I. THE RATE OF PROTEIN CATABOLISM[1].

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The whole question of the course of the breakdown of the protein molecule within the tissues is one of the most obscure in physiology. We have now a fairly good idea of the form in which the protein is absorbed from the lumen of the intestine but its immediate fate is still unknown. Apparently however definite evidence is now collecting [Folin, 1912] in support of the view that no immediate synthesis, analogous to that of fat, takes place. Unquestionably, irrespective of the form in which the protein material is conveyed to the tissues, there is, soon after the ingestion of food, a fairly complete disintegration of the protein molecule as evidenced by the increase in the output of nitrogenous substances in the urine. Of course it might be maintained, as it has been, that this material arises not from the newly ingested material but from "effete" protoplasm broken down and discarded when a new supply of repair material is available. If this be so then the material excreted ought to bear some definite percentage relationship to the normal protein of the body, for example the ratio of sulphur to nitrogen in the urine should approximate to that of the average tissue as obtained say by the study of the sulphur and nitrogen ratio in complete starvation.

It was thought that, by a careful study of the ratios of S:N after feeding with specially chosen foodstuffs, light might be thrown on:—

(1) The rate of protein catabolism.
(2) The nature of the material catabolised.

PREVIOUS WORK.

Little work has been carried out in this field probably because of the fact that until recently the difficulty of carrying on a long series of sulphur analyses was considerable. Since the introduction of the very excellent and rapid method of S. R. Benedict this difficulty has largely disappeared.

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[1] The majority of the results here published were given in the form of a communication at the Biochemical Society's Cambridge meeting, October 1911.

Siven [1901] found that the sulphur output ran closely parallel to the nitrogen output. He came to the conclusion that all the nitrogen excreted does not come from a purely protein source.

Sherman and Hawk [1901] also found that the nitrogen and sulphur outputs ran very closely parallel.

Von Wendt [1905] made a very thorough investigation into the output of both nitrogen and sulphur. He found that when protein was broken down in the body, the sulphur-rich digest products were the first to be catabolised, i.e. that the sulphur output preceded that of the nitrogen and that therefore the nitrogenous products which were retained in the tissues were comparatively poor in sulphur. Von Wendt maintained that it is only when the nitrogen and sulphur excretions are considered together that the true picture of the total protein exchange in the body can be obtained: individually considered they only tell whether certain decomposition products are excreted.

Ehrström [1906] pointed out that, although the nitrogen and sulphur excretions ran practically parallel, the sulphur output acted more rapidly with any change in the intake. He suggested that the sulphur-containing amino-acids were more readily oxidisable in the organism and that this might account for the accelerated rate of output of the sulphur in comparison with that of the nitrogen.

Falta [1906] also supported the view that the protein molecule was catabolised in a step-like fashion. He believed that certain nuclei were more labile than others and that this lability would account for the appearance of certain products before other constituent substances of the protein molecule. He further pointed out in a very clear way that even in the case of the nitrogen-containing moiety of the protein molecule the output of the excess nitrogen or waste nitrogen was not immediate but that it might—depending on the nature of the material ingested—be spread over several days. Thus after the ingestion of caseinogen an amount of nitrogen equivalent to that taken in was excreted in four days whereas after the administration of egg albumin the excretion was continued to the sixth day.

Hämäläinen and Helme [1907] as a result of their experiments with a superimposed diet as used by Falta came to the conclusion that the output of sulphur ran very closely parallel to the output of nitrogen. At the same time they held that their results bore out fully the contention of von Wendt that the sulphur-rich products arising from the catabolism of the protein molecule are more rapidly burnt and excreted than those which are sulphur-poor.

Since our work was completed, two papers by Wolf [1912] have appeared which practically cover the same ground as our own. Wolf found that in some instances the maximum of the output of sulphur preceded that of the nitrogen but that in others the sulphur appeared simultaneously with or was behind the nitrogen. He arrived at the general conclusion however that in the majority of instances the sulphur part of the protein molecule is the site of the first catabolic process.

METHODS.

