XIII. STUDIES IN NITROGEN AND SULPHUR METABOLISM.

By HARRY ELLIS CHARTER WILSON.

From the Institute of Physiology, Glasgow University.

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THE experiments to be described were undertaken to gain further insight into the course of protein metabolism and to extend the work recorded in a previous paper [Wilson, 1925] by the investigation of the following points:

1. What capacity has gelatin for sparing body protein when ingested during starvation?
2. Is the gelatin under such circumstances used immediately for energy purposes or does it become incorporated in the cell and influence protein metabolism?
3. What is the influence of meat extractives on protein metabolism when the body is on a nitrogen-free diet?
4. What is the influence of excess water ingestion on protein metabolism?

The quantitative effect of gelatin on the nitrogen output in starvation has been repeatedly investigated [Voit, 1881; Kirchmann, 1900; Krummacker, 1901]. These investigators found that gelatin, no matter the amount given, never reduced the fasting nitrogen output more than 37 %. The sparing effect of gelatin however on the endogenous nitrogen output, *i.e.* when the body is on a carbohydrate-fat diet, is almost nil [Robison, 1922]. The present experiments were directed chiefly to the qualitative effect of gelatin on the nitrogen metabolism in starvation.

The experiments were carried out on myself as subject and the 24 hours' urine was collected and analysed for total nitrogen, total sulphur, ammonia, urea, uric acid and phosphorus as described in the previous paper.

In Exp. 1 a nitrogen-free diet of tapioca and olive oil was taken until the nitrogen output in the urine amounted to about 3 g. *per diem* and represented approximately the endogenous wear and tear quota. Four days of starvation followed, on the second day of which 70 g. gelatin containing 11.8 g. nitrogen and 0.3368 g. sulphur with a S : N ratio of 1 : 35 were ingested in three portions during the course of the day. At the end of starvation the nitrogen-free diet was resumed until the nitrogen output had returned to its original level. Exp. 2 was a control in which no gelatin was taken. The excess nitrogen output above the wear and tear quota was found

Table I.

Exp. 1.		Total N	Urea-N	Ammonia-N	Uric acid	Total S	Total P ₂ O ₅	S : N
Date	Diet	g.	g.	g.	mg.	g.	g.	
1924								
Feb. 4	Basal	4.900	3.416	0.252	327	0.341	1.564	1 : 14.3
" 5	"	3.808	2.425	0.235	295	0.253	1.150	1 : 15.5
" 6	"	3.528	2.184	0.252	262	0.231	0.923	1 : 15.2
" 7	Starvation	3.248	2.072	0.168	270	0.247	0.979	1 : 13.1
" 8	70 g. gelatin	7.364	5.510	0.369	337	0.407	0.781	1 : 18.1
" 9	Starvation	8.176	6.597	0.319	252	0.390	1.547	1 : 20.9
" 10	"	9.324	7.974	0.482	257	0.555	2.059	1 : 16.8
" 11	Basal	6.636	4.592	0.392	440	0.357	0.682	1 : 18.3
" 12	"	3.920	2.240	0.664	350	0.253	0.866	1 : 15.5
" 13	"	3.360	2.072	0.298	307	0.231	0.823	1 : 14.5
Exp. 2.								
1925								
Feb. 23	Basal	5.096	3.584	0.337	389	0.357	1.405	1 : 14.2
" 24	"	4.458	3.178	0.350	412	0.324	1.391	1 : 13.7
" 25	"	4.144	2.968	0.336	389	0.346	1.278	1 : 11.9
" 26	"	4.816	3.364	0.336	350	0.527	0.951	1 : 9.1
" 27	"	11.008	9.394	0.742	389	0.774	2.442	1 : 14.3
" 28	"	13.196	11.920	1.260	475	0.802	3.436	1 : 16.4
Mar. 1	"	11.872	9.512	1.426	587	0.819	3.138	1 : 14.4
" 2	"	8.120	6.440	0.784	637	0.484	1.058	1 : 16.7
" 3	"	4.500	3.010	0.546	487	0.423	0.951	1 : 10.6
" 4	"	3.752	2.548	0.420	362	0.357	1.192	1 : 10.5

by subtracting the nitrogen output on the day before starvation from that of the starvation and subsequent days until the urinary output had reached the original value. Calculating we find:

Excess nitrogen above basal value =	17.5 g.
„ sulphur „ „ =	0.8244 g.
S : N ratio of excess =	1 : 21.2
Nitrogen ingested =	11.8 g.
Sulphur „ =	0.3367 g.

