THE EFFECT OF CHLOROFORM ON THE INTERMEDIATE PROTEIN METABOLISM OF THE DOG

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The administration of chloroform has a very marked effect on the chemical changes in the body. It causes a considerable acceleration in the protein metabolism, as evidenced by the increased excretion of nitrogen. It has a marked action upon the liver cells outside the body,¹ and after its administration by various channels to the living animal, Dr. Clark has shown that the liver cells show histologically very great degenerative changes.² Dr. Noël Paton³ carried out a series of experiments to determine the effects of chloroform on the metabolism with especial consideration of the manner in which the power of the liver to elaborate urea was affected. In both these papers the literature of the subject is very fully considered.

The immediate result found was a considerable fall in the proportion of nitrogen in the form of urea, which was followed some days later by a rise in the proportion of nitrogen occurring as ammonia. The proportion of undetermined nitrogen rose with the fall in urea and previously to the rise in ammonia.

From the methods employed by Noël Paton⁴ for the determination of the urea nitrogen it appeared probable that some of the undetermined nitrogen might be due to the appearance of amino acids in the urine, possibly along with some other intermediate products of protein metabolism. Since the nature of such intermediate products is a question of considerable interest as throwing light on the course of catabolism, the present detailed examination of the distribution of nitrogen in the urine of dogs after the administration of chloroform was undertaken.

1. Noël Paton, *Phil. Trans. Roy. Soc.*, Vol. CLXXXV, p. 248.

2. *Proc. Roy. Soc. Edin.*, Vol. XXVIII, 1908.

3. *Proc. Roy. Soc. Edin.*, Vol. XXVIII, 1908.

4. *Loc. cit.*

After my work was nearly completed, Howland and Richards¹ published the results of investigations on the metabolic changes in the case of three dogs, two of which died of delayed chloroform poisoning. They observed the same fall in urea nitrogen and increase in ammonia and undetermined nitrogen. Where delayed poisoning was induced the changes were the same in character, but more marked than when the animals made a normal recovery.

In the present experiments the chloroform was administered by Professor D. Noël Paton, and the conditions were kept exactly similar to those of his previous investigation. The animals were fed on porridge and milk, and on the day of administration food was usually withheld till after the dose had been given. The urine was collected in the metabolic cages used in this laboratory.

If given hypodermically two doses of 5 c.c. were administered on two successive days. If given by the mouth the dose was 25 c.c. chloroform in oil. In most of the experiments the chloroform was injected hypodermically, as, while the effect on the metabolic changes was the same as when given by the mouth, digestive disturbances were less likely to occur.

METHODS OF ANALYSES

The *total nitrogen* was determined by Kjeldahl's method.

The *urea nitrogen* was determined by the Mörner-Folin method.

The *ammonia nitrogen* was determined by Folin's method.

The *allantoin nitrogen* was determined by a combination of Folin's and the Mörner-Folin methods for the determination of urea as described by me.²

The *amino acid nitrogen*, including hippuric acid nitrogen, was determined by a combination of Bohland's phosphotungstic acid method for the estimation of urea, and Folin's method, also described by me.³

Creatine and creatinine nitrogen were determined by Folin's colorimetric method, using a Duboscq colorimeter.

Protein, if present in any quantity, was precipitated by adding an equal volume of 10 per cent. trichloroacetic acid in the cold before any of the above estimations were carried out.

1. *Journ. of Exper. Med.*, Vol. XI, 1909.

2. *Bio-Chem. Journ.*, Vol. IV, p. 448, 1909.

3. *Loc. cit.*

The question of the error involved in such a differential method is of importance. In each case control estimations were made, and the difference between the two titrations ranged as a rule from 0 to 0.5 c.c. N/10 acid. Taking the mean of the titrations, this difference corresponds to about ± 2 per cent. of the total nitrogen. In some exceptional cases—not more than five throughout the whole series of experiments—the difference between the two titrations gave rise to a difference of ± 5 in the percentage of total nitrogen. These experiments are noted with an asterisk.

The possibility of such an error would seem to throw some doubt on the results obtained by these methods. In no case, however, did this error occur in the same series of analyses in more than one of the three methods—Bohland, Folin, Mörner-Folin—by which the nitrogen was determined. We may therefore conclude that the error of the method does not vitiate the conclusions drawn.