In our experiments we also adopted the superimposition method of feeding introduced by Falta, i.e. we added on a particular day to a standard diet the special foodstuff the rate of catabolism of which we wished to establish. The standard diet was always started four or five days previous to the test in order to get the organism into a state of approximate equilibrium. (It may be remarked here that in the majority of instances, particularly those on a low protein intake, the lowest level of nitrogen was probably never reached.) On a set day there was then added to the ordinary diet, as a rule with the morning meal, the special foodstuff. Then on the following two, three or four days the original diet was continued without addition of any kind. As far as possible throughout the feeding period the intake of fluid was kept constant. The subject H. H. G. lived an ordinary quiet laboratory life, taking no undue exercise.

The diets which we employed were (1) a low nitrogen diet consisting of tapioca and cream or potatoes and butter; (2) a diet containing a medium amount of nitrogen made up of bread, butter and milk and finally (3) a diet rich in nitrogen consisting of eggs, cheese, milk and bread.

As we were only concerned with the rate at which the nitrogen and sulphur were excreted the output of total nitrogen and total sulphur were alone considered. As a general rule, as the diets were creatine- and creatinine-free, the opportunity was taken of studying the output of these substances under the different conditions of the experiments. The total nitrogen was estimated by the ordinary Kjeldahl method and the total sulphur by Benedict's method [1909], creatine and creatinine by Folin's method.

In order to get as perfect a picture as possible, the outputs of total nitrogen and of total sulphur were estimated every two hours during the day of the experiment. As a rule, for purposes of comparison the estimations on the preday, and sometimes on the day immediately following, were also carried out on the two-hourly plan. The output of the total nitrogen in the faeces was also determined.

GELATIN.

Three experiments were carried out with gelatin superimposed on various diets, (1) tapioca, (2) potato diet, (3) the egg diet.

The first experiment was carried out with the tapioca diet which had been given for two days previously. The amount of gelatin given was that present in 515 grams of a "table jelly," which contained seven grams of nitrogen and ·503 grams of sulphur, with therefore an S:N ratio of 1:13·9. The whole of the gelatin was taken with the breakfast meal at 9 a.m. The following table gives the result of the experiment.

TABLE I.

Gelatin feeding (tapioca diet).

Date and hour	Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Creatinine in grms.	Remarks
Sept. 16, 1910	9·78	·624	15·7	2·02	
,, 17, ,, 9–11	·568	·0356	16·0	·162	Two-hourly collection.
11– 1	·554	·0384	14·4	·168	
1– 3	·901	·0462	19·5	·181	
3– 5	·697	·0366	19·0	·162	
5– 7	·618	·0385	16·0	·177	
7– 9	·680	·0549	12·4	·182	
9–11	·506	·0292	17·5	·164	
11– 9	2·050	·1366	15·0	·718	
	6·574	·4160	15·8*	1·92	
Sept. 18, 1910, 9–11	·490	·0354	13·8	·170	Fed day.
11– 1	·643	·0505	12·7	·178	515 grms. "table jelly'
1– 3	·902	·0735	12·3	·177	=7 grms. N taken at
3– 5	·697	·0607	11·5	·166	9 a.m.
5– 7	·645	·0658	9·8	·166	
7– 9	·918	·0594	15·5	·187	
9–11	·838	·0395	21·2	·160	
11– 9	2·732	·1389	19·7	·693	
	7·865	·5237	15·0*	1·90	
Sept. 19, 1910, 9–11	·462	·0291	15·9	·162	
11– 1	·539	·0352	15·3	·180	
1– 3	·566	·0311	18·2	·170	
3– 5	·574	·0284	20·2	·168	
5– 7	·454	·0256	17·7	·162	
7– 9	·798	·0411	19·4	·176	
9–11	·532	·0286	18·0	·168	
11– 9	2·197	·1254	17·5	·706	
	6·122	·3445	17·7*	1·90	
Sept. 20, 1910	5·7	·3189	18·1	1·90	

* Ratio of totals.

When the two-hourly outputs of the preday and the fed day are compared it will be noted that in the case of the total nitrogen the alteration from the preday takes place late in the afternoon whereas with the sulphur the change occurs a few hours after the ingestion of the gelatin. From an examination of the S : N ratios the increased rate of the sulphur output over the nitrogen is clearly seen. Even at the end of the first two hours the ratio of sulphur to nitrogen has increased to 13·8 as compared with the ratio of the total outputs of the previous day of 15·8. As will be noted the output of sulphur accelerated over that of the nitrogen until the ratio stood at 9·8. Later on however the rate of the output of nitrogen suddenly increased with the result that the apparent lag was almost done away with, the ratio of S : N falling to 21·2. This gives an average for the total outputs of the sulphur and the nitrogen for the day of 15·0, a result but little different from the preday. The postday shows a fall to 17·7. As regards the total output of the superimposed nitrogen and sulphur there is evidence of a marked retention of both substances. Of the ingested nitrogen only 18·6 °/₀ is excreted and of the sulphur 26·2 °/₀. On neither of the two postdays is there any evidence of the excretion of the retained nitrogen, 5·7 grams.

