XLIX. THE RELATION BETWEEN SULPHUR AND NITROGEN METABOLISM.

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THE purpose of the present paper was an attempt to elucidate the following controversial points in protein metabolism.

1. Is the breakdown of ingested protein immediate or is it spread over several days?

2. Is there any difference in the rates at which nitrogen and sulphur are excreted?

3. If ingested protein can be retained is it capable of covering the breakdown of body protein in the first few days of starvation?

4. What is the nature of the stored material?

Is ingested protein dealt with immediately? This question was repeatedly investigated by the older workers, but it was not until the introduction of the superimposition method of Falta that clear proof was obtained that a real delay in excretion may take place. Falta [1906] gave a subject a constant diet until nitrogen equilibrium was established and then on one day superimposed on this diet a known amount of the protein to be investigated. The course of its excretion was then followed for several days. The amount of the protein excreted in each particular day was found by subtracting the total nitrogen output on the basal diet from the nitrogen output on the day concerned. He found that the time of excretion varied with different proteins. Hämäläinen and Helme [1907], using this method, found the same delay in the metabolism. The general conclusion is that there is a retention in some form of the nitrogen of the food.

Is there any difference in the rate at which the nitrogen and sulphur of the ingested protein is excreted? Feder [1881], by following the outputs of nitrogen and sulphur in 2-hourly samples of the urine of a dog after meat ingestion, found an early excretion of sulphur and a delay in the nitrogen output. He also emphasised the importance of 2-hourly samples as opposed to the 24-hourly collection. Falta [1906] found, taking the 24-hour urine, an early excretion of sulphur after the ingestion of egg white and caseinogen. Hämäläinen and Helme [1907] obtained the same result with egg white, veal and proton (caseinogen). Wolf and Osterberg [1911] maintained that when egg white, either raw or coagulated, was superimposed sulphur was more slowly excreted

than nitrogen in the early hours. Lewis [1916] found a lag in the sulphur output as compared with the nitrogen on feeding meat to dogs after starvation. Cathcart and Green [1913], on the other hand, observed a nitrogen lag after the ingestion of gelatin and egg white. The evidence then as regards the rate of excretion of sulphur and nitrogen is on the whole rather contradictory. In regard to the nature of the material stored there has been a certain amount of inconclusive work. Falta thought the variation in the rate of excretion of the nitrogen and sulphur after the ingestion of different proteins was due to the differences in their resistance to breakdown. Ehström [1906], on the other hand, considered that the material was stored in some easily metabolisable form. Obviously, however, if there are differences in the rate at which nitrogen and sulphur are excreted then the stored material must differ in composition from the protein ingested. In the present experiments an attempt has been made to elucidate the problem by starving the subject after the superimposition of some definite protein and following the nitrogen and sulphur outputs.

The experiments described in this paper fall into four groups. These were all carried out on myself, a healthy subject weighing 60 kg. The first series was designed to find the effect of two different proteins on the nitrogen output during the first few days of starvation. In order to eliminate the effect of previous food a nitrogen-free diet was taken until the nitrogen excretion for 24 hours was below 3 g., which is about the lowest that can be reached. Then the protein to be investigated was superimposed for one day. Two days of starvation followed and on the third day the basal diet was resumed until the nitrogen output had fallen to the original value. In order to have a basis of comparison a starvation experiment was carried out without the superimposition of protein: this constituted Exp. 1 (Table I). In Exps. 2 and 3 gelatin and egg white respectively were the proteins superimposed. These two proteins were chosen because of the marked contrast in their sulphur content or S : N ratio. The nitrogen-free diet consisted of olive oil, 100 g., tapioca, 300 g., sugar, 100 g.

The second group consisted of two experiments in which gelatin and egg white were superimposed on a basal diet containing 6 g. nitrogen. The third group was the same except that the basal diet contained about 11 g. nitrogen. The fourth was the same as the first except that no starvation followed the taking of the proteins. In all these experiments the 24-hour output of urine was collected under toluene and analysed on the following day. Total nitrogen was determined by the Kjeldahl method, urea and ammonia by the urease method, phosphorus by the uranium acetate method and uric acid by the Hopkins-Folin method.

Referring to Table I it will be noted that the urinary constituents fell more or less evenly for the first few days. Taking the S : N ratios, however, a distinct rise is seen from 1 : 15·8 on the first day to 1 : 12·3 on the day before starvation. This means that when the body is on a nitrogen-free diet and

Table I.

