. to a mouse at an early stage of infection, was able to cut short the attack and prevent relapse.

Steiner and Fischl (1929) have used an aromatic organic gold derivative, di-sodium *p*-sulphomethyl-amino-*o*-auromercaptobenzene sulphonate, "Solganal," which is said to have the following structure :—



SOLGANAL

This substance can be given intravenously or intraperitoneally, while an adaptation of the same compound "A69" may be administered intramuscularly. Both preparations are said to have a higher chemotherapeutic index than neoarsphenamine.

Strain	Compound.	Injection.	Dose per 20 gm. mouse in gm.		Relapse after.	Therapeutic index.
			Dosis tolerata.	*Dosis curativa.*		
S. recurrentis .	Neoarsphenamine.	Intravenous	1/135	1/400	6 days	1 : 3
,,	Solganal .	Intravenous	1/100	1/1000	?	1 : 10
S. duttoni .	,,	Intraperitoneal.	1/100	1/2000	6 days	1 : 20
,,	A 69. .	Intramuscular.	1/70	1/1250	,,	1 : 18

In addition, residual infections of the central nervous system were entirely eliminated by these two gold preparations. An experimental relapsing fever in a paretic patient was cured by intravenous injections of solganal, the blood becoming negative after the second injection and the cerebrospinal fluid negative after nineteen days. Prophylactically, solganal is found to prevent, or at any rate to delay, the development of infection in mice.

Todd (1930) finds that in the relapsing fever, "tick fever," of Central Africa, neoarsphenamine is less useful than sodium potassium bismuth tartrate. While neoarsphenamine reduces the temperature within twenty-four hours relapses are quite common and two or even three injections of the drug may be required before the patient is entirely free from symptoms. Sodium potassium bismuth tartrate only reduces the fever in about thirty-six hours, but once down the temperature remains down and relapses are unknown. The drug, injected intramuscularly, is given in two consecutive doses of 0·2 gm. dissolved in 2 c.cm. of sterile water for an adult, while for a child of from two to ten years of age the doses are 0·1 gm.

Since relapsing fever is one of the few diseases in which cure can be brought about by a single sterilizing dose of an arsphen-

amine, thus fulfilling Ehrlich's ideal of a chemotherapeutic agent, much work has been carried out to determine the mode of action of arsphenamine in relapsing fever.

Kritschewski and his colleagues (1927–1928) believe that an intact reticulo-endothelial system is necessary for the full functioning of chemotherapeutic agents in relapsing fever. While blockade of the reticulo-endothelial system produces no result, splenectomy increases the mortality, the conclusion being that the protective function of the reticulo-endothelial system in relapsing fever depends not on phagocytosis of spirochætes by the reticulo-endothelial cells, but on the production of lytic antibodies. Feldt and Eisenmenger (1928) failed to confirm these findings.

The work of Levaditi, Lépine and Howard (1929) suggests that immune bodies interact in some way with the drug in killing the spirochætes, since the dose of neoarsphenamine necessary to bring about a cure is greater at the beginning of the disease, when spirochætes are few, than immediately before the crisis, when spirochætes swarm in the blood. An interrelationship of immune bodies and drug is also suggested by the work of Sagel (1928) in the treatment of experimental relapsing fever in paretic patients. In order to control infections due to *S. berbera* or *S. hispanica*, with neoarsphenamine it was essential to administer the drug when a number of febrile attacks had resulted in the development of a degree of natural immunity. If in addition the temperature were rising and spirochætes were present in the blood, the arsenicals had an immediate sterilizing effect, but if on the other hand these conditions were not fulfilled the spirochætes might be driven from the blood into the internal organs, often with the development of typhoid-like symptoms.

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SPIROCHÆTAL JAUNDICE

When guinea-pigs suffering from spirochætal jaundice are treated with arsphenamine, there is little or no effect on the progress of the disease. Colloidal silver, colloidal antimony, atoxyl and optochin were also found by Martin and Pettit (1916) to be without action. Sazerac, Nakamura and Kitchevatz (1927), however, obtained evidence that injections of bismuth sodium tartrate have both a curative and prophylactic action in guinea-pigs infected with *L. icterohæmorrhagiæ*. Guinea-pigs treated two or three days after inoculation with the leptospira were protected, as were animals treated with bismuth twenty-one days or even five months before infection. Similar results were obtained by Sazerac, Hosoya and Stefanopoulo (1927) with *L. icteroides*, 0·01 gm. per kilogram of body weight of bismuth sodium tartrate protecting guinea-pigs up to six days after injection of the leptospira. It is, of course, now recognised that *L. icteroides* and *L. icterohæmorrhagiæ* are serologically identical.

Uhlenhuth and Seiffert (1928 and 1929) have employed a large number of bismuth compounds in the treatment of experimental spirochætal jaundice in the guinea-pig. The most satisfactory was Bismuth-Yatren A, especially when given six or seven days after injection of the leptospira. It was found that when an infected guinea-pig is injected with this bismuth compound, leptospira disappear from the blood, but still remain in the internal organs, and on subinoculation these are found to be resistant to bismuth. Later, however, the leptospira disappear completely from the body and the animal is then immune, though it appears uncertain whether this is a true immunity due to the presence of antibodies or simply due to the retention of bismuth in the tissues. It seems probable that bismuth is not directly lethal to the leptospira, but reinforces the action of the immune bodies.

There is no published record of the treatment by bismuth of infective jaundice in man.

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OTHER SPIROCHÆTAL DISEASES

Other spirochætal diseases, such as Rat-bite fever, are readily cured by injection of the arsphenamines. Vincent's angina, which is generally regarded as being due to a spirochæte and *Bacillus fusiformis*, has also been treated with success, not only by intravenous injections, but also by local application of the arsphenamines. The influence of these arsenicals upon the saprophytic spirochætes of the mouth is very doubtful, and the *rôle* of spirochætes in the production of bronchitis highly uncertain. In cases of pulmonary gangrene associated with the presence of spirochætes in the lungs, however, Kline and Berger (1925) claim excellent results from the injection of arsphenamine.

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CHAPTER XI

THE CHEMOTHERAPY OF MYCOTIC DISEASES

THE chemotherapy of mycotic infections has up to the present received but little serious attention, with the result that the treatment of the majority of these infections is still in the stage of empiricism.

Schamberg and Kolmer (1922), however, have conducted a number of tests with various dyes and mercurials incorporated in Saboraud's medium to determine their relative efficiency in restraining the growth and in killing off various pathogenic fungi. The two mercurial compounds, mercurophen and mercuric chloride, exhibited the strongest killing effect, with iodine ranking third. Brilliant green prevented the growth of certain fungi, but was generally less toxic than crystal violet. Mercurochrome-220 compared favourably with the other mercurials, and had superior penetrating qualities.

Myers and Thienes (1925), in studying the lethal effect of various substances on a fungus isolated from fruit canners in Oregon, found that thymol, oil of cinnamon and oil of cloves were particularly effective. A mixed spirit composed of 5 per cent. thymol and 2 per cent. cinnamon oil painted on the sites of this and other mycotic infections brought about rapid cure. The action of chinosol (hydroxyquinoline sulphate) on pathogenic and saprophytic fungi was investigated by Lortat-Jacob and Bidault (1926), who found that pathogenic fungi, with the exception of *E. inguinale*, were all killed in twelve hours by a dilution of 1 in 400. Lesions due to *E. inguinale* were, however, rapidly destroyed by the following mixture :—

Chinosol	5	5 parts.
Alcohol	50	1 part.
Glycerine	20	
Water	150	

In an extensive report on the mycoses of Madagascar, Fontoynont and Boucher (1923) observed that a severe infection due to *Cryptococcus mena*, which was unaffected by potassium iodide, yielded to 0·10 gm. daily of methylene blue internally and the daily external application of a 1 per cent. solution of the same drug. For infections due to *Sporothrix beurmanni*, the intravenous injection of iodine as recommended by Ravaut (1921) was found to be the most satisfactory.

Castellani (1928) has suggested a paste containing phenol and fuchsine for the treatment of certain mycotic infections of the toes. The paint has the following composition :—

Saturated alcoholic solution of basic fuchsine	10 c.cm.
Aqueous 5 per cent. carbolic acid	100 ,,

The solution is filtered and boric acid 1 gm. is added. Two hours later 5 c.cm. acetone is added and after two hours 10 gm. resorcin. The paint should be kept in a dark-coloured bottle with a glass stopper.

Various drugs have been recommended for the treatment of blastomycotic infections; while successful results have been claimed for the intravenous injection of potassium iodide and gentian violet, there have been many cases in which these drugs entirely failed.

Madura foot has very occasionally been cured. Audrain (1924) alternated weekly injections of 0·75 gm. neoarsphenamine and 10 c.cm. of 1 per cent. mercurochrome with success in one case. Palmer (1926 and 1928) gave intravenous injections of bismuthyl tartrates, and obtained an apparent cure in two cases. Voizard and Leroy (1928) gave intravenous injections of lugol, from 1 to 10 c.cm. at a time, a total of 87 c.cm. being given in seventy-eight days.

In the treatment of actinomycosis, the only chemotherapeutic agent which has met with any success is potassium iodide injected intravenously in large doses. Colebrook (1921), however, in a long series of cases, was unable to confirm the good effect of this drug. Chitty (1926 and 1929) has recently found that iodine in milk, or preferably in cream, had a remarkable effect on eight cases of

actinomysosis: 5 to 10 minims were given in half a cup of milk three times a day. Pow (1929), however, gave colloidal iodine intravenously without success.

Epizootic lymphangitis is a chronic infectious disease of horses and mules which occurs in various parts of Europe, Asia, Africa and South America, and is due to infection with a type of yeast, *Blastomyces farciminosus* (*Cryptococcus farciminosus*). It is characterised by a purulent inflammation of the lymph vessels and regional lymph nodes of the subcutaneous tissues.

Nainsouta (1926) has found that mercuric iodide administered intravenously is a highly effective treatment. He gives 0·2 gm. twice a week for five weeks, or in severe cases 0·5 gm., each dose being suspended in 50 c.cm. of distilled water. These results were confirmed by Kelser (1928), who used 0·5 gm. of mercuric iodide daily either suspended in 30 c.cm. of distilled water, or dissolved with 0·5 gm. of potassium iodide. In nineteen cases of epizootic lymphangitis the results, after ten daily injections, were excellent.

The use of thallium acetate in the treatment of ringworm of the scalp also merits brief notice. Richet (1899) was the first to show that thallium has the curious power of producing epilation. Though thallium acetate was employed in the treatment of ringworm some thirty years ago by Sabourard, its use was not continued, possibly because too large doses were administered The more recent introduction of thallium acetate for the treatment of ringworm is due to Fiocco (1925) in Italy, and Buschke and his colleagues (1925) in Germany. A single dose of 8 mgm. per kilogram of body weight given by mouth in sweetened water produces epilation in from eight to nine days. Both normal and infected hairs are removed, the infected stumps falling out rather less readily than the healthy.

Various toxic symptoms such as headache, loss of appetite, pain in the joints, and albuminuria, have been recorded.

Dixon (1927) suggests that the depilatory power of thallium is due to its action on the autonomic nervous system, as the result of which normal stimuli are greatly exaggerated.

It is obvious that much remains to be done before the chemo-

therapeutic treatment of mycotic infections is placed on a rational and scientific basis.

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CHAPTER XII

THE CHEMOTHERAPY OF LEPROSY

THE modern treatment of leprosy dates from 1897 when, as a result of the First International Scientific Congress on Leprosy held in Berlin, the theory of hereditary transmission was finally rejected and the old mediæval belief that it was contagious was reaffirmed.

Any estimation of the value of treatment in leprosy is fraught with considerable difficulties owing to the self-curative tendency of the disease, the seemingly spontaneous periods of quiescence and activity apart from any treatment, the influence on the course of the illness of season and climate, and the inability to find a lower animal in which *Mycobacterium lepræ* will produce a condition resembling that seen in man. It is therefore difficult, if not impossible, to say that any particular case of leprosy is cured, for even though no signs of activity of the disease are present and the patient may be apparently free from active lesions for several years, recurrences are liable to take place, as the result of any lowering of the general resistance. It is thus of the greatest importance that, apart from specific treatment, the patient's general environment should be as good as possible, while all intercurrent diseases, such as malaria or syphilis, should also receive attention. Muir (1928), in fact, has drawn up a special course of treatment for malarial, syphilitic and other lepers with a view to getting them into good condition for leprosy treatment. Since arrest rather than cure is all that can be hoped for, it is essential to have certain criteria by which to judge of the arrest. Muir (1927) suggests that treatment having been continued for at least six months after the disappearance of all active lesions, the following should be taken as evidence that the leprosy has been arrested :—

(i.) Inability to find *M. lepræ* upon repeated examinations of the skin by clip smears, of the nasal mucosa by scraping, of the

lymph nodes by puncture or in any other way to isolate lepra bacilli during a period of six months.

(ii.) The disappearance of erythema from macules and the absence of all changes in lesions, such as an increase or decrease of anæsthesia for a period of six months.

(iii.) The patient must be negative when examined at stated intervals for at least two years. He must, however, be warned that active signs of the disease are liable to recur at any time.

Although with modern methods of treatment there is reason for optimism, nevertheless there are numerous factors which militate against even an arrest of the disease, such as non-recognition of the disease in its early stages, limitation of treatment to drugs alone without regard to food and environment, and failure to treat intercurrent infections. On the other hand, an apparent cure may be only the subsidence of an acute reaction such as occurs in the absence of any chemotherapeutic treatment.

It must be recognised therefore that when cases are described by various workers as cured, all that can be safely said is that the disease is arrested.

So far as the chemotherapeutic treatment of leprosy is concerned, there are two chief classes of drugs :—

(i.) Various fatty acids and their derivatives.

(ii.) Metallic preparations.

CHAULMOOGRA AND HYDNOCARPUS OILS

The use of chaulmoogra oil in the treatment of leprosy is by no means new, for it has been known in India for many centuries. Rock (1922) relates that in the "Mahawin," the history of the Buddhas and their Rahandas, there are certain passages relating to the kalaw or chaulmoogra tree. According to the Burmese legend Rama, King of Benares, being a leper, retired to the jungle, where he lived on herbs and fruits and in especial on the fruit and leaves of the kalaw tree. Recovered, he found in the jungle the Princess Piya, also a leper, whom, having cured with the same fruit, he subsequently married.

Certain passages in the " Ayur Veda " or " Knowledge of Life " are also said to refer to the value in leprosy of chaulmoogra under the name of " tuvaraka." Unfortunately the date assigned to Sushruta, who wrote the " Ayur Veda " at the inspiration of the divine Dhanvantari, is uncertain, and may vary from 327 B.C. to 750 A.D., a period which embraces the golden age of Hindu medicine.

Chaulmoogra oil was first introduced into western medicine by Mouat (1854), who reported improvement in a case of leprosy as a result of the oral administration and local application of chaulmoogra.

Up till 1900 it was believed that chaulmoogra oil was derived from the seeds of *Gynocardia odorata* R. Brown. Prain, however, showed that true chaulmoogra oil was obtained from the seeds of *Taraktogenos Kurzii* King, a tree grown in Burma and Assam. The seeds of *Taraktogenos Kurzii*, though very similar to those of *Gynocardia odorata*, may be distinguished by the fact that the radical of the seed is terminal, while in Gynocardia it is lateral. As a rule, also, the fruits yielding chaulmoogra oil are distinguished from those of Gynocardia by containing many seeds, packed so closely that they are facetted by mutual pressure, and hence are of very varying shape.

In addition to *Taraktogenos Kurzii*, certain other trees belonging to the natural order *Flacourtiaceæ* also yield oils having a composition closely akin to that of true chaulmoogra oil. The most important of these species are :—

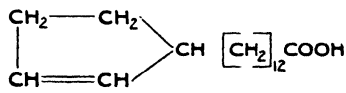
Hydnocarpus Wightiana	Blume.	Habitat	Malabar coast of India.
H. venenata . .	Gaertner.	,,	Ceylon, Deccan and Burma.
H. castanea . .	Hook, F. and Thoms.	,,	Burma.
H. anthelmintica . .	Pierre.	,,	Siam, French Indo-China.
H. Curtisii . .	King.	,,	Penang.
H. Hutchinsonii . .	Merrill.	,,	Philippine Islands.
H. subfalcata . .	,,	,,	,, ,,
H. alcalæ . . .	de Candolle.	,,	,, ,,
H. Woodii . .	Merrill.	,,	India.
H. alpina . .	Wight.		
Asteriostigma macrocarpa . . .	Beddome.	,,	Travancore.
Onchoba echinata .	Oliver.	,,	Sierra Leone.

It must, however, be realised that at present only the oils derived from *Taraktogenos Kurzii*, *Hydnocarpus Wightiana H. alcalæ* and *H. anthelmintica* are of proved therapeutic value.

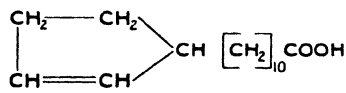
The successful cultivation of *H. Wightiana* and *H. anthelmintica* trees from seed distributed by the British Empire Leprosy Association has now been reported from tropical Africa, British Guiana, the West Indies and Fiji.

The principal fatty acids in the oils from *Taraktogenos Kurzii* and closely related species of Hydnocarpus are of a peculiar type known as the chaulmoogric acid series, having the general formula $C_nH_{2n-4}O_2$ and a closed ring made up of five carbon atoms. As a result of this structural peculiarity the acids of the chaulmoogric series have the power of optical rotation. The chief chemical constants of the chaulmoogric, hydnocarpic and allied oils derived from various sources are shown in the table on page 434.

The nomenclature of the characteristic acids derived from chaulmoogra oil was initiated by Moss (1879) who, believing that the oil was obtained from *Gynocardia odorata*, gave the name "gynocardic acid" to a crystalline preparation melting at about 20° C. Power and his collaborators (1904–5), on the other hand, obtained from true chaulmoogra oil, derived from *Taraktogenous Kurzii*, the characteristic acids which they termed chaulmoogric and hydnocarpic and the constitution of which they successfully determined.



CHAULMOOGRIC ACID



HYDNOCARPIC ACID

Chaulmoogra oil itself is either a brownish yellow liquid or a soft solid, which melts at about 22° to 30° C. It possesses a characteristic odour and a somewhat acrid taste. The chaulmoogric and hydnocarpic acids are present as the glyceryl esters, but can be obtained as crystalline colourless leaflets. Chaulmoogric acid—$C_{18}H_{32}O_2$—melts at 68·5° C. and is dextrorotatory, having $[\alpha]D + 62·1°$ (in chloroform). Its iodine number is 90·1. The methyl ester (methyl chaulmoograte) distils at 227° C. under a

Characteristics of the Chaulmoogra and Hydnocarpus Oils (Perkins and Cruz, 1923, modified)

Oil.	Specific gravity, $\frac{30^\circ\ C.}{30^\circ\ C.}$	Refractive index $\mu\ \frac{30}{D}$.	Freezing point °C.	Rotation 100 mm. 30°/D.	Iodine number hanus.	Saponification number.	Acidity as per cent. oleic.	Fatty acids freezing point, °C.	Fatty acid specific rotatory power $[\alpha]\ \frac{30}{D}$.
Gynocardia odorata . .	0·929	1·4743	4	0	160	198	2·7	20	0
Hydnocarpus alcalæ .	0·948	1·4763	24	48·3	94·0	202	6·7	55	40
Hydnocarpus anthelmintica	0·952	1·4630	16	44·2	84·5	201	3·6	36	50
Hydnocarpus Hutchinsonii	0·943	1·4743	23	44	83·5	199	5·3	43	50
Hydnocarpus subfalcata .	0·951	1·4761	21	49·1	89·0	206	6·6	41	36
Hydnocarpus venenata .	0·947	1·4769	20	46·4	90·7	191	1·2	47	49
Hydnocarpus Wightiana .	0·947	1·4763	11	51·2	97·0	207	6·7	40	54
Hydnocarpus Woodii .	—	1·473	18	45·9	68·5	192	5·9	43	53
Taraktogenos Kurzii .	0·951	1·4771	9	43·5	104	215	3·4	32	43
Asteriastigma macrocarpa.	0·955	—	—	48·1	95·2	198	—	—	—

pressure of 20 mm. as a colourless oil, which on cooling forms a solid mass of needles melting at 22° C. The ethyl ester is also a colourless oil which boils at 230° C. under a pressure of 20 mm. Hydnocarpic acid—$C_{16}H_{28}O_2$—melts at 60° C. and is also optically active, having [α]D + 68·1°. Its iodine number is 100·2. Its methyl and ethyl esters are both colourless oils, which boil at 200° to 203° C. and 211° C. respectively under a pressure of 19 mm. The methyl ester solidifies to a mass of colourless crystals which melt at 8° C. Besides the above-mentioned acids, chaulmoogra oil contains a small amount of palmitic acid and, as Wrenshall and Dean (1924) have found, another highly unsaturated acid with an iodine number 168·3, which is probably a chaulmoogric acid with a second double bond, having [α]D + 53·1°. Its presence was first suggested by Power and Cornall (1904). Its probable formula is $C_{17}H_{29}$. COOH.