Unfortunately no estimation of the nitrogen excreted by way of the faeces was carried out as previous experiments with other forms of superimposed foodstuffs had shown that no marked excretion took place by that channel. Still the retention here might have been only apparent, i.e. the bulk of the nitrogen might have been excreted by way of the faeces. Of course at the same time it was possible that a true retention of nitrogen had taken place especially as the subject was on a tapioca diet where a special demand for protein might be presumed.

In order to test this possibility the gelatin in the following experiment was superimposed on a diet rich in protein. The diet consisted of 10 eggs, 4 ozs. of cheese, 2 pints of milk, 8 ozs. of bread and 3 ozs. of butter. The gelatin employed was the same as in the previous experiment and the same amount was given. The only difference was that the subject had so much difficulty in consuming it with his otherwise abundant morning meal that about one third had to be left till the midday meal. Again, in order to get nitrogen equilibrium on the diet previous to the superimposition there were six predays of feeding (the urine on the sixth day being collected two-hourly). As will be seen from the following table the effect of the superimposition was not that of the previous experiment. Here there is neither a marked rise in the output of either the nitrogen or the sulphur nor is there an increase in the S:N ratio; indeed there is a slight decrease.

TABLE II.

Gelatin feeding (egg and cheese diet).

Date and hour		Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Creatinine in grms.	Remarks
Oct. 9, 1911		16·0	1·231	13·0	—	Weight 70·7 kilos.
„ 10, „		17·0	1·270	13·4	—	„ 70·9 „
„ 11, „		16·7	1·292	13·0	1·97	„ 71·0 „
„ 12, „	9–11	1·46	·1091	13·4	·165	„ 71·6 „
	11– 1	1·40	·0996	14·0	·178	Two-hourly collection.
	1– 3	1·54	·1036	14·8	·176	
	3– 5	1·48	·1042	14·2	·178	
	5– 7	1·46	·1041	14·1	·176	
	7– 9	1·76	·1295	13·6	·178	
	9–11	1·58	·1315	12·0	·165	
	11– 9	6·93	·5720	12·1	·755	
		17·61	1·349	13·0*	1·97	
Oct. 13, 1911,	9–11	1·55	·1179	13·2	·170	Weight 71·2 kilos.
	11– 1	1·66	·1266	13·1	·174	515 grms. "table jelly" with 7 grms. N taken $\frac{2}{3}$ at 9 a.m., $\frac{1}{3}$ at 1 p.m.
	1– 3	1·91	·1259	15·2	·168	
	3– 5	1·90	·1213	15·7	·176	
	5– 7	1·89	·1135	16·7	·178	
	7– 9	1·92	·1122	17·1	·183	
	9–11	1·63	·1136	16·9	·179	
	11– 9	7·82	·5793	13·5	·736	
		20·28	1·410	14·4*	1·96	
Oct. 14, 1911,	9–11	1·61	·120	13·3	·179	Weight 71·6 kilos.
	11– 1	1·69	·113	14·9	·182	
	1– 3	1·62	·110	14·7	·179	
	3– 5	1·55	·112	13·0	·184	
	5– 7	1·52	·112	12·7	·176	
	7– 9	1·88	·143	13·1	·180	
	9–11	1·20	·133	10·9	·176	
	11– 9	7·60	·654	11·6	·775	
		18·67	1·497	12·4*	2·03	
Oct. 15, 1911		19·0	1·448	13·1	2·04	
„ 16, „		18·6	1·389	13·4	1·99	
„ 17, „		18·8	1·392	13·5	1·97	Weight 71·6 kilos.

* Ratio of totals.

If the normal output of nitrogen for the predays be taken as 17·1 gram, the mean of the three predays preceding the superimposition, then practically the whole of the ingested extra nitrogen was excreted at the end of the second day after the feeding. 45·4 % of the extra nitrogen was excreted on the day of feeding, 22·4 % on the following day and 27·1 % on the second day after. For some inexplicable reason the output of nitrogen does not return to its old level.

