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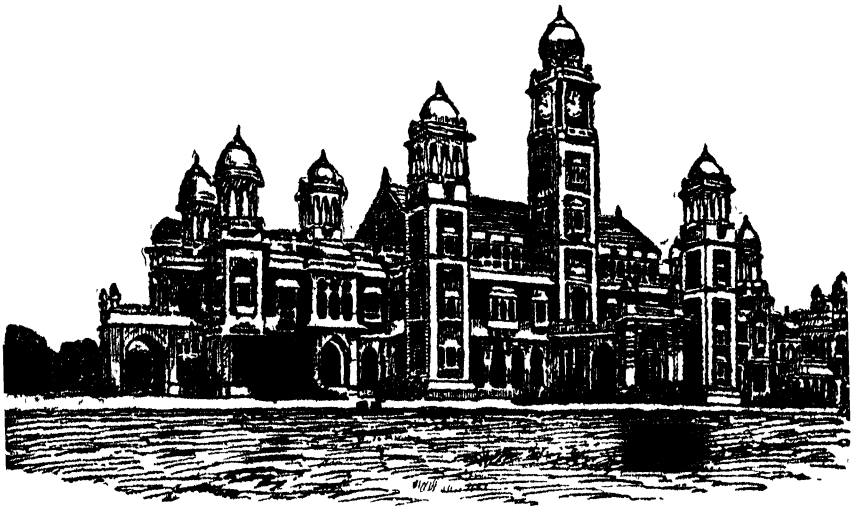

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CHEMISTRY SECTION

EPIDEMIC DROPSY & MUSTARD OIL—PART I*

BY

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In one of our previous publications (Palit & Basu—Jour. Ind. Med. Association, Vol. 7, No. 4, Page 1, Jan. 1939), we have made a systematic investigation on the aetiology of 'beri-beri' or epidemic dropsy. In the same publication, we have mentioned that the analysis of several samples of 'Bazar Mustard Oil' revealed the presence of hydrocyanic acid and that we were carrying on some more feeding experiments with laboratory animals using pure as well as Bazar samples of mustard oil. We have also mentioned that we were studying the chemical changes in the properties of the substance or substances as obtained by the decomposition of the several samples of mustard oil when heated to high temperatures, *i.e.*, to their boiling points.

PART I.

In this communication (Part I) we shall give an account of the results of the analysis of the various samples of mustard oil, before heating as well as after heating as obtained from different sources. In our next communica-

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tion (Part II), we shall give a full account of the feeding experiments carried on in this laboratory with animals and the field experiments, using mustard oils, pure as well as adulterated along with their diet.

The aetiology of the disease 'Beri-Beri' or epidemic dropsy is still shrouded in mystery. The investigations by various workers have mainly centered round three theories, which have superseded other minor theories. The aetiological agents according to these three theories are:—

1. Rice Theory.
2. Mustard oil Theory.
3. Infection Theory.

The studies of Dr. Lal and his collaborators (Ind. Jour. Med. Res. **25**, 1, 239—260, 1937) and that of Drs. Palit and Basu (Jour. Ind. Med. Association, Vol. 7, 4, pages 1—10, Jan. 1938) throw doubt on the validity of the 'rice theory', point out certain difficulties in accepting the contagion theory and provide suggestive evidence for holding mustard oil as the probable aetiological agent. Thus they tried to narrow down the issues but they could not offer positive evidence as to the nature of the aetiological agent. Hence, each of these hypothesis remains to be tested under well controlled conditions.

The findings in certain consignments of mustard oil of a fairly large amount of hydrocyanic acid by Dr. S. B. Dutt (Sci. & Cult., **3**, 255—257, 1937) and the suggestions put forward regarding the association of this constituent with epidemic dropsy deserve attention. The data presented by Dr. Dutt need close examination in the light of modern knowledge about cyanide poisoning. According to Dr. Dutt (loc. cit), the symptoms commonly associated with epidemic dropsy are similar to those produced by 'cardiac poisons like thevatin, digitoxin, strophanthin, hydrocyanic acid and cyanides.' But Mukerji (Sci. &

Cult., **3**, 441, 1938) has pointed out that "physiologists, pharmacologists and clinicians will find it hard to agree with such a statement. Though the aetiology of epidemic dropsy has not yet been finally worked out, sufficient work (Chopra, Choudhry and De—Ind. Med. Gaz. **72**, Jan. 31, Chopra and Choudhry—Antiseptic Feb. 1937; Chopra, Choudhry and Sen Gupta,—Ind. Med. Gaz. **72**, 281, 1937; De and Chatterji—Ind. Med. Gaz. **79**, 489, 1937) has been reported on the clinical, radiological and pathological aspects of the disease and the evidence available is conclusive enough to warrant the statement that the circulatory manifestations in epidemic dropsy are typical and characteristic and do not agree with the symptoms produced by drugs of the digitalis group. Hydrocyanic acid has been classed in the same category as the digitalis group of drugs. It is really a poison to tissue respiration and interferes with cellular oxidations, and the circulatory symptoms associated with cyanide poisoning are really secondary to asphyxia of the tissues. Digitalis like drugs are all characterised by an increase of tone excitability, contractibility and refractory phase of the cardiac muscle with showing of conduction through the auriculo-ventricular bundle. Such manifestations are seldom, if at all, met with cyanide poisoning."

The maximum concentration of hydrocyanic acid as recorded by Dr. Dutt in a sample of mustard oil from Benares is 0.7%, which is equivalent to 7 mgs. of HCN per c.c. of mustard oil. Now the fatal dose of HCN varies from 34—50 mgs. (De and Chatterji—Ind Med. Gaz. **70**, 489, 1935; Solmon—A Manual of Pharmacology, page 761, 1936; Sydney Smith—Canadian Public Health Reports—page 382, May 1933; Hug—Compt. rend Soc. Biol. pages 87, 112, 114, 511 and 947) for an adult weighing about 150 lbs. So about 6 or 7 c. c. of such an adulterated oil, which is not infrequently consumed by a large section of the population, is enough to produce a fatal

effect. Even smaller amounts will produce toxic manifestations but no evidence is available which may indicate that epidemic dropsy cases develop symptoms resembling acute cyanide poisoning by taking adulterated mustard oil. Apparently, therefore, hydrocyanic acid is not introduced in the system in such a high concentration. Hence we found it worth-while to reinvestigate the problem and to analyse various samples of mustard oil as obtained from several sources.

Experiments.

The method of analysis of mustard oil:—

Exactly 50 c. c. of mustard oil are taken by pipette into a separating funnel of about 250 c. c. capacity. 50 c. c. of 60% alcohol are added to the oil in the separating funnel and the contents are vigorously shaken for 15 minutes. The emulsified product is then allowed to stand for half an hour whereby a complete separation of two layers will be effected, the oil settling to the bottom and the alcoholic layer on the top. The mustard oil layer is then removed from the bottom into another separating funnel of the same capacity and is again shaken with another 50 c. c. of dilute alcohol as before and the alcoholic layer separated. The combined alcoholic layers are then filtered once or twice until a clear filtrate is obtained. Call this filtrate A.

Method I.—To the whole of this clear filtrate A, 2 c. c. of 20% nitric acid and then an excess of silver nitrate (2% solution) are added and silver cyanide is precipitated. The sulphides and mercaptans whose occurrence is definitely known in mustard oil in small amounts, are also precipitated with silver nitrate along with silver cyanide. These precipitates are now treated with ammonia in which only silver cyanide is soluble and the insoluble silver sulphide is removed and washed well. The silver cyanide is then reprecipitated by acidifying with nitric acid. This

precepitate is filtered, washed, dried out and weighed and the cyanides are calculated as hydrocyanic acid. The results of estimation of hydrocyanic acid in several samples of the mustard oil obtained from different sources are given in column I of the Table.

Defect of this method of estimation :—This method, however, is not free from defects. It cannot be assumed that only the cyanides and sulphides are precipitated with silver nitrate. Traces of cyanates have also been detected and silver cyanate is as soluble in ammonia as silver cyanide and these two are inseparable. Moreover, the aromatic and aliphatic nitriles and cyanhydrins cannot give a precipitate of silver cyanide with silver nitrate, because the cyanogen group is not present in the free or dissociable state. Hence, the above method (I) has to be modified and the modification adopted for the estimation of the total cyanides is as follows:—

Method II (*a*) :—An aliquot part of the alcoholic extract A is taken, made alkaline with caustic soda and distilled cautiously on water-bath to remove the alcohol. A little more alkali is then added and the mixture is heated with a reflux condenser for $\frac{1}{2}$ hour. Proper care should be taken during the alcoholic extraction process to see that no oil comes out with the extract, because the oil will cause saponification at this stage of the process and considerably vitiate the process. Hence the alcoholic filtrate A must be clear and free from oil. The inorganic cyanides and cyanhydrins which are present in the oil in large amounts are converted into sodium cyanide by this process of treatment with caustic soda thus:—

$$>C<\begin{matrix}OH\\CN\end{matrix}+NaOH=>C=O+NaCN+H_2O$$

(cyanohydrin)

Now this mixture is acidified and distilled carefully. The hydrocyanic acid is evolved and is then absorbed in a

flask containing a standard solution of caustic soda of about N/1000 kept in ice. The hydrocyanic acid combines with the alkali forming sodium cyanide. This mixture of sodium cyanide and caustic soda is then made upto a known volume. A standard solution of pure sodium cyanide (Merck's) of about N/1000 is prepared and is standardised by silver nitrate and then made slightly alkaline with ammonia. The Prussian Blue colour is developed in equal volumes of the known and unknown solutions by the usual process and the blue colour so developed is then matched in a colorometer and the amount of cyanides is then calculated as hydrocyanic acid. This determines only the inorganic cyanides and the cyanogen content from the cyanhydrins.

(*b*) The nitriles present in mustard oil in very minute quantities do not give or produce sodium cyanide on hydrolysis by alkali (Method II (a)) but form the corresponding sodium salts of carboxylic acids. These nitriles do not come in the quantitative colorometric estimation just described above (Method II (a)). Hence for the estimation of these nitriles, the following method is adopted which is based on the following reaction:—

$$R - C \equiv N + 2H_2O \xrightleftharpoons{\text{alkali hydrolysis}} R.\,COOH + NH_3$$

where R is the aliphatic or the aromatic group. We thus find that carboxylic acids as well as ammonia are formed. Hence for the estimation of these, another similar portion of the alcoholic extract A is taken, made strongly alkaline, refluxed and aspirated for 5-6 hours. This process will cause the decomposition of the nitriles evolving ammonia which is absorbed in standard solution of dilute sulphuric acid catch and the amount of nitrogen estimated colorometrically by Nessler's reagent, comparing against a standard solution of ammonium sulphate. From this the amount of nitrogen due to nitriles is calculated as HCN. Hence the total amount of cyanides as

obtained by the above two processes (a) and (b) of Method II will be the sum total of the result of these two processes.

Estimation of allyl-iso-thiocyanate.—The method of estimating allylisothiocyanate in mustard oil is a modification of that adopted by the Association of Official Agricultural Chemists, U.S.A. It may be briefly described as follows :—

10 grams of oil are weighed in a flask and 20 c. c. of 95% alcohol are added. The mixture is distilled in steam for ½ hour, collecting about 100 c. c. of the distillate into a 250 c. c. measuring flask containing 10 c. c. of 30% ammonia. Care is taken that the end of the condenser remain dipped below the surface of the solution. 20 c. c. of N/10 silver nitrate solution are added to the distillate and allowed to stand overnight. The contents are then heated to boiling over a waterbath for 15 minutes, cooled, made up to 250 c. c. and filtered. 100 c. c. of this filtrate are then acidified with 5 c. c. of concentrated nitric acid and titrated with N/10 ammonium sulphocyanide solution, using 3 c. c. of 10% Ferric Alum as indicator. Allylisothiocyanate is then estimated from the volume of silver nitrate used up. Thus 1 c. c. of N/10 $AgNO_3$ = 4·956 Mg. of allylisothiocyanate.

Lal, Ahmad and Roy (Ind. Jour. Med. Res. **26,** 1, 215—220, 1938) have conclusively shown by a series of experiments that this method gives a fairly accurate estimation of the amount of allylisothiocyanate present in the oil.

