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STUDIES ON THE PHYSIOLOGY OF PROTEIN RETENTION.

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The question of protein retention is one of considerable interest and practical importance; for while the storage of subcutaneous fat may be looked upon as a more or less inert reserve the storage of protein brings other issues to the fore. In the first place it may be asked whether there is a retention in the form of active metabolic tissue or whether there exists an inert deposit protein capable of being drawn upon like glycogen and body fat. There seems to be a fairly general agreement that even if the body stores protein it does so in limited amount.

It has long been known from the work of Voit [1881] that increasing the nitrogen intake leads to an increase in the output until within a few days equilibrium is established. Voit [1881] on the basis of his experiments held that there were two types of protein in the organism, namely, circulating protein and organized protein. The first is supposed to be built up directly from the food and is relatively labile in that it is broken down quickly in the first few days of starvation. According to this conception food protein does not first become converted into organized living tissue before utilization. Liebig [1870] held that all food protein became organized into living tissue before being metabolized. Pflüger [1893] vigorously maintained this point of view by stating that all food protein first became an integral part of the living cell before being metabolized. He repudiated the conception of a circulating protein, as this tended to imply that it was metabolized in the blood. Pflüger [1875] was definitely convinced that all metabolism took place in the cell. It should, however, be pointed out that the words "become an integral part of the living cell" are open to a variable interpretation. Cell life exists only if all the necessary constituents are present, and it is probable that any constituents, whether they are inorganic ions or complex nucleins, are equally important integral parts of the cell.

The fundamental difference between the views of Voit [1881] and

Pflüger [1893] lies in the fact that Voit believed the organism to be essentially static, metabolizing the foodstuffs as they were ingested—a metabolizing agent in short. According to Pflüger the organism was dynamic and in a state of flux, in that the tissues were transforming and being transformed by the food ingested. This conception was later elaborated by Kassowitz [1904], who maintained that all food protein became built up not merely into protein but into living protoplasm before being catabolized.

The present investigations were carried out in order to gain more information as to the nature of the protein retained. In order to achieve this object the intake and output of both nitrogen and sulphur (hereafter N and S) were estimated. By this means an attempt was made to determine if different types of retention were characterized by a difference in the lability and stability of the retained material. The method employed in those observations was that of the superimposition experiment. The work was carried out on the author, a healthy male subject weighing 65 kilos and 30 years of age. Total N (Kjeldahl) and total S (Denis) were estimated.

A basal diet of the following composition was ingested until equilibrium was attained (Exp. 1, Table I): 470 g. bread, 125 g. butter, 200 g. jam, 1 apple. Total calories 2500.

In period I 250 g. beef were superimposed for 4 days; in period II another 250 g. were added (500 g. in all); in period III the intake was reduced to 250 g. beef and in period IV the basal diet alone was ingested. As was to be expected, in periods I and II with an increasing N intake there was a retention, while in periods III and IV when the intake was reduced there was a loss of N.

In the first place some information can be obtained by noting the total amount of N and S excreted in excess of the N and S outputs on the basal diet. The figures employed as the basal N and S outputs are the averages of April 15th and 16th and May 3rd, viz.: basal N 6·206 g., basal S 0·572 g.

The following shows the total N and S and S : N ratio of the excess outputs in each of the periods:

Period I (four days) 250 g. beef: 13·039 g. N, 0·863 g. S; S : N 1 : 15·1.
Period II (five days) 500 g. beef: 56·331 g. N, 3·947 g. S; S : N 1 : 14·27.
Period III (four days) 250 g. beef: 28·519 g. N, 1·748 g. S; S : N 1 : 16·31.
Period IV (four days) basal diet alone: 6·258 g. N, 0·335 g. S; S : N 1 : 18·68.

The average S : N ratio of the beef ingested was 1 : 15·65 and it will be seen that the material metabolized in each period was not the

TABLE I. Exp. 1.

