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# AN INTRODUCTION TO THE BIOCHEMISTRY OF NITROGEN CONSERVATION

BY

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This Volume includes the substance of the lectures  
delivered at the Patna University by the Author  
as Sakhraj Ray Reader in Natural Science.

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## PREFACE

The following volume is based on the author's former work entitled *An Introduction to Bacteriological and Enzyme Chemistry*, which has now been out of print for some years.

In considering with the Publishers the question of a second edition the suggestion was made that in order to keep the size of the book within reasonable bounds, and at the same time to give space for the inclusion of fresh matter necessary to bring the book up to date, certain sections of the subject might be omitted, more especially the chapters on organic chemistry, and many of the details of laboratory practice which will find their place more suitably in a special laboratory handbook.

Recently the author was requested to give a course of lectures as Sakhraj Ray Reader in Natural Science to the Patna University. He chose as his subject The Biochemistry of Nitrogen Conservation since the main objective of his scientific and professional life's work has been the solution of the problem of the satisfactory utilization of nitrogenous waste material.

As the preparation of the lectures progressed it became evident that their subject alone would provide adequate material for a book without the inclusion of any of the other topics dealt with in the original volume. On placing this suggestion before the Publishers, they fully agreed, and the present volume, in consequence, is more of the nature of a specialist monograph than of a general handbook on Biochemistry and its applications.

For this reason a considerable proportion of space has been devoted to researches for which the author has been personally responsible, or which have come specially under his consideration.

Sufficient references are, it is hoped, given in the text, or in

the short bibliography at the end, to enable the reader to follow up the subject in any desired direction.

The author desires to thank the Honourable the Vice-Chancellor and Syndicate of the Patna University for nominating him as Sakhraj Ray Reader, and for their courtesy in facilitating arrangements for the publication of the present volume.

His thanks are also due to the Director of the Indian Institute of Science for permission to use the blocks illustrating nightsoil conservancy in Shanghai; to Dr. V. Subrahmanyam, Professor of Biochemistry in the Institute of Science, for arranging to have the drawings of micro-organisms made by the artist to the Department; to Messrs. Activated Sludge, Ltd., for the illustrations and diagram in Chapter IX; and to George L. Harvey, Esq., for the photographs of compost heaps in Chapter X.

G. J. F.

BANGALORE,  
S. INDIA.

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# AN INTRODUCTION TO THE BIOCHEMISTRY OF NITROGEN CONSERVATION

## INTRODUCTION

**Some Characteristics of Nitrogen.** From many points of view nitrogen is one of the most interesting of the elements, and one, moreover, of the utmost importance to humanity. We commonly think of nitrogen as an inert constituent of the atmosphere whose main function seems to be to moderate the excessive activity of its companion element oxygen. Actually, however, nitrogen once it is liberated from its quiescent condition as a free gas may exhibit very different properties. Thus the earliest of the published researches undertaken by the author when he was quite a young student, was concerned with certain silver compounds in the preparation of which silver oxide had to be dissolved in strong ammonia. While warming this mixture cautiously over a flame, there was noticed a black substance suddenly forming on the neck of the flask, and a moment afterwards a violent explosion occurred and the student was left with the neck of the flask in his hand and the remainder of it scattered about the laboratory as a fine powder. Mercifully, so rapid is the reaction even of a young chemist to danger, none of it entered his eyes, and in fact he was quite unhurt.

What had happened was that under the conditions of the experiment a small quantity of nitride of silver or fulminating silver had been formed which is only stable if left severely alone. It is possible that most young students of chemistry have amused themselves and annoyed their neighbours by smearing damp iodide of nitrogen on the handles of doors and so forth, while the even more violent chloride of nitrogen has

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only been seen by most of us as a lecture preparation. The author is old enough to have had the privilege of listening as a first-year student to the delightful lectures of Sir Henry Roscoe, who spared no pains to illustrate by specimen or lecture-experiment every important fact referred to. Chloride of nitrogen was no exception.<sup>1</sup> A small quantity of this compound collected in a leaden dish, was touched off by the addition of a drop of turpentine at the end of a long fishing rod. The imperturbability of the stately College porter of those days, Sergeant Nish, when the explosion went off like a gun immediately behind his back, was something to be remembered.

We have, alas! too great cause to be aware of the violent nature of nitrogen in certain of its combinations when we know that it was the ability of Germany to convert the placid nitrogen of the air into high explosive that made it possible for her to carry on the War. It is of interest to the author that early work of his on the chemistry of iron nitride in part of which he was associated with a good friend of India, then Mr., and now Sir Philip Hartog, played some part in building up the technique of the catalytic action of iron in the Haber process, now employed to furnish ammonia compounds for the peaceful service of agriculture.

Since those days, however, the author has been mainly concerned with the quieter activities of nitrogen in the permutations and combinations which it undergoes in the world of nature. For twenty years he was occupied with the problem of the transformation of nitrogen from its potentially offensive combinations in the raw sewage of Manchester, into harmless nitrates which could be discharged into the Ship Canal with actual benefit to that rather insalubrious water-way.

Since taking up his permanent residence in India from 1916 to the present time a large proportion of his scientific effort has been directed to the utilization of the waste products of human and animal life for the benefit of agriculture. It has

<sup>1</sup> Cf. Ruskin, *Unto This Last*, p. 3 : " We made learned experiments upon pure nitrogen, and have convinced ourselves that it is a very manageable gas : but behold ! the thing which we have practically to deal with is its chloride ; and this, the moment we touch it on our established principles, sends us and our apparatus through the ceiling."

thus been necessary for him to study closely some of the numberless transformations which take place in the journey of nitrogen from the plant in the field through men and animals back to the soil again. In his opinion there are few lines of inquiry to which the chemist can devote himself of more fascinating interest or of greater importance to humanity.

**The Problem of Nitrogen Conservation.** It is evident that the nitrogen we are accustomed to in the air we breathe is something very different from nitrogen in combination with

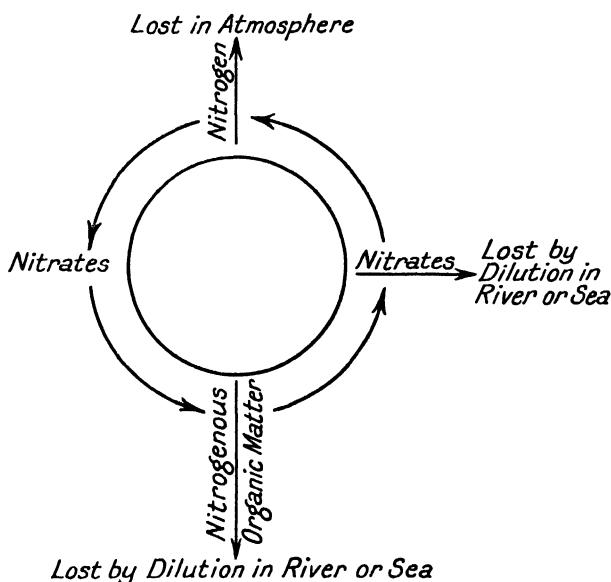


FIG. 1.

oxygen as nitrate, which can serve as food for plants, or from nitrogen in combination with carbon, hydrogen and oxygen with possible smaller quantities of sulphur and phosphorus, when as protein it is the foundational food for men and animals. We thus see how necessary it is to hold nitrogen in some such state of combination and prevent it from becoming inert through its return to the atmosphere. The problem of retaining nitrogen in combination and preventing its return to the free state and of bringing it back into combination, should

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such return to the free state take place, is the problem of *Nitrogen Conservation*.

The conservation of nitrogen may indeed be said to be the most important problem confronting the human race. Without an adequate supply of nitrogen the soil loses its fertility, and food ceases to be produced. Long before this complete exhaustion takes place, however, limited supply will become evident in poverty and stress of competition, which may explode in war.

It may fix our ideas if we visualize the problem for a moment in a simple diagram (Fig. 1). This cycle may quite properly be termed the wheel of life, for it will be seen that if the whole of the nitrogen passes off at the periphery, life will cease. Life is retained in proportion as the nitrogen is retained, and is more intense in proportion as the cycle of changes takes place rapidly or slowly, or as it might be expressed, as the wheel revolves with greater or less rapidity. This appears in practice in the fact that land which is intensively cultivated will support a larger population per acre than where primitive methods prevail. The problem of the conservation of nitrogen will be solved when the whole cycle is so far under control that adequate food supply can be assured for any desired population.

**The Warnings of Liebig and Crookes.** Some seventy years ago Liebig wrote as follows of ancient Rome :

The sewers of the immense metropolis of the Ancient World engulfed in the course of centuries the prosperity of the Roman peasants, and when the fields of the latter would no longer yield the means of feeding her populations, these same sewers devoured the wealth of Sicily, Sardinia, and the fertile lands on the coast of Africa.

Describing mediæval agriculture in England, Prothero writes :

There was little to mitigate, either for man or beast, the horrors of winter scarcity. Nothing is more characteristic of the infancy of farming than the violence of its alterations. On land which was inadequately manured, and on which neither field-turnips nor clovers were known till centuries later, there could be no middle course between the exhaustion of continuous cropping and the rest cure of barrenness.

Considerations such as these, applied to the conditions existing in his time, led to the famous warning of Sir William Crookes in 1898 as to the crying need for nitrogen conservation. Happily this warning has been heeded and modern chemical technology has come to the rescue, and by the expenditure of very considerable physico-chemical energy in the various processes of what is known as nitrogen fixation has succeeded in obtaining nitrogen from the air in simple combinations suitable for use as fertilizer such as ammonium salts, nitrates, calcium cyanamide, etc.

These modern methods of recovery of nitrogen from the air have so developed, especially since the War, that according to the latest statistics there would seem to be little danger of nitrogen famine. In fact there is a possibility of a glut of nitrogen compounds. Nevertheless, the problem is not, even so, entirely solved since it has been found that mineral nitrogen in the form of ammonium compounds and nitrates, or amino compounds such as cyanamide and urea, is not fully adequate to meet the needs of plants. Just as nitrogen in the form of gelatine was long ago found inadequate to supply the requirements of human food, and as all modern researches have indicated, certain accessory substances are necessary to maintain proper nutrition, so in the case of plants it has been found by age-long practical experience that something more than mineral nitrogen is required for healthy crop development. The experience of forty centuries of Chinese agriculture, and of more than half a century of scientific experiment at Rothamsted, has been confirmed by the researches of the Madras Department of Agriculture working in conjunction with the Deficiency Diseases Inquiry under Lieut.-Colonel Sir R. McCarrison, who together have shown that in order to obtain increased crop yields and to secure seed which will give a vigorous crop in the next generation, organic manure is essential. McCarrison has shown by feeding tests that wheat grown on a crop which received cattle manure was 15 per cent. more nutritious than wheat produced from a crop manured with artificials alone. Thus we find that the problem of agriculture and of nitrogen conservation cannot at present be met by the provision of mineral compounds on however vast a scale modern technology can produce them.

In India there is the further handicap of the poverty of the peasant and his lack of scientific knowledge which, even if he can afford mineral fertilizers in quantity, would make it almost certain that much of them would be wasted by inefficient application.

**Warnings from Modern Germany.** The extreme importance of the maintenance of what has come to be termed the "humus capital" of the land is being stressed in the modern literature of scientific agriculture in Germany.

In a review of recent literature on organic manure by Dr. G. Ruschmann, published in July, 1931, in *Biedermann's Central Blatt*, the following statements are made :

*The Humus Question.*—The humus economy [wirtschaft] is not only for Germany but for all civilized European States with their extensive agriculture [Ackerbauwirtschaft] an over-burning question. The evils of one-sided measures for manuring assume continually more obvious forms. However much mineral manures serve to increase the yield for the moment, so much the less can they safely maintain their position. This knowledge is spreading both among scientists and practical men. In spite of constant or increasing use of mineral manures, yields are decreasing.

The increase of soil fertility which is the aim of all modern scientific and practical effort cannot be attained by mineral manures. These by accelerating the breaking down of humus are actually detrimental. Increase of crop by improving the soil properties, and greater returns by addition of plant foods, are two different things, which are often confused. The latter can be effected by mineral manures which act immediately. On the other hand, to build up a good soil is a more lengthy process. While it is relatively simple to maintain the fertility of soil rich in humus, it is difficult in a soil which is mainly mineral to build up the necessary humus.

Arable soil [ackerkrume] is a living thing. The complaints of insufficient or completely negative results with mineral manure are rapidly increasing.

The humus capital of German soil has, according to Lohnis, a value of 30 milliards of Reichs-marks [German Mark currency] although Germany possesses mainly sandy soil. To increase this capital by skill is the important task of both the agricultural and business community. Humus capital puts every other kind of soil wealth on one side. Directly or indirectly all plant and animal life is made possible by the soil humus. To its increase may be systematically employed all those organic materials which at present are virtually wasted. The greatest attention should be devoted to the albuminous or nitrogen-containing organic dejecta and residues of human and animal life which

are admirably suitable for increasing the formation of humus in the soil. Unfortunately we are to-day still far from the general knowledge of what great importance attaches to all organics and the energy contained in them which comes to us through the sun's rays, and which is set free by the decomposition of these substances in the soil.

**The Needs of India.** Some ten years ago the author, as one of the representatives for India of the League of Red Cross Societies, received a questionnaire asking for a brief general statement as to what he considered the most important sanitary problem in the country. His reply was in part as follows :

The great difficulty in India in my view is the poverty and ignorance of the people. Religious prejudice prevents the systematic use of human excreta for manure as in China, and much animal manure, e.g. cow dung, is sold for fuel on account of the dearth of wood and coal, and much nitrogen from oil cake is lost to the country owing to the export of oil seeds, so that the soil is becoming unfertile and unable to sustain life at a high level.

There is consequently little power of resistance to diseases and for the bulk of the population the provision of a more ample and nutritious diet seems to be the most important sanitary problem.

He was confirmed in these views by economists such as Dr. Slater and Mr. Dubey and by agricultural authorities such as Mr. Anstead and Dr. Norris, formerly of the Madras Agricultural Service.

**Indian Crop Yields.** More recently important data have been put forward by Sir Alfred Chatterton in a lecture on the fertilizer problem in India,<sup>1</sup> from which certain outstanding statements may be quoted. The average yield of wheat lands in India is 605 lb. per acre, compared with 1,526 lb. in Japan, 1,530 lb. in Egypt, 1,246 lb. in Canada, and 2,202 lb. in Great Britain.

For rice the figures are as follows :

India	.	.	.	.	.	1,295 lb. per acre
Japan	.	.	.	.	.	3,040 „ „
Egypt	.	.	.	.	.	2,783 „ „

<sup>1</sup> "The Fertilizer Problem in India." A Lecture by Sir Alfred Chatterton, Kt., C.I.E., F.C.G.I., on the 8th April, 1931, at the Central College, Bangalore.

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The yield of cotton per acre is :

India . . . . .	87 lb.
America . . . . .	155 ,,
Japan . . . . .	181 ,,
Egypt . . . . .	371 ,,

While for sugar comparative returns are :

India . . . . .	2,400 lb.
Japan . . . . .	3,340 ,,
Egypt . . . . .	3,378 ,,
Java . . . . .	11,988 ,,
Hawaii . . . . .	18,799 ,,

These typical crops serve to show the low standard of cultivation prevalent in India as compared with other countries, in some of which, such as Egypt, Japan and Java, the system of small holdings also prevails.

It is clear that the prime necessity for India is to ensure a greatly increased return for the cultivation of the soil. Only by more efficient agriculture can the standard of living be raised, and a market be found for the products of those industries which we all desire to see established in this country.

Increased crop production is manifestly conditioned by the amount of plant food applied in the form of fertilizer. The amount thus applied can be to a large extent measured by the ratio of the expenditure on fertilizers to the value of the crop. According to Sir Alfred Chatterton, the value of agricultural produce raised in Great Britain is about £250 million a year, and the expenditure on fertilizers and feeding stuff is roughly £50 million a year, or 20 per cent. of the gross out-turn. In India the equivalent figures are only roughly known, an estimate for the value of crops in an average year being 1,200 crores of Rupees, while the expenditure on the purchase of fertilizers and feeding stuffs is insignificant. In this connection we must not forget the use by the cultivator in India of green manures, but, as will be shown later in this volume, there are great possibilities of improvement by the more scientific utilization of this source of nitrogen and organic matter.

It is true, as Sir Alfred Chatterton points out, that the potential resources of India in respect to the generation of hydro-electric energy are such as to make possible the economic production of nitrogenous fertilizers by the fixation of atmospheric nitrogen, but as has been already indicated, there are limits to the use of such products, and moreover there are possibilities of handling the less spectacular forces of biochemistry to produce nitrogenous fertilizers from materials at present wasted, and also from the air by processes which can be adopted in every town and village in India, with the assistance of a little intelligence and organization without the necessity for capital expenditure or complicated and highly technical machinery and plant.

There is, indeed, a great field for the energies of young chemists in studying and utilizing to the best advantage the sources of nitrogenous fertilizer which lie ready to hand, and the object of this volume will be attained if a stimulus of interest can be given to this important subject which may bear fruit ultimately in the village and the countryside.

**Subject Headings.** It is proposed to deal with the subject under the following heads :

1. The methods and reagents of biochemical research in its application to nitrogen conservation.
2. Sources of organic nitrogen.
3. Building up and breaking down of nitrogenous matter :
  - (a) In the soil,
  - (b) In manure and compost heaps,
  - (c) In sewage tanks and filter beds.
4. The assimilation of nitrogen by plants.
5. Practical application of the foregoing :
  - (a) Making of compost.
  - (b) Purification of sewage.
  - (c) Increase of crop yields.
6. General conclusions and recommendations.

## CHAPTER I

### METHODS AND REAGENTS OF BIOCHEMICAL RESEARCH IN ITS APPLICATION TO NITROGEN CONSERVATION

**Biochemistry a Kinetic Science.** When the student of systematic chemistry whether inorganic or organic takes up the study of biochemistry, he is confronted with a new set of conditions ; thus, in his efforts to make some new combination among the numberless derivatives handled by the organic chemist, he may not infrequently set aside an apparently intractable syrup and return after a short leave to find that it has set into the hoped-for crystalline mass. If on the other hand he should leave a solution in which the living reagents of biochemistry are present, either actually or potentially, he may return to find an overgrown and somewhat offensive agglomeration of living substance. By such experiences he soon learns that biochemistry is essentially a kinetic science, thus differing from the static conditions met with in reactions into which life activity does not enter.

No better illustration could be found of the difference between biochemistry and what may be termed "straight" chemistry than the incident of the croquet party in the English children's classic, *Alice in Wonderland*. There instead of hitting wooden balls with wooden mallets through iron hoops, which remained quietly in their places, Alice had for a mallet a long-necked flamingo who used to look up at her plaintively when she wanted to use it for hitting the ball. The balls, consisting of rolled-up hedgehogs, would uncurl themselves and run away at inconvenient moments. The hoops were formed by soldiers doing "touch toe" exercises, of which they often became tired ; altogether the game was rather difficult and strenuous. Real biochemical research,

however, is similarly concerned with conditions which are constantly changing in response to the vital activities of the particular organism or organisms which are under investigation. When the change ceases the result is death, and the study is no longer the study of biochemistry but of ordinary organic chemistry. Biochemistry, therefore, may be defined as the study of the chemical phenomena which accompany vital action. On the other hand, the simple study of the products of vital action, such as the colouring matters or sugars or alkaloids occurring in plants, belongs to pure organic chemistry. The tracing of the changes by which simple raw materials are built up into these complex end products is the task of the biochemist. The lectures which form the basis of the present book were concerned particularly with the transformations of nitrogen under the influence of vital action, and it is here that we can at once see the extraordinary difference between the methods which obtain in nature's laboratory, and those which are made use of in the modern factory to produce the same results.

**The Methods of Nature and Art Contrasted.** Thus if a dilute solution of carbonate of ammonia is allowed to percolate through a layer of soil containing the organisms necessary for fertility, the ammonia will be found to have oxidized to nitrate without any appreciable rise of temperature. To convert ammonia into nitric acid in the factory, platinum gauze is made use of, heated to  $500^{\circ}\text{C}$ ., or a low red heat.

The contrast is even more striking when we consider the conditions under which nitrogen as it occurs in the inert form in the atmosphere can be made to combine usefully with other elements with the ultimate production of the complex known as protoplasm. Thus we may sow some sweet-pea seeds in a sandy soil practically devoid of nitrogen, and they will germinate and produce lovely blossoms and long climbing stems and leaves and wispy tendrils, which on analysis will contain large percentages of nitrogen which can only have come from the atmosphere. In order to bring atmospheric nitrogen into combination with oxygen in the great works in Norway, temperatures of  $3000^{\circ}$  to  $3500^{\circ}\text{C}$ .

are made use of with an arc of 6500 volts, in order to burn the nitrogen of the air.

To produce cyanamide or nitrolin, calcium carbide is first obtained by heating carbon and lime in an electric furnace employing a current of 130 volts and 20,000 amperes, and the carbide thus formed is held in a current of nitrogen at 1100° C. to produce calcium cyanamide.

In the Haber process where nitrogen and hydrogen are caused to combine to form ammonia, a pressure of 200 atmospheres is employed with an iron catalyst heated to 600° C.

We may well inquire how does nature bring about similar changes by non-violent means.

**Nature's Methods.** A summary answer may be given by saying that nature achieves these results through the employment of vast numbers of living reagents, each bringing to bear efficient and powerful catalysts, and operating on a very extended front, that is to say, utilizing the effect of extended surfaces.

**Cell, Enzyme, Colloid.** We may perhaps define our living reagent as the *cell*, whether separate or conjoined. Our catalysts are the product of the life activity of these cells, and are known as *enzymes*, and our surface is the surface of a jelly or *colloid*.

**The Cell and the Amœba.** Some detailed attention may be devoted to the general characteristics of our living reagent, that is the cell. It is not strictly correct to say that the unit of life is the cell, since phenomena which are clearly characteristic of life are exhibited by masses of protoplasm which are unbounded by the wall which is characteristic of the cell. This simple non-cellular form of life is known as the *amœba*. That it is living and not dead protoplasm is clear from the fact that it exhibits the fundamental properties which distinguish living from non-living matter, viz. *assimilation* and *reproduction*. A crystal grows by accretion, it takes on additional substance and at the same time increases its size, but does not give out anything to its surroundings. The amœba takes in fresh substance and discharges waste. The crystal once formed does not reproduce itself

by giving rise to other crystals by division of its own substance. The amœba is capable, like other living things, of splitting off portions of itself which in turn possess the power of assimilation and reproduction.

A further characteristic of living matter which is presented by the amœba, and to a greater or less extent by all forms of life, is the power of spontaneous movement.

**The Cell.** Turning now to the *cell* which we may perhaps define as the unit of *structural* life, we find that all cells,<sup>1</sup> whether occurring separately or conjoined, that is whether present in unicellular organisms or in polycellular animals or plants, consist each one of a firm cell wall enclosing a cavity containing liquid plasma or sap, throughout which and lining the interior of the cell wall is a semi-fluid mucilaginous reticu-

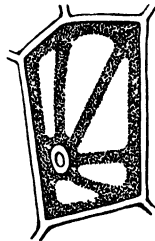


FIG. 2.—Typical Living Cell.

lated substance referred to as protoplasm. This is in a continual state of movement and of chemical change, and in the midst of it is a cell nucleus (Fig. 2).

It is clear that substances entering or leaving the cell must be possessed of certain physical properties if they are to pass through the cell wall, and particularly through the continuous layer of protoplasm which lines the interior of the cell wall, and adheres closely to it. Manifestly in the first place, insoluble matter will not even pass through the cell wall, but not all substances which are apparently in solution

<sup>1</sup> Recent research has shown that just as the unit of matter is no longer the atom but the electron, so the phenomena of life are first manifest in the "gene," an invisible point whose presence and position in the visible "chromosome" can be deduced from its effects on the character of the organism.

can pass quite freely through the layer of protoplasm which lines the cell. It is found, for example, that if certain plant cells are immersed in solutions, e.g. such as potassium nitrate, the protoplasmic layer tends to shrink away from the cell wall owing to the difference in the number of soluble ions within and without the cells and the consequent increase of *osmotic pressure* which forces away the protoplasmic layer from the cell wall.

**Plasmolysis.** This effect is known as *plasmolysis*. By observing the behaviour of selected plant cells in solutions of various concentration we can determine the osmotic pressure in the interior of the cell. This phenomenon is of very great importance in determining the optimum condition of life of practically all living things, thus a fresh water fish if plunged suddenly into salt water will die, but if the salinity of the water is increased gradually, the cells will adjust themselves to the difference in osmotic pressure, and the fish will continue to live under the new conditions. Such instances could be multiplied, and would concern such varied phenomena as the multiplication of bacteria in the soil, or the effect of high altitudes on the human organism.

While under greater or less pressure substances in true solution can find their way into the interior of the cell there to be worked up in Nature's laboratory into substances of increased complexity, it is otherwise with material which, although apparently in solution, is made up of particles or molecules of such a size that they will not pass through the membrane. Such material is said to be in the *colloid* state.

#### COLLOIDS AND CRYSTALLOIDS

Of late years the chemistry of colloids has developed to an extraordinary extent, in fact the study of biochemistry requires, at any rate, some elementary conception of the nature and properties of colloids.

It was first shown by Graham that by appropriate means solutions could be obtained which while devoid of visible particles were incapable of passing unchanged through a parchment membrane. Substances which were soluble and

which would pass while in solution through a parchment membrane Graham termed *crystalloids*. Substances which while soluble, as judged by ordinary physical tests, would yet not pass through a parchment membrane he termed *colloids*. A typical case illustrating the difference between a crystalloid and a colloid is the one selected by Graham, viz. silicate of soda. If a dilute solution of silicate of soda is carefully acidified with hydrochloric acid, no precipitation takes place. If the solution is now placed in a cylindrical vessel, one end of which is closed by a parchment diaphragm, and the whole immersed in clean water which is renewed from time to time, sodium chloride formed by the action of hydrochloric acid on the sodium silicate will diffuse through the parchment and eventually be completely removed. The silicic acid will remain behind in the cylinder. The sodium chloride in this case is the crystalloid and the silicic acid the colloid, the apparatus used in the experiment is known as a dialyser, and the process as *dialysis*.

**Permeable Membranes.** Considerable progress has been made of recent years in the technique of dialysis, membranes of varying permeability being obtained from collodion films of varying thickness and density according to the detail of technique of their preparation.

**Ultramicroscope.** Another line of investigation of colloids makes use of the ultramicroscope, an instrument the design of which is founded upon what is known as the Tyndall phenomenon, which is illustrated by the common observation of the lighting up by a sunbeam of the dust particles or cigarette smoke in the air of a room. The same phenomenon is observed with solutions; a solution practically free from suspended particles allows a beam of light to pass through and remain invisible; on introducing a colloidal substance such as gum mastic into the solution, the path of the beam at once lights up.

The application of this phenomenon to the study of colloidal matter has been the subject of very interesting researches by Zsigmondy and Siedentopf. Fig. 3 clearly illustrates the principle of the ultramicroscope. The solution to be examined is placed in the glass cell at *b* and is strongly illuminated by

a converging beam of light. On observing the lighted-up solution by the microscope at right angles to the path of the beam the colloidal substance present in the solution is visible as brightly illuminated particles. The methods of producing a brilliant converging beam of light and the construction of the observation cell have in practice been improved and rendered more compact and precise, but Fig. 3 sufficiently illustrates the principle employed.

A special condenser has been devised which can be attached to any microscope and which permits solutions to be observed under this strong beam of light.

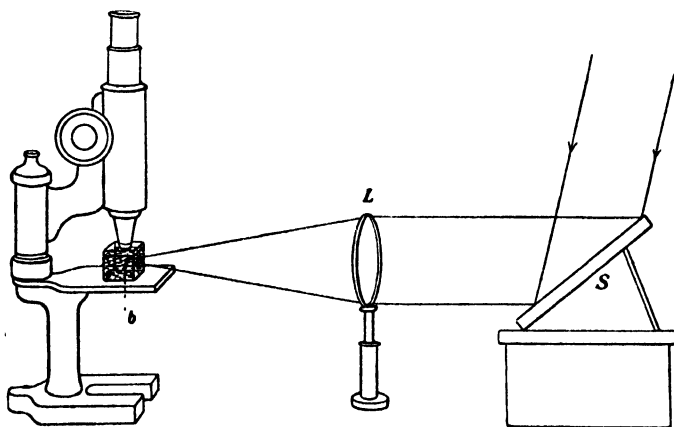


FIG. 3.—The Principle of the Ultramicroscope.

By means of the ultramicroscope particles are rendered visible which are far smaller than any that can be seen under the ordinary microscope. Thus, for example, if an ordinary blood corpuscle be represented by a circle 3 inches in diameter, a particle of colloidal gold at the same magnification would be barely visible as a minute dot, but yet it can be distinguished by means of the ultramicroscope. Colloid particles, therefore, are of sufficient size to be seen under the ultramicroscope. The difference in the size of the particles is probably the reason why certain colloids are described as irreversible and others as reversible. An *irreversible*

colloid is one which having been precipitated from solution cannot readily be brought into solution again or be redispersed, to use the term generally employed. A *reversible* colloid, on the other hand, readily passes into solution if the precipitating agent is removed, e.g. by dialysis, or if its effect is diminished by dilution.

A characteristic example of an irreversible colloid is the soluble silica, already referred to; on addition of hydrochloric acid or saline solution to the aqueous solution of silica, the latter gelatinizes and cannot readily be brought into solution again.

On the other hand certain colloids, notably enzymes, can be precipitated by alcohol from their aqueous solutions but will redissolve in water if the alcohol is removed by filtration. A colloid in solution is generally known as a "sol," when precipitated in a gelatinous or anhydrous form it is known as a "gel."

According to the solvent used, we may speak of a "hydro-sol" or a "hydrogel," or an "alcosol" or an "alcogel."

True colloids conduct electricity very slightly if at all, in fact under the influence of the electric current they move as a whole towards one pole or the other.

The precipitation of one colloid by another has been shown to be connected with the electrical condition of the respective substances. An electropositive colloid will precipitate an electronegative colloid, and vice versa. The condition of a colloidal solution depends indeed on the hydrogen ion concentration of the solution, that is on the electric potential of the solution. There is usually a specific hydrogen ion concentration, or as it is generally expressed a  $P_H$  value at which maximum dispersion or maximum aggregation or precipitation takes place. This factor is of great importance in the precipitation of colloidal matter in water supplies or in sewage by the addition of certain salts such as sulphate of alumina.

The flocculation or deflocculation of clay soils and the consequent effect upon the conditions of biochemical change therein, are controlled by their  $P_H$  value.

Colloids exercise a very low osmotic pressure, and since

this pressure has been shown to be proportional to the number of molecules in a given volume of solution, the molecular weight of a colloid is assumed to be very high. Colloidal particles, therefore, cannot pass directly into or out of the cell. The changes taking place in the cell must therefore consist in a breaking down of colloidal substances, such as starch and albumen, into crystalloidal substances, which escape from the cell, and the building up of complex matter from other crystalloidal substances which find entrance to the cell. Further, it is obvious that these changes must be analogous to those chemical changes which require the least complexity of chemical conditions, that is they must be of the nature of catalysis.

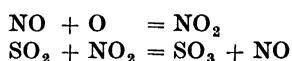
#### CATALYSIS

The characteristic of a catalytic change is that a very small quantity of the catalyst will chemically affect comparatively large quantities of reacting substances without itself apparently suffering any serious change. Thus if a thread of asbestos is soaked in a solution of platinum chloride, and the chlorine driven off by heat, a grey film of finely divided platinum is left on the asbestos. If such a thread of asbestos is covered with platinum in the manner described and then warmed and held in a stream of coal gas escaping, e.g. from an unlit Bunsen burner, the platinized asbestos will glow, and continue to glow so long as the gas passes over it. If the gas after passing over the platinized asbestos were analysed, it would be found to have undergone considerable changes in composition. Little change will be observed in the platinum.

In modern industry a vast number of different catalysts are used, in all these cases the acceleration of the chemical change is largely due to physical causes, the finely divided metal and the extended surfaces on which the reacting substances are brought into intimate union. Chemical agencies may be at work at the same time, e.g. the formation of unstable intermediate compounds, such as oxides or hydrides, but the physical conditions are probably the governing factor.

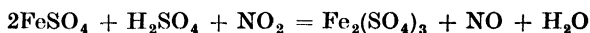
It is otherwise with certain other catalytic agents, notably

the combination of sulphur dioxide with oxygen through the intervention of nitric oxide which is the basis of the "chamber process" for the manufacture of sulphuric acid. Sulphur dioxide does not combine directly with oxygen, but when oxygen is presented to it in combination as nitrogen peroxide it is easily oxidized with simultaneous formation of nitric oxide. Nitric oxide, on the other hand, readily combines with the oxygen of the air, again producing nitrogen peroxide; the changes are expressed in the following simplified equation :



It will thus be seen that in presence of oxygen or equally of air a very small amount of nitric oxide, NO, is capable of converting an indefinite quantity of SO<sub>2</sub> into SO<sub>3</sub>, while itself remaining unchanged at the end of the process.

The catalytic action of nitrous fumes can be readily shown in the laboratory by shaking a solution of ferrous sulphate (green copperas) with a little sodium nitrite and sulphuric acid in a bottle nine-tenths full of air; the green colour of the copperas solution quickly changes to yellow owing to the formation of ferric sulphate according to the following equation :



The NO combines with the O in the air present, to form NO<sub>2</sub> and so continues the reaction.

Whether a catalytic reaction is brought about largely by physical conditions or by purely chemical combination, the general process is essentially the same.

The catalyst acts as an *accelerator* reducing the amount of energy necessary to bring about the reaction. A useful analogy which may be quoted as an illustration of catalytic action is an operation which can be frequently seen in the streets of Manchester when heavy bundles of cotton goods have to be transferred from a warehouse floor on the ground level to a lorry standing at the pavement curb. The effort to throw the bundle from the warehouse across the pavement on to the lorry would be rather too much for one man.

If a second man standing on the pavement can catch the bundle holding it only for an instant and then passing it to the lorry the operation is facilitated. The nitric oxide in the equation just given for the oxidation of sulphur dioxide plays exactly the part of the man on the pavement. In the case of an organic catalyst or enzyme the factor of surface plays a more important part, and instead of simple chemical combination as in the case of nitric oxide, the physical attraction of surface comes into play, giving rise to what is known as *adsorption*, such as occurs when a salt is adsorbed by a flocculent precipitate. The difficulty of washing the precipitate, e.g. of alumina, from adsorbed saline impurity is well known to every student of analytical chemistry. Such adsorption is more intense when precipitation is carried out in a concentrated solution, and it is frequently necessary in consequence to re-dissolve the precipitate and form it again in a more dilute solution before the adsorbed material can be removed.

**Organic Catalysts or Enzymes.** *Organic catalysts or enzymes* are of a colloidal nature and thus tend to adsorb reacting substances from the solution containing them, which is often spoken of as the *substrate*. At the same time the enzymes would appear to be possessed of a certain amount of definite structure since their reaction is specific and is not merely a simple matter of surface.

Whatever the exact and intimate facts of the case may be, and it is necessary to be guided largely by analogy, the result of the reaction is that changes take place in Nature's laboratory, i.e. in the living cell, with great rapidity and within very narrow limits of temperature and hydrogen ion concentration.

While it is possible to bring about these reactions by means of enzymes separated from the cell and which have none of the characteristic properties of living matter, yet these enzymes are, nevertheless, products of life, and the continuous and varied changes which are met with in nature find their full activity only when the cell is alive. While it would lead too far to attempt to define precisely what is meant by vital action it may be stated that the cell can be

looked upon as an energy *transformer* in which the energy, characteristic of living matter, and which may be termed *biotic energy*, is transformed into chemical activity, and eventually into heat, in the cell processes.

While a large number of fermentations are known which can be produced by the action of enzymes, there are others which so far have only been produced by the action of living organisms, i.e. it has not been possible hitherto in such cases to separate the enzyme from the living cell. A notable instance is the case of nitrifying bacteria, which are concerned in oxidizing ammonia to nitric acid. While this change can be brought about by inorganic catalysts, and probably by activated carbon, specific enzymes have not so far been isolated from the micro-organisms concerned.

**Digestive and Respiratory Processes.** It may here be pointed out that the activity of the cell is of a complex nature and in general the living organism is concerned in two distinct modes of activity, that is in maintaining its body substance and in developing energy for growth and reproduction. Thus broadly speaking, in the animal body the processes of digestion are concerned with maintenance of the body substance, the processes of respiration with the maintenance of energy. In both cases the chemical action is probably resolvable ultimately into similar factors though the nature of the products and the energy or heat changes are different in the two cases.

A simple case which illustrates the difference between what may be termed *digestive* and *respiratory* fermentation is afforded by the decomposition of urea in the presence of micro-organisms. The simple fermentative change consists in the transformation of the urea into ammonium carbonate by the addition of two molecules of water as in the following equation :



This change can be brought about by the enzyme *urease*, which is active even when the specific organisms bringing about the change are inactivated by alcohol, and which also occurs in, and can be separated from, the soya bean.

The conversion, therefore, of urea into ammonium carbonate is a comparatively simple catalytic reaction the conditions of which are not difficult to follow. At the same time, however, that the organism concerned with this fermentation is functioning in the manner described, it is also actively using a portion of the nitrogen present to maintain its body substance, and at the same time a corresponding amount of carbon dioxide is produced characteristic of respiratory change. This type of change is much more complex than the simple fermentative activity involved in the hydrolysis of urea, but although its conditions are not fully understood, it is probable that here also we have to do with a chemical change in which intermediate loosely compounded complexes are formed.

The utilization of nitrogen in this way is more characteristic of actual living matter than is the mere breaking down of more complex substances into simpler forms, by simple catalytic activity, nevertheless enzymes have been isolated by Croft Hill, Emil Fischer and others which are capable of bringing about synthetic or anabolic changes as distinct from breaking down or catabolic processes.

**Definition of Fermentative Change.** We may summarize the foregoing general discussion by defining biochemical or fermentative change, as the chemical change produced by the agency of protoplasm or of a secretion prepared from it.

#### NATURE'S REAGENTS

We have now to consider the reagents employed in nature in the environments and conditions under which they operate. The reagents, as we have seen, are enzymes and they are active in the environment of the cell which may be looked upon as Nature's laboratory or the unit of biochemical activity; we thus arrive at the place where we have to consider the organisms which function in the changes which have to be studied in connection with our main subject, the *conservation of nitrogen*. The organisms with which we are chiefly concerned are bacteria, yeasts, moulds, algæ and protozoa; we are also interested in the cellular activity

of higher animals and plants, but by the study of the simpler organisms we can better understand the more complex changes in plants and animals which belong to that portion of physiological chemistry with which the conservator of nitrogen has chiefly to do.

We may conveniently classify various types of cells and organisms which we are to study broadly as follows :

*Organisms which are Monocellular and Monoenzymic.* The majority of bacteria come under this head.

*Organisms which are Monocellular and Polyenzymic.* Under this category are included yeasts and protozoa.

*Polycellular and Polyenzymic Organisms.* Under this head are comprised moulds.

We shall also encounter numerous forms of *algæ*, and a somewhat indefinite class of organisms, intermediate between bacteria and fungi which are known as *actinomyces*. The more highly organized plants and animals may be termed polycellular and monoenzymic, in the sense that different cells exercise quite different functions, and secrete entirely different enzymes, the whole organism being, as it were, a controlled community whose members are exercising different functions working in harmony one with another. A simple illustration of what is meant by this description is afforded by an experiment which can be made on any dog and to which no objection can be taken either by the dog or his friends. If a dog is given a piece of toast he will chew it carefully and at some length before he swallows it ; if he is given a piece of meat, on the other hand, he will swallow it instantly without mastication. The dog presumably has no knowledge of biochemistry, but in fact he behaves in accordance with its laws, since the enzyme which dissolves the starch and carbohydrate of the toast is contained in his saliva, while the *pepsin* which breaks down the albuminoid of the meat is particularly active in his stomach, so much so that he has found preliminary mastication to be unnecessary.

## DESCRIPTIVE NOTES ON TYPICAL MICRO-ORGANISMS

After the foregoing general introduction we may now study in particular the micro-organisms with which we are specially concerned, and their conditions of life and development, taking each in turn.

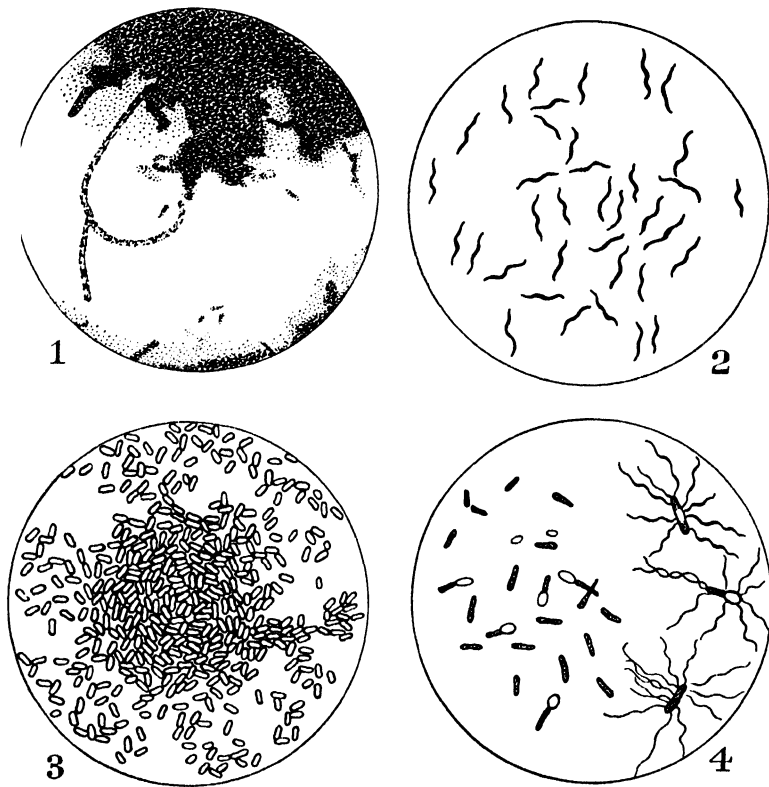


FIG. 4.—Typical Forms of Bacteria.

1. *Beggiatoa* (drawn from original photomicrograph by E. M. Mumford).
2. *Spirochaeta*.
3. *Bacilli*.
4. Spore-forming and motile bacteria, showing spores and flagella.

**Bacteria.** These are the lowest forms of vegetable life. Under a high-power microscope they appear as minute round dots or threads. They multiply either by splitting into two (that is, by fission or cell division), or by production

of small protuberances which separate eventually from the main organism and develop into fresh organisms similar to the parent organism ; this method of reproduction is known as *spore formation*. Bacteria are colourless, i.e. they contain no chlorophyll, they all possess an envelope or capsule consisting probably of cellulose or allied substances. For these various reasons they are classed among the fission fungi and from their method of reproduction are known as *schizomycetes*.

They are further divided according to their main differences into the following subdivisions :

1. Coccaceæ, round cells.
  2. Bacteriaceæ, rods and threads.
  3. Leptotricheæ
  4. Cladotricheæ
- } Higher Bacteria.

The round cells of the cocci and thread-like forms of the bacteria are exceedingly small, how small may be judged from the fact that if the largest single bacterial cell were magnified to the size of a man and the man magnified in proportion, he would be large enough to reach from Bombay to Calcutta. Among the cocci and bacteria those forms which transform urea into ammonium carbonate, and those which oxidize ammonia to nitrous and nitric acid, are of special importance in connection with our subject. The higher bacteria are distinguished by their growth in masses of long threads ; such organisms are characteristic especially of sewage polluted streams. In such a stream, especially if the pollution is fairly fresh and if it contains carbohydrates as, e.g., the waste from beet sugar factories, one may see the long waving plumes of *sphærotilus natans* which has attached itself to stones in the bed of the stream and whose threads wave with the movement of the water. If the pollution is less recent and putrefaction has begun to set in, instead of *Sphærotilus* may be seen the sulphur organism, *Beggiatoa*, whose threads when examined under the microscope are found to contain minute granules of sulphur, giving rise to unpleasant-smelling products should the growths decay.

In water supplies containing iron in solution either as

ferrous bicarbonate or in combination with some organic acid there may often occur luxuriant growths of numerous types of higher bacteria which have the property of oxidizing the soluble compounds of iron and of causing simultaneous precipitation of the separated ferric hydrate as a coating on the gelatinous sheath of the organism. Certain simple bacteria have also this property of precipitating oxide of iron, and it is probable that some of the great iron deposits in northern Europe owe their origin to such bacterial activity. One of these organisms was found to be present in some old colliery workings near Manchester, and was investigated by E. Moore Mumford under the writer's direction. In conjunction with traces of iron salts and in presence of artificial aeration it was employed to bring about clarification of sewage and so played an important part in the development of what afterwards came to be known as the Activated Sludge Process, which will be considered later as a means of nitrogen conservation.

All the various types of bacteria above mentioned are thus concerned in the changes which we have to study.

**Yeasts.** These are closely allied to the bacteria, differing mainly in size and shape of the cell, and their method of reproduction, which normally consists in the formation of small daughter cells or buds which are extruded from the parent cell in a process known as budding. Under certain conditions, however, yeasts are propagated from spores, and the various species of yeasts can be to a large extent differentiated by the character of their spore formation.

Speaking generally we may say that yeasts and spore-forming bacteria tend to form spores when there is a limited food supply, thus yeasts can be caused to spore by spreading a film of cells on the surface of a block of plaster of Paris kept moist by placing it in a shallow dish of water. Under these conditions on examining the growth after a day or so, sporing will be found to have taken place. In the case of spore-forming bacteria the culture is generally allowed to develop until all the food supply present is exhausted, after which spores will generally be found in the deposit present in the culture tube.

The importance of spore formation lies in the greater resist-

ance which these offer to adverse conditions so that while it may often happen that adult organisms are destroyed under certain adverse conditions the spores retain their vitality and become active again when conditions are more favourable. There is an analogy here to hibernating animals and

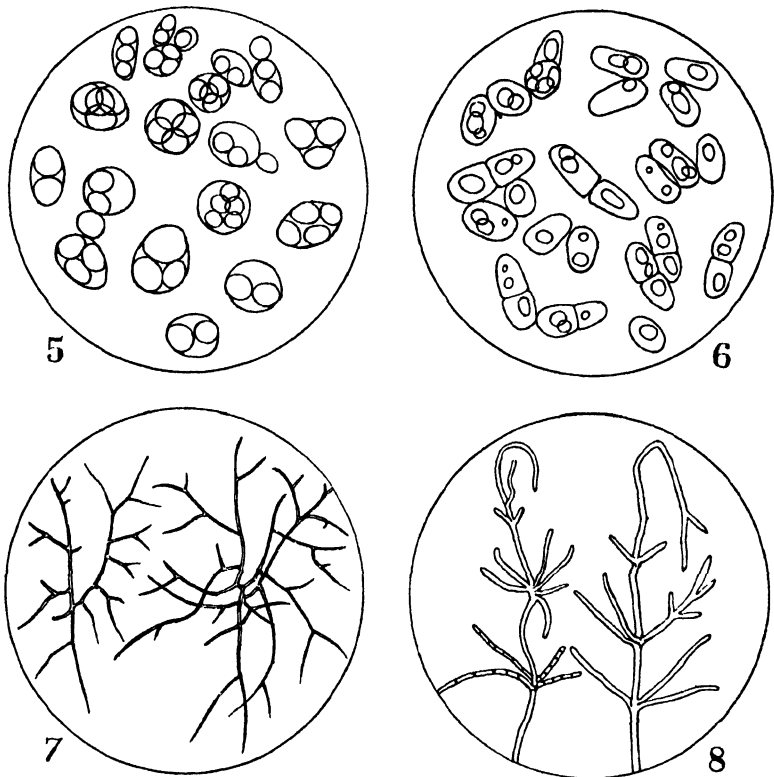


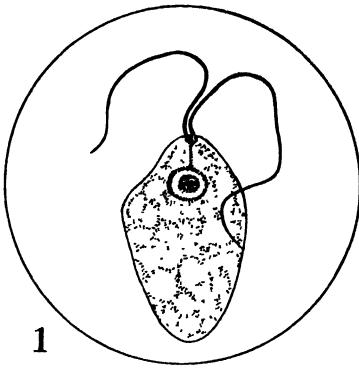
FIG. 5.—Yeasts and Actinomyces.

5 and 6. Yeasts showing spores and buds.

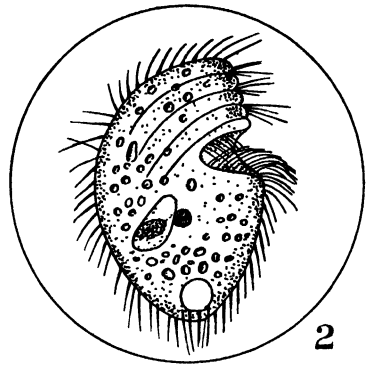
7 and 8. *Actinomyces* showing mycellal development.

resting seeds showing that the broad phenomena of life retain their characteristics throughout nature.

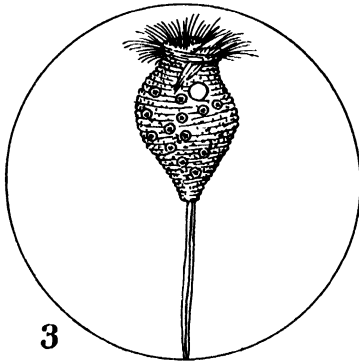
**Moulds.** These are more highly organized than the bacteria or yeasts, and they are sporing organisms; the spores or conidia give rise to long threads of cylindrical cells



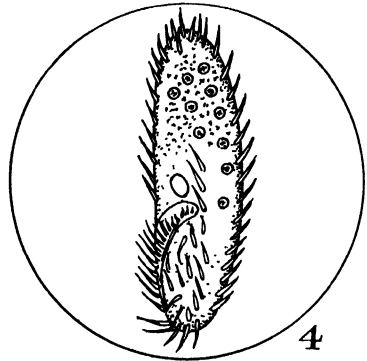
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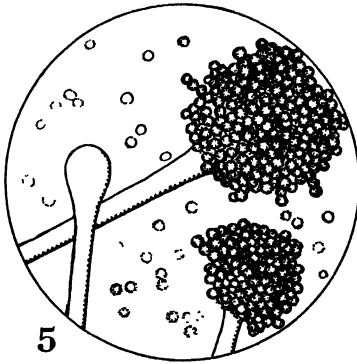
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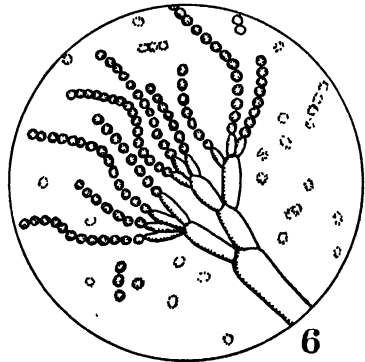
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FIG. 6.—Protozoa and Moulds.

1-4. PROTOZOA.

1. *Naegleria*.

2. *Colpoda*.

3. *Vorticella*.

4. *Gonostomum*.

5. *Aspergillus*.

5-6. MOULDS.

6. *Penicillium*.

forming a network known as mycelium. The individual threads are known as *hyphæ*. From these organs the moulds derive their general name of *hyphomycetes*.

A mould which at first has a fine thread-like appearance on further growth will be seen to be covered with minute dots which are often darker than the mycelium ; these on microscopical examination will be found to be clusters of spores or conidia ; in the case, e.g., of *aspergillus niger* they are black and in the case of *penicillium* they are green. According to the form of the hyphæ, the moulds are divided into four divisions, viz. :

1. Mucorineæ.
2. Aspergillinæ.
3. Pencilliacææ.
4. Oidaceæ.

1. *Mucor* is distinguished by richly branched mycelium with large club-like brownish-coloured sporangia.

2. *Aspergillus* as distinguished from *mucor* has a septate mycelium, i.e. the threads are divided into sections by septa or partitions though the conidia occur in club-like masses.

3. *Penicillium* is distinguished by a septate mycelium and brush-shaped as distinguished from club-like conidia-phores.

4. *Oidium*. The general shape of *oidium* is quite different from the foregoing, the mycelium, actually consisting of elongated *conidia*, being known then as *oidia*. *Oidium* would appear to be intermediate between true fungi and *actinomyces*.

Researches by Delepine have shown that a typical mould, e.g. *Aspergillus niger*, can thrive on a great variety of substances ; consequently we may properly define moulds as polycellular polyenzymic organisms.

**Actinomyces.** These are a group of organisms intermediate between bacteria and fungi. In their appearance they are difficult to distinguish from bacteria owing to the readiness with which the fine mycelia break up. The genus is characterized by the formation of a monocellular mycelium composed of hyphæ, which show true branching like that of the higher fungi. The aerial mycelium breaks up into

short fragments which resemble bacterial rods. They take an active part in the decomposition of organic matter in the soil.