Again 100 c. c. of each of the various samples of mustard oil were taken, heated to boiling for 5 minutes in open porcelain dish, then cooled in air and the estimations of cyanides and allylisothiocyanate were made by the methods just described. The results of estimation of cyanides and allylisothiocyanate and the iodine value are given in the following table :—

No. of Ex.	Name of the sources from where the oil was obtained.	Condition of the oil under which the Expt. was done.	Amount of cyanides calculated as HCN in gram in 100 grams of oil as estimated by.		Amount of allyliso-thiocyanate in miligram per 100 grams of oil.	Iodine value.
			Method I.	Method II (a & b).		
1	Inayat Khan, Allahabad.	(a) Cold normal oil.	0·0208	0·0041	300·18	110·2
		(b) Boiled oil.	Nil	Nil	Nil	75·2
2	Madan Stores, Allahabad.	(a) Cold normal oil.	0·024	0·0048	341·9	105·7
		(b) Boiled oil.	0·0054	in traces only.	9·74	74·2
3	Bhola Nath Oil Mill, Daryabad, Allahabad.	(a) Cold normal oil. Sample 1.	0·026	0·0052	267·6	100
		(b) Cold normal oil. Sample 2.	0·0298	0·006	338·49	...
		(c) Boiled oil. Sample 1.	In traces only.	Nil.	14·98	79·4
4	Ghee Bhandar, Nakhas Kona, Allahabad.	(a) Cold normal oil.	0·022	0·0044	301·32	106·0
		(b) Boiled oil.	Nil.	Nil	Only in traces	65·8
5	Chhotey Lal, Daragunj.	(a) Cold normal oil.	0·0322	0·006	420·27	104·0
		(b) Boiled oil.	Nil.	Nil	Nil	83·7
6	Thakur Ram, Allahabad.	(a) Cold normal oil.	0·0298	0·0059	396·5	110·5
		(b) Boiled oil.	Nil.	Nil	Only in traces.	79·5
7	Municipal oil from the Municipality, Allahabad.	(a) Cold normal oil, machine pressed.	0·012	0·0024	212·91	
		(b) Cold normal oil, hand pressed.	0·0218	0·0043	272·5	
8	Bhagwan din, Allahabad.	Cold normal oil.	0·027	0·0054	270·28	
9	Shankar Lal.	Do.	0·0264	0·0053	264·92	
10	Pure argemone oil.	Do.	Nil	Nil	93·0	

From the above table, it is seen that the amount of cyanides calculated as HCN is very small as estimated by Method II (a) and (b) combined, which is the most correct method and even this small amount of cyanides disappears or volatilises when the oil is heated to boiling. Similar is the case with allylisothiocyanate present in the oil, which volatilises also when the oil is boiled. The iodine value also drops down considerably when the oil is heated to boiling. It is interesting to note that in almost all the samples of the oil analysed, the amount of cyanides in the oil as estimated by Method II (a+b) is about 1/5 of the results obtained by Method I.

Discussion.

In our opinion the cyanide theory of Dr. S. B. Dutt does not seem to hold any ground on account of the following reasons:—

1. The nitriles and cyanhydrins hydrolyse with the acid of the stomach and form non-toxic non-poisonous carboxyllic acids thus:—

$$RCN + 2H_2O = R.COOH + NH_3 \quad \text{(i)}$$

and $$>C<^{OH}_{CN} + 2H_2O = >C<^{OH}_{COOH} + NH_3 \quad \text{(ii)}$$

2. The amount of hydrocyanic acid introduced into the system is so very small that it is almost immediately converted into a comparatively non-toxic compound of the type HSCN (Bodansky—Jour. Pharm. Exper. Therap—**37, 463, 1929**; Smith and Malcolm—*ibid.*, **40,** 457, 1930; Mukerji—Ind. Med. Gaz. June 7, 1936) or of the type HCNO (Voegtlin, Johnson and Dyer—Jour. Pharm. Exper. Therap—**27,** 467, 1926) through the mediation of sulphur compounds present in the body such as cystine, cysteine, glutathione, etc., thus :—

$$\begin{matrix} R-S \\ \quad | \\ R-S \end{matrix} + HCN = RSH + RSCN \quad \text{(i)}$$

and

$$\begin{matrix} R-S \\ \quad | \\ R-S \end{matrix} + KCN + H_2O = 2\,RSH + KCNO \quad \text{(ii)}$$

The experimental results show that in almost every case the amount of cyanides calculated as HCN in several samples of mustard oil is very small and that when the oil is heated to boiling, the same becomes almost nil. Hence, slow cyanide poisoning is not possible on theoretical grounds and our experimental results corroborate this statement.

3. All the cyanides and cyanates such as allylisothiocyanate present in mustard oil volatilise at 150°C or about that temperature and quite a large amount of these deleterious substances get volatilised in the process of cooking and the remaining portion left will not be in a concentration sufficiently high to produce any toxic and poisonous effect.

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EPIDEMIC DROPSY AND MUSTARD OIL—PART II.*

BY

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As mentioned before (vide part I), we will give in this communication, a full account of the results of the feeding experiments carried on with animals, using mustard oils—pure as well as adulterated—along with their diet and also some accounts of the field experiments that have been carried on here.

According to some, the occurrence of epidemic dropsy is due to poison present in mustard oil; the mustard seed is mixed with other seeds such as seeds of 'argemone mexicana' or 'Sialkanta' as an adulterant which acts as poison. The mustard oil theory is also ill-defined in as much as no definite views have yet been advanced as to the nature or source of the substance in oil which causes the symptoms. The experiments carried on by us (Palit and Basu—Jour. Ind. Med. Assoc.—Vol. 8, No. 4, 1 Jan. 1938) conducively prove that mustard oil as obtained from healthy and pure mustard seeds (yellow variety) has nothing to do with the causation of the disease and is not responsible for epidemic dropsy at all. In part I, we have shown on theoretical grounds that the cyanide theory does not seem to hold any ground and is not tenable. Now

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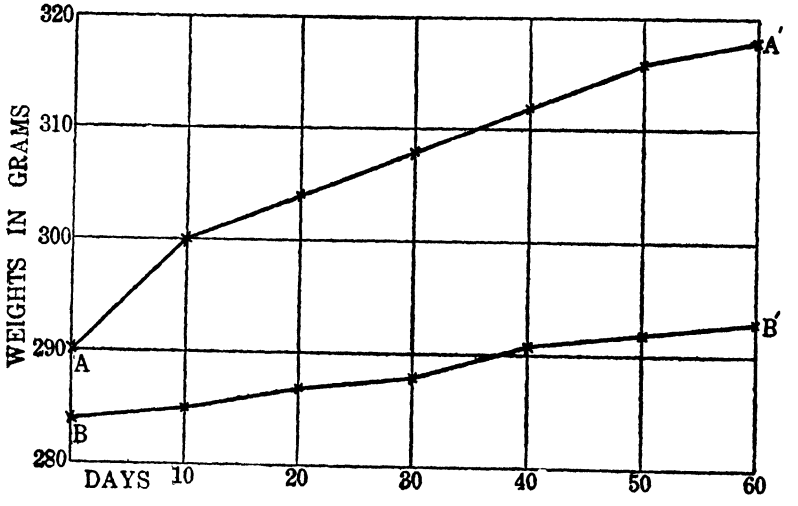
whatever be the loopholes in the cyanide theory, it cannot be discarded on mere theoretical grounds without studying experimentally the effect of cyanides on laboratory animals. However, we have made systematic investigations in this line with pigeons and studied the effect of different samples of oils containing different proportions of cyanides. The experiments were carried on as follows:—

FEEDING EXPERIMENTS.

Expt. No. I.—In this experiment the pigeons were divided into four different groups, each group having four pigeons. The diet for each group per day was as follows:

Group 1.—Bajra (Pennisetum typhoideum)—100 grams for four pigeons and boiled mustard oil free from hydrocyanic acid—1 c. c. to each pigeon.

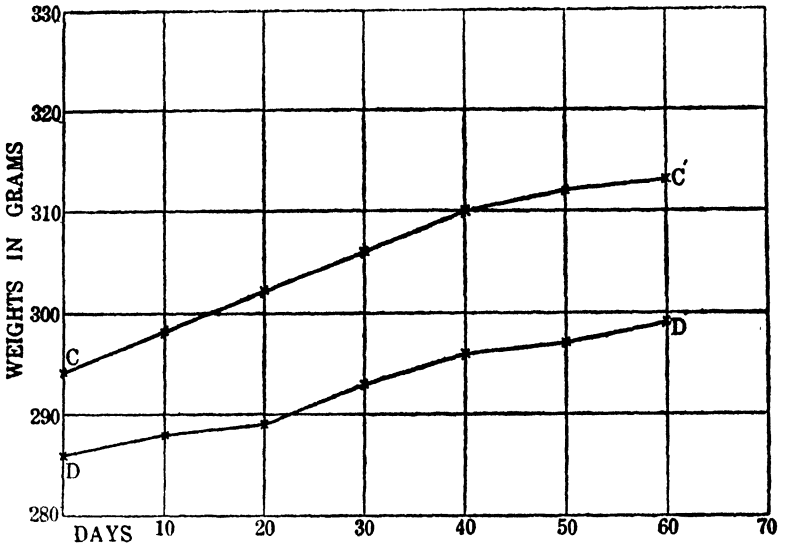
The Graph AA′ represents the experiment with Group 1 in Sunlight.
„ „ BB′ „ „ „ „ „ „ Dark.



Graph No. I

Group 2.—Bajra—100 grams for four pigeons and normal mustard oil (unboiled)—1 c. c. to each pigeon. (1. c. c. of this oil = 0.18 mg. of HCN).

The Graph CC′ represents the experiment with Group 2 in Sunlight.
„ „ DD′ „ „ „ „ „ „ Dark.

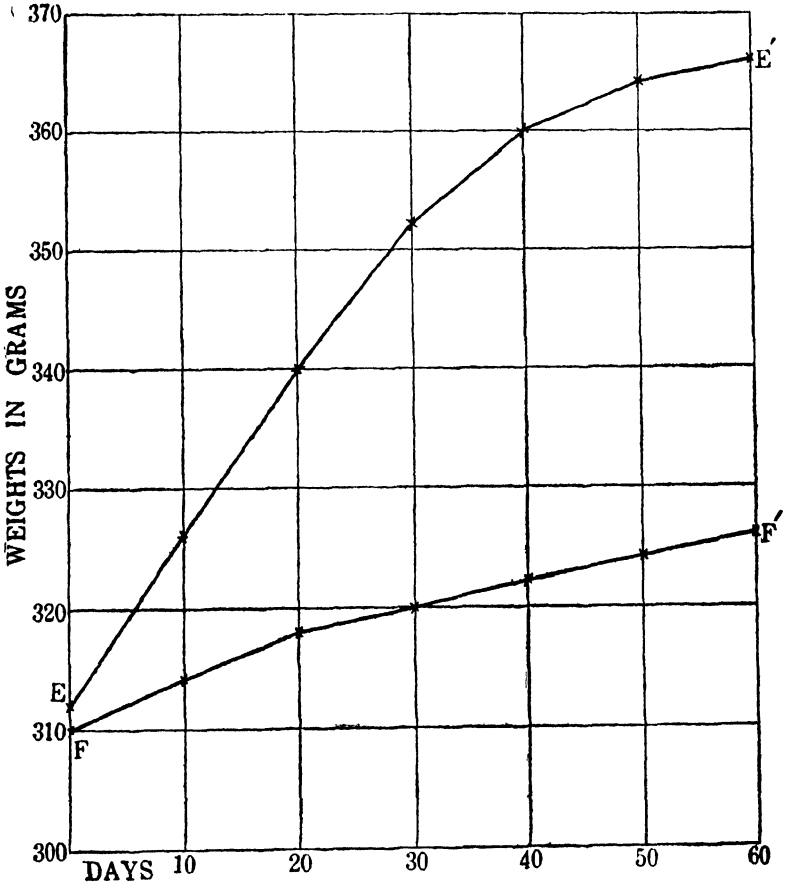


GRAPH NO. II

Group 3.—Bajra—100 grams for four pigeons and mustard oil containing excess of HCN prepared by

passing hydrocyanic acid gas in mustard oil—1 c. c. to each pigeon. (1 c. c. of this oil = 0.22 mg. of HCN).