Date	Diet	Total N (g.)	Total S (g.)	S : N	N above basal (g.)	S above basal (g.)	S : N above basal	N above upper basal (g.)	S above upper basal (g.)	S : N above upper basal	N retained or lost daily (g.)	S retained or lost daily (g.)	S : N of retention or loss	% N retained or lost each day	% S retained or lost each day
15. iv. 28	Basal	5·936	0·494	1 : 12·01	—	—	—	—	—	—	—	—	—	—	—
16. iv. 28	,,	6·123	0·527	1 : 11·61	—	—	—	—	—	—	—	—	—	—	—
Period I:															
17. iv. 28	250 g. beef	8·190	0·664	1 : 12·33	1·984	0·137	1 : 14·48	—	—	—	4·040	0·267	1 : 15·13	36·5	35·4
18. iv. 28	superimposed	9·301	0·747	1 : 12·46	3·095	0·220	1 : 14·06	—	—	—	2·929	0·184	1 : 15·91	26·4	24·9
19. iv. 28	,,	10·600	0·791	1 : 12·71	4·394	0·264	1 : 16·64	—	—	—	1·630	0·140	1 : 11·60	14·7	18·5
20. iv. 28	,,	9·772	0·769	1 : 12·70	3·566	0·242	1 : 14·73	—	—	—	2·458	0·162	1 : 15·18	22·2	21·5
Period II:															
21. iv. 28	500 g. beef	13·212	1·12	1 : 11·79	7·006	0·595	1 : 13·45	0·982	0·189	1 : 5·1	6·410	0·275	1 : 23·3	58·5	67·7
22. iv. 28	superimposed	16·480	1·318	1 : 12·50	10·274	0·791	1 : 12·98	4·250	0·387	1 : 10·9	3·142	0·077	1 : 40·4	28·6	18·9
23. iv. 28	,,	18·424	1·352	1 : 13·62	12·218	0·825	1 : 14·80	6·194	0·421	1 : 14·7	1·198	0·043	1 : 27·86	10·9	10·5
24. iv. 28	,,	19·824	1·406	1 : 14·09	13·618	0·827	1 : 15·49	7·594	0·473	1 : 15·9	—	—	—	—	—
25. iv. 28	,,	19·420	1·384	1 : 14·30	13·214	0·857	1 : 15·41	7·190	0·453	1 : 15·8	0·202	0·011	1 : 18·3	1·8	2·6
Period III:															
26. iv. 28	250 g. beef	15·540	1·055	1 : 14·83	9·334	0·528	1 : 17·67	3·310	0·124	1 : 26·6	3·310	0·124	1 : 26·6	60·7	88·5
27. iv. 28	superimposed	13·337	0·939	1 : 14·20	7·131	0·412	1 : 17·30	1·107	0·008	1 : 138·3	1·107	0·008	1 : 138·3	23·1	5·7
28. iv. 28	,,	11·866	0·923	1 : 12·55	5·660	0·396	1 : 14·29	—	—	—	—	—	—	—	—
29. iv. 28	,,	12·600	0·939	1 : 13·41	6·394	0·412	1 : 15·27	0·370	0·008	1 : 46·2	0·370	0·008	1 : 46·2	7·7	5·7
Period IV:															
30. iv. 28	Basal	9·819	0·791	1 : 12·42	3·616	0·264	1 : 13·69	—	—	—	3·616	0·264	1 : 13·69	57·6	78·8
1. v. 28	,,	7·756	0·532	1 : 14·57	1·553	0·005	1 : 310·0	—	—	—	1·553	0·005	1 : 310·6	24·8	14·9
2. v. 28	,,	6·935	0·560	1 : 12·38	0·732	0·033	1 : 22·1	—	—	—	0·732	0·033	1 : 22·1	10·0	9·5
3. v. 28	,,	6·560	0·560	1 : 11·71	0·357	0·033	1 : 10·81	—	—	—	0·357	0·033	1 : 10·8	5·7	9·5

TABLE II. Exp. 2.

Date	Diet	Total N (g.)	Total S (g.)	S : N	N above basal (g.)	S above basal (g.)	S : N above basal	N retained daily (g.)	S retained daily (g.)	S : N of retention	% N retained daily	% S retained daily
26. ii. 28	Basal	6·556	0·502	1 : 13·07	—	—	—	—	—	—	—	—
27. ii. 28	Basal—250 g. beef	8·204	0·681	1 : 12·04	1·638	0·179	1 : 9·15	3·861	0·255	1 : 15·14	24·8	26·9
28. ii. 28	,,	8·943	0·747	1 : 11·97	2·377	0·245	1 : 9·7	3·122	0·189	1 : 16·51	20·1	20·3
29. ii. 28	,,	9·204	0·798	1 : 11·52	2·638	0·295	1 : 8·94	2·861	0·139	1 : 20·58	18·4	14·9
1. iii. 28	,,	9·923	0·824	1 : 12·04	3·357	0·322	1 : 10·42	2·142	0·112	1 : 19·12	13·7	12·0
2. iii. 28	,,	10·200	0·824	1 : 12·37	3·634	0·322	1 : 11·28	1·865	0·112	1 : 16·65	12·0	12·0
3. iii. 28	,,	10·659	0·860	1 : 12·39	4·093	0·358	1 : 11·43	1·406	0·076	1 : 18·5	9·0	8·1
4. iii. 28	,,	11·802	0·890	1 : 13·26	5·236	0·388	1 : 13·49	0·263	0·046	1 : 5·71	1·6	4·9
5 iii. 28	,,	12·065	0·936	1 : 12·88	5·499	0·434	1 : 12·67	—	—	—	—	—

same as judged by the S : N ratio. The following figures show the S : N ratio of the excess output for periods I and II when the intake is being increased and for periods III and IV when it is being decreased:

Periods I + II: 69·37 g. N, 4·813 g. S; S : N 1 : 14·41.
Periods III + IV: 34·777 g. N, 2·087 g. S; S : N 1 : 16·37.
Total excess: 104·047 g. N, 6·896 g. S; S : N 1 : 15·09.

