I. THE FATE OF INORGANIC NITROGEN IN THE METABOLISM OF THE DOG.

BY WILLIAM CALDWELL
AND HAROLD REX SEPTIMUS CLOTWORTHY.

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These experiments were begun in January, 1913, and were undertaken with the object of confirming or otherwise the generally accepted view which prevailed at that time, namely, that retention of nitrogen takes place when the proteins of the food are replaced by inorganic nitrogen in the form of ammonium salts. As the investigation—the results of which are contained in Table I—proceeded, it was thought that it would be an interesting addition to the work to see in what forms the nitrogen was excreted, and if it was retained, to determine whether the retention was temporary or permanent.

The meaning of the expression “retention of nitrogen” is somewhat ambiguous. If the weight of the animal remains constant or increases, and some or all of the extra nitrogen given in the food or injected into the body is retained at the same time, then undoubtedly nitrogen is retained. On the other hand, if more nitrogen is excreted than is given with the food or injected into the body, although the weight of the animal increases, it is assumed that no nitrogen is retained. Again, if the weight of the animal decreases, and some of the extra nitrogen given is not immediately excreted, it is assumed that the decrease is due to a loss of water, and that in this case there is retention of nitrogen, though it may be temporary.

Peschek [1911] was the first to call attention to this problem. He fed an animal on food which contained a high percentage of nitrogen, and added ammonium acetate for certain periods to the food. His results, however, were contradictory—sometimes nitrogen was retained, and at other times it was lost.

The problem has since been investigated by a number of authors. Grafe and Schlaepfer [1912] assert that an animal has the power to retain nitrogen and use it to synthesise protein from nitrogen-free food (carbohydrates and fats) and inorganic nitrogen in the form of ammonium salts. Abderhalden [1912] also came to the conclusion that clear evidence was obtained of the formation of protein, when dogs were fed on a nitrogen-free food with the addition of inorganic nitrogen. But on an examination of his results it will be seen that in the first experiment there is a negative nitrogen balance and a continual loss in weight. Similarly in Exp. 2 there is a negative nitrogen balance and a continual loss in weight. In Exp. 3 a dog which had lost 2000 g. recovered its original weight rapidly—in 11 days—by feeding with the hydrolysed products obtained from flesh and blood. The dog was then fed on a nitrogen-free diet, and the animal lost weight, and the nitrogen balance was negative during this part of the experiment. Then ammonium acetate was added to the carbohydrate and fat food. The nitrogen balance was positive for five days and negative for the remainder of the period. The result of the feeding for the whole period was a retention of nitrogen and a slow but gradual decline in weight. In the last period of this experiment the amount of ammonium acetate given with the carbohydrates and fats was increased but the nitrogen balance remained negative during the whole period—5.34 g. nitrogen being lost—and the weight fell gradually but slowly. In Exp. 4 the nitrogen balance was negative during the whole of the experiment. Thus what Abderhalden has proved, apart from confirming the established fact, that the protein of the food may be replaced by the completely hydrolysed products obtained from flesh and blood, is, that the addition of inorganic nitrogen to a nitrogen-free food prevents the rapid loss of weight which takes place when an animal is fed on carbohydrates and fats alone. His experiments do not conclusively show a permanent retention of inorganic nitrogen and Abderhalden has lately admitted this. At the end of a long investigation [Abderhalden, 1913], he states that ammonium salts, and especially ammonium acetate, can influence at times the nitrogen balance in the sense of a retention, but, he adds, there are no grounds, from the results which he has obtained, for the assumption that protein can be formed from ammonia and nitrogen-free compounds, such as carbohydrates and fats. More recently the problem of the retention of nitrogen has been investigated by Taylor and Ringer [1913] who find that the presence of carbohydrates in the food is not a necessary factor in the retention of nitrogen from ammonium salts; and also by Underhill [1913, 1], whose communications are

important. He fed a dog on an adequate mixed diet. The ammonium salts of organic acids were given to the dog orally, and it was found that none of the added nitrogen was excreted as ammonia nitrogen. When however the ammonium salts of inorganic acids were given to the dog, the added nitrogen was excreted partly as ammonia nitrogen—with ammonium chloride 52 % of the added nitrogen, and with ammonium phosphate 64 % of the added nitrogen appeared in the urine. In a later paper Underhill [1913, 2] contests the conclusions of Grafe and Schlaepfer whose work he repeats but fails to find evidence of any of the inorganic nitrogen being used in the synthesis of proteins.