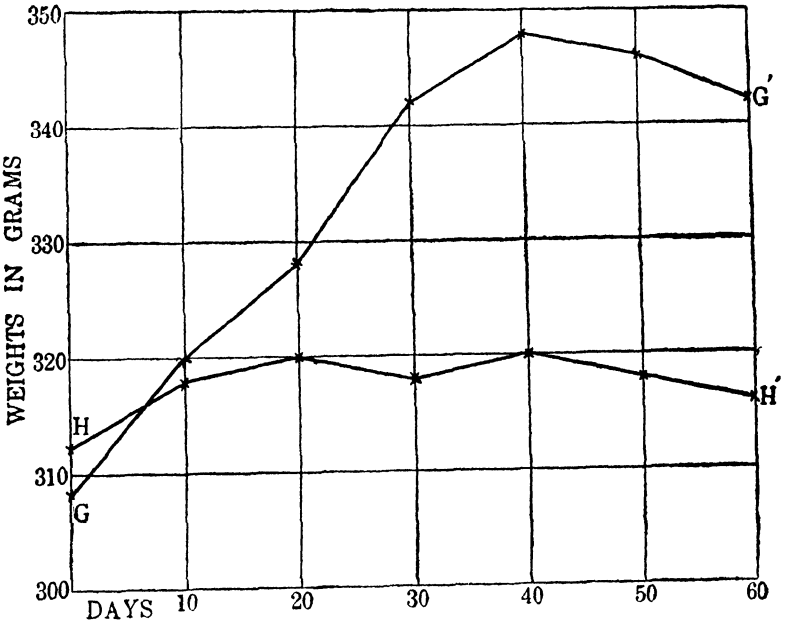
The Graph EE′ represents the experiment with Group 3 in Sunlight.
„ „ FF′ „ „ „ „ „ „ Dark.



GRAPH No. III

Group 4.—Bajra—100 grams for 4 pigeons and aqueous solution of HCN (dilute)—1 c. c. to each pigeon (1 c. c. of this HCN solution = 0.18 mg. of HCN).

The Graph GG′ represents the experiment with Group 4 in Sunlight
" " HH′ " " " " " " Dark.



GRAPH No. IV

The oils and the aqueous solution of hydrocyanic acid were directly administered to the pigeons by opening their mouths with a pipette. Each of these experiments from group 1 to group 4 were divided into two different sets—one carried on in sunlight and the other in the dark, side by side, thus having eight groups and using 32 pigeons. These experiments were started on the 2nd December, 1938 and then stopped on the 5th March, 1939. The pigeons in each group in sunlight as well as in the dark were weighed every tenth day and the change in their weights noted.

The results of the average weights of the four pigeons are given below:—

Set No. A-Sunlight.

Groups	Average weight of 4 pigeons taken every tenth day in Grams.						
	At start	10	20	30	40	50	60
Group 1. (boiled oil)	290	300	304	308	312	316	318
Group 2. (Unboiled oil)	294	298	302	306	310	312	318
Group 3. (Oil mixed with excess of HCN).	312	326	340	352	360	364	366
Group 4. (Solution of dilute HCN)	312	320	328	342	348	346	342

Set No. B-Dark.

Groups	Average weights of 4 pigeons in grams taken every tenth day.						
	At start	10	20	30	40	50	60
Group 1. (Boiled oil)	284	285	287	288	291	292	293
Group 2. (Unboiled oil)	286	288	289	293	296	297	299
Group 3. (Oil mixed with excess of HCN)	310	314	318	320	322	324	326
Group 4. (Dilute aqueous solution of HCN)	312	318	320	318	320	318	316

From the above experimental results, the graphs have been plotted (vide Graphs Nos. I—IV). In each of these feeding experiments in both the sets—*viz.*, in sunlight as well as in the dark—the average weights of the birds in all the groups with the exception of group 4 increased steadily as is quite evident from the graphs, though this increase became less and less as the experiments continued and finally the weights remained practically constant. In group 4, the pigeons in both the sets at first gained their

weights steadily for sometime and as the experiments continued, there was then a sudden drop in their weights, although this drop was not very great. From the graphs, it is also quite evident that the average weights gained by the pigeons in all the groups from 1—4 kept in the dark are much less than the weights in the corresponding groups kept in sunlight. This clearly shows that sunlight has a beneficial effect on the system and has got much influence on the healthy growth in animal and plant lives. None of the birds in any group in any set—sunlight or dark—developed any sign of disease suggesting epidemic dropsy and this is quite clear from the above experimental results, which show that the birds maintained steady growth and kept good health, the weights being increasing steadily for some time and then remaining practically constant at the end.

Now in Group 3 of both the sets in sunlight and in dark, the dose of the oil containing HCN was increased from 1 c. c. to 1.5 c. c. on the 20th January, 1939, and continued for six weeks. No symptoms of any disease developed in any case except that there was a remarkable change in the colour of their stools which were of bluish green colour. The dose of the oil was then increased to 2 c. c. on the 2nd March, 1939, and administered to three pigeons first kept in sunlight. All the three pigeons died one after the other within 5 minutes. The dose was not increased in other group kept in dark, as the pigeons in the set kept in sunlight died immediately after the administration of the oil in double doses. All the three pigeons which died were examined after post mortem and the results revealed that the metahaemoglobin detected in the heart—blood after post mortem, gave indication and signs of acute cyanide poisoning. It was found that the amount of lactic acid in the heart was also increased.

Similar operations were conducted with group 4 in which aqueous solution of hydrocyanic acid was adminis-

tered. Nothing happened when the dose of hydrocyanic acid was increased from 1 c. c. to 1.5 c. c., but when the dose was increased to 2 c. c., the results proved fatal. The post mortem examinations revealed the same results as in group 3, giving indications and signs of acute cyanide poisoning.

The poison appears to a certain extent, to get absorbed in the system of the birds by the formation of nontoxic substances. When the limit is exceeded, acute cyanide poisoning occurs. These experiments were stopped a little after 13 weeks which are held by some observers as the incubation period of the disease.

Expt. No. II—Another separate series of similar experiments were started on the 2nd February, 1940, polished rice being substituted for Bajra in their diet, other conditions remaining the same as in the previous experiments just described above. No symptoms of any disease or any remarkable change in their weights appeared. The respective dose of the oil and of the hydrocyanic acid solution was increased from 1 c. c. to 1.5 c. c. after a month. A number of casualties occurred in each of the Groups 3 and 4 in both the sets (sunlight as well as dark). A few of the dead pigeons after post mortem were examined for their variation in tissue structure of heart, liver and kidney and the tissue culture results showed changes which did not accord with the symptoms of epidemic dropsy but only showed signs of acute cyanide poisoning. These experiments terminated on the 8th March, 1940.

Expt. No. III.—These experiments with pigeons were started from the middle of January, 1940, with four different samples of oil which were collected from different places during the outbreak of epidemic dropsy in certain cities. The chief diet of the pigeons was as follows:—

(a) Bajra (Pennisetum typhoideum)
(b) Karkal (mixture of several grains)
(c) Polished rice (devoid of vitamin B).

In each group the diet was mixed with each of the four different samples of the oil obtained from different places. The experiments with each of the above three substances as diet were carried on separately. The quantity of oil first administered was 1 c. c., and then after three weeks, it was increased to 3 c. c. Karkal is a mixture of several kinds of seeds or grains containing very small particles of limestone or kankar, and is the main diet of birds. These small pieces of lime-stone or kankar are taken by the birds with great relish along with the various seeds and these are nothing but calcium carbonate and supply calcium to the system. The other conditions were the same as in the two previous experiments already described.

All these four samples of the oil were found, on analysis to contain, 'argemone oil', as each of them gave distinct indication for the presence of 'argemone oil' detected by the nitric acid test of Dr. Sarkar [Ind. Med. Gaz.—LXI, 62,1926; and also compare Ind. Med. Gaz.—LXXV, No. 5, 261, May 1940; also A Book of Public Health Practice by Stewart and Boyds, 1928 edition; cf. also Lewkowitsch and Warburton 1922]. The following results were obtained:—

(a) This experiment (with Bajra) was similar to Expt. I describe before with Bajra as the main diet. If any symptoms could develop. it must be certainly due to the presence of adulterated oil but no undesirable signs were exhibited. This experiment lasted for seven weeks and then terminated.

(b) In this experiment with 'Karkal', the same results were obtained as in the experiment (a) just described. After seven weeks, the experiment terminated without any positive result in favour of the cyanide theory.

(c) In this series, the birds were fed with vitamin free (polished) rice as their main diet mixed separately with each of the four different samples of the oil collected. A separate but quite a similar experiment was also started with birds using pure 'argemone oil' (1 c. c.) instead of the above oils, the diet and other conditions remaining the same. The experimental results show that after 10 or 12 days, several pigeons fed with pure 'argemone oil' got polyneuritis and developed symptoms of gastro-intestinal disturbances, while the other groups fed with the four different samples of the oil developed these symptoms a little later—in the middle of the third week. The results of this experiment are recorded below:—

Samples of oil.—Average weight of 4 pigeons in grams on the following dates.

	16.1.40	23.1.40	30.1.40	6.2.40	12.2.40	19.2.40
1. Oil No. 1—	316	302	288	262	238	256
2. Oil No. 2—	308	296	290	270	244	264
3. Oil No. 3—	304	298	278	258	250	268
4. Oil No. 4—	300	290	274	246	234	246
5. Argemone oil (1 c.c.)	314	284	264	238	210	228

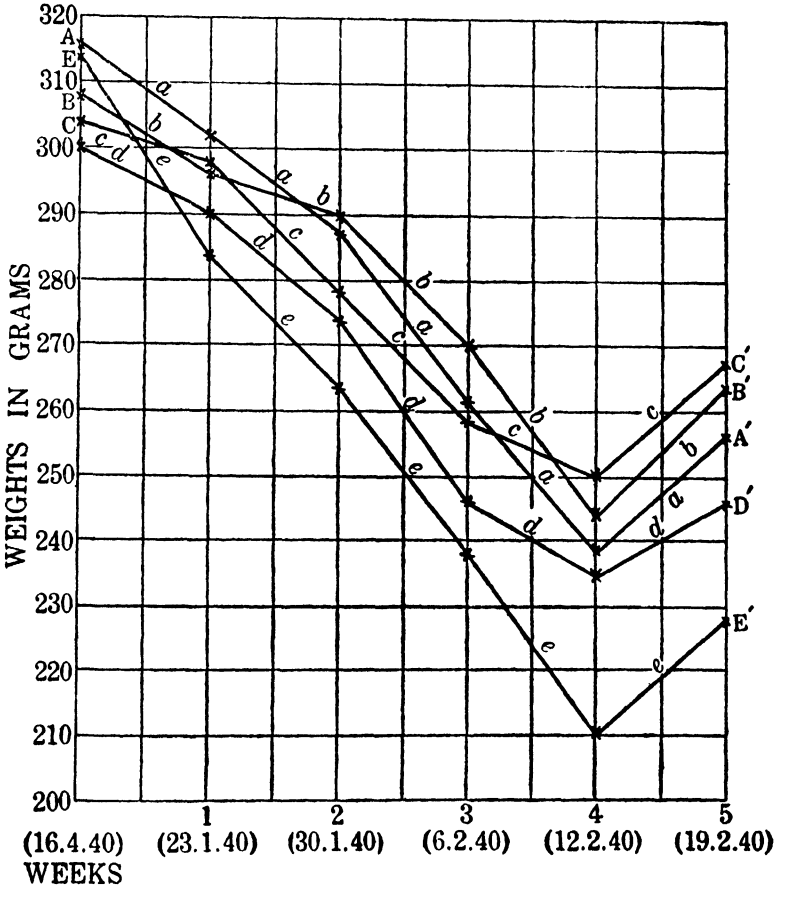
From the above results, the graphs have been plotted. The experiment was started on the 16th January, 1940, and stopped on the 12th February, 1940, when the ordinary diet Bajra and Karkal was restored to them.

From the graphs (vide Graph No. V), it is quite clear that the pigeons fed with polished rice and several samples of the oils collected and the 'argemone oil', began to loose their weights quite steadily and this decrease in weight was very much marked after the second week. In each case there was a sudden drop in their weights and

this drop was very high specially in the case with 'argemone oil' when the drop was from 164 grams to 210 grams in two weeks. In every case the pigeons went on loosing their weights as the experiments continued and began to fall ill one after the other. Immediately the

(1) The Graph AaaaaA' represents the experiment with Oil No- I,
(2) „ „ BbbbbB' „ „ „ „ „ No- II.
(3) „ „ CccccC' „ „ „ „ „ No. III.
(4) „ „ DdddD' „ „ „ „ „ No. IV.
(5) „ „ EeeeeE' „ „ „ „ „ Argemone.

AFTER THE 4TH WEEK, THE EXPERIMENT WAS STOPPED AND ORDINARY DIET WAS RESTORED TO THE PIGEONS



GRAPH No. V

administration of the four different samples of oils as well as 'argemone oil' was stopped and their diet—polished rice—was replaced by Bajra or Karkal along with calcium lactate and substances rich in vitamin B such as orange, sweet lemon, tomato, etc., in serious cases. All the birds showed definite signs of improvement, began to gain in weight and recovered after a few days as will be evident from the graphs. There were no casualties.