In period I the material metabolized has a ratio of 1 : 15·1, indicating a proportion of S slightly higher than in the beef. In period II the ratio shows that a material still richer in S is being metabolized. In periods III and IV the reverse is observed, the ratio falling first to 1 : 16·31 in period III and to 1 : 18·68 in period IV. There appears to be a distinct regularity in the four periods, which shows that the S : N ratio of the excess output is richer in S than the ingested protein when the N intake is rising (periods I and II), while the reverse is the case when the intake is being reduced. The total excess of N and S appears, however, to have a ratio approaching that of the beef ingested. The general trend of the S : N ratio shows that the higher the protein intake the richer in S is the excess material eliminated. In period I, for instance, with 250 g. beef the S : N ratio is 1 : 15·11, while in period II with 500 g. beef the ratio of the excess is 1 : 14·27. In order, however, to see the full significance of the results the N and S retention and loss in each period must be calculated. It is clear that in period I, when beef was superimposed, there was a retention for the first few days until equilibrium was attained; correspondingly in periods III and IV, when the intake was reduced, there was a loss until a balance between intake and output at a lower level was reached. The daily retention can be calculated from the balance between intake and output on the day concerned. The fæces were not, however, analysed for N and S, as the relatively small amount of S in fæces is difficult to estimate and may not be absolutely valid in view of the probable loss of S in the alimentary canal as H_2S. The following basis for calculation was therefore employed. It is assumed that when equilibrium is attained the N and S outputs in the urine represent the total N and S absorbed from the alimentary canal. In period I, with 250 g. beef, the maximum N output was 10·60 g. on the third day and 9·77 g. on the fourth day of superimposition. In period III, with the same beef intake, it was 11·87 g. and 12·60 g. on the third and fourth days respectively, and it is probable that the average of those is the more likely figure. The figures taken as representing the N and S absorbed in periods I and III are therefore the average of April 28th and 29th (12·232 g. N and 0·931 g. S).

Accordingly in period I, Table I, where it will be noted that there is a progressively decreasing retention each day until equilibrium is approximated, the retention for each day is calculated by subtracting the N and S outputs on that day from 12·232 g. N and 0·931 g. S respectively. Similarly in period II the total absorption of N and S is taken as the average of the last two days of that period, namely, April 24th and 25th (19·622 g. N and 1·395 g. S), and the retention is calculated using these figures. In period III as the N intake is reduced the output for the first few days is greater than that absorbed; correspondingly the figures employed in period I (12·232 g. N and 0·931 g. S) are subtracted from the total N and S outputs on each day on which they exceed this value. Period III represents a loss, as the N and S absorbed have been reduced from 19·622 g. N and 1·395 g. S to 12·232 g. N and 0·931 g. S. In period IV, when no beef was ingested, the loss is calculated by subtracting the original basal figures (6·206 g. N and 0·527 g. S) from the outputs on each day on which they exceed those figures. The following shows the retention or loss in each period:

Period I (four days): 11·057 g. N retained, 0·753 g. S retained; S : N 1 : 14·69.
Period II (five days): 10·952 g. N retained, 0·406 g. S retained; S : N 1 : 26·9.
Period III (four days): 4·787 g. N lost, 0·140 g. S lost; S : N 1 : 34·1.
Period IV (four days): 6·255 g. N lost, 0·335 g. S lost; S : N 1 : 18·6.

These figures show a definite symmetry as regards the S : N ratio. The material retained in period I with 250 g. beef is similar in composition to the beef ingested, namely, 1 : 14·69 as compared with 1 : 15·65 (beef). In period II, with 500 g. beef, the absolute retention due to the extra 250 g. beef is practically the same as in period I, *i.e.* 11 g. The quality of the material retained, however, at this higher level differs in that it is poor in S, with a S : N ratio of 1 : 26·9. Correspondingly in period III when the beef intake was reduced the S : N ratio of the loss is 1 : 34·1. In a short while the material lost in period III is less than half the retention in period II, qualitatively the substance lost in period III is approximately the same as that retained in the previous period (II), namely, a material poor in S. In period IV, which is the counterpart of period I, the S : N ratio of the loss approximates that of the retention in period I. Again the loss in period IV is less than the retention in period I, and as the same holds good for periods II and III there is a retention all over. The following shows the balance:

Periods I + II: 22·009 g. N retained, 1·159 g. S retained; S : N 1 : 18·91.
Periods III + IV: 11·042 g. N lost, 0·475 g. S lost; S : N 1 : 23·24.
Total retention: 10·967 g. N, 0·684 g. S; S : N 1 : 16·0.

The S : N ratio of the material retained at the end of the experiment, if compared to the S : N ratio of that retained up to the end of periods I + II, shows that the body has been more conservative in the loss of S than of N. In other words, the S : N ratio of the 22 g. N retained up to the middle of the experiment is 1 : 18·9, and the loss in periods III and IV has raised the ratio of the retained material to 1 : 16·0 by eliminating relatively more N than S. This selective retention of S is to be noted even in the individual periods. For instance, in period I the S : N ratio of the retained material is 1 : 14·69, while in period IV, its counterpart, the ratio of the loss is 1 : 18·6: similarly in period II the ratio of the retained products is 1 : 26·9, while in period III, its counterpart, the ratio of the loss is 1 : 34·4. In general, while the quality of the retained material at one level tends to be the same as that lost in a corresponding period of reduced intake, the loss in N is slightly greater than that of S.