The real loss of nitrogen above the basal value is therefore 5.7 g. and of sulphur 0.4877 g. with a S : N ratio of 1 : 11.7.

The total nitrogen output on the four starvation and two post-days (not subtracting the basal value) is 38.667 g. and that of sulphur 2.209 g., the S : N ratio being 1 : 17.8. If the nitrogen and sulphur of the gelatin are subtracted from these figures the total loss to the body is 28.8 g. nitrogen and 1.877 g. sulphur with a S : N ratio of 1 : 14.3, a figure which corresponds with the S : N ratio of muscle. It is clear that the tissues have not metabolised a sulphur-rich protein in order to improve the quality of the sulphur-poor gelatin. In Exp. 2, the control, we find the balance:

Excess nitrogen above the basal = 26.64 g.
 „ sulphur „ „ = 1.75 g.
 S : N ratio of excess = 1 : 16.2

The total output on the other hand is 53.512 g. nitrogen and 3.8 g. sulphur with a S : N ratio of 1 : 13.9, approximately that of muscle tissue. It would seem that the protein tissue utilised in starvation was muscle and that the giving of some other protein such as gelatin does not cause the body to break

down tissue protein of a different nature. The average S : N ratio of Cathcart's subject, Beauté, for the 14 starvation and 3 post-days is 1 : 13·7 [Cathcart, 1907]. Benedict's subject, Levanzin, for the first 14 days of his fast had a S : N ratio of 1 : 15·7, which Benedict considers to be due possibly to the breakdown of sulphur-poor material [Benedict, 1915].

It seems anomalous that the total nitrogen output in Exp. 2, when no gelatin was taken, should be greater than in Exp. 1. The result might suggest that, contrary to all previous findings, gelatin has a considerable sparing action on tissue breakdown in starvation. A possible and indeed probable explanation is the fact that in Exp. 1 the subject had just recently carried out a series of starvation experiments and was hence in a nitrogen-poor condition. Exp. 2 was carried out a year later following a period of normal diet. In addition it is possible that in starvation the absolute metabolism of protein is reduced. Hawk, Howe and Mattill [1911] noted such a reduction in a dog, the nitrogen output during a second fasting period being much lower than during the first. Benedict [1919] states that after a period of restricted diet the total metabolism is reduced; he considers that the loss of nitrogenous material from the fluids bathing the cells may diminish the specific stimulus to metabolism. In Exp. 2 the body had evidently a store of some labile nitrogen which was wanting in Exp. 1. From the great difference in the total nitrogen loss in the two experiments it is probable that gelatin has exercised some sparing action although it is difficult to say to what extent this has taken place.

An examination of the S : N ratio of the days following the taking of the gelatin shows that it was being metabolised up to the last day of the experiment. The ratio falls only to 1 : 14·5 on the last day of the experiment. In the control it has reached on the third post-day a value of 1 : 10·5. In a similar experiment (Exp. 1) in the previous paper [Wilson, 1925] it reached the value 1 : 10·4. A rise to 1·99 was also observed in Cathcart's subject, Beauté, on the third day of starch and cream following a fast [Cathcart, 1907]. The conclusion to be drawn is that some of the gelatin has been retained in the tissues for 6 days. It seems unlikely that it could have been circulating in the amino-acid form during this period and the conclusion may be warranted that it has been retained in some more complex form and that later this material became metabolised, possibly playing a part where a biologically complete protein was not essential.

As regards the uric acid output it will be seen that it is roughly proportional to the total nitrogen output; that is, it is greater in Exp. 2 than in Exp. 1. Another striking point is the immediate rise in uric acid output on the first post-day when the diet was resumed. This favours the idea that carbohydrate is necessary for the formation of the purine ring as suggested by Cathcart [1922]. The highest uric acid output was observed on the second post-day in Exp. 2 and is possibly accounted for by the fact that acetone was still present in the urine on the first post-day; the carbohydrates ingested

may thus have been stored on this day either as glycogen or possibly as a hexosephosphoric ester (it will be noted that the phosphorus output dropped on the first post-day in Exp. 2 from 3.13 to 1.05 g.). The sharp drop in the phosphorus output is partially accounted for by the reduction in acidosis, as is seen from the fall in the ammonia output.