The analogy between the plant cell and the animal cell does not seem to hold in respect to nitrogen; for, while syntheses are not denied to the animal cell, it has not yet been shown that it is able to synthesise complex organic substances from inorganic constituents. Again, we are much inclined to discuss results as if an animal were a perfect mechanical balance, which it is not; for, whilst approximate nitrogenous equilibrium can be attained, absolute nitrogenous equilibrium can scarcely be said to exist. A fair margin has to be allowed for temporary fluctuations on either side of the line of absolute equilibrium.

METHOD.

We fed a dog on a fixed diet containing a definite amount of nitrogen as determined by analysis, for a considerable time before the actual experiment began, to enable the dog to acquire a steady state or condition in which the amounts of nitrogen excreted per day should be approximately equal. When this steady state had been attained a known amount of an ammonium salt was added to the food—one-half to the food in the morning, and the remaining half to the food in the evening. This was continued for three or four days. An interval of three or four days was then allowed to elapse before a second ammonium salt was added to the food, and so on. Our feeding periods therefore extended for three or four days and the intervals after the addition of the extra nitrogen also extended for three or four days. The bladder was emptied regularly by a sterilised catheter at intervals of 24 hours. The day's urine was measured and a complete analysis was performed each day, whether during a period or an interval. The faeces were collected and grouped into periods corresponding to the experimental and the interval periods.

The amount of water which the dog drank was accurately recorded.

In some of the experiments the ammonium salt was injected subcutaneously in sterile condition—half of the volume was injected in the morning after food, and the other half injected in the evening after food.

These injections were made for us by Professor W. H. Thompson.

The diet consisted of 100 g. of potatoes, 100 g. dog biscuit, 50 g. flour, 10 g. dried milk, 10 g. lard, 2 g. salt and 250 g. water.

DOG XV.

The results obtained with dog XV are shown in Table I.

The investigation of the results of feeding with ammonium salts had been in progress for some time before analyses to ascertain the manner in which the extra nitrogen was excreted were undertaken. As a consequence the details of the early part of the experiment are in this respect not complete.

Period III. 0.617 g. nitrogen was added to the food as ammonium chloride and 0.939 g. appeared—0.312 g. or 33.2 % as ammonia nitrogen and 0.458 g. in the faeces.

Though there was a positive balance, yet compared with period II there was a loss of nitrogen.

Period IV. During this interval the amount of ammonia nitrogen decreased and became almost equal to the amount of ammonia nitrogen during period II. The period was a long one and may be compared with the succeeding feeding period.

Period V. 0.413 g. nitrogen was added to the food as ammonium carbonate. It will be seen that the amount of ammonia nitrogen has not been increased by the addition of ammonium carbonate to the food.

Period VI. The amount of ammonia nitrogen remained approximately steady.

Period VII. 0.315 g. nitrogen was added to the food as ammonium phosphate; more than this amount of nitrogen appeared, as may be seen on comparing the results with period VI or with period II. The amount of ammonia nitrogen was increased by 0.331 g.—an amount greater than the amount of nitrogen added.

Period VIII. During this interval the amount of ammonia nitrogen again became approximately normal, but on the addition of sodium phosphate to the food, the amount of ammonia nitrogen excreted was decreased, and the amount of urea nitrogen increased.

From this investigation it would appear therefore that the amount of ammonia nitrogen excreted depends on the nature of the ammonium salt

TABLE I. Dog XV.