**Algæ.** These are chlorophyll-containing forms of microscopic plant life. Together with certain groups of bacteria termed *autotrophic* they are the only micro-organisms in the soil that can synthesize organic matter from inorganic material. The autotrophic bacteria obtain their energy chemosynthetically, using inorganic substances as a source of energy, thus the nitrifying organisms can thrive in presence only of salts of ammonia and phosphoric acid. The algæ obtain their energy photosynthetically, using the energy of the sun's rays with which, for example, to utilize the carbon from carbon dioxide.

The algæ are represented in the soil by three large groups :

Cyanophyceæ.

Chlorophyceæ.

Bacillaraceæ.

The first are blue, green with violet, or brown in colour. The second grass green or yellow green, the third golden brown according to the particular combination of chlorophyll and related colouring matters present. They are essentially monocellular organisms and occur in numberless and varied beautiful forms.

**Protozoa.** These organisms are classified as coming under the animal kingdom, and they form numerically the most abundant group of the animal population of the soil, as many as 15,000 species being estimated as known; they are unicellular organisms varying in size from a few microns to 4 or 5 centimetres. They may also form colonies. Although they are essentially unicellular organisms, the single cell highly differentiated in structure, they are classified on the basis of locomotion into :

1. *Sarcodinia* or *Rhizopoda*, motility by means of pseudopodia, i.e. extensions, usually temporary, of the cell body.
2. *Mastigophora* or *Flagellata*, motility by means of flagella (whip at end).

3. *Ciliata* or *Infusoria*, motility by means of numerous cilia or short hair-like processes, present during the entire existence of the protozoa or during the embryonic stage only.

Most protozoa are free swimming, but some are attached by rigid or flexible stalks or pedicles. Protozoa are many of them carnivorous, larger forms in many cases actually devouring smaller protozoa, and quite generally feeding on bacteria. In absence of necessary food supply protozoa tend to encyst themselves, the cysts being usually small circular disc-like bodies.

**Worms, etc.** Besides the foregoing the organisms concerned with the conservation of nitrogen in the soil include numerous species of worms and insect larvæ. The study of these larger organisms leads to the problems of biology and physiology.

To the biochemist these various micro-organisms are living reagents and he is concerned with the chemical changes they effect.

#### CONDITIONS OF BACTERIAL ACTIVITY

In the earlier days of the study of bacteriology it was laid down by Pasteur that for each fermentation there was one corresponding organism ; we know now that this is only true of strictly monoenzymic organisms, the number of which is becoming fewer as their reactions are more carefully studied. By careful changes of environment, however, it is possible to bring into activity for practical purposes only one of the possible reactions which any given organism can bring about, thus in the case of *Aspergillus niger*, referred to above, if grown in a medium consisting largely of cane sugar, it will secrete *invertase*, and the medium can be used as an important source of this enzyme. On the other hand, if the same mould is grown on fat it will secrete the fat-splitting enzyme *lipase*. This has its analogy in the experience of travellers in the Arctic regions who under those conditions are able to consume quantities of fat which would be nauseating in a warmer climate.

**Occurrence of Micro-organisms.** All the above-mentioned classes of organisms, bacteria, yeasts, moulds,

## 32 APPLICATION OF BIOCHEMICAL RESEARCH

actinomyces, algæ and protozoa occur very widely distributed in nature. Bacteria, yeasts and moulds being, in a spore condition at any rate, microscopic in size, are easily carried by wind and in dust. Encysted forms of protozoa can also be carried in this way and thus attach themselves to vegetation or make their appearance in water supplies.

All micro-organisms are most abundant where there is the needful food supply. It is a matter of common knowledge that meat goes bad if long exposed to the air and jam if uncovered develops mould, that milk becomes sour, that sewage or excretal matter becomes offensive if allowed to accumulate. These changes are due to micro-organisms either originally present in the decomposing substance, or carried in air and deposited on substances capable of putrefactive change which themselves thus become sources of infection. The presence of bacteria or moulds in the air can be demonstrated by exposing a slice of potato for some time in a room. In the course of 48 hours or so small spots or centres of growth will appear, which can be recognized as colonies of bacteria or as moulds by methods shortly to be described.

Certain organisms are capable of producing chemical changes in the bodies of higher living organisms, and have been found to accompany the development of specific diseases; such organisms are termed *pathogenic*. Other organisms perform exceedingly useful functions. It is scarcely necessary to refer to the technical importance of yeasts in the brewing and baking industries. Special varieties of bacteria are concerned in the production of vinegar and the ripening of cheese or are useful at certain stages in the manufacture of leather in the tannery. The harmless disposal of refuse matter from men and animals is effected, largely by the activity of bacteria, and the processes of agriculture are increasingly found to depend upon the activity of the organisms in the soil; they are therefore well described by Percy Frankland as "our secret friends and foes."

In order to make the most efficient use of our allies among the population of the soil, we have to study the conditions under which they can best thrive or which are detrimental

to those organisms which we may term enemies. The following conditions are favourable to the development of micro-organisms :

Moderate warmth.

Moisture.

Organic fermentable matter.

Darkness.

Absence of Antiseptics.

The opposite of these conditions, viz. extremes of heat or cold, dryness, absence of organic fermentable matter, light and the presence of antiseptics, are all conditions unfavourable to the development of micro-organisms.

**Sterility.** The first lesson which the student of biochemistry in its relationship to micro-organisms has to learn is the acquirement of a strict sense of sterility. In the early days of bacteriological research there was a prolonged controversy on what is known as abiogenesis or spontaneous generation, that is the production of living forms from materials in which life was not originally present. Our space does not permit a detailed history of this subject, but it is sufficient to say that all experiments which have been brought forward in support of spontaneous generation have been found to be due to faulty technique, that is a failure to achieve complete sterility in the materials under investigation. If, therefore, a student finds an unexpected growth in his culture medium, it is not to be concluded that he has been favoured by the discovery of a new form of life, but rather that his sense of sterility has not been adequately cultivated.

That such a sense of sterility is unfortunately not possessed by many of our sanitary authorities is obvious from the quaint methods not infrequently used to allay the apprehensions of the public.

Actually it is by no means easy to render any medium really sterile, the reason, e.g., why meat "goes bad" near the bone is because the heat of cooking has not completely penetrated to that point.

Investigations in the Biochemical Department of the Indian Institute of Science showed that the ordinary methods

for cooking rice were by no means effective in producing a sterile pudding. The spores of the acetone bacillus which medical authorities believe to be associated with the symptoms of diabetes, can only be destroyed by heating for an appreciable period under at least 10 lb. pressure.

It is important, therefore, carefully to study the methods which are available for ensuring the sterility of the media to be employed in growing the organisms whose actions we desire to study. The methods for securing sterility comprise dry heat, moist heat and steam under pressure.

For *dry heat*, a hot-air sterilizer is used which is virtually a simple box of sheet copper heated by a gas flame to any desired temperature.

Sterilization by *moist heat* is easily effected by simply heating in a steam bath. By this method, however, it is not always possible to attain a temperature sufficiently high to destroy resistant spores, consequently it is customary when sterilizing in this way to repeat the process two or three times at 24 or 48 hours interval. In this way any spores which may have been left alive have time to develop into adult organisms which are killed in the next heating.

If *steam under pressure* is made use of so that a higher temperature is attained, sterilization may be effected in one operation. About 20 minutes' exposure to steam under 30 lb. pressure is sufficient to sterilize most materials, though certain exceptions may occur in practice.

For sterilization under pressure vessels known as *autoclaves* which are constructed to withstand high pressure are made use of. It is convenient in a bacteriological laboratory to have a number of autoclaves constructed to resist different pressures suited to the particular problem in sterilization which has to be dealt with.

Having learned the necessary conditions of sterilization and the methods of attaining them, the biochemist is now able to prepare media suitable for the particular micro-organism which he desires to study. The principles governing the preparation of such media may be illustrated by reference to certain typical culture media which are used in the investigations with which we are specially concerned.

Before describing these media in detail a short allusion to the history of bacteriological methods will be of value.

For the purpose of studying the precise chemical changes effected by a single organism, it is necessary to obtain it in pure culture in the laboratory, i.e. free from any admixture of any other organisms. The earliest method for accom-

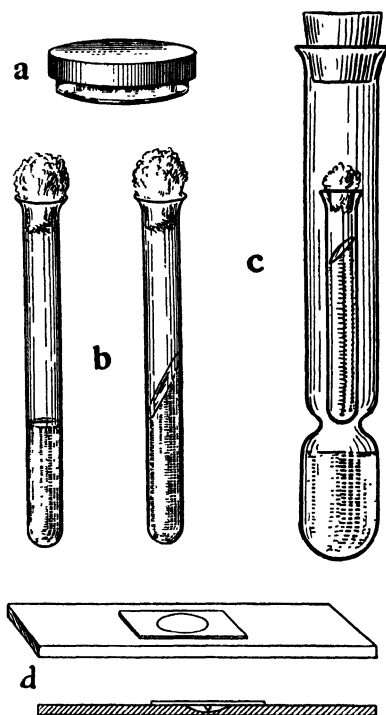


FIG. 7.—Apparatus for Bacteriological Culture.

plishing this, such as was used by Pasteur and Lister, was the method of *dilution*. A small portion of the solution containing the mixture of organisms was transferred to a second portion of the same solution rendered sterile by heat, and after development of the organisms a small portion of this solution was again transplanted and so on until a growth was obtained consisting only of one species of organ-

ism arrived at through a process of natural selection. Such a method is exceedingly tedious, but it is surprising what great advances in knowledge were made by this means. The method of *plate culture* described by Koch in 1881 is much more rapid and certain. Koch introduced a solution containing bacteria into a mixture of suitable nutritive substances thickened with gelatine, the mixture being kept at a temperature slightly above the melting-point of gelatine. On pouring the gelatine culture medium on to a plate and allowing the gelatine to set, wherever a micro-organism was present it developed *in situ*, forming a small opaque centre of growth or *colony*, which could be picked out and transferred to a similar gelatine culture medium and if necessary replated until only one species of organism was found to be present upon the gelatine plate. The form of plate now generally used is known from its inventor as a Petri dish, and consists of two shallow dishes fitting into one another, the larger serving as cover for the smaller, into which the gelatine is poured (Fig. 7a).

Different culture media have been found to be necessary for different organisms, but all require nitrogen in some form together with certain salts, especially phosphates.

**Direct Method of Culture.** Although the method of pure culture has added enormously to our accurate knowledge of the functions and reactions of innumerable special organisms, yet the method contains a fallacy when its results are applied to the study of the biochemical phenomena actually occurring in the soil. It is clear that the conditions obtaining in a pure culture in artificial media are not comparable with those of the free natural existence of any type of bacteria. The pure culture eliminates the struggle for existence. These considerations have been brought forward recently by Winogradsky, whose fundamental work on the nitrifying bacteria is esteemed by all soil biochemists. Winogradsky points out that bacteria as they have to live in the soil and bacteria in pure culture are analogous to wild and cultivated plants, and when it is considered that one year of bacterial life is equivalent to one or more centuries in the life of any phanerogam it is obvious that we must not

apply ideas derived from pure cultures directly to the conditions met with in the soil.

For this reason Winogradsky has brought forward what he terms a direct method of culture which aims at studying bacterial phenomena under conditions approaching those which occur in nature. Briefly, one gram of soil is shaken with successive lots of 3 c.c. of water, and the washings centrifuged yielding two suspensions and three deposits. These are fixed on slides with agar, if necessary, stained with erythrosin, and washed and fixed with absolute alcohol. So-called auxiliary cultures are made by inoculating minute particles of the deposits or suspensions into various media, thus by inoculating the earth into peptone, growth of proteolytic organisms may be obtained, which can be further grown on auxiliary plates. Similarly, in media containing urea, starch, mannose, nitrate, etc., the organisms affecting these substances tend to develop to the detriment of others.

These observations show that it is not the inherent qualities of a bacterium such as are exhibited in pure culture, which determine its rôle in the soil, but it is the struggle of the cells with unfavourable conditions which really governs the distribution of bacterial activity.

This new method is an attempt, in fact, to introduce a dynamic micro-biology and bears a similar relation to orthodox pure culture methods that these bear to the static conceptions of pure non-vital chemistry.

We may now consider in some detail the preparation of certain typical media used in the study of pure cultures of soil bacteria.

#### CULTURE MEDIA FOR BACTERIA

The basis of most media suitable for cultivating bacteria is broth or bouillon. The following formula has been found satisfactory as a general base :

Liebig's Meat Extract (Lemco).	.	.	9	grams
Peptone . . . . .	.	.	9	..
Sodium chloride . . . . .	.	.	4½	..
Distilled water. . . . .	.	.	900	..

This broth can be thickened with gelatine or with agar

(a Japanese product made from a species of marine alga). For work in hot climates or for organisms which grow best at a high temperature agar is preferable to gelatine. For a gelatine medium some 10 to 15 per cent. of gelatine is required to produce a stable jelly, whereas 2 per cent. or so of agar is generally sufficient. It should be remembered that gelatine is rich in nitrogen while agar is a carbohydrate.

To prepare nutrient gelatine, meat extract, peptone, salt and water are boiled for  $\frac{1}{4}$  hour and gelatine gradually added to the hot solution. The whole is allowed to cool (to 50° C. approx.) and neutralized with about 30 c.c. of a 4 per cent. solution of caustic soda (NaOH).

The white of an egg is mixed with an equal volume of water and added to the neutralized liquid. The mixture is placed in the steam bath for 1 hour and 8.5 grams soda crystals added.

For exact work the reaction of the medium should be brought to a definite  $P_H$  value depending on the characteristics of the organism to be studied. For this purpose a given volume of the approximately neutralized medium is titrated either with acid or alkali in presence of the indicator suited to the range of  $P_H$  required. The bulk of the medium is adjusted to the required  $P_H$  in accordance with the result of the titration.

After a further 40 minutes in the steam bath the liquid is filtered through a hot filter funnel.

The melted and filtered medium is carefully poured, preferably from a separating funnel, into a series of sterile test-tubes; about 10 c.c. are added to each test-tube, care being taken not to allow any medium to run down the sides. The test-tubes are plugged with cotton-wool, placed in wire cages and sterilized in the manner previously indicated. The medium should remain perfectly clear after sterilization and the tubes are then ready for use. (See Fig. 7*b*, p. 35.)

Agar medium is prepared in a similar way except that owing to its greater setting power it has to be filtered at a high temperature; this is generally accomplished by placing the whole filter funnel and subjacent flask in a large autoclave.

It may be noted that it is frequently necessary thoroughly to soak and wash agar free from adherent impurities. For work at specially high temperatures percentages of dry agar possibly up to 5 per cent. may be requisite.

To these "foundational" media, as they may be termed, various suitable additions may be made when special organisms are being investigated. Thus starch or sugars may be added, when organisms concerned with the breaking down of carbohydrates have to be studied, potassium nitrate when denitrifying bacteria are under observation and so on.

Certain organisms of great importance in the study of nitrogen conservation, namely those concerned with nitrification, i.e. the oxidation of ammonia to nitric acid, do not grow freely on ordinary nutrient gelatine, and a method was devised by Kühne and by Percy Frankland, in which gelatinous silica is used instead of gelatine, the medium being entirely free from organic matter.

The method of preparation is as follows :

Two solutions of the following composition are prepared.

(a) Ammonium sulphate . . . . .	0.4 gram.
Magnesium . . . . .	0.05 "
Calcium chloride . . . . .	trace
Distilled water . . . . .	50 c.c.
(b) Potassium phosphate . . . . .	0.1 gram.
Sodium carbonate . . . . .	0.75 "
Distilled water . . . . .	50 c.c.

These two solutions are rendered sterile and then mixed.

A sterile solution of dialysed silicic acid is now prepared as follows :

A solution of potassium or sodium silicate (1.05 to 1.06 sp. gr.) is poured into an equal volume of dilute hydrochloric acid (1.1 sp. gr.); the mixture is then placed in a dialyser the outside of which is kept surrounded with running water during the first day and subsequently with distilled water which is frequently changed until this yields no trace of turbidity with silver nitrate, thus showing the whole of the chlorides to have been extracted. The contents of the dia-

lyser, if the solution of alkaline silicate originally employed is not too strong, will be quite clear.

This liquid is then poured into a flask and concentrated by boiling until it is of such strength that it is found that on cooling a little of the solution and mixing it with  $\frac{1}{3}$  of its volume of the above mixed alkaline solution it readily gelatinizes on standing. When the solution of silicic acid is found to give this result it is cooled and  $\frac{1}{3}$  to  $\frac{1}{2}$  of its volume of the mixed alkaline solutions (*a*) and (*b*) are added, the solution is well mixed and at once poured into Petri dishes or flat-bottomed flasks. The medium should gelatinize in 5 to 15 minutes. The above method with possibly slight modifications depending on actual experience is the safest on the whole to adopt.

It is, however, possible to avoid the trouble of dialysing by mixing equivalent solutions of sodium silicate and hydrochloric acid directly in a Petri dish when a layer of gelatinous silica separates out. This can be thoroughly washed, and the necessary saline solution poured over the surface.

**Anaerobic Bacteria.** A certain number of bacteria are found to develop in absence of air; such organisms are classed as anaerobic in distinction to aerobic organisms which thrive in presence of oxygen or air. In order to cultivate such bacteria it is necessary to remove the oxygen from above the medium. This can be done most simply by enclosing the culture tube in a larger tube or receptacle containing alkaline pyrogallate, which has the property of rapidly absorbing oxygen. (See Fig. 7*c*, p. 35.)

An even simpler method is to fill the tube nearly to the top with medium and after inoculation to fill up the remaining space with vaseline.

In the case of anaerobic cultures in Petri dishes, it is customary to place these in an ordinary vacuum desiccator or specially constructed glass cylinder with a false bottom beneath which there is a layer of alkaline pyrogallate, the greater part of the air is removed by a vacuum pump and any remaining traces of oxygen are absorbed by the pyrogallate.

## CULTURE MEDIA FOR MICRO-ORGANISMS OTHER THAN BACTERIA

**Yeasts.** While most yeasts will grow on the same media which are utilized for bacteria, they grow more freely when supplied with nutriment specially suited to them. In this case instead of bouillon boiled hot extract of malt or wort should be diluted with water to a specific gravity of about 1050. The wort must be filtered until it is quite bright and should remain free from deposit after sterilization. Wort gelatine and wort agar are prepared in a similar manner to ordinary nutrient media, using wort instead of bouillon as a basis.

It is convenient where possible to obtain wort ready-made from a brewery. Where this cannot be arranged malt extract must be prepared by allowing a sufficient quantity of barley grains to sprout by spreading them on a surface of moist sand, and the growth stopped by heat as soon as the rootlets are about  $\frac{1}{4}$  inch long. The dried malt can then be ground and extracted with hot water to the necessary concentration.

**Actinomyces.** These will grow on ordinary culture media, special additions being made where required for purposes of differentiation.

**Moulds.** Moulds will also grow on nearly all the media so far considered, but the solution specially suited for their development is known as Raulin's solution. It is prepared as follows :

Water . . . . .	1,500 grams.
Cane sugar . . . . .	70 "
Tartaric acid . . . . .	4 "
Ammonium phosphate . . . . .	0.60 "
Magnesium carbonate . . . . .	0.40 "
Ammonium sulphate . . . . .	0.25 "
Zinc sulphate . . . . .	0.07 "
Ferrous sulphate . . . . .	0.07 "
Potassium silicate . . . . .	0.07 "

**Algæ.** The presence of algæ in the soil is readily demonstrated by moistening a small portion of soil with water and exposing to light in a Petri dish. By adding instead of ordinary water various saline solutions some differentiation in the growth of various species will be obtained. A

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pure culture can eventually be isolated by streaking out the crude material on the surface of an agar plate. The following two media have been largely used in the investigation of algæ :

Bristol's Solution.		Detmer's Solution.	
	Gram		Gram
NaNO <sub>3</sub> . . . . .	0.5	Ca(NO <sub>3</sub> ) <sub>2</sub> . . . . .	1.0
KH <sub>2</sub> PO <sub>4</sub> . . . . .	0.5	KH <sub>2</sub> PO <sub>4</sub> . . . . .	0.25
MgSO <sub>4</sub> , 7H <sub>2</sub> O . . . . .	0.15	KCl . . . . .	0.25
CaCl <sub>2</sub> . . . . .	0.05	MgSO <sub>4</sub> , 7H <sub>2</sub> O . . . . .	0.25
NaCl . . . . .	0.05	Tap water . . . . .	1,000 c.c.
FeCl <sub>3</sub> . . . . .	0.005		
Distilled water . . . . .	1,000 c.c.		

Detmer's medium is diluted to one-third of its strength and 0.01 per cent. FeCl<sub>3</sub> is added to the diluted solution.

**Protozoa.** The cultivation of protozoa and their observation in pure culture is a somewhat difficult and special technique since the majority of protozoa feed on bacteria and consequently need the presence of these in addition to various synthetic media. Thus infusions of hay and straw or horse-dung or even Lemco may be used. It is necessary frequently to transfer the culture to prevent the accumulation of injurious products, or of too rapid development of bacteria.

**Higher Organisms.** Apart from bacteria and other classes of micro-organisms which have been referred to, the changes which confront the biochemist are also influenced by larger organisms, especially various types of worms, small crustaceæ, arachnida, including mites, ticks and spiders, centipedes (myriapoda), termites and insects. Molluscs are also present in the soil and the chemical changes are also affected by the activity of mice and moles, and other burrowing animals.

The study of the latter classes of living things is the special task of the botanist and biologist rather than of the biochemist. The latter, however, should be able to perform the manipulative operations necessary for the study of the changes brought about by bacteria, yeasts and moulds including actinomyces whose reactions compose by far the greater number of those met with in the study of nitrogen conservation.

## EXAMINATION OF MICRO-ORGANISMS

It will be useful, therefore, briefly to describe the methods used for the isolation of these organisms and their examination under the microscope.

In transferring small quantities of material from one medium to another for the purpose of inoculation, short lengths of platinum wire mounted on glass rods are used. For minute quantities of liquid a wire with a small loop at the end is employed. With a little care loops can be made which will take up almost exactly a milligram, i.e. 0.001 c.c. of liquid. For transferring colonies of bacteria a small hook is made at the end of the wire. To inoculate a test-tube of gelatine or agar the cotton-wool plug is first sterilized by singeing in a Bunsen flame, is removed by a pair of forceps similarly sterilized and held between the first and second fingers of the left hand, while the test-tube is held between the first finger and thumb. The platinum wire after having been sterilized by passing through the flame is dipped into the solution to be examined and then inserted into the gelatine to about half the depth, quickly withdrawn, and the plug of cotton-wool again singed and replaced (Fig. 8a). Such a culture is known as a stab culture, and is chiefly used when inoculating from a pure cultivation. If the culture is a mixed one, the gelatine or agar medium is melted before removing the cotton-wool plug, by allowing the tube to stand for a few minutes in a beaker of water which has been heated to a temperature some 10 or 20 degrees above the melting-point of the medium. After inoculating and thorough mixing of the culture with the melted medium the latter is poured into a sterile Petri dish.

The medium is allowed to set in the Petri dish which is then placed in a moist chamber; the latter is a similar glass vessel of a much larger size in which some moist blotting-paper or a small Petri dish of water has been placed.

In order to accelerate the growth of organisms on the medium in the Petri dish, it may be necessary to place the latter in an incubator.

The incubator consists essentially of a water-jacketed

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chamber, the temperature of which is kept constant either by a gas flame regulated by a thermostat, or better, by controlled electric heating (Fig. 9). Modern electric incubators are designed to give very steady temperatures irrespective of slight fluctuations of current.

If the electric current can be relied on to be reasonably constant, incubators sufficiently accurate for preliminary investigations can be made by inserting an ordinary electric-

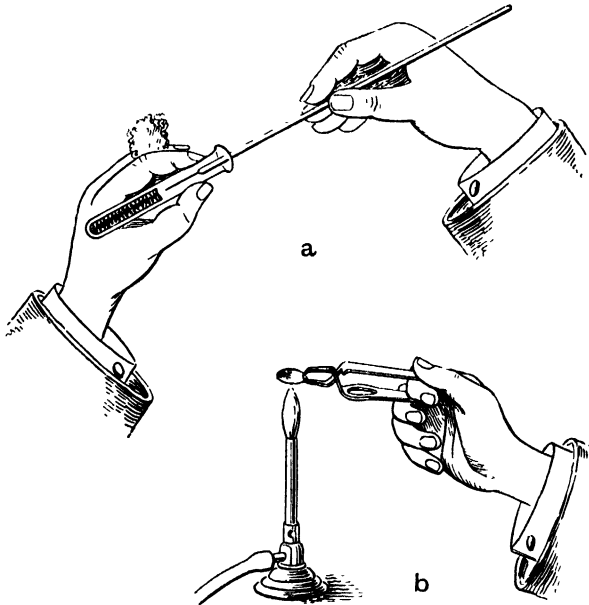


FIG. 8.—(a) Method of Inoculating the Culture Medium. (b) Fixing.

light bulb into a simple wooden box. The bulb should be covered with black paper to prevent the bactericidal effect of light.

After 24 hours in the incubator the Petri dish should be examined and signs of the development of colonies will then be probably apparent, though it is generally necessary to allow at least 2 days to elapse before making the stab culture. Specific sub-cultures are best made when the number of colonies on the plate does not exceed a hundred ; it is

generally, therefore, best to make two or three plates by transferring a loopful of the inoculated and melted medium from the first culture to a second and similarly to a third, plates being poured in each case. Well-defined colonies having been obtained on a plate culture, separate colonies can be removed by means of the platinum hook and transferred to a tube of gelatine or agar, there to develop.

For the proper carrying out of these operations manipulative practice is necessary in order to avoid accidental

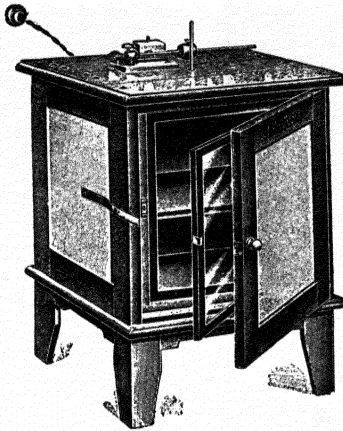


FIG. 9.—Hearson Biological Incubator.

infection by extraneous organisms from the air and also to acquire rapidity and dexterity of handling. It is wise to consider always that everything not actually sterilized is liable to be a source of infection. Thus a platinum wire after being laid down on the bench must be resterilized, and cotton-wool plugs re-singed after being held between the fingers. Such manipulative details become soon a matter of habit.

#### **Examination of Bacteria under the Microscope.**

Although every chemist should be familiar with the use of the microscope, experience shows that students when first introduced to the high-power instrument necessary for satisfactory work with living organisms, need to be

reminded of certain simple rules to be observed if risk of damage is to be avoided. These rules may be stated as follows :

1. When removing the microscope from its box or replacing it, it must be grasped by the upright portion of the stand, not by the microscope tube itself.

2. Focussing should always be done by moving the tube upwards rather than downwards, i.e. the lower lens or objec-

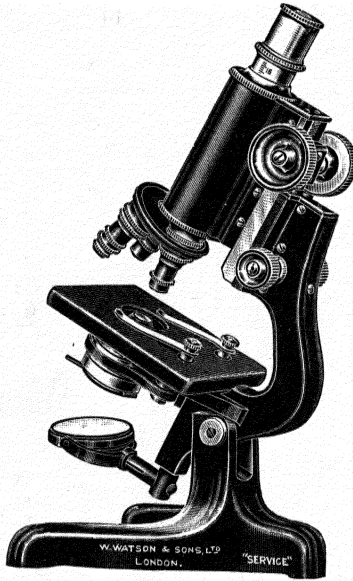


FIG. 10.—Bacteriological Microscope.

tive should be carefully screwed down till it just touches the object glass, and then should be carefully screwed away while observing the slide, stopping the movement when the object is in focus.

3. Preliminary focussing should be done with a coarse adjustment, and the fine adjustment only used for exact and final focussing.

4. All lenses should be carefully wiped clean from oil or Canada balsam between each observation or certainly before the instrument is put away.

For the satisfactory examination of bacteria a higher-power oil immersion lens ( $\frac{1}{12}$  inch) is necessary. With such a lens it is possible to see bacteria in a living condition in a drop culture. For stained preparations a  $\frac{1}{8}$  inch objective without oil immersion can be used but is not so satisfactory.

**Drop Culture.** To examine bacteria in drop culture a small portion of growth either from a plate or from a tube culture is removed by means of the platinum loop and quickly mixed with a drop of water on the under side of an ordinary microscopic cover glass, which is then placed on a specially made slide with a depression ground in it. On placing the cover glass with the drop on the under slide over the depression the bacteria can be observed. This method of examination is particularly useful in determining whether the bacteria are capable of movement or not, i.e. whether they belong to the class of *motile* bacteria (Fig. 7*d*, p. 35).

Bacteria are more simply observed when they are dried and stained with suitable dyes which render them more clearly observable. There are a number of methods in use for staining bacteria varying according to the media in which they are observed. Thus a special technique is necessary for staining bacteria when they occur in living tissue or in the condition of spores, and also for the purpose of bringing out such features as the flagella, or thread-like processes, which are characteristic of certain motile species, e.g. the typhoid bacillus.

**Staining.** It will be sufficient here briefly to indicate a simple method of staining a pure culture. A carefully cleaned cover glass is taken and held in a pair of specially constructed forceps, a drop of clean water is placed on the slip and a small portion of the culture mixed with the water, and spread in a thin film over the glass by means of a sterile platinum wire. The film is now carefully dried by passing the glass several times through a Bunsen flame with the film uppermost. The cover glass should never be made hotter than can easily be borne by the finger if the under side of the glass is pressed down on it. When the film is dried, a drop of stain is placed on the slide, ordinary magenta (rose-aniline) or gentian violet are commonly used. The

stain is allowed to remain for a minute or two in contact with the glass, and then washed off in a gentle stream of water or by immersion in a large volume of clean water. The preparation is again carefully dried and a drop of Canada balsam is dropped on the cover glass which is then carefully placed in contact with the ordinary mounting slide. With a little care only such a quantity of Canada balsam is dropped on the cover glass as will just suffice to reach to the edge of this when it is pressed down on the mounting slide. Care should be taken to remove all air bubbles from between the glass and the slide (Fig. 8*b*, p. 44).

**Yeasts and Protozoa.** For microscopical examination of yeasts and other larger organisms, special methods are used, which permit of isolation of actual single individuals. It is possible to observe protozoa in a living condition and count them by means of special devices such as are used for counting blood corpuscles.

In order to stain moulds it is necessary to remove a thin layer of fat on the surface of the mycelium, since otherwise they are not easily moistened with water. Before mounting, therefore, a portion of the mould intended for examination is immersed in alcohol to which a little ammonia has been added, the mould can then be stained with methylene blue, the filaments of the mycelium and hyphæ taking up the colour while the spores remain unstained. Special care must be taken not to overheat the spores by too rapid drying.

Instead of Canada balsam it is better to use glycerine in the case of organisms such as moulds and algæ, protozoa, etc., a cover glass being attached to the slide by a ring of shellac varnish.

## ENZYMES

**The Study and Preparation of Enzymes.** It has already been indicated that the various micro-organisms which we have to study effect the changes which characterize their activity through the agency of organic catalysts or enzymes. It will be of interest briefly to describe some of the methods used for isolating and studying these catalysts as distinct from the organisms which carry them.

As has been said, it was at one time believed that for every different chemical transformation there was a given organism, "one fermentation, one microbe," said Pasteur. This we have seen is not strictly true, but in the case of many species of bacteria and of yeasts it is roughly correct, and we may differentiate certain species of yeasts by their reaction with specific sugars or conversely different sugars may be identified by their fermentability or otherwise with certain species of yeasts. A similar differentiation is often employed in order to determine whether certain species or modified forms of *bacillus coli* are present in drinking water. According to the reactions observed after "putting them through the sugars" as it is termed, it is possible to decide whether the bacteria are characteristic of recent and therefore harmful contamination, or of old-standing pollution which has been found to be less dangerous.

It will be of interest at this point briefly to indicate the more important methods which are used for the separation and identification of the enzymes occurring in the organisms with which we have to deal.

The methods employed will vary according as the enzymes are intracellular or extracellular, whether, that is to say, they are diffusible through the cell wall and consequently can be detected without rupture of this, or whether they can only be studied separately from the organism by breaking the cellular structure and liberating the enzyme.

The procedure is simple in the case of *extracellular* enzymes. It is then only necessary to inhibit or destroy the vital activity of the organism by means of an antiseptic such as toluene and then to test the solution by appropriate means for the presence of the enzyme sought for.

For preparing the specific enzyme solution in quantity we may take as an example the enzyme secreted by any bacterium which liquefies gelatine; with a culture of such an organism the whole mass of gelatine will become liquid, generally within 24 hours. On filtering the liquid gelatine through a filter candle of porous porcelain, a solution passes through which, on microscopic examination, will be found to be free from living organisms; but if a portion of the liquid

is added to another batch of gelatine, the latter will be quickly liquefied, showing the liquefying enzyme has passed out from the living cell.

**Concentration of Enzymes.** From such a solution the enzyme can be obtained in a state of increasing purity by a number of methods which have been developed considerably in quite recent years.

A simple method of concentrating the enzyme solution is by freezing, and filtering off the concentrated solution of enzyme from the precipitated ice by means of a Buchner filter. On pouring the enzyme solution into alcohol the enzyme is precipitated along with other albuminous matter.

Willstätter has developed a method of systematic adsorption and re-solution of the enzyme, substances such as kaolin and precipitated alumina being used to provide an absorptive surface.

The extraction of enzymes when they occur in the *intracellular* condition is not so simple. In this case means have to be found for breaking the cells. In the case of bacteria they are sometimes frozen with liquid air and afterwards ground with fine sand, by pressing the product in a high-power hydraulic press the intracellular liquid is obtained and can be investigated as usual.

One of the earliest and most important intracellular enzymes to be separated and investigated was the *zymase* of yeast. It was Buchner who achieved this result by grinding the yeast cells with sand and then expressing the juice by means of a hydraulic press. This juice, although quite free from yeast cells, is capable of producing alcoholic fermentation in a solution of cane sugar.

A thorough study of the characteristics and the reactions of zymase and other enzymes present with it in the yeast cell is a chapter in biochemistry of the greatest fascination, with which, following Buchner, the names of Harden and his co-workers in England and Neuberg and others on the Continent are chiefly associated. They do not, however, closely concern our present subject.

An elegant method for determining the presence of a specific enzyme in a given organism is to grow it on a culture plate,

containing the substance or substrate as it is called, the effect on which it is desired to observe. Thus to detect the production of amylase by any organism, it may be grown on a plate of nutrient agar containing starch. After the colonies have developed if the plate is flooded with iodine it will be coloured blue except in the vicinity of the colonies producing amylase which will be surrounded by a colourless ring. By methods such as the foregoing enzymes may be detected and separated from the living cell whether it be of a mono-cellular organism, or the tissues of higher animals and plants.

**Enzyme Reactions.** It may be useful here to tabulate the most important reactions which we shall have to consider in the following chapters, indicating in each case the type of organism and enzyme concerned. (See Table on p. 53.)

**Conditions of Action of Enzymes.** At the same time a few general observations may be made on the conditions of action of enzymes in general. In many respects the action of the enzyme resembles the activity of the living organism, e.g. :

1. Enzymes are destroyed by heat.
2. They have an optimum temperature of reaction.
3. They are not exhausted by continuous activity.
4. They are greatly affected by alterations in the medium in which they act, e.g. by certain antiseptics and poisons.

On the other hand, enzymes can be differentiated from living organisms by certain other properties, i.e. some enzymes such as those of an extracellular type will, as we have seen, pass readily through a porous porcelain filter, which under similar conditions will retain the living organisms. Further, certain antiseptics which inhibit the action of micro-organisms are without effect on enzymes. Among these the most frequently used are thymol, chloroform and especially toluene.

In the case of living organisms, while the change which it effects is naturally subject in a degree to the number of individual organisms present (a slight inoculation, taking some time to develop, or possibly not developing at all, while a heavy inoculation brings about a rapid change), yet once

the reaction is started the living organisms continue to develop up to a maximum concentration so long as any of the medium remains unattacked.

In the case of the enzyme this power of development, which is characteristic of living organisms, is absent and the rate of reaction under certain conditions is simply in proportion to the mass of enzyme present. This condition is that the amount of substance to be acted upon is large in proportion to the quantity of enzyme used; under such conditions what is known as the law of proportionality obtains.

On the other hand, when the solution to be acted upon is very concentrated, it may often happen that the enzyme functions as a synthetic agent building up the substance from its products of decomposition. The first instance of this synthetic enzyme action was observed by Croft Hill, who found that if *maltase* was added to a very concentrated solution of dextrose a disaccharide was formed. Thus it appeared that while in dilute solutions there was a breaking down of larger into smaller molecules, in concentrated solutions there was a building up or synthesis of the simpler molecules into more complex. This would seem to indicate that all enzyme actions are potentially reversible, and the direction of the reaction depends on the concentration of the solution and the relative masses of the reacting bodies.

Subsequent to Croft Hill's researches other instances of reversible enzyme action have been discovered; thus Fischer and Armstrong have found that iso-lactose can be synthesized in the presence of the enzyme lactase from a mixture of equal proportions of glucose and galactose, and certain of the fat-splitting enzymes have been found to act reversibly, but the difficulty of working with such concentrated solutions limits the number of successful experiments in this direction. The importance of such researches and the synthetic activity of enzymes cannot, however, be overestimated, as they throw some light on the mechanism of those natural synthetic processes which take place in the living cell and which are of such fundamental importance in connection with the subject before us.

CLASSIFICATION OF ENZYMES CONCERNED WITH NITROGEN CONSERVATION

I. Hydrolytic Enzymes		
<i>(a) Carbohydrate Hydrolysis</i>		
Name of Enzyme.	Nature of Substrate.	Carrier of Enzyme.
Invertase . . .	Saccharose	Yeasts and moulds. Leaves, seeds and stems of plants.
Amylase . . .	Starch	Leaves and seeds of plants. Saliva and digestive tract of animals.
Cytase and Cellulase	Cellulose	Certain bacteria and many moulds. Seeds of plants.
Maltase . . .	Maltose	Yeasts, leaves and seeds of plants.
<i>(b) Fat Splitting</i>		
Lipase . . .	Oils and fats	Oil-bearing seeds, certain bacteria and many moulds
<i>(c) Protein Splitting</i>		
Pepsin, Trypsin .	Proteins in general.	Bacteria, yeasts and moulds. Leaves, seeds and fruits of plants, and digestive tract of animals.
<i>(d) Urea Splitting</i>		
Urease . . .	Urea	Certain specific micrococci and bacteria. Soya bean.
II. Fermenting Enzymes		
Zymase . . .	Invert-sugar	Yeast.
III. Oxidizing Enzymes		
Oxidase . . .	Tannin and fruit juices	Plant and animal tissues. Also certain gums and resins.

## CHAPTER II

### SOURCES OF ORGANIC NITROGEN

In the diagram referred to on page 3 it will be seen that there are three main forms in which nitrogen occurs in nature, others being intermediate forms :

1. Atmospheric nitrogen.
2. Nitrates.
3. Organic nitrogen, or nitrogen combined in complex organic substances, the immediate products of animal or vegetable life.

These various sources of nitrogen may now be considered from the point of view of their quantity and availability. The maximum world consumption of fixed nitrogen in 1920 was 1,300,000 tons per annum, this being a war-time figure. We may now consider in some detail these sources from which this nitrogen can be obtained.

#### 1. Atmospheric Nitrogen.

The volume of air over each square yard of the earth's surface contains 5·8 tons of nitrogen, and the nitrogen above one square mile of land amounts, therefore, to 20,000,000 tons, or enough to supply the world's present requirement for twenty years. Of this amount only a very small fraction, viz. about 0·000002, is actually in circulation among animals, plants and bacteria.

It is obvious, therefore, that in the atmosphere there is a reservoir of nitrogen of unlimited extent provided it can be brought back economically into the nitrogen cycle.

#### 2. Nitrates.

In 1917 the annual output of nitrogen as nitrate of soda (Chili saltpetre) from the Chilean deposit was 380,000 tons. This increased to a maximum of 464,000 in 1929-30. This

is still much less than the maximum annual output possible which has been estimated at 5,000,000 tons.

The annual production of nitrogen as Indian saltpetre (potassium nitrate) is only small, namely, 2,800 tons in 1917, which declined to less than half of that amount in 1924; later figures are not so far available.

### 3. Organic Nitrogen.

(a) **Nitrogen stored in coal, peat and river silt.** The nitrogen of ancient vegetation either of past or recent geological periods can be recovered as ammonia by various processes of distillation from the above materials.

The nitrogen contained in the actual annual coal output of the world amounts to 11,000,000 tons; of this only 425,000 tons is recovered as ammonium sulphate, which emphasizes the importance of by-product recovery from coal.

The recovery of ammonia from peat has not yet attained large proportions, though enough is present to justify its recovery. One ton of dry peat contains 1 per cent. of nitrogen, equivalent to 80 lb. ammonium sulphate per ton.

The mud of many rivers and estuaries contains much vegetable debris, such deposits in fact being comparable to some extent with those from which the coal measures were originally formed. Deposits of this kind occur in Germany and may contain from 2 to 4 per cent. of nitrogen.

In the innumerable creeks and waterways of China, vegetable mud accumulates and is regularly dredged out and used as a constituent of compost, in the making of which the Chinese farmer is a master.

From the point of view of Agriculture nitrogen as sulphate of ammonia is in practice almost as useful as nitrogen in the form of nitrate, as there is evidence that nitrogen in the form of ammonia can be absorbed by plants, and in any event it is converted into nitrate by the nitrifying organisms in well-aerated arable soil. The suitability of nitrate of soda or sulphate of ammonia, or any other of the numerous modern synthetic compounds of nitrogen, depends on price and local conditions, particularly on the character of the soil, and is a matter to be decided by the agricultural biochemist.

(b) **Slaughter-house Refuse.** This is a highly nitrogenous material consisting mainly of the entrails of animals, and is sometimes put on the land direct or is rendered down first to recover the fat, and the residue worked up into forms convenient to handle. The bulk of the blood is recovered as such and either used for various food preparations, or dried down and used as a fertilizer. The general washing-down liquors, which are also highly nitrogenous and are likely to be exceedingly offensive, are generally sent into the nearest sewer, if such exists, and become part of the town sewage.

(c) **Fish Meal.** This is an important source of nitrogen as there is much waste fish material at most fishing centres which can be properly used in this way. Moreover, as can be shown later, the nitrogen which exists as nitrate in sewage effluents and which may readily become unavailable by discharge into river or sea, can be specially conserved in the form of fish, and so brought back into circulation. No doubt much of the nitrogen escaping into river and sea does actually come back in this way in the long run, but the process is capable of being controlled.

In China and in many parts of the Continent, particularly Germany and Austria, the manuring of fish ponds with sewage is carried out as a definite industry, and shows considerable profits. An average fish meal contains the following percentages of important constituents :

Nitrogen . . . . .	9
Phosphoric acid ( $P_2O_5$ ) . . . . .	7
Oil . . . . .	10

(d) **Animal Debris.** Hair, leather, hoofs, horns, etc. These are sometimes used directly as fertilizer, but some form of preliminary treatment to render them more available is to be recommended. Thus by the action of high-pressure steam horns and hoofs become more friable and capable of being readily powdered, and consequently easily mixed with the soil. Under such circumstances these otherwise intractable materials form quite a useful fertilizer.

(e) **Green Manures.** Reference was made in the introduction to the capacity of certain plants in association with bacteria to fix nitrogen from the air. The majority of such

plants belong to the order leguminosæ, and the process known as green manuring is the utilization of such nitrogen-containing plants, by ploughing them into the soil when they are broken down by bacterial action yielding simple nitrogenous bodies which are again utilized by the growing plant.

Such plants can be grown as ordinary field crops or as trees or shrubs, whose leaves and twigs can be utilized as constituents of compost. Under this category such well-known plants as sunn hemp, ground nut and numerous species of vetches are employed. Among leguminous shrubs the following have given good results in India and other tropical countries, *Leucana glauca* and *Tephrosia candida*. The leaves of the tamarind-tree, which fall in large quantities in their season, are a very useful constituent of nitrogenous composts.

The biochemistry of the changes taking place when leguminous plants are ploughed into the soil or utilized in the production of composts is a very important field of research for the biochemist, who should assist the agriculturist to avoid as far as possible the losses of nitrogen which may sometimes occur if green manures are not properly handled.

(f) **Oil Seed Cakes.** The cake remaining after pressing the oil from the various oil seeds of course always contains an appreciable amount of nitrogen.

The figures for a number of Indian oil seeds are given below, the determinations having been made by Mr. S. R. Bhate, B.Sc., one of the writer's former students, partly at the Indian Institute of Science, and partly at the Government Industrial Laboratory, Hyderabad, Deccan.

Description of Oil Seed.	Name of Cake.	Percentage of Nitrogen.
Bassia latifolia . . . . .	Pressed	3.20
Castor, Mahboobnagar . . . . .	Pressed	5.60
Castor, Mahboobnagar . . . . .	Solvent extracted	11.50
Castor, Aurungabad . . . . .	Solvent extracted	9.72
Castor, Hyderabad . . . . .	Solvent extracted	10.66
Castor, Italian . . . . .	Solvent extracted	9.76
Ground Nut . . . . .	Pressed	6.85
Safflower . . . . .	Solvent extracted	9.28
Safflower . . . . .	Pressed	2.40

It will be seen that the percentage of nitrogen is appreciable in all cases and becomes considerable if the oil is thoroughly extracted from the cake by means of solvents.

It may sometimes, however, be found preferable to obtain the food value of the oil as well as of the other food material of the cake by feeding to cattle when at any rate a large proportion of the nitrogen will be also available as solid and liquid manure.

Owing to the export of oil seeds from India much of this nitrogen is lost to Indian agriculture and the question is receiving the careful attention of Indian agricultural advisers. It will be interesting shortly to consider the utilization as fertilizer of certain of the more important oil seed cakes, and in this connection the most recent information has been taken from the valuable report of Mr. A. F. Yuill on the vegetable oil industries of Hyderabad State.

*Castor Cake.* In certain parts of the country, notably the Bombay Presidency, castor cake is readily sold at good prices, and there is ample experience to show that it is an excellent fertilizer, having the highest percentage of nitrogen in any oil cake. It has some possibility as a cattle food and experiments in this direction have been made in the Harcourt Butler Technological Institute, Cawnpore, but the expense and difficulty of extracting both the oil and the *ricin* (a poisonous principle contained in the seed) make it questionable whether it is worth while to pursue investigation in this direction, especially as the prepared cake is not very readily taken by cattle.

*Ground Nut Cake.* Until recently ground nut cake has been fetching a better price in Europe than in India; in consequence, therefore, ground nuts have been imported into Europe instead of oil. The altered conditions caused by the recent drop in the price of ground nut cake and the difficulty which manufacturers in Europe have had in the disposing of this by-product, may lead them to consider the alternative proposition of importing ground nut oil instead of ground nuts. Mr. Yuill recommends that Hyderabad ground nuts should be crushed and the oil sent in tank cars to the nearest port, and from thence in tank steamers to Europe.

In order to avoid the necessity for exporting the cake he recommends that efforts should be made to find out alternative uses for oil cake.

It has been stated that in the northern portions of the Madras Presidency ground nut cake finds ready application as a cheap and useful manure.

*Mahua (mowhra) Seed Cake.* Experiments by Rege at the Indian Institute of Science showed that mahua cake contained a saponin called mowhrin, which had a distinctly bactericidal effect, practically inhibiting nitrification; thus on incubating comparative mixtures of soil with raw mahua cake on the one hand and mowhrin-free mahua cake on the other, the following figures were obtained for nitric nitrogen in the mixture after 3 months :

Raw mahua cake . . . . .	0.224	mgm.	per	100	grams	of	soil
Mowhrin-free mahua cake . . . . .	1.92	"	"	"	"	"	"

Some interesting results have been obtained in regard to this material both at Pusa and at Cawnpore. At Pusa it has been shown that preliminary fermentation of the cake, especially in presence of charcoal + 25 per cent. of soil, renders the nitrogen much more available. At Cawnpore it has been found that the mowhrin present in the cake is certainly detrimental to the growth of plants, if the cake is used without a previous treatment, or if the crop is planted immediately after the application of the cake to the soil. On washing out the mowhrin with water, however, the residual cake gives very good results, the effect being striking in regard to the flowers and seeds. The value of preliminary treatment in this way will depend on whether a use can be found for the extracted mowhrin, which as a saponin has some possible uses as a hair wash and as an ingredient of ordinary soap. It is certainly useful as a germicide, and according to Mr. Yuill, is thus used in Europe and America for destroying worms on golf courses and lawns.

The mahua cake can, however, be used without any preparation if added to the soil just before the monsoon, and if sufficient time is allowed for the rain to wash out the detrimental constituent.

*Cotton Seed Cake.* According to Mr. Yuill, undecorticated cotton seed cake contains about  $3\frac{1}{2}$  per cent. of nitrogen, 1.56 per cent. phosphoric acid, and 1.4 per cent. of potash. Basing its value on the present prices of the same ingredients in artificial manures the value of 1 ton of undecorticated cotton seed cake is not less than Rs. 40 per ton.

*Other Seeds.* Safflower seed, as can be seen from the table of analyses already given, is an excellent manure and has been fetching better prices, especially for sugar-cane growing, than such oil cakes as ground nut, til seed, etc.

It is probable that til seed, like linseed, is best used as cattle food.

(g) **Animal Manures.** The proper conservancy of animal manures, that is, the dung and urine of horses, cattle, sheep and goats, is one of the most important farming problems.

True economy would urge that all should go back to the soil, but in practice there is loss of nitrogen through improperly controlled fermentation in the manure heap, and in many places complete loss of the urine under the belief that it cannot be used satisfactorily for manurial purposes.

This belief has been shown to be erroneous by Professor Kendrick at Aberdeen, and by trials carried on at the College of Agriculture, Holmes Chapel, Cheshire, by the late Major (then Mr.) and Mrs. Mumford, and Messrs. Gaul and Chamney, under the present author's supervision ("The Effect of Liquid Manure on Crops," Special Reports to the Board of Agriculture and Fisheries, 1915 and 1917). The nitrogen in liquid manure exists mainly as hippuric acid, and laboratory experiments showed that the free acid is incapable of nitrification. The calcium salt, however, nitrifies readily, and consequently if lime is present in the soil, or if it is added to the liquid manure in adequate but not excessive amount, this material yields very good crops and there is certainly no warrant for allowing it to run to waste. In India the use of cattle manure is hampered by the frequent shortage of wood fuel which necessitates the burning of cow-dung instead of its return to the soil. The importance of the agriculturist having a ready access to cheap fuel wherever possible has been pointed out by Voelcker (*Improvement of Indian Agriculture*, Chap. 7).

Lander, Agricultural Chemist to the Government of the Punjab, states (*Agricultural Journal of India*, March, 1926, p. 124) that the annual loss to the Punjab from the burning of the dung cakes, based on the actual market value of farmyard manure, is 1.5 crores; taking the value at Rs. 7 per ton calculated from the increased yield over controls, the loss is about 8 crores.

It may be hoped that experiments which have already been suggested, to manufacture cheap briquettes for the use of agriculturists from the waste coal of Indian collieries, may be taken up in earnest. Cheap fuel for the agriculturist is one of the crying needs of agriculture.

Another step which would tend to reduce the consumption of cow-dung as fuel is the design of a cheap simple apparatus for heating milk in such a way that it will not be liable to spoil. At present this has been achieved by a slowly burning fire of cow-dung cakes.

Even under present conditions experiences at Gurgaon have shown that a little intelligent education and strenuous but sympathetic work in the villages can remove this great barrier to progress in Indian agriculture.

Moreover, there are great possibilities in the economical use of such cow-dung as is available, instead of allowing it to rot in masses without any scientific supervision, or to be mixed without care with miscellaneous plant and other residues; it is possible by careful management to get far more value out of it when it is used as a ferment than when it is used in bulk as a manure. If waste vegetable matter is piled in heaps and thoroughly moistened with a mixture of cow-dung and water, the necessary bacteria present in the cow-dung are evenly distributed over the waste materials.

In this way an active mass of fermenting compost is obtained which can be used as an *Activator* for further quantities of raw material, the whole being kept moist with quantities of soluble nitrogen added in the form of diluted cattle urine. By a little systematizing the process can be made continuous; at one end of the heap well-rotted compost can be taken away, while fresh material is added and

mixed, the whole being periodically turned over from end to end.