In this experiment the output of nitrogen in the faeces was followed. The average output for four predays (collected and estimated each day) was 3·13 grams. The output on the day of feeding was 3·15 grams, on the first postday 3·72 grams, and the average daily output for the three following days was 2·59 grams. Evidently then the retention of the nitrogen in the first experiment was due to the actual retention of the nitrogen within the tissues and not to an excretion by way of the intestine.

As regards the output of the sulphur as already noted there is a slight fall in the ratio on the day of feeding to be followed however on the first postday by a slight rise; on the other postdays the ratio practically returns to the normal ratio found previous to the day of feeding.

If the normal output of sulphur be taken as the average of the four predays, viz. 1·31 gram, then on the day of feeding 21·8 % of the ingested sulphur has been excreted, 35·8 % on the first post-day, and 29·8 % on the second. Thus the whole of the ingested sulphur, just as in the case of the nitrogen, is excreted within three days but also, just as with the nitrogen, instead of returning to its original level the output of sulphur continues slightly above normal. It is extremely difficult to offer any adequate explanation for this continued increase in the outputs of nitrogen and sulphur unless it be that the ingestion of the gelatin has stimulated protein catabolism.

Later a third experiment was tried in which there was a return to a lower nitrogen intake. The diet on this occasion consisted of boiled rice 10 ozs. (weighed dry), potatoes 1 lb. (weighed after peeling), butter 6 ozs., milk ½ pint, containing about 5·5 grams nitrogen. The day of superimposition was preceded by six days on the diet alone, the food being taken in four meals. On the seventh day of the diet 670 grams of a "table jelly" were superimposed. The subject found it absolutely impossible to consume this amount of gelatin at the first meal, and accordingly half the amount was taken at breakfast, one fourth at midday, and the other fourth with the afternoon meal. No attempt was made in this experiment to collect the urine two-hourly. The faeces were again examined for their output of nitrogen and the following figures show quite conclusively that no loss took place by this channel: average daily output on the predays 3·24 grams, day of feeding 3·22 grams, on days immediately following 3·12 grams and 3·24 grams.

As regards the output of nitrogen in the urine it is found that there is an immediate rise on the day of feeding and that there is also a distinct increase in the output during the three following days. The same statement holds

TABLE III.

Gelatin feeding (rice, potatoes, butter and milk diet).

Date	Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Creatinine in grms.	Weight, kilos.	Remarks
March 19, 1912	8·53	·658	12·9	—	—	
20	7·82	·678	11·6	—	72·2	
21	7·02	·556	12·4	1·94	72·3	
22	6·85	·582	12·3	1·92	—	
23	6·72	·547	11·7	1·92	72·0	
24	6·60	·564	11·7	1·92	71·8	
25	10·18	·883	11·4	1·91	—	On the 25th 670 grms. "table jelly" taken (= 9·12 grms. N) ½ at 8.30 a.m., ¼ at 1.30 p.m. & ¼ at 5 p.m. Urine collected from 8.30 a.m. to 8.30 a.m.
26	9·07	·663	13·7	1·96	72·0	
27	7·60	·663	11·5	1·94	—	
28	7·14	·582	12·3	—	—	
29	6·60	·560	11·8	—	71·9	

good for the output of sulphur during these days. There is a curious irregularity in the S : N ratio which cannot be accounted for. The results were all checked by repeat analyses and found to be correct.

As regards the percentage amount of the ingested nitrogen and sulphur which was excreted during the day of feeding and the three following days the table below gives the result very clearly.

TABLE IV.

	N	S
	Percentage	excreted
Fed day	37·9	59·0
First day after	25·7	18·5
Second „ „	9·6	18·5
Third „ „	4·6	3·9
	77·8	99·9

Thus it will be noted that there is again quite a well-marked retention of nitrogen although it does not take place in the case of the sulphur. This retention is not so conspicuous as that which took place in the first experiment. The difference in result may be due to the fact that in the second experiment although the protein content is comparatively low still the diet is a more mixed one. Both experiments demonstrate that the sulphur, as other observers have previously found, is excreted more rapidly than the nitrogen.

EGG ALBUMIN.

Two experiments were carried out with boiled egg-white superimposed in the first case on the tapioca diet and in the second on the potato and butter diet.