Period	Weight g.	Nitrogen given per day g.	Nitrogen excreted per day g.	Nitrogen balance g.	Nitrogen added g.	Nitrogen excreted g.	Urea nitrogen g.	Ammonia nitrogen g.	Creatinine mg.	Creatine mg.	Nitrogen in faeces g.
I. 6 days. Ammonium acetate (in food)	7,003	4.369	3.786	0.583 +	0.539	—	—	0.0726	192.9	44.7	0.942
II. 8 days. Interval ...	7,048	3.830	2.844	0.986 +	—	—	—	0.0749	199.8	43.7	0.317
III. 6 days. Ammonium chloride (in food)	7,156	4.447	3.783	0.664 +	0.617	0.939	—	0.3869	207.8	24.8	0.775
IV. 8 days. Interval ...	7,282	3.830	3.163	0.667 +	—	0.319	—	0.0847	208.0	26.8	0.634
V. 6 days. Ammonium carbonate (in food)	7,424	4.243	3.542	0.701 +	0.413	0.698 (0.379)	—	0.0737	197.1	25.3	0.713
VI. 3 days. Interval ...	7,535	3.830	3.562	0.268 +	—	0.718	—	0.0676	195.1	20.3	0.697
VII. 6 days. Ammonium phosphate (in food)	7,609	4.145	4.040	0.105 +	0.315	1.196 (0.478)	2.3599	0.3981	204.5	45.3	0.731
VIII. 3 days. Interval	7,779	3.830	3.490	0.340 +	—	0.646	1.756	0.0733	199.4	20.7	0.786
IX. 5 days. Sodium phosphate (in food)	7,824	3.830	3.620	0.210 +	—	0.776	2.153	0.0366	208.6	25.1	0.855
X. 3 days. Interval ...	7,929	3.830	3.524	0.306 +	—	0.680	1.977	0.0550	197.3	26.6	0.752

added to the food. The ions into which the ammonium salts are dissociated may or may not act as foreign substances in the body. In the case of ammonium carbonate, the body does not find it necessary to protect itself but in the cases of ammonium chloride and ammonium phosphate it would seem that the decomposition of the ammonium salt demands from the body some kind of protection from the hydrochloric acid or the phosphoric acid, and the ammonia produced is excreted; hence the increase in the amount of excreted ammonia nitrogen.

The addition of sodium phosphate to the food is somewhat interesting. Di-sodium hydrogen phosphate is slightly hydrolysed in solution and has an alkaline reaction. The effect of such a substance would seem to decrease the amount of ammonia nitrogen excreted and increase the amount of urea nitrogen excreted.

The conclusion we may legitimately draw from the foregoing investigation is that an ammonium salt of a weak acid has little or no effect on the amount of ammonia nitrogen excreted; whereas the ammonium salt of a strong acid increases very considerably the amount of excreted ammonia nitrogen.

While the nitrogen balance was positive during the whole experiment, the amount of nitrogen excreted increased during the course of the experiment. There was therefore no retention of the added nitrogen during this series of experiments.

DOG XVII. (TABLE II.)

Dogs XV and XVIII were reserved exclusively for the investigation of the metabolism of the ammonium salts mentioned in the tables which record the results. These dogs were kept for a very considerable time on a constant diet before the real investigation began. Dog XVII was not treated in such a systematic manner.

In this investigation the ammonium salts of lactic acid and citric acid were used. When the salts were injected there was not a very marked increase in the amount of the ammonia nitrogen excreted. The amount of urea nitrogen excreted increased when ammonium lactate was injected and decreased when ammonium citrate was injected. The behaviour of these salts on injection, however, is in strange contrast to the behaviour of the same salts when they are added to the food.

During period IV, 0.550 g. nitrogen was added to the food as ammonium lactate, and 0.480 g. nitrogen appeared as excreted nitrogen. Of this excreted nitrogen 0.436 g. or 90.8 % appeared as urea nitrogen. During period VI

TABLE II. Dog XVII.