By this method the original process of Richards and Hutchinson, now known as the ADCO process, is greatly accelerated. The value of this use of activator and cow-dung mixture is mainly to reduce the time necessary for production of fertilizer, but incidentally it would also appear that the product contains a greater percentage of nitrogen than is possible by less elaborate methods. For this reason the product has been given the name of Activated Compost, since it is produced on principles similar to those of the Activated Sludge Process to which reference will be made later.

Even when the raw material is simply rotted in heaps very valuable results are obtained, and it is stated in a paper issued a few years ago by the Director of Agriculture and the Agricultural Chemist of Bengal, that for the first time in its history the Government farm at Dacca has had an ample supply of manure ; what must it mean to the whole of India where resources in knowledge and materials, in general, are so much less ?

For centuries the Chinese have been accustomed to make compost out of every species of vegetable debris, large quantities of which are brought from considerable distances for the purpose. Interesting descriptions of these methods are given by King in *Farmers of Forty Centuries*, London, 1927.

The foregoing references are mainly concerned with India, but in estimating the world's resources of animal manures mention should be made of an enormous deposit of manure existing in the form of guano or bird excreta, on certain islands belonging to Peru. The annual production amounts to about 100,000 tons, of which 14,000 are nitrogen. A fresh deposit estimated at 10,000 tons has been discovered on an island opposite Campechi, Mexico.

(h) **Town Sewage.** In order properly to appreciate the nitrogen value of town sewage certain fundamental physiological data have to be considered. Various data are given by different authorities. Rübner gives 1.4 grams

per capita as faecal nitrogen (Richet, *Dictionnaire de Physiologie*). Metcalf and Eddy (*American Sewerage Practice*, vol. III, p. 153) give the following figures :

<i>Faeces</i> . Dry solids . . . . .	20.5	grams	per	capita	per	day
<i>Faecal Nitrogen</i> . . . . .	0.9	”	”	”	”	”
<i>Urine Nitrogen</i> . . . . .	7.0	”	”	”	”	”

The total amount to be dealt with in grams per capita per day apart from water and general household waste, i.e. on an efficient dry conservancy system, is as follows :

Faeces . . . . .	90
Urine . . . . .	1,170
Paper . . . . .	20
	1,280

In American *Sewage* the daily amount of nitrogen in grams per capita is as follows :

As free ammonia . . . . .	8.0
Other nitrogen . . . . .	7.0

(Metcalf and Eddy, Table 52, p. 194, vol. III.)

Indian latrine sewage does not average more than approximately 2 grams nitrogen per capita daily, but a large proportion of the urine fails to be included.

Taking Rübner's figure of 1.4 grams nitrogen per capita daily as faecal nitrogen, i.e. rather more than the figure 0.9 given as faecal nitrogen by Metcalf and Eddy, but less than that given by them for organic nitrogen, it follows that a population of 1,000,000 people will produce 1.4 tons approximately per day or about 500 tons per annum.

The purely faecal nitrogen, however, if recovered amounts to a large proportion of the world's needs as is shown by the table on page 64, the totals being arrived at by multiplying the millions of population in eight of the chief countries of the world by 500.

If to this the nitrogen in solution (urine nitrogen) is added, we get a total of 4,000,000 tons or more than three times the total world consumption of "manufactured nitrogen" for agriculture, viz. 1,300,000 tons per annum.

## 64 BIOCHEMISTRY OF NITROGEN CONSERVATION

Name of country.	Millions of inhabitants.	Tons of nitrogen produced per annum.
China . . . . .	400	200,000
India . . . . .	300	150,000
Great Britain . . . . .	45	22,500
Japan . . . . .	40	20,000
Germany . . . . .	60	30,000
Austria . . . . .	50	25,000
France . . . . .	40	20,000
U.S.A. . . . .	100	50,000

say 500,000

In an address to the first International Congress of Soil Science in Washington, Sir John Russell repeats the estimate of the value of the excreta of the population of the United Kingdom which he gave in war-time for a population of 45·2 millions, as amounting to a sum of £17,500,000 per annum or 7s. 9d., that is Rs. 5, per head ; very little of this finds application in agriculture.

The chief centres of the world each containing over 500,000 inhabitants represent a population of 35,000,000 which is equivalent to 17,500 tons of nitrogen in the solid form and 122,500 tons in solution per annum.

Further data may be quoted from a paper on nitrogen and sewage by Dr. McGowan, formerly Chemist to the Royal Commission on Sewage Disposal (*Surveyor*, May 7, 1920, p. 405), in which figures are given on the authority of Colonel M. Flack :

Daily nitrogen in urine :

Man . . . . .	16 grams
Woman . . . . .	13 „
Child . . . . .	8 „

Daily nitrogen in fœces :

Average . . . . .	2 grams
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According to these figures the average daily total nitrogen produced per million inhabitants in the United Kingdom is :

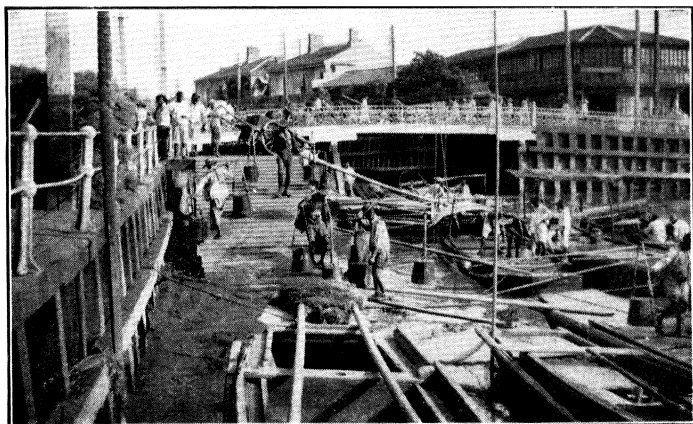
Nitrogen in urine . . . . .	12 tons
Nitrogen in fœces . . . . .	2 „

These figures are higher than those on which the foregoing calculations are based, so that it may be taken that the error,

PLATE I.



(i) IN THE FOREGROUND A COOLIE BRINGING HIS BUCKETS TO THE BOATS. THE CART ON WHEELS IS A WOODEN TANK USED FOR THE SAME PURPOSE. IT IS JUST BEING TIPPED UP FOR DISCHARGING.



(ii) A TYPICAL LOADING STATION.





(i) A TYPICAL GARDEN SHOWING POSITION OF "KONG" (ON EXTREME LEFT) IN RELATION TO CROPS.



(ii) A COOLIE APPLYING THE FERTILIZER TO A BED OF YOUNG CABBAGES



if any, lies in under-estimating the value of the nitrogen in human excreta.<sup>1</sup>

A striking proof of the value of this nitrogen is the fact that the city of Shanghai was at one time paid £40,000 per annum by contractors for its nightsoil, which would include a part but not the whole of the urine of the population. In addition to this large payment the contractors maintained an army of coolies and a fleet of nightsoil boats so that the city has no conservancy charges. The total value of nightsoil in Shanghai thus works out at something like Rs. 2 per head per annum.

Owing to the more general adoption of water carriage the receipts from the sale of nightsoil are not now as great as formerly, but in a recent letter from the Commissioner of Works, Municipality of Shanghai, he states that the Municipality still sells surplus nightsoil for 11,000 taels, i.e. about Rs. 22,000 net per month.

All the material for which the contractor pays so liberally is conveyed in a liquid state by boats along the multitudinous creeks of China to be used with meticulous care by the farmer.<sup>2</sup>

The methods in use in India form a somewhat melancholy contrast to the scientific thrift of the Chinese. Even when in the country certain fields are set apart for purposes of nature, no effort is made to incorporate the excreta with the soil, so that much of the manurial value is lost and highly insanitary conditions are created owing to the infection carried by flies and other insects which alight on the exposed excreta. The fact that children, even in towns, are not trained to sanitary habits is a cause of loss and danger to the community. Where pail conservancy is practised the collection is often imperfect and dilatory so that flies have time to deposit eggs on the exposed excreta, and are the cause of serious fly trouble when the nightsoil is brought to the fields for use as manure or even when it is disposed of by trenching. The ordinary methods of trenching, even when the contents of the trench are ultimately used for manure, result in great loss of nitrogen. Moreover, when reasonable sanitary efficiency is attained, religious or caste prejudice is so strong in many places that no product

<sup>1</sup> See also Appendices I, II and III.

<sup>2</sup> See Plates I and II.

of any sort if ultimately derived from nightsoil will be touched by the cultivator.

This caste prejudice can in these days hardly claim religious sanction while the bathing ghats at Benares are being increasingly polluted by the sullage from that holy city, and when the whole of the sewage of Cawnpore is allowed to pass unpurified into the Ganges.

The following extract from a note by Mr. Asharfi Lal Gupta, Executive Officer Municipal Board, Benares, dated 17th April, 1925, is a striking comment on this state of affairs. Mr. Gupta writes :

It is in the interests of sanitation as well as religious sentiment to save these waters from being polluted and rendered unfit for bathing. Rivers are not polluted even in countries where they are not considered holy or sacred by anybody ; for example, in England and Ireland, where no one worships any river, there are Rivers Pollution Prevention Acts under which any act by which the river water is polluted is an offence. Very probably at least one reason for investing such waters with "religious sacredness" here in India by long-continued tradition, was the very sanitary purpose of preventing persons from befouling them, but unfortunately this aspect of the question has been completely forgotten by the public.

In spite of the appalling waste involved one may have some sympathy with those who are reluctant to use any form of human waste as manure. When used without proper preparation it is an exceedingly unpleasant substance to deal with. The advent of the Activated Sludge Process, which briefly stated depends on intensive biochemical oxidation, makes it possible to use such material without any nuisance, with the production of an inoffensive brown humus containing 5 to 6 per cent. of nitrogen of great availability and a clear odourless solution of nitrates, which can be used for irrigation without danger of nuisance or of rendering the land "sewage sick."

During the last ten years striking progress has been made in the development of the Activated Sludge Process. Great cities such as Milwaukee and Chicago have expended millions of dollars on sewage works, to be followed by Indianapolis and in the near future by a portion of New York. Birmingham, Manchester and London have large schemes

in hand, and in India Bombay is actively preparing plans for the treatment of a portion of its sewage by this process. Shanghai, which formerly, as has been said, obtained an income of about Rs. 2 per head by the sale of its nightsoil, is now gradually bringing the whole of its human wastage to be treated in Activated Sludge Plants and the Chinese agriculturist is gradually accustoming himself to the use of the sludge instead of the offensive crude nightsoil which he hitherto valued so highly. Bulk for bulk, the Sanitary Chemist to the Shanghai Municipality reports that comparative analyses of a large number of samples of sludge and ordure show that an average sludge containing 6 per cent. of nitrogen on the dried matter, if de-watered to the condition of spadable mud, will provide two and a half times as much available nitrogen and other constituents of manurial value as the same bulk of ordure. It is much better, if possible, to use activated sludge in the semi-liquid state as is done by the Chinese farmer, who will be able to boat the material about the creeks of China as he used to convey his horrible cargoes of liquid nightsoil.

In India river creek communication is infrequent, so that transport of this material, if it cannot be used on the site of a works, will necessitate preliminary drying. For nine months of the year this should not be difficult and methods are at present under consideration for economically drying it by artificial heat. The advantage of this would be the elimination of hookworm which is a natural cause of anxiety to Directors of Public Health.

That the drying of activated sludge is no longer a matter of experiment, although capable of great improvement, is shown by the published reports from Milwaukee where they have now created an assured market for 30,000 tons of dried sludge having an annual value of £100,000 sterling. The nightsoil of India at present, instead of being a source of wealth as it is in China and as it will shortly be in the United States and in Europe, is a menace and a nuisance. It may be hoped that its proper utilization may be one of the subjects for consideration by the Imperial Research Council recommended by the Agricultural Commission.

### CHAPTER III

## BUILDING UP AND BREAKING DOWN OF NITROGENOUS MATTER

### OUTLINES OF THE CHEMISTRY OF PROTEINS

Most of the nitrogen added to the soil in the form of one or other of the organic fertilizers considered in the last section, is in the form of proteins and their derivatives. These substances cannot be assimilated by higher plants as such, but have to be first broken down into simple compounds. This process is carried out in the soil by the agency of micro-organisms, the final product of hydrolysis being chiefly ammonia. The latter is either used by the plants as such or is oxidized further to nitrates. Nitrates are either assimilated by plants or by micro-organisms, reduced by denitrifying bacteria, or washed out in the drainage water.

In order to follow the biochemistry of these changes it is necessary to study carefully the chemistry of proteins. All living organisms contain as an essential constituent a highly complex nitrogen-containing substance known as *protoplasm*; the most highly developed animal chemically considered is a vast aggregation of cells of different structure and function and all of them containing protoplasm in some form or other. Protoplasm is in no sense a chemical entity; with a definite composition such as may be ascribed to even highly complicated organic substances, it possesses a structure visible under the microscope and must be looked upon when alive as a constantly changing complex wherein loose combinations are constantly being formed and decompositions taking place. Protoplasm may indeed be regarded as a factory where raw material of various kinds is taken in, where finished products are delivered and where a certain amount of waste material is produced.

It will obviously be of little help to the understanding of the operations of such a factory simply to know the materials of which it is composed or even the bare enumeration of its contents in terms of iron and steel or bricks and mortar or weight of stores. The ultimate chemical composition of protoplasm therefore can tell us little of its real nature, though it is of interest to know that its invariable constituents include carbon and nitrogen, and almost universally phosphorus; it will obviously be more instructive to describe it, as it were, by stages classifying the chemical contents into substances of gradually decreasing complexity. Even then we shall only have obtained a vague idea of the constituents of dead protoplasm as we might make an inventory of the contents of our hypothetical factory after business had been shut down. Of the course of operations or of the economic conduct of the factory we should know little or nothing. Having obtained such an inventory, however, and presuming the factory began working again, by taking careful note of the material entering and leaving the factory we could form a much better idea of the processes carried on therein. The task which confronts the biochemist is to investigate in this kind of way the chemistry of protoplasm which, in other words, is the chemistry of life. In the present chapter an attempt will be made broadly to indicate essential facts with reference to the products of the activity of protoplasm. Substances which have been isolated as more definite chemical entities belong to the class known generally as albumins, proteid bodies or more recently as proteins. It will probably be simplest to take one or two of the most characteristic of these substances and study their properties and products of decomposition, after which will be given in brief summary an account of the principal bodies of this class which are known together with their decomposition products.

As a typical albumin *white of egg* may be made use of. If 1 c.c. of white of egg is poured into 50 c.c. of water, stirring meanwhile, a white precipitate is formed. This can be filtered off and a portion of the filtrate boiled, when a further precipitate is obtained.

It will thus be seen that the egg white can be separated

readily into two substances, one soluble and the other insoluble in cold water. The insoluble portion is known as *globulin*, the soluble substance as *albumin*.

As the word albumin is also used in a more or less generic sense it is better perhaps to refer to this body as *egg albumin*. A related substance can be obtained from blood serum and is known as *serum-albumin*, and also from milk, when it is known as *lact-albumin*.

These albumins give a number of characteristic reactions; thus on boiling with strong caustic soda an evolution of ammonia can readily be detected. If a few drops of lead acetate are added to a 20 per cent. solution of caustic soda a precipitate is formed which readily dissolves; if a little of this solution is boiled with a solution of egg-albumin it rapidly darkens owing to the formation of sulphide of lead; this indicates the presence of sulphur in the egg-albumin.

On warming with strong caustic soda and adding a few drops of dilute copper sulphate solution a violet colour is obtained; this is known as Piotrowski's reaction. If the boiling with caustic soda is prolonged, a rose pink colour is obtained on addition of copper sulphate; that is the *biuret* reaction. The formation of this colour is due to the production of biuret, or an allied substance. The biuret group, it will be noted therefore, is characteristic of the decomposition products of albumin, and the biuret reaction is a useful indicator of the extent to which decomposition has taken place.

On warming an albumin with strong nitric acid and subsequently adding ammonia, an orange colour is obtained; this is known as the *xantho-proteic* reaction.

**Precipitation of Albumins.** The albumins belong to the class of substances known as colloids, the general properties of which have been referred to earlier. Like other colloids the albumins can be precipitated by the addition of certain salts. Thus sodium chloride, magnesium sulphate, zinc acetate and especially ammonium sulphate may be employed for this purpose.

Albumin substances belong, as a rule, to the class of compounds known as *amphoteric*, i.e. they are capable of acting both as weak acids and weak bases. The solution of albumin

in dilute alkali is sometimes known as *alkali albumin*; if acid is added very carefully to such a solution, the albumin is first precipitated and then redissolved, forming so-called *acid albumin*. Thus albumin, it will be seen, is capable of forming salts, and of combining both with acids and bases.

Hydrated oxides such as aluminium hydroxide,  $\text{Al}_2(\text{OH})_6$ , or ferric hydroxide,  $\text{Fe}_2(\text{OH})_6$ , are capable of precipitating albumin from solution. This phenomenon is no doubt partly physical and partly chemical, physical in that one colloid body on separating from solution tends to attract other colloids by a process known as adsorption, and chemical in that the metallic hydroxide actually combines with the albumin. This tendency of colloidal precipitates to carry out of solution other colloids, especially those related to albumin, finds application on the large scale in the chemical precipitation of sewage and other polluted liquids.

The well-known household cookery recipe for clarifying soup, etc., by means of white of egg is an illustration of this same property.

What has been termed the *clarification test* was developed in the laboratories of the Manchester Sewage Works and depends on the fact that by carefully conducted precipitation either with hydrated alumina or ferric hydroxide it is possible to remove from solution all colloidal matter with results similar to those which are obtained by dialysis. Some measure of the oxidizable matters present, e.g. in a sample of sewage or effluent, either in the colloidal state or in true solution, is thus quickly reached.

Besides the hydroxides of aluminium and iron, hydrated copper oxide combines readily with albumin, and copper salts have been used on a large scale in the treatment of water supplies more especially with the object of preventing the growth of algae in reservoirs.

It is possible that the toxic action of copper in its various applications is due to the readiness with which insoluble compounds of copper and albumin are formed.

The qualitative tests which have been described show that on violently attacking albumin by such substances as strong caustic soda the presence of end products such as ammonia,

biuret and sulphuretted hydrogen can be detected. It is obvious, however, that such a procedure gives us but little information. Determinations by physical methods would indicate that the molecular weight of albumin is probably somewhere in the neighbourhood of 15,000. Its composition, according to ultimate analysis, can be expressed within the following limits :

Carbon . . . . .	50	to	55	per cent.
Hydrogen . . . . .	6.9	„	7.3	„ „
Nitrogen . . . . .	15	„	19	„ „
Oxygen . . . . .	19	„	24	„ „
Sulphur . . . . .	0.3	„	2.4	„ „

The information given by these figures is the same kind of information that would be obtained with regard to the construction of a watch if it was stated to be made up of a certain weight of glass and silver, of gold, of brass and of steel, together with a few precious stones. It is obviously necessary that in order to get some idea of the construction of the watch, it must be taken to pieces carefully, and each independent portion separately described. Similarly, in order to obtain even an approximate idea of the structure of the albumin molecule, means must be found to take it to pieces gradually and to identify the products thus obtained. In order to accomplish this two means are at our disposal, viz. in the first place the action of acids or alkalis, in the second place and especially, the action of so-called proteolytic enzymes, i.e. enzymes which are capable of breaking up protein substances. Of these two the chief are pepsin and trypsin.

**Pepsin** is the characteristic enzyme of the gastric juice. Ordinary "*liquor pepticus*" is prepared by macerating the mucous membrane of the stomach of a dog or pig with dilute hydrochloric acid, 0.2 per cent., and filtering the solution. The filtered solution contains pepsin.

By extracting with glycerine in absence of acid, a purer but less active product is obtained. The enzyme can be further purified by precipitation with sodium phosphate and calcium chloride, the calcium phosphate formed carrying down the enzyme. The enzyme is separated from the precipitate by solution in hydrochloric acid and the mineral salts removed



for the experienced organic chemist, and its details cannot profitably be discussed here.

The historic investigations of Emil Fischer and his colleagues have resulted in methods for separating and identifying amino acids, and in the synthesis of a number of polypeptides, whose complexity approaches in certain cases the complexity of the peptone molecule, and which are even capable of being broken down again into simple substances by the action of trypsin.

Certain American investigators have announced that they are able to synthesize peptone-like bodies by the action of trypsin on polypeptides. However this may be, it is clear that in this direction we must look for definite knowledge as to the ultimate structure of the albumin molecule or its derivatives, and a brief account of the chief products, separated or prepared by Emil Fischer and others, will be found interesting and valuable as affording a basis for the classification of the very numerous bodies relating to albumin.

The chief end products obtained by taking to pieces as it were the molecule of albumin may be roughly classified as follows :

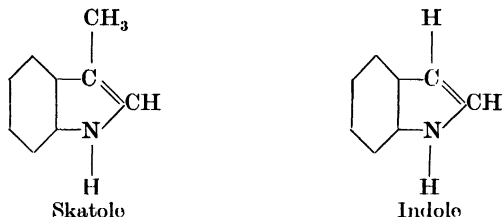
- Mono-amino acids.
- Hydroxy-amino acids.
- Di-amino acids.
- Amino-dicarboxylic acids.
- Sulphur derivatives.
- Purin bases.
- Ptomaines.
- Carbohydrates.

#### MONO-AMINO ACIDS

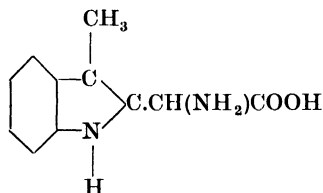
The following are the chief mono-amino acids :

1. **Glycocoll**, or amino-acetic acid,  $\text{CH}_2(\text{NH}_2)\text{COOH}$ . This is the simplest member of the mono-amino acids ; it is frequently termed *glycine* for the sake of convenience in describing its numerous derivatives among the polypeptides. Glycocoll is what is believed to be the mother substance of skatole, a substance occurring in excreta, the unpleasant smell of which is largely due to it. Skatole can be recognized in fresh sewage

by the pink colour which is obtained on warming with strong sulphuric acid. It has been shown to be methyl indole, the relation of the two bodies skatole and indole being shown by the following formulæ :



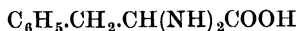
It probably occurs as a decomposition product of albumin in the form of skatole-amino-acetic acid,



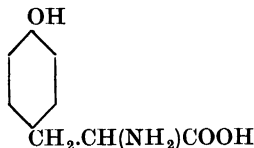
2. **Alanine**, or amino-propionic acid,



This acid has been shown by Emil Fischer to be widely distributed as a decomposition product of albuminoids. Its derivatives have, many of them, been well known for some time, especially phenyl-alanine, i.e.



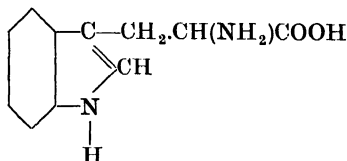
and para-hydroxy-phenyl-alanine, more commonly known as **tyrosine**,



Tyrosine is easily isolated on account of its sparing insolubility. It is one of the products of excretion of the animal body and occurs together with *leucine*.

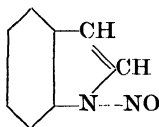
Other derivatives of alanine give rise to the very important substances indole and skatole already mentioned. The sub-

stance termed *tryptophane* has been isolated from the mixture of substances produced by the action of trypsin on albumin. It has been established by synthesis that tryptophane is an indole-amino-propionic acid of the following formula :



In this way we can see how indole may be produced by the decomposition of albumin substances. It is of interest to note that the formation of indole recognized by the red coloration it produces with nitrous acid is a characteristic reaction for certain bacteria, notably *B. coli*, and serves to distinguish this from the more dangerous typhoid bacillus. The *Cholera red* reaction given by the cholera organism depends also on the formation of indole.

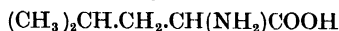
In both cases the red coloration is due to the formation of nitroso-indole,



formed by the action of nitrous acid on the *amino* group present.

### 3. Amino-valerianic acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ .

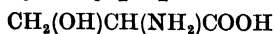
This acid is very interesting mainly on account of its derivatives the chief of which is known as *arginine*, to which reference will be made later, and *leucine* or *isobutyl- $\alpha$ -amino-acetic acid*, which has the following formula,



This is one of the earliest known of the decomposition products of albumin and readily crystallizes in scales or nodules with very characteristic appearance.

### HYDROXY-AMINO ACIDS

**Serin**,  $\alpha$ -amino- $\beta$ -hydroxy-propionic acid,



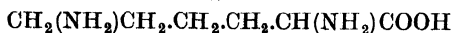
This acid is very specially interesting as being one of the chief decomposition products of silk. Emil Fischer has shown that it is a general product of the breaking up of albumin.

DI-AMINO ACIDS

There are three important di-amino acids which, according to Kossel, occur in all albumins in greater or less amount, and whose relative preponderance can therefore serve as a means of classification of albumin bodies. These three di-amino acids are termed by Kossel *hexone* bases, as they all contain 6 carbon atoms, and the basic character which is characteristic of all amino-acids predominates. These three substances are **arginine**, **lysine** and **histidine**. Arginine is guanidine- $\alpha$ -amino-valerianic acid; guanidine has the formula  $\text{NH}:\text{C}(\text{NH}_2)_2$ , and arginine is really a compound of guanidine with ornithine, a derivative of which is found in the urine of birds. Ornithine is a  $\alpha$ - $\delta$ -di-amino-valerianic acid, and arginine, therefore, may be written

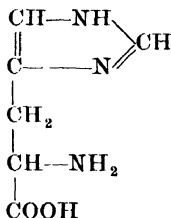


Lysine is a  $\alpha$ - $\epsilon$ -di-amino-*n*-caproic acid, i.e.



It occurs in greatest quantities in casein and gelatine.

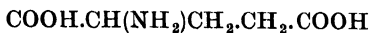
Histidine has a rather more complex constitution than either of the other two hexone bases. It is a condensation product formed by elimination of  $\text{NH}_3$  from arginine and its constitutional formula is :



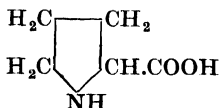
AMINO-DI-CARBOXYLIC ACIDS

Of these the following may be mentioned, **Aspartic Acid** or amino-succinic acid

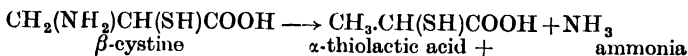
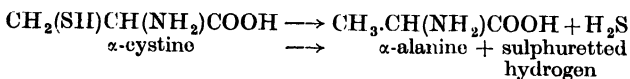


and **Glutamic Acid** or  $\alpha$ -amino-glutaric acid**PYRROLIDINE-CARBOXYLIC ACID OR PROLINE**

This has been obtained as a product of hydrolysis of casein and is of interest from the point of view of the synthetical experiments of Emil Fischer and others. It has the following constitution :

**SULPHUR BODIES**

**Cystine.** This is a very interesting substance as it is probably the parent body of the isomeric forms  $\alpha$ -cystine and  $\beta$ -cystine, which are very likely the parent bodies of the unpleasant-smelling sulphur derivatives of albumin. These two bodies are differentiated according to the products obtained when they are treated with hydrochloric acid under pressure, as indicated by the following formulæ :

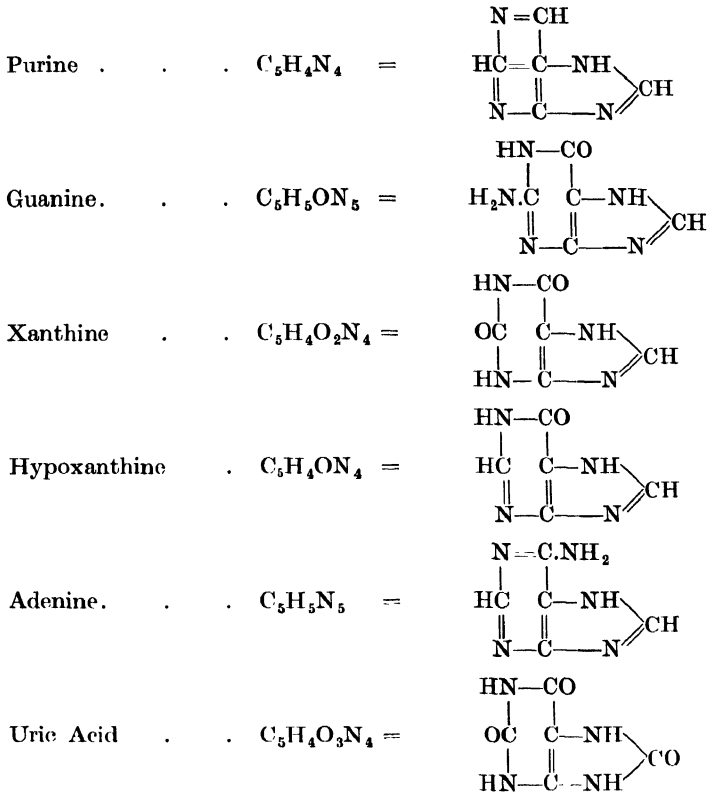


Probably both  $\alpha$ - and  $\beta$ -cystine contain at least two groups as given in the above equations, joined in each case by sulphur thus :

**PURINE BASES**

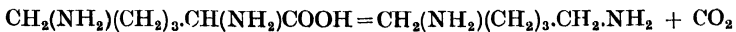
These important substances are obtained as decomposition products of nucleic acid, produced in its turn from so-called nucleo albumins. They are derived from a parent substance prepared by Emil Fischer, which he termed **purine**. The

relation of the purine bases to purine is shown by the following formulæ :

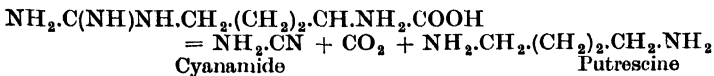


PTOMAINES

These bodies are products of putrefactive decomposition of albumin and are mostly strong bases, they can be obtained by splitting off  $CO_2$  from amino acids. Thus leucine gives rise in this way to pentamethylene-diamine or **Cadaverine** according to the following equation :

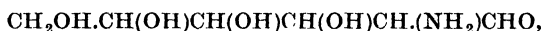


While arginine gives rise to *putrescine*, cyanamide being formed at the same time :



## CARBOHYDRATES

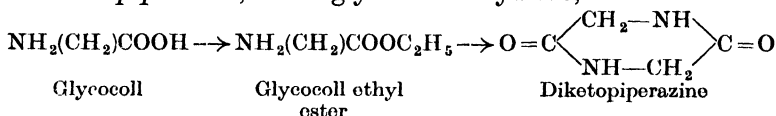
These occur among the decomposition products of certain albumins in the form of amino derivatives of which **glucosamine**,



is a characteristic example.

**Synthesis of Disintegration Products.** We are now in a position to understand something of the significance of the syntheses of the complicated bodies known as *polypeptides*, from the starting-point of the disintegration products which have just been described. It would lead too far to attempt to give these in any detail, but the simplest case will suffice to indicate the principle on which more complex substances may be built up. Glycocoll or glycine may be taken as a starting-point.

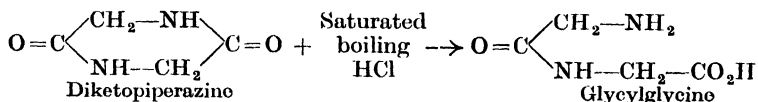
The ethyl ester is first prepared ; on long standing, condensation takes place, with formation of a ring compound known as *diketopiperazine*, or di-glycocoll anhydride,



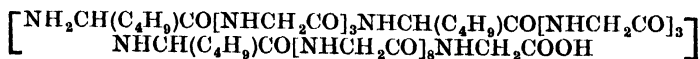
On saturating a boiling solution of this compound with gaseous hydrochloric acid, it is split up with formation of the simplest polypeptide, known as **glycylglycine** or



the group  $\text{NH}_2.\text{CH}_2.\text{CO}$  being termed *glycyl*. The reaction is expressed as follows :



It is readily seen that if glycylglycine is taken in its turn as a starting-point, and a similar set of reactions carried out, further similar complexes of higher molecular weight could be obtained. The most complex polypeptide so far synthesized has the constitution,





various substances leucine, tyrosine, aspartic acid, or lysine can be split off from such a complex. On oxidation with permanganate, these side chains are finally converted into oxalic acid and ammonia.

In ordinary animal metabolism, hydrolysis and oxidation go on together, with formation of urea as an end product. The constitution of individual albumins is by no means sufficiently well known to permit of a strict chemical classification according to their decomposition products. An attempt has, however, been made by Kossel, who divides albumins into four classes, according to their yield of the so-called hexone bases already referred to, viz. lysine, arginine and histidine. Kossel's classification was as follows :

1. *Protamines*, all rich in arginine, but differing in the amounts of other bases and of mono-amino acids.
2. *Histones*, Relatively high in arginine.
3. *Vegetable albumins*, Poor in arginine and no lysine.
4. All others containing all three hexone bases and most amino acids.

#### THE SEPARATION AND EXTRACTION OF ALBUMINS

It has already been seen while studying the precipitates of ordinary egg-albumin that it was possible to separate the albumin from the associated substance globulin by the insolubility of the latter in water. Further, it was found that whereas albumoses were precipitated by both alcohol and ammonium sulphate, peptones were precipitated by alcohol and not by ammonium sulphate. The method of precipitation by suitable salts and other substances, if carried out with care, can be used for separating the various albumins one from another ; such a process is known as *salting out*.

The salts chiefly used for preparations of the albumins are as follows, beginning with the least effective.

- Class I. Sodium chloride.  
           Sodium sulphate.  
           Sodium acetate.  
           Sodium nitrate.  
           Magnesium sulphate.
- Class II. Potassium acetate.

Class III. Ammonium sulphate.

Zinc sulphate.

The members of each class are more or less equivalent in precipitating power, but whereas sodium chloride will not precipitate egg-albumin, ammonium sulphate will not only precipitate egg-albumin but also its primary disintegration products, viz. albumoses.

Different albumins and their derivatives are precipitated within limits of concentration characteristic of the albumin, and the concentration and character of the salt used, and the separation of albumins one from another by fractional precipitation is thus an operation of considerable complexity.

In addition to the salts above mentioned albumins can be precipitated, as we have seen by colloidal metallic hydroxides.

Albumins also combine with numerous organic colouring matters, and advantage is taken of this in the various methods for staining tissues for microscopical examination.

For the precipitation of *peptones*, and to these may be added enzymes which we have seen have many of the properties of peptones and are allied to them in composition, substances such as phosphotungstic and phosphomolybdic acids may be used. Metaphosphoric acid, and also a mixture of potassium ferrocyanide, and concentrated acetic acid can also be used for precipitation of bodies of this class. Tannic acid also precipitates peptone bodies, and it is probable that the difficulty of extracting certain enzymes from plants depends on the fact that they exist in the plant in combination with tannic acid.

#### CLASSIFICATION OF ALBUMINS

We are now in a position better to appreciate the following classification of albumins and allied substances. Where the name of the substance does not indicate its source or characteristic properties, short explanatory notes are added.

**Albumins Proper.** These are naturally occurring substances and are all typical colloids :

#### GROUP I

1. Serum albumin, lact-albumin, egg-albumin.
2. Serum-globulin, lacto-globulin, cell globulin.

3. Plant globulin and vitellins.
4. Fibrinogen (occurs in the blood plasma of all vertebrates).
5. Myosin and allied substances (derived from muscle).
6. Phosphorus-containing albumins, caseins, vitellins and the protoplasts.
7. Protamines (these occur in the spermatozoa of fishes, etc.).
8. Histones (these do not occur in the free state but in combination with other complexes to form substances such as hæmoglobin).

#### GROUP II

##### **Disintegration Products of Group I :**

1. Acid albumins, alkali albumins.
2. Albumoses, peptones and peptides.
3. Halogen compounds of albumins, etc.

#### GROUP III

**Proteids.** These are compounds of albumins with other complex groups which have been termed prosthetic groups :

1. Nucleo proteids, compounds of albumin with nucleic acid.
2. Hæmoglobin and allied substances. (Hæmoglobin is the red colouring matter of the blood, and consists of an albumin compound with a prosthetic group, which in this case gives rise to colouring matter and is therefore called a chromatogenic group.)
3. Glycoproteids and mucins occurring in mucus. In this case the prosthetic group is a residue of a carbohydrate.

#### GROUP IV

**Abuminoids.** These are many of them rather ill-defined bodies which form part of the skeletal structure of the animal or plant organism. The classification is mainly anatomical.

1. Collagin, gelatine. (The substratum of bone and cartilage consists of collagen; on boiling with water it yields gelatine or glue.)
2. Keratin. (The chief constituents of the horny substances of mammals and birds.)
3. Elastin. (Occurs in certain fibrous animal tissue.)
4. Fibroin. (Occurs in raw silk.)

5. Spongin. (Forms the framework of the bath-sponge.)
6. Amyloid (a pathological product, sometimes found in the brain, liver, etc.).
7. Albumoids. (Sundry substances found in various animals, membranes, etc., difficult to classify.)
8. Colouring matters derived from albumins, e.g., melanin, the pigment substance of the skin of dark-skinned races.

It may be useful shortly to summarize the information in the foregoing chapter as follows :

*Albumins* or *Proteins* are complex nitrogenous colloidal substances occurring in animal or vegetable protoplasm, etc., and capable of being separated by their varying solubility in solutions of certain salts.

These yield on treatment with *dilute* acids or alkalis, solutions containing *acid* or *alkali*-albumins.

By heating with *acids* or by the action of *enzymes* such as *Pepsin* and *Trypsin*, albumins are gradually broken down, yielding successively :

1. *Albumoses*, precipitated by alcohol and by ammonium sulphate.
2. *Peptones*, precipitated by alcohol but not by ammonium sulphate.
3. *Polypeptides*, compounds which still give the biuret reaction, are capable of synthesis by the condensation of amino acids and can be further broken down to
4. *Amino acids*, and related substances known as primary disintegration products.

On account of the various constitutions of proteins the course of decomposition varies in different cases.

Moreover, besides the proteolytic enzymes already referred to, namely, pepsin and trypsin, there are others occurring in certain tissues of plants and animals which can be distinguished according to the conditions and range of their activity.

Thus *Erepsin* occurs in the intestinal tubes and is capable of decomposing casein, proteoses and peptones.

*Papain* which occurs in the latex of the fruit of the papaya (*Carica papaya*) is a powerful proteolytic enzyme attacking even living flesh.

## THE NUTRITIVE VALUE OF PROTEINS

It has long been known that the nutritive value of a protein was not dependent solely upon its quantity but upon its composition. It is on record that a French chemist in the early days of the nineteenth century, tried the experiment of feeding the people of Paris on gelatine obtained by boiling bones. The experiment was not a success. The true explanation of the failure had to await the results of the researches of the present century, which have shown that the nutritive value of albumins depends on the presence or absence of certain specific amino acids.

The investigations of Professor William C. Rose and his colleagues at the University of Illinois may be specially mentioned. Prior researches had shown that the gliadin of wheat was deficient in lysine, the zein of maize was practically devoid of lysine and tryptophane, and gelatine was seen to be lacking in tryptophane, tyrosine, cystine and iso-leucine.

Of the twenty generally recognized protein components Professor Rose states that the indispensable nature of only four has been positively established, viz. *lysine*, *tryptophane*, *cystine*, and *histidine*.

The importance of eight others is at the present time uncertain. Concerning the remaining eight, available information does not warrant their classification with respect to maintenance of growth.

Having regard to the prevalent vegetarian diet of India, the importance of thorough investigation of the vegetable proteins present in Indian food grains is obvious. According to McCarrison, whole wheat flour (atta) contains some suitable proteins but more that are less suitable. Oatmeal, barley, ragi, cholam, cambu, unpolished rice, peas, beans, dhal, gram, contain less suitable proteins. The proteins of wheat flour and maize are unsuitable. The best sources of protein for the vegetarian are milk, curds, buttermilk, cheese, green leafy vegetables such as spinach, lettuce, watercress and sorrel and the young shoots of edible plants. Careful and systematic studies are being made in the Department of Biochemistry at the Indian Institute of Science, Bangalore, of the composition of vegetable proteins occurring in Indian food grains.

Thus N. Narayana and Norris state that ragi contains an albumin, eleusinin yielding arginine, histidine and lysine, but not cystine.

The globulins of Bengal gram and horse gram were isolated and analysed, and it was found that the arginine content of the Bengal gram was much higher than that usually met with in the globulins of other Indian pulses. Both proteins were found to contain sufficient amounts of arginine, tyrosine and lysine, but were deficient in cystine and tryptophane.

P. S. Sundaram has investigated the proteins of the pigeon pea and finds evidence of two globulins, which he terms cajanin and concajanin. He is of opinion that no single seed is complete from the protein point of view, unless supplemented by either vegetable or preferably animal proteins.

The following tables, taken from an article entitled "A 4,000-Year Food Experiment," by William H. Adolph, Ph.D. (*Scientific American*, Dec., 1930), are of interest in the present connection :

TABLE I  
*Composition of the Chinese Dietary*  
(In percentage by Weight)

	China (North).	U.S.A.
Cereals and beans . . . . .	65	25
Vegetables and fruits . . . . .	27	20
Butter, fats, sugar . . . . .	1	14
Meat and fish . . . . .	4	18
Eggs . . . . .	1	5
Milk and cheese . . . . .	0	15
Other foods . . . . .	2	3

TABLE II  
*Composition of the Chinese Dietary*

Intake of protein, total energy value, and so on, per man per day

(Figures are for North China)

Total food . . . . .	1138.0 grams
Total protein . . . . .	86.4 "
Total fat . . . . .	34.1 "
Total carbohydrate . . . . .	537.0 "
Total energy value . . . . .	2794.0 calories
Weight of average man . . . . .	60.0 kilos.

TABLE III

*Meat Consumption of the Principal Countries of the World*

(Grams per capita per day)	
United States . . . . .	149
Great Britain . . . . .	130
France . . . . .	92
Belgium and Holland . . . . .	86
Austria-Hungary . . . . .	79
Spain . . . . .	61
Russia . . . . .	59
Italy . . . . .	29
Japan . . . . .	25
China (North) . . . . .	15

## BACTERIA AND PROTEOLYSIS

Any protein substance not immediately used by higher plants and animals is subject to attack by micro-organisms of all kinds.

The enzymes concerned are similar to those found in higher organisms, though they may vary to some extent according to the nature of the organisms concerned.

Thus yeast cells secrete an enzyme of a type similar to trypsin, while numerous bacteria are capable of breaking down protein in an acid medium.

If an ordinary plate culture is made from a small quantity of sewage the gelatine will be found to liquefy round several of the colonies; this liquefying action is not infrequently so rapid and intense that a few liquefying organisms will cause the whole plate to become liquid before the remaining colonies have time to develop. The method of separating the liquefying enzyme in this case from the bacteria has already been referred to and a considerable amount of research has been devoted in recent years to the detailed examination of the action of certain bacteria on toxic decomposition products of albumin.

Of particular interest in connection with nitrogen conservation are the researches by Messrs. Clark and Gage, of the Massachusetts State Board of Health. They compared some 300 cultures of sewage bacteria as regards their ability to produce ammonia in peptone solution, and to liquefy organic matter in the form of gelatine during an incubation period of 7 days.

The peptone solution consisted of 0·1 per cent. of Witte's peptone in distilled water which gave an organic nitrogen value (determined by the Kjeldahl method) of 14 parts nitrogen per 100,000. Nitrated peptone solution contained in addition nitrate equivalent to 10 parts nitrogen per 100,000. The liquefying power was determined by taking test-tubes of uniform bore filled to a depth of 100 millimetres with standard beef peptone gelatine. The entire surface was inoculated and the depth of liquefaction was measured after a given time.

The general result of these researches was to show that as a rule the liquefying power was synonymous with increased ability to reduce nitrates and to ammoniafy peptone.

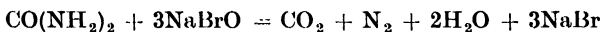
## CHAPTER IV

### THE CHEMISTRY OF AMMONIA FORMATION AND THE DECOMPOSITION OF PROTEINS BY MICRO- ORGANISMS

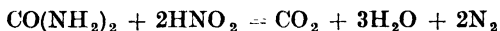
We have seen in the discussion on the chemistry of proteins that substances comprised under this term constitute the bases of both animal and vegetable living matter. We know that the nitrogen in our foodstuffs occurs mainly in the form of albumin, either animal or vegetable. A vegetarian, if he does not consume eggs, must add to his diet a considerable proportion of beans and peas which are rich in vegetable albumin. The actual amount of nitrogenous food needed for useful work is, as has been seen, a complicated question. Our present object, however, is to follow out the chemical history of the nitrogen, whether large or small in quantity, used as food. We have already learned that peptic and tryptic digestion of albumin leads by gradual stages to the formation of end products largely consisting of amino acids. These one would not expect to be excreted as such from the body, they are built up again into the body substance through biotic energy, and a portion also is used up as fuel for maintaining that energy, consequently therefore we do not find in the products of excretion of the animal body just those amino acids and polypeptides arising from albumin digestion by pepsin or trypsin under laboratory conditions.

Some of these substances, it is true, are found amongst the products of excretion, thus leucine and tyrosine have been mentioned as occurring under certain conditions in human urine, and ornithine is so named from its occurrence in the urine of birds. Skatole and indole are characteristic constituents of fœces. In the case of flesh-eating animals, however, by far the greater portion of the nitrogen which is not used up

in adding to or maintaining the body substance is excreted in the form of *urea* contained in the urine. Urea is a comparatively simple substance of which the chemical formula is  $\text{CO}(\text{NH}_2)_2$ . Chemically it is known as carbamide, being the amide of carbonic acid,  $\text{CO}(\text{OH})_2$ . The proportion of urea in the urine is, in fact, an index as to whether proper physiological equilibrium is being maintained, and its determination in the urine is a routine test in medicine. Its estimation depends on the fact that it is decomposed by sodium hypobromite with liberation of nitrogen according to the following equation :



Urea is also broken up in a similar manner by nitrous acid obtained by adding a mixture of sodium nitrite and sulphuric acid to the solution containing the urea. In this case nitrogen is evolved both from the nitrous acid and the urea in equal proportion according to the following equation :



This reaction is of far-reaching importance, since under certain conditions it may represent one method by which the nitrogen originally consumed as an albumin food finally reappears as free nitrogen.

In the case of animals whose diet is wholly vegetable, the greater part of the nitrogen is excreted as so-called **hippuric acid**, or benzoyl glycocol, which has the formula :



We have now to consider how these two main end products of nitrogen metabolism, viz. urea and hippuric acid, are reabsorbed in the cycle of nature. They are not in themselves directly available for plant food, and the first stage in their reabsorption by plants whose nitrogen may serve again as food for animals consists in their conversion into ammonia. That the conversion of urea into ammonia was a fermentation process and therefore due to life agency in some form, most probably to bacteria, was first suspected by Pasteur, and also by Van Tieghem. Subsequent investigation showed that numerous organisms can induce ammoniacal fermentation ; the most active of these is a micro-coccus known as *Micrococcus*

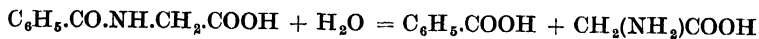
*ureæ*, and also a bacillus, *Bacillus ureæ*. These organisms are very widely distributed, and consequently urine if left exposed to the air very rapidly becomes ammoniacal, and the strong smell of an ill-kept urinal, or of ground which has become saturated with urine, is thus accounted for. In normal health it has been shown that the organisms causing urea fermentation are not present in freshly excreted urine.

It has been shown that ammoniacal fermentation of urea can take place both under aerobic and anaerobic conditions, the organisms of ammoniacal fermentation belonging therefore to the class known as facultative aerobes. This circumstance is of considerable importance in connection with the purification of sewage. It is rare that urea appears in quantity in sewage after it has passed through any distance of sewers, since it is very rapidly converted into ammonia.

The reaction which takes place is a simple hydrolytic change resulting in the formation of ammonium carbonate thus :



It has been found that the organism which brings about the conversion of urea into ammonia will also decompose uric acid with production eventually of ammonia, and hippuric acid, with formation of benzoic acid and glycine :



Like all other fermentations the ammoniacal fermentation ceases when a certain concentration is reached ; in this case the fermentation proceeds until the ammonium carbonate formed reaches a concentration of 13 per cent.

The ammoniacal fermentation belongs to an increasing number of changes which can ultimately be referred to the activity of a non-living enzyme.

In 1874, Musculus found that if ammoniacal urine was filtered through filter paper and the filter paper was washed and dried and afterwards placed in a neutral solution of urea, ammoniacal fermentation takes place. This also happened if the filter paper was washed with strong alcohol, showing that the activity was due to something other than the living organism. Although not absolutely conclusive, the evidence at present available indicates that the micro-organisms secrete an enzyme

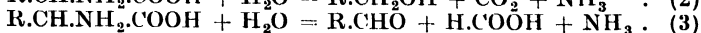
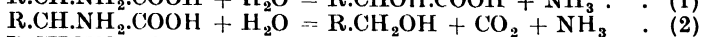
which has been termed *urease* ; it can be precipitated by alcohol and is destroyed by acids. Sheridan Lea, in 1885, obtained a rapid ammoniacal fermentation of a 2 per cent. solution of urea by incubating it at 38° C. with the alcoholic precipitate obtained from pathological urine.

The conditions for obtaining an active precipitate of this kind are not always easy to realize and consequently Sheridan Lea's conclusion that urease was soluble in water after the cells had been killed by alcohol, but that otherwise it was insoluble, is hardly conclusively established.

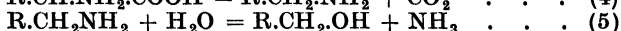
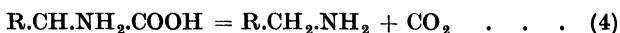
It has been shown by Takeuchi that very active urease occurs in soya bean, and Armstrong has employed this source of the enzyme in a series of researches concerned with the careful study of this type of enzyme action.

The various amino acids from protein decomposition can all evidently be broken down to simpler bodies by organisms which secrete enzymes of a character similar to urease. Enzymes which hydrolyze amides are known as amidases. Norris and Thakur have shown that certain fungi secrete an amidase capable of breaking down products of protein decomposition finally to ammonia. Subrahmanyam has also investigated the aminase present in various Indian and Rothamsted soils capable of breaking down amino compounds in the absence of living bacteria. The following summary of the more important reactions resulting in the formation of ammonia is based on Waksman's *Principles of Soil Microbiology*, page 481 and *seq.*

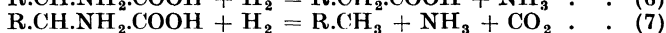
(1) **Hydrolytic Decomposition.** The hydrolysis of an amino acid may result in the formation of a lower fatty acid and ammonia, of an alcohol, CO<sub>2</sub> and ammonia, or of an aldehyde, lower acid and ammonia, as shown by the general formulæ :



These processes are carried out by various aerobic organisms, formula (2) is of common occurrence among bacteria, fungi and yeasts.

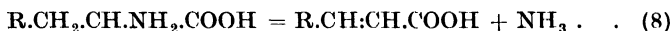
**(2) Decarboxylation :**

This process of amino acid decomposition through the amine stage with the formation of alcohol and ammonia has been described for yeasts and fungi; the first part of the process, namely the formation of amines, is characteristic of the so-called putrefaction process.

**(3) Reductive Deamination :**

This process of reduction is carried on by anaerobic bacteria which reduce the amino acids with the formation of saturated fatty acids and ammonia.

**(4) Anaerobic Bacteria** may produce ammonia from amino acids without reduction :

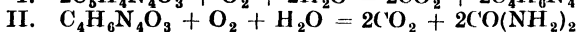
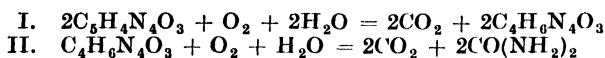
**(5) Oxidative Deamination :**

This process is carried out by aerobic organisms, especially by fungi.

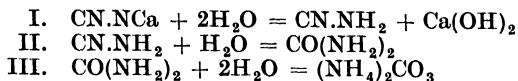
Aeration conditions have an important influence upon the nature of the products formed from the decomposition of the amino acids. Products formed under aerobic conditions such as hydroxy acids may prove unstable under anaerobic conditions and vice versa.

The acids formed in the process of deamination give rise to calcium salts. These are broken down to carbonates and the ammonia is oxidized to nitrates; the amines formed from decarboxylation are, however, more resistant to bacterial action. In the case of optically active acids both forms are attacked at equal or unequal rates.

The decomposition of uric acid is a little more complicated; in the following scheme it is first changed to allantoin, and then to urea.



The transformation of cyanamide takes place likewise through urea. The chemical substance contained in the fertilizer called *cyanamide* or *nitrolim*, is calcium cyanamide; this separates quickly in moist soil into calcium hydroxide and cyanamide. The latter is then rapidly changed by soil colloids to urea and this by bacteria to ammonium carbonate:



No bacteria but some fungi are known to be able to attack cyanamide directly; under natural conditions, however, the quicker action of soil colloids prevents their becoming active. The urea derived from cyanamide is not transformed by the typical urea bacteria but by various other kinds which seem to be less susceptible to the poisonous character of the intact cyanamide and of some of the by-products present in the commercial fertilizer. Among these a substance known as dicyanodiamide may be present, or may be formed by polymerization in the soil. This substance does not nitrify and is even toxic to nitrifying bacteria, but is not toxic to ammonia-forming organisms. For this reason it is necessary to exercise some care and judgment in the use of cyanamide as a fertilizer; time should be allowed for the necessary transformations to take place before it is actually needed as a plant food.