The conclusions as to the influence of meat extractives on protein metabolism have been rather conflicting. Burgi [1904] found, on giving Liebig's extract to starving dogs, that it exerted no sparing action on the nitrogen output and in addition was mostly excreted unchanged, 5 % only being unaccounted for. Rubner [1904] came to the same conclusion but qualified it by stating that if the tissues are poor in certain constituents present in the extract a retention might occur. He also showed that the carbon-poor extractives were excreted before the carbon-rich. Thompson [1910] stated that extractives improve the digestibility of the food taken with them and promote nitrogen retention. In the present investigations (Exp. 3, Table II) 25 g.

Table II.

Exp. 3.

Date 1924	Diet	Total N g.	Urea- N g.	Ammonia- N g.	Uric acid mg.	Total S g.	Total P ₂ O ₅ g.	S : N
May 25	N-free basal	5.152	3.711	0.241	350	0.522	1.630	1 : 9.8
" 26	"	3.948	2.844	0.207	342	0.341	1.164	1 : 11.5
" 27	"	3.388	2.290	0.202	330	0.258	0.956	1 : 13.1
" 28	"	2.744	1.717	0.274	242	0.247	0.908	1 : 11.2
" 29	"	3.416	0.789	0.252	580	0.313	2.101	1 : 10.9
" 30	+ 25 g. extractives	2.917	1.601	0.190	387	0.231	1.562	1 : 12.2
" 31	"	2.576	1.400	0.168	350	0.247	1.107	1 : 10.4

meat extractives were superimposed on a nitrogen-free diet when the daily nitrogen output had fallen to 2.744 g. It will be noted that the output rose on the day of ingestion to 3.416 g. and on the second post-day had fallen below the value of the pre-day. As the basal value on the day before taking the extractives might be too high (the endogenous output having scarcely reached its minimum figure) the basal value was taken as the mean between the pre-day and the last post-day. The excess nitrogen output due to the taking of the extractives was 1.029 g. and the sulphur 0.0494 g. with a S : N ratio of 1 : 20. The extractives taken contained 2.689 g. nitrogen and 0.1236 g. sulphur with a S : N ratio of 1 : 19.7. The amount stored is thus 1.661 g. nitrogen and 0.0742 g. sulphur with a S : N ratio of 1 : 22.0. If on the other hand the pre-day figure alone be utilised as the basal value a retention above the basal value of 1.844 g. nitrogen and 0.742 g. sulphur with a S : N ratio of 1 : 24.8 is found. Under the conditions of the experiment the tissues have retained a material which is definitely sulphur-poor. It will be noted that, on the nitrogen-free diet, the S : N ratio tends to rise, *e.g.* Exps. 2 and 3; this signifies a relative retention of nitrogen or, expressed otherwise, a lag in the excretion of nitrogen behind the sulphur. It seems extremely probable that it is this tendency for nitrogen in preference to sulphur retention which

has favoured the storing of sulphur-poor material from the ingested extractives. It will also be noted that the urea output on the day of ingestion is markedly reduced both relatively and absolutely. The relative decrease is to be explained by the elimination in the urine of nitrogen-containing extractives; the uric acid output, for instance, is doubled. The absolute decrease is peculiar because on the first post-day it returns to its pre-day value; there is no compensatory increase in the ammonia output to account for it. The suggestion is made that the extractives must have influenced the formation of urea, probably by diminishing the deaminising processes. The conclusions to be drawn are, firstly, that under suitable conditions, such as nitrogen starvation of the tissues, meat extractives can be stored up to 70 % of their nitrogen content, and, secondly, that the extractives do not stimulate protein metabolism but, on the contrary, seem rather to inhibit it and thus favour storage.