Date	Diet	Total N g.	Ammonia N g.	Uric acid mg.	Total P (as P_2O_5) g.	Total S g.	S : N	P_2O_5 : N
9 Jan.	Basal	4·788	0·3640	450	1·7892	0·3022	1 : 15·8	1 : 2·6
10 ,,	,, ...	4·382	0·4134	327	1·8316	0·2748	1 : 15·9	1 : 2·3
11 ,,	,, ...	3·052	0·3080	212	1·4200	0·2528	1 : 12·0	1 : 2·1
12 ,,	,, ...	2·996	0·3024	210	1·1644	0·2418	1 : 12·3	1 : 2·5
13 ,,	Starving (Exp. 1)	2·492	0·1848	205	0·7524	0·2143	1 : 11·6	1 : 3·3
14 ,,	,, (,,)	4·872	0·3360	290	1·3488	0·4286	1 : 11·3	1 : 3·6
15 ,,	Basal	6·944	0·3808	337	1·2498	0·4012	1 : 17·3	1 : 5·5
16 ,,	,, ...	4·480	0·3966	225	1·4340	0·2638	1 : 16·9	1 : 3·1
17 ,,	,, ...	2·996	0·3920	257	1·1369	0·2857	1 : 10·4	1 : 2·6
18 ,,	Gel. supsd (Exp. 2)	5·901	0·5460	307	0·8520	0·2473	1 : 23·8	1 : 6·9
19 ,,	Starving	3·984	0·2352	327	1·0648	0·2693	1 : 11·0	1 : 3·7
20 ,,	,, ...	6·804	0·3024	302	1·4624	0·4836	1 : 14·0	1 : 4·6
21 ,,	Basal	8·284	0·5656	437	1·0224	0·5660	1 : 14·5	1 : 8·1
22 ,,	,, ...	4·228	0·3024	262	0·9088	0·2528	1 : 16·7	1 : 4·6
23 ,,	,, ...	3·332	0·2912	285	0·9228	0·2253	1 : 14·7	1 : 3·6
24 ,,	Alb. supsd (Exp. 3)	3·920	0·4088	312	0·7952	0·4876	1 : 8·0	1 : 4·9
25 ,,	Starving	5·628	0·2800	365	0·7808	0·5660	1 : 9·9	1 : 7·3
26 ,,	,, ...	7·112	0·3360	292	1·4484	0·4506	1 : 15·7	1 : 4·9
27 ,,	Basal	7·532	0·4368	415	1·1070	0·5056	1 : 14·8	1 : 6·8
28 ,,	,, ...	3·900	0·2912	299	0·8662	0·2693	1 : 16·7	1 : 4·5
29 ,,	,, ...	2·996	0·2856	270	0·9230	0·2583	1 : 11·5	1 : 3·2

drawing on its tissue protein there is either a breakdown of some sulphur-rich protein or the retention of the nitrogen of some protein of average sulphur content, *e.g.* muscle. If, however, there were a breakdown of, say, muscle tissue along with a nitrogen retention, this nitrogen should ultimately be excreted and bring down the S : N ratio. This is exactly what happened, as, on the return to the basal diet, the nitrogen rose almost 50 %, while the sulphur output fell a little. Corresponding to this the S : N ratio fell to 1 : 17, but by Jan. 17th rose again to 1 : 10·4. If the total nitrogen and sulphur output of the two starvation and two following days be taken, the S : N ratio of the sum is 1 : 14·3, which corresponds to the average value found for muscle. Apparently then there is either an alternating breakdown of sulphur-rich and sulphur-poor protein or else a breakdown of some protein such as muscle with a nitrogen retention. The sudden elimination of the retained nitrogen on the first day after starvation is probably due to the stimulating action of the food.

Had food been taken constantly the excretion of this retained nitrogen would have increased until the S : N ratio was 1 : 14·3. At this point the body would have a certain quantity of temporarily retained nitrogen in it but its excretion would equal the rate of formation and hence a S : N ratio of 1 : 14·3 would result if muscle were the source. The same rise in the S : N ratio was seen in Cathcart's subject [1907]. The S : N ratio rose from 1 : 14·7 on the last day of starvation to 1 : 9·9 on the third day of a nitrogen-free diet.

Both these experiments, however, show that the influence of carbohydrate on the sulphur moiety is more marked than on that of the nitrogen. This is to be expected, for, if the sulphur of the protein molecule is the first to be broken down and eliminated, the sparing effect of carbohydrate on protein breakdown should be directed primarily at the sulphur-containing group.

From the data of Exp. 1 it is possible to calculate the nitrogen and sulphur loss due to starvation alone by subtracting the basal value (the nitrogen and sulphur output on the day before starvation) from the total nitrogen for each day on which the nitrogen output exceeds the basal value. In this case the two starvation and two subsequent days are involved. The first starvation day gives a positive balance however.

Excess nitrogen output above basal value = 6·804 g.
„ sulphur „ „ „ „ = 0·3646 g.
S : N = 1 : 18·6.

This low ratio does not necessarily mean that sulphur-poor protein has been used for starvation metabolism. The explanation is that on the one hand the S : N ratio of the basal value is high, which shows up the early excretion of sulphur and the retention of the nitrogen. The S : N ratio of 1 : 18·6 on the other hand shows up the elimination of the retained nitrogen. As was stated above, the S : N ratio of those four days is 1 : 14·3. The conclusions drawn from this experiment are:

1. That the sulphur fraction of the protein molecule is broken down and eliminated in advance of the nitrogen. This applies to the breakdown of tissue protein when on a carbohydrate-fat diet.

2. The sparing action of carbohydrates on the breakdown of body protein is exerted first on the sulphur-containing moiety. Carbohydrate has no effect on the temporarily retained nitrogen and this cannot be used for synthesis as there is no adequate supply of sulphur present to make up the full complement necessary for the formation of protein.

The same procedure was carried out in Exp. 2, with the exception that 70 g. gelatin containing 11·8 g. nitrogen and 0·3367 g. sulphur with a S : N ratio of 1 : 35 were superimposed on the day before starvation. It will be noted that the same basal value as in Exp. 1 had been reached on the day before gelatin was taken. Gelatin was chosen as a type of sulphur-poor protein, as will be seen from the S : N ratio. On the day of superimposition the S : N ratio fell to 1 : 23 instead of rising. There is no advanced output of sulphur even, although the S : N ratio for the day (1 : 23) is higher than that of the gelatin taken, because the total sulphur output on the day of superimposition is actually lower than that of the previous day. The sulphur has been completely stored, while some of the nitrogen has been eliminated. On the first starvation day the S : N ratio showed a sharp rise to 1 : 11. The probable explanation of this change is that the first day of starvation produces a very definite reduction in the material metabolised. The nitrogen drops considerably, while the sulphur shows a slight rise. This slight rise is probably due to the metabolism of protein stored the previous day—possibly in an unstable form. Evidence of such an instability will be seen in later experiments. It is not considered, however, that the low S : N ratio on the day of superimposition and the high ratio the following day are to be attributed to a sulphur lag.