Among the other nitrogenous substances of interest found in the soil may be mentioned *chitin* which occurs as a constituent of the cells of micro-organisms, especially fungi, and is constantly added to the store of soil organic matter. It is a polymer of mono-acetyl-glucosamine giving acetic acid and glucosamine upon hydrolysis. Certain bacteria and actinomyces decompose chitin in the soil by means of an enzyme *chitinase*.

## CHAPTER V

### NITRIFICATION

The practice of nitre farming is of ancient origin, probably having been first brought to a state of proficiency in China, and thence imported to Europe in 1686. Judge Samuel Lewell copied upon the cover of his *Journal* a practical man's recipe for making saltpetre beds in which it was directed among other things that there should be added to it "mother of petre," that is soil from an old nitre bed.<sup>1</sup> Cavendish brought about oxidation of ammonia on surfaces in 1777. In order to produce nitre for the armies of France so-called *nitrières* were established in which organic matter was allowed to oxidize in presence of air and moisture, and with intermixture of arable soil, the last being found to be essential for the success of the process.

In 1838 Kuhlmann developed Cavendish's experiments and obtained nitrate of ammonia in presence of porous substances. Boussingault later showed that in the formation of nitrates from organic matter there was no absorption of nitrogen from the air as was supposed by some. He showed that there was no increase in nitrogen but only a change from a slightly diffusible and almost insoluble form to a completely soluble and very diffusible condition. Organic matter was only oxidized in presence of earth; when only sand was present no change took place.

It was Pasteur who first suggested that the oxidation of ammonia to nitric acid which evidently takes place in nature was really due to micro-organisms, and two French chemists, Schlösing and Muntz, actually proved that this was the case. They found that if solutions containing ammonia were allowed to percolate through soil which was well aerated at regular intervals, the ammonia was mainly converted into nitrate,

<sup>1</sup> *Farmers of Early Centuries*, by F. H. King, p. 252.

but that if any living energy in the soil was paralysed, e.g. by the introduction of chloroform vapour, or by other anti-septics, no nitrification takes place. The study of the conditions of nitrification has engaged the attention of a great number of workers both in England and on the Continent, and is of the greatest importance from the point of view of agriculture and the kindred subject of sewage purification.

The sequence of changes which takes place due to oxidation of organic matter to nitrates can be readily followed in the laboratory in the following manner :

10 c.c. of urine may be added to a litre of water in a Winchester bottle together with about a gram of good garden mould and the solution, which will occupy rather less than half the bottle, may be continually aerated either by drawing air through by means of a Bunsen water pump, or by attaching the bottle to a shaking machine.

At intervals of 2 or 3 days about 20 c.c. of the solution may be examined by suitable tests for ammonia, nitrites and nitrates.

It will be found that a progressive change takes place ; first of all a formation of ammonia will be noticed, with no nitrite or nitrate ; this attains a maximum, and then decreases with simultaneous appearance of nitrite, but little or no nitrate ; finally the nitrites disappear and there is left a solution containing only nitrate. During the course of the experiment the bottle should be kept as far as possible in darkness to prevent the formation of green algal growths which combine with the nitrogen of the ammonia or the nitrate and so confuse the progress of the reaction.

Experiments of this kind were carried out by Munro in 1886 who showed that practically all forms of nitrogenous organic matter were capable of undergoing this series of changes.

Munro's paper (*J.C.S.*, 1886, 49, 632) contains a very important observation in which the principle of "activation," which is the basis of the Activated Sludge Process of sewage purification and kindred operations, is clearly set out. Munro writes :

The less nitrifying ferment there is present the longer does it take

to multiply to an extent sufficient to attack the relatively enormous proportion of ammonia presented to it. But once the ferment is sufficiently developed, it will produce nitrification in a fresh solution *without* incubation, *provided* fermentable organic matter be absent (or perhaps if all reducing organisms can be rigorously excluded). When the nitrified solution last mentioned, e.g., was poured off the 5 grams of soil, and replaced by 150 c.c. fresh ammonium chloride of the same strength, nitrification commenced in less than 24 hours, and lasted 20 days instead of 30. When this was over the solution was poured away, the wet soil divided into 2 equal portions, and each of them covered with 50 c.c. ammonium chloride solution of half the former strength. The bottles were also heated to 80° to 85° F. (to one of them a little sterilized solution of Rochelle salt was added). The one without tartrate commenced nitrifying in *less than 7 hours* and finished in 9 days, here the suppression of incubation was complete.

Here Munro develops scientifically what the Chinese nitre farmer had long ago discovered in what he termed "mother of petre." Later in this volume the rediscovery of this principle and its application to what is known as the Activated Sludge Process will be discussed.

We are indebted to the labours of Warington for the exhaustive study of the conditions under which nitrification occurs.

He showed that the power of nitrification could be communicated to solutions which otherwise did not nitrify by inoculating them from solutions in which nitrification was taking place.

He further confirmed the results of Schlösing and Muntz by showing that nitrification could be inhibited by the introduction of antiseptics such as chloroform and carbon bisulphide.

The following were the conditions which Warington found to be essential for nitrification and his results are in harmony with those of other observers among whom may be specially mentioned Munro and Winogradsky.

1. It was found that phosphates are the essential element of the food of the organism of nitrification. In fact the very interesting observation was made that these organisms could thrive on purely inorganic material and even that the presence of organic matter appears to have an inhibiting effect. This question will be further considered in the light of more recent investigations.

2. The presence of oxygen is essential to the activity of the nitrifying organisms.

3. The presence of a base is also essential to neutralize the nitrous and nitric acids as they are formed ; at the same time there must not be an excessive alkalinity.

4. Like other organisms the nitrifying organisms have an optimum temperature of activity. They will produce effects at as low a temperature as 3° or 4° Centigrade (37° or 39° F.). They are fairly active at 12° C. (54° F.), but they work best at 37° C. (99° F.). Still higher temperatures begin to be prejudicial and like other organisms they are apt to be destroyed by strong sunlight. The latter circumstances, it may be mentioned, are believed by Clemesha to account for the absence of nitrates in certain surface waters in India.

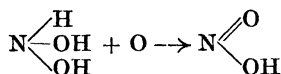
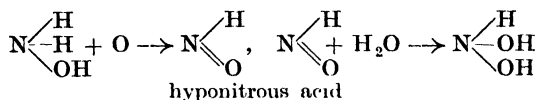
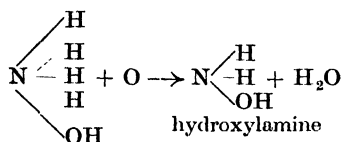
These facts have a very important bearing on the processes of agriculture, and especially also on those of sewage purification. The experiment which has just been described indicates clearly that nitrification proceeds in two stages, the ammonia being first oxidized to nitrite and then to nitrate.

Experiments made under the author's direction in the Frankland laboratory of the University of Manchester, and later at the Indian Institute of Science, Bangalore, indicate clearly that there is at least a third stage in the oxidation of ammonia to nitric acid. Thus Beesley examined the rate of nitrification of the following substances: urea, uric acid, asparagine, glycine, methylamine, acetamide, ammonium oxalate, ammonium sulphate. He employed dilute solutions of the various compounds in the presence of suitable mineral nutrients inoculated with nitrifying organisms, and allowed them to stand for many days, samples being taken at fairly long intervals ; aeration was carried on for a few minutes every week. It was found that all the various substances examined nitrified at practically equal rates, moreover the ammonia was evidently almost quantitatively converted into nitrate. At certain points on the curve of oxidation, however, there appeared to be an actual loss of nitrogen which was later recovered. This pointed to the formation of hydroxylamine, which was afterwards definitely detected in solution by Mumford.

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Beesley's results were also confirmed by a set of experiments made by Deo, in which it was shown that ammonia disappeared much more rapidly than the nitrous nitrogen made its appearance.

The theory of the progressive hydroxylation of ammonia appears to be accepted by recent workers, e.g. Kluyver and Donker (Waksman, p. 526), who assume the possible formation, in addition to hydroxylamine, of hyponitrous acid according to the following stages :



It has been suggested by workers at Pusa that the temporary apparent loss of ammonia during nitrification is due to its absorption by the organisms which convert it into protein and then re-excrete it as nitrate. Adeney, however, has shown that the amount of nitrogen required by the nitrifying organisms for their multiplication is infinitesimal, and it is hardly to be expected that the exact amount of nitrogen absorbed should in each case reappear.

Apart indeed from the direct evidence supplied by Mumford, the hydroxylation theory would seem most probable on the ground of analogy with other biochemical oxidations, for instance, of alcohol to acetic acid, while the classic experiments of Bone on the progressive hydroxylation of methane in contact with heated surfaces, lead to the same conclusion.

So far the biochemical oxidation of ammonia to nitric acid has been shown to be the work of at least two separate organisms.

Warington was not successful in separating either of these,

partly for the reason that nitrifying organisms will not grow on gelatine. Winogradsky in Russia, and Percy Frankland in England, independently made use of gelatinous silica as a means of cultivation ; the solution used with which the silica was gelatinized had the following composition in the case of the nitrous organism :

2.0 grams ammonium sulphate ;  
0.5 ,, magnesium sulphate ;  
2.0 ,, sodium chloride ;  
0.4 ,, ferrous sulphate ;  
1,000 c.c. of water.

The nitric organism is more difficult to isolate even than the nitrous, as it is much smaller. Winogradsky, however, succeeded in 1891 ; he made use of the following solution :

1 gram potassium hydrogen phosphate,  
 $\frac{1}{2}$  gram magnesium sulphate,  
Trace of calcium chloride,  
2 grams sodium chloride,  
1,000 c.c. water.

20 c.c. of this solution were placed in a flat-bottomed flask, and a little freshly washed magnesium carbonate added. The flask was closed with cotton-wool and sterilized. Two c.c. of a 2 per cent. solution of ammonium sulphate were then added and the whole inoculated with a little soil. When nitrate development had taken place sub-cultures were made on to silica jelly.

Frankland worked independently on similar lines to Winogradsky, and their researches have been confirmed by other investigators.

From the detailed work of Boullanger and Massol, it appears that there are two well-defined organisms which oxidize ammonia to nitrous acid ; *nitrosomonas*, which is a fairly large spherical organism, exists in two varieties, one the form usually found in Europe and the other being present in certain soils occurring in Java. There is also a similar form known as *nitrosococcus*.

The *nitric* organism is a very small bacterium whose length somewhat exceeds its breadth.

These two organisms, the nitric and the nitrous, work

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together in nature and neither can do its work without the help of the other ; the nitric organism is incapable of directly oxidizing ammonia and the nitrous organism cannot carry out oxidation of ammonia further than the stage of nitrite. A very important consequence of this differentiated action is seen in the changes which take place when sewage matters discharge into sea water ; the nitrifying organism under these conditions is either actually destroyed or rendered inactive. Dr. W. E. Adency gives the following figures for the results of spontaneous oxidation of sewage and comparative mixtures of sewage and fresh water and sewage and sea water respectively :

	Parts per 100,000.		
	Sewage.	Sea Water Mixture.	Fresh Water Mixture.
At Commencement.			
Nitrogen as ammonia . .	0.825	0.165	0.165
Nitrogen as nitrites . .	0.0	0.0	0.0
Nitrogen as nitrates . .	0.0	0.01	0.01
Organic nitrogen . .	0.675	0.135	0.135
-----			
At Conclusion.			
Nitrogen as ammonia . .	0.02	0.0	0.0
Nitrogen as nitrites . .	0.0	0.14	0.0
Nitrogen as nitrates . .	0.5	0.0	0.142
Organic nitrogen . .	0.5	0.072	0.076

The author confirmed these observations in experiments made for the purpose of tracing the changes taking place when sewage sludge is discharged into sea water. He found not only that the ultimate product of oxidation of nitrogen was nitrite rather than nitrate, but also that the actual oxidation of ammonia took place more slowly in sea water than in fresh water.

The same phenomena of the production of nitrite rather than of nitrate have also been noted in a case where sewage effluent was being discharged into a stream containing large quantities of calcium chloride from an ammonia soda works.

These observations have had some interesting confirmation in recent experience in India. In the absence of sufficient fresh water an installation for the purification of latrine sewage by the Activated Sludge Process at the Madura Mills, Tuticorin, could only be operated by means of sea water; nevertheless, excellent results have been obtained although oxidation could not be carried further than the stage of nitrite, large quantities of which were found in the effluent.

At Coimbatore, similarly, the water on the estate of the Agricultural College contains abnormal quantities of calcium and magnesium salts; nevertheless, satisfactory purification of the local sewage has been obtained in the new Activated Sludge installation. It remains, however, to be seen how far the presence of rather excessive quantities of nitrite in the effluent will affect its use when applied to the fertilization of crops. It is likely in such a case that under the conditions normally met with on the land the final oxidation to nitrate will be rapidly attained.

**Nitrification in presence of organic matter.** So far we have considered primarily the oxidation of solutions containing ammonium salts with no admixture of organic matter and with more or less pure cultivations of nitrous and nitric organisms. In nature, however, such conditions do not obtain, we have there to do with organic matter in different stages of decomposition and with mixtures of numerous organisms. The conditions under which the final nitrification then takes place have been worked out by Adeney in a series of careful researches. His method of research consisted in exposing solutions either of definite chemical compounds such as urea, asparagine, ammonium tartrate, etc., or less defined organic matter such as town sewage or infusions of peat to the prolonged action of oxygen in the presence of the usual organisms to be found in natural waters. This was accomplished either by mixing the solution with a known volume of aerated tap water, or by shaking periodically with known volumes of air. Not only were the products of decomposition and oxidation determined, such as ammonia and nitrous and nitric acid, but also the carbonic acid resulting from the oxidation of the carbonaceous matter present as well as the resulting change

in composition of the dissolved gases. For this purpose Adeney devised a special form of gas analysis apparatus which enabled him to analyse the gases obtained on boiling out the solutions *in vacuo*. He discovered the source of error in previous determinations, viz. the fact that the carbon dioxide formed by oxidation of organic matters, present largely as carbonate, is only fully recovered from the solution if the latter is acidified before boiling. The oversight of this fact led Sir Edward Frankland to conclude that the rate of oxidation, e.g. of sewage matter when discharged into a stream, was much less than was actually the case. As a result of prolonged investigation Adeney arrived at the following conclusions :

Oxidation of organic matter proceeds in two well-defined stages which may be briefly described as the *carbon* oxidation stage and the *nitrogen* oxidation stage.

In the carbon oxidation stage carbon dioxide, water, ammonia and excretory substances are produced. In the second or nitrogen oxidation stage the last-named bodies are further fermented, the products being nitrites, nitrates and comparatively small quantities of carbon dioxide. He confirmed the conclusions of previous observers by showing that in solutions of organic matter the nitrous organisms thrive while the nitric organisms lose their vitality. He also found that the nitrous organisms cannot carry oxidation beyond the stage of nitrite whereas the nitric organisms only oxidize nitrites to nitrates. He added a further important conclusion that the presence of peaty humus matter appears to preserve the vitality of the nitric organisms during the earlier stages of the fermentation process and to establish the conditions whereby it is possible for the nitric organisms to thrive simultaneously with the nitrous. The latter conclusion has an important bearing on the oxidation of organic matter in nature, and especially under the controlled conditions which obtain in modern processes for the biological purification of sewage.

In the researches of Beesley already referred to, in addition to the important conclusions as to the rate and character of the oxidation changes which take place when nitrogenous substances are fermented to nitrate, one or two interesting

exceptions to the general course of reactions are worth recording as throwing light on possible changes which protein matter may undergo when subject to the disintegrating influences present, e.g. in nightsoil. Beesley's work confirms the ready nitrifiability of most organic nitrogen compounds containing amino groups. Researches by Ardern and Lockett, working under the author's direction in the laboratory of the Manchester Sewage Works, showed also that ammonium sulpho-cyanate could be readily oxidized to nitrate and sulphate. On the other hand Beesley, and later Mrs. Mumford, showed that thio-urea was entirely unaffected under similar conditions. This would seem to indicate a possible method of utilizing bacteria as reagents for determining the differences in constitution between isomeric compounds.

The case of aniline is also of interest as indicating the possible course of oxidation of protein decomposition products containing a benzene ring. Beesley found that although change took place under the influence of bacterial action resulting in the production of ammonia, no nitrification occurred during the period of the experiment. It was assumed that simple hydrolysis took place resulting in the production of ammonia and phenol, but that the latter is destroyed by bacterial action as soon as formed as it could not be detected in solution. The possibility of the bacterial oxidation of phenol had been shown previously by Ardern, Lockett and the author.

The researches of H. N. Batham contribute important facts concerning the relative nitrifiability of protein decomposition products in the soil. His method was to mix solutions of the various compounds studied each containing 15 mgm. of nitrogen with 100 gm. portions of air-dried soil, together with 0.5 gm. of pure calcium carbonate and sufficient sterile distilled water to afford the optimum moisture content. The mixture was incubated at room temperature for 30 or 40 days. The moisture content was maintained throughout the incubation period, and two controls were simultaneously observed, one containing equivalent nitrogen in the form of ammonium sulphate and one without added nitrogen.

He found that ammonium sulphate was more effectively nitrified than any of the amino acids. Tryptophane contain-

ing both ring and chain nitrogen was more readily nitrifiable than compounds containing only chain nitrogen. Amino acids belonging to the mono-amino mono-carboxylic acid series are nitrified at about the same rate, the nitrification being apparently independent of the nitrogen-carbon ratio. This is confirmatory of Beesley's results.

Tyrosine and cystine are less easily oxidized than phenylalanine, leucine,  $\alpha$ -alanine and tryptophane. The presence of sulphur in cystine appears to depress the nitrification of its nitrogen.

Batham further states that the amount of nitric nitrogen in the soil fluctuates regularly, and the rise and fall of the nitrifying bacteria take place at different periods of the year. The nitrifying bacteria remained active in the soil even when kept in stock for more than a year.

**Surface aeration.** In all researches on the nitrifying organisms which have been referred to here, the conditions have been essentially those of the laboratory where the solutions of organic matter have been exposed to air, so to speak, in bulk, either by simple exposure of the solution in a flask, by shaking with air, or by bubbling the air through; the element of surface action has been brought only partially into play. In the case, e.g., of Batham's soil mixtures, the surface action between the soil particles and the oxidizable material was essentially static. It is clear on reflection that if the solution to be nitrified could be passed in a thin film over a large surface with free circulation of air, the conditions for oxidation would be very much more favourable. For not only would the presence of ample oxidation be assured, but also the extended surface would afford a substratum for a greatly increased development of the necessary organisms. It was the application of these principles which led to great developments in the art of sewage purification.

In 1869 Sir Edward Frankland, acting on behalf of the Royal Commission on Sewage Disposal then sitting, made his classical experiment on the so-called intermittent filtration of sewage through soil. He employed cylinders 15 feet in height filled with sand or earth and dosed them with definite quantities of sewage, allowing intervals of aeration between each

dose. By this method he was able to purify much greater quantities of sewage on a given surface area of soil than by the so-called broad irrigation processes formerly in vogue. At that time, however, the true explanation of the oxidation change which took place was not properly understood and it was considered to be a purely chemical phenomenon. Later on the Massachusetts State Board of Health took up the subject in the light of the researches of Warington, Winogradsky and Percy Frankland, and they worked out the conditions for the successful oxidation of sewage matter by percolation through sand filters. They showed that the results depended essentially upon the presence of oxygen, and upon the time allowed for the change to take place. They confirmed Warington's conclusion that it was necessary for a base of some kind to be present to combine with the nitrous and nitric acid produced by the oxidation of ammonia; all other conditions, they considered, were secondary to these three.

It was Stoddart who showed in 1893 that the time factor could be gradually decreased if filters of more open material than sand were used and if care was taken to distribute the nitrifying solution in such a way that a thin film only was exposed to the action of the air. By allowing a solution of ammonium carbonate (one part nitrogen in 10,000) to drip on to a column of coarsely powdered chalk properly inoculated with nitrifying organisms, he was able to obtain highly efficient nitrification.

This experiment of Stoddart's is really the original of the modern trickling or percolating sewage filter.

Scott Moncrieff, in 1898, by employing superimposed trays of filtering medium for the final purification of sewage which had undergone preliminary ammoniacal fermentation in a so-called "cultivation tank," obtained a high degree of nitrification. A very interesting result was also demonstrated by these experiments, viz. that the nitrification was a progressive phenomenon, and its course was interfered with considerably if after it had once been established the sequence of the trays was altered, the lowest tray, e.g., being substituted for the highest, in which case the nitrification was considerably impeded until the original conditions were re-established.

The bacteriological conditions obtaining in sewage filters of this description were worked out later by Boullanger and Massol at the Pasteur Institute at Lille, by Schulze-Schulzenstein in Germany, and by Dr. Harriette Chick of the Lister Institute. All these investigators agree that the nitrifying organisms found in ordinary sewage filters are the same as those which occur in soil. Boullanger and Massol found an explanation for the seeming discrepancy between the results of Winogradsky and those which are obtained on sewage filters. According to Winogradsky, it will be remembered, the activity of nitrifying organisms is inhibited by the presence of ammonia or of organic matter. Boullanger and Massol concluded from their experiments that while the presence of large quantities of ammonia or of organic matter may impede the original development of the nitric organism, yet if the growth of this is once established its activity is unaffected by these conditions. These results are in harmony with Adeney's conclusion that the presence of peaty matter is of assistance in maintaining the activity of the nitric organism. In a sewage filter the extended surface enables an abundant growth of nitrifying organisms to take place; at the same time it is well known that if the maximum load of sewage matter is put upon the filter in its early stages before nitrification is established, it is difficult, if not impossible, for the right conditions to be set up later. It is consequently necessary to "ripen" the filter by putting on only comparatively small quantities of sewage at first, increasing the quantity as nitrification becomes established.

Dr. Chick found that in sewage filters as in the experiments with solutions the nitrification took place in two well-defined stages, first nitrites and then nitrates being formed. The length of time required for complete nitrification to become established depended on the amount of ammonia present either actually as ammonium carbonate or potentially as unfermented organic matter in the sewage applied. Temperature also has a marked effect, as might be expected, in determining the time necessary for nitrification to be established; for this reason it is advisable always if possible to bring new sewage filters into work during the warm months of the year.

Finally, mention may be made of the importance of the

character of the material used in the construction of the filter beds. Practical experience has shown that better results are obtained with a medium which offers a maximum of surface ; thus irregular material such as clinkers gives better results than when a smoother material such as gravel is used. Experiments by Percy Gaunt and the author showed that in addition to the effect of surface in giving an extended habitat for bacteria the majority of vesicular or porous materials have the power to a greater or less extent of retaining ammonium salts either in their smaller pores or in their larger interstices. Such materials therefore afford a somewhat longer time for the nitrifying action to take place, when a solution containing ammonium salts is brought into contact with them.

The purely physical side of this question has also been carefully investigated by W. Clifford ; he allowed known amounts of water to trickle through media of different kinds and dimensions and at different rates. When equilibrium was established between the rate of flow and outflow, a known amount of sodium chloride solution of known strength was run on to the filter. The amount of chlorine emerging from the medium was determined at definite intervals. He afterwards allowed the medium to drain and measured the amount of drainage water and finally dried the medium and determined the loss of moisture. He thus measured for each class of medium :

- (a) The amount of water passing through in a given time.
- (b) The amount of water held in the large interstices.
- (c) The amount of water retained in the pores.

This experiment showed generally that the time of percolation through clean filter material varies inversely as the rate of application of the water, and directly as the amount of water taking part in the water movement through the bed. This latter obviously depends on the size of particles and the physical character of the medium.

These results find expression in the following formula :

$$c = \frac{I}{RT}$$

Where  $c$  is a constant,  $I$  the interstitial water per cubic yard,  $R$  the rate of sprinkling per square yard per hour, and  $T$  the average time of trickling through 3 feet of medium.

Experiments in the laboratory of the Manchester Sewage Works showed that when a nitrifying solution made up after Winogradsky's recipe is allowed to drip on laboratory filters composed respectively of quartz particles about  $\frac{1}{8}$  inch in diameter and of broken clinker of the same dimensions nitrification is established much more rapidly in the case of the clinker medium than in the case of the quartz.

### Intensive Nitrification.

Ammonia can be readily oxidized to oxides of nitrogen and finally to nitric acid by passing the gas over a surface of heated platinum gauze. In this way nitric acid is formed from the ammonia obtained by combining atmospheric nitrogen with hydrogen by the Haber process.

While natural or biochemical processes can hardly operate at such high concentrations, yet by careful regulation of the conditions of nitrification, concentrations of nitrate much higher than normal can be obtained, and the study of these conditions is of considerable theoretical and practical interest.

Reference has already been made to the use of so-called *nitrières*, in the time of the Napoleonic wars, these being based on the old-world practice of nitre farming.

The first experiments on modern lines directed to obtain in this way large yields of nitrate capable of commercial application appear to be due to Muntz and Lainé (*Ann. de la Science Agronomique*, 1906). They stated that the nitrifying organisms operating at a temperature of 30° Centigrade were active up to a concentration of more than 1 per cent. of ammonium salts.

Muntz and Lainé considered peat to be the best medium on which to carry out the nitrification process, and they claimed to be able to nitrify per day and per cubic metre 1,000 litres of solution containing 7.5 grams ammonium sulphate per litre. They finally obtained a solution containing 5 or 6 per cent. of calcium nitrate per hectare of peat filter per day.

These experiments were taken up and developed with great care by E. Boullanger (*Ann. de l'Inst. Pasteur*, 1921, 35, 575 and 1922, 36, 395). Boullanger's experiments were carried out both in the laboratory and on a semi-technical scale, using

other media besides peat, the most satisfactory of which was found to be natural pozzuolana in granules the size of cherry stones.

Boullanger's conclusions are of great interest and value, and the more important of them may be noted here. It is necessary to begin the process of intensive nitrification very gradually as the nitrifying organism is paralysed by excess of ammonia. Nitrates also have an inhibitory action at the beginning of the process and therefore ammonium sulphate was used as raw material at the outset, and ammonium nitrate only later. This salt was obtained either by direct solution in water or by double decomposition of ammonium carbonate with calcium nitrate formed by the process. It was found best to use bicarbonate or sesquicarbonate of ammonia rather than sulphate, and so avoid formation of insoluble calcium sulphate.

The strongest solution obtainable was found to be 138.2 grams of calcium nitrate per litre, and this was slightly inhibitive. A regular output was obtained with 120 grams, i.e. 12 per cent. calcium nitrate.

A very careful and special technique, for details of which the original paper must be consulted, was necessary to obtain these results, the underlying principle being to maintain the necessary minimum of ammoniacal nitrogen while allowing gradually increasing concentration of nitric nitrogen. In order to obtain this concentration it was necessary to dissolve each fresh addition of ammonium nitrate in a portion of the liquor from the previous operation, fresh water being added equivalent to the amount of nitrate solution withdrawn for recovery of the dissolved nitrate.

All the conclusions reached in the laboratory were verified on the semi-industrial scale. If the proper conditions are fulfilled liquors can be obtained containing 11 to 13 per cent. calcium nitrate by the use of solutions containing 15 to 18 grams nitric nitrogen and 1.5 ammoniacal nitrogen per litre. Under these conditions about 50 grams of nitric nitrogen per day, corresponding to 286 grams ammonium nitrate or 293 grams calcium nitrate, can be obtained per cubic metre of medium. A nitrifier of one hectare with a working depth of 1.8 metres or 18,000 cubic metres would give per 24 hours

rather more than 5 tons of ammonium or calcium nitrate. This is about one-eighteenth of the figure given by Muntz and Lainé whose results could not be maintained in continuous practice.

Boullanger gives a detailed financial estimate of the cost of nitrate production in this way and arrives at a price of 2,200 francs per ton of ammonium nitrate, the ordinary price at that date (1922) being 1,600 francs. Except as a war emergency, therefore, the process could not be considered economical.

Joshi (*Agri. J. of India*) has made a number of interesting experiments at Pusa on the use of an intensive nitrifying bed as a means of preventing nitrogen losses from cattle urine. Joshi's results are somewhat better than Boullanger's, probably owing to the nitrogen in the urine being in a form more readily attacked by micro-organisms than in the simple ammoniacal solution used by Boullanger. He was able to oxidize a solution containing 1.5 grams ammoniacal nitrogen per litre at the rate of 100 litres daily per cubic metre of medium or 0.15 grams ammoniacal nitrogen daily per litre of space, which is higher than the figure given by Boullanger. In the hot weather Joshi reached the figure of 0.2 gram ammoniacal nitrogen oxidized per litre of space.

Since the discovery of the Activated Sludge Process, a number of experiments have been made to see how far it is possible to accelerate the process of nitrification by operating on the principle of this process.

At this point, therefore, a short description is necessary of what is known as the *Activated Sludge Process*. The oxidation of ammonia so far referred to has been brought about either by the slow absorption of air from the surface of solutions, by static oxidation at the surface of moist soil particles, or by the passage of solutions over surfaces of various kinds so as to obtain more intimate contact between the air and the liquid.

The Activated Sludge Process differs from any of the above methods in that the organisms concerned with nitrification are moved through the solution to be nitrified instead of passing the solution over a fixed surface on which the organisms

were developed. The Activated Sludge Process was developed in Manchester under the author's direction, with the assistance mainly of Messrs. Ardern, Lockett and Mumford.

At that time the important observations of Munro were not consciously present to the mind, although his paper had been studied years before ; actually it was his principle which was made use of in the pioneer experiments of the Activated Sludge Process. The history of these is given in some detail later in Chapter IX. These experiments showed that if air is allowed to bubble through a sample of sewage a number of changes progressively take place.

The first to be noticed is the gradual conversion of the colloidal matter which gives the turbid appearance to sewage into brown granular particles which readily settle, leaving a sparklingly clear liquid. At the same time the progressive change already referred to from ammonia to nitrate also takes place.

The complete oxidation of sewage by the action solely of air in conjunction with the bacteria originally present in the sewage occupies a considerable time, probably many days, and for this reason the purification of sewage by air alone was for a long time deemed impracticable. It was found, however, that if the brown granular deposit which forms is allowed to settle out from the liquid and the latter decanted away in accordance with Munro's experiment and a further quantity of sewage aerated in contact with the brown deposit and this process repeated, the brown deposit being retained at the end of each operation, then as the quantity of the deposit increases the time required for the purification of the sewage decreases. Finally, when the deposit has accumulated to the extent of about one-fifth of the total volume of the sewage the latter can be purified in a few hours' time and the process becomes a practical proposition. The brown deposit is termed *Activated Sludge*.

In the early years of the development of the process a good deal of laboratory work was done in the Manchester Sewage Works and in the Frankland Laboratory of the Manchester University, some of the later experiments bearing on the present subject of intensive nitrification.

The experiments of Beesley already referred to were repeated by intensive aeration in the presence of Activated Sludge.

For these and other laboratory experiments where the effect of Activated Sludge upon sewage or other substances has to be studied, and fairly large volumes employed, the sludge is suspended in water contained in an earthenware cylinder with a porous false bottom through which air can be forced under pressure in small bubbles. A modification of this apparatus was later devised in which discs of porous material could be inserted in inverted bell jars, any crevices in which sludge might possibly settle being filled up with wax.

It will be seen that the sludge being thus kept in constant motion by the stream of air bubbles presents a constantly changing surface and an intensive bacterial action results.

The experiments in the Frankland Laboratory were carried out by Mrs. Mumford. Urea, hydroxylamine, glycine, asparagine, uric acid, hippuric acid were all readily nitrified by Activated Sludge in presence of sufficient calcium carbonate to neutralize any acidity produced, and in most cases no appreciable loss of nitrogen took place, in so far that the greater part of the nitrogen originally present in solution was recovered as nitrate. An exception occurred in the case of hydroxylamine, and in one case with ammonium sulphate loss of ammonia took place when an excess of calcium carbonate was added to the solution.

Further experiments with activated sludge, designed if possible to obtain concentrations of nitrate which would be technically recoverable, were carried out later in the Department of Biochemistry in the Indian Institute of Science, Bangalore. The experiments were started in 1920 before the publication of the results of Boullanger or of Joshi, and although a number of interesting results were obtained in the early stages it was not until a careful study of Boullanger's paper had shown some of the necessary conditions for success that results of technical importance were reached.

The earlier experiments were undertaken by M. B. Roy with the special object of obtaining some quantitative knowledge of the effect of various substances, such as might be present in the soil, on the nitrification process. Among

these the following may be mentioned : sulphates of manganese, iron, calcium, magnesium and potassium, and solutions of glucose and peptone. It was found that both peptone solution and iron salts considerably increased the activity of the nitrification process as compared with the blank. Manganese accelerates the process up to a point and afterwards exercises a depressing effect. Potassium salts exercise a depressing effect ; glucose may be almost said to inhibit nitrification owing doubtless to its affording favourable conditions for the activity of denitrifying organisms.

It is reasonable to conclude that the stimulating effect of peptone is due to its furnishing necessary food for the organisms and also in the possibility of its decomposition products being capable of conversion into nitrates.

The action of iron salts is doubtless catalytic and has been confirmed in subsequent experiments by Norris and Ranganathan.

The experiments with calcium sulphate were extended to a concentration of 14 per cent. in the solution under experiment without any appreciable effect on the rate of nitrification : this is of interest in connection with the use of ammonium sulphate as a fertilizer on limed soils.

In view of the statement by Muntz and Lainé that the nitrifying organism will tolerate concentration of nitrate up to 20 per cent., this amount of calcium nitrate was added to the nitrifying solution, with the result, however, of immediately inhibiting the process.

A series of experiments was undertaken later by Y. N. Kotwal to ascertain whether by the application of the Activated Sludge principle, i.e. the circulation of appropriate bacteria in presence of air through a solution of substances to be nitrified, solutions of nitrates could be produced sufficiently concentrated to make the recovery of the dissolved nitrates by evaporation economically possible.

A further attempt was made to nitrify ammonia in presence of high concentration of nitrate by bubbling air through a 15 per cent. solution of calcium nitrate containing an efficient activated sludge in suspension, and adding gradually a 4.5 per cent. solution of ammonium hydrate. No evidence of nitrification either by loss of ammonia or by development

of nitrite was obtained. This result confirmed the observation of Roy, but it is a little difficult to understand in view of the fact that under natural conditions nitrification appears to take place in slightly moist soils with the production of actual crystals of nitrate. Some condition in nature evidently remains to be investigated. It may be that the ammoniacal fermentation of urea takes place slowly and ammonia is oxidized *pari passu*.

Shortly after this failure the papers of Boullanger came to hand and were studied, and on proceeding further on the lines therein described, positive results were obtained. The general method of experiment was as follows :

500 c.c. of activated sludge in good "condition" was mixed with 1,500 c.c. of effluent in a tall narrow graduated cylinder of 2,000 c.c. capacity. Air was drawn through by means of a filter pump, and the solution to be nitrified slowly added from a tap funnel.

Under the best conditions Kotwal succeeded in obtaining a slightly higher rate of nitrification than that reached by Joshi. Joshi was able to oxidize 0.2 grams of ammoniacal nitrogen in 1 day per 1 litre space, while in his later experiments Kotwal obtained a rate of 0.25 grams ammoniacal nitrogen per litre per day.

The subsequent experiments of Norris and Ranganathan seem to indicate that this high rate is not maintained as the concentration of nitrate in the solution increases. Ranganathan confirmed the observation of Roy on the beneficial action of traces of iron salts, but the maximum concentration of calcium nitrate obtained in the laboratory was 7.5 per cent.

It was noted that the rapid rate at which oxidation is brought about by activated sludge is due to two causes. In the first place, the extended surface at which the oxidation can occur owing to the flocculent state of the sludge, and secondly, the oxidizable material probably absorbed on the sludge particles is brought into immediate contact with relatively large quantities of oxygen by the aeration employed.

It was not therefore surprising to find that the rate of nitrification in an activated sludge suspension could be increased by adding substances such as silt or charcoal which increased

the area of the oxidation surface. Experiments with silt, animal charcoal and ordinary charcoal indicated that animal charcoal assisted the process to the greatest extent, in fact animal charcoal alone was able to bring about some nitrification even in absence of activated sludge, the relative efficiencies being as follows :

Sludge alone (control)	.	.	.	.	.	100
Sludge and animal charcoal	.	.	.	.	.	136.5
Animal charcoal alone	.	.	.	.	.	82

These experiments bear out the results of Warburg and others in so far as they demonstrate the power of animal charcoal to bring about biological oxidations. Working with cystine Warburg showed that animal charcoal alone could oxidize this substance to ammonia and sulphuric acid. He did not, however, obtain any further oxidation of the ammonia to nitrite or nitrate such as was observed in the experiments just described.

Contrary to the contention of some investigators that thrice or even more times the theoretical amount of calcium carbonate is necessary for efficiency, nitrification experiments indicated that no advantage at all resulted by increasing the amount of calcium carbonate above that theoretically required to neutralize the acid produced in the oxidation ; on the other hand, high amounts tended to bring about a loss of nitrogen and were therefore detrimental.

Special observation showed that the nitrifying organisms thrive on media of  $P_H$  ranging from 5.8 to 8.0 but functioned best when the  $P_H$  lay between 7.0 and 8.0.

The outstanding result of this experimental work is to show the necessity in the first place for very carefully cultivating organisms of special activity, and also for the maintenance of certain limiting conditions of concentration, if the most satisfactory results are to be obtained. These have great importance in connection with the practical technique and economical working of Activated Sludge plants employed for the purification of sewage, especially where highly concentrated liquors have to be used. The possibility is also indicated of employing activated sludge tanks for producing nitrates from

various nitrogenous raw materials as a preliminary to their application to the purposes of agriculture.

While these researches, especially the observations of Ranganathan on the effect of animal charcoal, show that biochemical oxidation of ammonia to nitrate can be accelerated by the presence of catalysts, and while it has been shown by Dhar and Gopala Rao and others that a certain amount of oxidation of ammonia can take place by photo-chemical action in presence of certain catalysts, yet the quantity of nitrate formed in Nature by chemical agencies alone is, in the author's opinion, insignificant and of little importance in the soil.

## CHAPTER VI

### DECOMPOSITION OF NON-NITROGENOUS ORGANIC MATTER BY MICRO-ORGANISMS

We have seen how complex organic matter known as protein or albumin is broken down by various stages to ammonia, and how the latter is oxidized to nitrate. We have now to consider the possibilities of the reverse change whereby nitrate may be reduced to ammonia with possibilities of liberating nitrogen in the free state. Further, we shall have to study the biochemical process by which this free or gaseous nitrogen can be brought back into the cycle of life. To understand these changes it will be necessary to consider the non-nitrogenous organic substances which take part in them since it is by interaction with these substances that the transformations of nitrate into gaseous nitrogen, and gaseous nitrogen back to protein, are brought about. The ratio of carbon to nitrogen is indeed of first importance among the factors which determine the general course of bacterial action.

The framework of the vegetable world, broadly speaking, consists of what is generally known as cellulose, and when the vast quantity of vegetable matter on the face of the globe is considered, a knowledge of the changes which accompany its decomposition and absorption into the cycle of life is seen to be necessary for the proper understanding and control of the processes of nitrogen conservation. Closely associated with cellulose are a number of substances which also play an important part in variation of structure or anatomy. We shall, in addition, have to consider non-nitrogenous substances such as starches and sugars, tannins, oils, fats and waxes which are products of the metabolic changes continuously occurring in the living plant.

Cellulose and its allied bodies may be looked upon as constituting the bones of the vegetable world. Starches, sugars,

tannins, oils, fats and waxes, together with protein and its derivatives, may be looked upon as the flesh.

In considering the changes which accompany the life cycle of the plant we may take these two broad divisions separately, though, from the point of view of our main theme, the conservation of nitrogen, it is probable that the changes which are undergone by cellulose and allied compounds are the more important if for no other reason than that they constitute a much larger portion of the total weight of non-nitrogenous matter available. We will first deal in outline with the more purely chemical characteristics of the various materials under consideration, and afterwards examine the processes by which they fulfil their function in the cycle of life.

#### THE CHEMISTRY OF CELLULOSE

The structural constituents of plants can be classified into five general groups :

1. *True celluloses* not acted upon by dilute acids.
2. *Hemi-celluloses* readily hydrolyzed by dilute mineral acids.
3. *Pectins*, different in composition, but related to the hemi-celluloses.
4. *Lignins*, forming complex physical absorption compounds or mechanical incrustations with celluloses which constitute the woody tissues of plants.
5. *Corky or cutinized lamelle*, not well defined chemically.

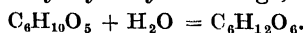
The woody parts of the plant or the plant cell membranes are usually referred to as "crude fibre." This is made up of cell walls composed principally of cellulose ; they are accompanied by various other substances dependent upon the nature of the plant and the difference in the amount and kind of impurities present. In the case of cotton, flax and hemp, pectin forms the chief impurity. In the case of straw, wood and jute, lignin is the impurity, hence the terms Pecto-Cellulose and Ligno-Cellulose.

Cellulose can be obtained as a residue after dissolving out the other constituents of plants. For this purpose a solution of powdered chlorate of potash in cold nitric acid of specific gravity 1.1 is used (30 grams of the salt to 520 c.c. of nitric acid), when any vegetable debris, leaves, stems, etc., are allowed

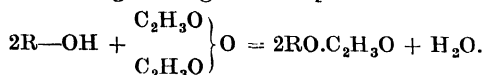
to remain in this mixture for several weeks at a temperature not above 20° C., a perfectly white skeleton remains behind.

Pure Swedish filter paper (acido hydrochlorico et fluorico extracto) is practically pure cellulose.

The empirical composition of cellulose is expressed by the formula  $(C_6H_{10}O_5)_n$ ; it is therefore seen to be a carbohydrate, i.e. a compound of carbon with hydrogen and oxygen in the proportion to form water. We are indebted in large measure for our knowledge of the chemistry of cellulose to the long-continued and careful researches of Cross and Bevan. In their researches the main reagents used were strong acids and alkalis which bring about conversion into sugar by the ordinary hydrolytic change, i.e.

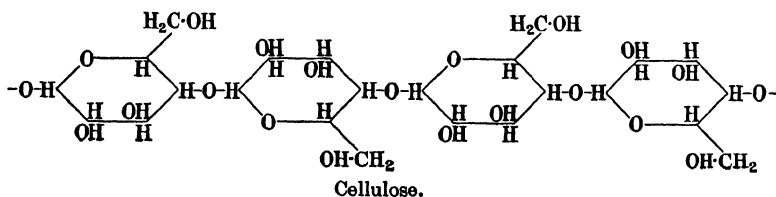


Acetic anhydride combines with any OH groups which may be present according to a general equation :



True celluloses are insoluble in simple solvents, soluble in ammoniacal copper solution (Schwizer's reagent), in zinc chloride ( $ZnCl_2$ ), and strong acids ( $H_2SO_4$ ), and give a dark brown to violet colour with zinc iodide. They are very resistant to biochemical change, but they can be hydrolyzed by certain specific micro-organisms. Cotton contains 87 to 91 per cent. cellulose, cereals, say, 35 per cent.

Various formulæ have been suggested from time to time to account for the chemical structure of the cellulose molecule. Recently Haworth and Hirst have investigated the polysaccharide xylan which has been found to be closely associated with all forms of cellulose, even after careful purification. They have obtained clear evidence of the close chemical relationship between xylan and cellulose, and as a result of their work Meyer has proposed the following formula for cellulose :



This has derived confirmation from X-ray analysis, radiographs of different types of cellulose, e.g. ramie, flax, esparto and cotton, all indicating that the cellulose molecule is a long chain of glucose residues, some 200 at least, alternately pointing in opposite directions.

**Hemi-Celluloses.** Cross and Bevan classified celluloses into three classes according to their behaviour with reagents. The true celluloses referred to in the last paragraph and which are represented by the cellulose of cotton fibre, do not contain, according to Cross and Bevan, any directly active CO groups, i.e. the CO is not easily oxidized and does not combine, e.g., with phenylhydrazine.

The other types of cellulose classified by Cross and Bevan according to their greater or less resistance to hydrolysis are now generally placed together under the heading of hemi-celluloses or pentosans.

The hemi-celluloses are amorphous poly-saccharides, but are distinguished from the true celluloses by their easy solubility in dilute alkalis and in hot dilute acids, such as 1 per cent. HCl.

They are differentiated from starches by being unattacked by amylase.

On hydrolysis hemi-celluloses give hexoses or pentoses or mixtures of these. They are therefore often referred to as pentosans, a possible formula being  $(C_5H_8O_4)_2$  yielding on hydrolysis  $C_5H_{10}O_5$  or pentoses.

Pentosans are present in the cell walls of well-grown plants, in the woody fibres of trees, in most fungi, seeds and fruits.

The following percentages of pentosans have been found to be present in typical materials :

	Per cent.
Straw . . . . .	23.0 to 29.0
Corn cobs . . . . .	32.0
Pine Needles . . . . .	6.8
Oak leaves . . . . .	10.3
Sugar cane fibre . . . . .	32.04

Old tissues contain larger quantities of pentosans than younger ones. The pentosans are probably not reserve materials like starch but are of importance in the formation of the woody skeletal structure of various plants.

**Pectins.** Allied with hemi-celluloses are kindred bodies belonging to the pectin group.

Pectose is the name given to the parent substance of such bodies as pectin, pectic acid, etc.

Pectin can be obtained by filtering the juice of a ripe apple or pear through muslin, and adding an equal bulk of alcohol. The pectin precipitates as a stringy gelatinous mass which can be reduced to a white powder soluble in water.

A solution of pectin gelatinizes on standing probably by the action of the enzyme *pectase* contained in the fruit juice.

The general result of the large amount of study which has been given in the past and renewed recently to the chemistry of pectin bodies is to identify the numerous forms described with one or other of the following modifications :

*Pectose.* The insoluble modification present in association with cellulose in the cell walls of tissues which is the precursor of the soluble form.

*Pectin.* The soluble form now recognized to be a methoxylated form of pectic acid.

*Pectinic Acids.* The primary hydrolysis products of pectin, with varying methoxy content.

*Pectic Acid.* Completely demethoxylated pectin ; it may be regarded as the basal molecule of pectin as a complex galacturonic acid<sup>1</sup> combined with arabinose and galactose.

*Metapectic Acid.* The final stable product of hydrolytic decomposition of pectin.

Pectose derivatives can be distinguished from cellulose by being insoluble in Schweizer's reagent and being unstained by iodine.

The study of pectin has recently assumed great practical importance owing to the extension of the jam and allied industries requiring stable jellies.

The main sources of pectin are citrus fruits, beets, apples, carrots and turnips ; possibly the highest percentage is to be found in the rind of citrus fruits. Recently papaya has been found to be rich in pectin.

<sup>1</sup> The term uronic acid is given to an aldehyde acid formed by the mild oxidation of poly-saccharides and glucosides, the aldehyde group in such cases being shielded from attack by linkage.

The pectin is extracted from the materials by subjecting them after mincing to the action of super-heated steam or by heating them with water under pressure. Some investigators recommend extraction with dilute acid, e.g. oxalic or better tartaric acid. In all cases, however, the underlying principle is the same, namely the hydrolysis of pectose with the production of pectin.

Pectin for commercial use is prepared either in the form of concentrated extract or as a dry powder obtained by precipitating the aqueous extract with alcohol.

**Lignins and ligno-celluloses.** Lignin is the non-carbohydrate portion of the lignified tissue after it has been freed from fats and waxes, tannins and resins, and is next to celluloses and hemi-celluloses the most abundant constituent of plant tissues. Lignin occurs mainly in combination with cellulose as ligno-cellulose.

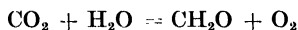
It is a matter of common observation that the stems of plants become tougher and more resistant with advancing growth; this process is called lignification and is accompanied by a transformation of pure cellulose into ligno-cellulose.

Lignin can be prepared from plant material either by treatment under pressure with concentrated acids in which it is insoluble or by concentrated alkali in which it dissolves and from which it can be precipitated by acid. A complicated constitution has been suggested for lignin containing three closed carbon chains.

### **Non-skeletal Constituents.**

**Sugars.** The term sugar as popularly understood generally refers to cane sugar or preparations made from it. Chemically, however, the word has much wider application and comprises a large number of substances all of which are classified as carbohydrates since they contain carbon combined with hydrogen and oxygen in the proportion to form water. It is a familiar fact in nature study that starch, which is also a carbohydrate, is the first visible product of plant assimilation in the leaf. It is also to be observed that if a plant is exposed to sunlight under water saturated with  $\text{CO}_2$ , gas is rapidly evolved which is found to be oxygen. It would appear

likely that the great family of carbohydrates as found in nature may originate in the first instance from formaldehyde, which is evidently the simplest carbohydrate, its empirical formula being  $\text{CH}_2\text{O}$ . The reaction under which this would be formed is a simple one, viz, as follows :



A suggestion of this sort appears reasonable even when we simply look at the empirical formulæ of the important classes of carbohydrates generally termed the mono-saccharoses, the di-saccharoses and the poly-saccharoses. These terms are not altogether satisfactory because the so-called mono-saccharoses include a large number of substances of differing molecular formulæ, all of which have the general formula  $\text{C}_n\text{H}_{2n}\text{O}_n$ . The best-known members of this group are, however, the hexoses of the general formula  $\text{C}_6\text{H}_{12}\text{O}_6$ , and the di-saccharoses are so named because by the addition of a molecule of water they give rise to two molecules of a hexose. The general formula, therefore, of a di-saccharose is  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ . The poly-saccharoses are much more complicated substances whose molecular formulæ are unknown, but they have the general formula  $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ .

Taking now for the sake of comparison an even number of carbon atoms in all three cases we get the following relationship :

Mono-saccharoses (2 or more molecules)	.	$\text{C}_{12}\text{H}_{24}\text{O}_{12}$
Di-saccharoses . . . . .	.	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$
Poly-saccharoses ( $n = 2$ ) . . . . .	.	$\text{C}_{12}\text{H}_{20}\text{O}_{10}$

We can thus see at a glance how these important naturally occurring groups are generally related. The mono-saccharoses can evidently be considered as built up by the combination of a number of molecules of formaldehyde. By elimination of water from one or two molecules of mono-saccharoses, di-saccharoses result, and by further elimination of water poly-saccharoses are obtained.

The study of the intimate structure of the very numerous members of the sugar family is a special chapter in pure organic chemistry much of which is due to the classical researches in the last century of Emil Fischer and his school, and more

recently to the work of Irvine, Haworth and their colleagues. For the purpose of our present subject it will be sufficient simply to mention some of the most important sugars which occur in nature, and which afford pabulum for micro-organisms. Among the mono-saccharoses may be mentioned grape sugar and lævulose. Among the di-saccharoses cane sugar occurs in the sugar cane and in beetroot, from which two sources the world derives practically the whole of its sugar. Milk sugar or lactose occurs in the milk of all mammals to the extent of about 4 per cent., and maltose is obtained from starch by the action of the enzyme known as *amylase*.

Among the poly-saccharoses cellulose has already been considered as a skeletal constituent of plants. In a following paragraph reference will be made to starch.

**Gums.** Gums are highly resistant bodies which often occur in association with resins and terpenes. Gums are compounds of sugars such as arabinose, xylose, and galactose with complex organic acids.

**Starches.** Starch occurs widely in the vegetable world; in the leaves of plants it is the first visible product of assimilation in the presence of chlorophyll.

The starches are predominantly reserve materials forming 60 to 70 per cent. of the dry weight of cereal grain. They also occur in tubers, especially potatoes, and in the rhizomes or roots of such plants as Jerusalem artichokes and cassava or manioc.

Examined under the microscope starch, which in the mass is a white powder, is seen to consist of small granules which have the power of polarizing light. Different species of starch vary greatly in the size of their granules. Under high magnification, especially after treatment with dilute alkali, the starch granules can be seen to consist of a series of layers arranged round a nucleus.

Investigation has shown that the nature of the material comprising the inner portion of the starch granule differs from that of the outer portion. The former of these is known under different names, among which are granulose, amylocellulose or amylose, and the name amylopectin has been given to the outer material.

Amylopectin is not so readily attacked by enzyme action

or by acids as the soluble starch or granulose. If the solution of starch is boiled for some time with dilute acid the solution will become clear and it will be possible by suitable tests to recognize the presence of the sugar in the solution. The same reaction is brought about if a small amount of extract of malt is added to the starch. This reaction, which is known as the hydrolysis or saccharification of starch, has been found to be due to an enzyme which has been termed *amylase*.