Period	Weight g.	Nitrogen given per day g.	Nitrogen excreted per day g.	Nitrogen balance g.	Nitrogen added g.	Nitrogen excreted g.	Urea nitrogen g.	Ammonia nitrogen g.	Creatinine mg.	Creatine mg.	Nitrogen in faeces g.
I. 5 days. Interval ...	8,208	3.83	3.908	0.078 -	—	—	2.615	0.155	261	12	0.875
II. 5 days. Ammonium lactate (injection)	8,293	4.09	4.326	0.236 -	0.260	0.418	2.707	0.178	264	20	0.9298
III. 4 days. Interval ...	8,341	3.83	3.504	0.326 +	—	—	2.371	0.163	233	41	0.643
IV. 5 days. Ammonium lactate (in food)	8,372	4.38	3.984	0.396 +	0.550	0.480	2.807	0.148	240	58	0.656
V. 3 days. Interval ...	8,419	3.83	3.905	0.075 -	—	—	2.465	0.148	232	55	0.920
VI. 5 days. Ammonium citrate (in food)	8,494	4.38	4.283	0.097 +	0.550	0.378	2.741	0.144	234	49	0.833
VII. 3 days. Interval ...	8,548	3.83	3.660	0.170 +	—	—	2.381	0.132	239	49	0.777
VIII. 3 days. Ammonium citrate (injection)	8,561	4.10	3.996	0.104 +	0.270	0.336	2.308	0.168	242	51	0.931
IX. 3 days. Interval ...	8,590	3.83	3.685	0.145 +	—	—	2.393	0.146	238	53	0.816

0.378 g. nitrogen appeared as excreted nitrogen after the addition of 0.550 g. nitrogen to the food as ammonium citrate, and of this excreted nitrogen 0.276 g. or 73 % appeared as urea nitrogen.

DOG XVIII. (TABLE III.)

Period I. The steady state is represented by:—3.830 g. nitrogen given per day, and 3.545 g. nitrogen excreted per day.

Period II. 0.47 g. nitrogen was added to the food as ammonium acetate, and of this 0.424 g. appeared as excreted nitrogen; 0.383 g. or 90.3 % of this excreted nitrogen was in the form of urea nitrogen.

0.046 g. nitrogen was retained during the period, but this amount was lost in the succeeding interval. Not only so, but a loss of 0.914 g. nitrogen occurred in interval III and this nitrogen was excreted in the faeces of interval III. The added nitrogen therefore seems not only to be excreted, but to have caused some decomposition of nitrogenous substances. The result of feeding with ammonium acetate is therefore an unaccountable loss of nitrogen. It may be a washing out of temporarily retained nitrogen.

There was a decrease of 100 g. in the weight of the dog during the interval III.

Period IV. 0.283 g. nitrogen was injected as ammonium acetate, and 0.675 g. nitrogen appeared as excreted nitrogen. 0.443 g. or 60.6 % of this excreted nitrogen appeared as urea nitrogen, and 0.12 g. nitrogen appeared as excess in the faeces. There was thus a loss of nitrogen during the period.

Period V. During this interval there was a very slight loss of nitrogen compared with the state of equilibrium which existed at the beginning of the experiment.

Period VI. 0.61 g. nitrogen was added to the food as ammonium chloride and of this 0.490 g. appeared as excreted nitrogen. In comparison with the "steady state" at the beginning of the experiment, 0.297 g. or 60.5 % was excreted as urea nitrogen, and 0.327 g. or 66.7 % as ammonia nitrogen. But in comparison with interval V, 0.135 g. or 27.5 % appeared as urea nitrogen, and 0.354 g. or 72.2 % as ammonia nitrogen.

0.12 g. nitrogen was retained at the end of the period.

Period VII. During this interval not only was the retained nitrogen lost, but 0.416 g. extra nitrogen was excreted, principally in the faeces. The urea nitrogen excreted was 0.202 g. less than normal, whilst the nitrogen in the faeces was 0.67 g. more than in the normal condition. Most of this extra excreted nitrogen appeared on the day following the previous period.

TABLE III. Dog XVIII.