The inferences to be drawn from these experiments tend to show that two types of protein have been retained: (1) a labile material poor in S which is eliminated when the N intake is reduced: this corresponds to Voit's circulating protein; (2) a more stable material whose composition as judged by its S : N ratio approaches that of muscle. This material is held on to even when the N intake is reduced. It should be noted that the labile material has no definite S content, it appears to be poorer in S the higher the N intake. It is possible that its low S content may condition its instability. The general tendency appears to be to store the stable material rich in S first and the circulating protein poor in S later.

A similar experiment (Exp. 2, Table II) was carried out to confirm this selective retention of a S-rich product first. A basal diet similar to that employed in Exp. 1 was ingested, and 250 g. beef superimposed daily until N equilibrium was attained. The time required to reach equilibrium was unexpectedly long, as the body was storing N for some unknown reason. The beef was superimposed daily for eight successive days until equilibrium was reached. The basal figures for the N and S outputs were those of the preceding day (February 26th): N 6·556 g., S 0·502 g.

The excess N and S outputs and S : N ratios were calculated for each day. It will be noted that the ratio of the excess in general tends to fall from 1 : 9·15 to 1 : 12·65; this indicates that relatively more S than N was being eliminated at the beginning than at the end.

The following is the total excess output for the eight days over basal:

28·472 g. N, 2·543 g. S; S : N 1 : 11·19.

The S : N ratio of the excess is definitely richer in S than that of the beef ingested (S : N 1 : 15·65). The figure is also considerably higher than the ratio of the excess in the corresponding period in Exp. 1. In period I, Exp. 1, the S : N ratio of the excess for the four days was 1 : 15·0. The retention of N and S during the eight days shows some points of interest. *A priori* as the S : N ratio of the excess is higher than that of the beef and higher than the excess S : N ratio in period I, Exp. 1, it is to be expected that the S : N ratio of the retained material would be lower than the retention in period I, Exp. 1, and lower than that of the beef ingested. This is exactly what has been found. The calculation is on a similar basis to that in Exp. 1, the total N and S outputs on March 5th, 12·065 g. N and 0·936 g. S, being taken as the daily absorption of those elements during the course of the experiment.

The following is the amount retained over the eight days:

N retained 15·520 g., S retained 0·929 g.; S : N 1 : 16·76.

The S : N of the material retained in period I, Exp. 1, was 1 : 14·69, slightly higher than that of the beef ingested, while in this experiment as the S : N ratio of the excess was high (1 : 11·19) it is only to be expected that the ratio of the retained material should be 1 : 16·76, *i.e.* lower than that of the beef (1 : 15·6). It will be noted that the S : N ratios of the all-over retention in Exps. 1 and 2 are 1 : 16·0 and 1 : 16·76, both indicating a material slightly poorer in S than the beef (1 : 15·6) or muscle tissue (1 : 15·0). In Exp. 2 the S : N ratio of the material retained daily has been calculated. It will be seen (Table II) that the ratio drops from 1 : 15·15 on the first day to 1 : 20·5 and 1 : 19·1 on the third and fourth days of superimposition and then tends to fluctuate for a day or two. This irregularity and the fact that equilibrium is attained only after eight days indicate possibly that two types of retention are going on. It is the general experience that N equilibrium when the body is relatively well supplied with protein is attained within a period of some three or four days: period II, Exp. 1, shows a striking example, the maximum N output being reached by the third day. These three days represent the time required to fill the circulating protein depots. If for any reason some of the protein is being retained in some more stable form, equilibrium or the filling of the circulating depots will be delayed. It seems possible that in this experiment both processes have been going on. The tendency for the first few days has been to retain a material relatively rich in S and later a product poor in S was stored. From the fact that the S : N ratio fluctuates for a day or so it appears that possibly both processes are at work. It is indeed possible that an equilibrium between both types might exist. If this assumption be true then once so much stable protein is built up its quota of circulating protein must be added to it. The point to note, however, is the tendency to retain the more stable material rich in S first, which is analogous to the different types of material stored in periods I and II in Exp. 1. In Exp. 1 the product rich in S was stored and in period II that poor in S.

The question still remains, however, as to what is the nature or function of the different retention products. Since Voit's time Rubner is the only worker who has attempted a classification of the different retention products based not on their chemical constitution but on their relative lability or stability in metabolism. Rubner's [1911] classification is as follows:

1. Organized protein (Organeiweiss, O.E.): protein retained during growth or convalescence: this was held to be a retention of organized or living material.
2. Improvement protein (Meliorationseiweiss, M.E.): the criterion for the existence of this was the fact that a normal adult on a high protein diet may retain a certain amount of N which is not metabolized when the diet is changed to a N-free one.
3. Transitional protein (Übergangseiweiss, U.E.): this material was slowly metabolized over a period of eighteen days or so of a N-free diet in contrast to the improvement quota which was more stable.
4. Reserve protein (Vorratseiweiss, V.E.): Voit's circulating protein. The quantity of this material retained at any one time is dependent on the protein content of the diet. A certain amount of this circulating protein is retained or lost within three days or so of increasing or decreasing the protein intake.