These symptoms of polyneuritis and gastro-intestinal disturbances are evidently due to polished rice deficient in vitamin B and certainly not due to the oils. Polyneuritis in pigeons by polished rice has been definitely established long ago, since the administration of vitamin B concentrates indicates almost instantaneous improvements. The combination of rice with oil adulterated with 'argemone oil' makes a fertile ground for the development of epidemic dropsy.

Now we come to some of the actual cases of our field experiments carried on here, *i.e.*, the actual cases of persons suffering from an attack of epidemic dropsy commonly known as 'beri-beri' that we have come across and mention the system or the method of treatment based on the results of our biological investigations that have been followed:

Case I.—Patient A.—This patient was a washer-woman and aged about 50 years. She was, on examination, found to be suffering from severe cardiac complaints and eye-troubles. She had dimness in her vision and oedema with flushed rosy skin of the legs, which pitted on pressure. The spasms were so violent that it was expected that she would die of heart failure at any moment. History revealed the fact that she had also some sort of gastro-intestinal disturbances in the beginning. She was a very poor woman and could not afford to meet the expenses for her medical treatment. During spasms, she was given amyl nitrite capsules for inhalation. For the first few

days, she was given some fruit juices (supplied by us) rich in vitamin contents, specially vitamin B (complex) and lime water (2 drachm) twice a day after food. She was also given freshly fermented palm juice (4 oz.) every 4 or 5 hours, the total quantity of palm juice consumed during 24 hours being 20—24 oz. She was exposed to sunlight in the morning for half an hour every day and was rubbed olive oil (also supplied by us) all over her body while taking the sun-bath. After a few days, she was found to be a little better, when she was given some rice (Bhat) prepared from freshly hand-pounded rice with red pericarp over it, some milk, green vegetables and 'ghôle' or 'mattah' prepared by churning curd and then diluting with water, and a few 'Chapatis' or cakes prepared from Bajra. After taking Bajra and rice (Bhat) for a few days, she began to improve remarkably and was found altogether quite a new woman after a month. She began to progress quite satisfactorily and within a course of a month and a half she recovered completely and the symptoms of epidemic dropsy or 'beri'-beri' disappeared. All her troubles such as dimness of vision, oedema, heart troubles, etc., subsided. She is quite hale and hearty and is now attending to her profession.

Case II.—Patient B.—This patient was the son of patient A and was a young man of 25-27 years of age. He had oedema of his legs and erythma all over the lower part of his body and abdomen. His face as well as his body became quite dark and looked quite flushed. He complained of constipation and had severe breathlessness due to heart trouble. The treatment that was followed was exactly the same as that of his mother (Patient A). This patient recovered quickly. He has had no further trouble since then and is now keeping good health attending to his profession.

Case III.—Epidemic dropsy or 'beri-beri' broke out in a Bengali family consisting of (a) himself, (b) his wife,

(c) and (d) his two daughters and (e) his son. All of them had oedema of the legs with rosy skin, which became more prominent in the afternoon and the legs pitted on pressure. All of them had, more or less, some sort of cardiac and eye complaints:—

No. 1.—Two of them, the wife (No. b) and her eldest daughter (No. c) had large number sarcoids all over the lower parts of their bodies. These patients complained that the stools they were passing were of corrosive nature. The mother developed piles and fissures from which blood was constantly oozing out.

No. 2.—The other three patients, the father (No. a), his youngest daughter (No. d) and his son (No. e) were a bit constipated and had been complaining of breathlessness due to heart trouble. Their cardiac complaints were not so severe as in the above two casses (No. b) and (No. c).

The system of treatment that was followed was on the same lines as communicated in our previous publication (Palit and Basu—Jour. Ind. Med. Association—Vol. 7, No. 4, 1 Jan. 38):—

(i) Calcium lactate—15 grains twice daily to all the patients.

(ii) Acid Mixture—nitro-muriatic dilute or phosphoric acid dilute 15 minims twice daily after food.

(iii) Freshly fermented palm juice—4 oz., every 4 or 5 hours.

(iv) Exposure to the sun for half an hour in the morning. Each of them was rubbed with pure mustard oil all over the body while taking sun-bath.

(v) Exposure to ultraviolet radiation every day for 10-15 minutes at a distance of 3ft. from the lamp all over the body excepting the head

and face and also over the rectum in the case of mother (No. b) for 5-10 minutes from the same distance. For swelling and pain in the rectum she was prescribed the application of 'Hadensa' in the rectum.

(vi) Diet. Sufficient quantity of milk and ghôle. Bhat (cooked rice) prepared from freshly hand-pounded rice with red pericarp over it, green leafy vegetables such as 'Palang Sâk' (spinach olerace—Hindi Palak). Kalmi Sāk (Ipomeoea reptaus—Hindi Karmoha), Salad, Patal (Tricosanthis dioica—Hindi Parwal), Palta (leaves of Patal or Parwal), Dhenrosh (hibiscus esenlenties—Hindi Bhindi), bread prepared from whole wheat and the cakes prepared from Bajra, meat, liver and fruit juices, like orange, grapes, sweet lemon, pine apple, pomegranate, tomato, etc., and also some irradiated (either by exposing to the ultraviolet radiations for 15-20 minutes or to the sunlight for 1-2 hours) fruits such as cucumber, shaddock (Batapi Lebu) oranges, and apples cut in thin slices. Mustard oil from Bazar was totally avoided but the oil obtained from pure mustard seeds (yellow variety) from 'ghani' was freely used.

In several cases, the mother and her eldest daughter (Nos. b and c) were each given 'Makaradhwaj'—one grain with honey and extract of Arjun in the afternoon and were also given injections of calcium and 'beri-beri' vaccine every 4th or 5th day. The oedema of the legs and the sarcoids began to subside and finally disappeared. The swelling in the rectum (No. b) diminished and the inflamed painful piles also slowly subsided. The patient (No. b)

became quite normal in the course of a month and a half or so. All the patients recovered very quickly and gained much in weight. None of them had any further complication since then and all of them are now keeping good health.

Discussion

In our previous communication (Palit and Basu—Jour. Ind. Med. Association Vol. 7, No. 4, 1 Jan. 38. Palit and Dhar—Jour. Phy. Chem—**32**, 1263, 1928; **34**, 993, 1930; **36**, 2504, 1932), the effect of sunlight on animal body has already been discussed. Palit and Basu have shown that the light absorbed by the system accelerates the metabolism of food materials in the body and that by the absorption of light, the body cells are activated and thus increased oxidation of food materials takes place. The diseases like 'beri-beri', epidemic dropsy, ricket, gout, anaemia and so on, which originate with defective metabolism of food materials should be prevented or cured by light treatment. Hence light acts as a preventive and the disease is thus avoided in its presence. The increase in the metabolism of food materials is the most fundamental action of light which leads to the prevention and cure of diseases when a person is exposed to sunlight or ultraviolet radiations.

In the same communication (ibid.), we have also discussed the importance of calcium and iron salts. The uses of calcium salts in many body reactions have been accepted as an important agent in counter-acting infection and repairing the damage of the tissues caused by the disease. Moreover, it acts as a stimulant to the heart. In the treatment of metabolism diseases like 'beri-beri', epidemic dropsy, anæmia, etc., iron preparations specially of colloidal nature are found to be helpful and efficacious in the treatment of deficiency and metabolism diseases and

also the addition of minute traces of copper or manganese salts to iron is very helpful. We have already shown (Jour. Ind. Med. Assos. Vol. 7, No. 4, 1 Jan. 1938) that common leafy vegetables contain iron and hence the usefulness of green leafy vegetables in the prevention of deficiency diseases, is not only due to the presence of vitamins in them but is also due to their iron and other mineral contents. Recent researches (Palit & Dhar—Jour. Ind. Chem. Soc.—**13**, 502, 1936; **11**, 471, 1934; **11**, 666, 1934; science and culture—**1**, 363, 1935) have shown that traces of copper or manganese are of great importance in many animal and plant processes. These minute traces of copper or manganese stimulate markedly the oxidation of food materials. Hence in the treatment of metabolism diseases or anaemia, the addition of traces of copper or manganese salts to iron is very useful.

All the experiments carried on by us, as described in this communication, prove that mustard oil as obtained from healthy and pure mustard seeds (yellow variety) has nothing to do with the causation of the disease and is not responsible for epidemic dropsy or the so-called 'beri-beri'. We have constantly used and have still been using pure and freshly prepared mustard oil in families afflicted with disease with no ill effect at all.

From the feeding experiments with pigeons mentioned in this communication, it is found that none of the birds developed any signs of disease suggesting epidemic dropsy. The sub-lethal doses of cyanides that were being administered into their system, were probably being converted into non-toxic and non-poisonous substances as discussed in Part I. Another probable reason for assimilating poison may be due to Bajra itself which is the basic diet of the pigeons. Bajra contains some sulphur compounds, iron and calcium. The sulphur compounds as said before (vide part I) form non-toxic compounds with cyanides. The glutathione, a sulphur compound present in animal system,

is converted into disulphide form by its oxidation thus:—

$$2\ G-SH+O=G-S-S-G+H_2O,\ i.e.,$$

$$2\ G-S-H+O=G-S \mid S-G+H_2O$$

The disulphide compound such as cystine, etc., thus formed and also present in the system, react with hydrocyanic acid forming non-toxic and non-poisonous substances regenerating the original substances glutathione or cystein thus:—

$$G-S \mid G-S + HCN = G-S-H + GCNS,$$

Disulphide (Hydrosulphide) Thiocyanate derivative)

where G stands for the radicle $-CH_2.\ NH_2.\ CH\ COOH$ in cystein.

The whole reaction may be represented with cystein thus:—

$$\begin{array}{l} CH_2-S-H \\ | \\ CH\ NH_2 \\ | \\ COOH \end{array} + O + \begin{array}{l} H-S-CH_2 \\ | \\ CH.NH_2 \\ | \\ HOO\ C \end{array} = \begin{array}{l} S-CH_2\ NH_2\ CH.\ COOH \\ | \qquad\qquad\qquad + H_2O. \\ S-CH_2\ NH_2\ CH\ COOH \end{array}$$

β. thio-α-amino Propionic acid (cystein) (cystein) Disulphide compound (cystein).

and $$\begin{array}{l} S-CH_2.\ NH_2.\ CH.COOH \\ | \qquad\qquad + HCN = \\ S-CH_2.\ NH_2,\ CH.\ COOH \end{array} \begin{array}{l} CH_2,\ NH_2,\ CH.\ COOH \\ | \qquad\qquad + \\ S-H \\ \text{(cystein)} \end{array}$$

$$HOOC.\ CH.\ NH_2.\ CH_2.\ CNS$$

(thiocyanate derivative)

Now the thiocyanate derivative G. CNS is decomposed by dilute acid (hydrochloric) present in the stomach into carbonyl sulphide COS which in contact with water is immediately decomposed into carbon dioxide and hydrogen sulphide, the latter in turn forms hydrosulphide compound again or is oxidised by air into water with the precipitation of sulphur thus:—

$$GCNS+2HCl+H_2O=GCl+NH_4Cl+COS.....(i)$$

$$COS+H_2O=CO_2+H_2S... .....(ii)\ and$$

$$H_2S+O\ (air)=H_2O+S..... ... .\ (iii)$$

The oxidation of glutathione into the disulphide form induces the oxidation of cell constituents as is shown by the fact that more oxygen is taken in than is required for the formation of the disulphide compound. It is also revealed by the investigations of Meyerhof and Hopkins that the tissues are also capable of effecting the reduction of the disulphide compound into glutathione thus:—

$$\begin{matrix} G-S \\ | \\ G-S \end{matrix} \quad + XH_2 = 2\ G-SH + X$$

Nature of the compound XH_2 in the muscle tissues is not known yet. The glutathione is again converted as before into the disulphide compound and the disulphide again into glutathione so that the cycle is repeated.

Similar reactions occur with Bajra and with Karkal as well, which not only contains Bajra but also many other varieties of other small grains, mineral salts, iron, and calcium. In groups 3 and 4 of the feeding experiments with pigeons, in which excess of cyanide is used, iron seems to play a very important part by combining with the cyanides introduced in the system and forming 'Prussian Blue' which is indicated by the bluish green stools of the birds. Calcium and iron as is evident from the results maintain this steady growth. The possibility of a reaction to form a non-toxic compound of the nature above is seen in the fact that the pigeons have a high rate of metabolism, their body temperature being about 112°F.