Sorensen's formol titration method,¹ based upon the principle that the amino group in ammonia and amino acids reacts with formaldehyde, has been elaborated by Henriques² for the determination of the amino acid nitrogen.

This method I have used in several instances in this series of experiments as an alternative and check to the method of amino acid estimation above described.

Much work has recently been done on the accuracy of this method. Using it, Henriques,³ Frey and Gigon,⁴ Henriques and Sorensen,⁵ Tanzo Yoshida,⁶ all found that the urine normally contained a considerable percentage of amino acids.

Malfatti,⁷ the details of whose method vary considerably from those of Henriques, finds amino acids to be normally absent, or at most present only in traces.

Oehler,⁸ who employed β -naphthaline sulphochloride for the estimation of the amino acids, got similar results. Malfatti, in a later paper,⁹ in reviewing the previous work, criticises the use of litmus as an indicator in the first stage of the process.

1. *Biochem. Ztsch.*, VII, 1908.
2. *Ztsch. f. phys. Chem.*, LX, 1909.
3. *Loc. cit.*
4. *Biochem. Ztsch.*, XXII.
5. *Ztsch. f. phys. Chem.*, LXIII.
7. *Ztsch. f. phys. Chem.*, LXI.
6. *Biochem. Ztsch.*, XXIII.
8. *Biochem. Ztsch.*, XXI.
9. *Ztsch. f. phys. Chem.*, LXVI.

Jager¹ also questions the accuracy of the results obtained with litmus as indicator, and, like Malfatti, employs phenol phthalein alone. He discusses² the influence of urea on formaldehyde, but with the concentration of urea solution which occurs in the urine he concludes that as employed for the estimation of amino acids in the urine, the method gives, in practice, good results.

Since, however, the method I had devised had proved reliable, and had been used in the earlier experiments of the series, it was employed throughout this investigation.

RESULTS

Experiment I.—Collie dog. Twenty-five c.c. of chloroform in olive oil were given on the morning of the third day. This caused sickness, so the dose was repeated later in the day.

TABLE I

Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	2.747	0.133	—	—	—	0.137	0	0.108
2	3.567	0.218	2.762	0.041	0.139	0.156	0.006	0.245
3*	2.828	0.12	2.26	0.028	0.118	0.125	0.002	0.175
5	4.488	0.239	3.73	0.088	0.013	0.121	0.050	0.247
6	5.87	0.506	3.826	—	0.795	0.133	0.073	0.537
7	3.382	0.309	2.211	0.045	0.323	0.138	0.042	0.314
8	3.024	0.355	2.102	0.088	0.048	0.109	0.055	0.267
9	2.374	0.219	1.811	0.053	0.011	0.086	0.018	0.176
10	2.974	0.207	2.423	—	—	0.130	0.011	0.117
1	2.632	0.188	2.108	0.067	—	0.105	0	—

TABLE II

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	4.8	—	—	0	4.6	0	4.4
2	6.1	77.4	1.1	3.9	4.3	0.2	7.0
3*	4.2	80.0	0.9	4.1	4.4	0.1	6.3
5	5.3	83.1	1.9	0.2	2.6	1.2	5.7
6	8.6	65.2	—	13.4	2.2	1.3	9.3
7	9.1	†65.4	1.3	9.4	4.0	1.3	9.5
8	11.7	69.5	2.9	1.6	3.6	1.8	8.9
9	9.2	76.3	2.2	0.5	3.6	0.7	7.5
10	6.9	81.5	—	—	4.3	0.4	4.1
11	7.1	80.1	2.5	—	3.9	0	—

1. *Ztsch. f. phys. Chem.*, LXII, LXIV.
2. *Ztsch. f. phys. Chem.*, LXVII.

Throughout the curves, tracings represent as follows :—Urea ———. Ammonia Allantoin ———. Amino acids ———. Und. N ———.

EXPERIMENT I *

* The size of these charts is reduced by leaving out the middle part between 10 per cent. and the lowest percentage to which the urea nitrogen fell.

Experiment II.—Brown retriever weighing 17.9 kilos.

Twenty-five c.c. chloroform in oil were given on the morning of the third day, and food was withheld.

The dog took no food on the fourth and fifth days, and on these days there was no urine.

On the third day after the chloroform which was the first urine to be examined, there were a few granular tube casts, some albumin and bile pigments.