The process of saccharification of starch is by no means simple; shortly, it may be stated that the starch is converted first into compounds known as dextrans of varying complexity but of the same empirical formula as the starch, viz.  $(C_6H_{10}O_5)_n$ , and then into sugars, first maltose  $(C_{12}H_{22}O_{11})$  and finally glucose  $(C_6H_{12}O_6)$ .

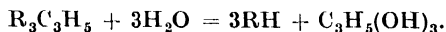
**Fats and Waxes.** Fats are defined chemically as esters of the so-called fatty acids and glycerine. Glycerine is an alcohol containing 3 hydroxyl groups with the formula:  $CH_2OH.CH(OH)CH_2OH$ .

Animal fat or stearin is a compound of glycerine and stearic acid, the latter having the formula:  $C_{17}H_{35}.COOH$ .

Stearin therefore, being a glycerolester of stearic acid, has the formula:  $C_3H_5(C_{17}H_{35}CO_2)_3$ .

Vegetable oils and fats occur in the numerous varieties of oil seeds and are compounds of glycerine with various complex organic acids.

Fats and oils can all be split up either by heating with acid or alkali or by the action of steam under pressure yielding a fatty acid and glycerine according to the following general equation where R equals the residue of the fatty acids:



If an alkali is used the corresponding alkali salt of the fatty acid is known as a soap, and the process is hence known commonly as saponification, though it is essentially a simple hydrolysis.

Nature's method for effecting the above change, which is of primary importance in the assimilation of fat by living organisms, or in the splitting of fat which occurs in soil and manure heaps, is less direct. In the plant or animal which

uses fat to build up its body substances, enzymes are produced known as lipolytic or fat-splitting enzymes which are generally referred to as an individual enzyme under the term *lipase* or *steapsin*.

Waxes are chemically related to the fats, being esters of alcohols of a higher molecular weight than glycerol, and of higher fatty acids; they are consequently more resistant to decomposition than are the fats, and their presence in the stems of grasses, in cereal straw and sugar cane partly accounts for the difficulty of its decomposition in the soil.

Waxes and fats are formed under certain conditions, by the action of various micro-organisms, e.g. moulds and yeasts, and their formation may to some extent account for what is known as sewage sickness of soil, the pores of the soil being clogged by such products and the normal processes of oxidation thereby hindered.

**Other Hydrocarbon Derivatives.** There may be present in the soil as products of decomposition of complex nitrogenous matter, and also occasionally through the actual addition of antiseptics, certain derivatives of hydrocarbons such as phenols, naphthols, etc. Even these under suitable conditions can be finally oxidized by micro-organisms.

#### THE DECOMPOSITION OF NON-NITROGENOUS ORGANIC MATTER BY MICRO-ORGANISMS

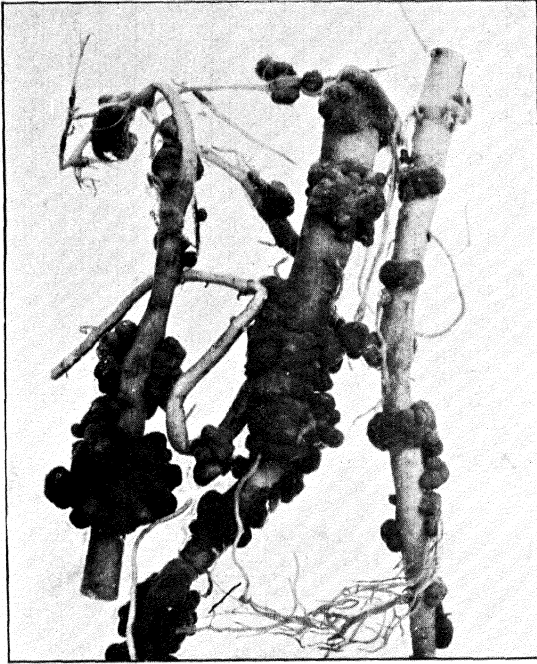
In the foregoing section the most important types of non-nitrogenous substances likely to be met with in the study of nitrogen conservation, i.e. those likely to occur in the soil, in a manure heap or in the sewage tank, have been shortly described.

We may now consider in outline the chief fermentation changes which these substances undergo when submitted to the attack of micro-organisms, in the first place independently of the presence of more than traces of nitrogenous compounds.

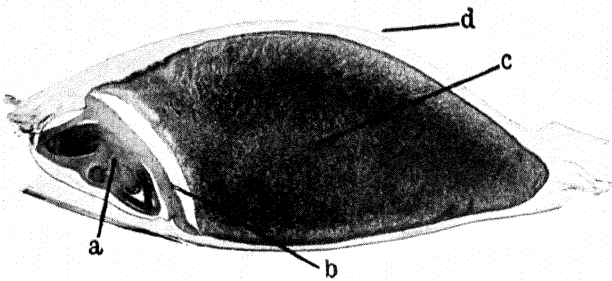
The most important of the changes are those concerned with cellulose. Cellulose is broken down in nature by three well-defined processes :

1. By the action of the enzyme *cytase* which is secreted by animals and plant cells and by various organisms.





(i) ROOT NODULES OF PEA.



(ii) SECTION OF BARLEY GRAIN.  
(a) Germ.  
(b) Scutellar epithelium.  
(c) Endosperm.  
(d) Husk.

2. By fermentation under anaerobic conditions through the action of certain specific bacteria.
3. By decomposition under aerobic conditions through the action of certain bacteria and moulds in presence generally of nitrates.

We can consider these processes in order :

(1) When barley or any other cereal grain is allowed to germinate, the contents of the grain become quite soft and the whole grain quite friable ; this is due in the first place to the action of the enzyme *cytase* which breaks down the walls of the cells of the endosperm of the grain facilitating the action of the starch-saccharifying enzyme *amylase* (Plate III (ii)).

Investigation by Horace T. Brown has shown that the power of grain-feeding animals to digest such food depends on the enzyme contained in the grain and not on any cellulose-dissolving power present in the secretion of the stomach of the animal.

The rotting of potatoes is preceded by the breaking down of the cellular tissue by means of *cytase*.

(2) The decomposition of cellulose by bacteria in *absence of air* has been very carefully worked out by the Russian chemist, Omelianski. For the purpose of his investigation he used Neva mud and a pure Swedish filter paper. He was able to isolate two different organisms, one of which produced marsh gas and the other hydrogen. They were found to be almost identical in appearance and both produced spores. They differed only in their optimum temperature of reaction and their resulting products. He was able to show that the products obtained completely accounted for the weight of paper originally taken, certain fatty acids being produced together with the gas. The fatty acids consist mainly of acetic acid together with smaller quantities of butyric acid.

The decomposition of cellulose by bacteria under both anaerobic and aerobic conditions has been the subject of numerous researches since Omelianski. At the Indian Institute of Science, Bangalore, a number of experiments were made with the object of discovering the best conditions for gas production from waste cellulose materials ; a necessary condition of success was that the fermenting mass of cellulose should

active in the breaking down of cellulose secrete an enzyme called *cellase* which breaks down the cellulose to a di-saccharide *cellobiose* which by another enzyme termed *cellobiase* is further broken down to glucose.

A specially active organism is the aerobic bacterium *Spirochaeta cytophaga* discovered by Hutchinson and Clayton. It uses cellulose as the only source of carbon and produces a pigment related to the carotin group and also some mucilage.

While, as has been stated, the hemi-celluloses and less resistant cellulose fibres break down fairly rapidly under the conditions described, anything of the nature of woody fibre, e.g., the lignified stems of plants, is very resistant to decomposition, and may remain practically unattacked for a long period of time in a disintegrated condition in such end products as peat, lignite, etc. These contain the somewhat ill-defined substance known as *humus* which is also formed by prolonged boiling of sugars with dilute acids. Humus bodies are generally of an acid character dissolving in alkalies to form brown solutions.

The final production of gases such as carbon dioxide and methane from cellulose is probably a sugar fermentation, since these gases as well as organic acids are produced by the fermentation of sugars by numerous bacteria.

Fatty acids are also decomposed by certain bacteria yielding under some conditions large quantities of methane or a mixture of methane and hydrogen.

The foregoing changes are of great practical importance since the fermentation of sewage sludge which contains large quantities of cellulosic debris, from undigested food in India and toilet paper in Europe, as well as a certain proportion of soap and fat, has been developed of recent years in such a way as to produce large quantities of power gas capable of being converted into power in large gas engines. These are modern developments. From the earliest days of septic tank treatment at the beginning of the present century at the Davyhulme Works of the Manchester Corporation, special experiments were made on the production of gas from sewage screenings consisting largely of paper and various debris. At one time a workman's cabin was lit by a mantle burner fed with gas from a small gas holder floating on the surface of a

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septic tank, the screenings being pushed under the gas holder from time to time. For many years the gas was collected from septic tanks at the Matunga Leper Asylum near Bombay and used in a gas engine for pumping the sewage as well as for lighting and cooking purposes. (See Plate IV (i)). Calculations by Messrs. Vokes and Townend confirm the common belief at Matunga, viz. that the gas production per head of population was sufficient to pump a normal volume of sewage per head.

Experiments made at Cawnpore and also by Professor H. K. Sen, of Calcutta, indicate that a large quantity of power gas can be obtained by the fermentation of water hyacinth. It would appear probable that by the fermentation of such material, e.g., in the sewage sludge of Calcutta, large quantities of power gas might be available; from the point of view of nitrogen conservation, however, careful consideration will have to be given to the relative values involved. In the next section the changes which take place when carbonaceous material is fermented in presence of nitrates with possible loss of nitrogen will be considered as well as the general question of the interaction of carbonaceous and nitrogenous material.

### DENITRIFICATION

Denitrification, as the name implies, is the reverse of nitrification. Denitrification changes are concerned with:

1. Reduction of nitrates to nitrites or ammonia.
2. The reduction of nitrates and nitrites to oxides of nitrogen, NO and  $N_2O$ , or
3. The reduction of nitrates and nitrites to nitrogen.

The first characteristic work on this subject was done by Gayon and Dupetit in 1882. They found that when a solution containing potassium nitrate together with sewage and a little urine was allowed to stand in absence of air the nitrate was reduced. When using nitrated broth containing asparagine they obtained an evolution of nitric oxide; they also noted the effect on the reaction of the addition of carbohydrates, tartrates, etc., and they concluded that denitrification was essentially the combustion of organic material by the oxygen of the nitrates. It thus naturally proceeded best in presence of

a minimum air supply. It could be shown, e.g., that in a given solution a greater amount of denitrification took place in the lower portion of the liquid than on the surface.

The subject of denitrification has been investigated by numerous workers, notably Percy Frankland and Beyerinck.

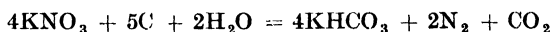
The latter describes an elegant experiment for the demonstration of the presence of denitrifying organisms in sewage :

0.1 per cent. of potassium nitrate and a little starch paste is added to nutrient gelatine and the whole sterilized and poured into a Petri dish. A little sewage, diluted say 20 times with distilled water, is poured on and off the plate which is then turned with the gelatine surface downwards and incubated at 20° C. When the colonies have developed, a dilute solution of hydrochloric acid and potassium iodide is poured over half the plate. Wherever nitrites have been formed, iodine will be liberated and will colour the starch blue. Colonies on the other half of the plate similar in appearance to those giving the blue starch-iodide reaction, and which will not have been killed by the acid, may be picked out and grown separately in suitable solutions.

For the study of the ultimate conversion of nitrate into nitrogen the following solution may be made use of :

River water	.	.	.	.	.	1 litre
Calcium tartrate	.	.	.	.	.	2 grams
Potassium hydrogen phosphate	.	.	.	.	.	0.5 gram
Potassium nitrate	.	.	.	.	.	0.1 „

This is sown with a little horse-dung or straw and incubated at 35° C. The general reaction taking place may be expressed by the following equation :



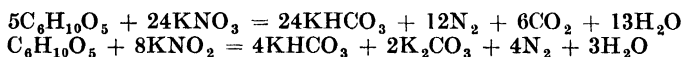
In the foregoing section reference is made to the breaking down of cellulose materials under aerobic conditions in presence of nitrogenous matter ; we may now revert to that subject as it has a very important bearing on the problem of the conservation of nitrogen.

Researches by van Iterson have shown that the carbon in the above equation can be supplied by cellulose. He found that in presence of nitrates certain organisms are capable of

oxidizing cellulose, utilizing the oxygen of the nitrate which is simultaneously reduced. The following experiment will illustrate this action :

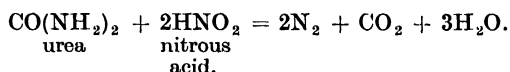
100 c.c. of tap water are placed in a 200 c.c. flask together with 2 grams of Swedish filter paper, 0.25 gram potassium nitrate, 0.05 gram potassium hydrogen phosphate ( $K_2HPO_4$ ), a few c.c. of sewage, and a little leaf mould.

The flask is then filled to the neck, plugged with cotton-wool and placed in an incubator at  $35^\circ C$ . In 15 days, if the conditions are successfully realized all nitrite and nitrate will have disappeared and 100 c.c. of the solution are then poured off and a further 100 c.c. of tap water containing the same quantity of potassium nitrate and potassium hydrogen phosphate as originally used are added. On incubation the nitrate will be found to disappear much more rapidly, and on further repetitions of the process van Iterson was able to reduce 0.5 gram potassium nitrate in 1 or 2 days. The paper in the meanwhile disintegrates and disappears, and potassium carbonate is found in solution. The evolution of nitrogen is observed but no trace of hydrogen, methane or nitrous oxide. The equations representing this change are given by van Iterson as follows :



Van Iterson found that the decomposition of cellulose in presence of nitrate as above described went on at much the same rate as the anaerobic change studied by Omelianski. He drew attention to the fact that the nitrite produced is readily re-oxidized by the organism of nitrification and consequently that in presence of air the nitrate is being continually reproduced and the conditions for the destruction of cellulose are therefore constantly maintained. This observation is of great importance in connection with the destruction of cellulose in the bacterial filter beds employed in the purification of sewage. From the point of view of nitrogen conservation it is obviously a condition of things to be avoided, and in the storage of farmyard manure and in the making of compost care should be taken to prevent if possible the production of nitrates.

Denitrification is by no means so restricted a phenomenon as nitrification, and quite a large number of organisms have been found which are capable of bringing about denitrification to a greater or less extent. Broadly speaking, these may be classified into two classes: true denitrifying organisms which are capable of pushing the reaction to its full limit and producing free nitrogen, and indirect denitrifying organisms which only reduce nitrates to nitrites when, through the interaction of nitrites with amido compounds in acid solution, we have the reaction typified by the case of urea:



Thus it was observed by Hulme, working in the Frankland Laboratory in the University of Manchester, that when a solution containing glucose and nitrate was inoculated with denitrifying organisms, the first gas to be evolved was nitrogen from the reduction of nitrate. When the nitrogen was exhausted, hydrogen gas was evolved showing that the reduction of the nitrate was in the first place due to nascent hydrogen arising from the fermentation of the sugar. In the course of his work Hulme was able to produce evidence that the denitrifying organism which he studied secreted a reducing substance of the nature of an enzyme. A culture in nitrate broth was filtered through a Chamberland filter into a sterilized bottle. The filtrate, the sterility of which was checked by plate culture, was tested for its action on nitrates, when it was found to exercise a distinct reducing action. A precipitate was also obtained with an excess of absolute alcohol and sodium chloride, the precipitate was washed with absolute alcohol and finally dissolved in distilled water. Equal quantities of these solutions were added to each of two equal volumes of 1 per cent. potassium nitrate solution, one of which was boiled when no action took place. In the unboiled solution a very strong reducing action was obtained. This experiment is of interest as being a somewhat isolated case of the separation of the active body (whether strictly an enzyme or not can hardly be said to be established) from bacteria of this type.

With regard to what may be termed *indirect* denitrifying

organisms the reaction typified in the well-known urea equation does not appear in the light of recent investigations to be so common as might be supposed. A large number of experiments were carried out by Kotwal in the Biochemical Department of the Indian Institute of Science using solutions of a concentration comparable to those likely to be present under natural conditions but without the presence of bacteria. Under these circumstances little or no evidence of loss of nitrogen in the free state could be found. The experiments tried included the effect of temperature on the stability of dilute solutions of ammonium nitrite both alone and in presence of carbon dioxide, and also on the interaction of dilute solutions of nitrites with urea and with a mixture of various amino compounds resulting from the decomposition of proteins in presence of dilute solutions of various acids. Even in presence of appreciable quantities of mineral acid only a very small quantity of gaseous nitrogen was obtained under such conditions. Subsequently V. Subrahmanyan, now Head of the Department of Biochemistry at Bangalore, working at Rothamsted on the chemistry of water-logged soils, found little evidence, if any, of loss of nitrogen, although the combinations in which it was present were undergoing change.

Other researches by Kotwal at Bangalore on the conditions of loss of nitrogen from activated sludge indicate that this only takes place if the material is in an imperfectly oxidized condition when brought in contact with nitrates. In fact it has been proposed as a method of distinguishing between sludge which is in a well-“conditioned” state and sludge which is imperfectly conditioned to incubate the material in contact with a dilute solution of nitrates when, if the sludge tends to be at all putrefactive, nitrogen will be evolved. This would indicate that thorough aeration is desirable if loss of nitrogen is to be avoided.

It is difficult to recall any evidence of loss of nitrogen in the form of gas in any natural process other than through the reduction of nitrates. Loss occurs in this way undoubtedly in the so-called contact bed used in the purification of effluents, where the bed is alternatively filled and emptied. During the period when the bed stands empty there is a formation of

nitrate, and when it is standing full this nitrate is reduced by the organic matter of the effluent with the result of loss of nitrogen in the free state.

Quite recently, however, S. H. Jenkins, in a study of the oxidation of sugar and carbohydrate matter on percolating filters, brings forward evidence to show that loss of nitrogen in these filters is not necessarily due to the formation of ammonia or to the production of nitrite and nitrate and subsequent denitrification. It would appear possible that the liberation of nitrogen from ammonia and organic compounds of nitrogen may be carried out entirely within the cell of the living organism taking part in the biological oxidation process. It may be noted that ammonium salts, sodium nitrite and sodium nitrate were equally effective as sources of nitrogen for the maintenance of the biological activity, and the best results were obtained when the ratio of carbon to nitrogen in solution was about 15 to 1.

It is well known that there is often considerable loss of nitrogen during the storage of farmyard manure ; the origin of this has never been quite clear, but Niklewski contends that the loss of nitrogen from manure heaps is due to the formation of nitrates in semi-dry portions of the heap and interaction of these nitrates with other portions. He stated that there is no loss of nitrogen if an ample supply of ammoniacal nitrogen is present as urine so that the process of nitrification is checked.

Loss of nitrogen has been reported through the excessive liming of peat soils or by the excessive application of ammonium sulphate to normal soils.

It is evident that more research is needed on this question. It was at one time thought that free nitrogen was given out from septic tanks, but although there is change in the form of combination there is no evidence of actual loss in the septic tank process.

Akin to the conditions in a septic tank are those in water-logged paddy soils and yet from them little loss of nitrogen has been observed.

Subrahmanyam has recently shown that there is a gradual absorption of dissolved oxygen from water which covers alluvial soil, the amount of dissolved oxygen being naturally

most at the surface and less in immediate contact with the soil. It would appear quite likely from this and from other evidence connected with the operation of activated sludge tanks that nitrates may be formed in the upper portion of the aqueous layer and reduced to free nitrogen at greater depth below the surface. It has been suggested consequently that the depth to which paddy fields may be flooded is one of serious agricultural importance on account of these possibilities of nitrogen loss. This point was left somewhat obscure by the researches of Harrison and Subramania Ayyar. In their experiments copper sulphate was added in order to eliminate the action of the surface film of algæ, but it would appear possible that oxygen of the air can take part and nitrates be formed without the intervention of green algæ.

Reference may finally be made to a rather striking experiment described by Beyerinck and Minkmann. They filled a bottle with bouillon containing 8 per cent. of potassium nitrate, to which 10 to 20 grams of garden soil were added. After incubation at 37° C. for a day or two a considerable froth forms which forces out the liquid by a capillary action between the stopper and the neck of the bottle, the gas evolved remains in the bottle, and at the end of 48 hours on applying a glowing chip it will burst into flame through the action of the nitrous oxide present. This has been found to amount to as much as 90 per cent. of the gases evolved.

## CHAPTER VII

### NITROGEN FIXATION

From the considerations discussed in the last chapter it is clear that there is often a large percentage loss of nitrogen from manure heaps, from the operations of agriculture and from most methods of sewage purification. This escape of nitrogen into the atmosphere if continued indefinitely would evidently result in the ultimate depletion of the stock of nitrogen available for life as a certain percentage of the nitrogen of all organic matter would be permanently lost in this way. Fortunately means exist for bringing back this escaped nitrogen once more into the cycle of life. A certain small quantity is returned as nitric acid through the combination of nitrogen and oxygen brought about by the electrical discharge of lightning.

Reference was made in the introduction to modern industrial methods of recovering nitrogen from the air, and these were contrasted with the methods employed by nature.

For the sake of completeness a brief reference may be made to these industrial processes which supply a very large portion of the nitrogenous mineral fertilizers used in agriculture.

These processes may be roughly classified as :

1. Electrical,
2. Thermo-electrical,
3. Thermo-chemical.

They may be shortly described in the following paragraphs :

1. **The Arc Process.** In this process the oxygen of the air is caused to combine with nitrogen in a high-tension electrical arc with formation of oxides of nitrogen which are afterwards absorbed by water to form nitric acid of 33 per cent. strength. There is some discussion whether this process should be classed as a purely thermal or as an electrical process, but the balance of experimental evidence appears to be in favour of considering it as mainly electrical.

There are several processes by which the electrical arc is produced; the largest production is by the Birkeland and Eyde process operated in Norway by hydro-electric power. The electrodes consist of U-shaped copper tubes carrying a current with a voltage of 6,500; the temperature of the flame between the electrodes is 3,000° C. to 3,500° C. The gases from the furnace pass under boilers, then through coolers of aluminium tubes cooled by water, from thence to oxidizers where any unoxidized NO is converted into NO<sub>2</sub> and finally into absorption towers 90 feet high.

The absorption towers are filled with pieces of quartz. The absorbing liquid circulates in the towers until it contains, as stated, 33 per cent. nitric acid; it is then run into granite channels containing limestone, the solution of calcium nitrate thus obtained is concentrated *in vacuo* and then poured into iron drums or into shallow iron pans.

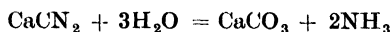
Any nitric oxides which escape absorption by water pass into towers fed with soda solution. The sodium nitrate here formed is concentrated in a similar manner to the calcium nitrate.

**2. Thermo-electrical Processes.** The chief of these processes consists in the production of *cyanamide* or *nitrolim*. The process comprises the following stages:

1. Calcium carbide is formed by heating lime and coke in an electric furnace.
2. Calcium carbide and nitrogen at a red heat produce calcium cyanamide according to the following equation:



Calcium cyanamide comes directly on to the market and when applied to the soil gives rise to ammonia by the following equation:



It should be noted that during the production of calcium cyanamide there is a possibility of the formation of certain secondary products, as is also the case when it is added to the soil. Among these products should be mentioned dicyanodiamide, (CNNH<sub>2</sub>)<sub>2</sub>. Cyanamide CNNH<sub>2</sub>, and urea CO(NH<sub>2</sub>)<sub>2</sub>, are also formed by the decomposition of nitrolim.

As the first of these compounds has a prejudicial effect upon bacteria, nitrolim should be applied to the soil some time before the sowing of the crop, so that complete decomposition into calcium carbonate and ammonia may first be effected.

**3. Thermo-chemical Processes**<sup>1</sup>. Of these the Haber process is the most extensively employed. By this process pure nitrogen and hydrogen are caused to combine in presence of an iron catalyst at a temperature of 600° C. and a pressure of 200 atmospheres.

The hydrogen is obtained by passing water gas ( $\text{CO} + \text{H}_2$ ) and steam over a catalyst of ferric oxide rendered active by a certain percentage of chromium oxide  $\text{Cr}_2\text{O}_3$  at a temperature of 400° to 500° C. The  $\text{CO}_2$  produced in the reaction is washed out with water and any unacted upon CO with ammoniacal copper formate.

The nitrogen is obtained from a Linde liquid air plant.

The reaction takes place in steel bombs 6 metres long and 80 centimetres in internal diameter and with walls 18 centimetres thick. The ammonia produced is dissolved in water under pressure.

It is of interest to note that at the Oppau Works of the Badische Co. the personnel comprised at one time 1,500 labourers, 3,000 mechanics, 350 clerks and 350 chemists.

#### BIOCHEMICAL PROCESSES

The natural biochemical processes of nitrogen fixation go on silently at ordinary temperatures and pressures and in the aggregate fix vastly more nitrogen than all the great installations operating the artificial industrial methods.

The nature of the natural activities and the possibilities of their utilization and control may now be discussed.

The micro-organisms concerned with nitrogen fixation may be broadly classified under two groups :

- (a) Organisms working in symbiosis with higher plants or other micro-organisms.
- (b) Free living organisms.

It will be convenient to deal with these in order.

<sup>1</sup> See also Appendix II, p. 251.

**(a) Symbiotic Organisms.**

It is of interest to note that Roman agricultural writers some 2,000 years ago were fully aware of the fertilizing effect which may be realized by the cultivation of leguminous plants. In China and Japan this knowledge was available from still earlier times.

It has also been known for a long time that leguminous plants contain more nitrogen than others and that this nitrogen can be used for food for other crops when the nitrogen-containing plant is used as green manure. How the plant came to have this nitrogen, and how it often thrives on a soil almost devoid of nitrogen, was for a long time unexplained.

The first exact experiments were made in 1838 by the French chemist Boussingault: these indicated that the nitrogen must be fixed in some way from the air.

These experiments were confirmatory of earlier ones by Priestley, who in 1771 had stated that certain higher plants were capable of utilizing the free nitrogen of the air.

Nevertheless, up to 1879, the general opinion of agriculturists was against nitrogen fixation. Lachmann, however, in 1858 had drawn attention to the existence of nodules on the roots of leguminous plants, and had suggested that they were concerned with the nutrition of the plant, and actually showed that they contained "vibrionartige" organisms. W. O. Atwater, in 1881 and 1882, drew attention to the large quantities of nitrogen taken up from the air by peas, and later (1885) suggested that they "might favour the action of nitrogen-fixing organisms."

It was, however, Helriegel and Wilfarth, in 1885, who first showed definitely the part played by bacteria. They treated plants with (a) distilled water, (b) an extract of soil sterilized by boiling, (c) an extract of soil unsterilized. The growth was much more marked when the plant was treated with the unboiled extract, showing that in some way bacteria assisted the growth of the plant.

They noted the presence of nodules or tubercles on the roots of the plants, and that these were much more numerous in soil poor in nitrogen; they also showed that the nitrogen was not stored in the tubercle.

Their conclusions were confirmed by Bréal who compared the growth of rootlets of lupin inoculated directly with the liquid contents of the root nodule with the development of similar rootlets which had not been inoculated. The growth and percentage of nitrogen in the resulting plant was much greater in the former than in the latter case.

It was Beyerinck (1888-91) who carefully studied nodule bacteria and gave them the name, *Bacillus radicicola*. His results were confirmed by Prazmowski in Poland and Mazé in France. The observation of these workers that the nodule bacteria could fix nitrogen in pure culture independently of the host plant has not always been confirmed, but under conditions where the metabolic products are promptly removed from the bacterial growth, it appears proven that fixation does take place apart from the plant.

The life cycle of the nodule bacteria has been carefully worked out in the Rothamsted laboratories, Bewley and Hutchinson having studied the organisms in pure culture and Thornton and Gangulee under natural living conditions in the soil; they are thus described by Russell (*Soil Conditions and Plant Growth*, p. 346):

Beginning with the non-motile cocci: these swell, develop flagella, and become motile (the "swarmers" of the older workers); next they elongate and develop more flagella, becoming motile rods; finally they lose their flagella, become non-motile and vacuolated, taking on a banded appearance; they may be straight or branched. These then break up, releasing the cocci and so the cycle starts again.

The chemistry of the changes by which the free nitrogen is caused to combine and to be assimilated by the plant is not fully known, but it probably enters the plant in the form of protein. There is evidence of the formation of a bacteriophage which dissolves the bacteria and so makes their contents available to the plant.

It is important to note that the association of the nodule bacteria with the legume is of mutual benefit, and thus is truly symbiotic. Weak unhealthy plants produce only a few nodules. For vigorous nodule development it is necessary that abundance of plant food other than nitrogen, viz. phosphorus, potassium and calcium, should be present in the soil.

A typical development of root nodules is shown in Plate III (i), p. 128.

There are numerous types of nodule bacteria, varying according to the host plant. This variation probably depends on the reaction of the cell sap of the plants in question. The limiting  $P_H$  values at which various strains of *B. radicicola* will grow vary from 5.0 to 3.2 on the acid side and 9.0 to 10.0 on the alkaline. The optimum reaction is  $P_H$  5.5 to 7.0. The optimum temperature is 25° to 28° C., with 0° and 50° as the limits.

In addition to the fixation of nitrogen by bacteria living in the root nodules of leguminous plants, other symbiotic micro-organisms have been discovered which live in and on the roots of certain non-leguminous plants and which also assimilate nitrogen. Further, in the leaves of certain tropical plants small beadlike nodules are noticed which are filled with bacteria, which, like the *Bacillus radicicola* of leguminous plants, are found to fix nitrogen even in pure culture. These leaves are often used for green manuring.

In addition to the foregoing types of biological nitrogen fixation, certain fungi and algæ play their part in increasing the nitrogen supply, although according to the researches of Bristol-Roach the algæ are not capable themselves of fixing nitrogen but do in some way increase by their presence the activity of ordinary nitrogen-fixing organisms.

Many attempts have been made artificially to induce nitrogen fixation by inoculation of the soil or of the plant seeds.

Among the earlier attempts, nitrogen-fixing bacteria were grown by Nobbe on gelatine and the cultures sold as Nitragin. It was found, however, that the gelatine contained too much nitrogen for efficient growth of these bacteria which are most active in fixing nitrogen from the atmosphere when deprived of any other source of supply.

To avoid this difficulty American bacteriologists endeavoured to transport active cultures on cotton-wool. Small-scale experiments on these lines had some success, but it was really only when the actual earth containing necessary bacteria was transported to soils poor in nitrogen that any marked effect on a large scale was produced.

More recently Stoklasa speaks very favourably of a special

material produced by inoculating sterile turf with suitable organisms and adding finely powdered phosphate and a small percentage of kainit, a mixture which he terms humophosphate, large quantities of which have been used with good results in Czecho-Slovakia, increases of the crop yield from 30 per cent. to 130 per cent. being reported.

Russell recommended the inoculation of seed by special cultures grown in milk.

One very interesting result which was given out from Rothamsted in recent years is that of the importance of traces of boron in the soil for establishing the necessary link between symbiotic nitrogen-fixing organisms and the corresponding plants.

Mention should finally be made of the important fact which has long been known in practice, and has been confirmed by careful scientific experiment, that when a legume such as peas is grown with a non-legume such as oats, the percentage of nitrogen in the latter is increased. The total amount of nitrogen fixed from the air under such conditions would seem to be greater than when the legume is grown by itself, owing to the removal of nitrogenous products from the soil by the non-legume.

### Free Living Organisms.

The fact that there is a general tendency even in barren soils gradually to recover their fertility would indicate that there is some source of nitrogen generally available. Many purely chemical hypotheses have been advanced to account for this gain in nitrogen, but it was only in 1885 that Berthelot suggested the activity of bacteria and confirmed his suggestion in 1893 by investigating nitrogen fixation with pure cultures of soil bacteria.

This important discovery was confirmed about the same time by Winogradsky. The bacteria studied by Berthelot were aerobic, whereas Winogradsky's organism, which he called *Clostridium pastorianum*, was active under anaerobic conditions. It was indeed only a variety of the common anaerobic butyric acid bacillus, *B. amylobacter*, which is generally present in soil and which under suitable conditions always fixes some nitrogen.

The most active free living organism would seem to be the aerobic bacterium, *Azotobacter chroococcum*, which was described by Beyerinck in 1901.

The medium generally used for developing these organisms has the following composition :

Water	.	.	.	.	.	100.00	grams
Mannite	.	.	.	.	.	2.00	„
K <sub>2</sub> HPO <sub>4</sub>	.	.	.	.	.	0.02	„

Beyerinck and Van Delden also found that numerous other bacteria occurring in the soil were capable of fixing nitrogen, notably a species which they named *Azotobacter agile*, and various forms of the genus *Granulabacter*.

**Occurrence of *Azotobacter*.** Apart from the normal occurrence of nitrogen-fixing organisms in the soil, the discovery of their presence and ability to thrive in animal and human excreta is of considerable importance from the point of view of nitrogen conservation.

During the years 1915-16 and 17 a number of Special Reports were made to the Board of Agriculture and Fisheries, London, on the effect of liquid manure on crops. The experiments were carried out at the College of Agriculture, Holmes Chapel, Cheshire, by Mr. (later Major, deceased) and Mrs. Mumford, and Messrs. Gaul and Chamney, working under the general supervision of the present writer. The results of the main investigation have been referred to in Chapter II, page 60, *re* animal manures. Incidental to these researches a number of interesting experiments were carried out on the then newly discovered product, "Activated Sludge," produced by the scientific methods of oxidation of town sewage. These experiments which are recorded in appendices to the Reports referred to, and were described with others in a paper published by the present writer in 1920, are here summarized.

### Experiments at the University of Manchester. ;

The following experiments were carried out by Mrs. Mumford in the Frankland laboratory :

Two lots of 50 c.c. each of activated sludge were taken, one to act as check, while to the other was added 1 per cent. o glucose. The samples were aerated in wash bottles, using

suitable precautions to prevent evaporation and to avoid loss of nitrogen by evolution of ammonia. There was an undoubted increase in total nitrogen in the sample containing glucose, as compared with the check.

Another type of experiment was arranged in which activated sludge remained in contact with air in a large separating funnel with occasional shaking. Analysis of the air at the end of a fortnight indicated that an appreciable percentage of nitrogen had been fixed by the sludge.

Further experiments arranged to imitate the ordinary process of purification of sewage by means of activated sludge were made to determine whether there was an increase of nitrogen under such conditions. The results, although not entirely conclusive as to increase of nitrogen, showed at any rate that with adequate aeration no loss of nitrogen occurred. This conclusion has been frequently confirmed since in large-scale practice.

The fact that nitrogen fixation takes place on aeration of activated sludge in presence of a little calcium carbonate, but without any special addition of carbohydrate, was confirmed by E. Gaul in 1917.

**Rothamsted Experiments.** Gaul's experiments were largely due to a suggestion from Sir John (then Dr.) Russell, and the method employed was similar to that used by E. H. Richards in his experiments demonstrating the presence of nitrogen-fixing organisms in the faeces of animals, viz. in horse and cow dung.

Among Richards's conclusions are the following :

Under the most favourable conditions 1 gram of dry matter in the faeces will fix 4 milligrams of nitrogen.

Evidence is adduced to show that fixation is brought about by a mixed culture of *Azotobacter* and *B. lactis aerogenes*. Of these the latter is normally present in faeces; *Azotobacter* is not, but readily infects faeces. Both organisms were present in the soil used and will fix nitrogen in raw faeces but not in sterile faeces.

**Experiments at the Indian Institute of Science.** Experiments were carried out by Sarangdhar and Nayak in 1916 in which 500 c.c. of activated sludge and 1,500 c.c. of raw sewage

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served as a starting-point, after which a known daily volume of clear effluent was withdrawn and a known volume of fresh sewage put in. After continuous aeration for 5 days there appeared to be a clear gain in total nitrogen.

Finally, mention may be made of experiments by R. R. Deo, in which the following medium was used, similar to that used by Walton in his work at Pusa on nitrogen fixation, except that di-potassium hydrogen phosphate was substituted for the mono-potassium salt :

Mannite . . . . .	20	grams
Di-potassium phosphate . . . . .	0.3	..
Magnesium sulphate . . . . .	0.2	..
Sodium chloride . . . . .	0.2	..
Calcium sulphate . . . . .	0.1	..
Water . . . . .	1000.0	..

A number of small flasks containing this medium were inoculated with activated sludge which had been prepared at Jamshedpur and stored in a tin for upwards of a year.

Some of these were placed in an incubator at 35° C. and incubated without artificial aeration. Air was bubbled through others at laboratory temperature for about 10 hours daily with precautions against gain or loss of ammonia.

In every case there was a gain of nitrogen.

In the incubator without any artificial aeration the gain in nitrogen was about 15 per cent. in 2 weeks rising to 26 per cent. in 6 weeks. With aeration at laboratory temperature there was a gain of 25 per cent. in 4 weeks.

The foregoing experiments unite in showing that an increase of nitrogen, greater or less according to circumstances, unquestionably takes place when activated sludge is kept in contact with air and moisture, especially if the necessary carbohydrate medium is present.

These experiments have since been confirmed in large-scale practice. While it is true, as pointed out by several observers, that loss of nitrogen may occur during the purification of sewage by activated sludge, yet this is always under conditions of intermittent aeration ; if aeration is continuous such loss does not take place.

When activated sludge is allowed to ferment under aerobic

conditions together with carbonaceous materials, as in the production of compost, there is clear evidence of nitrogen fixation. Fixation has also been shown to occur during the preparation of compost from cow-dung and vegetable refuse. The effect of humus on nitrogen fixation will be referred to in the next section.

The foregoing has reference to aerobic organisms of the *Azotobacter* type. It has been pointed out by Dr. N. Kunjan Pillai, of Travancore, that in a large class of soils in which rice is cultivated, conditions are anaerobic, and there organisms like *Clostridium* are likely to play the chief part in fixing nitrogen. He has himself found such a species in some rice soils of Travancore.

The characteristic features of these two classes of nitrogen-fixing bacteria and the chemical changes accompanying their activities may now be considered.

In the original experiments of Winogradsky the two organisms *Azotobacter* and *Clostridium* were observed to be present together, and only under these conditions was *Clostridium* able to fix nitrogen in presence of air, the oxygen being taken up by the associated bacteria. In pure culture *Clostridium* could only fix nitrogen under anaerobic conditions.

The fixation of nitrogen by *Clostridium* under anaerobic conditions requires less expenditure of energy than when oxygen is present, since in the former case the energy is derived from the partial decomposition of carbon-containing compounds, whereas in presence of oxygen these are more completely oxidized to  $\text{CO}_2$ . Thus it has been found that an anaerobic organism produced 45 per cent. acid and 55 per cent. gas out of the sugar consumed, as compared with 33 per cent. acid and 67 per cent. gas in the case of the aerobic organism. The aerobic organism fixes more nitrogen in proportion to the sugar consumed, but on the other hand liberates more energy in the process than does the anaerobic organism.

A careful investigation of the chemical changes accompanying the fixation of nitrogen by *Azotobacter chroococcum* has been made by Norris and Ranganathan, an account of which may serve to illustrate the methods of study employed in this field of research.

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The organism was isolated from ordinary garden soil by inoculating 100 c.c. of Ashby's mannite medium with 1 gram of the soil.

Ashby's medium has the following composition :

Distilled water . . .	1,000 c.c.	NaCl	0.2 gram
Mannite . . . . .	15 grams	CaSO <sub>4</sub> .2H <sub>2</sub> O	0.1 „
K <sub>2</sub> HPO <sub>4</sub> . . . . .	0.2 gram	CaCO <sub>3</sub>	5.0 grams
MgSO <sub>4</sub> .7H <sub>2</sub> O . . . . .	0.2 „		

In the experiments concerned with the study of the chemical reactions accompanying nitrogen-fixation, dextrose was used instead of mannite.

The  $P_H$  of the mannite medium was maintained between 7.0 and 7.4. In about 3 days a heavy growth was obtained, a loopful of which was taken and subcultured in the same medium until microscopical examination showed it to be comparatively pure when it was plated out and subcultured on a mineral salts-mannite-agar medium, until nothing but milky white colonies of *Azotobacter* were to be found. After about 10 days' growth the colonies became brownish black owing to the pigmentation which is characteristic of the organism.

For the experiments on nitrogen fixation, as has been said, dextrose was used as the source of carbon, 1,000 c.c. of sterile dextrose-Ashby medium being inoculated with 10 c.c. of fresh culture of *Azotobacter*. The sugar was completely fermented in 8 days, and more than 50 per cent. of the nitrogen fixed from the atmosphere was assimilated within the first 3 days.

Special experiments were directed to determine the relation between concentration of sugar and nitrogen fixation and also of the effect of phosphates on the process. It was found that there was no regular increase in nitrogen fixation with increasing concentration of sugar and that phosphates accelerate the process of nitrogen fixation, but do not economize sugar consumption.

The balance between the carbon in the original sugar used, and the amount recovered as carbon dioxide and present in the residual growth and the solution containing the by-products of fermentation, was determined by carrying out the fermentation in a current of air and collecting and weighing the carbon dioxide evolved.

The carbon in the residual growth, and remaining in solution after the fermentation, was determined by wet combustion according to the latest methods. The results indicate that rather more than 70 per cent. of the carbon due to sugar utilized appeared as carbon dioxide, and about 12 per cent. was assimilated by the bacterial cells, leaving about 18 per cent. constituting the carbon of the fermentation products other than carbon dioxide. These by-products were found to consist of ethyl alcohol, aldehyde, and formic, acetic, lactic and tartaric acids.

Special experiments indicated that aldehydes and acids were first formed, and that by-products of these arose at a later stage.

The mechanism of the nitrogen fixation was studied by removing samples from the fermenting flask every alternate day, and examining for total ammoniacal nitrite and nitrate, mono-amino and di-amino nitrogen. The results pointed to the first product of atmospheric nitrogen being ammonia; neither nitrites nor nitrates were formed. As the culture grows older the ammoniacal nitrogen gradually decreases and there is a corresponding increase in the complex nitrogen. Similarly the mono-amino nitrogen content gradually decreases with the increasing age of the culture. It therefore seems likely that the first product of nitrogen fixation by *Azotobacter* is ammonia, which is gradually worked up into more complex forms of nitrogen.

Preliminary investigation of the composition of the *Azotobacter* cells indicated that they contain about 30 per cent. protein matter, together with a considerable amount of fat and phosphatide material.

An examination of the *enzymes* present in the cells was made by grinding these with quartz sand and a little glycerol. The mass was centrifuged and the supernatant liquid used in the tests. Evidence was obtained of the presence of the following enzymes, de-aminase, carboxylase, oxidase and catalase.

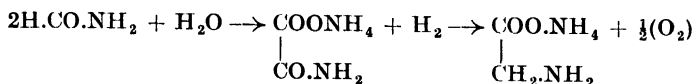
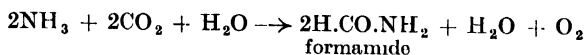
Experiments to determine the effect on nitrogen fixation of the association of *Azotobacter* with certain fungi were carried out and it was found that the presence of *Aspergillus* or *Citromyces* did appear to increase the nitrogen fixation. Both these species break down dextrose very vigorously and it is

possible that *Azotobacter* can more readily utilize the intermediate products so formed than it can dextrose itself.

Norris and Ranganathan's conclusion that ammonia is the first product of nitrogen fixation is in accordance with the observations of earlier workers and with what might be expected from the energy conditions. The combination of nitrogen and hydrogen is exothermic, the reaction evolving 24.0 cal., while the formation of nitric oxide absorbs 43.2 cal.

Winogradsky believed that in the case of anaerobic *Clostridium* the bacterial plasma produces ammonia out of the nitrogen gas and nascent hydrogen with which it comes into contact. The hydrogen is formed in the butyric fermentation of the sugar initiated by the organism. The ammonia is immediately assimilated and converted into protein.

The actual assimilation of the nitrogen may take place according to the following reactions :



Other workers have observed the stimulating effect of the presence of other organisms on the fixation of nitrogen by *Azotobacter*. The simultaneous activities of *Azotobacter* and *Clostridium* have already been referred to and have been found to lead to an increased fixation of nitrogen. The interaction of protozoa and *Azotobacter* has been studied by Cutler and Bal, and Cutler attributes this to :

1. Increased individual efficiency, resulting from the smaller size of the population.

2. The removal by the protozoa of the older cells of azotobacter leaving the field clear for the younger and more vigorously growing organisms which are the most active fixers of nitrogen.

3. The removal of the nitrogen fixed by azotobacter by transferring it from the bodies in which it chiefly occurs to the bodies of amœbæ feeding upon them, in which form it can accumulate without hampering the fixation process.

Whether these suggestions are really in accordance with

fact or not, the actual end result is of considerable interest in connection with the Activated Sludge Process, where the presence of protozoa in a vigorous state of activity has been found to be characteristic of "healthy" sludge, containing a high percentage of nitrogen.

Among other factors influencing nitrogen fixation in the soil must be mentioned iron, which in the form of its salts has a definitely favourable influence upon the development of *Azotobacter*, both by playing a part in its metabolism and exerting a favourable influence through its colloidal condition. The effect of iron is more marked in presence of "humus" or soil organic matter, when indeed it is in the form of an organic compound. This is a fact of considerable importance in the stimulation of nitrogen fixation in the soil and in composts, to which reference will be made in later chapters.

A great deal of research has been carried out by Bottomley and others on the effect of "growth-promoting" substances (vitamins, auximones) upon *Azotobacter*. The results are still subject to discussion, but it would seem proven that "auximones," present in humus material, do tend to increase nitrogen fixation.

The series of changes which has been discussed in the foregoing chapters may be usefully summarized in the diagram (Fig. 11, p. 154), which is self-explanatory.

The application of the facts and conclusions discussed in this and previous chapters to the problems of sewage and refuse disposal, and of agriculture in its relation to food production, will form the subject of the remaining portion of this volume. Opportunity will thus be afforded for the further consideration of the conditions governing efficient nitrogen conservation.

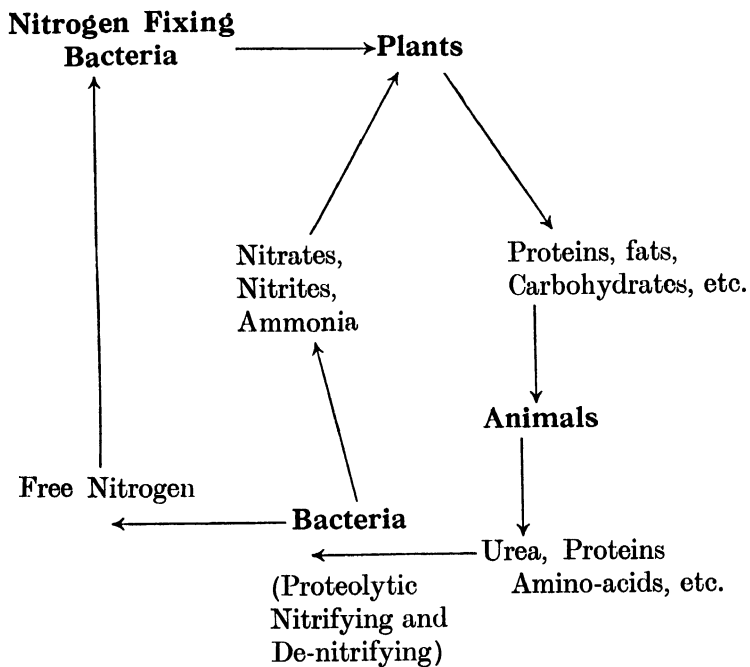


FIG. 11.—The Nitrogen Cycle.

## CHAPTER VIII

### THE CHEMISTRY OF SEWAGE PURIFICATION

The problem of dealing with the waste products of human life becomes increasingly difficult as mankind desires to live in large cities. Thus in ancient days in India it was the custom to vacate temporarily or evacuate entirely, a village which had been lived in for a sufficiently long time, the population then re-establishing itself in a fresh place in order that the impurities of many years might finally be disposed of by natural agencies. The sites of these old towns and villages have been converted into nitre deposits which in many cases yield profitable returns from the nitrate recovery.

As the conditions of life become less simple what is known as the water-carriage system is generally introduced, i.e. a system of underground pipes or sewers whereby waste matters are carried away at least from the houses. Already in many large towns even in India such a network of pipes and sewers exists, or provision is made for the carrying away of liquid waste by means of open channels. The liquid flowing along these channels consists mainly of wash water and urine, and is known as *sullage*. Solid excreta are generally removed by hand. A complete water-carriage system employs a network of closed pipes and sewers, into which the solid excreta may be discharged together with the same liquid waste which by itself constitutes *sullage*. The liquid discharged from closed sewers and which includes solid excreta is known as *sewage*. The problem of sewage purification is concerned with the conversion of these liquids, and the impurities carried by them, into inoffensive end products. The re-absorption of these products into the cycle of life is an important problem in nitrogen conservation.

In western towns and cities the provision of a complete

water-carriage system is almost universal, and large sums have also been spent in various methods of sewage purification.

In India and the East the more general condition of things is the provision of sullage drains leading to sullage farms, and the separate disposal of nightsoil by means of trenching or otherwise.

The larger towns in the East, however, are now finding the complete provision of water carriage more and more a sanitary necessity.

It is of very great importance, therefore, for the proper carrying out of any of these sanitary methods that the changes which it is intended to bring about by their means should be thoroughly understood by those responsible for their construction and efficient operation.

Although the actual construction of a modern sewage works or the laying out of a modern sewage farm is largely a matter of engineering the design depends upon careful adaptation of means to ends, and the bacteriological chemist and the engineer should here work in collaboration. The object of the works, as already indicated, is to convert objectionable waste products, which if left to themselves would be a source of nuisance and danger, into other substances which are incapable of producing such unpleasant and unhealthy developments. In the course of the necessary transformation at one point or another practically all the typical chemical changes which have been considered in the previous chapters are met with, and the consideration of the processes carried on in a modern sewage works forms, therefore, an excellent illustration of the application of bacteriological and enzyme chemistry. The most modern processes, in addition to the purification of the sewage, have also as their aim the recovery of a large proportion of the nitrogen present for the use of agriculture.

It will be necessary in the first place carefully to consider the composition of ordinary town sewage. For this purpose reference will only be made to domestic sewage, the question of the treatment of trade effluents, or mixtures of trade effluents and sewage constituting a special problem.

The main constituents of domestic sewage may be described as follows :

(i) *Matters in Solution.* (Mainly derived from urine.)

Nitrogenous Substances, e.g. urea and kindred compounds ;

Mineral Salts, chiefly sodium chloride together with phosphates.

(ii) *Matters in Suspension, Emulsion or Colloidal Solution.*

Nitrogenous Substances of complex character containing sulphur (mainly derived from fæces).

Cellulose (disintegrated paper) and vegetable debris.

Soap and Fat.

(iii) *Sedimentary Matters,* silt, clay, sand, etc.

There cannot, of course, be sharp lines drawn between these various classes of substances. It will depend, e.g., on the hardness of the water, how much, if any, of the soap is present in solution or suspension ; substances in Class (ii) will also be partially carried down by the quickly sedimenting mineral matters of Class (iii).

In general, about twenty gallons may be taken as the usual water supply per head per day, in which the above constituents are disseminated.

The complete purification of the sewage, which is effected by bacterial treatment, results finally in the production of some or all of the following substances :

(i) *Gases :*

Methane (marsh gas).

Hydrogen.

Nitrogen.

Carbon dioxide.

(ii) *Soluble Salts :*

Nitrates.

Phosphates.

Sulphates.

Chlorides.

(iii) Insoluble residual matters conveniently termed "Humus."<sup>1</sup>

<sup>1</sup> Strictly speaking the term "humus" should be reserved for organic residual matter of special chemical characteristics. For the sake of brevity the term is used here to include organic matters of somewhat indefinite composition which remain undecomposed at the

## TREATMENT OF SEWAGE BY "DILUTION"

It is possible to transform sewage products by direct oxidation, through the agency of the requisite organisms, in the presence of air.

Thus, if a sample of sewage be shaken in a bottle with an excess of water saturated with air, and allowed to stand a sufficient time, under conditions which allow of an excess of oxygen being always present, it will be gradually transformed, and eventually nothing will be left in the bottle but a solution of the above salts, with some brown particles of "humus," and some carbon dioxide in solution.

Although it can be shown by careful analysis that the sewage suffers a regular sequence of changes, yet at no point are offensive gases evolved under these conditions—and neither marsh gas nor hydrogen is produced.

In the above case the sewage is purified under strictly *aerobic* conditions. In practice such conditions are met with when sewage is discharged into a stream or body of water, of such a volume that an excess of dissolved oxygen is always present, over that necessary to oxidize the sewage.

It is rare, however, to find conditions under which it is possible to deal with sewage in this way, by what may be termed the *dilution* method. A favourably situated outfall must admit of the sewage being quickly mixed with a large excess of water, so that at all times the oxygen content is maintained at such a point that offensive products cannot be produced.