In the first experiment the tapioca was given for two days previously, the urine on the second day being collected two-hourly. On the third day 8 ozs. of boiled egg-white containing 4·75 grams nitrogen and ·594 gram sulphur, thus with a S : N ratio of 1 : 8, were taken with the first meal. The collection of the urine was continued on the tapioca diet for still another day. The results obtained are given in the following table.

TABLE V.

Egg-albumin feeding (*tapioca diet*).

Date and hour	Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Remarks
Aug. 18, 1911	8·81	—	—	—
„ 19, „ 9–11	·68	·0374	18·0	Two-hourly collection.
11– 1	·64	·0394	16	
1– 3	·63	·0344	19	
3– 5	·83	·0436	19	
5– 7	·78	·0428	18	
7– 9	·72	·0507	14	
9–11	·38	·0266	14	
11– 9	2·17	·1317	16	
	6·83	·4066	17*	
Aug. 20, 1911, 9–11	·344	·0266	13	225 grms. boiled egg-white (=4·75 grms. N) superimposed at 9 a.m.
11– 1	·406	·0340	12	
1– 3	·589	·0437	13·5	
3– 5	·813	·0710	11·4	
5– 7	·675	·0580	11·7	
7– 9	·874	·0827	10·6	
9–11	·788	·0691	11·4	
11– 9	2·070	·1870	11·1	
	6·56	·5721	11·4*	
Aug. 21, 1911, 9–11	·399	·0392	10·2	
11– 1	·382	·0416	9·2	
1– 3	·580	·0419	14·0	
3– 5	·473	·0409	11·5	
5– 7	·468	·0353	13·3	
7– 9	·474	·0366	13·0	
9–11	·672	·0425	15·8	
11– 9	2·090	·1356	15·4	
	5·54	·4136	13·4*	

* Ratio of totals.

It will be noted that the total output of nitrogen is actually less on the fed day although there is an increase in the output of sulphur. When the two-hourly outputs of the preday and the feeding day are compared it is seen that the diminution in the output of the nitrogen is due to some apparent retention during the early hours of the day. Although the rise takes place on both days about the eighth hour, on the fed day instead of a diminution following there is a marked increase, particularly at the twelfth and fourteenth hours. During the night period however there is less nitrogen excreted than during the night of the preday.

As regards the sulphur output on the fed day there is also less sulphur excreted during the first four hours than during the same period on the preday but thereafter there is a well-marked rise in the output. This acceleration in the rate of the output of sulphur is particularly noticeable when the S:N ratios are compared with those of the preday. The actual maximum on the preday is 14, whereas on the fed day it rises to 10·6.

There is no rise in the output of nitrogen on the day following although the output of sulphur still shows some increase.

In this experiment it is clear that under the conditions employed there has been a complete retention of the 4·75 grams of nitrogen given, but, of the ·594 gram sulphur, ·173 gram has reappeared, i.e. 29 °/₀ of the amount ingested.

A second experiment was carried out in which 11 ozs. of boiled egg-white containing 6·5 grams nitrogen and ·81 gram sulphur with a S:N ratio of 1:8 were superimposed on a potato and butter diet containing about 4 grams of nitrogen (3 lbs. potatoes and ½ lb. of butter). In this experiment four days of the diet were carried out and then the egg-white was taken with the morning meal on the fifth day. The diet was continued and the urine collected for two days more.

The results will be found in Table VI (page 11).

Here it will be noted that there is not a complete retention of the ingested nitrogen although as in the previous experiment there is quite a well-marked rise in the output of the sulphur. When the two-hourly outputs of the fed day and the preday are compared it is found that the outputs of nitrogen bear a very close resemblance to one another. In each the maximum output takes place about the eleventh and twelfth hours. In the case of the sulphur the output rapidly rises and continues well above the preday throughout. This accelerated output of sulphur is also clearly demonstrated when the S : N ratios are examined. The maximum ratio on the preday is 10 whereas on the fed day it rises to 7·6, the total ratio for this day compared with that of the preday being 9·8 to 12.

On the day following there is no continued rise in the output of nitrogen and the sulphur output also falls back practically to the preday level, although the S : N ratio is still high, 10·7, indicating a continued acceleration in the output of the sulphur. The second day after the feeding shows a further fall in the output of nitrogen and also in that of the sulphur. The S : N ratio is however still high, being 10.

TABLE VI.

Egg-albumin feeding (potatoes and butter diet).