Period	Weight g.	Nitrogen given per day g.	Nitrogen excreted per day g.	Nitrogen balance g.	Nitrogen added g.	Nitrogen excreted g.	Urea nitrogen g.	Ammonia nitrogen g.	Creatinine mg.	Creatine mg.	Nitrogen excreted in faeces g.
I. 5 days. Interval ...	6,735	3-830	3-545	0-285 +	—	—	2-2861	0-1919	176-76	68-02	0-790
II. 5 days. Ammonium acetate (in food)	6,887	4-300	3-969	0-331 +	0-47	0-424	2-6692	0-1878	175-62	96-10	0-7878
III. 4 days. Interval ...	6,787	3-830	4-459	0-629 -	—	0-914	2-2673	0-1857	176-85	38-15	1-741
IV. 3 days. Ammonium acetate (injected)	6,837	4-113	4-220	0-107 -	0-283	0-675	2-7292	0-1608	182-6	21-3	0-912
V. 3 days. Interval ...	6,771	3-830	3-576	0-254 +	—	0-031	2-4482	0-1658	170-6	26-4	0-9037
VI. 5 days. Ammonium chloride (in food)	6,830	4-440	4-035	0-405 +	0-61	0-490	2-5834	0-5190	181-2	32-1	0-825
VII. 3 days. Interval ...	7,016	3-830	3-961	0-131 -	—	0-416	2-0849	0-2081	177-8	21-0	1-461
VIII. 5 days. Ammonium chloride (injected)	6,980	4-418	4-217	0-201 +	0-588	0-672	2-4746	0-4150	189-8	27-7	0-855
IX. 2 days. Interval ...	6,938	3-830	3-075	0-755 +	—	0-470 (Retained)	2-3040	0-1925	184-7	78-8	0-3495
X. 3 days. Ammonium phosphate (in food)	7,107	4-281	4-210	0-071 +	0-4508	0-665	2-2941	0-3989	191-6	32-0	1-267
XI. 2 days. Interval ...	7,122	3-830	3-635	0-195 +	—	0-090	2-0741	0-2429	192-7	27-1	1-063
XII. 3 days. Ammonium phosphate (injected)	7,268	4-334	3-680	0-654 +	0-504	0-135	2-4377	0-3103	203-4	40-3	0-584
XIII. 2 days. Interval ...	—	3-830	—	—	—	—	2-0594	0-3136	179-3	71-9	—

Period VIII. 0.588 g. nitrogen was injected as ammonium chloride, and 0.672 g. nitrogen appeared as excreted nitrogen. In comparison with the "steady state" 0.1885 g. or 28 % appeared as urea nitrogen, and 0.2231 g. or 33.2 % as ammonia nitrogen; whereas in comparison with the previous period 0.3897 g. or 58 % appeared as urea nitrogen, and 0.2069 g. as ammonia nitrogen—an amount very slightly in excess of the added nitrogen injected.

Period IX. During this interval a comparatively large amount of nitrogen was retained from the regular food, 0.755 g. in comparison with 0.285 g. during the equilibrium stage at the beginning of the experiment. The amount of urea nitrogen became normal, and so also did the ammonia nitrogen, whilst the amount of nitrogen excreted in the faeces was 0.45 g. less than during the first period.

Period X. 0.4508 g. nitrogen was added to the food as ammonium phosphate, and 0.665 g. appeared as excreted nitrogen. The urea nitrogen excreted remained normal, but the ammonia nitrogen was 0.207 g. (or 31 % of the excreted nitrogen) in excess of that of the equilibrium stage. 0.47 g. nitrogen was excreted in the faeces more than the normal amount. This amount and the amount of extra urea nitrogen excreted make up the total amount excreted.

Period XI. During this interval some nitrogen was lost—0.09 g. The urea nitrogen excreted was 0.22 g. less than in the normal state, but against that the faeces contained 0.27 g. nitrogen in excess of that of the first period.

Period XII. 0.504 g. nitrogen was injected as ammonium phosphate and 0.135 g. appeared as excreted nitrogen. 0.15 g. appeared as urea nitrogen, and 0.12 g. as ammonia nitrogen, whilst 0.21 g. nitrogen less than the normal amount was excreted in the faeces. In this period a very considerable amount of nitrogen is retained.

It was noted during the experiment that the amount of ammonia nitrogen varied directly as the acidity of the urine, determined by titration with N/10 alkali, using phenolphthalein as indicator. The urine itself, as was to be expected, was never alkaline to phenolphthalein. The exact determinations will be published in a later paper.

An interesting point is to compare the periods of feeding with the periods of injection in the foregoing experiments.