The extensive investigations of Letts and Adeney<sup>1</sup> on the pollution of estuaries and tidal waters have resulted in the suggestion of various standards, in relation to the amount of oxygen available in the mixed sewage and tidal water, to meet the different conditions of discharge.

end of the ordinary processes of purification of sewage. They are generally associated with a fair proportion of mineral matter, especially phosphates, and lime and alumina compounds.

<sup>1</sup>Royal Commission on Sewage Disposal, Fifth Report 1908, Appendix VI: "Report on the Pollution of Estuaries and Tidal Waters," by E. A. Letts and W. E. Adeney.

The methods and results of these researches have been published by Dr. Adeney in his valuable and interesting monograph on *The Dilution Method of Sewage Disposal*, which has been consulted in the writing of this section.

It is at the outset of first importance that the conditions of solubility of oxygen in water should be fully understood. Actually oxygen is very sparingly soluble in fresh-water under atmospheric conditions and still less so in sea-water. Thus, according to Adeney, it requires but 1.0 gram of oxygen to saturate 100,000 c.c. (22 gallons) of fresh-water at 65° F., while only 0.8 gram of oxygen is required to saturate a similar volume of sea-water at the same temperature under atmospheric pressure.

Adeney and his co-workers have shown that aeration of a body of water is greatly increased by the "streaming" of thin films from the surface, owing to the increased density of the surface film produced by evaporation and cooling. This effect is naturally greater in the case of sea-water owing to its higher saline content. Experiments arranged to eliminate this streaming effect indicated that the rates of absorption of oxygen by fresh and sea-water were practically identical when these values are expressed in percentages of saturation.

Adeney gives five conditions upon which the mechanism of the aeration of fresh and sea-waters, under open-air conditions, primarily depends. They are :

- (1) Area of water exposed to the air.
- (2) Temperature.
- (3) Humidity of the air.
- (4) Salinity (in the case of tidal waters).
- (5) Wind, causing both an increase in area exposed and an acceleration of evaporation per unit area.

The sparing solubility of oxygen in water, even when the foregoing conditions are all favourable, limits the rate of oxidation of all but the more soluble of the above mentioned constituents of sewage. The finer suspended or colloidal matters are in general attacked by the higher forms of life present in natural waters, whose oxygen demand in proportion to the organic matter consumed, is less than that of bacteria.

These higher forms of life are comprised under three categories.

(a) "Plankton" (floating organisms), i.e. micro-organisms, such as algæ, protozoa and minute crustaceæ, which are carried about passively by water currents.

(b) The "Benthos" (depth of the sea) include those animals which are attached or practically so to the bed of a body of water such as the annulosa, mollusca, and the larger forms of crustaceæ.

(c) The "Nekton" (swimming) comprises fish and other forms of animal life which freely traverse wide areas of water.

It is important to note that "plankton" life is the main food of fish, and so in the cycle of nature we have bacteria which are consumed by the protozoa and these again by fish. As fish, therefore, soluble nitrogenous matter again becomes insoluble and capable of utilization by man.

These changes can be successfully controlled in the aquatic sewage farm, just as on land the nitrogen is made to pass from bacteria to plant and plant to animal.

It is clear that the conditions under which sewage matter may be discharged into any body of water without offence will depend on several factors, among which the following may be mentioned :

(a) The concentration or "strength" of the polluting discharge.

(b) Its volume in relation to the body of water with which it is mixed.

(c) The cleanliness and the oxygen content of this body of water.

In the light of the investigations of Letts and Adeney the Royal Commission on Sewage Disposal conclude that with a dilution of over 500 volumes crude sewage may be discharged subject to certain safeguards. For dilutions below 500 various kinds of preliminary treatment become necessary, differing in completeness in accordance with the amount and nature of diluting water available.

The Commission concluded that the consumption of dissolved oxygen by a polluted liquid was the most trustworthy chemical index of its liability to cause nuisance or danger

The now well-known "Royal Commission Standard" which they recommend for effluents which have received complete purification, requires that the effluent should not contain more than 3 parts of suspended matter per 100,000 c.c. and that, including its suspended matters, it should not take up more than 2 grams of dissolved oxygen per 100,000 c.c. in five days at 65° F. (18.3° C.).

In addition to the researches of Letts and Adeney, many other investigations have since been made by numerous authorities, whose publications constitute a very considerable literature. Among these the Reports of the Metropolitan Sewerage Commission of New York, 1910-14, are worthy of special mention for their very complete and extensive character. The observations in particular of the dissolved oxygen content of the water of New York Harbour at numerous critical points have been continued up to the present time by Kenneth Allen, Sanitary Engineer to the Board of Estimate and Apportionment of the City of New York.

Elaborate studies of the pollution and natural purification of the Ohio River have been published by the United States Health Service, and a critical review of the whole subject of purification by dilution is given in *Public Health Bulletin*, No. 173, under the title of "The Oxygen Demand of Polluted Waters."

Among the valuable researches of the recently constituted Water Pollution Research Board of the Department of Scientific and Industrial Research of the British Government, the survey of the River Tees has extended our available knowledge, especially of the algal life of polluted streams.

A modern application of the dilution method to the purification of the sewage of towns in the thickly populated valley of the Ruhr River in north-western Germany is of great interest. The sewage which enters the tributaries of the Ruhr is freed from grosser solids by carefully designed settlement tanks. A large impounding reservoir is then constructed at the junction of the tributary with the main stream, and in this impounding reservoir final purification takes place by means of the natural agencies above described.

The first impounding reservoir known as Lake Hengstey

was completed in 1928, the second called Lake Harkort came into operation early in 1931. Six more are in contemplation varying in surface area from 100 to 590 acres. These reservoirs are great assets from the point of view of sanitation, economics and public recreation.

The conditions for dilution and aeration make it possible to complete the purification by natural agencies of any residual sewage matter present, and obviate the necessity for final purification works of the kind shortly to be described. By ingenious engineering methods the water is pumped at night to a high-level reservoir from which it is available to generate electricity during the hours of peak load.

Finally the lakes afford recreational opportunities, boating, bathing, and open-air enjoyment for the industrial population of the valley.

In the majority of cases, even where the dilution method is resorted to, and still more when it is a question of purification by application to land or artificial filter beds, some form of *tank treatment* is required, and the chemistry of this process may now be considered.

#### TANK TREATMENT OF SEWAGE

When sewage passes through a tank of any description deposition of the heavier matters present will take place with greater or less completeness, according to the method of construction of the tank, the rate at which the sewage passes through, and the addition or otherwise of chemical coagulants to facilitate the deposition of the matters in emulsion.

We may consider tank treatment according as it is directed to effect one or other of the following results :

- (a) Simple sedimentation.
- (b) Chemical clarification.
- (c) Anaerobic decomposition.
- (d) Aerobic decomposition.

##### (a) Simple Sedimentation.

In this case we shall expect all the heavier matters in the sewage to be deposited, i.e. the mineral substances, sand and silt, etc., together with paper, fæces, grease and soap. The

character of the effluent will depend on the dilution strength of the original sewage and the distance between the sewage works and the source of the sewage. The passage of sewage through sewers results in the mechanical breaking up and emulsification of faecal matter and the setting up of ammoniacal fermentation of the urea present in the urine. The extent to which these changes take place depends on the length of sewer to be traversed and also the state of construction of the sewer. New sewers with smooth surfaces will not readily allow the formation of adhering accumulations of sewage matter which tend to facilitate fermentative change.

With well-laid sewers only the initial stages of fermentation of nitrogenous matter will have set in by the time the sewage reaches the works, and such sewage should therefore be comparatively inoffensive. Moreover, in designing tanks for simple sedimentation they should be of such a size that the sewage will not remain in them sufficiently long for any but the preliminary stages of decomposition to take place.

The *sludge* or deposit from such a sedimentation tank will, in consequence of what has been said, also need to be very frequently removed if offensive decomposition is not to take place, and not only must it be quickly removed, but it must, for the same reason, be quickly disposed of, e.g., by burying in trenches, by spraying and harrowing over the surface of agricultural land, or by other methods some of which will be referred to later.

#### (b) Chemical Clarification.

In the chapter on the chemistry of albumins it was shown that colloidal substances of this nature could be coagulated and precipitated by addition to their solutions of hydrated precipitates such as those of iron and aluminium hydroxides. This precipitation is made use of for the clarification of sewage. The chief precipitates used are aluminium sulphate (alumino-ferric), ferric sulphate, and lime and ferrous sulphate (green copperas) used in conjunction. The choice of precipitant depends on the relative market price of the particular chemicals and on facilities available for their efficient use.

The right adjustment, e.g., of lime and copperas so as to keep the lime always slightly in excess requires constant attention, whereas salts of alumina are precipitated directly by the carbonate of ammonia present in the sewage.

The development of modern processes for the complete purification of sewage without the aid of chemicals has rendered their use infrequent except in cases where exceptional amounts of particular trade effluents are present the removal of which is necessary before purely biochemical processes can be usefully employed. Thus at Wakefield (Eng.) where the sewage contains large quantities of wool scouring waste, as well as dye waste and other refuse from textile factories, ferric salts have been used with advantage. At Bradford, where wool scouring is the main industry, the sewage is treated directly with sulphuric acid in order to liberate the wool fat, and the resultant sludge is treated in filter presses at a temperature sufficient to liquefy the grease which is collected and sold. The residual sludge cake containing more than 2 per cent. of nitrogen finds a ready market as a fertilizer, especially after it has been subjected to a process of natural fermentation which takes place largely through the agency of moulds when the sludge cake is piled in heaps.

For the removal of sulphides and sulphur compounds such as occur in the effluents from sulphur dyeworks, and from certain artificial silk processes, lime and copperas have been recommended as precipitants prior to final purification of the soluble matters by filtration or otherwise.

All processes of chemical precipitation while they are capable of yielding effluents containing less suspended matter than other methods of preliminary tank treatment, result in the production of considerable quantities of sludge which needs special care in its disposal, as its constituents are still capable of undergoing offensive decomposition.

The processes of simple sedimentation or of chemical precipitation are not, strictly speaking, processes of purification, but only of disposal, since the offensive matters removed from the liquid are merely collected in the sludge and have to be ultimately purified, that is re-absorbed without offence into the cycle of nature by further processes occurring either

in the soil or in certain methods of sludge treatment to be described later.

### (c) Anaerobic Decomposition.

A tank designed to facilitate anaerobic decomposition differs from a sedimentation tank chiefly in being relatively larger and so allowing time for decomposition to take place under anaerobic conditions. Such a tank has been variously termed a cesspool, a septic tank, a liquefying tank or a hydrolytic tank. The differences in design are mainly structural to facilitate deposition and removal of the solid matters and to control more or less the character and extent of the chemical changes taking place. These chemical changes may at this point be usefully considered in detail.

*Decomposition of Cellulose.* The anaerobic decomposition of cellulose has been considered in a former chapter; it was there shown to be due to two organisms, one of which produced mainly hydrogen and the other marsh gas. In both cases carbon dioxide ( $\text{CO}_2$ ) and fatty acids are also produced as by-products. The production of gases is a visible indication that fermentation is taking place in the sewage. The evolution of nitrogen has often been regarded as taking place in septic tanks; the author's experience, however, would tend to show that such nitrogen if it is produced arises either from the air dissolved in the incoming sewage, or from the reduction of nitrates present therein, and not from the anaerobic decomposition of nitrogenous matter. Studies which were made by the author in 1906, led to the conclusion that the gases which are evolved in the septic tank arise chiefly from the decomposition of cellulose. The researches of Omelianski, already referred to, showed that the optimum temperature for this fermentation was above  $90^\circ \text{F}$ . For this reason the activity of septic tanks is specially marked in tropical countries. In localities where the temperature is seldom less than  $70^\circ \text{F}$ ., and often much higher, a quite extraordinary development of gas may take place. The illustration on Plate IV (i), p. 132, is from a photograph taken by the author at an installation at that time attached to the Leper Colony at Matunga, near Bombay.

Here the tanks were provided with gas-tight iron covers and

the gases were drawn from below these into a gas holder. The carbon dioxide was removed by lime purifiers and the inflammable marsh gas and hydrogen used for driving the engine which pumps the sewage, and also for lighting and cooking purposes. The gas holder, lime purifiers and engine house are shown in the photograph.

In the early days of the adoption of the septic tank in England some attempt was made to utilize the gas evolved for lighting and heating purposes on a modest scale. It was not, however, until research was directed towards the fermentation of the sludge as apart from the whole of the sewage, both liquid and solid, that large-scale application of the anaerobic process of fermentation has been successfully employed. One of the earliest plants for collecting and utilizing the gas in this way was at Parramatta, New South Wales, but it has been largely through the researches of F. R. O'Shaughnessy at Birmingham, assisted by Mr. J. D. Watson and his successors, that successful utilization of the gas has been achieved. There is now an extensive literature on the subject mainly from Germany and the U.S.A. The important facts which emerge appear to be the importance of raising the temperature, which, as only the sludge has to be considered and not the whole sewage, is now a practical proposition, and the control of the  $P_H$  value by judicious additions of lime as may be required.

An important observation has been made by Buswell and his co-workers in Illinois, that a large proportion of the gas from European and American sewage arises from the decomposition of grease and soap. This sewage also contains a greater proportion of cellulose residue from paper than is present, e.g., in tropical sewage. Altogether, therefore, the yield of gas from sludge in non-tropical countries is greater than from Indian sewages, especially when artificial heating is resorted to.

Numerous ingenious designs of tank have been employed to increase the facility of separation and separate fermentation of the sludge, but this aspect of the subject is chiefly of engineering interest. From the point of view of nitrogen conservation the most important fact to be considered is that although there is no actual loss of nitrogen in the gaseous state in the

course of these fermentations, there is certainly a very great decrease in the amount of nitrogen recovered in the sludge since, under anaerobic conditions, the protein substances in the sewage are broken down into amino acids and finally into ammonia, so that the proportion of ammoniacal derivatives is distinctly greater in the effluent from these tanks, than in the soluble portion of the incoming sewage.

*Ammoniacal Fermentation.* As already mentioned, the greater part of the nitrogen of sewage is present as urea and the ammoniacal fermentation readily sets in. In many cases, indeed, it may be almost complete before the sewage reaches the purification works. A great deal of discussion as to the necessity or otherwise of preliminary anaerobic treatment of sewage has been confused by the failure clearly to distinguish between absolutely fresh sewage and sewage which has passed, it may be, through several miles of sewers. The consideration of the conditions necessary for nitrification which found its place in Chapter V shows the necessity for a preliminary conversion of urea and allied substances such as amino acids into ammonia before nitrification takes place. The author has found beyond question that if absolutely fresh sewage is to be put upon a filter, considerably more filter space is requisite to convert the nitrogen into nitrates than if time is first allowed, e.g., by retention in tanks for ammoniacal fermentation to take place. It is however rarely in the case of ordinary town sewage works that sewage is met with in such a fresh condition. As already stated, ammoniacal fermentation nearly completes itself in the sewers, or at any rate during a comparatively short tank treatment. It is doubtful whether urea would ever be found in an ordinary sample of town sewage.

The case may be somewhat different where absolutely fresh sewage has to be dealt with, e.g., from the many mill latrines which are at work in India. In such a case a certain provision of preliminary tank space, even if small, in which some preliminary breaking down may take place, is desirable, since it has been shown that considerably less provision is then necessary either in the nature of filters or of aeration tanks for the production of an inoffensive effluent. Such preliminary tank space, whether designed simply to retain the faecal matter

for a short time while it undergoes partial breaking down or "maceration," or mechanical disintegration by vigorous aeration or otherwise, plays the part of a prolonged flow through a length of sewer.

Apart from the urea, however, the other nitrogenous constituents of sewage have to be considered ; these are of a very complex character. Briefly speaking, all the various decomposition products of albumin will be represented in some form or other, together with undigested portions of nitrogenous food. Apart from undigested food it has been shown that the bulk of faeces consists of intestinal secretions, epithelium, detritus, etc., and masses of bacteria. Under the conditions maintained within the anaerobic tank these will all gradually break down, and an important consideration here arises, viz. as to how far this breaking down is to be carried. We have seen that eventually by the decomposition of albuminous matter, evil-smelling substances such as hydrogen sulphide, indole, skatole and various amines are produced. At one time there was a tendency to design septic tanks of too large a capacity so that the decomposition of nitrogenous matter was carried more or less to its limit with the production in many cases of serious nuisance. The so-called hydrolytic tank of Travis and the Emscher-Brunnen of Imhoff were designed with the object of rapidly depositing solid matter and retaining it for a period sufficiently long to enable it to be broken down as far as is economically desirable, while the liquid portion of the sewage was led quickly away, sufficient time only being allowed for ammoniacal fermentation and incipient proteolysis to take place therein. These tanks were virtually settling tanks with a separate chamber for the deposited sludge. Of recent years it has become customary for the sludge to be removed entirely into a separate digestion tank, and recent research has shown that the more concentrated the sludge which is thus removed, the less is the liability to nuisance since it has been shown that volatile sulphides are not produced when the quantity of water in contact with the sludge is restricted to 95 per cent. or less. When this dense sludge is handled in the crude or unfermented state the odour is that of indole, skatole and various acidic bodies ; if the dense sludge

has undergone methane fermentation under alkaline reaction, then the only volatile matters possessing odour are traces of volatile paraffins and ammonia. These produce a faint tar-like odour associated with separate sludge digestion. From the point of view of nitrogen conservation it is important to recognize that whether the sewage is submitted to anaerobic fermentation as a whole, or whether the sludge is fermented separately, the tendency is for rapid proteolysis to take place and for nitrogen to go into solution in the form of ammonia and amino-derivatives which pass away in the effluent or in the liquid drawn away from the sludge. These nitrogenous products are only recovered in a practical sense if they are applied to land and used for the irrigation of crops. There are, however, special difficulties in using highly concentrated nitrogenous liquids of this kind for broad irrigation, and it is generally necessary to dilute such an effluent with clean water.

There is no evidence that any appreciable quantity of nitrogen is evolved in the gaseous state either from ordinary septic tanks or from sludge digestion tanks.

*Decomposition of Fats.* There is evidence that besides the decomposition of cellulose and nitrogenous products a considerable change takes place in the fatty constituents of the sewage in the anaerobic tank. In European sewage, fat is always present in household refuse, the washing of plates and dishes. All the soap which is used finds its way into the sewage and partially digested fatty matter is often present in fæces. Free fat is readily broken up by bacterial or enzyme action yielding fatty acids and glycerine. The higher fatty acids thus produced may be further broken down into soluble fatty acids of lower molecular weight. Soaps also are capable of change but only very slowly.

In the case of small installations attached to institutions such as sanatoria, asylums or colleges where a separate laundry exists, the author's experience strongly favours the separate retention of the soap by treatment with lime salts and collection of the precipitated lime soaps in specially devised intercepting traps. In such cases also it is desirable to retain the grease waste from the kitchen which is quite capable of being

readily and economically worked up into soap on the spot. The retention of fats apart from its economic aspect greatly simplifies the operations of the sewage works where insoluble soaps are likely to be formed causing accumulations in the tanks and clogging of the filters which receive the tank effluent. The decomposition of fat also gives rise to an extremely objectionable rancid odour due to the formation of butyric acid.

The much greater facility with which sewage can be purified in India and other Eastern countries is among other things due to the small proportion of soap and fat present in the sewage so that the colloidal matters flocculate much more readily and the decomposition changes are much less complicated.

To summarize therefore, the changes which take place in the anaerobic tank are mainly the decomposition and gasification of cellulosic derivatives, the ammoniacal fermentation of urea, the breaking down to a greater or less extent of more complex nitrogenous substances and the splitting of fats. These changes are almost wholly due to bacteria and to enzymes, the latter in all probability being present in faeces. The changes can be followed by analysis of the sewage, and of the deposited sludge. In the liquid sewage it will be found that the free ammonia increases at the expense of the albuminoid. The oxygen absorbed from permanganate while possibly not greatly differing at the beginning and end of the process in its total amount, will be found to be distributed in different proportion between the easily oxidized matter and the more difficultly oxidizable substances, or in other words, while the oxygen absorbed in 4 hours by the so-called "4 hours' test" may not greatly decrease, the oxygen absorbed in 3 minutes will probably increase.

Whether there is an increase or decrease in *colloidal matter* depends upon circumstances. With highly concentrated sewages such, e.g., as are met with under conditions of very limited water supply, actual solution of colloidal matter undoubtedly takes place owing to the breaking down, e.g., of albuminoid substances into amino acids; on the other hand, it may readily be the case that the colloidal substances in the

effluent from the tank receiving dilute sewage increase, owing to the washing out of colloids from the sludge present in the tank. In considering the changes taking place in anaerobic tanks the exact conditions of the tank must always be carefully borne in mind.

It was at one time considered essential that anaerobic tanks should be closed in order to prevent access of light and air. Numerous experiments have shown that this condition is not necessary. It is obvious that beneath the immediate surface the conditions must be anaerobic, and the covering of tanks is only called for by reasons of sightliness or to render more permanent the scum which generally forms owing to the throwing up of solid matter by the gases evolved during the fermentation of the sludge. A cover, of course, is necessary if the fermentation gases are to be collected and utilized, but is of very little use simply for the prevention of nuisance unless the escaping gases are collected and passed through a washing tower, e.g., of coke down which a spray of water passes. Such an arrangement used to be in operation in connection with a septic tank installation serving the residential suburb of Ghizireh, Cairo.

Mention may be made here of the so-called pit latrines which are becoming very popular in connection with the sanitation of villages and isolated houses in Southern India and elsewhere in the tropics. These are simply holes sunk in the ground to a depth of some 12 feet and the excreta are deposited directly into them. No water other than ablution water is used, yet there is apparently little or no nuisance. It would appear that a gradual process of decomposition sets in unaccompanied by the evolution of objectionable gases in quantity, just as the fermentation of ordinary sewage sludge has been shown to be reasonably inoffensive if sufficiently concentrated.

#### **(d) Aerobic Clarification.**

In the earlier experiments of Mr. Dibdin at Sutton, in Surrey, England, during the later nineties the attempt was made directly to treat crude sewage on a coarse contact bed, i.e. a tank filled with large pieces of coke, burnt clay or other

material. Such a tank is first filled up with the sewage, when the insoluble and colloidal matters are for the most part deposited upon the surfaces of the pieces of material in the tank and the liquid allowed to run off from the bottom of the tank. Air enters the interstices of the medium to replace the liquid. The tank is allowed to remain empty for some hours and opportunity is afforded for the deposited organic matter to be oxidized; the tank is then filled again with the sewage and the cycle of operations repeated. A tank of this kind may be termed an aerobic tank and the changes which go on in it are essentially different from those taking place under anaerobic conditions as described in the previous section.

The process used at Sutton acted well in so far that the heavier suspended matter in the sewage was largely removed and converted in course of time into an inoffensive residuum. The main drawback to the process was the gradual blockage of the interstices of the medium and the difficulty of cleaning it without complete removal from the tank. This difficulty Dibdin sought to avoid in a later invention known as the *slate bed*. In this case instead of the tank being filled with irregular lumps of material, super-imposed horizontal layers of slate are made use of, separated by distance pieces about 2 inches in depth. On filling the tank with sewage the suspended solids deposited themselves on the slates and were gradually oxidized in the same manner as in the Sutton process. It was possible, however, to remove the deposit from time to time from the surface of the slates by flushing out and so retaining the water-holding capacity of the tank undiminished. The writer had occasion to examine with some care the changes which went on in these slate beds. He found that the oxidation of the organic matter, and especially of the fatty constituents, was largely due to masses of nematode worms with protozoa, etc., and of course bacteria. The deposit on the slates in course of time assumed a liver-like consistency and could be stripped off in pieces and examined; the smell was not offensive but similar to that of an exposed river bank.

If some of the material was placed in a glass tube and air led over it considerable volumes of  $\text{CO}_2$  were given off through

the respiration and other changes of the organisms present. If the deposit was covered with water and air excluded it very soon putrefied and became offensive; it was evident, therefore, that in the working of such tanks care had to be taken that the conditions at no time became anaerobic. When the sewage first entered the tank a considerable amount of air was dissolved as the liquid trickled through the interstices between the slates and a further quantity was trapped underneath the slate surfaces. This sufficed to maintain aerobic conditions for an hour or two which was the length of time allowed to elapse before the tank was emptied. On flushing out the deposit and allowing it to drain and weather in the air it was gradually converted into a brown inoffensive mass resembling garden mould; this material was found to be an excellent nitrogenous fertilizer, the nitrogen content varying from 2.6 to 3.6. The process therefore handled the ordinary sludge problem in an inoffensive manner. The effluent, however, required further purification before it could be finally discharged.

Although it did not come into very extended use, the present writer can testify that the slate bed played its part in the development of completely aerobic methods of purifying sewage which ultimated in the Activated Sludge Process.

**Summary.** The choice of one or other of the foregoing methods of preliminary tank treatment depends on local conditions. In the case of small communities where constant attention cannot be given, and also where the fall is limited, anaerobic tanks find useful application. In certain cases also, notably at Birmingham and to some extent at Manchester, anaerobic treatment has been found useful; in the first case it produces an inoffensive sludge and in the second case it tends to neutralize the effect of antiseptic trade effluents present in the sewage before the latter is finally treated on filter beds. In both these cases, however, the presence of considerable quantities of iron salts in the sewage diminishes the chances of nuisance, owing to the combination with the dissolved iron of any sulphuretted hydrogen produced, with formation of black ferrous sulphide. The anaerobic process of recent years has been carried out chiefly in tanks designed

as already mentioned, confining the anaerobic fermentation to the deposited sludge only.

It was at one time thought that the clarification of sewage by means of chemicals must give place entirely to biological treatment of one sort or another.

At the date of the Report of the Royal Commission on Sewage Disposal their conclusions, in the author's opinion, were quite correct that many cases existed where the former method of purification was still to be preferred. Where the large amount of sludge produced by chemical precipitation could be easily and cheaply disposed of and where the space available for the final filtration was limited, the total expense involved was probably less by this method than by any other, owing to the longer life of the final filter beds in consequence of the smaller amount of suspended solids passing on to them. A typical case of the application of chemicals was afforded by the conditions of the sewage works at Salford. Here the available area of filters was necessarily very restricted owing to the site of the works. On the other hand, the sludge could be shipped to sea and the standing charges of the sludge steamer having always to be maintained, an increase in the sludge production would not necessarily mean a proportional increase in the cost. A somewhat similar set of conditions existed at the London outfalls.

With the advent of the Activated Sludge Process, however, it was possible with no greater provision of tank space to obtain sludge of a high nitrogen content and a really clarified or even fully oxidized effluent.

The use of chemical precipitants, therefore, is now mainly of value for treatment of special trade effluents or of sewage containing a large proportion of trade effluents. Thus at Bradford, where most of the wool-washing industry of Yorkshire is concentrated, there can be little doubt that the decision to treat the whole of the sewage with sulphuric acid resulting in the production of a sludge from which the wool grease could be extracted by hot-pressing has been amply justified. Even where the Activated Sludge Process is in use some preliminary treatment of trade effluents is often called for where these are present in large proportion.

## FINAL PURIFICATION OF SEWAGE

In general, as has been explained, some form of preliminary treatment is necessary before sewage can be finally mineralized in biological filter beds. It is possible, however, under special conditions to treat crude sewage directly on filters. Where the sewage is dilute and where considerable fall is available the liquid after efficient screening and removal of the coarser solids, sand, etc., in catch pits, may be directly sprayed upon coarse percolating filters of considerable depth. In this case the oxidation of the suspended and colloidal matters takes place by much the same agencies as were at work in the slate bed above described, and the resulting granular residue passes out at the bottom of the filter and can be retained in catch pits, or on the surface of sand strainers. Works at Rothwell in the West Riding of Yorkshire were successfully designed on such lines. The conditions differ in such a filter from those obtaining in the slate bed in that the liquid portion of the sewage passes in a thin film over the filtering medium, and its soluble impurities are therefore oxidized as well as the matters in suspension. If the rate of filtration is not too high it is even possible completely to oxidize fairly strong sewage in this way. A periodical renewal of a portion of the filtering medium is, however, likely to be called for in such a case. A good instance of such treatment of strong sewage was to be seen at Little Drayton, where a filter known from its designer, Colonel Ducat, as a Ducat filter was in use for many years, and gave good results as the writer can testify.

In the majority of cases where filter beds are used it will probably be necessary, or at any rate profitable, to adopt some form of preliminary treatment of the sewage before its final purification on filter beds, and we may now consider the changes which take place when such previously treated sewage is applied to filters.

The artificial filters in general use are of two types which may be broadly divided according as the sewage is applied intermittently or continuously into

- (a) contact beds,
- (b) percolating or trickling filters.

**(a) Contact Beds.**

The general design of a contact bed is seen in Fig. 12. It consists of a water-tight tank, generally of concrete, filled with filtering material carefully screened and graded to a definite size. The essentials of such a material are that it should be durable, i.e. not likely to crumble on use and should expose as large a surface as possible. Hard well-fused clinkers fulfil this condition most perfectly, but other materials may also be used if good clinker is not available. Effluent from the preliminary process is passed on to such a bed, and allowed to remain in contact generally for about 2 hours and then run off and if not sufficiently purified submitted to similar treatment on another bed at a lower level. The material in the second contact bed must be of smaller dimensions than

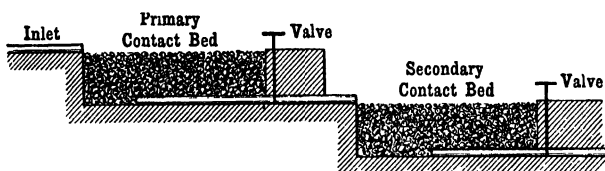


FIG. 12.—Contact Filter Bed.

that in the preliminary bed if the best results are to be obtained. In special cases a third treatment on still more finely graded material, e.g., sand, may be called for.

Although the contact bed has been largely replaced by the percolating filter, years of experience in Manchester and Sheffield, England, have shown that they are capable of sustained use at a cost, when capital and revenue charges are both taken into account, which compares quite favourably with percolating filters and their appurtenant machinery and humus tanks. One great advantage of the contact bed, especially on a large scale, is its entire freedom from nuisance, either from odours or flies. Provided a contact bed is not overworked, i.e. when the load put upon it does not exceed a certain limit, it has been proved capable of being maintained in adequate operation for a period of at least twenty years

without renewal of the material. Even when operated at higher rates, involving renewal or washing of the material at the end of a period of five or six years, the cost is still not prohibitive. It will therefore be of interest to consider with some care the biochemical changes which take place in this form of filter bed, especially as they illustrate in a clear way the changes which are likely to take place under various conditions of application of nitrogenous fertilizers to the soil, more especially the possibility under certain conditions of loss of nitrogen in the gaseous state.

The following are the principal changes which take place in a contact bed. The suspended and colloidal matter still present in the liquid to be treated is mechanically retained by the filtering medium. It is evident, therefore, that the fineness of this medium must increase as the amount of suspended and colloidal matter decreases if this mechanical effect is to be obtained. Besides the mere mechanical straining, a species of absorptive action also takes place between the surface of the medium and the constituents of the sewage, which increases within limits as the slimy layer thus formed on each fragment of filtering material becomes more well defined. This slimy layer also acts as a sponge, retaining an appreciable proportion of the liquid applied together with its dissolved constituents. A considerable amount of purification will therefore take place by purely mechanical and absorptive action immediately the liquid is applied to the filter. This action, however, would very soon cease, and the contact bed become clogged and foul were it not for the biological activities which are set up within it. These activities are exceedingly various and depend not only on the life of the bacteria, but on many higher organisms, notably small worms, many species of protozoa and even certain insects. Researches carried out at the Manchester laboratories and also at the Government experimental station in Berlin, have emphasized the functions of these higher organisms, and it is here that the choice of preliminary treatment, whether by simple sedimentation, by anaerobic or aerobic tanks, or by chemical precipitation, needs careful study. If a sample of sewage be collected in a sterile bottle, and allowed to stand

freely exposed to air but protected from infection by a plug of cotton-wool, a film of organic life generally makes its appearance. If this is carefully examined under the microscope after the lapse of some days or even weeks, numerous forms of life are generally visible. This life, potentially present in the sewage, is probably an important initial source of population of the sewage filter beds. The effect of the different methods of preliminary treatment, above referred to, upon this organic life has been even yet only imperfectly studied. We should expect, a priori, that effluents from simple sedimentation or from an aerobic tank would be the most favourable to the existence of aerobic organisms of this sort, as contrasted with either anaerobic treatment, which might destroy them, or chemical precipitation, which would tend mechanically to remove them. It is not unlikely that the organic life of sewage will vary according to the amount of subsoil and surface water drainage entering the sewers. The author has indeed found when investigating the conditions of purification of sewage obtained in an absolutely fresh condition, without admixture of surface water, that decomposition and nitrification take place with extreme slowness when the sewage is allowed spontaneously to oxidize in a bottle. Inoculation with a portion of medium from the filter greatly accelerated the rate of oxidation. He has further found that the effluent from an aerobic tank oxidized spontaneously more quickly than the effluent from chemical precipitation containing an equivalent amount of oxidizable matter.

Whatever the preliminary source of the population of a sewage filter bed may be, whether derived from the original sewage or from bacteria naturally present in all unsterilized material such as is likely to be used for the construction of such filters, there is no doubt that in course of time countless numbers of bacteria and other organisms of the nature specified above establish themselves in the filter. During the period when the contact bed is empty and when consequently its interstitial spaces are full of air, these organisms act upon the suspended and dissolved impurities retained by the filtering medium. Unbroken-down albuminoid matter is further peptonized and ammonia is oxidized to nitrite and to nitrate.

Fatty acids and other carbonaceous matters are finally oxidized to  $\text{CO}_2$ . This can be verified if the gases in the interior of such a bed are drawn off and analysed when a marked increase in the  $\text{CO}_2$  over that present in the atmosphere will be noted. Moreover, if a portion of the filtering material is carefully removed from the bed without disturbing its coating of slime, and is placed in a closed vessel provided with a manometer, an appreciable rise in the mercury may be observed owing to the absorption of the oxygen in the containing vessel. The presence of nitrates can be determined by washing the material with water free from nitrate, and testing for the presence of the latter in the washings.

The changes just described take place when the bed is standing empty, and are characterized by the predominance of nitrification. When the bed is again filled with liquid a somewhat different set of conditions arises. Mechanical absorption of the more insoluble matter takes place as already described, but oxidation also occurs through interaction of these substances and other impurities in solution with the preformed nitrates. In this way finely divided cellulose may be oxidized as explained in Chapter VI. During these changes, which may be grouped together as *denitrification changes*, loss of nitrogen occurs, as has been shown in Chapter VI, either as free nitrogen or it may be as nitrous oxide,  $\text{N}_2\text{O}$ , this gas having actually been discovered by Letts in solution in the liquid contents of a contact bed.

The proper working of a contact bed can be controlled by having regard particularly to the amount of nitrate present in the effluent, especially in the first discharge after a long period of rest. The nitrates present represent the overplus left after denitrification has taken place. Within limits the longer the period of standing empty, the greater will be the amount of nitrate found, but if nitrates are present at all it is evident that the conditions are still mainly aerobic and therefore suited to the maintenance of organic life. If a contact bed becomes clogged and water-logged, not only will the nitrates be absent in the effluent but often crowds of worms will emerge at the surface of the bed seeking their necessary air supply.

An interesting application of denitrification was made by Letts in Belfast, with the object of producing an effluent containing as little nitrogen as possible, either in the form of ammonia or nitrate, in order to minimize the growth of *Ulva latissima*, which was found to derive its nitrogen equally well from either of these sources. Letts purified a portion of the effluent by means of trickling filters in order to obtain as high a yield of nitrate as possible. This nitrified effluent was then mixed with the remainder of the unfiltered effluent, and the mixture treated on a denitrifying bed. The nitrates in the one portion interacted with the organic matter in the other, with elimination of nitrogen and production of a purified effluent containing a minimum of nutriment for the *Ulva*.

#### (b) Trickling or Percolating Filters.

The operation of a trickling filter differs from that of a contact bed in that the liquid is applied to it in such a way that it flows over the fragments of filtering medium in a thin film and the oxidation process is consequently continuously proceeding. It is in this sense that the trickling filter may be spoken of as a continuous filter as distinct from a contact bed whose operation is intermittent and clearly divisible, as we have seen, into two distinct processes. It is doubtful whether a really continuous filter, considered simply as a mechanism, has yet been constructed. When a sewage effluent is sprinkled upon a trickling filter by an ordinary rotary distributor, the operation is in fact discontinuous, each element of surface receiving a dose of liquid at given intervals of time, depending on the speed of rotation of the sprinklers.

It is not necessary here to describe in detail the various methods of applying sewage effluent to trickling filters. An account of them will be found in text-books dealing with the engineering side of the problem. It is necessary only to mention that distribution may be effected by simple intermittent discharge on to a surface of fine material, by rotary distributors such as are indicated in Plate IV (ii), p. 132, by

spray jets (Fig. 13), and by other mechanical devices of greater or less complexity.

Plate IV (ii) shows a set of trickling filters at the Accrington Sewage Works which will sufficiently indicate their general appearance.

We have here carefully to consider, assuming equable distribution of the liquid upon the filtering material, what the physical and mechanical conditions are which result in the production of a purified effluent.

The efficiency of a sewage filter depends on the total effective surface area of the filtering material, together with a sufficient air supply. By effective surface area is here meant the sum of the surface areas of the fragments of material. The surface area of the filter may be spoken of as the upper surface area ;

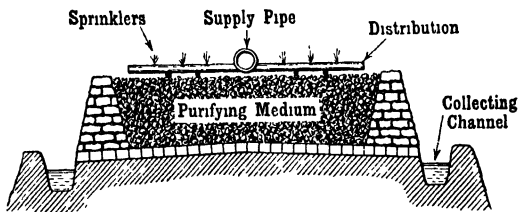


FIG. 13.—Percolating Filter with Sprinklers.

thus if large size material is used a greater depth of it is necessary in order to obtain the same total or effective surface area. On the other hand, the material may be so far subdivided that the interstices rapidly fill up with gelatinous matter which in its turn holds up water so that the interstices become reduced and the circulation of air is interfered with.

In general, therefore, it will be found economical to use the smallest material which allows of free circulation of air. The main direction of air in a trickling filter is probably from above downwards, the air being drawn through the filter by the percolation of the liquid. An exception to this rule may occur in cold weather when the higher temperature of the filter as compared with the outside air may induce an upward current.

It is obvious that filtering material must be avoided which tends to weather and break down as the interstices will tend to be filled with small pieces of broken-down material and air circulation will be impeded. In order to obtain the greatest possible surface area material of an irregular character such as hard furnace clinker gives the best results, but other available material can be used so long as it is not so smooth as to exert little or no retaining power or retentivity upon the gelatinous matter deposited upon it.

Further, thorough drainage is essential, otherwise water will tend to be held up by capillary attraction in the bottom layers of the filter, and will interfere with air circulation. For this reason a concrete bottom for the filtering medium is advisable, the thickness of which will depend on engineering conditions. These conditions of efficiency apply equally to contact beds and to trickling filters.

It is obvious that if the filtering material is to be fully made use of, efficient and equable distribution of the effluent over every part of the filter is essential ; it may, however, be pointed out that in certain circumstances, especially in small works, it is well to have an ample surface area of material so that efficiency of the process shall not be too dependent on the exact operation of mechanical devices. In other words, a large factor of safety should be provided.

The physical conditions giving the rate of the passage of liquid through trickling filters have been studied by W. Clifford in the researches referred to on page 109.

We are now in a position to follow the changes which take place in such a filter. In the first place, as in the contact bed, a purely mechanical action is exerted and the suspended and colloidal matters deposit themselves on the surface of the medium. This will take place obviously to a greater extent in the upper layers of the filter, and there is consequently a limit to the depths of such filters, owing to the concentration of deposited matter in the upper layers which will take place if such effluent is poured upon them at a very high rate. For this reason also trickling filters are better adapted to deal with large volumes of dilute effluent rather

than with more concentrated liquid the application of which results in a rapid accumulation of undigested organic matter in the upper layers of the filter. In course of time forms of life establish themselves in these filters, worms, larvæ, protozoa, and bacteria which maintain the cycle of changes. Albuminoid substances are broken down to amino compounds, and finally oxidized to nitrates. The trickling filter differs from the contact bed primarily in the predominance of nitrification, owing to the constant presence of oxygen in its interspaces. No doubt some denitrification takes place in the interstices of the medium, but, speaking generally, a greater proportion of the nitrogen is recovered as nitrate than in the case of the contact bed. A further great advantage possessed by the trickling filter is that the effluent passing away from it is constantly saturated with dissolved oxygen, and consequently the effluents from these filters contain generally a greater reserve of oxygen available for purification in the stream into which the effluent may flow. On the other hand, owing to their method of operation there is a greater tendency for incompletely oxidized nitrogenous matter to pass away from them either in solution in the colloidal state as granular residual "humus," or as debris of growths formed in the filter. In the contact bed, as has been shown, the oxygen of the nitrate interacts with the undecomposed oxidizable matter during the period of standing full.

It is necessary in the case of trickling filters that means should in all cases be provided for arresting suspended matter which continuously passes away from them. For this purpose either so-called "humus tanks" or sand strainers may be employed; especially are these necessary after the filter has had a period of rest. The colloidal matter deposited on the filtering medium suffers oxidation during such periods and is rendered granular and readily detaches itself in consequence from the filtering medium. Therefore, the effluent will then contain abnormal quantities of solid matter. This is also the case in spring-time when the organic life of the filter is particularly active. The "humus" which has been stored during the previous months is then to a large

extent ejected from the filter. This phenomenon is further instructive as showing that the changes taking place in these filters are by no means instantaneous, but take place over a long period of time.

## CHAPTER IX

### THE ACTIVATED SLUDGE PROCESS

Those who are responsible for the purification of the sewage of towns and cities will agree that the sewage problem is essentially the sludge problem. Whatever the type of process adopted, the inoffensive disposal of the insoluble and colloidal constituents of sewage which give rise to various forms of sludge is the real difficulty.

From the earliest days of the Contact Bed fears had always been entertained of the ultimate clogging of the medium. Even percolating filters unless very open and consequently very deep tend slowly to choke up. In any event they necessitate a second series of tanks to arrest the so-called "humus."

As long ago as 1897 a series of experiments was carried out in the Laboratories of the Davyhulme Works of the Manchester Corporation, by the present writer, on the purification of the effluent from the chemical treatment, which was then in operation, by employing various methods of aeration, either by exposure in shallow vessels or by bubbling air through the liquid. In every case the turbid colloidal matter readily clotted and settled out as a granular precipitate. Analysis of the mixture of clarified effluent and sediment by customary methods showed very little reduction of oxidizable impurity. The work was therefore abandoned for the time; if only the clear liquid had been decanted off and the liquid and sediment separately analysed, great progress would have been made; indeed the Activated Sludge Process might have at once emerged. At that period, however, it was the general belief that sewage could be completely oxidized or fermented into soluble or gaseous products by the action of bacteria of various sorts. A paper embodying these views as applied to sewage filters constructed of fine materials, was

even read before the Royal Society by Dr. Reid, Medical Officer of Health for Staffordshire.

The earlier trials with septic tanks had also led to the belief that a greater proportion of the impurities present were converted into inoffensive soluble and gaseous products than was actually the case. Exact measurement of these changes was not easy in the laboratory, and on the large scale necessitated very careful observations over a long period.

The true significance of the 1897 experiments at Davyhulme was not appreciated at the time. Nevertheless, this experiment drew attention to the importance of the colloidal matter present in the sewage and the part which it played in the clogging of the filters. Special studies were therefore made of these sewage colloids in the Davyhulme Laboratories and methods of analysis were published by which a measure could be obtained of the extent to which they were present in any sample of sewage or effluent. These studies awakened great interest, and many papers were published by other workers both in England and on the Continent. Important among these was one by Dr. Travis, of Hampton-on-Thames, in which he put forward what he termed the "Hampton Doctrine," according to which the action of bacterial sewage filters was mainly mechanical, the apparent purification being considered to be merely the physical adsorption of colloidal matter by the extended surfaces of the filter medium. In the Travis tank, the precursor of the Imhoff tank, he introduced what he called "Colloiders" of lattice work by means of which the colloids were retained at any rate to an appreciable extent. The retention of colloidal organic matter, by means of moving lattice work, has been further studied in recent times by Buswell and others in the United States.

Among the suggestions as to the cause of the initial and apparently rapid reduction in impurities which takes place when sewage or effluent is sprayed upon a percolating filter, that of enzyme action was suggested by the late Dr. H. Maclean Wilson, then Chief Inspector to the West Riding of Yorkshire Rivers Board. Experiments in the Davyhulme laboratory, using the humus from percolating filters as a purifying agent, were not, however, very successful. Nevertheless, the

minds of the Manchester workers had been focussed for many years on the changes taking place in the colloidal matter of the sewage, and the means of dealing with the problems involved. A preliminary trial was given to a mechanical filter with reversible washing. Incidentally a remarkable observation was made that after the raw effluent had been passed for some time through a three-inch pipe leading to the filter, a gelatinous growth developed which completely choked the pipe. Experiments were tried in the laboratory by Dr. J. F. Thorpe, who used to enjoy taking duty occasionally during the absence of the present writer on holiday. He found that filtration through porous porcelain would remove colloidal matter, but apparently at impracticable cost.

At this time although the paper by Munro referred to on page 97 had been read several years previously, the description of what was the experimental demonstration of the principle of activation had not been appreciated and was not brought to light until years afterwards, until indeed the writer had begun his work at the Indian Institute of Science, Bangalore.

Meanwhile old experiments by Waring and by Lowcock on the forced aeration of filter beds had not been forgotten and were taken up again under various conditions by Major Black and Professor Phelps in the United States.

In 1912 the present writer was called to New York along with other specialists to report upon the proposals of the Metropolitan Sewerage Commission of New York for disposing of the sewage of greater New York which amounted to the enormous volume of 1,000 million gallons per day. To treat such a volume on percolating filters with the possibility of flies and odours seemed impracticable. Chemical treatment of such volumes also had its special difficulties. It was while considering this problem that the present writer visited the Lawrence experimental station of the Massachusetts State Board of Health. There experiments were in hand on the forced aeration of sewage in presence of algal growths which it was anticipated would facilitate oxidation through the evolution of nascent oxygen from their living cells. The experiments were so far successful that sewage could be purified in 19 hours' aeration. The point which immediately

impressed the writer was that the adherent growths and heavy deposit were not thrown out from the bottle in which the sewage was being aerated but were retained to assist in the purification of the next dose of crude sewage. The old experiments of 1897 were recalled in which the deposit was thrown out with the clarified liquid, "the baby with the bath" in fact, and also the experiment on the purifying power of filter humus when no attempt had been made to aerate the mixture and which gave an unsuccessful result came back to mind. Clearly some combination of conditions was required. The Lawrence experiments did not fulfil the requirements of the problem entirely since expensive surfaces were still needed. They represent, in fact, an improved Lowcock filter. The goal aimed at was the purification of sewage *in an open tank*. As the Chairman of the Commission remarked, "Can you not clot it out?"

Upon returning to Manchester work was begun along more than one line, with the object first of clotting out the colloidal matter by some form of enzyme or bacterial action, and if possible of finally purifying the soluble constituents as well. At first the attempt was made to clarify the sewage only to such an extent that the colloids would be removed and there would be less danger of clogging of the filter beds.

At that time work was in progress in the Frankland Laboratory of the Manchester University, which was then under the direction of the present writer, on the treatment of certain trade effluents, and it had been noticed that the filters developed growths of higher bacteria, which tended to collect oxide of iron on their sheaths.

Mr., afterwards Major, E. M. Mumford, who was then Chief Research Assistant in the Frankland Laboratory, visited some old colliery workings at Worsley, near Manchester, in search of this organism in the yellow iron-laden pit water. He did not find the particular organism sought for, but brought back what was found to be an almost pure culture of a bacillus which had obviously the power of precipitating dilute solutions of iron in which this element was dissolved in presence of or in combination with organic matter. The experiment was at once tried of aerating sewage in presence of this organism

with a trace of iron salt added. Complete clarification resulted and a small model plant was constructed in which several gallons per day of sewage effluent could be treated. The clear effluent from this plant could be finally purified at a very high rate on a model percolating filter.

The process came to be known as the M7 Process from the first letter of the name of the discoverer of the bacillus, and the fact that this was numbered 7 among the special cultures kept in the Frankland Laboratory. A paper was read on the subject at the Congress of the Royal Sanitary Institute at Exeter in 1913, which aroused considerable interest. A paper on the specific properties of this organism was also published by E. M. Mumford in the *Journal of the (London) Chemical Society* in which he showed that it secreted a substance capable of precipitating iron when present in solution together with organic matter. Thus the enzymic precipitation of sewage was in a measure at any rate attained.

Large quantities of culture solution of the pure organism were made in carboys, using lentil broth as medium, and this culture was used to inoculate quantities of sewage effluent up to 10,000 gallons. It is believed that this is the only case in which large quantities of a pure culture of an organism have been used to inoculate sewage. Although the process did not come into practical use the experience, both engineering and biochemical, acquired during the experiments was invaluable in connection with subsequent developments ultimating in the Activated Sludge Process.

Early in 1913, and as an outgrowth and continuation of the M7 work, it was suggested by the present writer, who, as Consulting Chemist to the Rivers Committee, was ultimately responsible for the purification of the Manchester, England, sewage, to Messrs. Ardern and Lockett, chief chemist and chief research assistant, respectively, at the Davyhulme Works of the Manchester Corporation, that further experiments should be made on similar lines to those seen in the Lawrence Laboratories, with the important exception that precautions should be taken by protecting the bottles from light, to prevent the development of algal growths, since the objective was a process which could be operated in an open tank. Out of these

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researches of Ardern and Lockett, together with the ideas arising from the study of the M7 organism, and with the engineering guidance of Mr. Walter Jones and his associates of the firm of Messrs. Jones & Attwood, Ltd., the Activated Sludge Process in all essentials was developed.

The earliest experiments revealed that the most efficient way of applying air was by the use of so-called diffusers which delivered the air in extremely fine bubbles, so that aeration was rapid and complete, and every particle of the moving sludge was kept fully aerated. The following table, taken from Part II of Messrs. Ardern and Lockett's paper <sup>1</sup> on the researches on the oxidation of sewage, without the aid of filters, shows this effect very clearly :

RATE OF OXYGENATION OF RAW SEWAGE  
Dissolved Oxygen in Parts per 100,000

Length of Time of Aeration in Minutes.	Strong Sewage.		Dilute Sewage.			
	Excess of Air.		Average Amount of Air.		Minimum Amount of Air.	
	Nozzle. 2	Diffuser. 3	Nozzle. 4	Diffuser. 5	Nozzle. 6	Diffuser. 7
0	0.06	0.06	0.24	0.24	0.23	0.23
5	0.23	0.53	—	—	0.21	0.29
10	0.42	0.63	—	—	0.26	0.46
15	0.49	0.62	0.42	0.73	0.23	0.50
30	0.56	0.66	0.49	0.74	0.22	0.59
45	0.56	0.64	0.56	0.76	0.22	0.66
60	—	—	0.57	0.77	0.09	0.56
90	0.57	0.63	0.63	0.79	—	—

In this table columns 3, 5 and 7 show the improved aeration obtained from diffusers, as compared with plain nozzles in columns 2, 4 and 6.

This is especially marked with a minimum amount of air (see column 7), where the aeration for 15 minutes with a diffuser produced a high degree of saturation, whereas the same amount

<sup>1</sup> *Journal Soc. Chem. Ind.*, vol. xxxiii, p. 1123.

of air through a plain nozzle (see col. 6) produced no aeration whatever.

Coincident with the laboratory work conducted at the Davyhulme works, Walter Jones, in collaboration with the writer, carried on large-scale experiments at the Davyhulme works and elsewhere which resulted in the early activated sludge plants which were put down by Messrs. Jones & Attwood at Davyhulme and elsewhere in England. To this firm, and particularly to its managing director, the late Walter Jones, the credit is due for the ultimate success of this process.

Since that time improvements in detail have been introduced, especially the Clifford Inlet, devised by W. Clifford, of Wolverhampton, at one time on the staff at Davyhulme. Alternative types of mechanism for effecting aeration of the sewage have been worked out since, by Howarth, of Sheffield, Bolton, of Bury, and Imhoff and Kissener, on the Continent, and a very large literature on the technical, economic and scientific aspects of the process has accumulated but no fundamental advance on the work of the pioneers has yet been reported. Nevertheless, this does not imply that the process is entirely understood or is incapable of further improvement. Far from it. In spite of hundreds of papers which have been published fundamental work is still required, and the Water Pollution Research Board, with Dr. Calvert as Consulting Adviser, is now engaged in the study of the colloid matter of sewage. In a subject of such urgent public importance as the purification of sewage, practice must of necessity often precede theory, since it is only under working conditions in many cases that the real problems emerge. It is at this point that the purely scientific worker is most helpful.

In the remaining portions of this chapter a description will be given of a typical Activated Sludge Plant, and the most important biochemical processes which take place therein will be briefly discussed, with reference particularly to the subject of nitrogen conservation.