Date and hour	Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Creatinine in grms.	Remarks
Sept. 3, 1911	9·8	—	—	—	
„ 4, „	8·3	·804	10·4	2·06	
„ 5, „	7·6	·685	11·1	1·97	
„ 6, „ 9–11	·700	·0668	10·5	·174	
11– 1	·767	·0688	11·1	·182	
1– 3	·526	·0536	10·0	·170	
3– 5	·743	·0597	12·4	·180	
5– 7	·637	·0626	10·2	·180	
7– 9	·980	·0653	15·0	·182	
9–11	·764	·0584	13·1	·186	
11– 9	3·100	·2482	12·5	·850	
	8·217	·6834	12·0*	2·10	
Sept. 7, 1911, 9–11	·768	·0604	12·6	·177	312 grms. boiled egg-white (=6·5 grms. N) superimposed at 9 a.m.
11– 1	·788	·0653	12·1	·171	
1– 3	·663	·0664	10·0	·170	
3– 5	·786	·0974	8·1	·181	
5– 7	·786	·1027	7·6	·173	
7– 9	1·037	·0933	11·0	·173	
9–11	·781	·0871	9·0	·169	
11– 9	3·394	·3436	9·9	·737	
	9·011	·9162	9·8*	2·00	
Sept. 8, 1911, 9–11	·722	·0612	11·8	·168	
11– 1	·755	·0728	10·4	·178	
1– 3	·636	·0584	9·2	·176	
3– 5	·522	·0538	9·7	·174	
5– 7	·593	·0608	9·7	·182	
7– 9	·805	·0755	10·6	·188	
9–11	·590	·0584	10·1	·169	
11– 9	2·721	·2442	11·1	·734	
	7·344	·6851	10·7*	1·97	
Sept. 9, 1911	6·70	·676	10·0	1·97	

* Ratios of totals.

Thus of the 6·5 grams of nitrogen taken in (the mean of the three pre-days' output of nitrogen being 8·01 grams and of the sulphur for two days ·684 gram) there has only been 1 gram or 15·3 % excreted and that on the day of feeding. Of the sulphur ·232 gram or 28·6 % was excreted on the day of feeding and a mere trace on the first day after.

These results do not agree very well with those obtained by Wolf. He found that the sulphur output lagged behind that of the nitrogen. He had previously observed the same curious inversion of the output rates in his feeding experiments with uncoagulated egg albumin. This delay in the output of the sulphur did not take place however when predigested egg albumin was fed; in this case the sulphur output preceded that of the nitrogen.

We had also carried out a couple of experiments with uncoagulated egg albumin but we had used instead of the ordinary raw material from eggs a finely powdered dry product (Egg albumin extra fine powder, Merck). In both experiments the albumin was superimposed on a tapioca diet. As these experiments were amongst our earliest they were not so thoroughly carried out as those previously described. In the first, in which 6·7 grams of nitrogen were given, there was a retention of at least 5·8 grams nitrogen. (The sulphur output was not measured.) In the second experiment, in which the same amount of nitrogen was given, there was a retention of about 6·0 grams. In neither case was the urine collected on any day following the feeding. As regards the output of sulphur in the second experiment the total amount excreted rose above that of the preday, the S : N ratios altering from 14·3 on the preday to 11·5 on the day of feeding. In this experiment, although it was not very definite, there was some support for Wolf's contention that when uncoagulated egg albumin is fed the sulphur is at first not so rapidly excreted as the nitrogen, but that later, when the excretion does start, it proceeds at a faster rate than that of the nitrogen. Wolf believed that the sulphur complex in the uncoagulated egg albumin has some power of withstanding the onset of the ferments.

PLASMON.

Only one experiment was carried out with this substance in which it was superimposed on a bread, butter and milk diet. After twelve predays of feeding, 5·9 grams of nitrogen in the form of plasmon were superimposed daily for eight days with the result that there was a consistent retention of nitrogen throughout. The mean daily output of nitrogen before the plasmon was added was 15·6 grams in the urine and faeces and despite the daily extra intake of 5·9 grams during the plasmon period the mean daily total output was only about 17·4 grams, giving a retention of nitrogen during the whole plasmon period of about 33 grams nitrogen. The four days, which made up the after-period, did not show very conspicuously the slow fall, which was to be expected from previous work, in the output of nitrogen to the original level. It may be noted that although the subject was in very good condition throughout this experiment there was a steady although slight gain in body weight. The sulphur output showed nothing abnormal. Wolf in his plasmon experiment also found that there was no definite evidence in this case that the sulphur output preceded the output of nitrogen.