The explanation suggested is that in the storage of protein the sulphur fraction is stored first and the nitrogen moiety later. In other words it is the theory of the nitrogen lag applied to the anabolism of protein as well as to its catabolism. One can imagine the movements of the nitrogen fraction always following those of the sulphur. In protein catabolism the sulphur is eliminated first and then the nitrogen follows, whereas in protein anabolism the sulphur is built up first, then the nitrogen. When the influence of carbohydrates on the breakdown of body protein is considered, the effect is seen first on the sulphur output and later on the nitrogen output (Exp. 1). It will be noted that on the return to the basal diet the nitrogen and sulphur outputs both rose proportionately, as there was little change in the S:N ratio. The S:N ratio fell, however, the next day to 1:16·7, so that evidently there was some retained labile nitrogen in the body, though the S : N ratio never returned to its original high value. It is probable that the intake of sulphur-poor protein in the form of gelatin has saturated the tissues to their maximum capacity for holding sulphur-free nitrogen bodies; hence the failure to reach the previous high value of the S : N ratio. If these figures are treated as in Exp. 1 we find:

Excess nitrogen above basal value	= 14·557 g.
,, ,, ,, ,, ,, in Exp. 1 (pure starvation)	= 6·804 ,,
,, ,, ,, that in Exp. 1 = 14·557 − 6·804	= 7·753 ,,

Hence by taking 11·8 g. nitrogen in the form of gelatin before starving, 7·753 g. nitrogen were excreted above the output due to starvation alone, and hence 4·047 g. nitrogen were stored or utilised to cover the breakdown of body protein in starvation. In the case of the sulphur output the same calculation is made.

Excess sulphur above basal value	= 0·3704 g.
,, ,, ,, ,, for Exp. 1	= 0·3646 ,,
,, ,, ,, that in Exp. 1	= 0·0580 ,,

As 0·3363 g. were ingested it follows that 0·2783 g. were stored, or covered the sulphur output in starvation. The S : N ratio of the stored material is 1 : 14·5, while that of the gelatin was 1 : 35. If one be permitted to draw a deduction from the nitrogen and sulphur content there would seem to have been a selection among the various amino acids in gelatin in order to retain a substance corresponding to muscle protein.

In the case of phosphorus the total output in Exp. 2 is less by 0·0288 g. P_2O_5 than in Exp. 1. Taking the total nitrogen output in Exp. 1 from the day before starvation to the second post day we find it to amount to 21·77 g. nitrogen. In Exp. 2 it amounts to 29·8 g. nitrogen, but as 11·8 g. nitrogen were ingested the real loss to the body is 29·8 − 11·8 = 18 g. This is 3·77 g. nitrogen less than in Exp. 1; the stored phosphorus is 0·0288 g. P_2O_5 and the P_2O_5 : N ratio of this is 1 : 130. From these figures it can be seen that 58 % of the excess nitrogen output due to starvation can be spared by gelatin.

This is a higher value than those found by the older workers, and is possibly due to the different method of calculation employed. The method of calculation used in the present experiments narrows the question to finding the effect of gelatin on the excess output of nitrogen above the basal value due to starvation alone.

Experiments on the capacity of gelatin to cover the minimum wear and tear output on a nitrogen-free diet were carried out by Robison [1922]. He found that gelatin had very little sparing effect even although a large excess was taken. It would seem to follow that protein of a more specific nature is required to cover the endogenous wear and tear than to cover the excess output above this occasioned by starvation.

Exp. 3 consisted in superimposing egg white under the same conditions. Unfortunately in this experiment the basal value was a little higher on the day before superimposition than on the corresponding day of the previous experiment. 495 g. egg white, containing 8·87 g. nitrogen and 0·960 g. sulphur, with a S : N ratio of 1 : 9·2, were distributed evenly over the three meals on the day of superimposition. The egg white was obtained directly from fresh eggs and taken in the soft-boiled state. It will be noted that this is a sulphur-rich protein. On the day of superimposition the nitrogen output rose 0·59 g., while the sulphur output was nearly double that of the previous day. The S : N ratio accordingly rose to 1 : 8. On the first day of starvation the sulphur and nitrogen outputs both rose, which is in marked contrast to Exps. 1 and 2, where a fall was the rule. A possible explanation of this variation will be found in some of the later experiments. It is probably associated with a peculiar instability of egg white. The S : N ratio on this day is still high. On the second day of starvation the S : N ratio fell to 1 : 15·7, the sulphur output falling a little, while the nitrogen output rose. On the first post day, when the basal diet was resumed, there was the same rise in the nitrogen output though much less than in the previous experiments. The sulphur output also rose a little, but the S : N ratio fell to 16·7, apparently an excess output of nitrogen in relation to sulphur. On the last day the ratio rose to 11·5. In all three experiments a certain amount of temporarily retained nitrogen seems to be eliminated after starvation, but in Exps. 2 and 3 this occurs on the second post day, as deduced from the low S : N ratio, whilst in Exp. 1 it occurs mostly on the first post day. Owing to the fact that the original basal value was not reached in this experiment, there are two figures on which to base the calculations, either the actual basal value obtained for this experiment or the original one of 2·9 g. nitrogen. Taking the former we get:

Excess nitrogen above basal (3·3 g.)	= 11·432 g.
,, ,, ,, ,, in Exp. 1	= 6·804 ,,
,, ,, ,, that in Exp. 1 = 11·432 − 6·804	= 4·628 ,,

Hence 8·87 g. nitrogen in the form of egg white caused an excess output above that found in pure starvation of 4·628 g. nitrogen. As 8·87 g. nitrogen

were ingested it follows that 4·142 g. nitrogen were stored, or covered the breakdown of body protein during starvation.