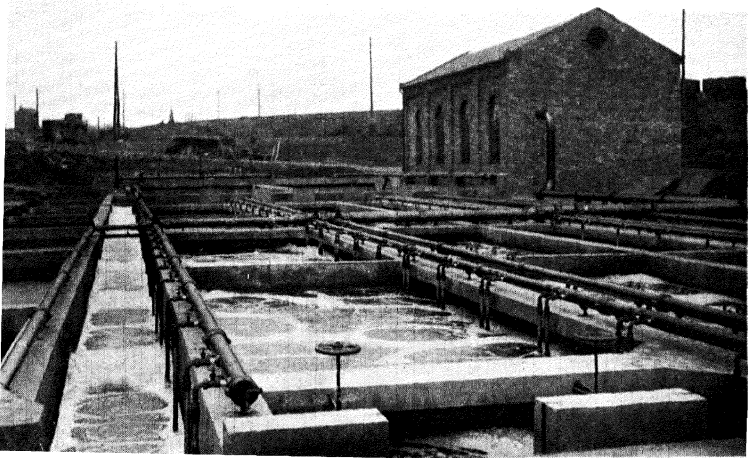
### **General Description of Activated Sludge Tanks.**

The details of the process can be seen from the folding plan. It is most important that all heavy grit and large floating

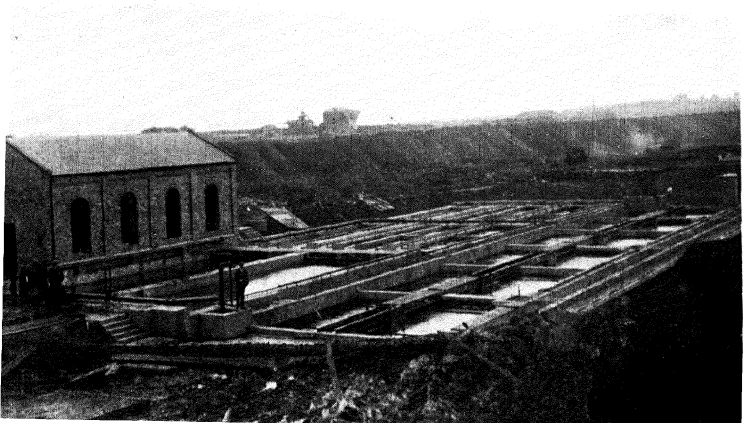
solids should be removed before the sewage enters the aeration tanks. The design of such detritus tanks requires considerable engineering skill so that while all heavy mineral matter is retained, the greater percentage of the valuable organic matter goes forward into the treatment tanks. After passing through a coarse screen the sewage enters a further preliminary tank in which all solid matter is broken up into small pieces, either by a natural process of maceration or by violent agitation by means of compressed air. It then goes forward into the treatment tanks. Immediately at the entrance of the first treatment tank the sewage meets a percentage of previously re-aerated sludge from the re-activation channel. The volume of activated sludge varies according to circumstances, 5 to 20 per cent. according to the nature of the sewage and the degree of purification required. The mixture of sewage and activated sludge, sometimes called the "mixed liquor," passes along the aerating channels to the final settling tank. According to the latest practice the diffusers run longitudinally in V-shaped depressions which extend the whole length of the channel.

The air rises from the diffusers in fine bubbles forming an emulsion of air and water which rises to the surface, and after liberating a certain proportion of air returns back to the diffuser to meet the stream of air again. The air thus fulfills a threefold purpose; it keeps the sewage in a constant state of agitation, it helps to coagulate the colloids and supplies oxygen for the life of the aerobic organisms, through the activity of which the purification takes place. The air pressure depends on the depth of the tank and need only be about  $\frac{1}{4}$  lb. per square inch more than the pressure due to the liquid.

From the treatment tanks the sewage passes to the settlement tank which it enters by means of the Clifford inlet. This consists of a vertical pipe discharging down into an eddy bucket which is supported inside a guard chamber. The object of this is to break the force of the incoming sewage so that the settlement of the sludge is undisturbed. The sludge being thoroughly flocculated is deposited very quickly, and in one hour settlement is complete. The effluent is drawn off over suitably constructed weirs or by bell mouths either directly



(i) Aeration Tanks.



(ii) Compressor House, Preliminary Settlement Tanks, Aeration Tanks and Final Settlement Tank.

ACTIVATED SLUDGE PLANT AT COWDENBEATH.



to the outfall or for further treatment on filters or land according to circumstances.

The sludge is continuously being forced out from the bottom of the settling tank by the hydraulic pressure of the liquid above it, the rate of flow being regulated by a penstock. It then enters the re-aeration channel from which it is lifted by means of an air lift to join the incoming sewage. The surplus sludge not required for treating the sewage can be lifted out of the plant in a similar manner by compressed air and either disposed of directly as a fertilizer by discharge on to the land, or on to drying beds or other means for converting it into a portable fertilizer. The general appearance of an Activated Sludge plant can be seen from the photographs on Plate V.

The actual course of the Activated Sludge Process appears to be divided roughly into two stages :

1. **The clotting out stage**, during which coagulation of the colloidal matter held in the sewage takes place and the pollute is transferred from the liquid to the sludge. Clarification of the sewage is thus obtained to a large extent, but the pollute is still held practically unoxidized in the sludge. This reaction can be looked upon as purely physico-chemical.

2. **The oxidation stage**, which embraces the breaking down of carbonaceous and nitrogenous matters and their subsequent oxidation to carbon dioxide and nitrite and nitrate. This stage can be considered as purely biological.

To a certain extent the two stages overlap, but as a rule very little oxidation can be detected as measured by the production of carbon dioxide until at least an hour has elapsed.

The oxidation of nitrogenous compounds is slower than carbon oxidation, and as a rule does not begin until the clarification stage is complete. The effluent obtained from the Activated Sludge Process is remarkably pure, it is not only extremely stable but is clear and free from colloids owing to the clotting action of the activated sludge. The clotting or clarification which occurs almost immediately is a sign that purification is taking place correctly ; it also enables the sludge to separate rapidly from the liquid, and after coagulation is complete, separation will usually take place in less than one

hour. When sufficiently oxidized the effluent without further treatment will pass any of the usual standards, including those recommended by the Royal Commission on Sewage Disposal.

**Organisms Present in Activated Sludge.** A number of workers have studied carefully the organisms which abound in the Activated Sludge tank, notably Swaminathan, Stewart, Lockett and others. According to Swaminathan, the bacterial population includes the following types :

Ammoniafying,

Nitrifying,

De-nitrifying,

Nitrogen fixing.

These were demonstrated by inoculating suitable media and by showing the several changes characteristic of the respective types.

The non-bacterial life ranged from minute protozoa to worms, insect larvæ and insects :

1. *Protozoa*.—Amœba, Paramœcium, Vorticella, Styloichia, Leucophrys, Cœnomorpha, Actinophrys and Acineta.
2. *Trochelmintes*.—Brachionas, Philidina and Distyla.
3. *Vermes*.—Dero and Auginlilla.
4. *Arthropoda*.—Diptera, Chironomus and Culex.

Microscopic study showed whether the sludge was in condition or not, the presence of ciliated organisms being evidence of ample aeration while flagellate forms denoted defective air supply. This was in accordance with the results obtained by Richards and Sawyer at Rothamsted, and by Lockett in Manchester.

Seasonal variations of the different types were remarkable, Amœbæ, Styloichia, Leucophrys and Nematodes were invariably noticed in the sludge from October to March. Paramœcia and Rotifera were observed to be more numerous from July to October though they were found throughout the year. Vorticella and Acineta developed very rapidly with ample air-supply and appeared in all seasons.

Apart from the appearance of non-bacterial life in the sludge a study of the relationship between the higher forms and the bacteria present was of profound interest. From continued observations under the microscope it is believed

that a major proportion of these higher organisms use the bacteria in the sludge as their food supply. In the case of vorticella and rotifera it is easy to see the actual movements of the cilia with which they direct sludge particles and probably countless bacteria into their mouths.

In order to discover whether the reaction of the protozoa upon the bacteria impairs the activity of the latter, experiments on partial sterilization on the lines of Russell and Hutchinson, Fairbrother and Renshaw and others were carried out using as sterilizing agents methylene blue, acridine yellow and Meldola's blue. There was, however, no clear evidence that the absence of protozoa results in an increase in the bacterial population; certainly special experiments gave no indication that nitrification took place more rapidly in absence of protozoa. In this connection several observations by Cremer are of interest. According to him, the protozoa in the activated sludge tank when they reach a certain stage of development finally die and disintegrate, and the contents of the cells thus afford further food for the bacteria. The protozoa therefore perform a useful function in synthesizing organic matter which would otherwise remain in a colloidal non-stable state. He recommends the daily careful and intelligent microscopic examination of the sludge as the most effective means of diagnosing operating conditions and difficulties.

Among these difficulties that of " Bulking " has often given trouble. When this occurs the volume of sludge found in the aeration tanks suddenly becomes abnormal and increases with considerable rapidity during several days. The sludge is also changed in physical character, is slow in settling with less than 50 per cent. purification owing to the large quantity of suspended solids in the effluent.

Microscopical examination reveals the immediate cause of bulking to be large masses of filamentous growths, higher forms of life being rare. Bulked sludge is grey and does not resemble a normal healthy sludge, which should be granular, brown in colour and settling readily. When bulked sludge is allowed to settle there appears a white slimy mass above the sludge proper. The supernatant liquid in such a case carries white specks in suspension, which pass away in the effluent.

The only remedy so far found for bulking is to reduce the flow of sewage and increase the air supply for a prolonged period when the filaments break up and are finally destroyed.

Bulking, however, is always a sign of deficient air supply. This condition may not be noticed for some time and then bulking suddenly occurs much in the same way that an individual may be in poor health for a long period owing to defective environment and may then suddenly fall ill.

The following extract from a letter published by the writer in the *Surveyor* apropos of a paper read by W. T. Lockett before the annual meeting of the Sewage Works Managers' Association in 1924, summarizes the conditions which have to be observed in the efficient operation of Activated Sludge tanks :

These researches led clearly to the conclusion that the changes which go on in an activated sludge tank are essentially the same as those taking part in arable soil. The researches of Mr. and Mrs. Howard and others have shown the immense importance to plant life of thorough aeration of the soil. In the absence of sufficient tillage or aeration the normal processes in the soil are arrested and the plant suffers. In extreme cases where aeration is stopped through water-logging or other causes the plant may wilt and die. The effect of sewage sickness on land is well known. Precisely similar phenomena occur in the activated sludge tank, and a very striking way of testing the conditions in an installation is to suspend a living plant growing in sand in the final aeration tank. The plant has been found to respond promptly to the varying conditions of aeration and consequent activity of the sludge. Further, if fresh activated sludge is used as a manure and if the soil with which it is mixed is insufficiently stirred it is found that though the plants at first grow very quickly they are liable to droop suddenly, such drooping being found to coincide with excessive quantities of carbon dioxide in the soil. On removal of this excess by suitable disturbance of the soil the plant again thrives. Activated sludge contains forms of life almost identical with those found in fertile soil. Seasonal variations among these are noted similar to those which have been observed in the course of prolonged studies of soil phenomena at Rothamsted. The effect of selective antiseptics in inhibiting the growth of protozoa with the consequent increase in the bacterial population is much the same in activated sludge as in soil. Both soil and activated sludge must be considered as living systems if they are to be properly "managed."

Bearing these facts in mind it is possible to deduce Mr. Lockett's results in a large measure from a priori considerations. Thus while a

living organism requires a certain minimum amount of air in order to live, any excess over a certain maximum will not increase its vital activity. On the other hand an excess of products of respiration or excretion will be deleterious and will depress vital activity.

Thus it can be seen that even an excessive amount of air cannot accelerate the process beyond a certain maximum rate, as the respiratory activities of the organisms are incapable of increase beyond their natural limit.

Further, it is evident that by the use of an intermittent air supply the processes in the tank, though not arrested, will take longer to complete.

It would have been of interest had it been possible to determine the biological condition and the agricultural value of the sludge produced under different conditions of aeration. This may be suggested as a future line of investigation.

### **The Agricultural Value of the Activated Sludge Process.**

The older method of utilizing sewage for the benefit of agriculture was by the direct treatment of the sewage on land and the simultaneous growing of crops. This is the method chiefly employed in India for the treatment both of sullage and sewage.

Apart from any question of carelessness and inefficiency there is a fundamental difficulty which has nearly always to be faced in both sullage and sewage farms, viz. that the crop requires water and manure at stated intervals only, whereas of necessity the stream of sullage or sewage flows continuously. It is not unnatural, therefore, that in the absence of an adequate area of suitable land and thorough supervision, the liquid is allowed to go to waste when not actually needed, with consequent possibility of nuisance and danger, as well as the economic loss.

Careful experimental work on a large scale at Poona and Lyallpur, and on an experimental scale at the Indian Institute of Science in Bangalore, have shown that undiluted sullage or septic tank effluent cannot long be treated direct on land without clogging of the latter, and is also too strong for the healthy growth of crops. Dilution up to three times with irrigation water has thus been found necessary.

It is obvious, however, that such dilution water is only rarely available and also it at once introduces a difficulty,

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viz. the increased burden of actual liquid to be added to the soil.

The effluent from the Activated Sludge Process should be free from colloidal matter and therefore does not tend to clog the pores of the soil; it has, moreover, correspondingly less nitrogen per gallon than raw sullage or raw septic tank effluent as can be seen by the following analyses where the Activated Sludge Process was tried under exactly similar conditions to the septic tank.

	Parts per 100,000	
	Ammoniacal Nitrogen.	Albuminoid Nitrogen.
Sewage . . . . .	12.6	4.2
Septic Tank Effluent . . . . .	9.44	1.5
Activated Sludge Effluent. . . . .	Nil	0.06

The most labile forms of nitrogenous matter contained in the colloid portions of the sewage have been coagulated in the course of the process and accumulated in the sludge which in consequence has a much higher nitrogen percentage than sludge from settling or septic tanks. The following figures give the limits of analyses of activated sludge with different types of sewage.

TABLE OF ANALYSES OF ACTIVATED SLUDGE

(Last three columns from A. J. Martin's *Activated Sludge Process*, p. 18.)

	Combatore.	Withington.	Manchester.	Salford.
Loss on ignition. . . . .	64.1	70.4	64.7	54.5
Mineral matter . . . . .	35.9	29.6	35.3	45.5
Acid insoluble . . . . .	14.4	—	—	—
Total nitrogen . . . . .	5.88	6.0	4.6	3.95
P <sub>2</sub> O <sub>5</sub> . . . . .	3.18	4.2	2.6	2.1
K <sub>2</sub> O . . . . .	0.674	—	—	—
CaO . . . . .	7.625	—	—	—

Sludge from sedimentation tanks or septic tanks seldom contains more than 2 to 3 per cent. of nitrogen, while the

sludge remaining as a residue in sludge digestion tanks of the Imhoff type contains even less.

The effluent, therefore, from an activated sludge plant can be used for irrigation of crops in much the same way that a diluted septic tank effluent or sullage can be used, though owing to the normal saline content which no process of sewage purification can remove, periodic flushing of the land with clean water may be necessary unless this is adequately provided for by rain.

An activated sludge effluent which is diverted from the land on this account can be led into ponds where its nitrogen ultimately reappears in fish. The manuring of fishponds with sewage has long been practised in China, and more recently at many centres on the continent of Europe. The use of raw sewage for this purpose has its possible dangers, and in some cases the fish before being sent to the market for human consumption may have to be kept for a while in clean water. Where activated sludge effluent has been used, however, this has not been found necessary.

The exceptional value of activated sludge as nitrogenous fertilizer has been demonstrated by numerous experiments in different parts of the world.

In the early days of the Activated Sludge Process, Bartow and Hadfield, of the University of Illinois, found that with equivalent quantities of nitrogen activated sludge gave better results than such nitrogenous fertilizers as dried blood, nitrate of soda, sulphate of ammonia and gluten meal. Since that time Bartow and his colleagues have shown that numerous organic bases and nucleo-proteids can be extracted from activated sludge.

At Jamshedpur the activated sludge from a population of 6,000 was subjected to careful experiment. Trials on the production of green fodder, especially lucerne, showed that 1 ton of activated sludge used under the right conditions is the equivalent of 2 tons of sulphate of ammonia or 50 tons of cow-dung. When effluent and sludge were used for irrigating sugar cane astonishing results were obtained, in fact nearly three times the yield produced at Lucknow with untreated sewage. This confirms the results obtained in other

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parts of the world by the use of nightsoil as nitrogenous manure.

Experience reported from Shanghai where large activated sludge installations are in operation, shows that an average sludge containing 6 per cent. nitrogen on the dried matter if dewatered to the condition of a spadeable mud will provide two and a half times as much available nitrogen and other constituents of manurial value as the same bulk of nightsoil.

All these statements confirm the report furnished to Government during the war by Sir John Russell, Director of the Rothamsted Experimental Station, that the value of the sludge when dried was at that time equal to £3 10s. per ton. Since then thousands of tons have been dried in Milwaukee, and sold at about that price.

The drying of activated sludge presents special difficulties since the masses of organic living matter of which it consists and whose structure resembles minute sausage skins hold up water very tenaciously. Any successful drying method must take cognizance of this fact. The disadvantage of drying in the open air on ordinary sludge drying beds is that the protein in the sludge readily ferments, yielding soluble amino-derivatives which escape with the drainage water, thus causing loss of nitrogen. In the same way if activated sludge is allowed to remain more than a short time in the treatment tanks without aeration, loss of nitrogen may take place in a similar way, or even under certain conditions it may escape in the free state (see Table—Coimbatore).

### COIMBATORE

Table showing Loss of Nitrogen from Sludge on Stoppage of Aeration  
(a) Sludge.

	Loss on Ignition.	Total Nitrogen.
Day aeration was discontinued . . . . .	63.1	5.1
3rd day . . . . .	57.4	4.8
4th day . . . . .	56.3	4.53
5th day . . . . .	55.0	4.32
6th day . . . . .	55.0	4.25

(b) Effluent.

	Oxygen Absorbed.		Am. N.	Albd. N.	Nitrous N.	Nitric N.
	3 mins.	4 hrs.				
Day aeration was discontinued .	0.08	0.85	0.003	0.001	0.04	0.01
3rd day . . .	0.75	2.00	0.033	0.027	0.002	trace
4th day . . .	0.75	1.84	0.035	0.037	0.002	nil
5th day . . .	0.82	1.84	0.042	0.046	0.001	nil
6th day . . .	0.87	1.84	0.064	0.05	0.0008	nil

When, however, the process is controlled from beginning to end in such a way as to maintain the necessary scientific conditions for successful operation it permits the whole of the nitrogen present in the sewage of towns to be recovered for the use of agriculture and thus constitutes a valuable means of increasing the real wealth of the world.

## CHAPTER X

### THE DISPOSAL OF SOLID REFUSE

The former chapters have dealt with the purification of the liquid waste matters of towns, viz. sullage and sewage. The dry or semi-dry waste known generally as *towns' refuse* is now becoming as serious a problem as sewage disposal, owing largely to the higher price of coal and consequent greater economy in its use, so that there is hardly sufficient calorific value in household refuse to make the installation of "destructors," or specially constructed furnaces, an economic proposition. Consequently the accumulation of refuse in "waste heaps" or "dumps" is becoming more frequent, constituting a menace to health and well-being owing to the production of objectionable smells and the breeding of flies and rats. The scientific and economical utilization of this material is the subject of the present chapter.

From time immemorial the chief source of fertilizer for the world's agriculture has been waste organic matter of various kinds.

The farmers of China and Japan are masters of the art of utilizing such material. Their methods are fully described in the fascinating volume by Professor F. H. King, entitled *Farmers of Forty Centuries*.

In addition to the careful conservation of nightsoil, every kind of waste vegetable material is brought into service. Whole areas of forest land are placed under contribution to furnish green manure and compost material. This material, consisting largely of green herbage which would be otherwise useless, is systematically rotted down together with mud from the bottom of creeks and other organic matter to form an excellent manure. In this and other ways the agriculture of China and Japan has been carried on and a vast population

maintained for centuries without the necessity for a single ton of so-called artificial manure.

In India also agriculture has been maintained by means of natural fertilizer, but by no means so efficiently as in China and Japan. Religious prejudice has largely vetoed the use of nightsoil, at any rate until after long burial in pits and trenches it has become inoffensive. This often means that at least 18 months must elapse before it can be used, in the course of which a large proportion of the nitrogen is either washed away or escapes into the air in a gaseous state.

Much of the organic matter which would be useful as humus or which would improve the texture of the soil likewise disappears or is converted into a resistant form.

House refuse, or "katchra" as it is termed in India, is often employed as a manure, when it is either spread upon the land and afterwards burnt, or it is ploughed in without burning, or thirdly it is made into compost by fermentation. In the first case, although the soil may be improved to some extent by heating, and enriched by the plant food constituents of the ash, all the valuable organic matter is lost.

Where the katchra is ploughed direct into the land, it may actually have a deleterious effect upon the crop owing to the excessive amounts of carbonaceous matter present.

By the third method, which is better than either of the two foregoing, the refuse is allowed to rot in a pit for some time, with the addition of a certain amount of cow-dung, until it is converted into a compost, a product in which the original components are broken down to a fairly homogeneous mass, having a texture suitable for direct use as manure. If the fermentation process is conducted with care and with proper attention to the necessary conditions for success, the results are very good, but loss often occurs through washing out of the pits by rain, or, on the other hand, by allowing the material to become dry, when no fermentation can take place. Moreover, at no time does the temperature become very high, and therefore the germinating power of seeds of weeds is not destroyed.

The process at best takes a very long time for the required changes to be brought about, with possible loss of valuable

ingredients and locking up of capital represented by the value of the manure.

It is well known that the greater portion of the cattle manure of India is used as fuel, and that the even more valuable cattle urine is almost wholly wasted.

Calculations by Dr. Lander, of the Agricultural College at Lyallpur, indicate the annual loss to the Punjab by the burning of dung cakes, based on the agricultural market value of farmyard manure, to be 1.5 crores. If the value is taken at Rs. 7 per ton as calculated from the increased yield over controls, the calculated loss is about 8 crores. This does not take account of the value of the cattle urine which is largely lost. From data based on the careful observations of Dr. F. J. Warth, Physiological Chemist to the Imperial Institute of Animal Husbandry (according to whom the nitrogen voided as urine per head of cattle may vary from 15 grams up to 100 grams), the loss of nitrogen to the Punjab alone would amount to more than 2 crores of rupees per annum. The importance of recovering as much of this nitrogen as possible is therefore obvious. If it can be used in conjunction with waste vegetable matter to produce a compost of high nitrogen content, and with a good percentage of humus, the gain to the agriculturist would be enormous.

The subjects of the proper utilization of green manure and the preparation of composts have been investigated by several workers in the various agricultural departments of India. Reference may be made to the pioneer work of Hutchinson and Milligan who advised partial preliminary decomposition under controlled conditions before using green plants as manure. Hutchinson has also carried out experiments on the nitrogen-fixing power of plant residues. The Howards have described numerous experiments with various kinds of composts, and have also emphasized the importance of the fermentation of plant residues prior to use as manure. This work, which has been largely developed at Indore at the Institute of Plant Industry, has recently been published by A. Howard and Y. D. Wad, under the title of *The Waste Materials of Agriculture*. Further reference to this work will be made later.

Mention may also be made of the work done at Dacca by Carbery and Finlow, which has resulted in greatly increased provision of manure for the Dacca Agricultural Farm.

All these researches and those to be described later have received a great impetus from the important work of E. H. Richards and H. B. Hutchinson at Rothamsted. They clearly indicated the principles which must be followed if the preparation of artificial farmyard manure, shortly termed "compost," is to be successful. They showed that there must be a definite ratio between the nitrogen and carbon in the raw materials used. They found also that the organisms responsible for the breaking down of straw and other carbonaceous material functioned best in the presence of air, that is they are aerobic. It is necessary to apply the nitrogenous compounds at suitable concentration, and under neutral or slightly alkaline conditions. They stated that the highest concentration of soluble nitrogenous compounds for rapid breakdown is appreciably below that of the weakest undiluted urine. They found that soluble nitrogen thus applied is temporarily rendered insoluble, forming probably insoluble albuminoids. They speak of the nitrogen as being "immobilized," or fixed by the straw, and the amount the straw is capable of thus fixing is equal to that necessary for pronounced rotting. The soluble nitrogen thus fixed, or more correctly locked up, by 100 parts of rice straw, is termed the "Nitrogen Factor"; any excess remains dissolved or is lost by volatilization or leaching. The great loss observed in producing ordinary farmyard manure can thus be easily explained. If the nitrogen, either as urine or in another soluble form, is in excess there will be almost certain loss. On the other hand, it must be remembered that excess of carbonaceous matter, while it may prevent loss of nitrogen, may nevertheless spoil the quality of the resultant manure, owing to its tendency to interfere with subsequent nitrification.

In their original patent of what is now known as the ADCO process, Richards and Hutchinson claim to activate carbonaceous material by maintaining it in contact with a solution of soluble nitrogen for a sufficient period of time, e.g., not

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less than 90 hours or more. It is clear that for the production of an activator not only must suitable nitrogen compounds and carbonaceous materials be present but also the specific organisms in sufficiently quantity to cause the process to develop rapidly. In the laboratory investigations in Bangalore it was found that, of the total nitrogen present, the percentage of "available" nitrogen in undecomposed "katchra" or refuse was much less than in cattle manure, the figures being as follows :

Refuse . . . . .	17.25
Cattle manure . . . . .	24.8

Moreover, the cattle manure gave a much higher bacterial count than the refuse :

	Bacteria per gram.
Refuse . . . . .	30,000 to 70,000
Cattle manure . . . . .	800,000 ,, 1,900,000

It was assumed that activated sludge would contain all the organisms naturally present in good arable soil in which vegetable organic matters rapidly break down and that it would be a better starter or activator than cattle manure ; this proved to be the case. Large piles of katchra and fallen leaves collected on the estate of the Indian Institute of Science were systematically moistened with sullage and a certain amount of activated sludge from the installation in operation at the Institute was added. The leaves and other organic materials began to break down with gradually increasing rapidity until when the heap was fully ripe a gunny-bag which was buried in the refuse was completely rotted in about a week.

These changes were carefully investigated in the laboratory by Rege, whose original paper should be studied for details.

It was found that by aerating a mixture of sulphate of ammonia, calcium carbonate and grass powder with activated sludge, the ammonia at first was rapidly converted into nitrate, while on further aeration the nitrate disappeared with, however, no loss of total nitrogen. From this point repeated additions of ammonium sulphate and of grass powder were made, when it was found that disappearance of nitrate from the solution took place more and more rapidly with successive additions.

Finally, the additions of ammonium sulphate and grass powder were made simultaneously, and at this stage the whole process of nitrate formation and disappearance took place within 24 hours.

These results indicate the possibility, under scientifically controlled conditions, of withdrawing soluble nitrogen from solution, i.e. of "demineralizing" nitrogen, with production of what may be termed artificial organic manure in a very short period of time, the conditions being the presence of a suitable activator, carbonaceous material of the right character, and carefully controlled addition of soluble nitrogen compounds.

An attempt to apply these results on a practical scale was subsequently made at the Municipal depot at Nasik under the direction of the present writer. The contents of a nightsoil cart was used both as a source of soluble nitrogen and to produce the requisite activator, and was thoroughly mixed with a sufficient quantity of katchra. The mixture was stirred two or three times a day for some days until all was quite odourless and the nightsoil had entirely disappeared. More nightsoil was then added, and the stirring process repeated until the change became rapid, and the straw and other fibrous material of the katchra was obviously breaking down. More katchra was then added and thoroughly mixed with the fermenting material together with a further addition of nightsoil and the whole again mixed. This process was repeated, as much nightsoil being added at a time as was necessary to keep the whole mass moist but not wet, and not so much as to cause any objectionable odour more than a foot or so away. In this way the katchra was rapidly broken down and a satisfactory manure of good texture obtained in the course of a few weeks.

Simultaneously with the experiments at Nasik a research was carried out by Rege at Rothamsted, where he was then a research scholar, in which he showed among other interesting results that moulds played an important part in the breaking down of carbonaceous matter. Three different species of mould were isolated in pure culture, each of which was active at comparatively high temperatures, the maximum in one case (i.e. *Acremoniella*) being about 60° C.

Following on the above-described researches a systematic

attack upon the problem was made in the General Applied Chemistry Department of the Harcourt Butler Technological Institute, Cawnpore, under the direction of the present writer, in association with Mr. P. B. Richards, Entomologist to the U.P. Government, who arranged for the necessary equipment and service from funds at his disposal, and himself made a number of observations on the conditions affecting the development or otherwise of insect life during the formation of composts. Other officers of the Agricultural Department assisted in field trials of the compost produced. The results of these researches, together with those already mentioned, have been collected in a Review written on the invitation of the Imperial Council of Agricultural Research and published in the *Agricultural Journal of India* for September, 1930. Some of the outstanding results observed may be summarized in the following paragraphs.

#### RAW MATERIALS

**Prickly Pear.** The percentage of nitrogen in prickly pear is small, varying in air-dried samples of uncultivated varieties from 0.77 to 0.86. In the cultivated spineless variety there may be as much as 1.4 per cent. of nitrogen. The bulk of the substance of prickly pear consists of mucilage composed largely of galactan. This undergoes acid fermentation apparently to butyric acid, and for this reason prickly pear is unsuitable for use as a green manure without preliminary treatment. The ryot's custom of burying prickly pear for a long time prior to use is well founded. It may be assumed that time is thus allowed for the butyric-acid fermentation to complete itself and for the gases accompanying anaerobic fermentation, i.e. hydrogen and carbon dioxide, to disappear. If adequate carbonate of lime is present, the calcium butyrate formed may be further fermented and its final oxidation to carbonate of lime may furnish energy for nitrogen fixation, a reaction shown to be possible by the experiments of Miss Mockeridge (*Bio-Chem. J.*, 1915, 9, 272). In laboratory experiments at Bangalore, distinct evidence of nitrogen fixation was obtained if the conditions were not allowed to become too acid.

**Fallen Leaves.** The importance of a careful study of the

breaking down of fallen leaves is manifest, when it is remembered that much of the manuring of forest areas is derived from the leaves which fall each year. Preliminary experiments at Bangalore indicated that the leaf proteins are a valuable source of nitrogen, and that leaves disintegrate more readily and produce more humus when allowed to ferment in the presence of air than when air is excluded. More recent experiments in Bangalore on the fermentation of leaves under aerobic conditions with nightsoil and cow-dung have confirmed this conclusion. Leaves naturally vary in the rapidity with which they ferment, mango leaves and Java fig leaves being particularly tough and resistant. Soft leaves from crops such as maize and fresh grass and weeds break down much faster, consequently a mixture of such material is to be recommended when possible. The nitrogen percentage in the resultant moisture-free compost varied from 0.7 with green vegetable leaves and weeds to 1.38 with pongamia leaves and flowers. Mixed dried leaves averaged 1.05 per cent. nitrogen.

**Towns' Refuse (katchra).** The utilization of towns' refuse for compost making is of very great importance both to the agriculturist and the health departments of towns since it furnishes a valuable source of manure to the former and removes a serious nuisance from the latter with the additional possibility of its becoming a source of revenue. As already mentioned, the earlier experiments at Nasik made use of towns' refuse and the subject has been further pursued at Cawnpore. The material consisted of street sweepings, including pieces of brick and kankar, wood and bamboo strips, pieces of cardboard, paper, cloth, straw, etc. The bricks and stones were sorted out and weighed 6 per cent. of the original material. It was not easy to obtain a satisfactory sample for analysis from the mixed ingredients above mentioned, but from such analysis as was possible it may be assumed that the nitrogen percentage in ordinary towns' refuse will not exceed 0.15 per cent., while there may be as much as 60 per cent. of mineral dust. For the economical preparation of compost a large proportion of this latter should be removed by preliminary screening; nevertheless, by systematic fermentation with a decoction of cow-dung for about 5 weeks, a material was

obtained which gave the following figures on analysis even after being allowed to remain undisturbed during the whole of the rainy season (4 months).

Moisture . . . . .	11.7
Ash. . . . .	71.7
Nitrogen . . . . .	0.8

**Mahua Flowers.** The flowers of the mahua tree (*Bassia latifolia*) contain from 50 to 60 per cent. of sugar, which the distilleries extract systematically by solution in water, the solution being fermented to alcohol. The "spent" mahua flowers contain less than 0.5 per cent. sugar, and consist mainly of cellulose or hemi-cellulose material, and are mixed with extraneous matter, e.g., sand, stones, fragments of wood, etc., present in the original flowers. The original flowers before extraction of the sugar contain about 0.63 per cent. nitrogen. During extraction of the sugar 50 per cent. of this nitrogen goes into solution. The remaining 50 per cent. is in the spent mahua, which latter is 14–15 per cent. of the original flowers. Thus the nitrogen content of the spent flowers calculated on dry material approximates to 2 per cent. The fresh spent mahua flowers as they come wet from the diffusion battery amount in weight to 95 per cent. of the original flowers. In large distilleries the disposal of spent mahua flowers presents, therefore, a formidable problem. Owing to the excessive moisture the bulk is large, and, if the weather conditions are favourable, acid fermentation sets in throughout the mass and proves very offensive.

The most satisfactory method for the utilization of spent mahua flowers is their conversion into organic manure. Special precautions have to be taken if a satisfactory result is to be obtained. When wet spent mahua flowers are heaped up they decompose very slowly. Acid fermentation sets in and the mass becomes very slimy and so closely packed that it becomes almost impervious to air. Even after standing two or three years, the central portions possess the original colour of the spent mahua which remains practically unchanged. When, however, the material was mixed with a sufficient proportion of cow-dung and cattle-shed sweepings containing straw litter

soaked in urine in the proportion of five parts of spent mahua to one part of cow-dung sweepings the temperature rose to 55°–60° C., and the mass quickly became a dark colour. By aerating the heap by stirring every 2 or 3 days a perfect manure was formed within 10 to 15 days' time, not a trace of the original flowers remaining, and the manure had a clean earthy smell. The nitrogen content was 1.5 to 2.0 per cent.

Spent mahua flowers being heavy and sodden, the manual labour involved in the necessary stirring is rather excessive. By fermenting the heaps in alternate layers of spent mahua and dry grass rubbish about 9 inches thick, the labour was much diminished, as the layer of rubbish admitted sufficient air to keep the process aerobic with moderate stirring at intervals.

**Banana Waste.** Banana stems and leaves are commonly thrown away by the cultivators after collecting the fruit every year. Experiments at Cawnpore have shown, however, that by passing the stems through a chaff-cutter, a material is obtained which can be readily fermented to humus, since the stems and leaves contain in addition to a large proportion of heavy cellulose material, exceptional proportions of potash and phosphorus; the final product is of special value as an all-round manure, the following being the analysis of the dried material in percentages :

Ash . . . . .	38.1
Nitrogen . . . . .	1.87
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) . . . . .	0.43
Potash (K <sub>2</sub> O) . . . . .	0.45

The above figures are after 3 months' storage.

**Leguminous Plants.** For the production of organic manure, rich in nitrogen, the use of leguminous plants is obviously advantageous if they can be obtained in sufficient quantities. Such plants can be grown as ordinary field crops or as trees or shrubs, whose leaves and twigs can be utilized as constituents of composts. Satisfactory results were obtained at Cawnpore with sun hemp (*Crotalaria juncea*), green-pea stalks and the leaves of *Leucaena glauca* and *Tephrosia candida*.

## INOCULANTS

Materials which are biochemically active either from the actual presence of fermentation organisms, or which are immediate products of their activity, and therefore may contain active enzymes, may in relation to the formation of organic manure be termed *inoculants*. Such materials are the following :

Nightsoil,  
Cow-dung,  
Cattle urine,  
Activated sludge.

The first two materials were generally used in the form of a thin decoction so that every portion of the plant residues might be uniformly infected with the necessary organisms.

The common method of carelessly mixing lumps of half-dried cow-dung roughly with vegetable debris in a bed is manifestly unscientific, and results in loss of nitrogen and in increased lapse of time before the manure is ready.

Experiments at Nasik with towns' refuse, and at Cawnpore with banana stems, clearly indicate that nightsoil produces quicker fermentation than cow-dung ; the reason for this is not at once apparent. It may be suggested that the nitrogen compounds present in nightsoil lend themselves more readily than the hippuric acid derivatives, likely to be found in cow-dung, to the changes both catabolic and anabolic which are concerned in the production of organic manure. Nevertheless, dilute cattle urine is unquestionably a very valuable agent in building up a product rich in nitrogen.

For making the decoction *fresh* cow-dung should always be used. The cow-dung manure in common use has a very low nitrogen content, analyses of various samples giving percentages of 0.28 to 0.4 only.

**Urine.** Both human urine and cattle urine as stated, when suitably dilute, are excellent sources of nitrogen for the production of organic manure. Unfortunately in India both are largely wasted. Of the former, apart from what is voided by the roadside, etc., and so is largely irrecoverable, much that might with profit be recovered from household latrines

is allowed to run to waste and soak into the ground with production of a most abominable and ubiquitous nuisance.

An experiment with a servants' latrine at Nasik showed that by the simple provision of a small heap of kankar under the outlet pipe from which the urine commonly escaped, aeration and subsequent nitrification took place with complete absence of nuisance; actual crystals of nitrate could be observed. Such small heaps could be easily renewed, and the kankar with the accumulated nitrates, powdered and added to the compost heaps.

The earliest experiments of Rege show that the nitrate nitrogen can be "mobilized" in the compost heap, provided aerobic conditions are maintained. At Nasik alone there must be hundreds of such latrines now poisoning the atmosphere, which would furnish large quantities of nitrates. The late Dr. Munsiff, Director of Public Health to the Government of Bombay, took much interest in these possibilities.

In order to utilize cattle urine to the best advantage straw and vegetable debris should be spread under the cattle at night in sufficient amount to absorb the urine.

Experiments at Cawnpore indicated that the highest figure was obtained when the litter was allowed to remain under the cattle for 40 hours. In the Indore process, to be referred to later, the floor of the byres is made of stamped earth which is called urine earth, and at intervals a layer of this earth is removed and ground to powder in an ordinary mortar mill and either added direct to the soil or employed as an ingredient of compost, fresh earth being placed under the cattle.

**Activated Sludge**, which contains, as a rule, about 5 per cent. of nitrogen, was used very successfully as an inoculant in the early experiments at Bangalore. The results of numerous quantitative experiments on the production of compost from towns' refuse have been recently published by J. Jagannatha Rao and V. Subrahmanyam.

A number of nitrogen compounds, including raw sewage, ammonium sulphate, calcium cyanamide and nitrate of soda were used as sources of nitrogen in comparison with activated sludge. In every case, except when activated sludge was used, distinct losses of nitrogen occurred, varying from 7 to 35 per

cent. In the two experiments where activated sludge was used, there was a gain, in one case of 8.5 per cent., in the other of 16.5 per cent.

The evidence given in Chapter VII of the presence of nitrogen-fixing organisms in activated sludge is thus confirmed.

Activated sludge, however, is itself a manure of such value that it is undesirable to use it in this way if it can be separately dried and marketed. In the absence of facilities for producing this dried product the nitrogen in the sludge can be conserved by composting the wet sludge with towns' refuse, care being always taken to maintain aerobic conditions throughout the process. In any case a certain percentage of sludge can be used as inoculant, leaving the bulk to be dried by other means. Just as when cow-dung is economically used in the manner already described a large proportion is still available if desired by the Indian agriculturist for the purposes of fuel when other fuel supplies cannot be obtained.

#### GENERAL PRINCIPLES OF COMPOST MAKING

The experiments described in the foregoing paragraphs were based on the fundamental researches undertaken by Rege under the direction of the writer, at the Indian Institute of Science, Bangalore. The object was to accelerate the fermentation by building up a mass of activator in the first place, and by systematically adding successive small quantities of fresh material to the mass while removing an equivalent portion of the finished product, to develop a continuous process identical in principle with the Activated Sludge Process of purifying sewage. Compost made on these lines has therefore been termed *Activated Compost*. The operations involved in the production of such compost are illustrated in the photographs in Plate VI.

In the Indore process described by Messrs. Howard and Wad, comparatively small quantities of inoculant are added to large quantities of raw material; their process therefore takes a longer time to carry out than the Activated Compost process, just as the activated sludge tank could not operate rapidly if a fresh quantity of activated sludge had to be built up for every day's supply of sewage. Moreover, by maintain-



(i) Activated Compost Heaps showing progressive Breaking Down of Raw Material.



(ii) Turning the Heaps for Aeration.

COMPOST MAKING FROM CATTLE AND HORSE DUNG AND URINE, FALLEN LEAVES AND HOUSEHOLD REFUSE. (CENTRAL HOTEL, BANGALORE.)



ing a large mass of actively fermenting material in continual fermentation, stable conditions are set up for fermentation which increase the efficiency of the process.

Both the Indore process and the Activated Compost process require certain fundamental conditions to be observed. The most important of these are the following :

(a) *A proper mixture* of the various available materials so that the nitrogen content and the average rate of fermentation remain reasonably constant. The material should be broken or cut up into pieces sufficiently small to be easily manipulated, and to afford adequate surface for attack by the various organisms present.

(b) *A high initial temperature* is essential if the special organisms concerned with the fermentation are to function. It results, further, in the elimination of all insect larvæ and other deleterious forms of life, including the seeds of weeds. The presence of these is one of the chief objections raised by farmers to the use of unscientifically prepared organic manure. An experiment is quoted by Howard and Wad, in which 25 lb. of grass seeds were mixed with two lots of fermenting composts. Germination tests of the ripe manure gave negative results in each case.

(c) *Aerobic conditions* must be maintained throughout. If this is successfully achieved there is no loss of nitrogen ; in fact, often an actual gain by fixation from the air. If the conditions are allowed to become anaerobic through too close compacting of the material or through over-watering, loss of nitrogen is sure to occur and also nuisance from smell and possible fly-breeding.

(d) *The mass should never become sodden.* More water is absorbed by the material in the early stages when it is loose and open than later, when owing to the fermentation process it becomes more spongy in texture. The moisture content should not exceed 60 per cent. or be less than 50 per cent.

(e) Besides the thermophilic bacteria, *moulds* play a large part in the breaking-down process and their presence should be carefully maintained. Howard and Wad speak of them as the "storm troops of the compost process."

**Fixation of Nitrogen.** During the composting process, as

mentioned above, there is evidence that fixation of nitrogen takes place, if the right conditions are maintained, more particularly if care is taken that there is always ample air supply. As explained in Chapter VII, the main condition of nitrogen fixation is the oxidation of a sufficient amount of carbon to furnish energy for the fixation process, provided of course that the necessary organisms, in this case the free living species, are present.

E. H. Richards, at Rothamsted, has shown that these organisms are present in cow-dung, and research at the Indian Institute of Science has demonstrated their presence in activated sludge. The conditions, then, in a properly operating compost heap are highly favourable to nitrogen fixation, and Howard and Wad state that under such circumstances nitrogen fixation is almost always observed.

In the Cawnpore experiments one or two quantitative observations were made. Thus, in one experiment when sann hemp was fermented with cow-dung and urine the calculation of the nitrogen balance on an original total quantity of 14.79 lb. showed an increase of 4.47 lb. of nitrogen at the end of the experiment.

In a blank experiment in which sann hemp was fermented with activator without addition of cow-dung and urine, there was an actual loss of 0.688 lb. of nitrogen for an equivalent quantity of sann hemp and activator.

Another experiment utilizing sun-dried weeds, together with cow-dung, and cow-dung and activator, showed an increase of nitrogen where activator was present. The heaps were raked daily for aeration. In the absence of cattle urine the rotting was not very satisfactory in either case, but a distinct gain in nitrogen was shown in the heap to which activator had been added.

The two experiments afford fairly conclusive evidence that nitrogen fixation does really take place under favourable conditions during the fermentation changes accompanying the production of artificial organic manure. The simultaneous presence of cattle urine and activator appear necessary, the former to hasten the rotting process and the latter to supply necessary organisms.

Reports from Coimbatore confirm the experience at Cawnpore and Indore that nitrogen fixation does take place during the fermentation of compost. The importance of this fact is obvious since, e.g., if every village in India was able to fix a certain quantity of nitrogen from the air to be immediately available as plant food the product would be equivalent to the output of a very large modern factory for the production of mineral fertilizer from the nitrogen of the air.

Further detailed and large-scale research is still needed on this important subject. The experiments of Jagannatha Rao and Subrahmanyan are referred to on page 213.

That the conversion of nightsoil and towns' refuse into compost is a practicable proposition is shown by the enterprise of the Municipality of the City of Mysore where the waste materials of the population of some 100,000 people is made into compost, considerable quantities of which are being taken up by the Agricultural Department of Mysore State for the manuring of large areas of sugar cane and paddy, irrigated by the Irwin Canal.

Mention should also be made of the growing attention which is being paid in Germany to the production of composts, particularly by the Krantz hot fermentation process (Edelmist). It is being increasingly realized that the use of mineral fertilizers unless accompanied by adequate quantities of organic manure is likely seriously to deplete the "humus-capital" of the soil.

It may be hoped that these results will encourage many other towns more favourably situated than Mysore to convert their rubbish and sewage sludge into a valuable manure for which there should be a large demand for the allotments which the Government is encouraging in England and Wales.

It should certainly pay sea-coast towns to treat their sewage before discharge into the sea, and utilize the sludge for compost making with towns' refuse. In this way foreshore pollution will be prevented, and valuable nitrogen restored to the land for the benefit of agriculture.

In conclusion the following percentage analyses of the Mysore compost made by the Agricultural Chemist to the Mysore Government may be quoted :

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Organic matter	.	.	.	.	.	.	17·18
Nitrogen (total)	.	.	.	.	.	.	0·81
P <sub>2</sub> O <sub>5</sub>	.	.	.	.	.	.	0·87
K <sub>2</sub> O	.	.	.	.	.	.	1·26

The product as sold is a brown granular powder, readily transportable in bulk or in sacks. It is entirely inoffensive, having a pleasant odour of leaf mould.

## CHAPTER XI

### THE ASSIMILATION OF NITROGEN BY PLANTS

**Chemical Changes in Plant Cells.** When a plant is burned its organic constituents disappear mainly as carbon dioxide, nitrogen and water. Its mineral constituents remain behind in the ash, the growing plant builds itself up again out of these products of its combustion. The mineral constituents and water, it takes in through the roots; the carbon and oxygen through the leaves, the nitrogen ultimately being supplied from the sources already discussed. All the complex physical and chemical processes involved in building up a plant are controlled ultimately by the vital energy of the plant cells, together with the energy of sunlight. The correlation of these processes is the task of physiological botany, and a knowledge of this is obviously indispensable if the plant is to be grown under the best conditions and supplied with its right food. Enzyme chemistry forms the foundation knowledge of physiological botany. It is clearly necessary to have some understanding of the primary chemical changes taking place in specific cells before establishing their general relations.

The initial impulse to plant growth is to be found in the potential biotic energy of the seed, or more properly speaking, of the embryo. This, of course, like all forms of life with which we are acquainted, has its origin in pre-existent life, but the difference between the changes taking place in the seed and those which occur in the leaf is that the former are not directly dependent upon sunlight, though indirectly it is true they are dependent upon it for warmth.

The seeds of all plants contain in the endosperm a store of reserve material which has been elaborated by the growing plant (see Plate III (ii), p. 128). The embryo has the power of

secreting various enzymes, e.g., *cytase* which breaks down the cell walls of the endosperm, and *amylase* which converts starch into sugar. There are also *proteolytic enzymes* which break down the stored albumin of the seeds, and in the case of oil-containing seeds, e.g., those of the castor-oil plant, *lypolytic enzymes* are present which hydrolyze the oil into fatty acid and glycerine. All these changes it will be seen are essentially concerned with the breaking down of material already elaborated, i.e. they are what is known as *catabolic* changes, and unless fresh nutriment is supplied on the one hand, and fresh energy on the other, growth will cease. Nutriment is supplied to the plant, as already stated, by the roots and by the leaves. Energy is supplied by the leaves only, and it is in the leaf cell, therefore, that we have to look to find what we may describe as the power house of the plant. The chemistry of the leaf cell is one of the most fascinating problems which has occupied the attention of chemists, but in spite of numerous rescarches by highly qualified workers it is still only imperfectly understood.

The simple beginnings and ends of the process have been known for a long time; they may be studied without difficulty and indeed form one of the subjects of most "Nature study" classes. The following experiments are easily carried out:

A portion of American pondweed, *Elodea canadensis*, is placed in water in a cylindrical vessel with a little earth at the bottom for root attachments and the whole set in the sun; bubbles of gas soon rise from the leaves, and may be readily collected. If a glowing splinter of wood be held in the gas it will burst into flame, showing that the gas consists for the most part at any rate of oxygen.

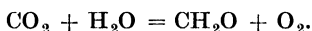
On the other hand, if the plant is placed in darkness, and air freed from  $\text{CO}_2$  by passing through potassium hydroxide solution is led over the plant and then passed into baryta water, the latter will become turbid from formation of barium carbonate.

From these experiments it is clear that two main processes go on in the leaves, the evolution of oxygen in sunlight and of carbon dioxide in darkness. These two changes as a matter of fact take place at all times, but the preponderance of one over the other depends on the presence or absence of sunlight. The evolution of oxygen is a building up or *anabolic* process

arising from the decomposition of carbon dioxide in the air, the plant utilizing the carbon and giving off the oxygen. On the other hand, the evolution of carbon dioxide is essentially a process of respiration or a *catabolic* process where the carbonaceous constituents of the plant are broken down with the production of carbon dioxide and water. The volume of the oxygen given out in the assimilation process is practically equal to the volume of carbon dioxide taken in, sufficiently indicating that the changes involved are of a fairly simple order.

The problem for the chemist is to discover how the carbon taken in by the plant as  $\text{CO}_2$  is built up into starch and cellulose, and by what stages these latter are re-converted into carbon dioxide and thus the life-cycle maintained. Microscopic observation indicates that starch is the first visible product appearing in the leaf cell, but it is evident that between a simple substance such as carbon dioxide, and a complex molecule such as starch, the chemical steps must be very numerous.

In 1870 von Baeyer put forward a very suggestive hypothesis in regard to the first of these steps; the simplest carbohydrate is formaldehyde,  $\text{CH}_2\text{O}$ . Von Baeyer suggested that in the simultaneous presence of light and of chlorophyll carbon dioxide and water may react according to the following simple equation :



An explanation is here indicated of the equivalence alluded to above between  $\text{CO}_2$  decomposed and the oxygen evolved. It is easy to conceive further that the formaldehyde by a series of polymerizations can build up more complex carbohydrates such as starch. This hypothesis derives confirmation from the fact that on standing in contact with a dilute solution of lime, water formaldehyde actually does become gradually converted into a mixture of hexoses.

A great deal of research has taken place on this subject since Baeyer's time, but interesting as these researches are our immediate concern is with nitrogen assimilation, and the production of carbohydrate in this connection concerns us only so far as it is linked up with the production of nitrogenous products.

The question before us is how does the plant convert nitrogen when presented to it in the form of ammonia and nitrates

into the more complex amino derivatives and finally into albumin or protein substance. It has been shown that an increase in protein occurs partly with the accumulation of carbohydrate in the presence of light. This protein synthesis occurs in the leaves. It has been further shown that the nitrates present in the soil eventually reach the leaves through the circulatory system of the plant. Only very small quantities of nitrate can, however, be actually detected in the leaves, and it would seem therefore that they are very quickly transformed into more complex substances. That the building up of these substances is connected with the photosynthetic assimilation of carbon would appear evident from the fact that accumulation of nitrates occurs in plants that have been kept in darkness, and these salts are used up afterwards when the plants are exposed to light. This conclusion is further supported by the fact that the disappearance of nitrate is coincident not only with the presence of chlorophyll, but that in the case of chlorotic leaves which are devoid of chlorophyll no transformation of nitrates occurs in the light. Finally, a very interesting experiment has shown that if variegated leaves, such as those of the croton, are left in the dark, nitrates accumulate both in the green and white portions. After subsequent illumination the nitrates are found to have disappeared in the green or coloured portions only, in the colourless parts the amount of nitrate remains unchanged.

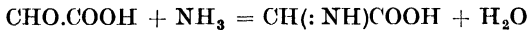
That the simultaneous presence of carbohydrate is necessary to protein synthesis has been shown by experiments in which darkened leaves were supplied with carbohydrates by means of a nutrient solution, in which case protein synthesis took place even in the dark. It would thus appear that light is only indirectly responsible for the energy required for protein synthesis, in so far as it is directly concerned with the production of carbohydrate. It is likely, however, that with an adequate supply of carbohydrate in both cases protein synthesis will go on more rapidly in light than in darkness.