The first two were classified as static and the other two as alimentary types of retention. The improvement quota (M.E.) was supposed to be retained in all the tissue cells, regardless of their function, their nutritive condition being thus raised. The transitional protein (U.E.), on the other hand, was a temporary retention of material possibly concerned with the assimilation and metabolism of protein food. On a N-free diet this reserve was not essential, and hence it was slowly metabolized over a period of days as in Thomas's [1910] experiment on himself. In this connection it is of interest to note that Seitz [1906] found that the livers of ducks and hens, which were fed on a high protein diet, increased in weight proportionally more than the rest of the body, for instance 100 p.c. as compared with 15 p.c. Similarly Mackay, Mackay and

Addis [1928] and Mendel [1924] observed a hypertrophy of the kidneys in rats fed on a high protein diet. As both these organs are intimately concerned in protein metabolism it is not improbable that transitional protein (U.E.) is associated with the hypertrophy of the liver and kidneys and possibly certain other tissues. As regards circulating protein (V.E.) Rubner [1911] holds that it is simply a prerequisite for N equilibrium. If there were no temporary reserve such as this, equilibrium would be impossble with an intermittent food intake. The characteristic feature of transitional protein (U.E.) and circulating protein (V.E.) is their relative lability. The latter is metabolized within three days or so after the N intake has been reduced or stopped, while the former requires some eighteen days (depending on the previous feeding) of a N-free diet before it is completely metabolized. Thomas's [1910] experiment on himself shows the difference between circulating (V.E.) and transitional (U.E.) protein. A N-free diet was ingested until the endogenous wear and tear output of 2·2 g. N was reached; four days of high protein followed during which 66 g. N were retained; the N-free diet was then resumed. Thomas calculated that all the N eliminated over the endogenous wear and tear level came from this retained material. The following figures are taken from his paper and show the trend of the N output on a N-free diet after high protein feeding:

	N in urine (g.)
Last day of high protein diet	77·0
1st day of N-free diet	28·3
2nd "	10·7
3rd "	5·15
4th "	5·16
5th "	4·72
8th "	3·6
20th "	2·23

It will be noted that the material metabolized from the third day of the N-free diet is relatively stable, in that it is slowly excreted from the third day on: by the twentieth day the endogenous wear and tear is being covered entirely by what is considered to be true body tissue, possibly improvement quota (M.E.). These figures show the difference in the stability of transitional (U.E.) protein and circulating protein (V.E.).

Two experiments were therefore devised in order to gain some information as to the separate existence of an improvement quota (M.E.) and a transitional protein (U.E.) and further to see if there is any difference in S content between them. It will be remembered that the material finally retained in Exps. 1 and 2 had a rather lower S : N ratio than muscle tissue, and the problem is which of the above mentioned proteins this was. *A priori* it might be anticipated that the improvement quota (M.E.), which is the more stable, would have a ratio corresponding to muscle and the transitional protein (U.E.), which is intermediate in stability between improvement quota and circulating protein, might have a ratio slightly lower than muscle tissue. The following basal diet was ingested for three days: 500 g. beef, 50 g. cheese, 50 g. butter, 400 g. bread, 3 eggs.

The total N intake was approximately 26 g. In Exp. 3 (Table III) there followed a two-days' fast (except for water) in Exp. 4, on the other hand, a N-free diet was ingested for four days; the N-free diet consisted of 300 g. tapioca, 100 g. olive oil, 100 g. sugar, 1 apple.

TABLE III.

Exp. 3.

Date	Diet	Total N (g.)	Total S (g.)	S : N
22. x. 28	Basal	19·460	1·417	1 : 13·72
23. x. 28	,,	24·948	1·505	1 : 16·56
24. x. 28	,,	25·676	1·461	1 : 17·56
25. x. 28	Fasting	13·608	0·692	1 : 19·65
26. x. 28	,,	17·056	0·912	1 : 18·69

Exp. 4.

Date	Diet	Total N (g.)	Total S (g.)	S : N
11. iv. 29	Basal	17·184	1·450	1 : 11·84
12. iv. 29	,,	20·664	1·549	1 : 13·33
13. iv. 29	,,	20·748	1·527	1 : 13·57
14. iv. 29	N-free diet	10·437	0·527	1 : 19·78
15. iv. 29	,,	5·572	0·296	1 : 18·07
16. iv. 29	,,	4·592	0·269	1 : 17·05
17. iv. 29	,,	4·454	0·291	1 : 15·29

In Exp. 4 on the first day of the N-free diet, it will be noted that the N output dropped to a half, while the S output dropped to a third of their values on the previous day on the high N intake, and correspondingly the S : N ratio fell from 1 : 13·5 to 1 : 19·7. On the second day of the diet the N output dropped 50 p.c., while the S output dropped 40 p.c. of their values on the day before; the S : N ratio was still however low. On the third and fourth days of the diet the N and S outputs fell extremely little, while the S : N ratio tended to rise to a value of 1 : 15·2. It is this type of slow fall which Thomas observed on himself in a similar experiment and which is due, according to Rubner, to a gradual metabolizing of transitional protein (U.E.). A further analysis of the figures may, however, be made in two-day periods.