Excess sulphur above basal	= 1·1792 g.
,, ,, ,, ,, in Exp. 1	= 0·3646 ,,
,, ,, ,, output in Exp. 1	= 0·8146 ,,

As 0·960 g. sulphur was ingested, 0·1454 g. was retained or covered the breakdown of body sulphur. The S : N ratio of the retained material is 1 : 31·7 —a low value. The storage of sulphur is only 0·1454 g. in this experiment, while in Exp. 2 with gelatin it was almost twice as much. It is rather an anomaly that the sulphur retention should have been so low with the sulphur-rich protein as compared with the sulphur-poor gelatin. It is unlikely that some other limiting factor is associated with egg white, such as a minimal percentage of some essential amino acid, especially as it is a much more perfect protein than gelatin. There is the possibility, however, that egg white brought about the temporary retention of some unstable sulphur-rich protein which was quickly broken down. It has recently been shown to contain only 15 % of its sulphur in the form of cystine, so that the effective content of egg white in this amino acid might be low [Harris, 1923]. This presupposes that cystine is the only form of sulphur which the body can utilise. It is questionable however if the sulphur in gelatin is in the form of cystine [Dakin, 1920], though it was, nevertheless, well utilised. The percentage retention of the nitrogen of the white was nearly 50 %, whilst in the case of gelatin it was only 31 %. There is, however, an inconsistency in the above calculations. The excess for this experiment was calculated with 3·3 g. nitrogen as a basal value. The excess output was then compared with the control Exp. 1 in order to see the effect of the egg white on starvation. The excess output on pure starvation (Exp. 1) was calculated on a basal figure of 2·9 g. nitrogen. Hence, to make the comparison more valid, the excess output for pure starvation should be recalculated with 3·3 g. nitrogen as a basal figure. This result should then be subtracted from the excess output found in Exp. 3 with 3·3 g. nitrogen as a basal figure. This recalculation gave an excess output for pure starvation of 5·66 g. nitrogen. The excess output above this in Exp. 3 then becomes 3·098 g. nitrogen: applying the same method to the sulphur we find that 0·1867 g. was stored. The S : N ratio of this stored material is 1 : 16·5, which appears more reasonable than the ratio based on the first calculation. If we take 2·9 g. as the basal figure, a storage of 2·562 g. nitrogen and 0·2960 g. sulphur is found. The S : N ratio in this case is 1 : 9·6. This would bear out the hypothesis that the body stores sulphur first.

These three methods of calculation have each yielded a different S : N ratio, namely, 1 : 31·7, 1 : 16·5 and 1 : 9·6. It is most likely that the value 1 : 16·5 is the correct one but it is possible that the long period of nitrogen starvation may have so altered conditions that a sulphur-rich or a sulphur-poor protein has been stored. From the last two experiments it cannot be definitely

stated whether the body stores protein whose composition corresponds to that of the food on the one hand or to that of muscle on the other. On the whole the evidence from Exp. 2 with gelatin favours the view that the protein stored corresponds in its sulphur content to muscle tissue. The same can be said of Exp. 3 if we accept the basis of calculation which gives a S : N ratio of 1 : 16·5. There is a certain amount of evidence that in the process of storing the sulphur may be retained first and the nitrogen later, and thus, although the end result is to have a protein of average S : N ratio, for a short time during storage a sulphur-rich protein may exist; and *vice versâ* in the breakdown of protein.

Table II.

Date	Diet	Total N g.	Ammonia N g.	Uric acid mg.	Total P (as P_2O_5) g.	Total S g.	S : N	P_2O_5 : N
23 Feb.	Basal	5·460	0·3472	280	1·491	0·3737	1 : 14·6	1 : 3·6
24 „	„	5·544	0·3640	322	1·526	0·3242	1 : 17·1	1 : 3·6
25 „	„	5·768	0·4480	302	1·562	0·4396	1 : 11·9	1 : 3·6
26 „	„	5·537	0·4256	370	1·278	0·4836	1 : 11·1	1 : 4·0
27 „	„	6·552	0·3752	467	1·278	0·5056	1 : 12·9	1 : 5·1
28 „	Gel. supsd (Exp. 4)	9·240	0·6992	457	1·313	0·7969	1 : 11·5	1 : 7·03
29 „	Basal	6·860	0·5992	312	1·171	0·5605	1 : 12·2	1 : 4·0
1 March	„	6·468	0·6608	322	1·242	0·4461	1 : 14·4	1 : 5·2
2 „	„	6·244	0·6104	462	1·207	0·4561	1 : 13·6	1 : 5·1
3 „	„	6·076	0·5040	407	1·936	0·4561	1 : 13·6	1 : 5·8
4 „	Alb. supsd (Exp. 5)	7·252	0·4984	462	1·312	0·7912	1 : 9·1	1 : 5·5
5 „	Basal („)	8·344	0·5432	565	1·312	0·7473	1 : 11·1	1 : 6·3
6 „	„	6·076	0·4816	360	1·278	0·4467	1 : 13·6	1 : 4·7
7 „	„	6·272	0·4312	542	1·3845	0·4506	1 : 13·9	1 : 4·5