TABLE VII.

Veal feeding (bread and butter diet).

Date and hour	Total nitrogen in grms.	Total sulphur in grms.	N : S ratio	Creatinine in grms.	Weight, kilos.	Remarks
Feb. 19, 1912	9·76	—	—	—	72·3	
„ 20, „	8·62	·625	13·8	1·89	72·4	
„ 21, „	7·90	·662	11·9	1·96	72·5	
„ 22, „	7·01	·616	11·4	1·96	72·5	
„ 23, „	7·73	·664	11·6	1·93	72·6	
„ 24, „ 8–10	·575	·0436	13·2	·162	72·8	
10–12	·731	·0586	12·5	·178	—	
12– 2	·582	·0477	13·0	·172	—	
2– 4	·605	·0452	13·4	·176	—	
4– 6	·583	·0460	12·7	·178	—	
6– 8	·732	·0662	11·1	·176	—	
8–10	·680	·0663	10·3	·180	—	
10– 8	2·910	·2583	11·3	·779	—	
	7·40	·629	11·7*	1·99		
Feb. 25, 1912, 8–10	·579	·0482	12·0	·175	73·0	226 grms. stewed veal (=10·6 grms. N) superimposed at 8.30 a.m. † Trace of creatine present.
10–12	·847	·0592	14·3	·206	—	
12– 2	·991	·0574	17·3	·206†	—	
2– 4	·964	·0614	15·7	·196	—	
4– 6	1·131	·0773	14·6	·200	—	
6– 8	1·360	·0945	14·4	·191	—	
8–10	·983	·0801	12·3	·179	—	
10– 8	3·577	·3380	10·9	·750	—	
	10·43	·8081	12·9*	2·10		
Feb. 26, 1912, 8–10	·882	·0710	12·4	·179	73·0	
10–12	·929	·0702	13·2	·184	—	
12– 2	·838	·0611	13·7	·180	—	
2– 4	·778	·0616	12·6	·181	—	
4– 6	·692	·0583	11·9	·177	—	
6– 8	·809	·0710	11·4	·180	—	
8–10	·723	·0625	11·4	·182	—	
10– 8	2·932	·2658	11·0	·792	—	
	8·58	·7215	11·9*	2·05		
Feb. 27, 1912	8·16	·668	12·2	1·96	72·7	
„ 28, „	7·87	·630	12·5	1·92	72·6	
„ 29, „	7·34	·596	12·3	1·90	72·6	

* Ratios of totals.

VEAL.

In this experiment 226 grams of veal containing 10·6 grams of nitrogen and ·664 gram of sulphur with a S : N ratio of 1 : 16 were superimposed on a bread and butter diet which was taken for six days previous to the day of feeding. The sixth preday urine was collected two-hourly. Following the day of feeding there were four postdays. Table VII (p. 13) gives the general result.

Here it will be noted that there is a well-marked rise in the excretion of nitrogen as the result of the feeding, an excretion which is spread over the day of feeding and the three following days. In the case of the sulphur there is also a rise in the output but the normal is regained at the end of the third day after feeding. When the percentage amounts of the intake of both nitrogen and sulphur are compared it is found that here there is no, or perhaps only a very slight, acceleration in the output of the sulphur as is seen from the following table.

TABLE VIII.

	N	S
	Percentage output	
Day of feeding	28·6	27·0
First day after	11·2	14·0
Second ,,	7·2	5·0
Third ,,	4·4	—

Thus of the nitrogen and sulphur taken in, 56·1 °/₀ (including 4·7 °/₀ in the faeces) of the nitrogen is excreted in four days, and 46 °/₀ of the sulphur in three days.

This result fully substantiates the result obtained by Wolf who found that about 45 °/₀ of the nitrogen taken in the form of veal was re-excreted within two days. He also found that the nitrogen and sulphur output ran nearly parallel, although the output curve of the sulphur was a little steeper than that of the nitrogen, indicating that the former output was slightly more rapid. It does not however agree with the results obtained by Hämäläinen and Helme, who did not find any marked retention of the nitrogen and who found a much more rapid excretion of the sulphur than of the nitrogen.