The effect of water ingestion on protein metabolism has been a subject of investigation since Voit's time. Voit [1881] noted an increase in the urea output of a dog after the ingestion of excess water. He concluded that it was not due to a washing out as there was no increase in the salt output which, owing to its higher concentration in the blood than urea, should have been increased if washing out were the cause. His conclusion was in favour of an increase in protein catabolism. Heilner [1907] came to the same conclusion and noted that the effect was not observed in a well-fed animal but only in a starving one. Hawk [1910] in his experiments on human subjects showed that excess water ingestion improved digestion and assimilation and promoted the retention of protein. Orr [1914] found that it increased the absolute and relative amount of urea and concluded that water stimulated the catabolism of protein and promoted subsequent retention of nitrogen. The present experiments are an attempt to elucidate whether water ingestion causes a mere washing out of the tissues or stimulates protein metabolism. It was thought that the consideration both of the sulphur and the nitrogen output would aid the solution of the problem. Three series of experiments were therefore performed. In series A (Table III) a constant diet of bread and cheese was taken until nitrogen equilibrium was obtained. The S : N ratio was not very constant but had an average of 1 : 14. Two litres of water were ingested on two separate days and the effect on the output noted. On the second water-drinking day the day and night urines were collected and estimated separately. It will be noted that on each occasion the ingestion of water brought about a rise in the nitrogen output and a fall in the S : N ratio. An examination of the day and night urine of the second water-drinking day shows that the increase in nitrogen output occurred during the day. On each occasion the S : N ratio rose on the following day along with a retention of nitrogen. The sulphur on the other hand scarcely shows any rise at all; nevertheless on the subsequent day there was a retention of sulphur. The results of these experiments were not definite but if anything,

Table III.

Series A.

Date 1924	Diet	Urine cc.	Total N g.	Uric acid mg.	Total S g.	Total P ₂ O ₅ g.	S : N
Nov. 3	Basal	660	8.592	488	0.615	2.399	1 : 13.9
" 4	"	640	8.540	512	0.604	2.428	1 : 14.1
" 5	"	640	9.072	540	0.654	2.442	1 : 13.8
" 6	"	630	8.932	546	0.665	2.338	1 : 13.4
" 7*	"	1600	9.982	537	0.670	2.712	1 : 14.7
" 8	"	750	8.708	540	0.604	2.634	1 : 14.3
" 9	"	640	8.848	536	0.610	2.385	1 : 14.5
" 10	"	550	8.736	545	0.621	2.442	1 : 14.0
" 11	"	560	8.764	505	0.626	2.343	1 : 13.9
" 12* Day	"	1196	5.339	231	0.317	1.378	1 : 16.8
" Night	"	630	4.284	—	0.383	1.278	1 : 13.6
" Total		1826	9.625	—	0.630	2.656	1 : 15.2
" 13	"	900	8.036	461	0.588	2.201	1 : 13.7
" 14	"	700	8.492	526	0.560	2.560	1 : 14.4
" 15	"	700	8.456	532	0.615	2.428	1 : 13.7

Series B.

Date 1925	Diet	Urine cc.	Total N g.	Urea- N g.	Ammonia- N g.	Uric acid mg.	Total S g.	Total P ₂ O ₅ g.	S : N	Urea-N % of total N
Oct. 23	Basal	900	9.800	8.287	0.494	547	0.593	2.428	1 : 16	84
" 24	"	1000	9.772	7.985	0.414	500	0.637	2.130	1 : 15.3	81
" 25	"	800	9.800	7.940	0.571	612	0.665	2.272	1 : 14.7	80
" 26†	"	2600	10.080	8.636	0.604	615	0.676	2.424	1 : 14.9	85
" 27	"	800	9.204	7.834	0.398	370	0.632	2.130	1 : 14.5	85
" 28†	"	3200	10.136	8.758	0.538	520	0.615	2.840	1 : 16.9	86
" 29	"	900	8.988	7.431	0.437	472	0.626	2.456	1 : 14.3	82
" 30	"	800	9.268	7.558	0.364	487	0.593	2.399	1 : 15.7	81

Series C.