Exps. 4 and 5 were carried out with the same two proteins, superimposed in this case on a diet containing about 6·4 g. nitrogen, but without subsequent starvation. In Exp. 4, where the nitrogen output on the pre-days is unfortunately rather irregular, 70 g. gelatin containing 9·939 g. nitrogen and 0·3750 g. sulphur with a S : N ratio of 1 : 26·5 were superimposed. The S : N ratio was 1 : 12·9 on the day before superimposition (Table II). This ratio rose on the day gelatin was taken, which is in contrast with Exp. 2. The ratio rose to 1 : 11·5. The nitrogen and sulphur outputs both fell to their basal value by the fourth day, whilst the S : N ratio fell a little below its basal value. It will be noted that the S : N ratio was highest on the day of superimposition and lowest on the third day, so that there was clearly a nitrogen lag. The output of both nitrogen and sulphur was highest on the day of superimposition. The balance is calculated by subtracting the total nitrogen output on this diet from the output on the day of superimposition and subsequent days.

Nitrogen excreted in excess of basal = 2·996 g.
„ ingested = 9·939 „
„ retained = 9·939 − 2·996 = 6·943 „
Sulphur excreted in excess of basal = 0·3459 „
„ ingested = 0·3750 „
Sulphur retained = 0·3750 − 0·3459 = 0·0291 „
S : N ratio of stored material = 1 : 22·8.

In this case the material stored from gelatin is much poorer in sulphur than in Exp. 2. The conditions however are not quite identical. In Exp. 2 the body had lost protein and was in a relatively sulphur-poor condition, as is seen from the high S : N ratio before the gelatin was superimposed. In Exp. 4, on the other hand, the body is in nitrogen equilibrium with a protein of high sulphur content as is seen from the high S : N ratio on the day before superimposition (1 : 12·9). It is possible that the body was relatively saturated with sulphur groups and retained the nitrogen of the gelatin in order to lower the S : N ratio of the circulating protein to an average.

It will be noted that 6·9 g. of the 9·9 g. nitrogen of the gelatin ingested was retained. This is a high value for gelatin, which is considered to be a protein of low biological value, so that in all probability the basal diet improved the quality of the gelatin as a protein and made it suitable for storage.

Exp. 5 was carried out in the same way with egg white, 500 g. of this containing 19·296 g. nitrogen and 1·059 g. sulphur, with a S : N ratio of 1 : 9·6, being superimposed. On the day of superimposition the nitrogen output rose only 1·2 g., whilst the sulphur output did not exceed that on the day gelatin was taken; nevertheless the S : N ratio rose to 1 : 9·1. On the next day the nitrogen output rose 2·3 g. above the basal value, whilst the sulphur output and the S : N ratio fell a little. The maximum output of nitrogen is on the second day in contrast to what was seen in Exp. 4. This late rise seems to prove the extreme lability of the material stored from egg white. This also probably accounts for the rise in nitrogen output in Exp. 3 on the first day of starvation, instead of the fall seen in Exps. 1 and 2. This lability is possibly conditioned partly by the high sulphur content and partly perhaps by the nature of the sulphur moiety of egg white. The material stored and broken down again is evidently sulphur-rich, as the S : N ratio is high for the first two days, whilst on the third day, when the nitrogen output has reached its basal figure, the S : N ratio is 1 : 13·6, a little below the average for the experiment. It will also be noted that the sulphur has preceded the nitrogen output. The following figures show the balance.

Nitrogen excreted above the basal	= 3·440	g.
,, ingested	= 10·206	,,
,, retained = 10·206 − 3·440	= 6·766	,,
Sulphur excreted above the basal	= 0·6262	,,
,, ingested	= 1·059	,,
,, retained = 1·059 − 0·6262	= 0·4332	,,
S : N ratio of the stored material	= 1 : 15·2.	

In this experiment there has been a much better utilisation of sulphur than in Exp. 2. Again the body has selectively retained a material corresponding in its nitrogen and sulphur content to muscle tissue. It is necessary to remark that, although 0·4332 g. sulphur was retained, it does not follow that it was the sulphur of the egg white. It is quite possible that most of

the egg white sulphur was excreted and the sulphur of the basal diet stored in order to build up a protein with the nitrogen of the egg white. A later experiment lends support to this view.

Table III.

Date	Diet	Total N g.	Ammonia N g.	Uric acid mg.	Total P (as P_2O_5) g.	Total S g.	S : N	P_2O_5 : N
22 March	Basal	11·116	0·504	495	1·948	0·610	1 : 18·2	1 : 5·7
23 ,,	,, ...	10·500	0·504	440	1·5713	0·560	1 : 18·7	1 : 6·6
24 ,,	,, ...	10·416	0·5152	482	1·929	0·599	1 : 17·3	1 : 5·3
25 ,,	,, ...	11·284	0·470	462	2·214	0·588	1 : 19·1	1 : 5·0
26 ,,	Gel. supsd (Exp. 6)	16·520	0·739	607	2·692	0·901	1 : 18·3	1 : 6·1
27 ,,	Basal	14·448	0·750	600	2·258	0·626	1 : 23·0	1 : 6·3
28 ,,	,,	13·272	0·694	565	2·498	0·670	1 : 19·7	1 : 5·3
29 ,,	,,	11·200	0·560	487	2·857	0·615	1 : 18·1	1 : 3·9
30 ,,	,,	11·680	0·539	467	2·333	0·582	1 : 20·0	1 : 5·0
31 ,,	Alb. supsd (Exp. 7)	13·888	0·649	590	2·491	1·198	1 : 11·6	1 : 5·5
1 April	Basal	15·232	0·772	565	2·737	1·187	1 : 12·8	1 : 5·7
2 ,,	,,	11·312	0·694	402	1·944	0·7364	1 : 15·3	1 : 5·7
3 ,,	,,	11·704	0·660	467	1·884	0·747	1 : 15·6	1 : 6·7