UREA.

Finally a test with urea was carried out on a comparatively low protein diet to see if, when a nitrogenous material was used which we presumed could not take the place of protein, retention of nitrogen took place. After giving a potato and butter diet for four days twelve grams of pure urea containing 5·6 grams of nitrogen were superimposed on the breakfast meal. We found that over 95 % was excreted during the day of feeding and on the subsequent day. There was thus practically no evidence of retention. Wolf obtained a similar result: he found an excretion of nearly 97 % in the first two days. This result does not favour the idea that urea can replace to a certain extent the protein of the diet. If this were possible it would have been expected that under the particularly favourable conditions of our experiment, urea given on a comparatively low protein diet, a certain amount of retention of nitrogen would have occurred. The ingestion of the urea had no influence on the output of sulphur.

CREATININE OUTPUT.

The opportunity was taken in the course of the above experiments to follow the output of creatinine as it was thought that the effect of the addition of a nitrogenous substance free from creatine and creatinine to a diet poor in protein might throw some additional light on the course of the metabolism of creatine and creatinine. Our results show that the output of creatinine was scarcely affected by such additions, except of course in the case of the veal which contained creatine in small amount. This substantiates the statement of Folin that the output of the creatinine is maintained practically at a constant level.

DISCUSSION OF RESULTS.

From these experiments then, and from others carried out by Wolf, etc., it may be concluded that Falta is right in his contention that the breakdown of protein takes place in a more or less step-like fashion. The fact that in nearly every instance there is an attempt by the cells of the organism to retain a certain amount of the nitrogen and to get rid at the same time of the excess of sulphur points to the fact that the form in which the nitrogen is retained, and apparently it is retained, although perhaps to a very limited extent, is a special one. The evidence for the storage of protein in the body is very scanty and has previously been discussed by one of us [E. P. C., 1912] at some length. Briefly it may be said that the general opinion is that a certain amount of retention can take place although the form in which the material is retained is still a matter of dispute. Some of the most interesting work in this connection is that of Müller [1907] who fed dogs on a definite low protein diet and then amputated a limb. Subsequently the animal was fed on a high protein diet and killed after a long period of feeding. An analysis of the tissues obtained from the amputated limb on the low protein diet compared with the analysis carried out after the high protein diet showed that a certain amount of retention had taken place. Recently Diesselhorst [1911] repeated this work of Müller under practically the same conditions but making more elaborate analyses. He also found that there was a certain gain in the amount of nitrogenous material after feeding on the protein-rich diet. Grund [1910] also investigated the alterations in the composition of the tissues in starvation and after feeding. He discusses this question of the retention of reserve protein in the cells and comes to the conclusion that there is a certain amount of evidence in its favour, although he at the same time maintains that if this retention does take place it cannot play a very important part in the total metabolism. He further holds that there is a general tendency both in periods of starvation and of feeding for the tissues to maintain the same relative composition.

In conclusion we believe that from the study of the S : N ratios it is clearly shown that the increase in the output of nitrogen and sulphur which as a rule follows the ingestion of a protein meal is due to the catabolism of the protein actually ingested and not to the displacement of "effete" protoplasm from the tissues. It will be noted for example after the superimposition of the egg albumin with a S : N ratio of 1 : 8 (see Table VI) that the ratio obtained approximates very closely to the ratio of the ingested protein. This is particularly noticeable in the variations in the ratio in the two-hourly collection of the preday and the fed day. One of us [E. P. C., 1907] has previously shown that the S : N ratio in starvation when all the nitrogen and sulphur which is excreted in the urine must come from an endogenous source is about 1 : 15. If then the extra nitrogen and sulphur excreted after the ingestion of the egg albumin had come from the displacement of "effete" tissue protein it would have been expected that the S : N ratio would be nearer 1 : 15 than the 1 : 9·8 found.

CONCLUSIONS.

1. The sulphur-containing moiety of the protein after ingestion is, as a rule, more rapidly catabolised and the sulphur more rapidly excreted than the nitrogen.

2. When protein is superimposed on a low protein diet a retention of part of the nitrogen superimposed takes place.

3. The retained material is apparently stored in the tissues as a pabulum of uniform composition.

4. The rise in the output of nitrogen and sulphur after a protein meal is due to the catabolism of the actual material ingested.

5. The superimposition of protein has, in these experiments, no effect on the output of creatinine.

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