Moreover it has been shown by experiments on pigeons that when 'argemone oil' is heated till it 'fumes' well, the toxicity of the oil greatly diminishes to that of bland oils like olive oil, although the heated oil still gives the positive test with nitric acid. It has been shown (Chopra, Pasricha, Lal, Goyal and Sen—Ind. Med. Gaz. LXXIV, No. 4, 193-195, April 1939; Chopra, Pasricha and Banerji—LXXIV, No. 12, 733-735 and 751-752, 1939. LXXV, No. 5, 261, May 1940) by the feeding experiments

in man that the oil expressed from the seeds of 'argemone mexicana' along with milled rice as diet can produce signs and symptoms of epidemic dropsy and that the aetiologocal reason for the causation of the disease is the 'argemone oil' which is largely used as an adulterant of mustard oil. Chopra, Pasricha and their collaborators have found from their feeding experiments during an outbreak of the disease that when rice and the mustard oil that gave positive test for the 'argemone oil' were each withdrawn from the diet and respectively replaced by bread and ghee, the disease disappeared and the patients recoverèd quickly. As far as rice used in diet is concerned, all the experimental results of the above investigators just corroborate the results which we have obtained and mentioned in this communication. But when these experiments are carried on with Bajra omitting rice altogether, we did not find any sign or symptom whatsoever of the disease like epidemic dropsy although the oils containing cyanide and 'argemone oil' were freely used to a certain limit. Our experimental results conclusively prove that rice with mustard oil adulterated with argemone oil makes a fertile ground for the development of epidemic dropsy and when the rice and oil are omitted from the diet, the disease disappears. Moreover from the results of our feeding experiments and the actual field experiments, we come to definite conclusion that Bajra has both preventive as well as curative properties and the disease is thus avoided. We can confidently say that whatever the aetiological reason for the causation of epidemic dropsy may be, the disease can altogether be avoided by taking 'Chapatis' or cakes prepared from Bajra and calcium with plenty of green vegetables and fruit juices rich in mineral salts, iron contents, calcium and vitamins specially vitamin B (complex). We are also of opinion that with the outbreak of epidemic dropsy, vitamin deficiency is caused and not that the disease is caused by the deficiency of vitamins.

Hence it is necessary to replenish the system with vitamins.

In the last epidemic, one of the authors (Dr. S. N. Basu, Chairman of the Medical Relief Committee) got the form (vide Appendix A) printed for the investigation of the causation of the disease. We requested Major D. N. Chakravarti, I.M.S., to help in the investigation and to carry on the work in collaboration and our request was gladly accepted by him. The Municipal Board was also pleased to help us in this investigation by offering the temporary services of an assistant chemist for a few months and by supplying us oils collected from different infected areas. The above form has been practically drawn up by Major Chakravarti. Uufortunately he had to leave the work as no further help would be given by the Board and he had to leave India on active service. One of the authors (Dr. Basu) got the circulars distributed amongst the public and got pure mustard oil pressed from the yellow variety of mustard seed under the supervision of the Public Health Department of the Municipal Board, and sold at cost price to the public and to the patients. He also requested Mr. Venkatesh Narain Tewari, the Parliamentary Secretary to the Premier, to get a supply of pure unadulterated palm juice just fermenting for the use of patients which was being supplied from the Public Health office. Our information is that the toddy that is sold in toddy shops is adulterated with various ingredients to make it more intoxicating and so the use of toddy from toddy shops was prohibited by us; but we advocated the use of palm juice just fermenting. The reasons why we advocate the use of just fermenting palm-juice are :—

(i) Palm-juice is nothing but pure glucose in solution in its natural form which is more easily absorbed in the system than chemical glucose solution which we give.

(ii) 'Just fermenting' means that vitamin B is being formed which we found to be very efficacious in toning up the heart, ameliorating the symptoms of pain and digestive troubles so often complained of by the patients suffering from epidemic dropsy. We used injections of vitamin B along with calcium injections with quite satisfactory results.

Pasricha and his collaborators (1936) claim to have discovered antibodies in the blood of epidemic cases against a gram-positive spore-forming bacillus isolated from their stools, while such antibodies are said to be absent in the healthy controls.

Recently we have been using 'beri-beri' vaccine prepared from the stools of 'beri-beri' patients by the Clinical Research Association Ltd., Calcutta, with far better results than with calcium and vitamin B alone. The vaccine is very efficacious in gastro-intestinal disturbance cases.

In our opinion, the epidemic dropsy is nothing but a group of large number of various diseases. Hence no single treament or causation can fit in for the epidemic. We request the other investigators to work on the line of (a) infection, (b) Chemical Poison and (c) Metabolic deficiency. We also request them to keep their mind open and not to be carried away by any dogma.

In conclusion we offer our sincere thanks to Major D. N. Chakravarti, I.M.S., D.T.M. & H., for his results of the post mortem examinations of the dead pigeons carried on by him in his laboratory and hospital. We also offer our sincere and heartfelt thanks to Pt. Amaranatha Jha, M.A., F.R.S.L., the Hon'ble Vice-Chancellor of the University of Allahabad, to accord his kind permission to carry on this investigation at the Biochemical Laboratory of the University in collaboration with well

qualified and experienced medical men. Our thanks are also due to the Municipal Board, Allahabad, for offering the services of an assistant chemist (on tomporary measure) for this investigation.

Biochemical Laboratory.

APPENDIX A

Research Form—A.

ALLAHABAD MUNICIPALITY

BERI-BERI AND EPIDEMIC DROPSY RESEARCH

FAMILY SCHEDULE

1. Patient :—(*a*) Name
   (*b*) Caste
   (*c*) Sex
   (*d*) Age
   (*e*) Diagnosis
   (*f*) By whom (give name)

2. Name, address and occupation of the head of the family.

3. Number of persons in the family. Total
   (*a*) Sufferers
   (*b*) Non-sufferers.

   Ages { (*a*) / (*b*)

   Sex { (*a*) / (*b*)

4. Dietetic habits of the family :—
   Vegetarian or Non-vegetarian
   Intake of fruits or raw vegetables

5. Names, ages and relationship with the head of the family, of sufferers and the duration of the disease:—

Name	Sex	Age	Relationship	Duration	Lapse of time after first case in the family

6. Names, ages and relationship with the head of the family, of persons with previous attacks of the disease :—

Name	Age	Sex	Date of last attack	Present condition

7. Visitors during last six months :—

Date of visit	Age	Sex	Beri-Beri or Epidemic Dropsy case Yes or No	Beri-Beri or Epidemic Dropsy case contact Yes or No	Present condition if known

8. Number of rooms occupied by the family :—

Are the rooms particularly damp, Yes or No.

9. Any two or more sufferers living in the same room, give names if yes :—

Names	Were they sharing the same bed Yes or No	Serial Number of their attack within the family

10. Any history of sore throat and/or diarrhœa prior to or after the attack amongst the members of the family including the patient. Give details :—

Names	Sore throat Yes or No	Diarrhœa Yes or No	Duration prior to attack	Present condition

11. Any particular history of gastric trouble or colic prior to or during the attack. Give details :—

12. Sanitary condition of the house (if possible give floor space per head available and state of ventilation).

Also specify if there is water carriage system or served privy :—

13. Economic status. (Average income) per head *i.e.*, total income divided by the number of persons in the family, average feeding expenditure of the family :—

Total Income	Number of members	Per head income (monthly)	Total Expenditure on foodstuff	Remarks

14. Source of rice (sample of rice alleged to have been consumed by the family prior to and during the attack to be sent for examination) :—

Source	Quality Atap or par boiled	Hand or Machine Milled	Duration of consumption prior to attack

15. Method of rice storage adopted by the family.

Quantity of rice stored in the family at a time, particularly during the rainy season, and for how long and under what conditions :—

Quantity	How stored	How long

16. Source of mustard oil. Supply samples of mustard oil used:—

Source	Name of Brand	Machine or hand pressed	Duration of consumption prior to attack

17. Method of storage of mustard oil :—

Quantity	How stored	How long	Is it consumed raw Yes or No

18. Any other food material regularly stored by the family and had been consumed by the affected persons. Give details and forward samples if available :—

19. Are there any neighbours affected with the disease, if so give details. Do the two families visit each other frequently?

20. Any recent history of sore throat or diarrhœa amongst neighbours or in families regularly visited. Give details with dates if known.

21. Is there any history of a case in the same house prior to occupation by the family? If so, how long ago (give dates):—

22. Has any member of the family visited endemic areas or affected families. If so, give names with dates:—

23. Use of intoxicants, *e.g.*, Ganja, Charas, Wine, Opium, etc. Give details if any:—

24. Any other points of interest (Here note details of points not mentioned above, also give details of the patients' symptoms and treatment taken):—

Signature and Designation (of the person filling this form).

Date

STUDIES ON THE DEPENDENCE OF OPTICAL ACTIVITY ON CHEMICAL CONSTITUTION PART XXIII-THE ROTATORY DISPERSION OF SALTS OF CAMPHOR-β-SULPHONIC ACID WITH ANILINE, *o*-, *m*-, AND *p*-TOLUIDINE, α-, AND β-NAPHTHYLAMINE AND *ar*-TETRAHYDRO-α-NAPHTHYLAMINE*

BY

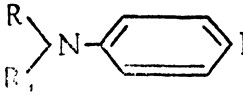
BAWA KARTAR SINGH, ONKAR NATH PERTI, AND BIRENDRA NARAIN SINGH.

Reychler's camphor-β-sulphonic acid was introduced by Pope as a powerful weapon in determining molecular asymmetry and enantiomorphism in organic bases. It is a much stronger acid than tartaric and, therefore, its salts are less likely to undergo hydrolysis. It forms only one kind of salt, being a monobasic acid. The products are generally crystalline. The acid itself is free from the risk of racemisation.

The molecular configuration of nitrogen compounds with 3-covalent nitrogen atom as in ammonia (NH_3) or an amine (Nabc), having a valency group of 8 electrons, of which 6 are shared, remained for long as one of the out-standing problems in stereochemistry.[1] These compounds (N R R′ R″) have resisted all attempts at resolution which would indicate their planar configuration. The physical properties of ammonia or amines, on the other hand, support the view that in these compounds the nitrogen atom does not lie in one plane with the three attached groups,[2] and that a tetrahedral configuration must be assigned to the 3-covalent nitrogen as in the case

* Reprinted from the Proceedings of the Lahore Philosophical Society, 1944, VI, 15.

of 4-covalent nitrogen atom.[3] Again it has been proved on stereochemical considerations based on the structure of oximes established by Mill's work, that the valencies of 3-covalent nitrogen as in ammonia or amines do not lie in one plane.[1] It, therefore, follows that under suitabe conditions the unsymmetrically substituted p-phenylenediamine derivatives of the formula,



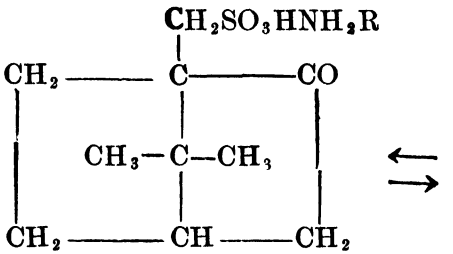
should be capable of resolution with the aid of optically active acids of the type of camphor-β-sulphonic acid.

Schreiber and Shriner[4] found, however, that the primary amine salts of Reychler's acid of the above type underwent mutarotation in non-aqueous solvents which may be due to the formation of diastereoisomerides of the two forms of the amine,

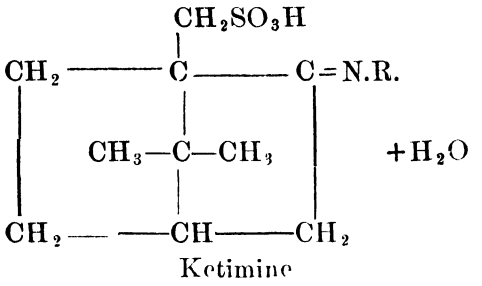
$$dl\text{B} + 2\ \ d\text{-A} \rightleftarrows d\text{A}d\text{B},\ d\text{A}\ l\text{B}$$

with the *d*-camphor-β-sulphonic acid, indicating that resolution has taken place. On liberation of the free bases from the solution of these salts, they were, however, found to be optically inactive.