Exps. 6 and 7 (Table III) were the same as the preceding, but the basal diet was much richer in nitrogen. In Exp. 6 gelatin was taken in the same quantity as in Exp. 4. The average S : N ratio of the pre-days was 1 : 18·3, but the actual value on the day before superimposition was 1 : 19·1. On the day of superimposition it rose to its average value of 1 : 18·3. On the next day it fell to 1 : 23 and had returned to its average value two days later. It will be noted that there was a distinct nitrogen lag. The maximum output of nitrogen occurred on the day of superimposition and four days elapsed before it fell to the basal value. Five days elapsed before the sulphur came down to its basal value. The following is the balance for the period.

Nitrogen excreted above the basal value = 10·574 g.
,, ingested = 9·939 ,,
,, lost = 10·574 − 9·939 = 0·635 ,,
Sulphur excreted above the basal value = 0·4605 ,,
,, ingested = 0·3750 ,,
,, lost = 0·4605 − 0·3750 = 0·0850 ,,
S : N ratio of material lost = 1 : 10·2.

This small loss of both nitrogen and sulphur may either be real and due to the stimulating effect of gelatin on protein metabolism such as was observed by Cathcart and Green [1913], or it may simply fall within the limits of experimental error. The most important point is that when gelatin is superimposed on a basal diet of high nitrogen content, it is apparently completely rejected. In Exp. 7, 500 g. egg white containing 9·8 g. nitrogen and 1·204 g. sulphur with a S : N ratio of 1 : 11·6 were superimposed. On the day of superimposition the output of sulphur was nearly doubled, whilst that of the nitrogen rose only 2·4 g. The S : N ratio correspondingly rose to 1 : 11·6, almost double that of the pre-day (1 : 20). On the next day there is a repetition of what was seen in Exp. 5, the sulphur output falling a little whilst the

nitrogen output rose nearly 4 g. The same sharp fall is seen on the subsequent day but the sulphur never returned to its basal value. The S : N ratio continued to fall but failed to reach the original value. Taking as the basal value an average of the pre-days as in Exp. 7, there is the following balance:

Nitrogen excreted above the basal value	= 7·0 g.
,, ingested	= 9·8 ,,
,, stored = 9·8 − 7·0	= 2·8 ,,
Sulphur excreted above the basal value	= 1·5171 g.
,, ingested	= 1·2040 ,,
,, lost = 1·5171 − 1·2040	= 0·3131 ,,

In this case there is a considerable loss in sulphur with a small retention of nitrogen. It will be noted that the best retention of egg white both as regards the nitrogen and the sulphur was in Exp. 5, where the basal diet contained about 6 g. nitrogen. Possibly at the high level of protein intake in this experiment the body is at its maximum capacity for retaining nitrogen. In Exps. 4 and 5 the total nitrogen of the faeces rose in each case (about 1 g. above the average) on the day after superimposition. In Exps. 6 and 7, where the daily average output in the faeces was 2·236 g., the rise was rather more than 1 g. This increase in the nitrogen of the faeces may be due to non-absorption, in which case there would probably have been a lack of absorption of a corresponding quantity of sulphur. This would reduce the calculated quantity of material stored, but its quality would remain as found.

Table IV.

Date	Diet	Total N g.	Ammonia N. g.	Uric acid mg.	Total P (as P_2O_5) g.	Total S g.	S : N	P_2O_5 : N
14 April	Basal	4·312	0·408	327	2·101	0·3902	1 : 10·9	1 : 2·0
15 ,,	,,	4·256	0·3584	412	1·505	0·2528	1 : 16·8	1 : 2·8
16 ,,	,,	3·276	0·358	360	1·177	0·1978	1 : 16·5	1 : 2·7
17 ,,	,,	3·024	0·364	350	1·079	0·2088	1 : 14·4	1 : 2·8
18 ,,	,,	2·688	0·336	336	1·079	0·2088	1 : 12·8	1 : 2·4
19 ,,	Gel. supsd (Exp. 8)	4·564	0·476	345	0·568	0·4506	1 : 10·1	1 : 8·0
20 ,,	Basal	4·340	0·442	370	0·837	0·2528	1 : 17·1	1 : 5·1
21 ,,	,,	3·668	0·341	445	0·894	0·2528	1 : 14·4	1 : 4·1
22 ,,	,,	3·192	0·285	370	0·695	0·2308	1 : 13·8	1 : 4·5
23 ,,	,,	2·912	0·280	345	0·624	0·2253	1 : 12·9	1 : 4·6
24 ,,	Alb. supsd (Exp. 9)	3·696	0·330	472	0·781	0·4506	1 : 8·2	1 : 4·7
25 ,,	Basal	3·528	0·375	370	0·454	0·5001	1 : 7·0	1 : 7·7
26 ,,	,,	3·836	0·383	332	0·695	0·3792	1 : 10·1	1 : 5·5
27 ,,	,,	3·388	0·296	420	0·569	0·3627	1 : 9·3	1 : 5·6
28 ,,	,,	3·276	0·263	375	0·582	0·3462	1 : 9·4	1 : 5·6