Lauric acid has recently been isolated from *Hydnocarpus Wightiana* oil by Cole (1929) who has also obtained a new optically active liquid fatty acid from the same source, but not in sufficient purity to determine its composition. It is probably, however, a lower homologue of hydnocarpic acid.

An interesting occurrence of chaulmoogric acid has been recorded by Goulding and Akers (1913), who obtained it from the fatty oil of "Gorli" seed—the seeds of *Oncoba echinata* Oliver. It was found to be present to the extent of 84·5 per cent. in the mixed acids, the remainder consisting of liquid acids having a higher iodine value and being therefore more unsaturated. André and Jouatte (1928) have recently found lauric, myristic, palmitic and stearic acids present in Gorli oil in addition to chaulmoogric acid.

The first hydnocarpus oils to be completely investigated were those expressed from the seeds of *Hydnocarpus Wightiana*, Blume and *H. anthelmintica*, Pierre. Power and Barrowcliff (1905[1, 2]) found that they resembled chaulmoogra oil very closely, consisting to a large extent of the glyceryl esters of chaulmoogric and hydnocarpic acids. De Wolff and Koldewijn (1912) determined the physical constants of the oil from *Hydnocarpus alpina*, Wight, and found them to agree closely with those of chaulmoogra oil. The

oil from *H. venenata* Gaertner was shown by Brill (1916) to contain both chaulmoogric and hydnocarpic acids, and the same investigator (1917) has stated that more than 90 per cent. of the free acids from the oil of *H. alcalae*, de Candolle, consists of chaulmoogric acid.

Brill (1917) believed that the composition of the oil from the seeds of *Pangium edule* Reinwardt from the Philippine Islands, was very similar to that of chaulmoogra oil, but Perkins and Cruz (1923) were unable to determine the presence of either chaulmoogric or hydnocarpic acids.

The oil expressed from fresh seeds of *Gynocardia odorata* was shown by Power and Barrowcliff (1905[1]) to differ completely from chaulmoogra oil, both in its physical characters and in its chemical composition. Gynocardia oil at ordinary temperatures is a pale yellow liquid, having an odour resembling that of linseed oil. It is completely devoid of optical activity and contains the following well-known acids :—

(1) Linolic acid, or isomerides of the same series, constituting the largest proportion of the oil.

(2) Palmitic acid in considerable amount.

(3) Linolenic and *iso*linolenic acids, the latter preponderating.

(4) Oleic acid in small amount.

Gynocardia seeds contain, in addition to the fatty oil, a crystalline cyanogenetic glucoside, $C_{13}H_{19}O_9N$, which has been designated gynocardin, and an enzyme, termed gynocardase.

During the past few years numerous derivatives, chiefly simple or complex esters of the mixed acids from chaulmoogric and hydnocarpic oils, or of pure chaulmoogric or pure hydnocarpic acid, have been made by Perkins (1924), Santiago and West (1928), de Santos and West (1929[1, 2]), and Santillan and West (1929). So far none of these has proved to have any clinical advantage over the raw oils, ethyl esters or sodium salts.

Adams and his fellow workers (1925–1929) have succeeded in synthesizing a large number of acids, homologous with chaulmoogric and hydnocarpic acids or closely related to them, and have tested their action on the growth of an organism which they describe as *Mycobacterium lepræ*, but which at the present

juncture must be regarded as merely one of the acid-fast bacilli. Finally, while Perkins and Cruz (1927[1,2]) have synthesised *dl*-chaulmoogric acid, Stanley and Adams (1929) claim to have synthesized from hydnocarpic acid a substance which agrees in every respect with the naturally occurring chaulmoogric acid. Although at present this work is only of chemical interest, yet with advancing knowledge there is no reason why in the future the active therapeutic substances should not be satisfactorily prepared synthetically in the laboratory.

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THE TREATMENT OF LEPROSY BY CHAULMOOGRA AND ALLIED OILS

Various methods have been used for the administration of chaulmoogra and other oils in the treatment of leprosy. Oral administration, inunction, subcutaneous, intramuscular and intravenous injections have all been advocated from time to time.

Oral Administration.—When given by mouth the oils tend to produce nausea and vomiting, so that their prolonged use is in many cases impossible. Attempts have been made to overcome this irritant action by giving the oil in keratin-coated capsules or, as suggested by Denny (1929), by the addition of benzocaine. Wayson and Badger (1928) employed a preparation of the esters which can be given without inconvenience by mouth—an emulsion of acacia and a simple syrup of equal parts of the mixed esters of chaulmoogra oil and cod-liver oil to which iodine is added to the extent of 0·06 per cent. The mixture was given in doses of from 2 to 40 c.cm., corresponding to from 0·5 to 10 c.cm. of the esters per week per 100 lbs. body weight. Sufficient time has not yet elapsed to determine the therapeutic value of these preparations.

Travers (1926), in the Federated Malay States, has employed an old Chinese treatment which consists in giving the powdered whole nut of *Hydnocarpus anthelmintica* two parts, with *Cannabis indica* one part. The powder is taken in doses of 0·5 drachm twice daily after food. It must be made up freshly every week, as otherwise it tends to become rancid. As a result of this "Tai Fong Chee" treatment, 218 out of 275 cases are said to have shown improvement. Although the nuts of *H. Wightiana* are cheaper and more readily obtained, they are apt to produce gastric irritation and nausea. Similar results were obtained by Lindow (1927), 229 out of 345 cases improving, but none showing complete disappearance of the lesions. Rodriguez (1925) also found improvement with the Tai Fong Chee treatment, which does not produce toxic reactions. With the oral administration of chaulmoogra oil good results have been reported from South America by de Aguiar Pupo (1926). Of eighty-eight well-

developed cases, 20 per cent. improved; but of eighty-two incipient cases, 17 per cent were reported cured and a further 4 per cent. showed disappearance of the lesions, thus clearly indicating that the oral administration of chaulmoogra oil does have definite therapeutic effects. Read (1925), who reviews the various methods of oral administration, suggests that a dose of six drops daily of freshly prepared chaulmoogra oil is sufficient to produce improvement.

Intramuscular Injection.—The first effort to produce a preparation of chaulmoogra oil which on intramuscular injection would not prove too irritant was made by Mercado (1914), who used a mixture of 60 c.cm. of chaulmoogra oil, 60 c.cm. of camphorated oil to deaden the pain, and 4 gm. of resorcin as an antiseptic. Heiser (1914), who treated a small series of cases, reported only 11·1 per cent. of apparent cures and, according to Wade and Lara (1927), the majority of cases treated with the Mercado mixture ultimately relapsed, for in 1913 only four treated cases were released in the Philippine Islands after being negative for two years. This treatment, owing to the irritation which it produces in the tissues, has now been largely abandoned, as patients refuse to submit to it. The fact, however, remains that the partial success resulting from it stimulated further work with, ultimately, a far greater measure of success.

The greater success now attained in the treatment of leprosy is due in the first place to the work of Rogers in India, and Dean and Macdonald in Hawaii, the former introducing the use of the sodium salts of the fatty acids with low melting points derived from chaulmoogra oil, the latter preparing the ethyl esters of the fatty acids.

Intravenous Injection.—Rogers (1916), with the assistance of Ghosh (1916), prepared the sodium salts of the fatty acids of chaulmoogra oil and showed that those of the lower melting point fatty acids could be safely injected intravenously, the intravenous route being superior to the subcutaneous or intramuscular, which were attended with considerable pain. As the result of four and a half years' work on fifty-one cases treated for six or more months, Rogers (1921) was able to state that 41 per cent. were apparently cured and another 39 per cent. greatly improved, only one

advanced case being no better. It should, however, be mentioned that this series consisted for the most part of cases from the out-patient department of the Calcutta Medical College Hospital, so that they were not nearly so advanced as those usually seen in leper asylums. None of a small series of late European cases treated with less frequent injections at the Calcutta asylum was completely cleared of lesions. The main drawback to this form of treatment, however, is the gradual obstruction of the veins as a result of the intravenous injections.

In the meantime, Dean (1919), in Hawaii, had prepared four fatty acid fractions from chaulmoogra oil, which on conversion into the ethyl esters were injected intramuscularly. A group of patients was placed on each of the fractions, but as all did equally well after several months trial, fractionation was abandoned, and McDonald (1920) then gave weekly intramuscular injections of the ethyl esters of the entire fatty acids of the whole oil with 2 per cent. of iodine by weight, chemically combined ; the dose to begin with was 1 c.cm., which was increased by 1 c.cm. at every second or third injection until a maximum dose of from 2 to 6 c.cm. was reached according to the age and weight of the patient. In addition, mixed fatty acids with 2·5 per cent. iodine were given by mouth. The injection of the ethyl esters was unattended by unpleasant results, as in the course of 6,924 injections there was only one case of abscess formation; seventy-eight cases were apparently cured after an average stay in hospital of only fifteen months.

In a subsequent communication, McDonald and Dean (1921) investigated the effects of injecting the ethyl esters of pure chaulmoogric and hydnocarpic acids. The results with both acids were excellent, though hydnocarpic appeared to be somewhat more effective than chaulmoogric acid. Rogers (1921) also believes that hydnocarpic acid is the more active substance. De Vera and Lara (1929), however, find that ethyl chaulmoograte and ethyl hydnocarpate are each preferable to the ethyl esters of total fatty acids of *Hydnocarpus Wightiana* oil, but are identical in action. This view is in opposition to that generally entertained and recently confirmed by Stévenel (1929), who finds, as does

Muir, that undistilled mixed esters are more active than distilled mixed esters. Distilled mixed esters consist almost wholly of a mixture of ethyl hydnocarpate and ethyl chaulmoograte, the ethyl esters of the other acids not being volatile under the conditions of distillation. De Vera and Lara should therefore have found that the two single acid esters and the mixed esters were equally active. Further research is obviously needed on this point.

Treatment with the ethyl esters of the fatty acids of chaulmoogra oil has constituted the chief medicament in use at Culion, in the Philippine Islands, according to Wade and Lara (1927). The esters are given intramuscularly in doses of from 2 to 5 c.cm. once a week, for the most part with iodine added to reduce irritation. Cole (1929) has recently shown that too long or too short a period of heating the esters with iodine increases the irritant properties. The irritant properties can, however, be eliminated by controlling the heating with iodine by a time-temperature factor. In the most recent report from the Culion Hospital, Lara (1928) states that the total ethyl esters of oil from *Hydnocarpus Wightiana*, with 0·5 per cent. iodine, are given in doses of from 0·5 to 5 c.cm. intramuscularly once a week, while in addition small doses are given intradermally. By the end of 1927 some 900 cases or 16 per cent. of the advanced cases had been paroled. The percentage of bacteriologically negative cases, according to the type of leprosy, was 0·79 cutaneous, 6·0 mixed, and 50·7 nerve type. The best results were obtained in children, the period of most active sexual life being comparatively unfavourable, especially in males. Cases showing reactions were less influenced than those without reaction, possibly because so many of these patients were in an advanced condition.

The ethyl esters of hydnocarpus oil have also been used to a considerable extent in China by Fowler (1922), Wilson (1924), Read and Feng (1925) and others. Some workers have preferred to add 25 per cent. of camphor to the mixture.

Since their introduction by McDonald and Dean, ethyl esters have been prepared commercially on a large scale and sold either alone (*e.g.*, moogrol (British), antileprol (German), anti-

lebrine (Italian)), or with various proportions of fatty oils or disinfectants. In the experiments of Rangel (1926[1,2]), in Brazil, the commerical preparations were found to be of considerable value. Engel-Bey (1926) and Nägelsbach (1926) obtained similar results.

In India, Muir (1921) has popularised what is known as the E.C.C.O. mixture, as it appears to be less painful, more effective and more economical than the ethyl esters. The composition of the mixture is as follows :—

Ethyl esters of fatty acids of oil of *Hydnocarpus Wightiana* . .	1 c.cm.
Camphor	1 gm.
Creosote	1 c.cm.
Olive oil	2·5 c.cm.

Muir (1923) does not believe in the advantage of adding iodine to the mixture. Maire and Pinto (1927) find the E.C.C.O. mixture of benefit in nodular leprosy, but of little value in nerve cases.

Muir (1927) now adopts the following routine treatment. Hydnocarpus oil prepared from *Hydnocarpus Wightiana* and containing 4 per cent. of creosote is injected intramuscularly or intradermally in doses of from 4 to 10 c.cm. in order to test the reaction. A 1 per cent. or 2 per cent. solution in saline or sodium hydnocarpate is then given intravenously twice weekly, beginning with 2 to 4 c.cm. and rising to 10 c.cm. These injections are continued until a reaction occurs or until a vein becomes blocked, when intramuscular injections are again instituted. In the second stage great care has to be exercised to avoid reactions. Gradual thrombosis of the veins at the site of injection was formerly the great disadvantage of the intravenous method of administration, but recently Muir (1927) has found a means of overcoming this difficulty. Before actually injecting the sodium hydnocarpate into the vein, an equal quantity of blood is withdrawn from the vein and thoroughly mixed with the hydnocarpate solution in the syringe. The mixture can then be injected into the vein without causing that proliferation of the intima and narrowing of the lumen, which eventually causes reduction of the

vein to a thin fibrous cord. Incidentally, the sodium hydnocarpate prepared from *H. anthelmintica* and *H. alpina* is less likely to lead to blocking of the veins. Exposure of the hydnocarpates to ultra-violet light before injection also lessens the tendency to cause thrombosis. Wilson (1926), in Korea, used intramuscular injections of the oil derived from *H. Wightiana* in preference to the esters, as there was a complete absence of pain after the injections. Very favourable results were reported from a trial in 300 cases, the cheapness of the whole oil being an additional advantage when large numbers of poor patients had to be dealt with. In Fiji the whole oil has been given intravenously in many cases, but the proceeding is not without danger as compared with the intravenous injection of the soluble sodium salts.

Recently, Rogers (1927) has used the sodium salts of the fatty acids of *H. Wightiana*, prepared by Dr. T. A. Henry, those acids being selected which are of low melting point. This preparation, "Alepol," therefore corresponds roughly to that originally used by Rogers (1916) in Calcutta. On intramuscular or subcutaneous injection a 3 per cent. solution of alepol is found to be quite painless while, provided Muir's technique is used, intravenous injections may be given with impunity. As Muir (1927) points out, 700 doses of alepol can be made up from 100 gm. of powder, at a cost approximately one twentieth that of a good ethyl ester. Very favourable reports of extensive trials of this new preparation have already been received. Thus Cochrane (1929) found that after seven months' treatment, eighteen of twenty-four cases were improved, and two were entirely free from symptoms. As much as 10 c.cm. of a 6 per cent. solution could be given intramuscularly without pain twice a week.

Welch (1927) finds that cases which have failed to react to other preparations improve rapidly after the intravenous injection of from 1 to 10 c.cm. of a 1 to 2 per cent. solution of alepol with 0·5 per cent. carbolic acid. Alepol has also been used by Donaldson (1929), Markianos (1929) and others.

Intradermal Inoculation.—The plancha, or infiltration, method as employed at Culion, consists of intradermal injections of the usual antileprotic drugs directly into the leprous skin lesions.

Table of Cases Treated with Injection of Chaulmoogra Oil Derivatives

Place.	Observers.	Years.	Cases.	Apparently cured.	Percentage cured.	Much improved. Per cent.	Improved. Per cent.	Not improved. Per cent.	Remarks.
Manila Culion	H. W. Wade. C. B. Lara .	1921–5 1921–6	(2,990) 6,000	356 629[1]	— 10·5	— —	— —	— —	(1,511 sent to Culion.) Average duration 2·65 years. (Average duration 8 years.)
Honolulu	U.S. Report .	1920–4	394	124	31·47	—	—	—	Paroled, only 13 relapsed.[2]
Calcutta	E. Muir .	—	203	43	21·18	—	—	—	Cases treated 3 months or more.
			123	38	31·0	—	—	—	Cases treated 6 months or more.
Dichipali	I. Kerr .	1923–4	180	30	17·0	45·0	35·0	3·0	63 per cent. became uninfective.
		1925–6	?	—	19·0	—	—	—	
Japan . .	K. Shiga .	1921–3	211	23	10·9	14·2	—	—	Discharged, improved.
Siam . .	O'Brien .	2 years	357	—	—	21·7	48·6	29·6	Duration 1 to 3 years.
						23·3	49·4	28·2	,, + 3 years.
New Caledonia	Genevray .	—	70	6	8·75	31·4[3]	11·4	48·6	After 18 months, only 5 relapsed.
Korea . .	R. W. Wilson	—	—	75	—	—	—	—	—
Calcutta .	L. Rogers .	1915–19	51	21	41·2	39·2	17·6	2·0	Sod. hydnocarpate intravenously.
India . .	R. G. Cochrane	7 months	24	2	8·3	75	—	—	Alepol.

[1] By the end of 1927 the apparently cured would number 900. [2] Seven of the thirteen relapses did not continue treatment after parole. [3] 17·14 classed as "nearly cured."

The number of injections to be given in any case is determined by the size of the lesion to be infiltrated; each injection should produce a wheal over an area not more than 1 cm. square, and not more than 4 or 5 c.cm. should be given by this method in a single dose. Lara and Nicolas (1929) have treated five early cases and one slightly more advanced case of leprosy with the iodised ethyl esters of oil from *H. Wightiana*. Four of the early cases became bacteriologically negative in one month and one in two months. The slightly more advanced case was negative after five months of treatment.

In attempting to assess the value of the newer treatment of leprosy, some difficulty arises from the fact that the type of patient varies considerably in different institutions. For instance, at Culion, in the Philippines, the cases are nearly all of long standing, with an average duration of not less than eight years. The result, according to Lara (1928), is that only 16 per cent. of the cases have been cured. Nevertheless, Wade and Lara (1927) point out that "for large scale work the modern treatment methods are decidely superior to the older ones and seem to be particularly effective in the early cases." In the fifteen years, from 1906 to 1921, only forty-seven cases were paroled as negative, but in the five and a half years up to September, 1927, 587 were paroled, 39 had died in the colony after becoming negative, and 257 more negative cases were awaiting their discharge. Moreover, clinical relapses were rare and only some 5 per cent. relapsed after having been kept under observation for two years. In children, at Culion, Nicolas and Roxas-Pineda (1928) obtained even better results. In seventy confirmed cases in children treated with the ethyl esters, 18 or 50 per cent. of the males, and 20 or 58·8 per cent. of the females became negative. While thirty-one out of forty early cases cleared up, only one out of three late cases became negative.

As these figures are based on the treatment of nearly 6,000 cases at Culion, and 2,990 at Manila, they can leave no doubt but that a great advance has been made in the treatment of leprosy. In India, where there is an opportunity of subjecting cases to treatment at an earlier stage, the results have been even

more striking. At Dichipali, Kerr (1927), for instance, reports that 18 per cent. of cases were apparently cured, 45 per cent. were much improved, and only 3 per cent. were entirely unaffected.

Provided, therefore, that the cases come under treatment at an early stage, the newer methods ensure that a considerable number of cases will be successfully cured.

It is somewhat unfortunate that on the chemical side there is great confusion owing to the practice of calling the preparations of the total fatty acids of hydnocarpus oil sodium hydnocarpate and ethyl hydnocarpate and those from the total fatty acids of chaulmoogra oil, sodium chaulmoograte and ethyl chaulmoograte. Ethyl chaulmoograte has unfortunately been used officially in this wrong sense in the United States of America. The terms hydnocarpate and chaulmoograte in this connection should be reserved for preparations of the pure individual acids. The preparations of the total fatty acids from either oil should be given trivial names or called " sodium salts " or " ethyl esters " of the fatty acids of chaulmoogra or hydnocarpus oils. The following short names may be suggested as examples :—

Chaulmoogric ethyl esters.
Hydnocarpic ethyl esters.
Chaulmoogric sodium salts (total acids).
Chaulmoogric sodium salts (low melting acids).

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OLEUM MORRHUÆ AND OTHER OILS

In addition to chaulmoogra and hydnocarpus oils and their derivatives, various other oils have from time to time been used in the treatment of leprosy.

Cod-liver oil and its derivative, sodium morrhuate, were at one time somewhat extensively used in treatment, but have now been practically discarded in favour of the hydnocarpates. Neff (1929) has recently employed the ethyl esters of the oil extracted by the native method from the fruit of *Calophyllum bigator*, the "dilo" tree of Fiji and "tamanu" of Tahiti. Intramuscular injections of from 5 to 8 c.cm. a week caused some improvement in 70 per cent. of cases, but the results were not as good as those obtained with alepol or the ethyl esters of *Hydnocarpus Wightiana*.

The ethyl esters of *Calophyllum bigator* were, however, specially useful in cases with much nerve and joint pain when they can be given in association with the ethyl esters of hydnocarpus oil.

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TOXIC REACTIONS FOLLOWING THE ADMINISTRATION OF CHAULMOOGRA OIL AND ITS DERIVATIVES

The nausea and vomiting which result from the oral administration of chaulmoogra oil in the large doses required to obtain therapeutic results have been already mentioned. They would seem to be due, for the most part, to local irritation.