Sugar was absent.

There was a trace of protein on the next two days, but no tube casts. Gmelin's test also remained positive.

TABLE III

Day	Total	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N	N
1	5.346	0.107	4.5	—	—	0.079	0.073	0.282
2								
3*	4.889	0.203	4.128	0.043	0.049	0.135	0.030	0.301
6	8.946	0.257	8.003	0.140	0.067	0.232	0.022	0.225
7	5.2	0.147	4.119	0.049	0.021	0.120	0.010	0.657
8	4.648	0.137	3.867	0.028	0.011	0.136	0.003	0.466
9	4.424	0.262	3.49	0.157	0.004	0.122	0	0.389
10	3.36	0.202	2.808	0.081	0	0.119	0	0.150
11	4.427	0.219	3.4	0.308	0.046	0.131	0.004	0.319
12	4.585	0.140	3.57	0.266	—	0.130	—	0.438

TABLE IV

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N
1	2.0	84.1	—	—	1.4	1.3	5.2
2							
3*	4.1	84.1	1.2	1.0	2.7	0.6	6.3
6	2.8	89.5	1.5	0.8	2.5	0.3	2.6
7	2.8	79.2	0.8	2.1	2.3	0.1	12.7
8	2.9	83.2	0.6	0.2	2.9	0.1	10.1
9	5.9	78.9	3.5	0.1	2.9	0	8.7
10	6.0	83.2	2.7	0	3.5	0	4.6
11	4.9	76.8	7.0	0.9	2.9	0.1	7.4
12	3.0	77.9	5.8	—	2.8	—	9.4

EXPERIMENT II

Experiment III.—The same dog as in *Experiment II*.

During the course of the experiment the animal's weight fell from 19.3 to 17.6 kilos.

Five c.c. chloroform were given hypodermically on the afternoon of the first day, and the same dose again on the morning of the second.

The dog took its food regularly each day.

The urine was very dark on the second day after the chloroform, and showed faint trace of bile pigments on the next two days.

TABLE V

Day	Total	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N	N
1*	2.94	0.165	2.18	0.189	0.005	0.094	0.006	0.301
2*	4.55	0.160	3.585	0.203	—	0.114	0.020	0.58
3	5.705	0.213	4.634	0.137	—	0.115	0.008	0.617
4	5.355	0.302	4.213	0.147	0.036	0.116	0	0.541
5	3.815	0.171	2.699	0.218	—	0.115	0.003	0.589
6	3.724	0.271	2.613	0.028	0.012	0.098	0.013	0.689
7	3.808	0.148	2.932	—	—	0.108	0.001	0.718
8	4.06	0.141	3.191	0.173	—	0.116	0.002	0.555

TABLE VI

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N
1*	5.6	74.1	—	0.1	3.1	0.2	10.5
2*	3.5	78.8	4.4	—	2.5	0.4	12.8
3	3.7	81.2	2.4	—	2.0	0.1	10.9
4	5.6	78.7	2.7	0.8	2.1	0	10.3
5	4.4	70.9	5.6	—	3.0	0.1	15.6
6	7.2	70.2	0.7	0.3	2.6	0.3	18.7
7	3.8	77.0	—	—	2.8	0	19.0
8	3.4	78.6	4.6	—	2.8	0.1	13.8

EXPERIMENT III

Experiment IV.—The same dog as in *Experiment I.*

During the experiment the animal's weight rose from 14.6 to 15.4 kilos.

Five c.c. chloroform were given hypodermically on the afternoon of the second day, and repeated on the morning of the next.

Protein was present in small amount on the sixth and seventh days.

A trace of bile pigments was observable on the fifth, sixth and seventh days.

There was no reduction of Fehling's solution.