Since mutarotation was also exhibited by analogous salts from symmetric primary bases, it became clear that an asymmetric nitrogen atom was not involved in this chemical change. On the other hand, mutarotation resulted from a structural change in the salt into the ketimine or anil of the following type:



Salt



Ketimine

Serious difficulties may thus arise in interpreting the results of experiments in which camphor-β-sulphonic acid is used in resolving primary racemic amines. We have, therefore, undertaken a study of the primary amine salts of this acid as well as its laevo and racemic forms. The bases used are aniline, *o*-,*m*-, *p*-toluidines, α-, and β-naphthylamines and *ar*-tetrahydro-α-naphthylamine. In each case we have found that the salts exhibit mutarotation in organic solvents, but not in water. The mutarotation arises from the anil formation.

Our studies are also made with the object of determining the influence of position isomerism, the nature of the solvent, and the wave-length of the light on rotatory power.

The Effect of Position Isomerism on Rotatory Power

The sequence of the rotatory power of the position isomerides of the aniline and toluidine salts of camphor-β-sulphonic acid (Table 3) is m<o<p<un, in all the five solvents. This is neither in agreement with Frankland's "lever arm" hypothesis,[5] nor with the electrostatic modification suggested by Rule,[6] as according to both of these rules, the meta isomer should always be intermediate between the *ortho* and *para*. The rotatory power of α-naphthylamine salts is less than that of the β-isomerides in all the solvents except water where the reverse obtains. In the case of the *ar*-tetrahydro-α-naphthylamine salt, its rotatory power is greater than that of the salt from the

unreduced base in ethyl alcohol and pyridine, but less in methyl alcohol and water.

The above mentioned deductions are made from comparison of rotatory power for a single wave-length, namely, Hg 5461. The study of dispersion of organic compounds, however, enables us to overcome this limitation, especially in the case of those compounds which exhibit 'simple dispersion' and obey the one-term equation of Drude, $[\alpha]=\frac{K}{\lambda^2-\lambda_0^2}$. In this equation, K is the rotation constant for that value of λ, for which $\lambda^2-\lambda_0^2=1$ square micron, always in the infra-red region beyond 10,000 A.U. It may be taken as a measure of the rotatory power and is termed the absolute rotation of the compound, and is independent of the wave-length. These values of the rotation constant (K) are given in brackets in Table 3 and are found to lead to similar conclusions except for the following minor differences: in pyridine the order of the increasing rotatory power of the position isomerides is un<m<p<o, and in chloroform o<m<p<un.

Effect of Solvent on Rotatory Power.

The order of decreasing rotatory power of the compounds given in Table 3 in different solvent is chloroform> pyridine>ethyl alcohol>methyl alcohol>water and runs parallel in the reverse sense with the sequence of the dielctric constants of these solvents.

Physical Identity of Enantiomers

The values of rotatory power of the *d*—,and *l*—forms in different solvents (Tables 4 to 11) are identical within the limits of experimental error. Out of about 742 observations recorded in this paper, in as many as 270 cases, difference in the numerical value of the specific rotatory

power of the opposite and active forms corresponds to a difference of 0·01° or less in the observed angle of rotation, and in 390 cases, the corresponding angle lies between 0·01° and 0·02°, which is the limit of experimental error allowable in such measurements. Only in 82 cases the difference corresponds to between 0·02° and 0·04° in the observed angle of rotation. All these differences are of the nature of casual experimental errors. These

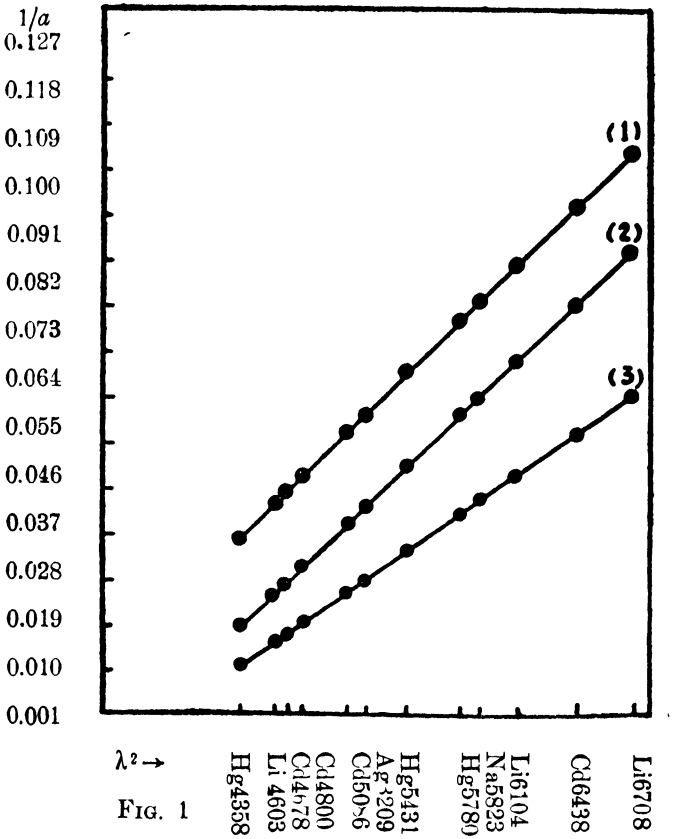


FIG. 1

(1) *ar*-TETRAHYDRO-α-NAPHTHYLAMINE-*d*-CAMPHOR-β-SULPHONATE IN WATER

(2) ANILINE-*d*-CHAMPHOR-β-SULPHONATE IN WATER

(3) β-NAPHTHYLAMINE-*d*-CAMPHOR-β-SULPHONATE IN METHYL ALCOHOL

results further support Pasteur's principle of molecular dissymmetry regarding the identity of rotatory power of the active and opposite isomers.[7] In the tables of rotatory dispersion (Tables 4 to 11) the differences (o − c) between the observed values of the rotatory power and those calculated from the simple dispersion formula of Drude, $[\alpha] = \frac{K}{\lambda^2 - \lambda_0^2}$ are omitted for the sake of economy of space, but in all cases the simple one-term formula fits in with the numerical data. A less rigid test than the above mentioned stringent numerical verification consists in plotting $\frac{1}{\alpha}$ against λ^2 when a straight line is obtained in each case. A few such illustrative typical curves are given in Fig. 1.

Nature of the Racemic Modification

In addition to the enantiomorphous salts studied in this paper, their racemic modifications have been investigated in an earlier communication.[8] The racemic form may be (*i*) a mixture of the *d* −, and *l* − form in equimolecular proportions, or (*ii*) a compound of these two forms, or (*iii*) a solid solution of the dextro and lævo forms due to the enantiomorphs being isomorphous, each crystal containing both the forms. The last case differs from the first in constituting a single phase, unlike a mixture. By applying Roozeboom's Freezing—Point method,[9] it was settled that the racemic modifications of all these salts were *dl* − compounds. These results derived from physical considerations are supported by a biochemical study of these compounds, the results of which will be published shortly.

The Molecular Rotatory Dispersion of the Salts of Camphor-β-Sulphonic Acid in Aqueous Solution, and the Value of Molecular Rotatory Power of Camphor-β-sulphonate Ion in Water deduced therefrom: The following

Table 1 gives the values of $[M]_\lambda$ for λ varying from 4358 A. U. to 6708 A. U. for the different camphor-β-sulphonates. If the salts were completely ionised in dilute aqueous solution, their molecular rotations would be practically the same for a given wave-length, the rotations being independent of the inactive basic ion : in concentrated solutions, the observed molecular rotation may be greater or less than the value in dilute solution, because it is due both to the active ion and to the non-ionised molecule. Our results given in Table 1 divide the salts into two groups : those derived from sodium hydroxide, aniline, *o*-,*m*-, and *p*-toluidine and β-naphthylamine give values of $[M]_D$ ranging from 48° to 54°, whereas the salts derived from α-naphthylamine and *ar*-tetrahydro-α-naphthylamine give higher or lower value than the mean value of $[M]_D = 52°$ for the acid ion.[10] It, therefore, follows that the former group of salts are fairly completely electrolytically dissociated even at a concentration of 4%, whereas the latter are not so even in 1% solution. This is undoubtedly due to the lower solubility in water of the latter group of salts. Graham[11] has recorded the molecular rotations of several metallic salts of camphor-β-sulphonic acid, but he has arrived at a different conclusion, namely, the rotation is not determined by the electrolytic dissociation alone but principally by the nature of the metallic atom. It is possible, just as in our case, that the results of Graham may be due to the different degrees of dissociation of his salts at equal concentrations but for a strict comparison a knowledge of the degree of ionisation of salts with dilution will be necessary. It will be, therefore, fair to compare the values of rotations of the salts for concentrations at which they are equally ionised.

TABLE 1.

Camphor-β-Sulphonates of:	Concentration in gms./100c.c.	Temperature	$[M]^t_\lambda$ in aqueous solution											
			Hg_{4358}	Li_{4603}	Cd_{4678}	Cd_{4800}	Cd_{5086}	Ag_{5209}	Hg_{5461}	Hg_{5780}	Na_{5893}	Li_{6104}	Cd_{6438}	Li_{6708}
1. Sodium ...	4 0056	33-34°C.	—	140 0	123·7	110 5	82·79	76·19	64 00	51·71	48 26	41·91	36·07	32·00
2. Aniline ...	4·0056	26°C	187·2	135·7	127 5	110 5	85·31	78·00	66 64	55·64	52 38	43 87	38·59	34·13
3. *o*-Toluidine ...	4 0032	27-28°C	181·8	132·9	124·7	109 1	87·70	77·84	67 36	55·54	50·86	46·44	40 24	34 31
4. *m*-Toluidine ...	4 0040	29-30°C	173·9	130·3	118 6	107·1	84·55	76·61	63 74	54 24	49 83	44·75	37·96	33 90
5. *p*-Toluidine ...	4 0040	29°C.	188·8	140 3	127 2	113·9	88 82	79·68	68 48	56·75	52 88	48 14	40 93	35·59
6. α-Naphthylamine	1 0008	35°C.	187·4	151 7	142·3	133 0	108·6	99 24	86·16	74·92	69·24	61·80	54 29	48 67
7. β-Naphthylamine	1 0000	35°C	178 1	138 8	127·5	—	93 74	84 37	71·25	59 99	54 96	50·62	45 00	39·38
8. *ar*-tetrahydro-α-Naphthylamine	1·0000	35°C.	108·0	90 95	85 27	81 47	68 22	64·42	56·84	49 26	4 547	43 58	37 90	34·10

EXPERIMENTAL

The *l*-,and *dl*-camphor-β-sulphonic acid were prepared in the same way as Reychler's acid[12] (*d*-camphor-β-sulphonic acid).

d-acid : found M.P. 197-8°C; eq. wt.=230·0;
l-acid : found M.P. 197-8°C; eq wt. = 231·8;
dl-acid : found M.P. 202-3°C; eq. wt.=235·0;
$C_{10}H_{15}O\ SO_3H$ requires eq. wt. =232·0;

Sodium-Camphor-β-sulphonates. A weighed amount *d*-, *l*-, or *dl*-camphor-β-sulphonic acid was dissolved in water and was exactly neutralised by an equivalent amount of sodium hydroxide solution. The solution was then evaporated to dryness on a water bath and finally in the air oven. The *d*-, *l*-, and *dl*-salts have the same crystalline form. They are extremely soluble in water but sparingly so in organic solvents.

Found: *d*-salt, Na=9·21%; *l*-salt, Na=8·96%;
dl-salt, Na=9·18%; $C_{10}H_{15}O\ SO_3Na$
requires Na=9·06%.

The amine salts of camphor-β-sulphonic acids. Molar amounts of each of the amines and the camphor-β-sulphonic acids were dissolved separately in hot ethyl acetate. The two solutions were then mixed and allowed to stand for a few hours when the salts crystallised out. They were then repeatedly recrystallised by solution in absolute alcohol and addition of an equal volume of ethyl acetate. They were dried at 80° in an air oven and then in vacuum. The camphor-β-sulphonates of aniline, *o*-, *m*-, *p*-toluidines, α-, and β-naphthylamines and *ar*-tetrahydro-α-naphthylamine are long colourless needles. The aniline, *o*-, *m*-, and *p*-toluidine salts are freely soluble in ethyl alcohol, methyl alcohol, chloroform, pyridine and water; sparingly so in

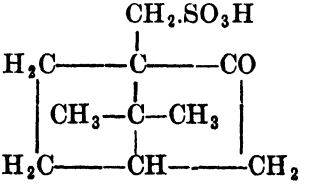
acetone and benzene; and insoluble in ether and ethyl acetate. α-Naphthylamine, β-naphthylamine and *ar*-tetrahydro-α-naphthylamine camphor-β-sulphonates are freely soluble in pyridine, less so in water, ethyl alcohol and methyl alcohol; sparingly so in acetone, benzene and chloroform, and insoluble in ether and ethyl acetate.