More important are the various types of reaction which follow the intramuscular or intravenous administration of chaulmoogra oil derivatives. These have been studied exhaustively by Wade, Lara and Nicolas (1924) at Culion. Of 2,818 cases treated by the intramuscular injection of chaulmoogric ethyl esters, 1,985 or 70 per cent. complained of some form of reaction.

Immediate Effects.—These consisted of a choking sensation, dizziness, and pains in the chest. Dizziness was the most common symptom, but was usually of short duration, though occasionally headache ensued. Severe cases were associated with dimness or even temporary loss of vision, while very rarely there were localised twitchings or general muscular convulsions. The cause of these immediate reactions, which were never fatal, is unknown. Certain patients appeared to be especially susceptible; in others the condition was possibly due to entrance of the drug into a vein during the intramuscular injection.

Local Effects.—Some slight degree of local reaction to the injected material was of constant occurrence. Induration, pain and abscess formation were more common after subcutaneous than after intramuscular injection, and though this form of reaction is generally localised to the site of injection, it may be found at a distance of 10 to 20 c.cm. below the point of injection as a result of the effects of gravity on the drug. Occasionally the regional lymph glands became enlarged and even ulcerated.

General Symptoms.—These consisted of headache, malaise, weakness, fever, insomnia, anorexia and abdominal pain, together with a peculiar sensation of general heat.

Respiratory Tract Symptoms.—Pain and a sense of oppression in the chest were by no means rare, with or without cough.

Hæmoptysis occurred in four cases of Wade's series, but all symptoms involving the respiratory tract necessitate careful investigation as so large a percentage of the inmates of a leper asylum present evidence of active or latent tuberculosis. The ethyl esters of chaulmoogra, if injudiciously used, seem clearly to activate the tuberculosis.

Renal Symptoms.—Albumin and urinary casts were also not uncommon as the result of treatment with chaulmoogra. In some instances there was actual nephritis, the death rate from which has apparently increased in leprosy as the result of treatment with the ethyl esters of chaulmoogra.

While a sensation of choking was noted in some 4·7 per cent. of cases, thoracic pain and oppression were present in 15 per cent. and induration in 21·3 per cent.

Wade (1924) found that the addition of 0·5 per cent. of iodine to the ethyl esters of chaulmoogra oil reduced the irritation at the site of injection and produced fewer complaints of general symptoms. The addition of 10 per cent. of creosote (1925) also reduced the local irritation, though not to quite the same extent as did the 0·5 per cent. iodine. Creosote, however, tended to increase the number of cases with irritation of the respiratory tract.

The So-called "Leprous Reaction."—This consists usually of fever and cutaneous eruptions, but neuritis, arthritis, orchitis and inflammatory reactions of the eye may occur in the absence of the more usual symptoms. The eye symptoms consist of iritis and iridocyclitis often associated with intense pain.

The cutaneous reaction may be regarded as an acute exacerbation of the disease and has been summarised by Muir (1927[2]) as follows :—

Swelling and erythema of existing lesions.

The appearance of fresh, rose-coloured nodules which are often painful.

Apparent granulation of lepra bacilli in lesions.

Acceleration of blood sedimentation rate.

An increase in the serum globulin—Wade (1925).

This acute phase of the disease may occur from time to time

in both treated and untreated cases. It is observed, however, more frequently in patients undergoing treatment with chaulmoogra oil and its derivatives. McDonald and Dean (1921) reported that about 10 per cent. of a large series of leper patients so treated suffered from reactions. A reaction incidence of 14·2 per cent. among 2,000 patients treated with ethyl esters of chaulmoogra is recorded by Wade, Lara and Nicolas (1924). In a similar series, Rodriguez (1925) reported the occurrence of " lepra fever " in 23·6 per cent. Green (1929) found that at Kuala Lumpur where, in addition to injections of ethyl esters of chaulmoogra, over 90 per cent. of patients had the Tai Fong Chee treatment (ground *Hydnocarpus anthelmintica* seed, two parts ; powdered *Cannabis indica*, one part) 17 to 20 per cent. of patients had one or more reactions.

The most striking cutaneous reactionary lesions are the new macules and nodules. The formation and subsequcnt progress of these lesions depend on several factors, the stage of the disease, the severity of the reaction, the previous degree of invasion of the skin surface as a whole by *Mycobacterium leprœ*, the depth of the bacilli beneath the epithelial surface, their numbers and the probable dissemination of fresh groups of *M. leprœ* by the blood-stream.

The following types of skin lesions were noted by Green (1929) :—

A rapidly occurring superficial series of lesions commencing as reddened macules and small nodules which quickly increase in size. Over these nodules blebs arise, the serum of which becomes infected with secondary skin organisms. This eventually leads to infection of the deeper layers of the skin, with subsequent scar formation. This is a fairly common type and is responsible for much eventual disfigurement.

A slower and more commonly seen type of cutaneous manifestation. In this, reddened flat semi-œdematous plaques of irregular size and shape make their appearance in various situations. These do not tend to break down, but the patient has the appearance of being covered with huge reddened mosquito bites.

A less common type in which deep painful subcutaneous nodules are rapidly formed. The skin is not reddened, but

appears to be stretched over the swellings, which do not break down and are slowly absorbed. This form is sometimes associated with arthritic pains and swellings.

An herpetic form of eruption described by Muir (1923).

In advanced cases of leprosy existing nodules may swell, become reddened, soften, and break down after a few days. Fever is always present, secondary infection sets in, and the surface of the nodules becomes deeply ulcerated.

The extent to which nerve lesions occur depends on the degree of previous involvement of the peripheral nerves, the stage of the disease, and the severity of the reaction. The patient may suffer from a localised or apparently generalised swelling of the peripheral nerves, the ulnar and peroneal nerves being most frequently affected. Any of the cutaneous lesions previously mentioned may be accompanied by pain, throbbing and swelling of presumably already involved peripheral nerves. On the other hand, " nerve reactions " occur without fever or attendant, freshly arising cutaneous lesions. The sudden and severe trophic disturbances seen in cases of advanced leprosy, leading to gangrene and loss of extremities, appear to have their origin in severe " nerve reactions."

The occurrence of leprous reactions may be induced by the administration of potassium iodide or by large doses of chaulmoogra oil given either by mouth or by injection. Denney and Hopkins (1922) find, for instance, that vaccination against smallpox causes the development of very severe reactions. Rogers and Muir (1925) and Green (1929) cite alimentary indiscretion as a predisposing cause.

The duration of the leprous reaction varies considerably. Fever, which may be absent, may continue for a few days or some weeks. Barrera and Chavarria (1924), in their series of cases, reported that the fever reached its height in the first three days and subsided by lysis after six to twelve or, more rarely, twenty days. As regards the skin manifestations, if these occur as part of the reaction in Muir's second stage, the freshly produced lesions tend to remain until the leprosy as a whole improves. In the other stages, however, these newly formed skin lesions

may begin to subside in from two weeks to six months or more. The average period is from three weeks to one month.

The question naturally arises as to whether the leprous reaction is beneficial to the patient or not.

Wayson (1921) found that after treatment with potassium iodide the lepers suffered considerably, the neuralgic pains and loss of sleep, however, being endured for the compensatory improvement.

Wade, Lara and Nicolas (1924) stated that on the whole leprous reactions were not beneficial. Four deaths due to the leprous reaction were reported by Pineda (1925) among 500 autopsies on lepers. Rodriguez (1925) also expressed the opinion that the leprous reaction is of doubtful value to the patient. In 2,951 patients under treatment, 695, or 23 per cent., had one or more attacks of lepra fever. Of these patients, 40·5 per cent. were improved, 40·6 per cent. remained stationary and 18·9 per cent. became worse. On the other hand, of those who did not have lepra fever, 50·3 per cent. were improved, 39·6 per cent. remained stationary and 10·1 per cent. became worse.

It must, however, be remembered that the majority of the patients at Culion are advanced cases and according to Muir (1927[2]) the stage of the disease has an important bearing on the value of reactions. In the earliest stages of leprosy no reaction occurs, as there is not enough lepromatous tissue to break down and cause a reaction. In the second stage a reaction is accompanied by the setting free of bacilli in the blood-stream and, with the low immunity accompanying this stage, free bacilli may lead to the formation of new lesions. Later on, a third stage is reached when immunity against the newly liberated bacilli is high and the reaction is followed by beneficial results. During the last and fourth stage, the bacilli are reduced in number, those that remain being confined to the nerve trunks.

Reactions are thus particularly dangerous during the second stage and in advanced or fourth stage cases may cause severe trophic lesions.

Various theories have been held as to the causation of the leprous reaction.

Rogers and Muir (1925) regard patients suffering from the leprous reaction as sensitised to the lepra bacillus and its toxins. Wade (1926) came to the conclusion that the reaction was anaphylactic in nature.

In this connection Green (1929) has found that the nodules or raised skin plaques formed during the reaction are of a semi-œdematous character and may arise in the course of two or three days. The newly formed nodules contain but few *M. leprœ*, as Hopkins (1926) has shown. The intradermal injection of *M. leprœ* in reaction cases is followed within twenty minutes by swelling and surrounding hyperæmia, and eventually gives rise to an infiltrated plaque, similar to those occurring as a result of the leprous reaction and subsiding only when the other reaction plaques begin to disappear.

It is therefore probable that the primary factor in the production of the cutaneous nodules and reddened macules is an allergic state of the tissues towards *M. leprœ* which are already present in small numbers in the skin or, newly disseminated from the original lesions, are carried to the skin by the blood-stream.

Treatment of the leprous reaction is to a certain extent connected with the general treatment of leprosy. Rogers and Muir (1925), however, found small injections of hydnocarpus esters in 0·25 c.cm. to 0·5 c.cm. doses beneficial. Many other drugs have been tried for relief of the condition. Thus Rodriguez (1925) injected intravenously doses of 20 c.cm. to 30 c.cm. of a 2 per cent. solution of calcium chloride in distilled water. Hexamine by mouth, sodium bicarbonate and antipyrin and phenacetin have also been used. Hopkins (1926) believes that Fowler's arsenical solution and quinine are valuable drugs during this reactive phase. Potassium antimonyl tartrate in doses of 0·02 gm. intravenously every second day was used by Muir (1927[1]) to control leprous reactions which had been induced in selected cases by potassium iodide. He also found that pain in the nerves was relieved by injections of adrenaline and normal saline. Wheatley (1927) and Green (1926 and 1927) have also used adrenaline with success.

In 1928, Muir and Chatterjee reported the relief of nerve pains by the oral use of ephedrine sulphate. It was noted also that

ephedrine appears to reduce the severity of these actions. Cochrane and Mittra (1928) and Green (1929) have also found that doses of ½ to 1 gr. of ephedrine hydrochloride three times a day have a definite effect in reducing nerve pains.

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MODE OF ACTION OF CHAULMOOGRA AND HYDNOCARPUS OILS IN LEPROSY

As in the case of other chemotherapeutic remedies, there is as yet no agreement as to the mode of action of the chaulmoogric series of acids.

Some workers believe that these acids have little or no specific action on the leprosy bacillus, merely producing a reaction with fever, as a result of which the lepra cells are ruptured, thus liberating bacilli which act as antigens and increase the immunity response. Any condition which produces fever might conceivably act as well as the chaulmoogra derivatives and, in fact, Muir (1927) has found that kala-azar complicating leprosy has in certain cases a remarkable effect in improving the leprous lesions. On the other hand, the majority of workers are now agreed that leprous reactions are to be avoided except perhaps during Muir's third stage.

The possibility of a direct action of the chaulmoogric acids has also been suggested. Read (1924) found that in toxic doses the hydnocarpates produce hæmolysis of red blood corpuscles, renal irritation with hæmoglobinuria and anorexia associated with nausea and vomiting. In therapeutic doses, however, these effects are not observed. Read (1925) believes that the increase in large mononuclear leucocytes following an injection of chaulmoogra oil assists in the transport of the oil throughout the body. One suggestion is that as the result of treatment with chaulmoogra the blood lipase is increased; *Mycobacterium lepræ* thus has its fatty coat removed and is more easily destroyed by the tissues. Walker and Sweeney (1920), on the other hand, believe that chaulmoogra oil contains bactericidal substances which, when tested *in vitro* on bacilli of the acid-fast group of bacteria, are found to be about 100 times more active than phenol, though the bactericidal effect is specific for acid-fast organisms. The active substances in chaulmoogra oil were the fatty acids of the chaulmoogric series, chaulmoogric and hydnocarpic acids and possibly lower isomers of the same series. Schöbl (1923 and 1924[1, 2]) has confirmed these results, but has found that *in vitro* certain

other oils, such as cinnamon, have an inhibitory effect on the growth of *Mycobacterium tuberculosis*. Unsaturated fatty acids did not necessarily exert an inhibitory effect because they were unsaturated, though in the chaulmoogric acid series the inhibitory action was bound up with the degree of unsaturation, since saturated chaulmoogric acid was found to have lost its inhibitory action. Schöbl also obtained some evidence to show that acids of the chaulmoogric series containing short side-chains are more active *in vitro* than those having long side-chains. Acid-fast bacteria were, however, quite able to adapt themselves to chaulmoogric acids and were then able to withstand much greater concentrations of the acids.

Adams and his co-workers (1925–1929) have also studied the effect of various derivatives of chaulmoogric and hydnocarpic acids on the growth *in vitro* of an acid-fast bacterium which they state is *M. leprœ*, despite the fact that there is no satisfactory evidence that Hansen's bacillus has yet been cultivated *in vitro*. Several series of organic acids of the general formula $RCH(COOH)R^1$ were prepared where R is a cyclohexyl, cyclopentyl, cyclopentenyl or cyclopropyl group or is one of these groups substituted in the ω position of the alkyl group and where R^1 is an alkyl group. No very marked differences between three-, five- or six-membered rings were found, with the result that the supposition that a ring is an important factor for bactericidal action is probably incorrect. The acids which gave the best results in each series were those with from sixteen to eighteen carbon atoms. When the two substituted groups in the acetic acids were of approximately the same number of carbon atoms, the products in general had the most pronounced bactericidal action. Octadecanoic and hexadecanoic acids were also found to possess a bactericidal action so that the presence of a ring in the molecule is not necessary for bactericidal action. The results obtained from these experiments suggest that bactericidal action is not correlated with chemical specificity, but is attributable to a combination of physical properties common to many of these acids as a result of which there is adsorption or solution of the acid in the waxy coating of the bacterium.

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PROTEIN SHOCK IN THE TREATMENT OF LEPROSY

Since in certain cases improvement has followed the occurrence of leprous reactions, the possibility of benefit arising from the

reaction following the injection of foreign protein has been entertained. Thus improvement in early nerve cases has been recorded after the injection of tuberculin or of typhoid vaccines in doses of from 50 to 200 millions. Dyce Sharp (1927) even found that considerable improvement followed the intravenous injection of 0·5 c.cm. of sterile " Milkmaid brand " condensed milk, diluted to 5 c.cm. with distilled water. The immediate effects of the injection were giddiness, feeble pulse, intense pain and hyperpyrexia lasting for some forty-eight hours. In a few advanced cases there was distinct clinical improvement, with healing of ulcers and absorption of nodules. Much further experience is required to determine if the severe reactions which are associated with this course of treatment can be sufficiently controlled to ensure good results in suitable cases.

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POTASSIUM IODIDE

The administration of potassium iodide has long been known to lead to severe reactions in lepers. In 1927 Muir advocated the routine use of potassium iodide, in association with tartar emetic intravenously, in order to control the reactions which may arise. Potassium iodide is given in a dose of 1 gr. per day, the daily dose being increased by 1 gr. until there is a rise of temperature to more than 99° F., or until the skin lesions become red and swollen. When the temperature falls below 99° F. and the swelling begins to diminish, the iodide is continued, with the same dose as produced the fever and swelling, except that the drug is now given only once or twice a week. If the fever or a great amount of swelling continues for more than three days, 0·02 gm. of tartar emetic is given in 2 c.cm. of sterile saline intravenously every second day till the temperature falls, when the administra-

tion of iodide is resumed. More recently Muir (1929) has recognized the limitations of potassium iodide therapy. While valuable in severe skin cases, its use is dangerous in early cases. In any case it is essential to give only doses of such size and at such intervals that the lowering of the general resistance caused by any given dose has disappeared before the administration of the next dose. Any effort to press the treatment inevitably causes an exacerbation of the disease. Cochrane (1929), Lowe (1929) and others have, in fact, entirely given up the use of potassium iodide, for compared with treatment by chaulmoogra oil, potassium iodide has few, if any, advantages and many disadvantages.

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METALLIC PREPARATIONS IN THE TREATMENT OF LEPROSY

Various metallic preparations have been used in the treatment of leprosy. Mercury and antimony are now scarcely ever employed, but copper, gold, and more recently, tellurium, are all stated to have a beneficial action in leprosy.

Tellurium has been used by Stanziale (1929) in the form of a 10 per cent. suspension of the metal in 5 per cent. glucose, as the biniodide, 10 per cent. in oil, and as the iodo-tellurate of quinine, 5 per cent. in oil.

Cases of the tuberculo-anæsthetic type showed some improvement, but a macular case was unaffected.

Copper salts have been employed in Italy by Rho (1924), cuprocyan, a double salt of copper and potassium cyanide, and cuproiodase, a compound of copper, iodine and cholesterol having

both produced improvement in the leprous lesions. Matta and Devoto (1923) and Urbino (1925) have claimed similar results.

Palmer (1925), in Assam, has employed intravenous injections of copper citrate in doses, for an adult, of 1·5 gr.

Henderson and Chatterji (1928) have used copper chloride-*p*-diazoiminobenzene hydrochloride, prepared by Mr. W. H. Gray. This compound contains 14·3 per cent. of metallic copper and undergoes decomposition if kept for any length of time in strong daylight; it must therefore be freshly prepared. It cannot be sterilized by heat but the salt itself, it is claimed, is sterile. The solution is made up by dissolving 0·02 gm. in 10 c.cm. sterile normal saline, the dose for an adult being 1 to 10 c.cm. given intravenously twice or thrice a week. Apart, however, from its action in stimulating reactions it appears to have no action on the course of the disease even after prolonged and relatively heavy dosage. Rangel (1926) has also used copper preparations without success.

Gold compounds are also believed to have some curative action in leprosy and, according to Feldt (1928), a considerable number have been introduced since 1914.

Paldrock (1927) claims to have had excellent results from the use of solganal in association with the local application of carbon-dioxide snow. Amies (1929) has also used solganal in eight cases of leprosy; one remained stationary, but the others all improved, the eye symptoms especially being ameliorated. No reactions were produced.

Sanocrysin has been used by Puente and Pierini (1926) and by Paldrock and Rängel (1927) with disappointing results. Krysologan (the sodium salt of 4-amino-2-aurophenol-2-carboxylic acid) was found of value by Hoffmann (1927) and by Kupffer (1927); both writers emphasised the value of these preparations in the ocular manifestations of leprosy. They claim that pain and photophobia are lessened, while diffuse opacities of the cornea resolve under this treatment. Eubanas and de Vera (1927) also treated five cases with krysolgan and another five with triphal (the sodium salt of auro-thio-benzimidozo carbonic acid). The results obtained, though not conclusive, suggest that these drugs

exert a beneficial effect on leprosy; nevertheless their action cannot be compared with that of chaulmoogra and hydnocarpus oils.

Fibrolysin was found to be quite useless by Henderson and Chatterji (1928).

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THE ERYTHROCYTE SEDIMENTATION TEST IN THE CONTROL OF LEPROSY

Recent observations have shown that the sedimentation rate of the red blood corpuscles provides a simple test by means of which the effects of treatment in leprosy may be more or less controlled.

It has been known for some years that an increase in the speed

of sedimentation of the erythrocytes is caused by a number of conditions such as fever, malignant growths and all diseases associated with decreased viscosity and decreased cholesterol content of the blood, while the speed of sedimentation is decreased by polycythæmia, hypercholesterolæmia and cyanosis.

Puxeddu (1924) showed that the sedimentation rate of the blood of lepers was accelerated, the acceleration being even more marked when the leprosy is complicated by malaria. The general consensus of opinion is that the increased rate of sedimentation is due, not to any change in the red corpuscles themselves, but rather to a variation in the serum which brings about a change in electrical charge. The fact that the change occurred in the serum was conclusively shown by Rubino (1927), who found that the test could be carried out with the washed red corpuscles of the sheep and the serum of leprous patients. The increased rate of sedimentation may be shown by sera inactivated at 55–56° C. for thirty minutes; it does not occur at 56° C., but if the whole system is cooled to 37° C., the reaction takes place. Iturbe (1927) estimated the rate of sedimentation in lepers of various types: nodular leprosy gave a rapid reaction, nerve cases, or those on the way to recovery, a much slower reaction.

Muir (1928 and 1929), however, was the first to show that the rate of sedimentation was still further increased by any drug that caused a breakdown of leprous tissue. Thus a small dose of 5 to 20 gr. of potassium iodide produces an immediate increase in the rate of sedimentation, the rate returning to its normal level only after two or three days. When, in addition to being bacteriologically negative, the sedimentation index of a patient has fallen to below 25, and is not raised above that level by 240 gr. of potassium iodide given twice a week over a period of three to six months, all treatment for leprosy may be discontinued. The test is also of use in determining the dosage and the patient's tolerance for any particular treatment, provided the non-specific character of the reaction is fully recognised.