The last group of experiments (Exps. 8 and 9, Table IV) were carried out with the nitrogen-free basal diet employed in the first group of experiments. The experiments were exactly similar to the earlier ones, except that there was no subsequent starvation. In Exp. 8, 70 g. gelatin, containing 9·939 g. nitrogen and 0·3705 g. sulphur, with a S : N ratio of 1 : 26·5, were superimposed when the nitrogen output was below 3 g. It will be noted that there was the same tendency for the S : N ratio to rise on the nitrogen-free diet.

On the day of superimposition the ratio rose to 1 : 10·1, the reverse of what was noted in Exp. 2. The possible reason of this is that in Exp. 2 the body had been on a nitrogen-free diet for seven days and, in addition, had starved two days. Under such conditions the tissues would store protein more readily. The following balance was found:

Nitrogen excreted above the basal value	= 5·236 g.
,, ingested	= 9·939 ,,
,, stored = 9·939 − 5·236	= 4·703 ,,
Sulphur excreted above the basal value	= 0·3683 ,,
,, ingested	= 0·3705 ,,
,, stored = 0·3705 − 0·3683	= 0·0022 ,,

In this case the nitrogen only has been stored. It is interesting to compare this experiment with Exp. 4, where the same quantity of gelatin was superimposed on a basal diet containing 6 g. nitrogen. In Exp. 4, 6·643 g. nitrogen and 0·0291 g. sulphur were stored. *A priori* the body should have retained more of the gelatin on a nitrogen-free diet than on a nitrogen-containing one. The reason for the poorer retention possibly lies in the fact that the basal diet in Exp. 4 may have improved the quality of the gelatin. It is also to be noted that even on the fifth day after superimposition the nitrogen had not quite returned to its basal value. This seems to show that the tissues were in need of nitrogen and were trying to utilise to the utmost an imperfect protein. The early excretion of sulphur with nitrogen lag is again seen in this experiment. In Exp. 9 egg white, containing 9·9619 g. nitrogen and 0·9995 g. sulphur with a S : N ratio of 1 : 9·9, were superimposed. Taking 2·912 g. nitrogen as the basal output we get:

Nitrogen excreted above the basal value	= 1·0218 g.
,, ingested	= 9·9619 ,,
,, stored = 9·9619 − 1·0218	= 8·9401 ,,
Sulphur excreted above the basal value	= 0·9123 ,,
,, ingested	= 0·9995 ,,
,, stored = 0·9995 − 0·9123	= 0·0872 ,,

S : N ratio of stored material = 1 : 101.

Again there is a poor retention of sulphur. The sulphur retention to cover starvation output is much greater than this in Exp. 3. The best retention of the sulphur of egg white was in Exp. 5, where 0·4322 g. was stored. In Exp. 5, however, it is likely that it was really the sulphur of the basal diet that was retained in order to build up some protein with the nitrogen of the egg white. In such a case the sulphur of the white may have been almost completely eliminated. The figures in Exp. 5 show a retention of 0·4322 g. sulphur when the white was superimposed, but clearly it does not necessarily follow that it was actually the sulphur of the white which was retained.

In fact from Exp. 9 it would seem as if the real value of the sulphur of egg white was low. Exp. 9 is a stringent experiment as the tissues were in great need of protein as compared with Exp. 5. In Exp. 9 the retention of nitrogen, however, was nearly 90 % of the intake.

This nitrogen was held firmly as the nitrogen output had not reached its basal value by the fifth day after superimposition, when the experiment was stopped as the diet became rather trying to take. The extreme difference in the quality of the material stored will be noted in Exps. 5 and 9. In Exp. 5 the material stored had a S : N ratio of 1 : 15·2, corresponding to the average for muscle tissue, whilst in Exp. 9 the retained material consisted of nitrogen alone (S : N = 1 : 101). It will be noted that the highest output of sulphur is on the day after superimposition, whilst in the case of the nitrogen it is on the second day after taking the egg white. The same lability of the material stored is seen as in Exps. 5 and 7, but, owing to the poverty in nitrogen of the body the material is retained a day longer than in the two previous experiments. This experiment also shows clearly the delay in the nitrogen excretion after that of sulphur. The sulphur output never reached its basal value although it had been eliminated almost completely.

The results of these last two experiments taken in conjunction with Exps. 4 and 5 show the effect of a basal diet in improving the quality of such proteins as gelatin and egg white in such a way as to render them more suitable for storage.

Throughout all these experiments the output of uric acid was followed. In general it will be noted that the lowest output of uric acid was on the nitrogen-free diet, and the highest on the high-nitrogen diet. The total variation in the uric acid output is nearly 300 %, the lowest output being 205 mg. on the first day of starvation, and the highest, 600 mg. on the day gelatin was taken in Exp. 6. The output in general varies with the level of nitrogen equilibrium at the time. This indicates that one of the factors governing the output of uric acid must be the metabolism not only of the ingested protein but also of the ingested carbohydrate and fat. On returning to the nitrogen-free diet after starving a rise in uric acid output is seen. According to Cathcart the presence of carbohydrate is supposed to aid in the synthesis of uric acid or its precursor from some nitrogenous source, possibly arginine and histidine [Ackroyd and Hopkins, 1916]. Folin classed uric acid as the product of a more or less constant metabolic process which was little influenced by food ingestion. This position can possibly be held for creatinine which appears to be constant in output, whether on a high- or low-nitrogen diet. The function associated with creatinine formation is probably one which must go on at all costs and within narrow limits. The output of uric acid, however, appears to be intimately associated with the metabolism of the three energy-yielding foodstuffs.