A considerable amount of research has also been carried out on the intermediate products taking part in protein synthesis: among these hydrocyanic acid is believed to play

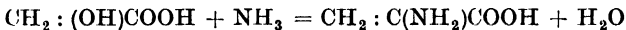
an important part since hydrocyanic acid is present in many plants in the form of glucosides either in the leaves or in the roots. The effect of light is to cause the formation of hydrocyanic acid which disappears when the plant is kept in the dark. When such leaves are supplied with nitrate and sugar in the dark, or with nitrate in the light, hydrocyanic acid is again produced.

It is now generally accepted that nitrogen is mainly stored in the plants in the form of amino acids or acid amides which are formed by the combination of ammonia or hydrocyanic acid with various groups such as CHO, CH<sub>2</sub>OH, CO<sub>2</sub>H, CHOH, etc., which in turn unite to form acids, aldehydes and carbohydrates. There is evidence, indeed, that such acids as glyoxylic acid and glycollic acid do occur in the leaves of plants.

The combination of ammonia with glyoxylic acid would give rise to an amino acid, thus :



It has however been recently suggested that the simplest building stone of protein synthesis is  $\alpha$ -amino-acrylic acid, formed by the condensation of enolic pyruvic acid and ammonia according to the following equation :



Reference may also be made to recent experiments by K. S. Varadachar at the Indian Institute of Science, where a solution of nitrate was injected directly into sunflower plants. There was evidence of continuous conversion of nitrate into other forms of nitrogen.

Although by far the majority of plants build up their protein from nitrates or ammonia, it is of interest to note that there are a number of carnivorous plants such as drosera, sun dew, and nepenthes, pitcher plants, which in various ways attract and entrap small insects whose bodies they digest and assimilate into their system.

**The Plant and the Soil.** In the foregoing paragraphs a short account is given of the changes which appear to take place in the plant itself in the course of nitrogen assimilation.

Closely connected with this are the preliminary processes

which go on in the soil, and which result in the preparation of plant food in a state suitable for assimilation.

The simple chemical theories of Liebig, according to which the needs of the plant could be met by suitable mineral salts, have been found to be inadequate and the rôle played by "organic matter" has proved to be of first-rate importance.

Our present knowledge on this subject has been excellently summarized by B. Viswa Nath in a monograph entitled *Some Aspects of Plant Nutrition*, published by the Society of Biological Chemists, India, in 1932. Most of the following salient points have been selected from this monograph and from the Report of a Symposium held by the above-mentioned Society on "The Rôle of Organic Matter in Soil."

The latest contribution to the subject of the chemical constitution of soil "organic matter" or "humus" is by Waksman and Narayana Ayyar. They consider that the organic matter of soils consists chiefly of two important chemical compounds, lignins and proteins. The former constitutes 40 to 50 per cent. and the latter 30 to 35 per cent. of humus. The remaining portion is made up of hemi-celluloses, fats and waxes.

Waksman and Narayana Ayyar bring forward experimental evidence in favour of the view that humus formation implies chemical interaction between carbohydrates and proteins. They succeeded in preparing ligno-protein complexes whose chemical and physical behaviour resembled closely that of humus extracted from soil.

Among the interesting facts recorded by these authors may be mentioned the important observation that the synthesized humus complexes containing iron had a highly beneficial effect upon the fixation of nitrogen by *Azotobacter*.

According to Waksman, the nitrogen passes through the stage of micro-organic protoplasm and after the death of the micro-organisms into protein. Page combines this view with that on the origin of humic matter from lignin and draws a picture of the course of conversion of organic residues into soil organic matter as consisting of two converging series of changes. The first consists in the conversion of the lignin into humic matter and the second in the conversion of non-

lignin substances, chiefly carbohydrates and protein, into micro-organic protein. These two products combine to form the humic matter protein complex.

The carbon : nitrogen ratio (C : N) of soils is no longer found to be constant at about 10 for all soils, as was at one time supposed. Leighty and Shorey found that while with the majority of soils examined by them, the ratios varied between 7 and 15, there were extremes from 3 to 35.

Experiments at Coimbatore and Bangalore lead to the general conclusion that when plant and animal residues are added to the soil there is a tendency to reach an equilibrium between carbon and nitrogen. The equilibrium point is different for different soils. In the efforts of the system at arriving at the equilibrium point nitrogen is either fixed or lost.

The practical importance of the carbon : nitrogen ratio in soil fertility and practice appears to be indeterminate.

There are however well-recognized limits to the amount of nitrogen which can be taken up by the plant under optimum conditions of growth since this is governed by the balance between the metabolic processes of carbon assimilation and respiration on the one hand and nitrogen assimilation on the other. A low carbon : nitrogen ratio encourages vegetable growth.

The magnitude of the ratio varies with the different crops, soils and climates. A good supply of fertilizers in the soil is conducive to vegetative activity, while a low supply may tend to reproduction. The study of the carbon : nitrogen ratio may assist in the control of vegetative and reproductive growth by internal and external agencies.

Numerous researches in different parts of the world unite in emphasizing the value of organic manure. Russell in the Rothamsted field experiments finds no combination of artificial fertilizers so effective as farmyard manure in steadying crop yields from year to year and attributes this effect to physical and physico-chemical actions on the soil.

The average nitrogen and carbon contents of Indian soils are 0.05 per cent. and 0.6 per cent. respectively, while the corresponding figures for European and American soils are

0.15 per cent. and 3 per cent. Experience shows that organic matter is very rapidly oxidized in Indian soils, and consequently emphasizes the necessity for maintaining the organic matter supply in Indian soil.

Investigations at Coimbatore show that in addition to enhancing yields, farmyard manure increases the ratio of grain to straw as compared with artificial fertilizers. Viswa Nath has found that organic manures assist the assimilation of chemical fertilizers in the soils tested by him. Further research is needed to ascertain the optimum proportion of organic manures and artificial fertilizers.

A very important observation made in the course of the investigations at Coimbatore is the effect of cattle manure on the quality of the seed. Viswa Nath and Suryanarayana have shown that manuring the parent crop influences the quality of the resulting seed in regard to its capacity for subsequent crop production.

McCarrison carried out animal nutrition experiments with the identical grains employed by Viswa Nath and Suryanarayana in their plant experiments, and found that, as in the case of seed vitality, the grain from the cattle-manured plot possessed higher nutritive value than the grain from either the unmanured plot or the mineral-manured plot. He attributed the better nutritive value to the higher "*vitamin* content of the grain."

These experiments and numerous others lead to the conclusion that in addition to the usually accepted functions of organic matter, it exercises also a subtle direct influence on the metabolic processes of the plant.

The earlier experiments of Bottomley and his co-workers which appeared to demonstrate the existence in organic manures of "accessory food factors," termed "auximones," performing similar functions in plant metabolism to those exercised by vitamins in animal economy, have been subject to criticism.

Nevertheless, in experiments at Bangalore where sunflowers were given injections of aqueous extracts of dried yeast, farmyard manure, dried blood and sewage effluent, striking response, particularly in increased flowers, was observed. The effect

was most marked in the case of yeast. These results were confirmed by a second set of experiments in which the plants were raised in washed sand.

The effect of organic matter on the nutritive value of seeds has received striking confirmation from the work of Rowlands and Wilkinson who compared the effect on rats, of grain seeds grown without manure and those grown in soil to which an extract of pig manure had been added. Although chemical analyses revealed little if any difference in composition between the two crops, the difference in nutritive value was markedly in favour of the seeds grown with traces of manure extract.

On the other hand, experiments by Clark with fresh and autoclaved manure, and with plant and bacterial extracts, under sterile and non-sterile conditions, showed that only under non-sterile conditions did stimulation take place.

It would appear, therefore, that bacteria play an important part in the cycle of events.

Just as animals obtain vitamins from plants, so it appears likely that the micro-organisms in the soil liberate either when alive or after death some active constituent which is absorbed by the plant and passed on to the seed.

## CHAPTER XII

### THE SULPHUR CYCLE

Everyone who has been confronted with a bad egg is aware of the unpleasant character of the final decomposition products of albumin. The product most easily recognized chemically is sulphuretted hydrogen or hydrogen sulphide,  $H_2S$ , whose presence is easily demonstrated by holding a paper soaked in a solution of lead acetate in its vicinity when the paper rapidly blackens. The smell of a rotten egg is mainly due to this gas. Hydrogen sulphide is therefore often described as having a smell like rotten eggs.

It has been shown in Chapter III that most varieties of albumin contain sulphur in greater or less proportion, and they are capable, like egg-albumin, when undergoing putrefaction, of liberating this sulphur as hydrogen sulphide. It is easily seen, therefore, that decomposing albuminous matter is capable of causing considerable nuisance from this source.

Sulphur appears to be an essential constituent of both animal and vegetable life, and a knowledge of its transformations as it passes from one to the other is of the greatest importance, especially in view of the possibility of nuisance being produced during the process. As, therefore, sulphur is closely associated with nitrogen in its transformations, some account of these is necessary to complete the subject of nitrogen conservation.

The transformations which sulphur compounds undergo bear a rough analogy to the transformations of nitrogen considered in previous chapters. Just as the plant takes up nitrate to furnish the nitrogen for vegetable albumin, and this nitrogen ultimately reappears, after passage through the animal organism, as urea and ammonia, to be finally again oxidized to nitrates, so the sulphur supplied to the plant as sulphates becomes part of vegetable and animal albumins,

which again break down, yielding hydrogen sulphide, and the latter is oxidized, either chemically or biologically, back to sulphate.

Moreover, just as nitrates are capable of reduction to form nitrites, and finally ammonia, so sulphates are capable of reduction to hydrogen sulphide.

In the sulphur cycle purely chemical reactions play a greater part than is apparently the case with nitrogen, but in all cases the sulphur transformations are capable of being facilitated by the activities of various organisms.

The chief workers on this important question of the natural sequence of combinations entered into by sulphur, have been Winogradsky in Russia, who has investigated the conditions under which sulphur is oxidized by certain specific sulphur organisms; Beyerinck and van Delden in Holland, who have studied particularly the reduction of sulphates; and Letts in Belfast, who, while repeating Beyerinck and van Delden's experiments, in conjunction with several of his students, made important original observations upon the conditions under which sulphuretted hydrogen is evolved, in the actual circumstances of certain estuaries.

It will perhaps be simplest to consider the subject under two heads:

- I. The production of hydrogen sulphide.
- II. The oxidation of hydrogen sulphide.

### **I. Production of Hydrogen Sulphide.**

Hydrogen sulphide can arise under natural conditions from the following sources:

- (a) The decomposition of albumin as already stated;
- (b) The reduction of sulphates.

Both these changes are due to the action of various organisms.

Letts and McKay also showed that carbon dioxide, itself produced by the decomposition of organic matter, can decompose sulphides, such as ferrous sulphide,  $\text{FeS}$ , yielding  $\text{H}_2\text{S}$ . Such sulphides can also be decomposed by fatty acids produced by other fermentations. The two sources, (a) and (b), of sulphuretted hydrogen may now be separately considered:

(a) **The Decomposition of Albumin.** The formation of hydrogen sulphide by the decomposition of albumin, through the action of bacteria, can be readily demonstrated. If a few drops of lead acetate solution are added to a small bottle full of sewage, the bottle closed, and placed in an incubator for a day or two, the solution turns black from the presence of lead sulphide. The actual organisms capable of decomposing albumin, with formation of hydrogen sulphide, can be recognized by an elegant method suggested by Beyerinck :

To ordinary nutrient gelatine, sufficient white lead is added to obtain a perfectly white plate ; when the medium is poured into the Petri dish, a little sewage diluted with distilled water is first added to the dish, and the whole mixed and allowed to set. After it is set, and as the colonies develop, black dots of lead sulphide will indicate the presence of these organisms, which are capable of breaking down albumin with production of hydrogen sulphide.

A very serious case of nuisance existed for a long time on the shores of Belfast Lough. Here great quantities of a seaweed, *Ulva latissima*, flourished. Professor Letts showed that this seaweed contained an abnormally high albumin content ; when deprived of its natural conditions of growth, the *Ulva* is capable of fermentation, apparently in two distinct and successive stages. The first stage results in the production of fatty acids, mainly propionic, together with carbon dioxide and hydrogen ; in the second stage of fermentation, in which a different species of micro-organism is concerned, sulphuretted hydrogen is produced.

It is not at present certain what are the exact sources, in the first place of the fatty acids, and in the second place of the sulphuretted hydrogen. They may both be due to decomposition of the albumin of the weed, or on the other hand sulphides may be produced by reduction of the sulphates in the sea water, or in the tissues of the *Ulva*, and these sulphides are then decomposed by the fatty acids produced in the first fermentation.

The evidence points to the hydrogen sulphide being derived from the reduction of sulphates, rather than from the decomposition of the albumin, inasmuch as when comparative tests

were made, by fermenting the *Ulva* in sea water and tap water, respectively, sulphuretted hydrogen was much more readily evolved from the sea-water experiment than from the tap water. The reduction of sulphates is clearly, then, a very important source of hydrogen sulphide.

Before considering this process in detail, however, it should be stated that the objectionable odour evolved, when organic matter is allowed to putrefy, is not solely due to hydrogen sulphide. Under certain conditions, very evil-smelling gases are evolved in which no trace of hydrogen sulphide can be discovered. These are probably organic sulphur compounds, such as mercaptan ( $C_2H_5SH$ ), also amines, and substances such as skatole, etc., which are also products of albumin decomposition. It has been further found that the yield of sulphuretted hydrogen can be increased in many cases if a small quantity of flowers of sulphur is added to the fermenting mixture.

(b) **Sulphate Reduction.** As already stated, this change has been studied by Beyerinck and van Delden. Beyerinck inoculated suitable solutions containing sulphates with small quantities of mud from the canals of Delft, and found that the best conditions for sulphate reduction were as follows :

1. No oxygen must be present.
2. No acid formation must take place, and consequently little or no sugar should be present in the culture media.
3. Phosphates and other suitable salts must be present.
4. Nitrogen compounds are only required in very small quantities ; sufficient indeed is contained in ordinary tap water.
5. The most favourable temperature for sulphate reduction is about  $26^{\circ} C$ . Beyerinck succeeded in isolating an organism which he termed *Spirillum desulphuricans* ; it is a strictly anaerobic organism, and this circumstance, in conjunction with its small need for nitrogenous nutriment, enables it best to grow in solutions which have been worked over by other organisms. These facts are of not a little practical interest. Those who have had to deal with samples of sewage and effluents will have noticed that such samples, if kept in stoppered bottles, may become in time practically clear, having only a small black sediment at the bottom ; and if they have

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been tightly stoppered, they may also retain considerable quantities of hydrogen sulphide. If this is removed by boiling, very little residual organic matter will be found to be present.

Stagnant polluted waters, e.g., the Manchester Ship Canal, show the same phenomenon. It is evident, in both these cases, that the nitrogenous organic matter is broken down by ordinary putrefactive organisms, and that finally sulphate reduction takes place. In such cases sulphides, or hydrogen sulphide, will be found to constitute almost all the oxidizable matter left.

To demonstrate the reduction of sulphates, the following solution was made use of by van Delden :

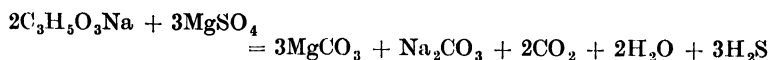
Tap water . . . . .	1,000 grams
Common salt . . . . .	30 "
Sodium lactate . . . . .	10 "
Crystallized magnesium sulphate . . . . .	8 "
Potassium phosphate . . . . .	0.5 gram
Asparagine . . . . .	0.5 "

This solution may be inoculated with a little sewage sludge from which sulphate-reducing organisms are seldom, if ever, absent.

Van Delden isolated an organism causing the reduction of sulphates in sea water, and found that it closely resembled *Spirillum desulphuricans* ; he named it *Microspira estuarii*. Both these organisms, although as above stated they do not need large quantities of nitrogen, are not inhibited in their growth by organic matter, if they are present in pure culture. Under natural conditions the presence of organic matter facilitates the growth of other organisms, to the detriment of the sulphate-reducing spirilla.

The reduction of sulphates is of special importance in relation to the discharge of sewage into sea water. There is no doubt that, in absence of sufficient dilution, putrefaction may set in, resulting, in the case of sea water, in sulphuretted hydrogen production ; so that the nuisance may be much greater in the case of discharges into sea water than into fresh water.

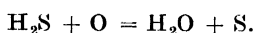
Sulphate reduction has been compared to de-nitrification ; it will be remembered that in the case of the reduction of nitrates the oxygen of the nitrate with the assistance of the de-nitrifying organism combined with the organic matter present. A similar reaction appears to take place in the case of sulphate reduction ; thus in the above-described solution. where the chief source at any rate of oxidizable material is sodium lactate, van Delden suggests the following equation :



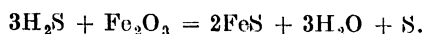
Experimental evidence supports the above equation fairly well.

## II. Oxidation of Hydrogen Sulphide.

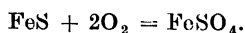
Unlike ammonia, whose direct oxidation by purely chemical means has been shown to take place to only a limited extent in nature, hydrogen sulphide readily oxidizes in a variety of ways. The simplest is the direct oxidation to water and sulphur according to the simple equation :



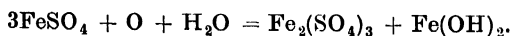
This change is hastened by the presence of certain metallic oxides, particularly those of iron and manganese ; thus in presence of oxide of iron the following changes may take place :



In presence of oxygen and moisture FeS may readily oxidize to ferrous sulphate,  $FeSO_4$ , thus :



And this may further oxidize with formation of ferric sulphate, thus :



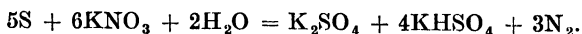
It is quite possible that pyrites, especially when found in coal, may owe its origin to the interaction of oxide of iron and the sulphides produced by the decay of vegetable matter. When such " coal brasses," as this form of pyrites is termed,

is exposed to the air, it oxidizes with formation of ferrous sulphate, or eventually, it may be, of ferric sulphate.

When black sewage mud is exposed to the air it turns brown and becomes acid, owing to the formation of hydrated oxide of iron and sulphuric acid.

How far hydrogen sulphide and sulphides are capable of being directly oxidized by solutions of nitrates does not appear to have been sufficiently studied. There is no doubt that nitrates are rapidly reduced in presence of sulphide mud; how far this is a purely chemical change and, if so, what is the exact cause of the change, has not been fully determined.

Beyerinck claims to have isolated an organism, *B. thioparus*, which brings about the following decomposition :



The most frequently occurring and obvious case of oxidation of hydrogen sulphide by bacterial agency is that effected by the higher bacteria, classified under the general term of *Beggiatoa*. These are the organisms which form the subject of Winogradsky's researches above referred to. They are found very often in sulphur springs and wherever putrefying sewage or such-like organic matter comes in contact with air, as, e.g., on the stones of a stream in the neighbourhood of a badly polluting discharge. The organism, as a matter of fact, grows between wind and water, but makes use of the sulphur either by decomposition of the  $H_2S$  present, or by actual absorption of the free sulphur formed by its spontaneous oxidation. If a strand of *Beggiatoa* is examined under a high-power microscope, very characteristic granules of sulphur are seen to be present throughout the organism, as is shown on page 30. This sulphur is the amorphous form soluble in carbon bisulphide. *Beggiatoa* is capable of absorbing large quantities of sulphur which it oxidizes to sulphates; for this purpose it is necessary that carbonates should be present in the surrounding liquid. Under its natural conditions of growth this will inevitably be the case, ammonium carbonate, e.g., being always present in decomposing sewage. *Beggiatoa* appears to use the sulphur as a source of energy rather than to increase its cell substance.

Winogradsky found that it could use up from two to four times its weight of sulphur without increasing in growth. Under these circumstances, comparatively small amounts of organic matter will suffice to sustain it, and thus it can flourish in sulphur springs, whose chief constituents, apart from hydrogen sulphide, are mineral salts.

To summarize the contents of the foregoing paragraphs, we may conclude that sulphur enters the cycle of living nature as mineral sulphates in the food of plants. By the decomposition of vegetable albumin, or at a further stage from the excretory products of animals, it may appear as hydrogen sulphide (sulphuretted hydrogen). This may be re-oxidized to sulphates, either directly by chemical means, e.g., oxides of iron, etc., or by the intervention of bacteria. Certain of these oxidize it directly to sulphate, while others make use of the presence of nitrates.

Sulphates are capable of being directly reduced to hydrogen sulphide by certain bacteria, in presence of small quantities of organic matter, but such changes only take place in absence of air. These various changes clearly indicate the importance of abundant supplies of oxygen, if the evolution of hydrogen sulphide, and the other less well-defined objectionable gases which accompany it, are to be avoided.

A striking example is afforded by the water supply of Madras derived from the Red Hills Lake, a supply which is characterized by absence of oxygen in the lower depths of the lake, and a high proportion of organic matter. As a consequence hydrogen sulphide develops in the sand filters, the filtered water at times containing 5 p. p. m. hydrogen sulphide. Aftergrowths of *Beggiatoa* and *Thiothrix* occur in the filter underdrains. The installation of percolating filters and other means of obtaining improved aeration are contemplated.

Since much of the foregoing matter was first written a number of interesting observations have been made in relation to the sulphur cycle and the practical application in particular of the activities of sulphur oxidizing bacteria.

Among the trade-effluents causing trouble in connection with sewage purification probably what is known as " ammonia

recovery liquor" has been the subject of the greatest amount of scientific and practical investigation.

When the ammonia liquor obtained in the course of production of gas from coal is distilled for the purpose of recovering ammonia, a residual liquor remains containing tarry matters, phenol and cresol derivatives and thiocyanates (saline compounds containing both nitrogen and sulphur).

Prolonged researches were carried out by the author in collaboration with the chemical staff at the Davyhulme Sewage Works of the Manchester Corporation and with the chemists at the gasworks of Manchester and Bradford (Yorks) which resulted in the devising of methods for purifying the waste liquors by bacterial oxidation. The whole subject has also in recent years been further investigated by the chemists of the Emscher Genossenschaft in connection with the purification of sewage in the Emscher valley (Germany).

Of immediate interest in relation to the sulphur cycle, however, is the fact discovered through the laboratory work at Davyhulme that potassium and ammonium thiocyanates could be oxidized in bacterial filters, and that about 50 per cent. of the nitrogen of the thiocyanate could be recovered as ammonia and nitrate and about 70 per cent. of the sulphur as sulphate.

It was found that no essential difference exists in the rate of oxidation of potassium and ammonium thiocyanates, and that solutions of thiocyanate up to a concentration of 18.7 parts CNS per 100,000 can be oxidized.

It is of special interest to note that denitrification changes do not play an important part in the oxidation of thiocyanates.

Arising out of this investigation W. T. Lockett, then research chemist on the Davyhulme staff, independently continued the investigation of the bacterial oxidation of thiosulphates which are also present in appreciable quantity in "ammonia recovery liquor."

In these as in the experiments with thiocyanates, small laboratory filters of granulated "clinker" were made use of. A number of very interesting conclusions were reached of which the following may be mentioned here:—

1. That dilute solutions of thiosulphate, trithionate, tetra-

thionate and pentathionate can be oxidized in bacterial filters.

2. That acid filtrates are formed during the oxidation of these compounds and that the rate of oxidation is accelerated by the addition of sufficient alkali to prevent the formation of free acid.

3. That these compounds are all finally oxidized to sulphate.

4. That solutions of dithionates cannot be oxidized on bacterial filters.

5. That solutions of thiosulphate up to a concentration of 500 parts  $\text{Na}_2\text{S}_2\text{O}_3$  per 100,000 can be oxidized.

6. That 70 per cent. of the sulphur of the thiosulphate is recoverable as sulphate.

In the course of this work Lockett was able by a careful and special technique to isolate an organism capable of oxidizing thiosulphate to sulphate in pure culture.

The oxidation of sulphur by soil organisms has been utilized by numerous workers as a means for solubilizing mineral phosphates in order to render the phosphoric acid more "available" as plant food.

Interesting investigations on these lines have been made in the Department of Biochemistry at the Indian Institute of Science, Bangalore, by Norris and Ramaswami Ayyar, using a suspension of activated sludge as a source of sulphur organisms.

The following is a summary of the main results obtained :

1. In the absence of a neutralizing agent the  $P_{II}$  fell to 1.6 and there was a rapid production of titratable acidity : with the introduction of the phosphate, although there was greater production of acid, this was utilized for the production of soluble phosphate, the  $P_{II}$  remaining at about 4.0.

2. In the absence of catalysts even though there was increased sulphur oxidation as indicated by the sulphate figures there was not a corresponding increase in the production of water-soluble phosphates, but with the sulphates of manganese, iron and aluminium the solubility rapidly increased.

3. With the rapid oxidation of elemental sulphur there was an accumulation of water-soluble sulphate. Generally the amount of sulphur oxidized in relation to the water-soluble

phosphate produced was found to be in the ratio of 4 of sulphur to 1 of mineral phosphate. The introduction of the catalysts reduced the ratio to 1 : 1.

During the progress of aeration and oxidation of sulphur there was decrease in the number of faecal and putrefactive bacteria and of chromogenic organisms. At a later stage, further specialization took place and only a few well-defined soil types persisted. When the  $P_H$  reached about 5.2 *Actinomyces* were the predominating organisms, dying out at a reaction between 3 and 4, when moulds appeared in large numbers. The latter survived until the  $P_H$  was about 2.1, *Fusarium* being usually the most resistant type. At this last stage, the sulphur-oxidizing organisms began to multiply vigorously; they were almost the sole survivors and continued to increase and function actively. Owing to the absence of nearly all other organisms, it was then easy to isolate the sulphur-oxidizing organisms in pure culture.

The organism develops on washed agar, silica jelly and gypsum blocks. It withstands  $P_H$  6.6 and can utilize carbohydrates to some extent, oxidizing more sulphur in their presence. Certain inorganic salts have also a favourable effect, the influence of sodium silicate being very marked.

Further experiments to determine the effect of sulphur-oxidation on ammonification and nitrification in suspensions of activated sludge, have shown that in presence of sulphur nitrification is completely inhibited, and with moderate oxidation ammonia production is also diminished.

The foregoing results taken together have an interesting practical bearing on the application of the activated sludge process to agriculture. The addition of sulphur to the aeration tanks would appear likely to decrease any possible excessive quantity of nitrogen in the effluent and concentrate it in the sludge (cf. Chapter IX, page 198). Moreover, the addition of mineral phosphates to the aeration tanks in presence of sulphur results in the solubilization of the phosphates which pass away with the effluent in a form available for plant food.

In this way the activated sludge tank becomes a veritable fertilizer factory. So far, however, such experiments have not proceeded beyond the laboratory stage.

These results agree with those of previous workers with soil composts, made up of soil, sulphur and rock phosphate, where abundant aeration and optimum moisture are found to offer favourable conditions; small amounts of ferrous and aluminium sulphates exert a stimulating effect. The uncomposted mixture cannot be added as such to the soil, since the phosphate will not become soluble under soil conditions. The reaction of the soil would have to be made acid before the transformation would take place.

On the other hand, the activity of sulphur-oxidizing organisms finds useful application in the reclaiming of alkali soils. A recent account of such a process is given in a paper submitted to the Second International Congress of Soil Science at Leningrad-Moscow in July, 1930, by W. L. Powers of the Oregon Agricultural Experiment Station, U.S.A. The author states that sulphur has proved the most effective single treatment for correcting "black alkali" where deep drainage and copious irrigation are employed. Less sulphur is needed for improving black alkali land if used in conjunction with bulky nitrogenous organic matter. The organic matter aids oxidation of the sulphur.

In the same paper it is stated that sulphur assists the formation of chlorophyll. Miller has shown that sulphur increases the protein content of alfalfa. It will be remembered that sulphur is a constituent of cystine, one of the products of the breaking down of the albumin molecule.

Feeding experiments in animals have indicated that the SH group increases cell-division in plants and animals. There is a possibility that animals cannot assimilate sulphur directly but that they require feeding by plants that can use this mineral. In view of the high cystine content of hair and the increased percentage of protein in alfalfa resulting from the use of sulphur as a fertilizer, it is suggested that such alfalfa, when used as green fodder for sheep, may produce an increased yield or better quality of wool, as compared with alfalfa grown in absence of such provision of sulphur.

Altogether it will be seen that the sulphur cycle is second only in importance to that of nitrogen.

## CHAPTER XIII

### SOME EXAMPLES OF NITROGEN CONSERVATION

Nature is lavish but not wasteful. The leaves fall in the forest, and are slowly changed to humus, which gives food for further growth.

Wood is burnt in the fire and the carbon dioxide formed feeds the leaves, and indirectly by its solvent actions in the soil, stimulates the roots and thus plays its part in the production of fresh vegetation.

The nitrogenous waste of man and animals is ultimately oxidized through the agency of micro-organisms in soil or water and is again taken up by plants, to enter once more into the cycle of life.

All is well so long as existence continues to be primitive and individuals are isolated one from another. So soon as community living begins, whether of bees or of men, measures of sanitation become necessary. In primitive ages the camp or village is vacated after a period of time, and the cleansing of the polluted soil is left to the operations of Nature. So bees and birds leave their old nests. Where this is impossible, as with many rocky colonies of sea-birds, vast accumulations of guano occur.

Until comparatively recent times, many towns, even in Europe, resembled the islands of the sea-birds. Portions of many towns and cities in the East are still little removed from that condition.

Nevertheless, the call for better things becomes insistent, and so sewers are made to remove offensive matter from sight. The trouble then reappears in polluted rivers and dying fish.

All this time the thrifty farmers of China and Japan have set the example to the Western world of how the waste of cities may be transformed into the wealth of the countryside.

The elementary methods by which this desirable result is obtained are, however, offensive to men of less practical mentality, and so the problem is left in Europe to the engineer, and in India to the "untouchable."

Hence arise sewage works and "trenching grounds" which may remove offence from the vicinity of dwellings, but add little to the wealth of the countryside.

Much was at one time hoped from the "Sewage Farm," but the high dilution of the plant food as it is present in sewage makes conditions for satisfactory cultivation very difficult to meet at all seasons and in all weathers.

Disposal by "dilution" into river or sea has been greatly favoured in America and to a less extent in England. This, too, has its limits. It is not easy to mix fresh water and salt, and so we see pools of sewage floating about within sight of Government House in Bombay.

Year by year at many points in New York Harbour, the dissolved oxygen approaches more nearly to a dangerously low limit. Along certain water fronts it is virtually absent, and the black stinking water, and its floating grease and debris, affords no cause for civic pride to the city whose great bridges and railway terminals are the admiration of the traveller.

Driftwood and more objectionable matters rob bathing resorts of their attraction. Estuaries, though free from obvious nuisance, require greater expenditure in dredging, if channels are to be kept open, since not only does even oxidized sewage sludge tend to form banks, but also the interaction of sewage and river silt accelerates deposition of more resistant mud.

It is true that some of the nitrogen thus "disposed" of may in time return in fish, but it is a long-period loan to Nature, and in the meantime local amenities suffer.

Finally, as civic populations increase even dilution becomes impossible. At one time by diverting the water of Lake Michigan into the Illinois River and finally into the Mississippi, by way of the so-called "Drainage Canal," it was possible to find sufficient dilution water to prevent the sewage of Chicago, which discharged into the Canal, from becoming offensive. With the extraordinary increase of population and of industry

which has taken place of late years, the amount of dilution water required has been so enormous as to threaten a lowering of the level of the Great Lakes, and has become a question of international importance. Millions, in consequence, have to be spent in works for "conserving" the nitrogen which was formerly merely "disposed" of.

North of Chicago lies Milwaukee, whose sewage at one time poured untreated into Lake Michigan, fouling the water supply of this city and its neighbours. Now an immense activated sludge plant converts all this impurity into clear effluent and into fertilizer, valued at £100,000 per annum. The sludge which used to defile the bright waters of the Lake now reappears as wheat on the wide acres of western prairies, or as smooth rich turf on countless golf greens.

In the construction of the works, costing a million sterling, workers of all grades—engineers, mechanics, chemists, architects and labourers—have found interesting and profitable employment, railways and ships have secured valuable freight. All these, added to the "invisible" returns of a reduced death-rate, make a total of real wealth giving ample value for the capital employed.

Milwaukee is perhaps the most outstanding example of nitrogen conservation by completely modern methods.

Shanghai, another great city, representing a nodal point between East and West, has long derived a handsome income from the sale of its nightsoil to the Chinese farmer, as described in Chapter II. Now in spite of this large profit, modern methods are being installed, and the Chinese farmer is coming to realize the worth of activated sludge, the nitrogen content of which (6 per cent.) is two and a half times as much as that of nightsoil analysed under comparable conditions of bulk.

At Jamshedpur, the town which has grown up round the Tata Iron and Steel Works, is one of the first activated sludge plants put down in India. It deals with the sewage from some 8,000 people. The sludge and effluent are used together to irrigate sugar cane. The resulting crop was three times that obtained at Lucknow where raw sewage was used for irrigation.

Experiments on the production of green fodder showed that 1 ton of activated sludge, used under the right conditions, is

the equivalent of 2 tons of sulphate of ammonia, or 50 tons of cow-dung. These results were confirmed with *lucerne* at Bangalore, thus showing the way to the production of excellent clean fodder for dairy farms.

Owing to the absence of colloidal matter in the effluent it can be stored in tanks without offence and used as required. In this way the fundamental difficulty already referred to as associated with sewage farming, viz. of either overdosing the land at times, or passing the sewage away untreated, does not arise.

Such effluent ponds, if they contain a certain amount of fresh water, are capable of supporting fish life; indeed fish-ponds, manured with raw or settled sewage, have long been used in China, and in many centres in Europe, notably Berlin. In the *Gesundheits Ingenieur* for December, 1929, a detailed account is given of a fish farm at Grafenwohr, where settled sewage is mixed with river water, which is then passed through tanks in which fish are grown. Details of cost of upkeep, fish food, etc., are given and compared with the receipts from the sale of fish, and a satisfactory profit is shown.

Imhoff states that in Germany fish-ponds are used as a final stage in the process of sewage purification in twelve cases—for example, Munich, Amberg—for a population of one million. The effluents are excellent in character. The area of the ponds is not more than one-tenth of that required for sewage farms to deal with the same quantity of sewage.

The writer well remembers the pride with which the caretaker spoke of the rapid growth of the blue carp which used to be cultivated in the fish-ponds at the Berlin Sewage Farm in the Malchow Division. According to authorities quoted by Adeney, *loc. cit.*, pp. 20-1 :

Six experimental ponds were constructed in the spring of 1890, each of 410 to 820 square metres, in all 3,710 square metres in area, and from  $\frac{1}{2}$  to 1 metre deep, provided with the necessary inlets and outlets for the effluent and means for emptying when required. These were stocked with white fish (Felchen), ordinary and rainbow trout, carp, and also Zander ova, as well as a number of small crustacea, such as cyclops and daphnia.

At the recently completed Esholt Works of the Bradford

(Yorks.) Corporation the effluent from a large area of percolating filters (see Chapter VIII, page 174) is passed into four ponds covering three acres, and having a combined capacity of  $4\frac{1}{2}$  million gallons. In these ponds the humus from the percolating filter effluent is deposited, and the clear effluent then passes into a large impounding lake  $14\frac{1}{2}$  acres in extent and containing  $10\frac{1}{2}$  million gallons of effluent. The depth varies from 4 feet to nothing, but is mostly shallow.

Preliminary experiments have shown that numerous forms of plankton life, especially cyclops, thrive abundantly in the effluent thus impounded. It is therefore confidently expected that fish will live freely in the waters. The fish introduced will be carp and tench.

Fish are grown in a tank on the estate of the Government Rifle Factory at Ishopore, near Calcutta, into which for some time the effluent from an activated sludge plant has been discharged. The fish have grown freely and healthily under these conditions, and are stated by European consumers to be of excellent quality.

In the foregoing ways the nitrogen in both effluent and sludge from the activated sludge tank is fully conserved.

New York and many other towns in the United States are now following at least in part the example of Milwaukee and Shanghai.

In England "disposal" has not yet given place to "conservation" at many centres, owing in part to the lack of large demand for organic fertilizers on the rich arable soil characteristic of the country.

A new outlet, however, is opening up in the development of allotments and market gardens, and in the combined utilization of sludge, particularly "activated sludge" and "towns' refuse," on the principles set forth in Chapter X.

R. D. Anstead, formerly Director of Agriculture, Madras Presidency, in a lecture before the Institution of Sanitary Engineers in May, 1932, quotes the following figures :

Area of small holdings in England and Wales 415,618 acres.

Area of allotments in England and Wales . 146,000 acres.

At 10s. a ton and 16 tons of manure per acre, this gives a prospective market valued at £2,808,000.

In India with its vast areas of land of low organic content, the demand for organic manure is practically unlimited.

For the area, 130,000 acres in extent, to be irrigated by the newly opened Irwin Canal in Mysore State, there is a demand for some 25,000 tons of nitrogenous organic manure per annum.

A small proportion of this demand is being supplied by compost produced from the nightsoil and rubbish of Mysore City.

The success attained in Mysore has stimulated other centres in India to undertake similar work. Bhopal in particular has obtained very encouraging results, and a scheme of co-operation with the railways has been set on foot, whereby the railway companies supervise small experimental plots at their fertilizer depots, and the ryots when bringing their crops to the railway, see the results obtained by the use of the compost and so are encouraged to take a return load to their farms.

In the villages themselves, progress is being made in the utilization of waste nitrogenous matter, and in the tea and coffee plantations much is being done.

In his evidence before the Linlithgow Commission on Indian Agriculture, the author strongly urged that a systematic survey of the nitrogen resources of the country should be made. This has been done in various isolated districts, notably at Nasik, near Bombay, and tentatively at various centres in the United Provinces and in the Punjab. At the time of writing the author is engaged, at the request of the Municipality of Madras, in making such a survey of the nitrogenous refuse of the city.

By systematic work on these lines, and by the application of the scientific principles, a knowledge of which it is the object of this book to impart, it is the author's hope that the menace of semi-starvation may be removed from the toiling millions of India.

With an increase in physical well-being will come a raising of the general standard of life, making possible a market for the products of mechanical industry, and so leading up to a better-balanced and more civilized existence for the nation as a whole.

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Nitrogen conservation, it will be seen, is a major factor in the world's prosperity; indeed, Nitrogen, thus conserved, together with the energy, mental and physical, liberated by an adequate food supply, is a real measure of the "Wealth of Nations."

## APPENDIX I

### DATA RE COMPOSITION OF HUMAN PHYSIOLOGICAL WASTES

(Taken from *Kleine Mitteilungen* für die Mitglieder des Vereins für Wasser, Boden, und Lufthygiene)

TABLE I

(K.M., Nos. 7-10, 1930, p. 252)

The daily amount of human urine may be taken on a wide average as 1500 c.c., the whole of the solid constituents as 60 g. The detailed composition of these solids is as follows (Neuberg) :

<i>Inorganic Constituents.</i> Total about 25 g.	<i>Organic Constituents.</i> Total about 35 g.
Salt (NaCl) . . . about 15.0 g.	Urea . . . . . about 30.0 g.
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) ,, 2.5 g.	Uric acid . . . . . ,, 0.7 g.
Sulphuric acid (H <sub>2</sub> SO <sub>4</sub> ) ,, 2.5 g.	Creatinine . . . . . ,, 1.5 g.
Potassium (K <sub>2</sub> O) . . . ,, 3.3 g.	Hippuric acid . . . . . ,, 0.7 g.
Ammonia (NH <sub>3</sub> ) . . . ,, 0.7 g.	Other organic matter ,, 2.1 g.
Calcium (CaO) . . . ,, 0.3 g.	
Magnesium (MgO) . . . ,, 0.5 g.	
Further mineral constituents . . . ,, 0.2 g.	

TABLE II

AVERAGE AMOUNT OF FÆCES AND URINE, CALCULATED PER HEAD OF POPULATION (VOGEL)

	In a Day.			In a Year.		
	Fæces. g.	Urine. g.	Total. g.	Fæces. Kg.	Urine. Kg.	Total. Kg.
In natural state . . .	133.0	1200.0	1333.0	48.50	438.00	486.50
Total dry matter . . .	30.3	54.0	84.3	11.10	19.70	30.80
Organic matter . . . . .	26.1	39.6	65.7	9.50	14.50	24.00
Ash . . . . .	4.2	14.4	18.6	1.50	5.30	6.80
Of these						
Nitrogen . . . . .	1.7	9.6	11.3	0.62	3.50	4.12
Phosphoric acid . . .	1.5	1.9	3.4	0.55	0.69	1.24
Potash . . . . .	0.5	2.3	2.8	0.18	0.84	1.02
Lime . . . . .	0.8	0.2	1.0	0.29	0.08	0.37

## APPENDIX I

TABLE III

(K.M., Nos. 11-12, 1930, p. 319)

TOTALS IN GRAMS PER HEAD PER DAY

	Total.	Water.	Ash.	Organic.	Nitrogen.
Fæces . . .	100	75	5	20	1
Urine . . .	1100	1045	33	22	11
	1200	1120	38	42	12

TABLE IV

(K.M., 1931, Nos. 1-4)

INFLUENCE OF NUTRITION ON THE COMPOSITION OF FÆCES AND URINE  
(Keller and Mori, *Centralbl. f. Agr.-Chem.*, 1889, p. 601)

	In %.	Water.	Organic Matter.	Nitrogen.	Ash.	Potash.	Lime.	Phosphoric Acid.	Chlorine.	Sodium Chloride.
JAPAN : Vegetarian diet	Fæces	88.58	9.58	1.04	1.84	0.34	0.05	0.36	0.37	0.61
	Urine	95.97	1.40	0.43	1.63	0.28	little	0.06	0.79	1.30
EUROPE : Mixed diet	Fæces	77.20	19.80	1.0	5.00	0.25	0.62	1.09	0.04	0.07
	Urine	96.30	2.40	0.6	1.30	0.20	0.02	0.17	0.50	0.82

It will be seen that with a vegetarian diet the urine nitrogen and phosphoric acid are lower than with a mixed diet, and therefore the chlorine and sodium chloride are higher.

A Bibliography of seventy-nine references is given in the course of these notes.

## APPENDIX II

### NITROGEN PRODUCTION AND CONSUMPTION

Statistics selected from Annual Report of British Sulphate of Ammonia Federation Limited for 1931-2 and other figures supplied by the courtesy of Imperial Chemical Industries Ltd.

TABLE I

WORLD PRODUCTION AND CONSUMPTION OF PURE NITROGEN FOR THE FERTILIZER YEARS (IN METRIC TONS)

**PRODUCTION :**

**Sulphate of Ammonia :**

	1924-5	1929-30	1931-32
By-product . . . . .	278,300	424,440	266,471
Synthetic . . . . .	255,000	442,100	519,120
	533,300	866,540	785,591
Cyanamide . . . . .	115,000	263,800	134,104
Nitrate of Lime . . . . .	25,000	130,500	79,519
Other forms of Nitrogen :			
Synthetic . . . . .	66,100	427,300	353,320
By-product . . . . .	47,400	51,400	31,170
Chile Nitrate . . . . .	367,500	464,000	170,000
Total production . . . . .	1,154,300	2,203,540	1,553,704

**CONSUMPTION :**

Manufactured Nitrogen . . . . .	786,800	1,586,904	1,421,580
Chile Nitrate . . . . .	363,000	363,893	138,208
Total consumption . . . . .	1,149,800	1,950,797	1,559,788
Agricultural consumption about . . . . .	1,020,000	1,750,000	1,409,000

It is stated in the Report that the total nitrogen producing capacity in the world at the date of publication, June, 1932, is estimated to be about 3,400,000 tons of nitrogen, exclusive of Chile. The "manufactured nitrogen" industry of the world thus operated at an average of about 42 per cent. of capacity.

## APPENDIX II

TABLE II

GEOGRAPHICAL DISTRIBUTION OF NITROGEN CONSUMED  
 WORLD CONSUMPTION OF PURE NITROGEN (IN METRIC TONS)

Continent.	Fertilizer Year.	Total.
Europe . . . . .	1926-7	909,329
	1929-30	1,170,576
	1931-2	1,014,834
Asia . . . . .	1926-7	155,860
	1929-30	270,209
	1931-2	247,900
America . . . . .	1926-7	242,506
	1929-30	417,980
	1931-2	215,372
World . . . . .	1926-7	1,366,335
	1929-30	1,950,797
	1931-2	1,559,788

In considering the question of the available food supply of the world it is of interest to note the statement in the Report that despite total nitrogen producing capacity in the world to-day being about 140 per cent. greater than present demand and 100 per cent. in excess of the record consumption of 1929-30, synthetic nitrogen plants are still being planned and constructed in a number of countries.

The table on page 251 shows the proportion of the total World Synthetic Ammonia Production turned out by the Haber-Bosch and similar processes as compared with other processes for the synthesis of ammonia.

The works of I.C.I. (Fertilizer and Synthetic Products), Ltd., a branch of Imperial Chemical Industries, Ltd., is situated at Billingham, near Stockton-on-Tees. The process employed is a modified form of the Haber-Bosch process, the hydrogen being obtained from water-gas, and producer gas being employed as a source of nitrogen. The works were designed for an output in 1930 of 560 tons per day of ammonia, or 150,000

TABLE III  
(THOUSANDS OF METRIC TONS OF NITROGEN)

Ammonia Process.	Fertilizer Years.	Total.
Haber-Bosch and similar processes	1926-7	479.5
	1929-30	907.9
	1930-1	974.2
Total (all processes).	1926-7	544.2
	1929-30	1284.9
	1930-1	1457.0

tons per year. A force of 5,000 men was originally employed, with an expectation of increase to 15,000.

There are subsidiary plants to convert ammonia into ammonium sulphate, ammonium nitrate, ammonium bicarbonate, nitro-chalk, etc. (*Chemical Trade Journal*, **81**, 1927, pages 52, 83, 603).

Imperial Chemical Industries, Ltd., maintains an extensive Research Farm and Organization at Jealott's Hill, near Bracknell, Berkshire (England), where the numerous problems connected with the use of mineral fertilizers are investigated by a large staff of scientific specialists.

It is of interest to note that Sir Max Muspratt, a Director of Imperial Chemical Industries, Ltd., has stated that the "fixation of atmospheric nitrogen through the intermediate stage of ammonia seems to have established itself as definitely superior to other methods of fixation" (Ernst, *Fixation of Atmospheric Nitrogen*, page 100).

For the manufacture of nitric acid and its innumerable derivatives for use in the explosives and colour industry it would appear likely that the synthetic ammonia process will come to be the chief source of supply of combined nitrogen.

On the other hand, Professor Haber, the inventor of the Haber process for the fixation of nitrogen by the combination of hydrogen and nitrogen to form ammonia, is reported to have remarked that the fixation of nitrogen in the future for soil fertilization would not be through great industrial plants, but rather through plant and soil bacteria (Ernst, *loc. cit.*, page 6).

## APPENDIX III

### TABULAR STATEMENTS OF NITROGEN PRODUCTION AND CONSUMPTION WITH SPECIAL REFERENCE TO INDIA

These figures have been supplied by Rao Bahadur B. Viswanath, F.I.C., Government Agricultural Chemist, Coimbatore, to whom the author's thanks are due, both for the Statements and for the explanatory notes sent in reply to the author's questions. (See also "The Case for the Electro-Chemical Fixation of Atmospheric Nitrogen in India," lecture delivered by B. Viswanath, F.I.C., at a joint session of the Indian Chemical Society (Madras branch), the Society of Biological Chemists (India) and the Association of Economic Biologists (Coimbatore) on the 8th October, 1932, under the Chairmanship of Major Howard, Chief Engineer, Hydro-Electric Development, Madras—*Madras Agricultural Journal*, Vol. XXI, 1933, p. 291.)

#### STATEMENT NO. I, GIVING THE WORLD'S ELECTRO-CHEMICAL FIXATION OF NITROGEN IN TONS PER YEAR FROM THE YEAR 1909 TO 1930

(After J. B. Lipman, Director, New Jersey Agri. Expt. Station, *Journal of American Society of Agronomy*, March, 1932)

Year.	Nitrogen Tons.
1909 . . . . .	569,250
1913 . . . . .	899,800
1917 . . . . .	1,205,000
1924-5 . . . . .	1,154,000
1928-9 . . . . .	2,113,000
1929-30 . . . . .	2,178,400

STATEMENT NO. II, SHOWING THE WORLD'S POPULATION AND THE CONSUMPTION OF NITROGEN AS FOOD AS CALCULATED BY LIPMAN FROM 1900-30

(After J. B. Lipman, in *Journ. Amer. Soc. Agron.*, March, 1932)

Year.	World's Population.	<sup>1</sup> Consumption of Nitrogen from Plant Sources only in Short Tons.
1900 . . . . .	1,500,000,000	Tons. 4,390,000
1910 . . . . .	1,523,000,000	4,460,000
1920 . . . . .	1,700,000,000	4,970,000
1930 . . . . .	2,000,000,000	5,850,000

<sup>1</sup>Lipman has worked out these figures on the basis that out of 100 grms. of protein requirements only 45 grms. directly come from plant sources in U.S.A.

STATEMENT NO. III, GIVING THE INDIVIDUAL AND TOTAL REQUIREMENT OF NITROGEN FOR CONSUMPTION AS FOOD, BY THE POPULATION OF INDIA, INCLUDING THE INDIAN STATES

Population.	Consumption of Nitrogen per head per day.	Consumption of Nitrogen per head per annum.	Total requirement of Nitrogen by the entire Population in a year.
353,000,000	Grms. 12	Lb. 9.66	Tons. 1,522,312

STATEMENT NO. IV, GIVING THE TOTAL AREA UNDER, AND THE OUT-TURN OF, THE PRINCIPAL FOOD GRAINS FOR THE WHOLE OF INDIA, INCLUDING THE INDIAN STATES, TOGETHER WITH THE AMOUNTS OF NITROGEN REMOVED BY THESE

SEASON, 1929-30

Grain.	Area (rounded to thousands).	Out-turn basis (husked) per acre.	Nitrogen in Grain.	Total Amount of Nitrogen for the whole Area.
	Acres.	Lb.	Per cent.	Tons.
Rice . . . . .	80,479,000	900	1·2	3,88,017
Wheat . . . . .	31,654,000	750	2·5	2,64,961
Barley . . . . .	8,787,000	750	2·5	71,185
Millets . . . . .	63,335,000	450	1·5	1,90,853
Maize . . . . .	8,724,000	560	1·5	31,822
Grain . . . . .	17,039,000	300	4·0	91,280
Other grains . . . . .	36,982,000	200	1·0	33,020
<b>Total . . . . .</b>	<b>247,000,000</b>	—	—	<b>10,71,138</b>

*Note.*—The average amount of nitrogen removed in a year by the above food grain is 9·71 lb. per acre.

STATEMENT NO. V, GIVING THE NUMBER OF LIVESTOCK FOR ALL INDIA, INCLUDING INDIAN STATES, FOR THE YEAR 1929-30 AND THE AMOUNTS OF NITROGEN THAT MAY BE CONSERVED FROM THEIR EXCRETA

Livestock.	Number (rounded to thousands).	Nitrogen likely to be produced by a single Animal in one Year.	Total Amount of Nitrogen.
		Lb.	Tons.
Human . . . . .	353,000,000	6·7	1,055,848
Bovine-cattle and buffaloes . . . . .	197,836,000	20·0	1,721,750
Bovine-goats and sheep . . . . .	90,123,000	12·5	502,918
Horses and ponies . . . . .	2,252,000	20·0	20,108
Mules, donkeys, camels, etc. . . . .	2,838,000	15·0	19,005
<b>Total . . . . .</b>	—	—	<b>3,319,629</b>

The foregoing Statements III, IV and V (IV, V and VI in the printed publication) are compiled by Rao Bahadur Viswanath, and the following explanatory note is based on his reply to the author's questions :—

Speaking generally, nitrogen requirement in terms of plant food should be multiplied by 5 to arrive at nitrogen requirement in terms of fertilizer requirement. This is because the nitrogen efficiency is only 20 per cent. ; i.e. when a fertilizer is applied only 15–20 per cent. of the nitrogen is in the food. For the sake of convenience the figure is given as 20 per cent., but on the average in India and elsewhere it is only 15 per cent. On this basis, for a requirement of 6 million tons of nitrogen in terms of food, 30 million tons of nitrogen in terms of fertilizers are necessary. On this basis it will be seen that for India 1·5 million tons of food nitrogen are required, and this is equivalent to 7 million tons of fertilizer nitrogen. Of this only 3·3 million tons are available as shown in Statement V, and if the nitrogen in human excreta is excluded—the reasons for doing so are explained in the reprint—there is an actual shortage of nitrogen.

The argument refers solely to food crops and to nitrogen consumption as food grains ordinarily used in India. The object is to show the inadequacy even in that matter of first importance, viz. food. This is the reason why sugar and sugar-cane have been excluded, which may be considered mainly an industrial crop. It will be noticed that oil seeds are not included in the calculations, as the nitrogen of these seeds is used for sugar-cane.

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