The slower the sedimentation rate and the quicker its fall after having been raised by the exhibition of iodides, the more rapidly can treatment be pressed.

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CHEMOTHERAPY AND THE PROPHYLAXIS OF LEPROSY

Recent advances in treatment have an important bearing on the prophylaxis and eradication of leprosy. Up till a few years ago the only prospect held out to a leper was imprisonment for life. It is not surprising, therefore, that compulsory segregation was evaded for as long as possible; with the result that but few lepers were segregated until they had suffered from the disease for, on an average, three years. As a result, a large number of contacts were infected by every leper. Since it is now possible to cure the majority of early cases, patients are beginning to come forward voluntarily, with the result that leper asylums are gradually being converted into sanatoria for the treatment of early infective cases. Leprosy is, for the most part, a house infection, and in most cases (80 per cent.) the incubation period does not exceed five years. Were it possible, therefore, to discover every fresh case of leprosy at an early stage, the examination of all contacts at intervals of six months for five years would ensure the detection of 80 per cent. of cases at a stage when they are easily curable, while but few would be left to act as fresh sources of infection.

Methods are therefore now available for the eradication of leprosy from all civilized countries, if not from the whole world.

CHAPTER XIII

THE CHEMOTHERAPY OF TUBERCULOSIS

ALTHOUGH many efforts have been made to find a specific chemotherapeutic treatment for tuberculosis, the results which have been obtained up to the present are disappointing. Treatments without number have been introduced and for a time have been attended with apparent success, it having been too often forgotten that any patient suffering from tuberculosis who is placed under medical treatment will improve in health, at least for a time, no matter what drugs, vaccines or specialities of treatment are employed. Improvement is due in this, as in other diseases, to efficient nursing, to the regulation of food, exercise and sleep, and to fresh air and sunlight.

The destruction of the tubercle bacillus in the body presents two special difficulties: (1) the fatty protective envelope of the bacillus, and (2) the poor blood supply of the tuberculous lesions.

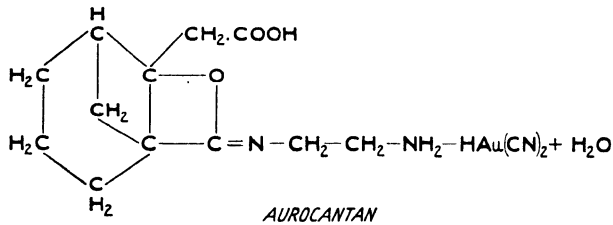
The only substances which have been found to possess a slight curative action are certain salts of the heavy metals. While the use of arsenic, silicon, mercury and cerium, however, has now been abandoned (Wells, de Witt and Long, 1923), derivatives of copper and gold are still employed.

When treatment by copper was first instituted almost all the reports were favourable to its use, but more extended observations both in animals and man with the sulphate, oleate, albuminates, various double compounds of cuprous cyanide, of which cyanocuprol is the best known, and other preparations have shown that little or no useful purpose is served by the administration of this metal in tuberculosis.

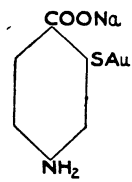
Gold has been employed in the treatment of tuberculosis for some considerable time. In 1890 Koch found that gold and potassium cyanide ($KAuCy_2$) had a lethal action on tubercle bacilli; it was subsequently shown, however, that although a

dilution of 1 in 1,000,000 prevents the growth of the bacilli in culture, yet the presence of blood serum interferes with the action and reduces the efficiency to 1 in 25,000. Gold chloride also inhibits the growth of tubercle bacilli in a dilution of 1 in 2,000,000, but has no action in controlling experimental tuberculosis.

Aurocantan was next introduced, but was found to be far too toxic for use. It was succeeded by "krysolgan," the sodium salt of *p*-amino-*o*-auromercaptobenzenecarboxylic acid, which was introduced by Feldt (1917).



AUROCANTAN



KRYSOLGAN

Although the results obtained in animals were disappointing, improvement was claimed in human cases (Feldt, 1924). De Witt (1918), from an analysis of the organs of healthy and tuberculous guinea-pigs treated with krysolgan, came to the conclusion, however, that gold has no specific affinity for tuberculous tissues.

In 1924 Moellgaard, of Copenhagen, introduced under the name of "sanocrysin," the double thiosulphate of gold and sodium (AuS_2O_3Na), $Na_2S_2O_3$, which had been prepared in 1845 by Fordos and Gélis.

When given intravenously to tuberculous patients, sanocrysin

not infrequently produces a severe reaction resembling that seen after the injection of tuberculin. At first it was thought that sanocrysin destroyed a large number of tubercle bacilli, thereby liberating endotoxins which produce the reaction. This view received support from the undoubted fact that the number of tubercle bacilli present in the sputum does diminish after treatment with sanocrysin. An antitoxic serum was therefore prepared and administered before sanocrysin in order to counteract the reaction, but its use proved of doubtful value and has now been abandoned. It seems, in fact, that the reaction following the injection of sanocrysin is in reality a sign of acute metallic poisoning.

At first when sanocrysin was used there were many fatal results, due to the fact that symptoms of acute poisoning were produced by the large doses which were given at intervals of from three to four days.

According to Moellgaard (1925), doses of from 1 to 4 gm. per kilogram of body weight had no effect in normal animals, although larger doses produced changes in the epithelium of the convoluted tubules of the kidneys. In patients with tuberculosis, however, a number of toxic reactions occurred and, when first introduced, a considerable number of patients died as a result of the treatment. The reactions may be classified as follows :—

Febrile reactions, which may be divided into three types :

(*a*) A sudden rise of temperature beginning an hour or less after the injection and lasting for a few hours. This is accompanied by malaise and sometimes rigor.

(*b*) An equally sudden rise of temperature, lasting, however, for a few days, with general malaise, headache and sometimes vomiting and jaundice.

(*c*) A rise of temperature beginning after three or more injections of sanocrysin and gradually increasing for five or more days. Hyperpyrexia may last for as long as seventeen days, and is accompanied by albuminuria with but little or no malaise.

Albuminuria. A very faint trace of albumin is found in most cases, there being a greater tendency for albuminuria to occur as treatment is continued. Usually the albuminuria is transient.

Gastro-intestinal symptoms. Vomiting and diarrhœa may occur, and a metallic taste and stomatitis have also been noted.

Aching in the limbs and rheumatic joint pains may at times be so severe as to necessitate discontinuance of treatment.

Erythematous skin rashes and, rarely, exfoliative dermatitis have been recorded.

Neuritis.

Œdema of the face and eyelids.

These toxic symptoms seem to be due to metallic intoxication rather than to any liberation of endotoxins from the tubercle bacilli. One curious condition sometimes noted during treatment is a well-marked eosinophilia.

By decreasing the doses and lengthening the intervals between them, severe reactions can be almost entirely eliminated. Faber (1925) gave 0·5 gm. intravenously for the first dose and 1 gm. for subsequent doses, with intervals of three days between the first two doses and five days between subsequent doses. If a reaction occurred, treatment was stopped until all signs of the reaction had gone. Very much smaller doses are now sometimes given, though it is doubtful whether any beneficial effect whatever is produced with minute doses. Burrell (1928) begins with 0·1 gm.; after three days this is increased to 0·25 gm., and then at weekly intervals 0·5, 0·75 and 1·0 gm. are given. If a reaction occurs, treatment is suspended until all reactive symptoms have disappeared, when the same dose that caused the reaction is repeated. A full course, therefore, lasts about six or seven weeks and consists of 5·6 or 6·6 gm. of sanocrysin. Experiments on mice also have shown that tolerance is raised by increasing the dose very gradually. The daily intravenous injection of 0·1 gm. of sanocrysin for a week, repeated after a week's rest, produces little or no improvement in acute cases.

When injected into the blood-stream sanocrysin is rapidly absorbed, nearly 50 per cent. having disappeared in from ten to fifteen minutes. At the end of twenty-four hours, however, some 25 per cent. of the gold originally injected can still be found in the blood. Excretion of the gold is slow, Eichelberger and McCluskey (1926) finding that at least thirty days are required

for all the gold to disappear from the urine. The same observers found that doses of 2 cgm. per kilogram of body weight had rather marked effects in normal dogs, causing vomiting, diarrhœa and albuminuria. To summarise, therefore, the present knowledge of the toxicity of sanocrysin, it appears that the drug in a pure state is not injurious to healthy mammals in doses of from 1·0 to 1·5 cgm. per kilogram of body weight. Tolerance is increased by gradual dosage, provided that there is a sufficient interval between the doses, but the tuberculous animal is much more liable to be damaged by sanocrysin than the healthy one.

Attempts to estimate the effects of sanocrysin treatment are surrounded by so many difficulties that as a result the most varied opinions have been expressed as to the value of the drug. (Reports of the Medical Research Council, 1925 and 1926.) It must be remembered that tuberculosis is an eminently curable disease apart from any specific chemotherapeutic treatment, and in order to arrive at any just appreciation of the effects of a particular treatment certain points must be carefully investigated. These are summarised by Walters (1927) as: the clinical state of the patient, the powers of resistance, and the general conditions of life and environment. The remedy must be applied to a sufficiently large group of patients in a definite and uniform manner and must be continued until there is reasonable probability of having attained the maximum therapeutic effect. Controls should be chosen of a similar nature, under similar conditions, excepting always the factor to be tested.

Efforts to fulfil these conditions have been made by Würtzen (1926), Bie and Andersen (1927), and more recently Clarke and Haddick (1929), with the result that, in so far as the immediate results are concerned, patients treated with sanocrysin seem to improve to a greater extent than the controls.

The most noticeable effect of treatment in man is a marked diminution and sometimes a complete disappearance of tubercle bacilli in the sputum. Often, but less constantly, there has been a rapid clearing up of the X-ray picture, heavy cottony shadows being replaced by well-defined markings and by signs of contraction of the diseased areas in the lungs. When these changes are

marked, the absence of bacilli from the sputum is likely to persist, but when the X-ray picture shows little improvement tubercle bacilli reappear in the sputum. The changes in the X-ray picture which follow sanocrysin treatment have been minutely described by several Continental observers, and the phrase "sanocrysin lung" has been coined to describe the shrunken fibroid lung often observed at the conclusion of a course of sanocrysin. This contraction of the lung is most likely to occur in cases where the disease is lobar in distribution, while in other exudative types of the disease resolution may take place without radiological evidence of much fibroid change.

The effect on fever which has not yielded to prolonged rest in bed is often very marked, and after a few injections the temperature may become normal. This improvement is followed later by a diminution of the pulse and respiratory rates.

In addition to improvement in the physical signs there is often a striking change in the aspect of the patient when treatment is successful. The phthisical facies disappears, and the flabby chest muscles become rounded and firm.

Lastly, there would seem to be a slightly lower mortality rate among patients treated with sanocrysin than among untreated patients. Thus Clarke (1929) found that, in a group of moderate severity, 80 per cent. of those treated with sanocrysin were alive, as contrasted with only 63 per cent. of those who did not have sanocrysin. In the advanced group 58 per cent. of the sanocrysin-treated cases were alive, as compared with 30 per cent. of untreated cases. Heap (1929) believes that, provided the cases have fairly good physique and no abdominal tuberculosis, the immediate results are good, but the remote effects are only encouraging in those cases which lose the tubercle bacilli from the sputum and become clinically arrested. Cummins (1926) found that in rabbits there was definite evidence of favourable effects, provided the sanocrysin was used at an early stage in the disease. The favourable effects varied inversely, however, with the virulence of the infecting bacilli and the size of the infecting dose—a conclusion of some importance in its bearing on the selection of human cases for treatment.

Sufficient time has now elapsed to give some indication of the type of case most likely to benefit from sanocrysin.

When cases are classified according to the X-ray picture into fibroid and exudative, it is found that the mortality is much higher in the latter group. Tuberculosis of the larynx does not seem to be a contra-indication, but with cachexia, intestinal tuberculosis or any form of kidney disease sanocrysin must be withheld.

In cases of chronic pulmonary tuberculosis there are often periods of comparatively good health alternating with periods when the disease progresses. Sanocrysin does not seem to prevent these relapses, but in many cases it checks an exacerbation of disease and brings about a period of arrest. In certain patients with chronic fibro-caseous tuberculosis the disease slowly spreads, and in these sanocrysin is sometimes of value.

In primary acute cases sanocrysin usually fails to produce any effect whatever, but rarely it checks the spread of the disease. When the disease is entirely unilateral and collapse therapy is indicated it is probably better to withhold sanocrysin, but in cases of bilateral involvement sanocrysin is of value in association with the complete collapse of one lung. The chief indications for sanocrysin treatment, according to Burrell (1929), are :—

(i.) To diminish the quantity of sputum and the number of tubercle bacilli.

(ii.) To check an acute spread of disease.

(iii.) To treat a patient who is gradually getting worse under other treatment.

(iv.) To treat bilateral disease in combination with artificial pneumothorax.

The effect of sanocrysin on tubercle bacilli grown *in vitro* has been investigated by Sweany and Wasick (1925). After twelve days tubercle bacilli grown in media to which were added dilutions of sanocrysin up to 1 in 20,000 were blackened. There was complete inhibition of growth in dilutions up to 1 in 200,000, partial inhibition up to 1 in 500,000, and slight inhibition up to 1 in 1,000,000. In another strain of more recently isolated tubercle bacilli, growth, however, took place in dilutions of 1 in 20,000.

After tubercle bacilli had been exposed to sanocrysin 1 in 1,000 in salt solution and serum they grew on Petroff's medium quite well, and even after sixty days' exposure to 1 in 2,000 sanocrysin the culture injected into guinea-pigs produced tuberculosis. Fry (1926) has also shown that concentrations of sanocrysin up to 1 in 2,500 in normal human blood, ox blood or plasma have no effect on the growth of the tubercle bacillus *in vitro*. Above this concentration the results were variable, but in some cases good growth was obtained in concentrations up to 1 in 250, and in one case up to 1 in 50. Tubercle bacilli grew as readily in the plasma of a tuberculous patient taken ten minutes or two days after a dose of 1 gm. of sanocrysin as in plasma drawn before the dose; similarly growth occurred in the plasma of a rabbit after a dose of sanocrysin equivalent to a dose of 3 gm. in a human being.

It is obvious, therefore, that sanocrysin has no direct action on tubercle bacilli either *in vivo* or *in vitro*. All that can be said at present is that in some way the defensive mechanism of the body is stimulated, for when given intravenously to a consumptive patient sanocrysin certainly does cause tubercle bacilli to disappear from the sputum with a rapidity unequalled by any other substance so far employed in the treatment of tuberculosis.

Other gold preparations which have been introduced by Feldt (1926 and 1927) for the chemotherapy of tuberculosis are the di-sodium salt of *p*-sulphomethylamino-*o*-auromercaptobenzene-sulphonic acid, "solganal," which has been used in the treatment of relapsing fever and leprosy, and contains 36·5 per cent. of gold, and "solganal B"—aurothioglucose, which is suitable for intramuscular injection and contains 50 per cent. gold. Other commercial gold preparations are triphal, lopion and allochrysine.

Experiments have shown that solganal has little or no action on tuberculosis in lower animals, but in man results similar to those obtained with sanocrysin have been recorded by Ernst (1928), Freund (1928) and other German clinicians. Solganal is said to be much less irritating to the kidneys than sanocrysin, when given in doses of 0·005 gm., slowly increasing to 0·25 gm. This compound has been but little used in this country.

The two other substances which have been used in the chemo-

therapeutic treatment of tuberculosis are calcium compounds and cod-liver oil. For upwards of a century "recalcification" methods in the treatment of tuberculosis have been advocated by French clinicians; nevertheless in this country, apart from its possible effect in controlling hæmoptysis, the results obtained by the use of calcium have been inconclusive. In the same way cod-liver oil has proved of somewhat uncertain value, while more recent efforts to treat tuberculosis with sodium morrhuate have proved entirely fruitless.

A clue to the value of cod-liver oil and calcium in tuberculosis may, however, be found in the vitamin content of cod-liver oil. Two vitamins, A and D, are known to be present in cod-liver oil. When animals are deprived of vitamin A they suffer from keratomalacia and broncho-pneumonia. This liability to infection is associated with changes in the Harderian and lachrymal glands and glandular epithelium of the bronchial mucosa. As shown by Fleming (1922), tears and mucus contain a bactericidal substance, lysozyme, and, as Findlay (1925) has pointed out, deprivation of vitamin A causes a reduction in the production of lysozyme. Vitamin D is chiefly associated with the retention and deposition of calcium, and when given in excess leads to the pathological deposition of calcium in the tissues. The therapeutic value of cod-liver oil would therefore seem to be two-fold: through its vitamin A content it guards against secondary bacterial infections, while through its vitamin D content it assists in the deposition of calcium. There is, however, no evidence *in vivo* or *in vitro* of any direct action of cod-liver oil on the tubercle bacillus.

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CHAPTER XIV

THE CHEMOTHERAPY OF GRANULOMA INGUINALE

THE ætiology of granuloma inguinale is still unsettled. Donovan, in 1905, observed within the macrophages obtained from smears made from the granulomatous lesions oval bodies measuring from 0·5 to 2 μ in diameter, either scattered throughout the cytoplasm or collected in small compact groups. These " Donovan bodies " were at first believed to be protozoa, but are now regarded as more probably of a bacterial nature. Their exact relationship to the ætiology of the disease is, however, uncertain ; while McIntosh (1926) has transmitted the disease from one individual to another, has constantly found Donovan bodies present, and has claimed that they can be grown in pure culture, typical lesions resulting from inoculation of the cultures, others regard a bacillus of the Friedlander group as the causal agent. Earlier methods of treatment such as surgical intervention, cauterization and irradiation by X-rays proved unsatisfactory, and the disease was regarded as incurable till 1913, when Aragão and Vianna found that antimony had a specific curative action.

Since then antimony has been used in a very large number of cases. In Dutch New Guinea, where a considerable proportion of the population suffers from granuloma inguinale, de Vogel (1927) states that 5,736 cases have been treated intravenously with tartar emetic, 86·1 per cent. being cured after one series of injections, 12·8 per cent. after a second series.

Numerous other workers in other parts of the world have obtained similar results. One curious fact, however, is that the amount of antimony necessary to bring about a cure varies considerably from case to case. Thus Manson-Bahr and Anderson (1927) found that healing might follow total amounts of antimony varying from 17·5 to 179 gr.

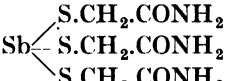
Giglioli (1928) and others have met with occasional cases where

there appeared to be a definite resistance to the action of tartar emetic. While the tartar emetic injections must always be given intravenously, the actual dosage has been varied by different workers. McGlinn (1926), for instance, gives 0·1 gm. dissolved in 10 c.cm. of sterile physiological saline at weekly intervals in ambulatory cases or every second day in hospital cases.

Others prefer to begin with 2 c.cm. of a 1 per cent. solution, followed at daily intervals by from four to ten injections, in doses of from 5 to 10 c.cm., according to the tolerance of the patient. In the majority of cases this treatment has been sufficient, but in very extensive or long-standing cases a larger number of injections may be necessary until as many as fifteen to thirty have been given. Having once begun the injections, it is essential to continue the course. Some workers believe that to ensure lasting cure an additional series of three or four injections should be given at weekly intervals after healing is apparently complete. As in the administration of tartar emetic for leishmaniasis and schistosomiasis, the chief inconvenience lies in the severe joint pains and in the possibility of damage to the kidneys. Very few fatalities have been recorded, although Johns and Gage (1924) report two deaths which they attribute to the use of tartar emetic.

In order to avoid the unpleasant symptoms which may attend the injection of tartar emetic various other antimony preparations have been employed.

Randall (1924) introduced sodium antimony thioglycollate and the triamide of antimony thioglycollic acid.



Triamide of antimony thioglycollic acid.

These compounds may be given either intravenously or intramuscularly, and were used with success by Shattuck, Little and Coughlin (1926) in three cases of the disease. Doses of from 5 to 20 c.cm. of a 0·4 per cent. solution of sodium antimony thioglycollate were given intravenously, while from 2 to 10 c.cm. of a 0·4 per cent. solution of the triamide were injected subcutaneously without causing any severe toxic reaction.

Pentavalent antimony compounds have only been used to a very limited extent in the treatment of granuloma inguinale. Manson-Bahr and Anderson (1927) found that stibosan, von Heyden 471, was even more efficacious than tartar emetic, while Giglioli (1928) used stibenyl with good results in a number of cases in British Guiana. The commencing dose was 0·1 gm. increased by 0·1 gm. to 0·6 gm., the drug dissolved in 20 c.cm. sterile normal saline being injected intravenously with extreme slowness. The first two doses were given on successive days, the others on alternate days. The total dosage varied from 2·70 to 5·80 gm. Improvement, as a rule, set in after the second injection ; certain cases, resistant to tartar emetic, were apparently cured by stibenyl. Toxic reactions, such as nausea, vomiting and hyperpyrexia, were only rarely encountered, and were of a mild character.