TABLE VII

Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	5.32	0.151	4.784	0	0.011	0.103	0	0.278
2*	4.34	0.148	3.422	0.154	0.05	0.086	0	0.48
3	5.04	0.151	4.469	—	0.081	0.109	0	0.37
4	7.42	0.190	6.25	—	0.414	0.126	0	0.706
5	6.09	0.246	4.531	0.235	0.339	0.092	0.035	0.39
6	6.23	0.353	5.177	0.042	0.115	0.119	0	0.424
7	4.312	0.289	3.351	0.011	0.110	0.108	0	0.443
8	3.948	0.193	3.279	—	—	0.093	0.010	0.218

TABLE VIII

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	2.8	89.9	0	0.2	1.8	0	5.4
2*	3.4	78.8	3.6	1.1	1.9	0	11.2
3	3.0	88.7	—	1.6	2.2	0	7.3
4	2.5	84.2	—	5.5	1.7	0	9.6
5	4.0	74.4	3.8	9.3	1.5	0.5	6.5
6	5.6	83.1	0.7	1.8	1.9	0	6.9
7	6.7	77.7	0.2	2.6	2.5	0	10.3
8	4.8	83.1	—	—	2.3	0.3	5.7

EXPERIMENT IV

Experiment V.—Dog. Whippet weighing 23 kilos.

The animal received 5 c.c. chloroform hypodermically on the first and second days of the experiment.

The urine of the second day contained vomited matter and faeces and therefore was not examined.

The dog took no food till the fifth day.

Protein was present from the third day till on the seventh a marked cloud on boiling with acetic acid was obtained.

The fourth day showed a slight reduction of Fehling's solution.

On the seventh day there was an abundance of granular cells but no tube casts. On this day the urine showed Pettenkofer's test for bile acids distinctly.

TABLE IX

Day	Total	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N	N
1*	4.091	0.172	3.38	0.182	—	0.184	0.007	—
3	16.432	0.340	12.155	0.189	1.232	0.311	0.095	2.11
4	7.665	0.315	5.46	0.651	0.192	0.165	0.060	0.822
5	5.365	0.276	4.171	0.375	0.081	0.190	0	0.332
6	6.412	0.419	4.397	0.134	0.924	0.200	0.011	0.327
7	8.669	0.242	6.864	0.091	0.099	0.183	0.052	1.269
8	6.783	0.215	5.192	0.044	0.384	0.195	0.008	0.932
9	9.975	0.302	8.238	0.392	0.269	0.289	0.005	0.764

TABLE X

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon.	Urea	Allantoin	Amino acid	Creatinin	Creatin	Und.
	N	N	N	N	N	N	N
1*	4.2	82.6	4.4	—	4.4	0.2	—
3	2.0	†74.0	1.2	7.4	1.8	0.6	13.0
4	4.1	71.2	8.5	2.5	2.1	0.8	10.8
5	5.1	†77.7	7.0	0.4	3.5	0	6.3
6	6.5	68.6	2.0	14.5	3.1	0.1	5.2
7	2.7	†79.2	1.1	1.0	2.1	0.6	13.3
8	3.1	76.6	0.6	5.6	2.8	0.1	11.2
9	3.0	82.6	3.9	2.6	2.8	0.1	5.1

EXPERIMENT V

Experiment VI.—Black retriever weighing 17·6 kilos.

4 c.c. chloroform were given hypodermically on the first and second days.

There was a small amount of protein present on the fourth and fifth days.

The dog took some food each day, but on several days did not finish all its food.

TABLE XI

Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	7·7	0·194	6·197	0·077	0·066	0·179	0·051	0·936
2*	6·475	0·229	5·143	0·060	0·119	0·167	0	0·759
3	10·087	0·446	6·869	0·740	0·162	0·188	0·007	0·675
4	7·91	0·498	5·312	0·924	0·232	0·179	0	0·765
5 6 }	6·581	0·412	4·958	0·416	0·060	0·149	0·018	0·536
7	5·6	0·316	4·374	0·266	—	0·146	0·025	0·644

TABLE XII

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	2·5	80·5	1·0	0·9	2·3	0·6	12·2
2*	3·5	79·4	0·9	1·8	2·5	0	11·9
3	4·4	68·1	17·2	1·6	1·8	0·1	6·8
4	6·2	†67·2	11·7	2·8	2·2	0	9·9
5 6 }	6·2	75·3	6·3	0·9	2·2	0·3	8·6
7	5·6	78·1	—	—	2·6	0·4	11·6

EXPERIMENT VI

GENERAL CONCLUSIONS

The results bear out those obtained by Professor D. Noël Paton.

Total nitrogen.—In every case there is a distinct increase in the total nitrogen excreted, an increase which is especially well marked in Experiments II, V, and VI.