The rotatory power determinations were made in a 2-dcm. tube. The value of λ_0 calculated from the dispersion formula is given in the Tables (4 to 11) and is expressed as μ or 10^{-4} cm. The temperature varied between 26° to 36°C. when the determinations of rotatory power were made in the case of optically active camphor-β-sulphonates of aniline, *o*-, *m*-, and *p*-toluidines but the temperature was maintained constant at 35°C when the determinations for rotatory power were made in the case of camphor-β-sulphonates of α- and β-naphthylamine and *ar*-terahydro-α-naphthylamine.

TABLE 2

Salts of *d*-,*l*- and *dl*-camphor-β-sulphonic acid with:		FOUND			CALCULATED	
		M P.	*Eq. Wt*	*Sulphur* (%)	*Eq. Wt*	*Sulphur* %
Aniline	*d*-salt ...	183·5°	323·5	9·84		
*($R.H_2N.C_6H_5$)	*l*-salt ...	183·5°	328·3	9·78	325·0	9·85
	dl-salt ...	170·0°	326·0	9·70		
o-Toluidine	*d*-salt ...	149·0°	340·1	9·21		
($R.H_2N\ C_6H_4.CH_3$)	*l*-salt	149·0°	335·2	9·31	339·0	9·44
	dl-salt ...	141·0°	338·4	9·44		
m-Toluidine	*d*-salt . .	149·0°	338 4	9·69		
($R.H_2NC_6H_4CH_3$)	*l*-salt	149·0°	341·1	9·59	339·0	9·44
	dl-salt ...	145·0°	336·0	9·85		
p-Toluidine	*d*-salt ...	170·0°	338·4	9·12		
($R.H_2N.C_6H_4\ CH_3$)	*l*-salt	170 0°	335·9	9·50	339·0	9·44
	dl-salt . .	155·0°	341 0	9·61		
α-Naphthylamine	*d*-salt ...	173 0°	375·4	8·52		
($R.H_2N.C_{10}H_7$)	*l*-salt ...	173·0°	377·5	8·59	375·0	8·53
	dl-salt ...	165 0°	377·3	8 19		
β-Naphthylamine	*d*-salt ...	165 5°	381·9	8·60		
($R.H_2N.C_{10}H_7$)	*l*-salt ...	165 5°	377·3	8·51	375·0	8·53
	dl-salt ...	183·5°	381·6	8·59		
ar-Tetrahydro-α-naphthylamine	*d*-salt ...	163·0°	381·3	8·51		
	l-salt ...	163·0°	381·9	8·88	379 0	8·46
($R.H_2N.C_{10}H_{11}$)	*dl*-salt ...	163 0°	383·1	8·09		

*R=$C_{10}H_{15}O.SO_3H$ or



We wish to make a grateful acknowledgement to the Univerity of Allahabad for the award of research acholarships to two of us (O.N.P. and B.N.S.), which has enabled them to take part in this investigation, and for providing research facilities.*

SUMMARY

The salts of camphor-β-sulphnic acid (*d*-, *l*-, and *dl*-) with organic bases have been prepared; and their rotatory dispersion determined. The effect of position isomerism, solvent and wavelength on rotatory power has been discussed. The application of Oudemans' law[13] regarding the rotatory power of the salts of the optically active acids with inactive bases has resulted in the division of the salts, described in this paper, into two groups, namely, those which are fairly completely dissociated and those which are not, at the given concentrations.

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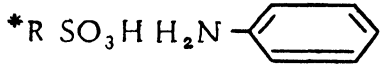
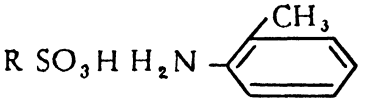
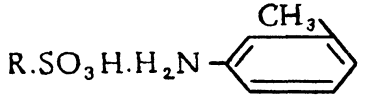
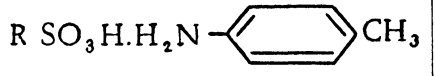
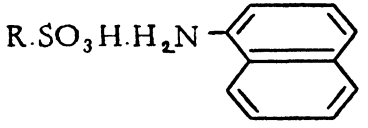
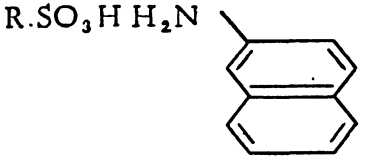
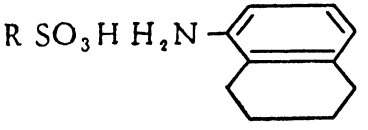
* Addendum: The experimental work on compounds (Table I) numbered 1, 6, 7 and 8 was carried out by O.N. Perti and on those numbered 1 to 5 by B. N. Singh [B.K.S.].

TABLE 3

Structural formula	Solvent				
	Water	Methyl alcohol	Ethyl alcohol	Pyridine	Chloroform
	20 50	32·40	36·40	36·10	45·30
	(3·400)	(6 284)	(6·997)	(7·57)	(9·276)
	19·87	29·60	34·74	36 20	43·45
	(3·367)	(5 685)	(6 88)	(7·93)	(8 81)
	18 80	28·90	34·10	35·50	42 70
	(3·31)	(5·661)	(6·79)	(7·74)	(8 91)
	20·20	30·47	35·50	37·60	44·10
	(3 39)	(5 96)	(6 95)	(7·81)	(9·12)
	22·98	28·00	30 00	33·97	
	(4 384)	(4 719)	(4 952)	(5·526)	
	19·00	31 42	34·50	35 98	
	(3·416)	(4·907)	(5·126)	(5·510)	
	15·00	24 97	36·47	39 00	
	(3 320)	(3·75)	(5·45)	(5 505)	

*R. = $C_{10}H_{15}O$, or

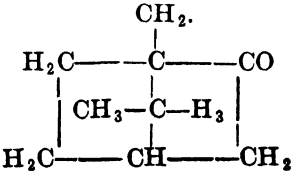


TABLE 4—Sodium-Camphor-β-Sulphonates in water

$$[\alpha] = \pm \frac{3\,933}{\lambda^2 - 0{\cdot}1401} \; ; \; \lambda_0 = 0\,3743$$

Temperature 33-34°C.

d-salt				*l*-salt	
Concentration in gms./100 c.c.	Observed [α]	Line	Calculated [α]	Observed [α]	Concentration in gms./100 c.c.
4·0056	+55·10°	Li4603	54·79°	−54·70°	4·0028
	48·70	Cd4678	49·92	49·20	
	43·50	Cd4800	43·56	—	
	32 60	Cd5086	33·16	32·24	
	30·60	Ag5209	29 98	30·12	
	25 00	Hg5461	24 88	25·50	
	20·20	Hg5780	20·28	20·12	
	19 36	Na5893	18 99	19·25	
	16·00	Li6104	16 92	17·10	
	14·20	Cd6438	14·33	14 50	
	12·60	Li6708	12 69	13 00	

TABLE 5—Aniline Camphor-β-Sulphonates

Solvent	Water		Methyl alcohol		Ethyl alcohol		Pyridine		Chloroform	
Concentration in gms/100 c.c. *d*-	4·0056		4·0024		4·0032		4·0036		4·0048	
Concentration in gms/100 c.c. *l*-	4·0044		4·0008		4·0028		4·0048		4·0052	
Calculated [α] =	$\pm\frac{3\cdot400}{\lambda^2-0\cdot1300}$		$\pm\frac{6\cdot284}{\lambda^2-0\cdot1045}$		$\pm\frac{0\cdot997}{\lambda^2-0\cdot1065}$		$\pm\frac{7\cdot57}{\lambda^2-0\cdot1000}$		$\pm\frac{9\cdot276}{\lambda^2-0\cdot09412}$	
λ_0	0·3606		0·3233		0·3263		0·3162		0·3068	
Line	*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]	
	d-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-
Hg4358	+57·60°	−57·10°	+73·60°	−74·20°	+83·37°	−84·10°	—	—	—	—
Li4603	41·75	42·20	—	—	66·60	66·20	—	—	+79·20°	−78·50°
Cd4678	39·20	38·90	54·08	55·60	62·50	62·00	+63·34°	−64·12°	75·00	74·12
Cd4800	34·00	34·25	50·00	50·20	56·00	56·20	57·60	58·10	68·50	68·25
Cd5086	26·25	26·50	40·80	41·20	45·87	46·20	47·60	47·90	55·70	56·10
Ag5209	24·00	24·25	37·60	38·10	42·50	42·00	44·60	44·10	52·10	52·50
Hg5461	20·50	20·25	32·40	32·70	36·40	36·60	38·10	38·32	45·30	45·62
Hg5780	17·12	17·00	27·35	27·10	31·10	30·80	32·48	32·72	38·40	38·75
Na5893	16·12	15·80	26·20	25·87	29·12	28·80	30·73	30·96	36·10	37·10
Li6104	13·50	14·10	23·46	23·60	26·00	26·20	27·61	27·95	32·70	32·37
Cd6438	11·87	12·25	20·10	20·60	22·87	22·60	24·36	24·00	29·45	28·75
Li6708	10·50	10·87	17·74	18·10	20·50	20·10	21·20	21·70	25·70	26·20
	No mutarotation.		*Shows mutarotation. Rotation for Hg5461 after 12 hours for:* *d*-, [α] = +23·00° *l*-, [α] = −23·20°		*Shows mutarotation. Rotation for Hg5401 after 12 hours for:* *d*-, [α] = +26·00° *l*-, [α] = −26·30°		*Solution turned dark after 12 hours.*		*Shows mutarotation. Rotation for Hg5461 after 12 hours for* *d*-, [α] = +29·40° *l*-, [α] = −29·50°	

TABLE 6.—*o*-Toluidine-Camphor-β-Sulphonates

Solvent	Water	Methyl alcohol	Ethyl alohol	Pyridine	Chloroform
Concentration in gms./100 c.c. *d*-	4·0032	4·0016	4·0060	4·0008	4·0024
Concentration in gms./100 c.c. *l*-	4·0048	4·0040	4·0044	4·0028	4·0044
Calculated $\frac{[\alpha]^{\lambda}_{\lambda_0}}{\lambda_0}$	$\pm\frac{3.367}{\lambda^2-0\,1269}$ 0·3562	$\pm\frac{5·685}{\lambda^2-0\,1039}$ 0·3223	$\pm\frac{6\,88}{\lambda^2-0\,1009}$ 0·3176	$\pm\frac{7\,93}{\lambda^2-0\,0780}$ 0·2793	$\pm\frac{8\,81}{\lambda^2-0\,0950}$ 0·3082
Line	*Obs.* [α]	*Obs.* [α]	*Obs.* [α]	*Obs.* [α]	*Obs* [α]
	d- *l*-	*d*- *l*-	*d*- *l*-	*d*- *l*-	*d*- *l*-
Hg4358	+53 60° —53 00°	+65 50° —66·10°	+77·30° —76 75°	— —	+93 10° —92·60°
Li4603	39·20 39·95	— —	61·60 62 10	— —	75 60 75·10
Cd4678	36·80 36 30	49 20 49 60	57 86 58·40	+56 60° —56 00°	70 80 71·30
Cd4800	32 20 32·60	45·20 44·70	53·20 52 80	51·80 52·20	65·10 64 70
Cd5086	25·87 25 50	37·00 36 60	43 60 43 20	43 70 43·45	53·70 53·40
Ag5209	22·96 23·30	33 70 34·10	40 20 40·60	40 70 41·10	50 00 49 50
Hg5461	19·87 20·00	29 60 29 38	34 74 35·00	36 20 35·80	43 45 43·20
Hg5780	16·38 16·10	24 60 24·80	29 96 29·20	30 94 31·10	36·45 36·80
Na5893	15·00 15·37	23 60 23·10	28 10 27·75	29 60 29 20	35·10 34 70
Li6104	13 70 13 35	21 50 21·10	25·10 25 50	26·60 27 00	32 00 31 60
Cd6438	11.87 11·36	18·00 18·47	21.60 22·10	23 70 23·30	27 60 27·90
Li6708	10 12 10 48	16·87 16·37	19 43 19 80	21 45 21·00	24·60 24 85
	No mutarotation.	*Shows mutarotation Rotation for Hg5461 after 12 hours for*: *d*-, [*a*] = +22 50° *l*-, [*a*] = —22 70°	*Shows mutavotation. Rotation for Hg5461 after 12 hours for:* *d*-, [*a*] = +26·00° *l*-, [*a*] = —26·20°	*Solution turned dark after 12 hours.*	*Shows mutarotation. Rotation for Hg5461 after 12 hours for:* *d*-, [*a*] = +24·20° *l*-, [*a*] = —24 00°