Hanschell (1929) has recorded healing in a rapidly spreading case of granuloma inguinale as a result of the intravenous injection of tartar emetic accompanied by a dose of 100 million bacteria of a stock typhoid, paratyphoid A and B vaccine, and followed later by intravenous injections of 0·3 gm. of neostam.

Gibson (1927), in a case in West Africa, failed with tartar emetic, but obtained rapid improvement after the oral administration of stovarsol.

There is at present no clue to the mode of action of tartar emetic in curing granuloma inguinale. McIntosh (1928) suggests that the activity of the large mononuclear cells is stimulated by the antimony. If the disease is really due to a bacterium, then it is unique, since there is no other known bacterial disease which reacts in this remarkable manner to the exhibition of antimony.

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CHAPTER XV

THE CHEMOTHERAPY OF ACUTE BACTERIAL INFECTIONS

WHILE a certain measure of success has been attained in the chemotherapeutic treatment of protozoal and spirochætal diseases, infections due to bacteria are at present largely uninfluenced by chemotherapy. This failure applies both to localised infections and to general septicæmic conditions. All the chemical antiseptics at present in use, in addition to combining with bacterial protoplasm, and so causing death of the bacteria, also enter into combination with other albuminous matter, and are in fact general protoplasmic poisons. As a result of this action, antiseptics tend not only to have an inhibitory action on the phagocytosis of bacteria by leucocytes, but, as Fleming (1924) has shown, they decrease the bactericidal power of the leucocytes, with the result that blood containing antiseptics allows the growth of more bacteria than normal blood. Intravenous injections of such antiseptic substances would therefore seriously damage the resisting power of the body were it not for the fact that the majority of antiseptics are rapidly removed from the circulation by combination with albumins. Even in the case of localised lesions, such as infected wounds, the strength of the antiseptic diminishes rapidly, so that though in the concentration usually applied antiseptics are capable of destroying bacteria with which they come in contact, this concentration is rapidly lost. This is perhaps as well, for pus cells, or emigrated leucocytes, are even more susceptible than bacteria to the toxic action of antiseptics. In this connection it is noteworthy that in the later stages of the war, when many different antiseptics had had very extensive trial in the treatment of septic wounds, those which enjoyed the greatest popularity were those which lost their potency most rapidly in the wound,

namely the hypochlorites. As Fleming (1919) has pointed out, if eusol or Dakin's fluid are applied to a wound, their potency is reduced in less than a quarter of an hour to such an extent that they cease to have an inhibitory effect on either bacteria or leucocytes. It is probable that this very rapid diminution in strength, with a corresponding shortening of the time during which the antiseptics are acting in a leucocidal concentration, has had much to do with their extended use.

It is therefore obvious that determinations of antiseptic action on bacteria *in vitro* throw little or no light on the value of a substance in combating bacterial infections *in vivo*.

It is, however, possible to define the qualities which must be possessed by the ideal antiseptic for use in bacterial infections. Such a substance should have a well-marked bactericidal action when brought into contact with bacteria in the presence of tissue fluids; it should be non-toxic to tissue cells; it should stimulate the bactericidal powers of the leucocytes; it should retain its bactericidal powers for a prolonged period when present in blood or tissue fluids, and it should readily diffuse from the blood into serous cavities and tissue spaces. It is obvious that the ideal antiseptic has yet to be found. In the meantime all that can be done is to describe in brief the many efforts that have been made to attain to the ideal.

The following groups of substances have been used as antiseptics for internal use:—

(i.) Aniline dyes.
(ii.) Ethylhydrocupreine and other cinchona derivatives.
(iii.) Compounds containing hypochlorites.
(iv.) Compounds of arsenic, mercury, silver and gold.
(v.) Phenols.

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ANILINE DYES

Very shortly after their discovery by W. H. Perkin, Senr., aniline dyes were used in histological staining by Beneke (1862) and Waldeyer (1863).

Intravital staining followed, while later the trypanocidal activity of such dyes as trypan red and trypan blue was demonstrated. Since then a vast amount of work has been carried out in an effort to discover dye-stuffs of bactericidal value. Many of the experiments have consisted in determining the concentrations of dyes necessary either to kill bacteria grown *in vitro* or to inhibit their growth in culture media. As a result many interesting facts have been brought to light, but, as previously pointed out, bactericidal action *in vitro* does not necessarily ensure chemotherapeutic activity *in vivo*, and while dyes without number have been investigated, the number of those which are of real value in chemotherapy is very small.

Natural dyes, such as indigo, cochineal, brazilin and litmus, have little or no bactericidal action either *in vitro* or *in vivo*, and the same is true of dyes belonging to the azo and oxyquinone groups. Certain nitroso compounds have, however, been investigated by Cooper and Haines (1929). *p*-Nitrosodimethylaniline kills cultures of *B. coli* in dilutions of 1 in 170,000 in forty-eight hours at 37° C. While the nitroso-compounds have little or no action on amino acids and proteins, they slowly react with nucleic acid, forming a dark green insoluble product. No tests have yet been carried out on their action *in vivo*.

The quinone-imide group of dyes includes :—

(i.) Indamines.

(ii.) Thiazines—thionine, toluidine blue and methylene blue.

(iii.) Oxazines—brilliant cresyl blue, Nile blue sulphate.

(*a*) Amido-azines—neutral red.

(*b*) Safranines—safranine 0, magdala red.

(*c*) Indulins—nigrosin.

The bactericidal action of many of these compounds has been studied by Fairbrother and Renshaw (1923), though, despite

their similarity in structure to dyes of the acridine group, their bactericidal action *in vitro* is small.

The phenyl-methane dyes have been more extensively investigated : in this group are included :—

(*a*) Diphenyl-methanes—auramine.

(*b*) Diamino-triphenyl methanes—malachite green and light green.

(*c*) Triamino-triphenyl methanes—basic and acid fuchsin, methyl violet, crystal violet (gentian violet).

(*d*) Hydroxy-triphenyl methanes—(rosolic acids)—aurine, coralline red.

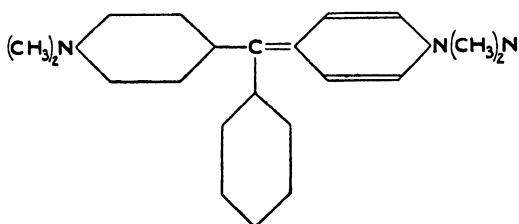
Xanthene dyes include pyronines, rhodamines, fluoranes, phenolphthaleins and sulphonphthaleins. Fluorescein enters into the composition of mercurochrome-220 soluble, but any action displayed by this compound is due to its mercury, and not to its dye content.

Acridine dyes include acriflavine, proflavine and rivanol.

In addition, a large series of derivatives of anil and styryl quinolines has been investigated by Browning, Cohen and their co-workers.

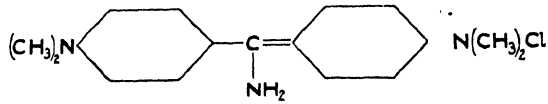
PHENYL-METHANE DYES

Attempts to correlate chemical composition and bactericidal action in the dyes of the phenyl-methane group have been made by Fairbrother and Renshaw (1923). Crystal violet was found to



CRYSTAL VIOLET

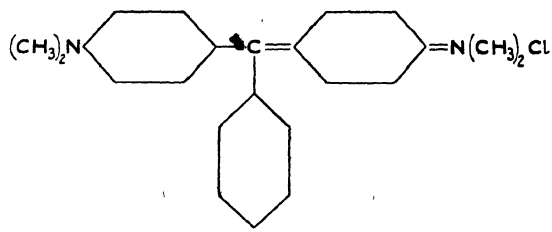
kill ten test organisms in a dilution of 1 in 1,000. Removal of a methyl group, as in methyl violet, led to no change in bactericidal action, but substitution of the methyl group by other side-chains, as in formyl violet, led to a definite reduction. More drastic simplification of the molecule led to the formation of auramine 0,



AURAMINE O

which was bactericidal in a dilution of 1 in 5,000. Auramine has unfortunately but little action *in vivo*, though it has been combined with emetine to form auremetine, which has been used in the treatment of amœbic dysentery.

Malachite green and its ethyl homologue brilliant green are slightly more active than crystal violet.



MALACHITE GREEN

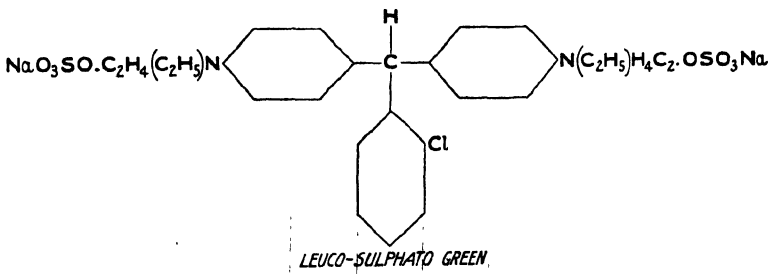
Brilliant green was much employed, on the recommendation of Browning, Gulbransen and Thornton (1917), as a dressing for war wounds, as it compared favourably with acriflavine in antiseptic properties. In bacteriology the dye is of considerable value as an enrichment for media used in isolating bacteria of the coli-typhoid group. Malachite green was also used to some extent as an antiseptic dressing during the war.

Considerable interest, however, has recently been taken in gentian violet, more especially in America, where, following the work of Churchman (1923 and 1925), gentian violet has been

employed in the treatment of septicæmia. Very varying results have been obtained from its use in septic conditions *in vivo*, and occasionally its administration has been followed by toxic symptoms (Vandecaveye, 1929). This is hardly surprising, for according to Conn (1929) the composition of gentian violet is extremely variable, and there are sold under this name various methyl violets with or without dextrine and crystal violet. Churchman (1923) believes that gentian violet is especially active against Gram-positive organisms. It seems very doubtful, however, whether the intravenous injection of gentian violet is followed by any beneficial result. Walker and Sweeney (1925) found that gentian violet was effective only against staphylococcal infections, and then only when brought into immediate contact with the bacteria, while Zau and Meleney (1928) reported that in dogs suffering from staphylococcal lesions the intra-arterial injection of this dyestuff had no action except to delay recovery.

Gentian violet has recently been used in the treatment of experimental clonorchiasis.

Coplans and Green (1926–1927) have investigated the chemotherapeutic possibilities of certain " sulphato " compounds of the triphenylmethane dyes and their leuco derivatives. These compounds owe their acidic character to the presence of sulphuric ester groups (HSO_4) connected to alkyl-amido radicals. The



LEUCO-SULPHATO GREEN

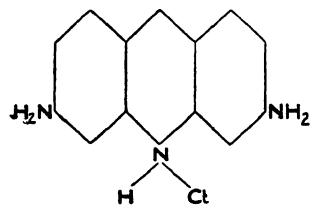
sulphato dyes and their leuco derivatives are non-toxic and non-bactericidal *in vitro*, but such compounds as leuco-sulphato green have the power of neutralising diphtheria and tetanus toxins *in vitro* and to a slight extent *in vivo*. When injected into animals

the sulphato compounds break up and render the urine, bile and, to a less extent, the blood, bactericidal.

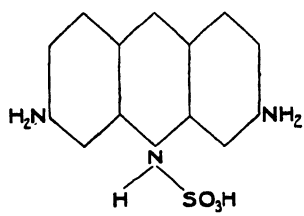
ACRIDINE DYES

The compounds of chemotherapeutic interest derived from the acridine nucleus are the quaternary salts formed from the diamino-acridine derivatives.

The parent substance is 3 : 6-diamino-acridine chloride.

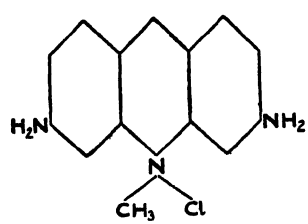


From this are derived 3 : 6-diamino-acridine sulphate—proflavine.



PROFLAVINE

and acriflavine, which is 3 : 6-diamino-methylacridine chloride.



ACRIFLAVINE

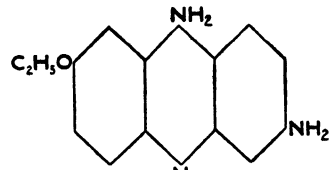
Both acriflavine and proflavine came into considerable prominence during the war, when they were recommended by Browning, Gulbransen and Thornton (1917) for the treatment of wounds. Although in many cases beneficial results were obtained, more especially from the use of acriflavine, there were many instances in which little or no benefit followed the employment of these dyes. Fleming (1924), especially, showed that acriflavine added to blood in dilutions of 1 in 500,000 interfered with the bactericidal action of the blood provided the acriflavine was allowed to act for five hours.

Zau and Meleney (1928) also have recently been unable to determine any bactericidal action in staphylococcal lesions from the intra-arterial injection of acriflavine. Acriflavine has recently been given intravenously in cases of undulant fever, but the beneficial results claimed have been unconvincing. Lawson (1927), however, has found that proflavine is of considerable value in the treatment of certain ophthalmological lesions requiring operation, while Blacklock (1929) states that antiseptics of the flavine series do not interfere with the growth of tissues *in vivo*, as is shown by the occurrence of mitosis in various types of cells 0·018 to 0·2 mm. below the growing surface of the wound.

Certain toxic effects have, however, been described as a result of the intravenous injection of acriflavine. The immediate symptoms have included syncope, nausea, congestion of the face, a bitter taste in the mouth, and local urticaria.

Delayed effects consisted of slight nausea, cardiac irritability, and staining of the skin. Murray (1930), who has recently failed to detect any good results following the intravenous injection of acriflavine in gonorrhœa, reports in about 11 per cent. of cases so treated the development of catarrhal jaundice eight or more weeks after cessation of the treatment. One case died from acute yellow atrophy of the liver.

Further investigations have been made by Morgenroth and his assistants (1921–1923) on the bactericidal action of the alkoxy derivatives of the diamino-acridines. The most widely known substance of this series is 2-ethoxy-6 : 9-diamino-acridine—rivanol.



RIVANOL

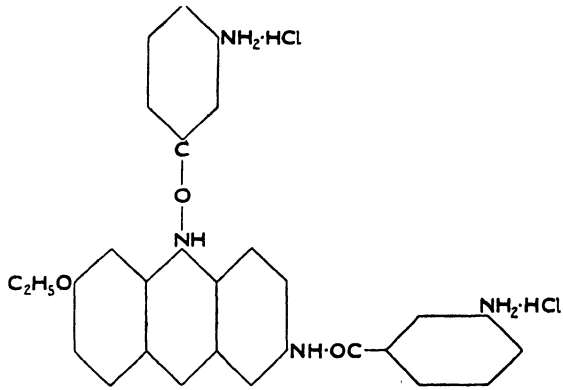
The contour of this substance inevitably suggests the quinoline portion of the quinine molecule, more especially the corresponding nucleus of optochin. As compounds of this type might also be endowed with bactericidal properties a series of alkoxy-ethanol-amino-acridine compounds was tested against a strain of streptococcus in the hope that there might be obtained an antiseptic with a selective action against this organism. The result of experiments on a single strain of streptococcus *in vitro* showed that the organisms were killed at the following dilutions in the presence of 10 per cent. of horse serum :—

Compound.	Highest bactericidal dilution.
2-methoxy-9-ethanolamino acridine . .	1 in 60,000
2-ethoxy-9-ethanolamino acridine . .	1 in 80,000
2-allyloxy-9-ethanolamino acridine . .	1 in 100,000
2-propyloxy-9-ethanolamino acridine . .	1 in 40,000
2-*iso*butyloxy-9-ethanolamino acridine . .	1 in 40,000
2-*iso*amyloxy-9-ethanolamino acridine . .	1 in 16,000

In vivo, however, the ethoxy compound was found to be much more active than the allyloxy compound, but with other strains of streptococci there was unfortunately little or no bactericidal action. Browning and Gulbransen (1928), for instance, found that both rivanol and acridine gave irregular results in the treatment of subcutaneous streptococcal infections in mice.

The chief use of rivanol at the present time is in the treatment of amœbic dysentery.

Supniewski (1926) prepared the di-*m*-benzoyl derivative of rivanol.



Di-m-AMINOBENZOYL-RIVANOL

This substance kills Gram-positive bacteria *in vitro* in dilutions of from 1 : 10,000 to 1 : 80,000, while in the subcutaneous tissues of mice streptococci are killed by dilutions of 1 in 30,000 and staphylococci by 1 in 10,000. Unfortunately the injection of maximal sublethal doses does not cure general septicæmias in animals due to streptococci or pneumococci, while parenchymatous nephritis frequently results in normal animals as a result of the injections. A further disadvantage in the use of acridine derivatives is the rapidity with which, according to Burke, Ulrich and Hendrie (1928), bacteria become resistant to these dyes.

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ANIL- AND STYRYL-QUINOLINES AND THEIR DERIVATIVES

Browning, Cohen, Gaunt and Gulbransen (1922) have investigated the antiseptic properties of a number of styryl-pyridine and styryl-quinoline compounds obtained by the condensation of the quaternary alkyl halides of 2-methylpyridine (α picoline) and 2-methylquinoline (quinaldine), or their substituted derivatives, with *p*-dimethylaminobenzaldehyde. It was found that a powerful antiseptic action was manifested. Subsequently, as the result of condensation of the quinaldine or picoline alkylhalides with *p*-nitroso-dialkylaniline, a series of *p*-dimethylamino-anil-6-methyl- or ethyloxy-quinolinemethochlorides was produced.

In the case of *Staphylococcus aureus* the sterilising concentration was found by Browning, Cohen, Ellingworth and Gulbransen (1924[1]) to be between 1 in 100,000 and 1 in 1,000,000, both in peptone water and serum, with the exception of 4-*p*-dimethylamino-anil-6-methylquinolinemethochloride and 2-*p*-dimethylamino-anilpyridinemethiodide, which were less active. Some members of the series were active against *B. coli* in dilutions of from 1 in 200,000 to 1 in 400,000. The same workers (1924[2]) also studied the antiseptic action of compounds of the apocyanine, carbocyanine and *iso*cyanine series, as well as the result of further variations in the quinol and benzene nuclei (1928). Certain of these compounds were also found to have a strong action against *B. coli*.

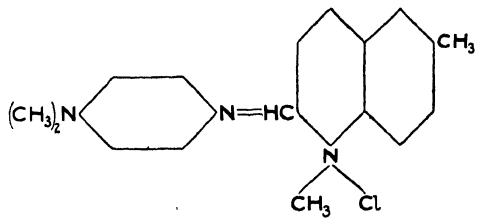
In 1926 Browning, Cohen, Ellingworth and Gulbransen continued their observations and showed that a number of derivatives of anilquinoline and styrylquinoline possessed strong antiseptic properties. There was found to be a close parallelism in the properties of the fundamental compounds 2-(*p*-aminostyryl) quinoline and 2-(*p*-aminoanil) quinoline methochloride.

While various changes in chemical constitution produced in general closely similar effects upon the antiseptic properties of

he two series, the aniline derivatives were as a rule more active *n vitro* against *B. coli*.

Owing to the instability of these compounds towards hydrochloric and other strong acids they are unsuitable for administration by the mouth unless special provision is made for protecting them in their passage through the stomach.

Quite recently Armitage, Gordon, Cohen, Ellingworth and Dobson (1929) have employed one of these anil-quinoline derivatives in the treatment of infective conditions in man.



(QUINANIL) 2 (p-DIMETHYLAMINO ANIL)-6-METHYLQUINOLINEMETHOCHLORIDE

Quinanil, 2 (*p*-dimethylamino anil)-6-methylquinolinemethochloride was found to be lethal to *S. aureus* in 1 per cent. peptone water, and in 20 per cent. serum-peptone water in dilutions of 1 in 500,000 and 1 in 400,000 respectively. Its lethal concentrations for *B. coli* were 1 in 3,500,000 and 1 in 300,000—considerably higher values than those obtained with acriflavine.

Toxicity tests showed that the mean dose tolerated by a 4½-lb. rabbit was in the neighbourhood of 9 c.cm. of a 1 in 200 solution in normal saline.

Quinanil has been used intravenously in cases of septicæmia, as a genito-urinary irrigant and as a local antiseptic. In septicæmia results of value were not obtained. Six cases of acute infective endocarditis, one pneumococcal and one staphylococcal septicæmia proved fatal, though one streptococcal septicæmia recovered.

As a local application, in strengths varying from 0·01 per cent. to 0·1 per cent., good results were recorded, while in genito-urinary conditions there is also said to have been definite improvement after the use of quinanil.

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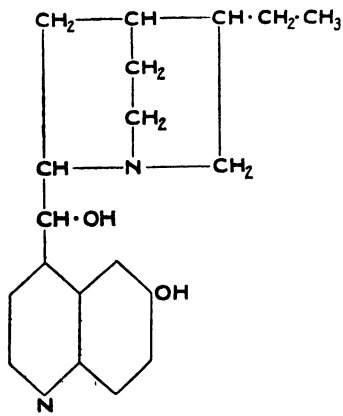
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ETHYLHYDROCUPREINE HYDROCHLORIDE AND *ISO*OCTYLHYDROCUPREINE HYDROCHLORIDE

It has been long and generally believed that quinine possesses certain curative properties in pneumococcal infections and, more especially, in pneumonia.