1. The experiments with ammonium chloride (Table III, periods V–VIII). On comparing the interval immediately before the feeding experiment with the feeding period itself, it will be seen that the extra nitrogen which was excreted appeared as 0.135 g. or 27.5 % urea nitrogen, and 0.354 g.

or 72.2 % ammonia nitrogen, whereas in the case of the injection of ammonium chloride, the extra nitrogen which was excreted appeared as 0.3897 g. or 58 % urea nitrogen, and 0.2069 g. or 30.8 % ammonia nitrogen.

The injection seems to reverse the magnitude of the amounts of excreted nitrogen as urea nitrogen and as ammonia nitrogen respectively, when compared with the feeding experiment.

2. The experiments with ammonium phosphate (Table III, periods IX–XIII). On comparing the interval immediately preceding the feeding experiment with the feeding experiment period, it will be noticed that the extra excreted nitrogen appears as ammonia nitrogen only, but in the case of the injected ammonium phosphate, the extra excreted nitrogen appears as 0.36 g. urea nitrogen and 0.07 g. ammonia nitrogen, a reversal of magnitude similar to the experiments with ammonium chloride.

3. The experiments with ammonium acetate (Table III, periods I–IV). On comparing the feeding experiment with the interval immediately preceding the feeding experiment, it will be seen that the extra excreted nitrogen appears as urea nitrogen only. The ammonia nitrogen is almost identical with that of the previous period. Similarly with the injected ammonium acetate. The extra excreted nitrogen appears as urea nitrogen only, but the ammonia nitrogen is 0.025 g. less than the previous period.

There is no such marked reversal as that noted in the cases of ammonium chloride and ammonium phosphate.

Taking the experiment as a whole there was no retention of the added nitrogen. The animal was excreting more nitrogen at the end of the experiment than during the "steady state" period; yet the weight of the animal had increased by more than 500 g. during the investigation, which lasted about nine weeks.

The health of the animal during the experiment was excellent.

DISCUSSION AND SUMMARY.

Speculations concerning the synthetic powers of the body under conditions such as we have been investigating are very attractive, but we hesitate to make positive assertions. Even when the extra nitrogen is given in the form of ammonium salts, and it appears as urea, we hesitate to say that the ammonia nitrogen is synthesised into urea. It is no doubt true that the ammonium salt is dissociated. That is evident when we consider the feeding experiments with ammonium chloride. The dissociation of the ammonium chloride into ammonia and hydrochloric acid and the separation of these

products involve a kind of protection on the part of the body, and the formation of a large amount of ammonia, which is subsequently excreted, to protect itself against the acidity of the hydrochloric acid. If the urea is produced from ammonia and carbon dioxide it will involve not only the dissociation of the ammonium chloride, but the oxidation of the ammonia and the reduction of the carbon dioxide in a fashion which we shall not discuss. We do not suggest that the nitrogen is not made use of in synthesis or replacement. It may be, though, that the ammonium salts have some other action, such as displacement, with decomposition of the substances which produce urea.

The chief interest in our work is the manner in which the extra nitrogen taken with the food or injected into the body is excreted. The form in which it is excreted depends on the ammonium salt employed, and apparently on the ions into which the ammonium salt is dissociated. For instance, the added nitrogen in the case of ammonium acetate is excreted in the form of urea. In the case of ammonium chloride and ammonium phosphate, it is excreted both as urea and ammonia. The acetic acid derived from ammonium acetate is a much weaker acid than the hydrochloric acid, or the phosphoric acid derived from the ammonium chloride and ammonium phosphate respectively. The body does not find it necessary to protect itself by the production of ammonia, to neutralise the acetic acid, but it is compelled to protect itself in the case of hydrochloric acid and phosphoric acid.

The form in which the added nitrogen is excreted depends also on whether the nitrogen is taken with the food, or injected into the body. When the ammonium salt is injected the output of urea is greatly increased, and the amount of nitrogen excreted in the faeces is much less than in the previous periods.

Professor W. H. Thompson very kindly performed the injections for us, and we are sincerely indebted to him for his valuable suggestions and advice during the course of the investigation.

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