TABLE 7—*m*-Toluidine-Camphor-β-Sulphonates

Solvent	Water	Methyl alcohol	Ethyl alcohol	Pyridine	Chloroform
Concentration in gms./100 c. c. *d*-	4·0040	4 0044	4.0016	4 0024	4 0008
l-	4 0060	4.0032	4 0028	4 0044	4 0032
Calculated $\frac{[\alpha]}{\lambda_0}$	$\pm\frac{3\,31}{\lambda^2-0\cdot1250}$ 0·3536	$+\frac{5\,661}{\lambda_2-0\,1006}$ 0.3172	$\pm\frac{6\,79}{\lambda^2-0\,1061}$ 0 3164	$\pm\frac{7\,74}{\lambda^2-0\,0824}$ 0.2871	$\pm\frac{8.91}{\lambda^2-0\,0906}$ 0 3010
Line	*Obs.* [α]	*Obs.* [α]	*Obs.* [α]	*Obs.* [α]	*Obs* [α]
	d- *l*-	*d*- *l*-	*d*- *l*-	*d*- *l*-	*d*- *l*-
Hg4358	+51·30°—50·80°	+61 87°—62 37°	+75 10°—75·80°	— —	+89 90°—89·40°
Li 4609	38 45 37·80	50 70 51·20	60·60 60 90	— —	73 20 73 70
Cd4678	34·94 35 40	48 20 47 87	57.30 56 70	+56 40°—57 00°	69 20 69·80
Cd 4800	31·60 32 00	43·80 43 48	52.20 51 80	52 50 52·70	63·35 62 90
Cd 5086	25 00 24 60	35·79 36·00	42 60 42 90	44 10 43·75	53·10 52·70
Ag5209	22 60 22 86	33·00 33 37	39 30 39·70	41·10 40 70	49 50 49 10
Hg5461	18 80 19·10	28 90 28 50	34.10 34·30	35 50 35·70	42 70 42·90
Hg5780	16·00 15 60	24·12 24·30	28 70 28 90	30 85 30 62	36 20 36 62
Na5893	14·70 15·10	23·20 22 87	27·10 27 50	28 87 29 10	35 00 35·50
Li 6104	13 20 13 60	21·00 20 74	25·10 24·70	26·87 26·40	31·35 31 70
Cd6438	11·03 11·60	18 12 17·87	21·70 21 30	23 10 23 50	26·90 27·20
Li 6708	10·00 10·40	16 38 16 00	19 60 19 20	20 70 21·10	24 50 24·70
	No mutarotation.	*Shows mutarotation. Rotation for Hg5461 after 12 hours for:* *d*-, [α] = +22 00° *l*-, [α] = −22 20°	*Shows mutarotation. Rotation for Hg5461 after 12 hours for:* *d*-, [α] = +24 70° *l*-, [α] = −24.50°	*Solution turned dark after 12 hours.*	*Shows mutarotation. Rotation for Hg5461 after 12 hours for:* *d*-, [α] = +19 60° *l*-, [α] = −19 80°

TABLE 8—*p*-Toluidine-Camphor-β-Sulphohates

Solvent	Water		Methl alcohol		Ethyl alcohol		Pyridine		Chloroform	
d- concentration in gms/100 c.c.	4·0040		4 0012		4·0040		4·0016		4 0044	
l-	4 0032		4·0032		4·0032		4 0028		4·0060	
Calculated $\frac{[\alpha]}{\lambda_0}$	$\pm\frac{3\cdot39}{\lambda^2-0\cdot1294}$ 0·3597		$\pm\frac{5\,96}{\lambda^2-0\cdot1038}$ 0 3222		$\pm\frac{6\,95}{\lambda^2-0\cdot1028}$ 0 3206		$\pm\frac{7\,81}{\lambda^2-0\,0913}$ 0·3022		$\pm\frac{9\cdot12}{\lambda^2-0\,0932}$ 0 3053	
Line	*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Osb.* [α]		*Obs* [α]	
	d-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-
Hg4358	+55·70°	−56·40°	+68·88°	−69·48°	+79 30°	−79 86°	—	—	+94 00°	−94·50°
Li4603	41·37	41 00	—	—	64·00	64 30	—	—	76·50	77·10
Cd4678	37·50	38·10	51.46	52 00	59 30	59·60	+60·60°	−61·30°	72·50	72 00
Cd4800	33·60	33·37	47·23	46·86	54·48	54·70	56·30	55·80	66·60	66·80
Cd5086	26·20	26 00	38·37	38 74	44·70	44 50	46·80	46·70	55·00	55·30
Ag5209	23 50	24·00	35·10	35·37	41·00	41·50	43·10	43 50	51·30	51·60
Hg5461	20 20	20·12	—	—	35 50	35 70	37·60	37·87	44·10	44·40
Hg5780	16·74	16·50	25 48	25 75	29·70	29 85	31·80	32·20	37·70	37·90
Na5893	15 60	15·75	24·12	24 34	28·50	28 20	30·60	30·38	35·60	36 00
Li6104	14·20	13 78	22·36	22·00	25.20	25 50	28·00	27·60	32 80	32·30
Cd6438	12·10	11 74	19·37	19·12	22 20	21·86	23·70	24·00	28·50	28·10
Li6708	10 50	10·87	17·00	17·35	20 00	20·20	21·30	21·86	25·10	25 36
	No mutarotation.		*Shows mutarotation Rotation for Hg5461 after 12 hours for*: *d*-, [α] = +24 40° *l*-, [α] = −24·60°		*Shows mutarotation. Rotation for Hg5461 after 12 hours for*: *d*-, [α] = +26 50° *l*-, [α] = −26 70°		*Solution turned dark after 12 hours.*		*Shows mutarotation. Rotation for Hg5461 after 12 hours for*: *d*-, [α] = +21·00° *l*-, [α] = −20·70°	

TABLE 9—α-Naphtylamine-Camphor-β-Sulphonates

Solvent		Water		Methyl alcohol		Ethyl alcohol		Pyridine	
Coneentration in gms. /100 c.c.	*d*-	1 0008		1·0000		1·0000		1 0008	
	l-	1·0000		1·0008		1 0024		1 0016	
Calculated	$\frac{[\alpha]}{\lambda_0}$	$\pm\frac{4\,384}{\lambda^2-0{\cdot}1043}+0{\cdot}3230$		$\pm\frac{4\,719}{\lambda^2-0{\cdot}1300}+0{\cdot}3606$		$\pm\frac{4{\cdot}952}{\lambda^2-0{\cdot}1363}+0{\cdot}3692$		$\pm\frac{5\,526}{\lambda^2-0{\cdot}1370}+0{\cdot}3701$	
Line		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]	
		d-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-
Hg4358		+49·96°	−49·50°	—	—	+98 50°	−98 76°	—	—
Li4603		40 46	40·00	+58·50°	−57·95°	66·50	66·33	+74·94°	−74 88°
Cd4678		37·96	38·00	52·50	52·46	59·00	58 81	66 44	66 39
Cd4800		35·47	35·50	48 00	47·96	52·50	52·37	60·45	61·40
Cd5086		28·97	28·50	37·50	37·97	40·50	40 39	44·96	44·92
Ag5209		26·47	26·00	33 50	33·97	37·00	36 86	40 96	40 93
Hg5461		22·98	23 00	28·00	27 97	30·00	30·42	33·97	33·94
Hg5780		19·98	20·00	23·50	22 97	25·50	25·44	27·97	27·95
Na5893		18·47	18·50	21 50	20 97	23·50	23·44	26·47	26·45
Li6104		16·48	16 00	19·00	18·97	21 00	20·94	23·47	22 96
Cd6438		14·48	14·50	16 50	16·98	17·50	17·46	19·97	19 92
Li6708		12·98	12·50	14·50	13·98	15·50	15·46	17·48	17·47

TABLE 10—β-Naphthylamine-Camphor-β-Sulphonates

Solvent		Water		Methyl alcohol		Ethyl alcohol		Pyridine	
Concentration in gms. /100 c.c.	*d*-	1 0000		1 0024		1 0000		1 0008	
	l-	1·0008		1·0016		1·0008		1 0016	
Calculated	$\frac{[\alpha]}{\lambda_0}$	$\pm\frac{3\,416}{\lambda^2-0\,1195}$ 0 3457		$\pm\frac{4\,907}{\lambda^2-0\,1416}$ 0 3763		$\pm\frac{5{\cdot}126}{\lambda^2-0{\cdot}1463}$ 0 3825		$\pm\frac{5\,510}{\lambda^2-0{\cdot}1439}$ 0 3793	
Line		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]	
		d-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-
Hg4358		+47 50°	—46 96°	+100·2°	—101·3°	+117 5°	—117·8°	—	—
Li 4603		37 00	36 97	68 83	69 36	76·00	75 43	+80 92°	—80 87°
Cd4678		34·00	33 98	62·35	61·92	69 00	—	72·97	72 88
Cd4800		—	—	55 31	55 42	62 00	62 44	63 94	63 92
Cd5086		25 00	24 48	41·89	41 93	47·00	46 96	47·46	47 92
Ag5209		22 50	22·98	37 86	37 94	41·50	41·46	43 46	43 43
Hg5461		19 00	19 48	31 42	31 48	34·50	34·48	35·98	36·44
Hg5780		16·00	15 99	25 94	25·95	27 00	26 98	29 98	30·49
Na5893		15 00	14 99	23 94	23·96	26 00	25·98	26 98	26 95
Li 6104		13·50	13 99	21 44	21 46	23 50	23·48	23 98	24·46
Cd6438		12·00	11·49	17·96	17 97	20·00	20 48	20·48	20·97
Li 6708		10·50	9·99	15 96	15·46	17 50	16 99	17 99	17·97

TABLE 11—*ar*-Tetrahydro-α-Naphthylamine-Camphor-β-Sulphonates

Solvent	Water		Methyl alcohol		Ethyl alcohol		Pyridine	
Concentration in gms. per 100 c.c. *d*-	1·0000		1·0003		1·0008		1·0000	
Concentration in gms. per 100 c.c. *l*-	1·0016		1·0000		1·0016		1·0024	
Calculated $[\alpha]$	$\pm\frac{3{\cdot}320}{\lambda^2-0{\cdot}07616}$		$\pm\frac{3{\cdot}75}{\lambda^2-0{\cdot}1483}$		$\pm\frac{5{\cdot}45}{\lambda^2-0{\cdot}1514}$		$\pm\frac{5{\cdot}505}{\lambda^2-0{\cdot}1562}$	
Calculated λ_0	0·2760		0·3851		0·3891		0·3952	
Line	*Obs.* [α]		*Obs.* [α]		*Obs.* [α]		*Obs.* [α]	
	d-	*l*-	*d*-	*l*-	*d*-	*l*-	*d*-	*l*-
Hg4358	+28·50	−28·45°	+90·44°	−90·50°	—	—	—	—
Li 4603	24·90	23·95	—	—	+90·35°	−90·35°	—	—
Cd4678	22·50	22·96	52·45	52·50	—	—	+88·50°	−88·78°
Cd4800	21·50	20·96	45·46	45·50	68·94	68·88	75·00	74·82
Cd5086	18·00	17·97	32·47	32·50	49·95	49·91	53·50	53·87
Ag5209	17·00	16·97	30·97	30·50	45·46	45·42	47·50	47·39
Hg5461	15·00	14·97	24·97	25·00	36·47	36·44	39·00	38·89
Hg5780	13·09	12·97	20·48	20·50	29·48	29·45	30·50	30·92
Na5893	12·00	12·47	18·48	19·00	27·98	27·45	28·50	28·92
Li6104	11·50	10·98	16·98	16·50	23·98	23·46	25·50	25·44
Cd6438	10·00	9·98	13·98	14·00	19·98	20·46	21·00	20·94
Li 6708	9·00	8·98	12·48	12·50	17·99	17·47	18·50	18·95
	No mutarotation.		*Shows mutarotation Rotation for Hg5461 for d-, [α] = +22·48 l-, [α] = −22·50 after 24 hours.*		*Shows mutarotation. Rotation for Hg5461 for: d-, [α] = +32·97 l-, [α] = −32·95 after 48 hours.*		*Solution turns dark after 24 hours*	

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