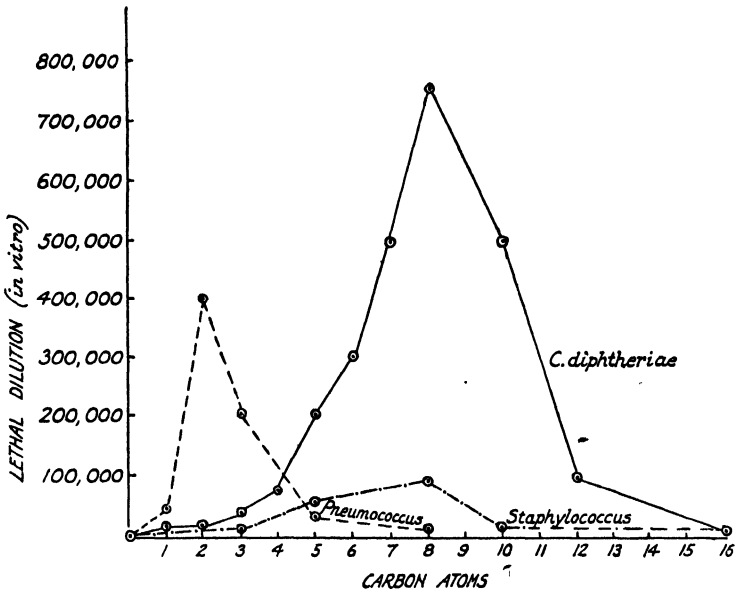
In order to investigate this question, Morgenroth and Levy



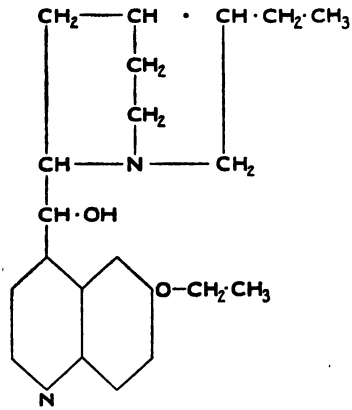
HYDROCUPREINE

(1911–1917) prepared, among other compounds, a series of derivatives of hydrocupreine, a substance which is readily obtainable, while cupreine itself is now difficult to procure.

The series was obtained by substituting various alkyl groups in place of the hydroxyl hydrogen of the parent substance, hydrocupreine hydrochloride. It was found that as the length of the carbon chain introduced with the substituent group increased, so the bactericidal activity also increased until a maximum was



reached beyond which any further increase in the length of the chain invariably diminished the activity. Another interesting feature was the selective action observed with different bacteria, as shown in the chart. Thus, although *iso*octylhydrocupreine hydrochloride, vuzin, is most active against *Corynebacterium diphtheriæ*, the most active substance against the pneumococcus is ethylhydrocupreine hydrochloride, which is twice as potent as any other derivative in this respect. The action of these compounds on staphylococcus is very small compared with that on



ETHYLHYDROCUPREINE

C. diphtheriæ, but the action on the two organisms runs parallel *in vitro*.

Experiments with optochin in experimental pneumococcal infections of the mouse showed that it had a beneficial action, but the difference between the minimum lethal dose and the minimum curative dose was found to be too small to establish a definite therapeutic effect once the pneumococcal infection had become systemic. The use of optochin in pneumococcal infections in man has had extensive trial. In pneumonia the investigations of Moore and Chesney (1917–1918), Manliu (1916), Wright (1912) and others have shown that its use is of little value. This rather discouraging conclusion is true despite an increase in the bactericidal power of the patient's blood and a negligible change in the opsonic index following the injection of optochin. Bier (1917) believed that optochin was of value in the treatment of superficial wounds, but uncertain results in pneumococcal meningitis of man were reported by Wolff and Lehmann (1914) and by Rosenow (1920). While Cordua (1921) recorded favourable results in cases of meningococcal meningitis in man, Kolmer and Idzumi (1920) were unable to detect any influence on experimental pneumococcal infections of the meninges in dogs and rabbits. Toxic amblyopia is also occasionally met with after treatment by optochin. The

use of optochin in pneumonia has thus proved unsatisfactory. Possibly a combination of optochin and specific immune serum may be of value, as suggested by the work of Stewart (1927) on pneumococcal meningitis.

Optochin has, however, been used with apparent success by a large number of workers in the treatment of eye diseases such as pneumococcal conjunctivitis and keratitis and in dacrocystitis, gonococcal and streptococcal conjunctivitis and trachoma. The drug is usually employed as a 1 or 2 per cent. solution applied directly to the conjunctiva. Others prefer to apply the drug as a powder (Bedell, 1920).

iso-Octylhydrocuprein (vuzin) has been used as a dressing for wounds, but has little or no beneficial action.

Various other cinchona derivatives have also been prepared and tested for their therapeutic effects. Thus Morgenroth has experimented with the substances obtained by replacing the methyl group of compounds containing an opened quinuclidine ring with longer aliphatic chains. Certain of such compounds have a more rapid bactericidal action than that of the closed ring compounds, but their actual developed action is not so great. Felton and Dougherty (1922) also studied the bactericidal properties of hydroquinine chloroacetanilide, hydroquinine *p*-chloroacetylaminophenol hydrochloride, hydroquinine *meta*-chloroacetylaminophenol hydrochloride and hydroquinine 4-chloroacetylaminocathecol hydrochloride. The curative results in mice were feeble, intravenous injection of the compounds apparently leading to a rupture of the natural defence mechanisms of the body.

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PHENOLS AND THEIR DERIVATIVES

Comparatively little fresh work has been carried out on the antiseptic action of the phenols owing to the difficulties that attend their use as internal disinfectants.

Caius, Naidu and Shamsher (1927) have, however, studied the bactericidal action of some of the commoner phenols and some of their derivatives on the growth *in vitro* of *Pasteurella pestis*.

The entrance of the hydroxyl group into benzene, with the formation of phenol, the first antiseptic used, causes a considerable increase in the antiseptic activity. The introduction of alkyl groups into the nucleus again increases the antiseptic activity, the three isomeric cresols being more powerful than phenol. The homologous phenols are, however, much less soluble in water than phenol itself, and the same is true of very many of its substitution products. Solution in alkali, however, either depresses or intensifies the bactericidal power, without apparent cause. Thus the bactericidal power of thymol (3-methyl-6-*iso*propyl phenol) is less, whilst that of its isomer carvacrol (2-methyl-5-*iso*propyl phenol) is greater than phenol. The entrance of a methyl group into the nucleus of sodium phenolate increases the bactericidal power of

1 : 2-methyl-hydroxybenzene, but depresses that of 1 : 3-methyl-hydroxybenzene. In the case of the polyhydric phenols, the bactericidal action on *P. pestis* is, as a rule, increased in alkaline solution.

Of the three dihydroxybenzenes, resorcinol is least toxic to *P. pestis*, while catechol is lethal in a dilution of 1 in 48,000 and quinol in 1 in 432,000.

The entrance of a methyl group into the molecule either of resorcinol, with the formation of orcinol, or into the molecule of quinol, with the formation of toluhydroquinone, depresses the bactericidal power.

In the case of the substituted phenols, the entrance of chlorine and bromine into the phenol nucleus increases the bactericidal power, 2 : 4 : 6-trichlorphenol being sixteen times more powerful than phenol. Nitration does not alter the bactericidal activity to any extent, but the entrance of either the carboxyl radical (COOH) or the sulphonic group (SO_2OH) depresses the activity. The bactericidal power is increased, on the other hand, by the entrance of an amino group—2 : 4-diaminophenol being twenty times as active as phenol, while 2-amino-4 : 6-dinitrophenol (picraminic acid) is five times as active as 2 : 4 : 6-trinitrophenol (picric acid).

Alkyl Resorcinols

The development of an antiseptic suitable for use in inflammatory conditions of the genito-urinary tract is a matter of some urgency. Hexamethylenamine and the many closely allied compounds which have been exploited under various names were thought for a time to be of promise, not only as urinary but as general internal antiseptics. Hinman (1913), however, pointed out the reasons for the complete failure of these substances as internal antiseptics and their limited usefulness as urinary antiseptics.

Owing to the time element necessary for the decomposition of these substances in acid urine, as much as to the necessity of an acid reaction, a large group of urologic patients are at once excluded from any possible benefit from their use. Among these patients may be mentioned all those with infections of the pelvis

of the kidney or incontinence of urine from any cause, for in neither of these groups does the urine remain in contact with the infected area for a sufficient time to allow of decomposition of the drug, and certainly not sufficiently long to allow the formaldehyde released to reach the concentration necessary to exert any bactericidal action. On the other hand, if and when the necessary concentration of formaldehyde is produced, it is extremely irritating to the inflamed urinary mucosa.

The ideal urinary antiseptic should fulfil the following conditions. It should be non-toxic to the body and chemically stable. It should be non-irritating to the urinary tract and should exert an antiseptic action in high dilution in urine at any reaction. It should be excreted by the kidneys in sufficient concentration to produce a local antiseptic action and at a rate which ensures continuous antiseptic action. Finally, it should be administrable by mouth.

These conditions, according to Leonard (1924), are fulfilled by hexyl resorcinol.

In 1913, Johnson and Hodge synthesized a number of the lower homologues of a series of alkyl resorcinols. These compounds were examined for bactericidal properties by Hale (1913)

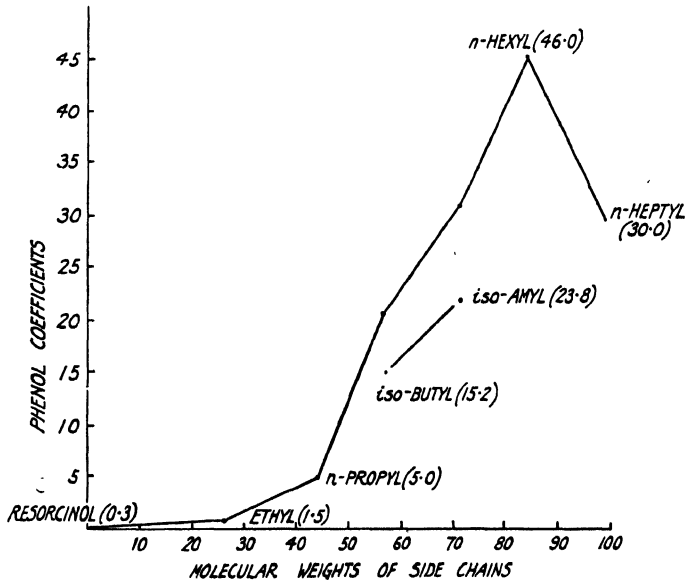


HEXYLRESORCINOL

and Leonard (1924), who found that alkylation of resorcinol decreases its toxicity for animals and at the same time increases the bactericidal power in direct proportion to the molecular weight of the alkyl chain, the maximum effect being reached in the six-carbon-atom straight-chain derivative—normal hexyl resorcinol. In man, doses varying from 0·33 to 1 gm. three times a day produce no toxic symptoms beyond a decided cathartic action due to intestinal irritation. The bulk of the drug excreted in

the urine appears in the conjugated form, which is inert: small quantities of unchanged hexyl resorcinol are present, however, in the urine and exert a bactericidal action.

Urinary tract infections due to *Staphylococcus albus*, *S. aureus*, streptococci and some strains of *Pseudomonas pyocyanea* are said to yield readily to oral administration of hexyl resorcinol without other treatment. Infections due to *Bacterium coli* are, however, less amenable. In a later communication Leonard and Feirier



(1927) recommend hexyl resorcinol not only as an irrigant of the urethra, bladder and renal pelvis, but as an application to the skin and in lesions of the ear, nose and throat, the solution consisting of 30 per cent. glycerine and 70 per cent. water in which is dissolved 1 mgm. of crystalline hexyl resorcinol per cubic centimetre.

Despite the fact that others have found hexyl resorcinol to be unpalatable and liable to produce gastro-intestinal irritation, remarkable improvement in urinary cases has been recorded by Henline (1925), Brown (1926), Wynne (1926), McCurry (1926) and

others. Eberbach and Arn (1927), as a result of the treatment of some 200 cases with hexyl resorcinol, have had less brilliant results. Nevertheless, about one-third of the cases were cured by hexyl resorcinol alone, while in an additional 20 per cent. there were symptomatic cures. The earlier the treatment is begun the more efficient is the drug, but whereas coccal infections respond rapidly, infections with *B. coli* are very resistant to treatment. According to Hampil (1928), *n*-hexyl resorcinol is more bactericidal in an alkaline medium.

Coulthard, Marshall and Pyman (1930) have studied 4-*n*-amyl-*m*-cresol in some detail. It is of low toxicity and appears suitable for use as a urinary antiseptic.

Ratcliffe (1929) has recently found that when fed to rats, *n*-butyl, *n*-hexyl, and *n*-octyl resorcinol produce a rapid change in the intestinal flora. One per cent. of the resorcinol derivatives added to a diet which normally promotes a predominance of *Lactobacillus acidophilus* had at the end of fifteen days almost entirely inhibited the growth of Gram- positive organisms. Gram-negative organisms, chiefly *B. aerogenes*, were predominant. The growth of intestinal protozoa such as *Trichomonas muris*, *T. parva* and *Endamœba muris* was also inhibited. Similar results have been obtained by Rettger, Valley and Plastridge (1929).

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MERCURY COMPOUNDS

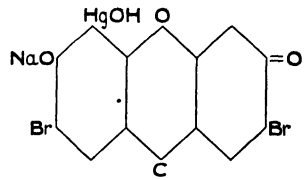
The antiseptic action of mercury compounds, more especially mercuric chloride, has been long known. As a result, many inorganic and organic mercury derivatives have been tested for their antiseptic action *in vitro*, in the hope that some compound might be discovered of low toxicity to the tissues which would yet prove of value in the treatment of septicæmia when administered intravenously.

Even mercuric chloride itself has not been without its advocates. Baccelli (1907), in Italy, recommended intravenous injections of mercuric chloride in the treatment of gonorrhœal rheumatism, while Vecki (1919) and others used it in septicæmia and influenza. More recently Dudgeon (1926) claims remarkable results in septicæmia, the drug being given in a dose of 5 c.cm. of a 1 in 1,250 solution in saline, the injection being repeated in from twelve to twenty-four hours if necessary. By allowing a little blood to flow into the syringe before giving the injection the danger of thrombosis is greatly decreased. Rigor may follow the injection, but the temperature is said to fall within twenty-four to forty-eight hours and, provided the injection is given at an early stage of the disease, there is definite arrest of the inflammatory process. Apart from thrombosis, diarrhœa, stomatitis and nephritis have all been recorded following the intravenous injection of mercuric chloride. Guillain and Gardin (1922) have in fact reported a fatal case with

acute nephritis and cardiac failure as the result of mercuric chloride given by this route.

Disodium-dibromohydroxy-mercurifluorescein. Mercurochrome-220 soluble

Probably the mercury compound which has aroused most interest in the chemotherapy of acute bacterial infections is mercurochrome-220 soluble, which was first prepared by White (1920), by the action of mercuric acetate on dibromofluorescein, as a disinfectant for the genito-urinary tract. The product, disodium - dibromohydroxy - mercurifluorescein, $C_{20}H_7O_5Br_2$-$HgOHNa_2$, consists of greenish iridescent scales or granules which are readily soluble in water and in saline solution. Its constitutional formula is :



MERCUROCHROME-220 SOLUBLE

Considerable differences have been found in the composition of the drug, and, as Eyre, Notton and Pope (1928) point out, slight modifications in the method of manufacture may lead to the presence of impurities such as dibromofluorescein, dibromo-dihydroxy-mercurifluorescein, mercury salts and sodium acetate.

Young, White and Swartz (1919), in their original paper on mercurochrome, stated that in rabbits, 10 mgm. per kilogram of body weight invariably caused death in twenty-four hours, with no definite post-mortem lesions, while 5 mgm. per kilogram of body weight caused albuminuria lasting a week. Young (1925) believes that the toxicity to rabbits per kilogram of body weight is about equal to that for men. Other observers find that the intravenous injection of mercurochrome with glucose is less toxic than mercurochrome alone, but Macht and Harden (1928) insist

that the glucose-mercurochrome mixture must be made immediately before use. Eyre, Notton and Pope (1928) found that the toxicity of various samples of mercurochrome varies, but a concentration of 0·4 per cent. is preferable to 1 per cent., the former allowing a dose of 25 mgm. per kilogram of body weight to be tolerated by rabbits.

Experiments on the inhibitory power of mercurochrome-220 on the growth of organisms *in vitro* show that in the case of *Bacterium coli* a solution of 1 part mercurochrome in 3,500 nutrient broth inhibits growth, whilst with a hæmolytic streptococcus, growth is inhibited by a 1 in 8,000 dilution.

Mercurochrome has now been used both intravenously and locally as a disinfectant in a large number of conditions.

Intravenously, brilliant results have been claimed in general septicæmia, puerperal fever, diffuse cellulitis, gonorrhœa and gonorrhœal arthritis, lobar pneumonia and post-influenzal pneumonia, typhoid, pyelitis and many other conditions.

Intravenous injections are not, however, entirely devoid of toxic effects. Small doses may cause no reaction beyond increased pulse rate and flushing, but larger doses frequently produce severe diarrhœa with rose-coloured stools, nausea, vomiting, rigor and rise of temperature. As a general rule these reactions subside in from twelve to twenty-four hours.

Locally, mercurochrome-220 soluble has been extensively used in the treatment of gonorrhœa and cystitis, in erysipelas and various skin conditions, and in ophthalmology.

The results which have been obtained following the intravenous use of mercurochrome-220 are somewhat contradictory. Dudgeon (1926) has used it intravenously in 150 cases of acute bacterial infection, and finds it of considerable value, the maximum dose being 10 c.cm. of a 0·5 per cent. solution in saline. The rigor which may follow intravenous injection can be prevented or greatly modified by keeping the patient warm during and after treatment and by giving 10 grains of aspirin with hot tea immediately after the inoculation.

Deekes and his colleagues (1924), in the report issued by the Medical Department of the United Fruit Company, record that

twenty-six cases of lobar pneumonia were inoculated intravenously with mercurochrome, with a mortality of less than 20 per cent., while in the same medical division the mortality from lobar pneumonia for a period of four years was 49·13 per cent.

Young (1926) has investigated the results of a number of recorded cases treated intravenously. Of 680 cases, covering an extremely wide range of local and general infections, 74·4 per cent. were cured or improved, in 2·6 per cent. the action of the drug was doubtful, 3·4 per cent. were temporarily improved, and in 19·6 per cent. treatment failed. The febrile reaction following injection seemed of importance in producing a cure, though with small doses (2 to 3 mgm. per kilogram of body weight) splendid results were obtained without any reaction. Certain individuals, it appears, display a hypersensitiveness to mercurochrome given intravenously. According to Redewill and Potter (1925), such patients excrete a proportionately greater amount of mercury by the saliva and gastro-intestinal tract than by the kidneys, and give a negative result with the following test: a little diphenylcarbazide and 0·5 c.c. carbon tetrachloride are shaken with the seminal fluid; a purple colour is produced with a minute amount of mercury. By this test and a mercurochrome kidney function test male patients not suited for repeated injections can easily be detected.

While the majority of successes with mercurochrome have been recorded as the result of clinical experience, both Walker and Sweeney (1926) and Sanner and Hill (1928) have brought forward evidence from experimental results which would seem to favour the rationale of this form of therapy.

On the other hand, a number of failures have been reported with mercurochrome-220, and it must be remembered that almost certainly many of the unfavourable results have never been recorded, because of a natural reticence in reporting failures and also because a series of failures is always small and the method is not continued. Horsley's results (1926) in septicæmia were not convincing. Simmonds (1926) could not be sure of any beneficial action. Meyer, Sommer and Eddie (1926) concluded that mercurochrome failed utterly to substantiate its claims

as a biliary antiseptic. Walker (1926) demonstrated that mercurochrome in the presence of fresh defibrinated blood was not antiseptic except in high concentration, and St. George (1925) decried the use of mercurochrome on the basis of autopsy findings in treated cases. Mercurochrome is quite ineffective in cases of plague.

Zau and Meleney (1928) have recently found that in dogs there is no evidence that the intra-arterial injection of mercurochrome-220 soluble in doses of 5 mgm. per kilogram of body weight, given twenty-four to seventy-two hours after the local injection of organisms into the leg, in any way affects the course of a local experimental hæmolytic *Staphylococcus aureus* infection otherwise than to prolong it. In fact the intra-arterial injection of mercurochrome-220 soluble in doses ranging from 1 to 16 mgm. per kilogram of body weight was regularly followed by damage to the kidneys or liver, which persisted for a month or more.

The advisability of administering mercurochrome intravenously has also been questioned by Colebrook and Hare (1927). They found that serum derived from human blood treated with mercurochrome (1 in 40,000 to 1 in 10,000) had no bactericidal power for staphylococcus or hæmolytic streptococcus, while defibrinated human blood which had received an addition of 1 in 10,000 mercurochrome had considerably less power to kill staphylococci than the same blood without mercurochrome. Bile derived from a rabbit which had received 5 mgm. of mercurochrome per kilogram of body weight possessed no bactericidal power for *Eberthella typhi* (*B. typhosus*), nor was the normal bactericidal power for *E. typhi* possessed by the serum of the rabbit increased by the injection of the drug.