Nov. 2	Basal	700	7.868	6.204	0.375	475	0.588	2.499	1 : 13.3	78
" 3	"	700	7.672	6.333	0.302	370	0.604	2.485	1 : 12.6	82
" 4	"	700	7.948	6.661	0.325	421	0.670	2.683	1 : 11.8	80
" 5†	"	2940	8.148	7.022	0.538	486	0.643	2.683	1 : 12.6	86
" 6	"	1100	7.098	5.630	0.370	487	0.610	2.619	1 : 11.6	79
" 7	"	980	7.364	6.338	0.330	482	0.626	2.627	1 : 11.6	86
" 8	"	620	7.600	6.120	0.347	555	0.648	2.556	1 : 11.7	80
" 9†	"	2300	8.064	7.168	0.588	516	0.626	2.641	1 : 12.8	88
" 10	"	700	6.748	5.432	0.319	487	0.626	2.201	1 : 10.7	80
" 11	"	660	7.128	5.678	0.342	482	0.621	2.485	1 : 11.4	79

* 2 litres water drunk.

† 2800 cc. water drunk.

except for the failure of the sulphur to increase, they favour the theory that it is a mere washing out process. Further experiments were carried out in which the basal S : N ratios were definitely divergent. Series B (Table III) consisted of a diet of bread and gelatin which had a low S : N ratio; series C consisted of a diet of bread and dried eggs with a high ratio. In addition urea and ammonia were estimated in series B and C. On each diet there were two water-drinking days when 2800 cc. water were taken. It will be noted that with the low S : N in series B the ratio fell on both water-drinking days but most definitely on the second water day. The slight change of

S : N ratio on the first day may be due to the fact that the increased nitrogen output is only 0.208 g. The urea output on the other hand increased 0.6 g. but the sulphur increased only 11 mg. On the second water day the ratio definitely fell. Although the nitrogen output increased about 0.8 g. the urea output increased over 1 g. The sulphur output on the other hand was reduced. In series C with the high S : N ratio the same phenomena are seen, a fall in the ratio and a rise in the nitrogen output, whilst the urea is increased both absolutely and relatively. The sulphur is decreased in both cases and there is a tendency to retention on the subsequent days which more than compensates for the loss on the day of water ingestion. It was hoped, if the effect of water was to stimulate tissue metabolism, that the gelatin series would show a rise; *i.e.* assuming that the water stimulates the breakdown of muscle tissue with a S : N ratio of 1 : 14.3, which would clearly tend to raise the low ratio of the gelatin series. In series C on the other hand one would expect the ratio to fall if tissue breakdown occurs. If, however, water stimulated the metabolism of the protein ingested at the time the ratio should remain the same. If it is a case of washing out, on the other hand, the ratio should fall in each experiment. Although the results would seem to favour the last view, there are three features which require explanation, firstly, the sulphur output nearly always tends to fall; secondly, the increase in the urea-nitrogen is greater than that of the total nitrogen; thirdly, the subsequent storage, which more than compensates the previous loss. The increase in the urea would suggest that the ingestion of water brings about a qualitative change in metabolism. The increased formation of urea must have been from materials which normally would have been excreted in some other form as residual nitrogen, amino-acids, purines etc. Howe, Mattill and Hawk [1912] have shown that the purine output is diminished in a starving dog after water ingestion. Orr [1914] concludes that water ingestion increases the catabolism of protein and promotes nitrogen retention. The present experiments also seem to show that water causes an increased catabolism of the nitrogen moiety of the protein molecule and simultaneously promotes storage. The fact of a small reduction in the sulphur output on the water day would suggest that the storage of sulphur commences on that day and favours the suggestion made in the previous paper [Wilson, 1925] that it is the sulphur which is apparently stored first.

SUMMARY.

1. A protein such as gelatin, when ingested during starvation, can be retained in part for at least six days. It is probable that the retention takes place in some complex form and not as the free amino-acids.
2. Meat extractives ingested during nitrogen starvation are in part retained. The material retained does not correspond in composition to muscle

tissue. Extractives further seem to exercise some kind of inhibitory influence on nitrogen catabolism.

3. Excess water ingestion increases the output of nitrogen in the urine by stimulating the catabolism of the nitrogen moiety of the protein molecule. The capacity of the tissues to retain protein also seems to be stimulated.

4. Further evidence is adduced in favour of the view that in the storage of protein the sulphur is retained first.

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