Conclusions.

The first question to be answered was whether there was a delay in the excretion of ingested protein, such as might lead to the conclusion that it was stored. This delay has been observed in the case both of gelatin and egg white. The ease with which these materials are stored and the duration of their retention in the body varies, however, with the nutritive condition at the time. In Exp. 4, 60 % of the gelatin was stored and the remaining 40 % was all eliminated in three days. This was with nitrogen equilibrium in the neighbourhood of 6 g. *per diem*. In Exp. 6, with nitrogen equilibrium at 11 g. *per diem*, the whole of the gelatin was eliminated in three days. In Exp. 8, where the body was on a nitrogen-free diet and when it was in the poorest nutritive condition as regards nitrogen of all three experiments, the amount stored was less than 50 %. It will be noted that it took at least five days for the rejected part to be eliminated. The possible explanation of this variation may be that in Exp. 6 the body was flushed to its maximum capacity with protein and was incapable of storing any more. In Exp. 4 the nitrogen condition of the body was below its optimum so that gelatin was stored and in addition its quality was improved by the nitrogen of the basal diet. In Exp. 8 the tissues, being poor in nitrogen, retained as much of it as possible. The amount stored was less than in Exp. 4 with a nitrogen-containing diet, because there was no protein in the basal diet to improve its quality for storage.

As regards the egg white it was best retained in Exp. 9, where the body was in need of protein, 90 % of the nitrogen intake being retained. The storage of sulphur, however, is different. In Exp. 9 the S : N ratio of the stored material is 1 : 101, practically no sulphur being retained. In Exp. 5 the S : N ratio of the stored material is 1 : 15·1, 40 % of the sulphur being retained. It was probably the sulphur of the basal diet which was mostly retained. The main points to be noted are:

1. The nitrogen of egg white can be stored independently of the sulphur, hence it follows that a sulphur-poor material may be retained in the tissues.

2. If the protein superimposed contain sulphur of proper quality much sulphur may be retained along with the nitrogen ingested to form a material whose S : N ratio is similar to that of tissue (muscle) protein.

It would seem to be clear from these experiments that the changes which occur in the protein molecule affect first the sulphur fraction and then the nitrogen. In the process of storage the sulphur is retained first and the nitrogen later. In breakdown the reverse occurs. If an attempt is made to visualise the possible changes which occur one cannot consider the sulphur-containing group as a nucleus around which sulphur-free nitrogen-containing bodies are built because in the catabolic phase one would require to assume that the sulphur moiety of the protein molecule would be attacked last and all the evidence is against such a view. Rather must the molecule be looked

on as a chain of amino acids with the sulphur-containing group interposed at certain links and conferring lability on the molecule.

It has been argued that the early excretion of sulphur is due to the fact that the breaking off of the sulphur molecule precedes the deamination of the amino group. Wolf and Osterberg [1911], however, found that when cystine was administered to an animal the nitrogen was eliminated before the sulphur. The natural inference from this experiment is that the sulphur was protected until the amino group had been removed. Another fact, which appears to support the view that there is no causal relationship between the lag in the output of nitrogen as compared with sulphur and the process of deamination and oxidation, is that a similar discrepancy in output occurs when anabolic phenomena predominate, as in the storage or retention of protein within the body. In this storage phenomenon it must be noted, however, that the primary preference in retention is given to the sulphur part of the molecule. In view of the fact that this lag in the nitrogen is associated both with the anabolism of food protein and the catabolism of protein (tissue protein or food protein built up into some complex), but not with the catabolism of food protein in the amino acid form, it would seem to follow that, when a differential delay in excretion is observed after the ingestion of food protein, it may be assumed that this food protein must have been built up into some form of protein in the tissues and not immediately metabolised as amino acids.

In this connection it must be born in mind that the nitrogen and sulphur ingested in the form of protein are not excreted forthwith, but may be retained in the body for three to five days. Hence it must follow that the retention takes place in some protected form and the normal assumption is that it has been taken into the cells and retained there either to become an integral part of the cell content or simply to be added on to form a kind of prosthetic group. The main point is that it cannot be regarded as remaining and circulating in the tissue fluids in a highly labile form.

As regards the sparing effect of gelatin on the nitrogen output in starvation it was found that 58 % of the excess output due to starvation could be spared by gelatin. This result is much superior to those previously given and may be due simply to the methods of calculation adopted in the present series, although it is believed that the present value is nearer the truth than the older ones.

Summary.

1. There is a delay in the excretion of ingested protein extending over several days. This occurs whether the basal diet contains nitrogen or not and even when the feeding is followed by a short period of starvation.

2. The sulphur moiety of the protein molecule is the first to be mobilised both in the storage and breakdown of protein. There is always a delay in the excretion of nitrogen as compared with sulphur. The delay in the excretion of sulphur, which is sometimes found, is not due to a delay in the utilisation of

the sulphur moiety of the protein. It is due to an active preferential retention of sulphur in the tissues.

3. There is a certain amount of evidence that the retained material can exercise a sparing action on protein breakdown in the first few days of starvation.

4. Ingested protein may be retained in the body in some relatively complex form.

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