In view of these findings, it seems clear that any therapeutic effect resulting from the action of mercurochrome-220 soluble is not due to a direct action on the invading bacteria. Colebrook and Hare suggest that the remarkable clinical improvements which are said to have occurred in some septicæmic cases are brought about by an auto-immunisation process initiated by the severe constitutional disturbances which may follow injection of the drug. The results would thus be analogous to those obtained

by " protein shock," which, as is well known, may sometimes produce a favourable effect on the course of bacterial infections.

Even in the case of localised infections, such as cystitis and urethritis, the beneficial results of mercurochrome therapy have been questioned, and from the variability of the recorded results it is obvious that mercurochrome-220 cannot be regarded as the ideal antiseptic.

Various other mercury preparations of dyes have also been produced, such as mercurochrome-565—a mercury derivative of rose-benzol 3B, and benzurin—a mercury derivative of benzopurpurin 4B. Experiments with these dyes have given inconclusive results.

Walker and Sweeney (1927) have studied the effect on staphylococcal infections in mice of benzene ring compounds containing mercury. Chemotherapeutic activity was conferred on the mercuribenzene ring by the introduction of the following radicals, $N(CH_3)_2$, $N(C_2H_5)_2$, OH and COOH. Other groups, which alone were inactive, were found to enhance the activity of the primary group when introduced in a certain position in the benzene ring. Thus SO_3H *para* to OH, Cl *ortho* to COOH, and NH_2 *ortho* to COOH increased chemotherapeutic activity. In every case, however, the therapeutic efficiency of the compounds studied was greatly reduced by indirect contact through the tissues and blood with the infecting staphylococci.

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ORGANIC ARSENICAL COMPOUNDS

Certain organic arsenical compounds have been tentatively tested in the treatment of septicæmia, though, inasmuch as arsenic has long been known to be a general protoplasmic poison, it is somewhat surprising that the arsphenamines have not been more extensively employed in the treatment of bacterial infections. As a matter of fact, arsphenamine was tried at an early stage in a few cases of erysipelas, glanders, puerperal fever and leprosy; very

remarkable results were also described in connection with the treatment of human anthrax with neoarsphenamine by Louw and Pijper (1922). Moreover, several papers appeared in the German medical press (Schiemann, 1915) dealing with the bactericidal action of arsphenamine and neoarsphenamine on the organisms of anthrax, glanders and swine erysipelas; the war emergency, however, seems to have brought these investigations to a premature end. In 1916, Douglas and Colebrook found that aqueous solutions of arsphenamine and neoarsphenamine exerted a considerable bactericidal effect upon staphylococci and that this effect was not much diminished when the drug was acting in human serum or blood. They also showed that the serum of a patient to whom one of these drugs had been administered an hour or two previously was capable of exerting a similar bactericidal effect upon staphylococci. These experiments attracted the attention of Allison (1918), in America, who repeated them, using hæmolytic streptococci, with similar results. A number of rabbits injected with these organisms were treated with arsphenamine, with the result that, while eight out of twenty-one controls recovered, seventeen out of twenty-five treated animals were cured. Following on these papers there appeared a number of reports of trials of arsphenamine in puerperal sepsis, and in one report at least, comprising a record of 260 cases, great claims were made (Joanny, 1923). The results obtained must be accepted with caution, however, as other remedies were employed and no record is given of any blood cultures. Colebrook (1928) has recently reinvestigated the action of the arsphenamines in the treatment of septicæmia.

Experiments have confirmed the fact that the arsphenamines exhibit certain properties which differentiate them rather sharply from almost every other known compound which has been used for the chemotherapy of bacterial infections. When injected into the animal or human body, these arsenicals confer upon the blood the peculiar bactericidal qualities which characterise their simple solutions, and these acquired bactericidal qualities are retained by the blood for a considerable time. It seems probable that, owing to the slowness of excretion of these drugs and the length of

time during which they enter into combination with the blood corpuscles and fixed tissue cells, a considerable fraction of the injected dose is retained for some time in the blood plasma. In common with other antiseptic agents these arsenicals are capable of a destructive action upon human leucocytes, silver arsphenamine being the most toxic. A considerable time, however, must elapse before this toxic action becomes manifest, for both leucocytes and streptococci are apparently unaffected during the first three or four hours of contact with solutions of arsenicals which afterwards destroy them. The arsphenamines differ, however, from the ordinary antiseptics in having a higher affinity for certain microbes, such as the hæmolytic streptococci and the pneumococci, than for the leucocytes.

In view of the slow action of the arsphenamines on hæmolytic streptococci, it is obviously desirable to retain a bactericidal concentration of the arsenical in the blood for a considerable time without, however, maintaining a concentration of sufficient strength to injure the patient's leucocytes, with consequent weakening of the natural defensive forces.

Colebrook now prefers to use sulpharsphenamine in preference to neoarsphenamine, since injections of the former can be given subcutaneously or intramuscularly, thus ensuring a more prolonged absorption into the blood-stream, and can be repeated at shorter intervals without undue risk of toxic reaction. The following plan of administration is suggested :—

	1st dose	0·3 gm.
	2nd ,,	0·3 gm., 6 hours later.
	3rd ,,	0·3 gm., 15 to 18 hours after the second.
(If	4th ,,	0·3 gm., 24 hours after the third.
necessary)	5th ,,	0·3 gm., 48 ,, ,, fourth.
	6th ,,	0·3 gm., 3 days after the fifth.
	7th ,,	0·3 gm., 4 ,, ,, sixth.
	8th ,,	0·3 gm., 5 ,, ,, seventh.

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GOLD

Apart from its use in the treatment of tuberculosis, gold has not been extensively employed in acute bacterial infections.

Various colloidal gold preparations have entirely failed to produce any beneficial results in acute septicæmia, while the same is true also of solganal. Landé (1927), however, claims to have obtained good results with solganal in sub-acute bacterial endocarditis, while reports have been made by Semon (1927) and others on the value of gold compounds in lupus erythematosus—a condition which, there is some evidence to suggest, may be due to a streptococcal infection.

It is not improbable that certain of the good results of injecting colloids may be due, not to the colloids themselves, but to protein shock, induced by the protein-like materials used as colloid protectors.

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OTHER SUBSTANCES EMPLOYED IN THE CHEMOTHERAPY OF ACUTE BACTERIAL INFECTIONS

Many other substances have also been employed in the treatment of acute bacterial infections. During the war, solutions liberating hypochlorites, such as eusol and Carrel-Dakin's fluid, enjoyed considerable popularity.

Various colloidal metals have also been used. In view of the findings of Steabben (1926), the value of colloidal therapy appears doubtful, for while the injection of colloidal substances into normal rabbits may temporarily increase phagocytosis and also raise the bactericidal power of whole blood and serum, the results are far from constant, and, in experimentally infected animals, the power to respond to this stimulation is lost as the infection advances.

The injection of a colloidal substance into an animal suffering from an acute infection may, in fact, cause a fatal reaction, analogous to " shock," or, if the disease is in a chronic condition, may cause a marked focal reaction and thus aggravate the symptoms.

Another compound which has recently excited interest is the symmetrical urea of *para*-benzoyl-*para*-amino-benzoyl-amino-naphthol-3 : 6-sodium sulphonate (S.U.P. 36). This drug was introduced by McDonagh (1926) in the belief that it would prove of value in therapeutics by stimulating the tissues of the host to overcome acute bacterial infections. Pearce (1929) has recently described its use in influenza ; in a series of more than eighty cases every alternate patient was given 0·005 gm. S.U.P. 36 intramuscularly, when the onset had occurred within the preceding forty-eight hours, and a further dose of 0·0075 gm. if necessary on the fourth day. The uninjected cases served as controls. It was found that the duration of the pyrexia, headache and muscular pain, as well as the total length of the illness, were about halved by the injections. Hall (1929) has also found the compound of value in doses up to 0·01 gm. in a variety of conditions such as acute pyelitis, cystitis, mastoiditis, osteomyelitis and broncho-pneumonia.

Others, however, have obtained less encouraging results. In any case the compound does not act as a prophylactic if injected when no acute infection is present.

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CHAPTER XVI

THE CHEMOTHERAPY OF VIRUS DISEASES

At present the treatment of diseases due to ultra-microscopic viruses and the various species of Rickettsia is, apart from the use of immune sera, entirely symptomatic.

Where no specific treatment has been found a large variety of drugs have, of necessity, been tested. Thus acriflavine has been given intravenously in encephalitis lethargica, while Young, Hill and Scott (1925) and others have obtained apparently beneficial results in a few cases of acute anterior poliomyelitis from the intravenous injection of a 1 per cent. solution of mercurochrome.

Herpes simplex and herpes zoster are unaffected by any known chemotherapeutic agent and the same is true of variola, varicella, measles and rabies.

Chemotherapy is equally powerless in the treatment of the ever-growing number of virus diseases of mammals and birds. Recently, however, Bridré, Donatien and Hilbert (1928) have found that stovarsol has a specific action on contagious agalaxia of the sheep and goat. A series of three injections of 5, 7·5 and 10 c.cm. of a 10 per cent. solution of stovarsol given at intervals of twenty-four hours effected a complete cure in eleven lambs in which the disease was complicated by simple or multiple arthritis.

Since a very large number of virus diseases can be produced in laboratory animals there is here a large field for the extension of experimental chemotherapeutic research.

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CHAPTER XVII

THE CHEMOTHERAPY OF CANCER

THE ætiology of cancer is still unknown. All that can be stated at present is that in response to certain forms of irritation, certain cells take on a new quality as a result of which they are enabled to grow in a manner that does not subserve the needs of the organism as a whole. The theory which would ascribe an ætiological *rôle* to some extrinsic organism, be it protozoon, bacterium or ultramicroscopic virus, has at present but little evidence in its favour. Nevertheless in one sense the cancer cell itself may be regarded as parasitic, since it lives at the expense of the normal tissue cells and eventually interferes with the due performance of their functions.

The aim of chemotherapy in cancer is therefore to destroy the malignant cell, a problem of the utmost difficulty, for, since cancer cells are primarily derived from normal cells, it follows that they have many qualities in common with the cells from which they originate. In cancer also it is essential to destroy all the malignant cells, for if any remain in the body they are liable to give rise to fresh new growths.

In order to destroy cancer cells by chemotherapy, three methods might be adopted :—

(i.) A direct lethal action of the drug on the cancer cells.

(ii.) A destructive action on the blood vessels of the new growth, thereby leading to defective nutrition and eventual death of the cancer cells.

(iii.) A stimulation of the fixed tissue cells around the growth resulting in an eventual sclerosis of the growth.

The production of a drug with a direct action on the cancer cells and no action on the normal tissues is of course the ideal, hence the importance of determining the metabolism of cancer cells in an effort to distinguish in normal and malignant cells

metabolic differences which would form the basis for a rational chemotherapy. Up to the present the only difference in metabolism which has been definitely established is due to the work of Warburg (1926), who showed that in the presence of oxygen, normal cells rely entirely on the oxidation of sugar for their supply of energy, while under anaerobic conditions they split sugar into lactic acid only to a very slight extent.

Cancer cells, on the other hand, obtain their energy supply not only from the oxidation of sugar, but also by splitting sugar into lactic acid both under aerobic and anaerobic conditions. As a result of this dual mechanism malignant cells enjoy a greater independence of an adequate supply of oxygen than normal cells. Warburg's work has been fully confirmed in this country by Crabtree (1928 and 1929) who, however, has found that the cells in certain pathological overgrowths, such as those induced by the viruses of fowlpox and warts, also exhibit the power of aerobic and anaerobic glycolysis, though these cells are in no way malignant.

Up to the present attempts to interfere with the oxygen or carbohydrate supply of tumour cells *in vivo* have not provided any method of chemotherapeutic attack.

In all earlier experiments on the chemotherapy of cancer the experimental material consisted of strains of transplanted tumour cells grown in rats or mice. Certain fallacies which need not now be discussed attended these experiments, and the results must therefore be received with caution, since they bear somewhat the same relationship to experiments on spontaneous tumours as *in vitro* bactericidal experiments bear to the treatment of septicæmia. The experimental production of cancer in mice by means of tar provides, however, far more suitable material on which to carry out satisfactory chemotherapeutic experiments.

One of the earliest chemotherapeutic attempts to treat cancer was made by Wassermann and Keysser (1911), who used selenium eosin compounds, with the idea that selenium might prove selectively destructive to cancer cells when brought to them by the eosin. Some cures were claimed, but later Keysser (1912) reported that the majority of the experimental animals had died, while other observers were unable to obtain any beneficial results from the use

of selenium eosin. In the meantime a number of other metals were tested for their curative action in cancer. Izar (1913), for instance, thought that injections of colloidal sulphur had some curative action on tumour-bearing rats: others used gold, zinc, platinum, tin, copper or a combination of selenium and vanadium with choline borate. In man also a large number of metallic compounds were injected without success.

COLLOIDAL LEAD

During the past few years, however, more satisfactory results have been claimed by Blair Bell (1922–1929), following the use of colloidal lead. It is unnecessary to enter fully into the theoretical reasons, probably erroneous, which led to the adoption of this substance in the chemotherapy of cancer; suffice it to say that it was believed that the cells of the chorionic villi are similar in certain respects to malignant cells, and since the well-known abortifacient action of lead appears to be due to its toxic action on the chorionic villi, it was argued that it might have a similar action on malignant cells. Incidentally lead as a local application to malignant growths appears to have been recommended by Goulard as long ago as 1760. Preparations of colloidal lead or of colloidal lead phosphate have now been extensively used both in this country and abroad.

Far the best results have been obtained by Blair Bell (1929), whose latest findings are recorded in the table:—

Table of Results

November 9th, 1920–November 9th, 1928

(1) Total number of cases treated	566
(2) Died of the disease before treatment could be completed	359
(*a*) received less than 0·25 gm. lead	198
(*b*) received more than 0·25 gm. lead, but less than 0·5 gm.	161

(3) Died of intercurrent affections after treatment had concluded	77
(4) Died of intercurrent affections while under treatment	7
(5) Died as result of extensive destruction of disease	5
(6) Complete treatment refused	22
(7) Too recent for results to be estimated	31
(8) Believed cured, but died of other affections	2+
(9) Disease completely arrested	12+
(10) Believed cured and treatment stopped	51+

Sixty-five cases have thus been definitely arrested as the result of intravenous injections of colloidal lead. It must, however, be remembered that though lead alone was used in certain cases, in others lead was combined with surgical intervention or with X-ray or radium therapy. Blair Bell (1929) insists that in selecting cases for treatment the general condition of the patient is of paramount importance, while the site of the tumour is of less consequence. Before beginning treatment it is essential therefore to investigate the condition of the liver and kidneys, as these organs bear the full brunt of the toxic action of the metal. In addition, cases of myocardial involvement and severe anæmia are unsuitable. So long, however, as the patient's general condition is good and there is a prospect of six months of life, treatment is justified.

The total dosage which should be given is from 0·5 to 0·8 gm. of metallic lead. In some cases less than 0·5 gm. has produced a cure, while in others more than 1 gm. of metallic lead has been followed by a scarcely appreciable amelioration. Toxic reactions following the injections are slight, consisting chiefly of headache and loss of appetite.

The results of other workers have, for the most part, been less satisfactory than those recorded by Blair Bell. Wyard (1928), using colloidal lead hydroxide, and Hume (1928) obtained highly unsatisfactory results, and the same is true of Roffo and Calcagno (1929) in South America, and of Simpson (1928) in the United States. These observers were unable to determine any improve-

ment following the injections, the condition of the patients in fact deteriorating rapidly. Thompson (1928), in an analysis of fifty-five cases treated with lead, found that the course of the disease was influenced favourably in fifteen, but the improvement, though definite, was in most cases only temporary, and in only two cases was there complete disappearance of all signs of the original disease. Very similar results have been obtained by Knox (1929), four out of forty cases showing definite improvement. X-ray therapy was, however, combined with the lead injections, so that it is difficult to evaluate correctly the effect of the lead alone. Soiland, Costolow and Meland (1929) also combined colloidal lead with X-rays and radium treatment, but did not obtain any cures in thirty-one patients, while Ullmann (1929) treated fifty cases with X-rays and colloidal lead phosphate; in one case of a bladder tumour, which had been largely removed at operation, lead therapy combined with X-rays was followed by complete disappearance of the growth. Brunner (1929) found some improvement in seven out of fifteen cases, although no case was actually cured.

Stone and Craver (1927) found eight out of twenty-one cases improved, while in two instances the results might be designated as a temporary clinical cure. Incidentally, a case of chorion epithelioma was uninfluenced.

In animal experiments, although diminution in the size of the growths has been obtained, complete cures have been rare. Thus Bang (1928) failed to arrest the growth of tar tumours in mice, while Marsh and Simpson (1927) obtained only occasional diminution in the size of the growths and no cures in spontaneous mouse tumours treated with colloidal lead. Toxic reactions were very common. The toxicity of lead given intravenously, however, appears to depend largely on the form of lead administered. Thus Bischoff, Maxwell, Evans and Nuzum (1928) find that in rabbits the most toxic compounds are ionic lead, colloidal lead hydroxide, metallic lead, glycerophosphate, oleate and stearate. The lead hydroxide and glycerophosphate are very soluble, while the metallic lead oxidises to the hydroxide in the blood-stream. All these compounds function potentially as ionic lead. The next

group, which, as Blair Bell and his co-workers have emphasised, is very toxic, includes colloidal lead oxychloride, oxycarbonate and carbonate. These compounds are very insoluble and are probably removed from the blood-stream before they have a chance to react with the constituents of the blood. From two to four times the dose of the second group must be given to cause a drop in the hæmoglobin comparable to that produced by the first group. The third group includes tetraethyl lead and triethyl lead chloride. These compounds bring about only a slight drop in hæmoglobin even when the lethal dose is approached. Since the lead is linked to carbon, these compounds are not capable of forming lead ions until hydrolysis occurs, this hydrolysis being a very slow process. The fourth group comprises trilead phosphate, dilead phosphate and lead sulphide, which do not have any demonstrable effect on the red blood corpuscles, probably because they are highly insoluble and stable at the *p*H of the blood.

In the preparation of colloidal lead it is obvious that the formation of ionic lead must be avoided if toxic results are to be prevented. Krause (1929) has also investigated the action of a number of organic lead compounds on transplantable mouse tumours. The most active compounds were tri-*n*-propyl lead fluoride, tri-*i*-butyl lead bromide, lead tetraphenyl and tricyclohexyl lead.

The combined use of colloidal lead and radiation in the treatment of tumours in man has already been mentioned. In animals, Mottram (1928) has shown that when mice inoculated with carcinoma are first injected with colloidal lead and three to four days later are exposed to radium, the growth of the tumours is inhibited. It is thus obvious that colloidal lead has a definite action in inhibiting the growth of certain tumours, but the conditions under which the lead acts and the reasons why certain tumours are destroyed, while others are untouched, is as yet unknown. There seems little or no evidence that lead acts directly on the tumour cells. Many attempts to estimate the amount of lead in tumours and in various organs have been made, but the results obtained have shown very considerable variations. Roffo and Calcagno (1929), in fact, found that malignant cells

growing in tissue cultures were no more susceptible to lead than normal cells. On the other hand, Wood (1926) has brought forward evidence to show that colloidal lead produces thrombosis in the blood-vessels of tumours. Since radium is also known to damage the blood-vessels of tumours, this may explain the enhanced results obtained from a combination of lead and radium therapy. Possibly the action of the colloidal lead is an indirect one on the tumour cells and is dependent on the fact demonstrated by Schulemann (1917) that metallic colloids are taken up from the blood-stream by the macrophages. Metastases in the lungs, around which, as a rule, there is a complete absence of macrophages, appear to be especially resistant to the action of metallic colloids. It is obviously highly desirable to gain further insight into the mode of action of these metallic colloids on malignant cells.

COLLOIDAL LEAD SELENIUM

Ever since the use of selenium eosin compounds by Wasser man and Keysser (1911), selenium has enjoyed a certain reputation in the treatment of cancer, Watson-Williams (1919), for instance, having been especially struck by its analgesic action.

Todd (1928), therefore, introduced the use of lead selenium colloids in the treatment of cancer, the preparation now in use—D_4S—having been prepared by Taylor and Lloyd (1928). The object of using a colloidal lead selenide was to avoid the formation of ionic lead. In addition to the selenide, the action of colloidal lead sulphides and tellurides was tested on transplantable mouse tumours. A definite inhibition of growth was obtained, and in some cases the tumours regressed entirely. In certain instances, however, tumours which had apparently disappeared returned after the cessation of treatment. This occurrence is strongly in favour of the view that these colloidal substances do not act directly on the tumour cells, but rather on the blood vessels surrounding connective tissues, thereby stimulating them into activity.