N output on first and second days of N-free diet	... ...	16·009 g.
S ,, ,, ,,	... ...	0·824

S : N ratio 1 : 19·42

N ouput on third and fourth days of N-free diet	9·046 g.
S „ „ „	0·560
S : N ratio 1 : 16·14.	
Total N output over the four days	25·055
„ S „ „	1·384
S : N ratio 1 : 18·09.	

It will be noted that the larger moiety excreted in the first two days is relatively poor in S, while the smaller fraction eliminated on the last two days approaches in composition to body or muscle tissue. If the results of Exp. 1 (Table I) are recalled it will be seen that when the N intake is reduced, as in periods III and IV, the material lost was poor in S; in period III the ratio was 1 : 34, while in period IV it was 1 : 18·0. This material lost in the last two periods of Exp. 1 was held to be circulating protein, and one would be justified in assuming that what is excreted in the first two days of Exp. 4 is largely from this source. The S : N ratio of the total retention in Exp. 1 was, however, 1 : 16·0, and the issue was whether to consider it as transitional protein (U.E.) or improvement quota (M.E.). In Exp. 4 the material excreted on the last two days of the N-free diet had a ratio of S : N of 1 : 16·1, and it may be questioned if this is transitional protein. If this assumption be true then transitional protein is characterized by its greater stability in that it was not metabolized in any amount on the first two days of the N-free diet. It can then be asked if its stability is conditioned by its S content, and if there is any justification for assuming such a type of protein retention as transitional protein (U.E.). These two queries are associated in that body tissue has a S : N ratio of 1 : 15–14 while the S : N ratio of what has been eliminated in the last two days is 1 : 16·1. If organized or body tissue had been drawn upon on those two days one would have expected a slightly higher S : N ratio. If the diet had been continued longer the N output would probably have fallen and the S : N ratio have risen to a value of 1 : 15, indicating that the endogenous wear and tear quota is derived from body tissue (O.E.). That this is the case has been shown by the writer in a previous paper [Wilson, 1925]. It then seems possible that there may be at least three types of protein retention, each one characterized by its degree of stability and S content. The following is a tentative classification:

1. Circulating protein (V.E.): this is unstable in that its retention is conditioned solely by the level of protein intake: it appears to be poor in S.
2. Transitional protein (U.E.): this is more stable than circulating

protein and appears to be intermediate in S content between circulating protein and body tissue.

3. Body protein (M.E.): this is the most stable of all, in that it is used most economically on a N-free diet to cover the endogenous wear and tear quota; its S content is that of body tissue.

If this classification can be held to give a relatively true picture of the nature of protein retention it would appear possible that the S content conditions the stability of each type. Further information regarding the retention products may be obtained by comparing Exp. 3 with Exp. 4 (Table III) where a two-days' fast followed the high protein feeding instead of the N-free diet as in Exp. 3. It will be noted that on the first fast day the N and S outputs are both half of that on the previous food day. This, so far as the N is concerned, is similar to Exp. 4, while the S has not fallen relatively so much. In Exp. 4 the S dropped from 1·5 g. to 0·52 g., while in Exp. 3 (fasting experiment) it dropped from 1·46 g. to 0·69 g. The influence of a day's fast as opposed to a N-free diet has extended in a small degree to the S output, while the drop in the N is relatively the same in each experiment. The S : N ratio on the first fast day is exactly the same as on the first day of the N-free diet in Exp. 4. On the second day's fast, however, the N and S outputs both rise, to 17·05 g. and 0·912 g. respectively, in contrast to the low figures of 5·573 g. and 0·296 g. on the second day of the N-free diet in Exp. 4. The S : N ratio is, however, the same on the corresponding day of each experiment. These figures show that the N-free diet has a very considerable sparing action on the breakdown of protein. The exact amount of N and S spared can be calculated by subtracting the outputs of those two substances on the second day of the N-free diet from the outputs on the second day's fast. The following are the figures obtained:

N output on second fast day	17·056 g.
N output on second day N-free diet	5·572
N spared	11·484
S output on second fast day	0·912
S output on second day N-free diet	0·296
S spared	0·616

S : N ratio of spared material 1 : 18·65.