Urea nitrogen.—In each case there is a distinct fall in the proportion of urea nitrogen, reaching its maximum on the third day after the administration of chloroform. In Experiment III a slight rise in the proportion of urea precedes the fall, and the maximum decrease is, in this case, not reached till the fifth day after the drug was administered.

Ammonia nitrogen.—The proportion of nitrogen in the form of ammonia rises, reaching its maximum as a rule on the fifth day after the administration of chloroform.

Allantoin nitrogen.—In Experiments I and II, in both of which the chloroform was administered by the mouth, the proportion of allantoin nitrogen shows a gradual but distinct increase, which in II does not reach its maximum till the sixth day after the administration of the chloroform. In the other experiments in which the drug was given hypodermically, the maximum increase is reached on the second day after (the fourth day in III), and is followed immediately by a sharp fall.

The significance of allantoin in the urine of the dog is not fully understood. Many observers consider that it is the end product of purin metabolism, while others are inclined to look upon it as an intermediate product which may undergo further change.

Wiechowski, for example, holds that it is an end product, and considers that the appearance of allantoin in the dog's urine is caused by the splitting of the uric acid. In man this decomposition occurs only to a slight extent, hence the replacement of allantoin by uric acid in the human urine. Sucklings, he states, seem to occupy an intermediate position with regard to this power of decomposing uric acid, and allantoin is found in their urines in considerable amounts. His observation that in the dog it is possible to recover completely the allantoin after injection confirms this view.

The fact that Pohl¹ found allantoin in the tissues of a dog poisoned with hydrazine, and Underhill and Kleiner² in the urine, but not in the normal animal, and the observation of Borissow³ that allantoin appears in the urine after diamido sulphate poisoning, seem to indicate either that it is an intermediate product, or that these poisons, by liberating nucleins, yield a larger supply of purins to be converted into allantoin. The increase of allantoin in the present experiments might be explained on either hypothesis.

Amino acid nitrogen.—The administration of chloroform causes in each case a rise in the proportion of amino acid nitrogen. In I and IV, the experiments on the collie dog, the rise is very marked; in II and III, on the contrary, those on the brown retriever, the increase corresponds rather to an appearance of amino acids, as in the normal urine of the brown retriever only a trace of amino acids could be found and on several days of the experiment they were completely absent.

The peculiar reaction of this dog is of considerable interest. Instead of the usual rise in mon-amino acids the undetermined nitrogen rose. This led to the consideration of the possibility of this being due to the appearance of products of the nature of diamino acids and polypeptides.

In Experiments II and III, therefore, where the amount of mon-amino acid nitrogen is small an attempt was made to ascertain approximately the amount of nitrogen present in the form of diamino acids.

The phosphotungstic acid precipitate was decomposed with barium hydrate and barium carbonate, the excess of barium removed with CO₂, and silver nitrate added. The nitrogen in the silver precipitate was estimated by Kjeldahl's method. It probably consists to a very large extent of the silver salts of the diamino acids.

	Und. Nitrogen	Ag prec. Nitrogen
Experiment II ...	12.7	4.9
	10.1	7.6
Experiment III ...	19.0	18.5

It would thus seem as if, in this animal, diamino acids rather than monamino acids formed intermediate products of catabolism. Gideon

1. *Archiv. f. exp. Pathol.*, XLVI, p. 367.

2. *Journ. of Biol. Chem.*, Vol. IV.

3. *Ztsch. f. phys. Chem.*, XIX.

Wells¹ found that in cases of delayed chloroform poisoning very considerable amounts of diamino acids could be isolated from the abnormal livers. Diamino acids also are said to be excreted in cases of cystinuria.²

Creatinine nitrogen.—There is no very marked change in the amount of creatinine nitrogen. The proportion appears to fall slightly on the day after the administration. This agrees with the work of Howland and Richards,³ who found creatinine remained fairly constant and consequently that there was a percentage decrease.

Creatine nitrogen.—The effect of chloroform is to cause the appearance of creatine in the urine two or three days after its administration at the time when the general disturbance in the metabolism is most marked. Howland and Richards observed a marked increase in the percentage of creatine in the two cases of delayed chloroform poisoning.

In Experiments III, IV, VI, the dog took its food each day, and the appearance of creatine which occurs in these may be considered as an effect of the chloroform. In Experiments I, II, V, in which food was refused for some days after the chloroform, the fact that starvation is a cause of the appearance of creatine must be taken into account. But in Experiment V the appearance of creatine takes place after the animal had resumed its food, when the starvation effect may fairly be held to be ended.