With the preparation known as D_4S, painful reactions were not marked so long as a clean injection into a vein was given. Lead colic, in spite of the large doses given, was rare and could be

partially allayed by the use of injections of ephedrine. Jaundice and mental weakening with delusions and hallucinations also very occasionally resulted from the treatment. Injections of D_4S are usually given at intervals of a week. At first 5·0 c.cm., then 7·0, 8·0 and 10·0 c.cm. are given. 15·0 c.cm. is probably the maximum which should be given at any one injection. After from eight to twelve injections a pause is made. Then, depending upon symptoms, the course is repeated or half a course given.

Subsidiary treatment is important. The bowels must be kept open, and liver extract is of importance in order to assist in blood regeneration. The condition of the blood, as judged by the percentage of hæmoglobin, must be carefully watched.

Todd (1928) gives a complete account of forty-four cases treated by colloidal lead selenium compounds. Very marked retardation of the growth occurred in eight cases and slight retardation in sixteen others. At present there is little indication of the type of case most suitable for this treatment. Peritoneal metastases from cervical carcinoma do not appear to be strikingly affected by the treatment, though the uterine masses, together with the hæmorrhage and pain, have been greatly ameliorated. Pulmonary metastases appear to be resistant and cases with marked cachexia or anorexia are unsuitable.

In a more recent paper Todd and Aldwinkle (1929) state that there is considerable danger of combining treatment by selenium lead compounds with exposure to radium, possibly owing to a too rapid disintegration of the tumour cells.

ANILINE DYES

From time to time attempts have been made to treat cancer with aniline dyes,the most recent having been the reputed successes obtained after injections of isamine blue. Marsh and Simpson (1927), however, have tested a very large number of aniline derivatives on mice bearing spontaneous tumours and have found that the growth of the tumours is entirely uninfluenced.

Recently, Copeman, Coke and Gouldesbrough (1929) have

claimed successful results with radium and X-ray treatment combined with painting by fluorescein. For some years it has been known that when *Paramecium* is exposed to daylight in the presence of a dilute solution of flourescein it is rapidly killed, whereas if kept in the dark no such lethal effect results. Since X-rays and radium have much greater penetrating power than ultra-violet light it seemed possible that a lethal action on cancer cells might result from the exposure of tissues containing fluorescein to X-rays and radium.

A 2·0 to 2·5 per cent. of sodium fluorescein is painted on the skin over the growth and, or, is administered intravenously. The patient is then treated with an adequate dose of X-rays or radium. These proceedings are repeated three times a week, after which three weeks' rest is allowed, when the treatment is again repeated. Mottram (1929) has also shown that dosages of radium and fluorescein, which alone are incapable of killing the cells of a transplantable rat sarcoma, are together able to exert such a summated action as to prevent growth of tumours in the majority of animals subsequently inoculated with the treated cells. It must, however, be remembered that secondary radiations have very low penetrating power and do not even penetrate effectively the chitin envelope of ascaris eggs, so that it seems doubtful whether they would have sufficient power to kill cancer cells embedded in the tissues.

It is thus obvious that though no satisfactory chemotherapeutic means has yet been found for the treatment of cancer, nevertheless it is possible to inhibit to some extent the growth of certain malignant tumours by means of colloidal lead. With further insight into the mode of action of these substances it may be possible both to enhance and to extend their therapeutic effects.

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INDEX

A 69 in relapsing fever, 420
4-Acetylamino-2-hydroxyphenylarsinic acid in trypanosomiasis, 250
Acetylarsan in relapsing fever, 419
in syphilis, 368
in trypanosomiasis, 254
in yaws, 412
Acridine dyes, 486
Acriflavine, 486
Agalaxia and stovarsol, 514
Albaspidin, 41
Albert 102, 317
Aldehyde test in kala azar, 188
Alepol in leprosy, 444
Alkalies in malaria, 127
Alkyl resorcinols, 498
Amino-stiburea, 185
4-Amino-2-hydroxyphenylarsinic acid in trypanosomiasis, 252
di-*m*-aminobenzoyl rivanol, 489
Amœbiasis, chemotherapy of, 77
Amœbic dysentery, auremetine in, 86
bismuth subnitrate in, 94
emetine bismuthous iodide in, 83
hydrochloride in, 82
periodide in, 85
ipecacuanha alkaloids in, 77
kurchi bark in, 93
rivanol in, 92
stovarsol in, 89
tréparsol in, 91
yatren in, 87
hepatitis, 96
decline of, since emetine discovered, 96
treatment of, by emetine, 96
Anæmia, aplastic, and arsphenamines, 344
Anil and styryl quinolines in bacterial infections, 491
in trypanosomiasis, 285
Aniline dyes, classification of, 482
in treatment of bacterial infections, 482
of cancer, 522
of helminthic infections, 74
Animal experiments and chemotherapy, 3
Ankylostomiasis—chemotherapy of, 15
treatment of, by ascaridole, 32
by β-naphthol, 37
by carbon tetrachloride, 15
by carbon trichloride, 26
by oil of chenopodium, 30
by tetrachlorethylene, 26
by thymol, 37
Anthelminthic action and chemical constitution, 37
Anthelminthics, classification, 13
critical testing, 10
history, 9
Antimony flocculation test in kala azar, 189
in treatment of dermal leishmaniasis, 216
of granuloma inguinale, 476
of kala azar, 168
of oriental sore, 216
of schistosomiasis, 55
of trypanosomiasis, 279
pentavalent compounds, advantages of, 206
classification, 177
sodium thioglycollate in granuloma inguinale, 477
thioglycollamide in granuloma inguinale, 477
Antimosan, 58
Arsalyt in relapsing fever, 419
Arsenic pentavalent compounds in amœbiasis, 89
in malaria, 145
in syphilis, 364
in trypanosomiasis, 221
in yaws, 410
Arsphenamine compounds, chemical structure, 313
in treatment of bacterial infections, 508
of malaria, 145
of relapsing fever, 418
of syphilis, 312
of Vincent's angina, 425

Arsphenamine compounds in treatment of yaws, 407
liver injury due to, 345
nomenclature, 312
pharmacology, 317
standardisation, 358
toxic effects, 325
Ascaridole in ankylostomiasis, 32
Aspidinol, 41
Atoxyl in trypanosomiasis, 248
Auramine O in bacterial infections, 484
Auremetine in amœbic dysentery, 86
Aurocantan, 467
Avenyl in leprosy, 395

Bacterial infections, acute, chemotherapy of, 480
treatment by acridine dyes, 486
by acriflavine, 486
by alkyl resorcinols, 498
by di-*m*-aminobenzoyl rivanol, 489
by anil and styryl quinolines, 491
by aniline dyes, 482
by arsphenamine compounds, 508
by auramine O, 484
by crystal violet, 484
by ethylhydrocupreine (optochin), 493
by gentian violet, 484
by gold, 511
by hexyl resorcinol, 499
by *iso*-octylhydrocupreine (vuzin), 493
by leucosulphato-green, 485
by malachite green, 484
by mercurochrome 220-soluble, 503
by phenol derivatives, 497
by phenyl-methanes, 483
by proflavine, 486
by quinanil, 492
by rivanol, 488
by S.U.P. 36, 512
Bayer 205 or Germanin, 258
chemical constitution, 258
identical with moranyl, 259
in treatment of experimental trypanosomiasis, 261
of human trypanosomiasis, 262
of trypanosomiasis of domestic animals, 268
pharmacology of, 274
prophylactic action, 271
toxic symptoms, 264
Benzisoxazine arsinic acids in trypanosomiasis, 230
Berberine in oriental sore, 218
β-naphthol and ankylostomiasis, 37
Bilharziasis. *See* Schistosomiasis.
Bismuth in treatment of amœbic dysentery, 94
of infective jaundice, 423
of relapsing fever, 420
of syphilis, 390
of trypanosomiasis, 284
of yaws, 414
pharmacology of, 374
preparations of, 380
toxicity of, 386
Blackwater fever and quinine, 154
and plasmoquin, 155
BR. 34 and trypanosomiasis, 257
BR. 34 A and trypanosomiasis, 257
BR. 68 and trypanosomiasis, 255

Calcium in tuberculosis, 474
Cancer, chemotherapy of, 515
aniline dyes in, 522
colloidal lead in, 517
selenium in, 521
Carbon tetrachloride in ankylostomiasis, 15
trichloride, 26
Carvacrol as anthelminthic, 37
Chaulmoogra oil in leprosy, 439
constitution, 433
distribution, 432
ethyl esters, 441
mode of action, 457
physical properties, 434
toxic reactions, 450
Chaulmoogric acid, 433
Chemotherapeutic action, 2
in amœbiasis, 97
in cancer, 515
in kala azar, 208
in leprosy, 457
in malaria, 138
in relapsing fever, 421
in schistosomiasis, 64
in syphilis, 406
in trypanosomiasis, 296
in tuberculosis, 473
in yaws, 407
Chemotherapy, animal experiments in, 3
clinical observations and, 4
definition of, 1
Ehrlich's theory of, 2
statistics in, 5

Chenopodium, oil of, 30
Cinchona alkaloids, 107
chemical structure, 108
pharmacology, 134
relative value of, 111
febrifuge, 111
Cinchonidine, 120
Cinchonine, 120
Clonorchiasis, chemotherapy of, 74
gentian violet in, 74
Cod liver oil in leprosy, 449
in tuberculosis, 449
Conessine in amœbic dysentery, 93
Copper in leprosy, 461
in tuberculosis, 466
Crystal violet and bacterial infections, 484
Cupreine, derivatives of, 123
Cyclosan, 236

Dermatitis, exfoliative, 338
Drug resistance in malaria, 118
in relapsing fever, 418
in syphilis, 401
in trypanosomiasis, 288
Dysentery, amœbic. *See* Amœbic dysentery.

E.C.C.O. in leprosy, 443
Ehrlich's theory of chemotherapy, 2
Emetamine, 79
Emetine, bismuthous iodide in amœbic dysentery, 83
chemical structure of, 78
effect on *Endamœba histolytica in vitro*, 97
hydrochloride in amœbic dysentery, 82
in amœbic hepatitis, 96
in oriental sore, 217
in pulmonary distomiasis, 72
in schistosomiasis, 68
periodide in amœbic dysentery, 85
in schistosomiasis, 69
pharmacology of, 79
toxic effects of, 80
Erythrocyte sedimentation rate in leprosy, 463
Etharsanol in trypanosomiasis, 227
Ethylhydrocupreine hydrochloride and pneumococcal infections, 493
Eugenol as anthelminthic, 39

Ficus laurifolia, latex of, and whipworms, 54
Filariasis, chemotherapy of, 12
Filicic acid, 41
Flumerin and syphilis, 394
4-Formylamino-2-hydroxyphenylarsinic acid (tréparsol), 252
Fuadin and schistosomiasis, 59

Gentian violet, composition of, 485
in bacterial infections, 484
in clonorchiasis, 74
Gold in bacterial infections, 511
in leprosy, 462
in tuberculosis, 466
Granuloma inguinale and antimony, 476

Hæmorrhagic encephalitis and arsphenamines, 333
Halarsol in treatment of yaws, 412
Helminthic infections, chemotherapy of, 9
Hepatitis. *See* Amœbic hepatitis.
Hexyl resorcinol and bacterial infections, 499
Hoechst 2754 in trypanosomiasis, 258
Hydnocarpic acid, chemical constitution of, 433
Hydnocarpus oil, ethyl esters of, 441
in leprosy, 439
physical properties of, 434

Immune bodies and chemotherapy of kala azar, 209
of malaria, 138
of relapsing fever, 421
of syphilis, 402
of trypanosomiasis, 302
Ipecacuanha alkaloids in amœbic dysentery, 77
chemical structure of, 78

Kala azar, advantages of pentavalent antimony compounds in, 206
aldehyde test in, 188
and antimony tartrates in treatment of, 168
and antimosan, 176
Mediterranean or infantile form, treatment of, 183
mode of action of antimony in, 208
serum antimony flocculation test in, 189
proteins in, 189
tests of cure in, 188

Kala azar, toxic reactions of antimony tartrates in, 175
toxic results of pentavalent antimony compounds and, 207
treatment by amino-stiburea, 185
by neostam, 184
by neostibosan, 186
by pentavalent antimony compounds, 182
by stibenyl, 182
by stibosan, 185
by urea-stibamine, 183
by urea-stibol, 185
by von Heyden 693...186
of experimental infections in hamster, 182
Krysolgan in tuberculosis, 467
Kurchi bark in amœbic dysentery, 93

Lead colloid in cancer, 517
selenium colloid in cancer, 522
Leishmaniasis. *See* Kala azar *and* Oriental sore.
dermal, and pentavalent antimony compounds in, 216
muco-cutaneous, treatment of, 218
Leprosy, action of chaulmoogra oil in, 457
chemotherapy of, 430
erythrocyte sedimentation rate in, 463
prophylaxis of, 465
treatment by alepol of, 444
by chaulmoogra oils, intradermally, 444
intramuscularly, 440
intravenously, 440
orally, 439
by cod liver oil, 449
by copper, 461
by E.C.C.O., 443
by ethyl esters of chaulmoogra and hydnocarpus oils, 441
by gold, 462
by hydnocarpus oil, 439
by Mercado mixture, 440
by potassium iodide, 460
by protein shock, 459
by sanocrysin, 462
by sodium salts of chaulmoogra oil, 440
by solganal, 462
by tellurium, 461
Leucosulphato-green and bacterial infections, 485
Liver abscess. *See* Amœbic hepatitis.
injury due to arsphenamine, 345
Lymphangitis, epizootic, mercuric iodide in treatment of, 428

Madura foot, treatment of, 427
Malachite green in bacterial infections, 484
Malaria, chemotherapy of, 106
drug resistance in, 118
mode of action of quinine in, 138
prophylaxis of, 132
treatment by alkalies, 127
by cinchona alkaloids, 111
febrifuge, 113
by cinchonidine, 120
by cinchonine, 120
by cupreine derivatives, 123
by methylene blue, 150
by plasmoquin, 156
compound, 158
by quinidine, 120
by quinine, 115
in pregnancy, 132
intramuscularly, 130
intravenously, 129
orally, 126
per rectum, 129
by quinine derivatives, 132
dimethylarsinate, 150
stovarsol, 149
troposan, 150
by quinoidine, 113
by stovarsol, 145
induced malaria, 118
of bird malaria, 125
of relapses, 121
Mercuric iodide and epizootic lymphangitis, 428
Mercurochrome-220 soluble and acute bacterial infections, 503
Mercury in syphilis, 394
Methylene blue in malaria, 150
in piroplasmosis, 309
Moranyl identical with Bayer 205...259
in trypanosomiasis, 267
Mycotic diseases, chemotherapy of, 426
potassium iodide in, 427

Neoarsphenamine, chemical constitution of, 315
in treatment of bacterial infections, 508
of syphilis, 315
nomenclature, 312
pharmacology, 317
standardisation, 358
toxic effects, 325

Neostam and kala azar, 184
Neostibosan and kala azar, 186

iso-**Octylhydrocupreine** hydrochlorine and bacterial infections, 493
Oriental sore, chemotherapy of, 216
antimony in treatment of, 216
berberine in treatment of, 218
emetine in treatment of, 217
phosphorated oil in treatment of, 217
Oroya fever and arsphenamine, 311

Phenol derivatives in bacterial infections, 497
Phenyl-methanes in bacterial infections, 483
Phenyl stibinic acid, chemical constitution of, 176
Phosphorated oil in oriental sore, 217
Piroplasmosis, chemotherapy of, 308
treatment of, by methylene blue, 309
by silver preparations, 309
by trypan blue, 308
Plasmoquin in treatment of blackwater fever, 155
of bird malaria, 156
of malaria, 157
toxic effects of, 161
Plasmoquin compound, advantages of, 164
in treatment of malaria, 158
Platinum in treatment of syphilis, 396
Polyneuritis due to arsphenamine treatment, 343
Potassium iodide in leprosy, 460
in mycotic diseases, 427
Proflavine in bacterial infections, 486
Proparsanol in trypanosomiasis, 227
Prophylaxis, chemotherapeutic, of malaria, 132
of relapsing fever, 420
of syphilis, 399
of trypanosomiasis, 246
of yaws, 412
Protein shock in treatment of leprosy, 459

Quinanil in bacterial infections, 492
Quinidine in treatment of malaria, 120
Quinine administration, methods of, 115
and plasmoquin, 158
and pregnancy, 132
Quinine, chemical structure of, 108
derivatives, 132
dimethylarsinate, 150
in treatment of blackwater fever, 154
of malaria, 115
mode of action in malaria, 138
pharmacology of, 134
prophylactic action in malaria, 132
relation to other cinchona alkaloids, 108
resistance in malaria, 118
stovarsol, 149
troposan, 150
Quinidine in treatment of malaria, 120
Quinoidine in treatment of malaria, 113

Relapsing fever, chemotherapy of, 418
mode of action of arsphenamines in, 421
treatment of, by A 69...420
by acetylarsan, 419
by arsalyt, 419
by arsphenamine, 418
by bismuth, 420
by neoarsphenamine, 418
by sanocrysin, 419
by solganal, 419
by stovarsol, 418
Ringworm and thallium acetate, 428
Rivanol in the treatment of amœbic dysentery, 92
of bacterial infections, 488

Sanocrysin, action of, on tubercle bacilli *in vitro*, 472
in treatment of experimental tuberculosis, 471
of leprosy, 462
of relapsing fever, 419
of tuberculosis in man, 467
mode of action of, 473
toxic effects of, 468
Santonin as anthelminthic, 43
constitution of, 46
derivatives of, 46
mode of action of, 44
toxic effects of, 51
Schistosomiasis and treatment by antimony tratrates, 55
by antimosan, 58
by fuadin, 59
Selenium lead colloid in cancer, 521
Serum proteins in kala azar, 189
Silver preparations in treatment of piroplasmosis, 309

Solganal in treatment of leprosy, 462
of relapsing fever, 419
of tuberculosis, 473
Spirochætal jaundice, chemotherapy of, 423
Statistics in chemotherapy, 5
Stibenyl in treatment of kala azar, 182
Stibosan in treatment of kala azar, 185
Stovarsol in treatment of agalaxia, 514
of amœbic dysentery, 89
of malaria, 145
of relapsing fever, 418
of syphilis, 370
of trypanosomiasis, 230
of yaws, 410
Styryl quinolines in trypanosomiasis, 285
S.U.P. 36 in bacterial infections, 512
Surra, chemotherapeutic treatment of, 246
Syphilis, chemotherapy of, 312
drug resistance in, 401
mode of action of drugs in, 406
prophylaxis of, 399
treatment of, by acetylarsan, 368
by Albert 102...317
by arsphenamine, 312
by avenyl, 395
by bismuth, 390
by copper, 397
by flumerin, 394
by mercury, 394
by neoarsphenamine, 315
by stovarsol, 370
by sulpharsphenamine, 316
by tellurium, 396
by tréparsol, 372
by tryparsamide, 364

Tellurium in treatment of leprosy, 461
of syphilis, 396
Tetrachlorethylene in ankylostomiasis, 26
Thallium acetate in treatment of ringworm, 428
Thymol in ankylostomiasis, 37
Tréparsol in treatment of amœbic dysentery, 91
of syphilis, 372
of yaws, 412
Trypanosomiasis, chemotherapy of, 221
drug resistance in, 288
mode of action of drugs in, 302
prophylaxis of, 246
treatment of, by 4-acetylamino-2-hydroxyphenylarsinic acid, 250
Trypanosomiasis, treatment of, by acetylarsan, 254
by 4-amino-2-hydroxyphenylarsinic acid, 252
by anil and styryl quinoline derivatives, 285
by antimony tartrates, 279
by arsacetin, 223
by atoxyl, 248
by Bayer 205 or Germanin, 262
by benzisoxazine arsinic acids, 230
by bismuth, 284
by BR. 68...255
by BR. 34 and 34A...257
by cyclosan, 236
by etharsanol, 227
by 4-formylamino-2-hydroxyphenylarsinic acid, 252
by Hoechst 2754...258
by moranyl or Furneau 309...267
by tryparsamide, 240
Tryparsamide and experimental trypanosomiasis, 239
chemical structure of, 238
in treatment of sleeping sickness, 240
of syphilis, 364
of trypanosomiasis of domestic animals, 245
prophylactic action of, 246
toxic effects of, 239
Tuberculosis, chemotherapy of, 466
treatment of, by aurocantan, 467
by calcium, 474
by cod liver oil, 474
by krysolgan, 467
by sanocrysin, 467
by solganal, 473
vitamin A in, 474
vitamin D in, 474

Urea stibamine in treatment of kala azar, 183
stibol in treatment of kala azar, 185

Vanadium in treatment of syphilis, 396
Vincent's angina, treatment of, by arsphenamine, 425
Virus diseases, chemotherapy of, 514
Vitamin A in tuberculosis, 474
Vitamin D in tuberculosis, 474

Whip worms, latex of *Ficus laurifolia* in treatment of, 54

Yatren in treatment of amœbic dysentery, 87
Yaws, chemotherapy of, 407
treatment of, by acetylarsan, 412
by bismuth, 414
by bismuth arsanilate, 415
Yaws, treatment of, by bismut o-yatren A and B, 415
by neoarsphenamine, 407
by stovarsol, 410
by tréparsol, 412

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