The N-free diet has hence been responsible for sparing the breakdown of some 11 g. N and 0·6 g. S, and from the S : N ratio of this product it might provisionally be called transitional protein (U.E.) as its ratio is below that of body tissue. Definite information, however, in favour of the existence of such a type of retention product may be obtained by comparing Exps. 3 and 4 with a somewhat similar experiment previously published by the writer [Wilson, 1925]. The question at issue is, is this 11 g. N and 0·6 g. S (which has been spared under the influence of the carbohydrate of the N-free diet) circulating protein (V.E.), transitional protein (U.E.), or true body tissue? Circulating protein may be dismissed for the following reasons. Circulating protein is known to be eliminated as soon as the N intake has been reduced; in general therefore on the first day of the N-free diet 80 p.c. of the circulating protein would be eliminated, on the second day 15 p.c. and on the third day 5 p.c. as was found by Grüber [1901]. On the second day of the N-free diet (Exp. 4) the N output was 5·572 g., and this figure would include any circulating protein in process of elimination, at most only a gram or two could remain to be eliminated on the third day of the N-free diet. The extra 11·486 g. N eliminated on the two fast days over that eliminated on the two days of the N-free diet cannot therefore be considered as circulating protein. It may then be assumed that this 11·486 g. N and 0·616 g. S which have been eliminated on the second day of the fast and spared catabolism in Exp. 4 must be derived from body tissue or transitional protein. A comparison with an experiment published will give evidence that it is probably the latter. In this particular experiment [Wilson, 1925] an N-free diet had been ingested until the daily N output in the urine was 2·996 g. At this low output it may be taken that all the circulating protein and most of the transitional protein, if such exists, had been metabolized. Two days' fast followed, the output of N being 2·49 g. and 4·8 g. respectively. This means that on the second fast day the output of N is about 2 g. above that of the day preceding the fast (2·9 g. on N-free diet). In Exp. 3 the N output is 11 g. above what it would have been on the N-free diet. It would appear that in Exp. 3 some material was present in the tissues which was not circulating protein but was more labile than organized protein, in that 11·48 g. extra N were metabolized on the second day of fast in contrast to the experiment just quoted where only some 2 g. extra N were drawn upon on the second day of fast. These figures would lend some justification to the view that there is a retention product which is more stable than circulating protein in that its rate of catabolism is spared by a carbohydrate rich N-free diet; further it appears to be more unstable than organized body protein in that it is more easily broken down in starvation. It is probably this slight instability which causes its slow breakdown over a long period of N-free feeding as Thomas [1910] observed. The S : N ratio of this 11·48 g. N and 0·615 g. S (1 : 18·65), which may be called transitional protein (U.E.), would favour the hypothesis that its slight instability in contrast to body protein is conditioned by its lower S content.

In the foregoing discussion of these experiments it should be kept in mind that it is improbable that the body metabolizes one type of protein exclusively until it is exhausted and then draws on the remaining reserves. It should further be remembered that it is impossible to fix the S : N ratio for any of those types of protein with the exception of body tissue. This ratio is a composite one and may simply express the average ratio of one, two or three proteins which are all being metabolized in varying degree. It is possible that instead of there being three distinct types of protein retention of different S content one type of a variable and plastic composition is synthesized. If the food ingested ultimately becomes protoplasm, as Kassowitz [1904] maintains, the lability and composition of this protoplasm may be conditioned by its S content.

The rôle of sulphur in metabolism. The writer [Wilson, 1925, 1926] has previously brought forward evidence in confirmation of the finding of Feder [1881], Falta [1906], and of Cathcart and Green [1912] that S is the first to be dealt with in the metabolism of protein. Evidence was further adduced, however, which tended to show that S plays the mobile rôle not only in catabolism but also in anabolism. The anabolic side of metabolism has been rather neglected, and this has probably been the cause of the disagreement among investigators [Hawk and Chamberlain, 1904] as to whether S or N was the first to be catabolized. If it is true that all food is first built up into some complex before it is catabolized a complete picture of the fate of ingested material can then be obtained only when both the anabolic and the catabolic phases are taken into consideration. The results obtained in Exps. 1 and 2 will tend to confirm the hypothesis that not only is S the first to be catabolized but that it is the first to be retained in the process of retention or anabolism. Exps. 1 and 2 show the advantage of a detailed analysis not only of the daily excess N and S excreted (catabolized) but also of the daily retention of those elements.