This disturbance in the creatine metabolism is almost certainly to be associated with the disturbance in the hepatic functions, since Mellanby⁴ found creatine was excreted in great amount in carcinoma of the liver; Underhill and Kleiner⁵ observed fatty degeneration of the liver and creatine excretion following injection of hydrazine sulphate; and Hoogenhuyze and Verploegh⁶ state that the amount of creatine present in the urine may be taken as an indication of the extent of the hepatic disturbance.

Undetermined nitrogen.—The proportion of undetermined nitrogen shows in each case a rise of from 5 to 9 per cent. Normally about 4 to 5

1. *Journ. of Biol. Chem.*, Vol. V, 1908-9.
2. Loewy u. Neuberg, *Ztsch. f. phys. Chem.*, XLIII.
3. *Loc. cit.*
4. *Journ. of Physiol.*, Vol. XXXVI.
5. *Loc. cit.*
6. *Ztsch. f. phys. Chem.*, LVII, p. 264.

per cent. of the total nitrogen is undetermined. The greatest increase occurs on the same day or the day after the maximum fall in the proportion of urea nitrogen, the rise in the proportion of nitrogen in the form of ammonia, allantoin and amino acids not being sufficient to counter-balance this decrease.

This fact, and the consequent increase in the undetermined nitrogen, leads to the question: To what is this undetermined nitrogen due? It occurs altogether in the phosphotungstic acid precipitate. Creatinine, which is also included, is separately estimated, the amount of purin nitrogen is quite negligible, so that in all probability this undetermined nitrogen consists of diamino acids and polypeptides.

With regard to the possible presence of polypeptides in the urine the formol titration method seemed to offer a convenient and speedy means for the estimation of the nitrogen in this form.

Henriques and Sorensen¹ have evolved a method whereby the polypeptides are decomposed by heating with hydrochloric acid, and the amino acids thus split off are estimated by formol titration. They by this method found polypeptides to be normally present in small amounts in the urine of dogs—0·0 to 1·6 per cent. of the total nitrogen.

The following table shows the results obtained which prove the presence of lower proteins and polypeptides in considerable amounts. The higher proteins present were first removed by precipitating with trichloroacetic acid:—

Experiment V:—

Day	Und. Nit. Grs. nit.	Polyp. Nit.	Und. Nit. % T.N.	Polyp. Nit. % T.N.	Still Und. % T.N.
3	2·11	2·23	13·0	13·6	— 0·6
4	0·822	0·451	10·8	5·8	5·0
5	0·332	0·397	6·3	8·9	— 2·6
6	0·327	0·259	5·2	4·9	0·3
7	1·269	1·035	13·3	12·0	1·3

Experiment VI:—

1	0·936	0·819	12·2	10·5	1·7
2	0·759	0·406	11·9	6·3	5·6
3	0·675	0·261	6·8	2·5	4·3
4	0·765	0·467	9·9	6·0	3·9
5	0·473	0·427	9·0	8·0	1·0
6	0·660	0·362	8·6	4·5	4·1

The disturbance in the nitrogenous metabolism revealed by these experiments appears to be primarily due to the disturbance in the

1. *Ztsch. f. phys. Chem.*, LXIII and LXIV.

function of the liver which is so clearly indicated by the structural changes described by Clark.¹

It has been maintained that they are due to an acidosis produced by the administration of chloroform. That such an acidosis occurs in the human subject there can be no doubt, but in the dog this is not manifest, although the changes in the distribution of nitrogen are quite marked. Underhill and Kleiner² got no evidence of the presence of acetone oxybutyric acid, etc., and Richards and Wallace³ found acetone was not present after cyanide poisoning. I myself also failed to find any indication of an increase of acetone and diacetic acid in the urine following on the administration of chloroform. Further work is at present being done on this question.

Hence it seems that the acidosis, when it occurs, is, like the disturbance in the distribution of nitrogen, secondary to the interference with the hepatic functions.

I take this opportunity of acknowledging my deep indebtedness to Professor Noël Paton, under whose guidance this work has been done, for carrying out the experimental work and for much help and criticism.

1. *Loc. cit.*

2. *Loc. cit.*

3. *Journ. of Biol. Chem.*, Vol. IV.