Exp. 1 has already been discussed in regard to the gross retention in each period. In Table I are given the daily excess outputs over the basal figures (no beef in diet) for each experimental period. In addition the excess outputs of N and S in periods II and III have been calculated using the figures of period III as basal. It will be remembered that in periods I and III 250 g. of beef were superimposed; the basal N output for those two periods was taken as the average of the last two days of period III (April 28th and 29th). The daily N and S outputs and the S : N ratios are tabulated along with the percentage of N and S retained each day in relation to the total amount of those elements retained in that particular experimental period. For instance in period I 11·057 g. N and 0·75 g. S were retained, and of this 4·04 g. N and 0·267 g. S were retained on the first day, *i.e.* 36·5 p.c. and 35·4 p.c. respectively. Similarly in periods III and IV the daily N and S loss is given and the percentage of each element lost daily in relation to each period separately. In period I (250 g. beef) the S : N ratio of the daily retained material is extremely constant except for the third day on which it rises to 1 : 11·6, and curiously there is a rise next day in the absolute amounts retained; correspondingly the percentage retentions of N and S are the same for each day except the third. In period II (500 g. beef) the S : N ratio of the material retained daily, though low as compared to the beef is higher on the first day (1 : 23·3) than on the second (1 : 40·4). This would appear to indicate that during retention the S is retained in advance of the N in spite of the fact that the S : N ratio of the total retention in this period is low (1 : 26·9). It is instructive to compare the daily excess N and S outputs of this period with the daily retention. As has been mentioned the excess output is given on two bases, namely, (1) excess over the output on basal diet; (2) excess over the output on 250 g. beef diet. The S : N ratio of the excess is seen to fall from 1 : 13·45 on the first day to 1 : 15·41 on the last day of period II on the first basis of calculation, while on the second basis of calculation it falls from 1 : 5·1 to 1 : 15·8. These figures indicate clearly that the S is catabolized in advance of the N, while the figures for the retention indicate that the S is retained in advance. The S : N ratio of the excess and of the material retained for each of the five days of period II are given below in order to show how the anabolic and the catabolic phases run concurrently; the excess calculated on the second basis has been quoted.

April	S : N excess	S : N retained
21st	1 : 5·1	1 : 23·3
22nd	1 : 10·9	1 : 40·4
23rd	1 : 14·7	1 : 27·8
24th	1 : 15·9	—
25th	1 : 15·8	1 : 18·3

These figures show the fallacy of estimating the S : N ratio of the excess output alone; in this period for instance the S content of the excess is higher than that of muscle, and a hasty conclusion would be drawn—correct in so far as it goes—that the S is catabolized in advance of the N. The material retained on the other hand is relatively poor in S, yet the S has preceded the N in the process of retention. In periods III and IV as the N intake is being reduced there is a diminishing loss until equilibrium is attained at a lower level. In period III the S : N ratio of the loss on the first day is 1 : 26·6 and on the second day 1 : 138·3, while the figures giving the percentage of N and S lost each day in relation to the loss in the whole period show that 88 p.c. of the S and only 60 p.c. of the N are lost on the first day. This again confirms the hypothesis that although the material lost is poor in S, the S precedes the N in catabolism. Period IV shows the same features in regard to the early catabolism of the S moiety. This selective retention of S is not observed in period I, Exp. 1; several factors might be responsible for this. In the first place it must be remembered that it is impossible to say how many days a retention process requires. It is possible that if a protein is superimposed for several days a certain fraction of the S is retained on the first day, while it may require perhaps two days for the corresponding N moiety to be retained. Under these circumstances the preferential retention of the S would be easily noted. In practice, however, on the second day of superimposition an additional fraction of S is retained, and the retention of its N is not completed until the third day. Similarly on the third and fourth days a diminishing amount of S is retained until equilibrium is attained and storage ceases. It is only to be expected under those circumstances that in following the retention of N and S a steady state is reached and the preferential storage of S obscured. Exp. 2 (Table II) illustrates this steady state in the process of retention. In Exp. 2, 250 g. of beef were superimposed on a basal diet similar to that employed in Exp. 1; eight days elapsed before equilibrium was attained and consequently a steadily decreasing retention was noted over the first seven days. The S : N ratio of the material retained over the seven days is 1 : 16·7, while the S : N ratio of the excess is 1 : 11·1. It will be noted that the S : N ratio of the daily excess tends to drop steadily from 1 : 9·1 to 1 : 12·6. The S : N ratio of the retained material is highest on the first day (February 27th) and from then on it tends to drop until the third and fourth days. The tendency however for the retention of a material relatively rich in S at the beginning and correspondingly poor in S later on is seen by comparing the S : N ratio of the total retention (1 : 16·7) with the S : N ratio of the material stored on the first day (1 : 15·1), which is slightly higher, with the ratio of the seventh day's retention (1 : 18·5) which is slightly lower than 1 : 16·7. This experiment then tends to confirm the contention that the S is the mobile unit not only in the catabolic but also in the anabolic phase. It is only here and there under certain experimental conditions that its rôle in metabolism can be observed.

It is hoped that the analysis just made serves to show that there is an order not only in the catabolism but also in the anabolism of protein. It would appear that in these two phases S or a moiety containing S is the mobile or one of the mobile units in the process. The results would appear to indicate that ingested protein is metabolized immediately after it has first been built up into some complex, possibly protoplasm, and hence becomes a part of the living structure before catabolism.

SUMMARY.

1. Evidence has been brought forward to show that the organism can store protein of varying composition as judged by its S : N ratio.

2. The stability of the retention product may possibly be conditioned by its S content; the higher the S content the more stable is the retained material.

3. Sulphur or a moiety containing S appears to be the mobile unit both in anabolism and catabolism.

4. The possibility is indicated that all ingested protein is not immediately metabolized as amino acids, but may first be built up into some complex before utilization.

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