XLII. THE INFLUENCE OF CARBOHYDRATE AND FAT ON PROTEIN METABOLISM WITH SPECIAL REFERENCE TO THE OUTPUT OF SULPHUR.

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In view of the fact that carbohydrate brings about a very definite reduction in the output of nitrogen in the urine and that fat tends to raise this output, when carbohydrate is present in small amount, it was of interest to find how the sulphur output would behave under similar conditions. The N : S ratio has now been repeatedly investigated, with special reference to the rate at which these two substances are excreted [Siven, 1901; Sherman and Hawk, 1901; von Wendt, 1905; Ehrström, 1906; Falta, 1906; Hämäläinen and Helme, 1907; Wolf, 1912; and Cathcart and Green, 1913]. These observers however do not all agree in their conclusions, some maintain that the nitrogen and sulphur are excreted at a parallel rate, whilst others hold that the sulphur appears before the nitrogen. Whatever the rate, the general conclusion of von Wendt is well founded, viz. that the only true picture of the total protein exchange in the body is obtained when the nitrogen and sulphur excretions are considered together.

As regards the partition of the sulphur present in the urine, Folin's [1905] conclusions may be accepted. He found that when the total sulphur excretion falls, the percentage of sulphur present as inorganic sulphates diminishes—these bear almost the same relationship to total sulphur as urea does to total nitrogen, i.e. they represent the products which are chiefly involved in quantitative changes of the total sulphur. The ethereal sulphates and neutral sulphur would seem to have an origin analogous to that of purines and creatinine. Folin found that the ethereal sulphate output becomes

more prominent when the food contains little or no protein and that the neutral sulphur represents products "which in the main are independent of the total amount of sulphur eliminated or of protein katabolised."

The method employed in the present experiment was that of superimposition, i.e. on a particular day there was added to a standard diet the special foodstuff to be tested, the content of which in nitrogen and sulphur was known. In different cases the diet was rich in fat and correspondingly poor in carbohydrate, or fat-poor and carbohydrate-rich. As the subject of the experiment a healthy bitch weighing approximately 13 kilo. was employed. The animal was kept in a metabolic cage in the usual way. She was catheterised each morning at ten and the urine added to that collected in the receiver of the cage. The urine, after the quantity, reaction, and specific gravity had been determined, was always made up to 800 cc. with water.

The diets employed were as follows:

Carbohydrate-rich				Fat-rich			
Oatmeal	...	...	70 g.	Oatmeal	...	...	70 g.
Tapioca	...	...	100 g.	Dry skimmed milk	...	...	30 g.
Cane sugar	...	...	40 g.	Margarine	...	...	90 g.
Dry skimmed milk	...	...	30 g.	Water	...	...	500 cc.
Margarine	...	...	20 g.				
Water	...	...	500 cc.				

The animal was allowed to have drinking water *ad libitum*.

The methods of analysis employed were: total nitrogen, Kjeldahl; total sulphur, Benedict; inorganic and ethereal sulphates, Folin. The faeces were not examined.

The standard diet was continued until the output of total nitrogen and total sulphur became fairly regular. Then an amount of the protein to be tested, which contained approximately 6 g. of nitrogen, was added to the standard diet at the usual feeding time. The standard diet was then continued until the output of total nitrogen and of total sulphur was again regular. The extra amounts of nitrogen and sulphur excreted were determined by the subtraction of the average amount of nitrogen and sulphur excreted on the three days immediately preceding the superimposition from that excreted after the addition.

Experiment I. Addition of Caseinogen.

The amount of caseinogen was 50 g., containing 6.21 g. total nitrogen, 0.482 g. of total sulphur (0.179 g. in the form of oxidised sulphur), thus giving an S : N ratio of 1 : 12.8. The result of this superimposition in the case of the two diets is seen in the following tables.

TABLE I. (*Carbohydrate diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.				Per cent. of T. sulphur			Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
v	470	acid	1010	2.43	0.166	0.079	0.012	0.075	47.6	7.2	45.2	1:14.6
vi	520	amph.	1009	2.41	0.166	0.071	0.013	0.082	42.8	7.8	49.4	1:14.5
vii	540	acid	1008	2.44	0.168	0.074	0.011	0.083	44.0	6.5	49.4	1:14.5
viii	560	"	1011	4.00	0.241	0.150	0.013	0.078	62.2	5.5	31.3	1:16.6 50 g. caseinogen
ix	640	"	1006	2.54	0.187	0.092	0.013	0.083	48.9	6.8	44.3	1:13.6
x	580	"	1009	2.21	0.163	0.075	0.009	0.080	45.6	5.4	49.0	1:13.8
xi	600	"	1008	2.16	0.160	0.072	0.009	0.079	44.8	5.9	49.3	1:13.5
xii	630	amph.	1006	2.18	0.146	0.064	0.011	0.071	43.6	7.5	48.8	1:15.0
xiii	630	acid	1006	2.16	0.150	0.066	0.011	0.072	44.4	7.6	48.0	1:14.5

 TABLE II. (*Fat diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.				Per cent. of T. sulphur			Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
iv	440	acid	1013	2.42	0.151	0.064	0.015	0.072	42.6	9.6	47.9	1:16.0
v	640	amph.	1008	2.54	0.160	0.072	0.016	0.072	44.7	10.1	45.2	1:15.9
vi	550	acid	1010	2.59	0.145	0.063	0.013	0.068	43.6	9.1	47.3	1:17.9
vii	1040	"	1007	5.55	0.341	0.233	0.018	0.089	68.5	5.3	26.2	1:16.3 50 g. caseinogen
viii	850	"	1005	2.99	0.178	0.090	0.015	0.073	50.6	8.4	41.0	1:16.8
ix	570	"	1012	2.64	0.168	0.080	0.014	0.073	47.8	8.4	43.8	1:15.8
x	530	"	1012	2.22	0.129	0.054	0.012	0.063	41.7	9.6	48.7	1:17.2
xi	480	"	1011	2.42	0.143	0.061	0.015	0.066	43.1	10.5	46.5	1:17.0

The extra-elimination of the nitrogen and the sulphur is shown in Tables I *A* and II *A*.

TABLE I *A*. (*Carbohydrate diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	1.57	0.074	0.075	0.001	0.002	1 : 21.1
First day	0.11	0.020	0.017	0.001	0.003	1 : 5.5
Total	1.68	0.094	0.092	0.002	0.005	1 : 17.9

TABLE II *A*. (*Fat diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	3.03	0.189	0.167	0.003	0.018	1 : 16.0
First day	0.47	0.026	0.024	—	0.002	1 : 18.8
Second day	0.12	0.016	0.014	0.001	0.002	1 : 7.5
Total	3.62	0.231	0.205	0.004	0.022	1 : 15.6

There is a definite rise in the output of total nitrogen and total sulphur after the superimposition, most marked on the day of feeding on both diets, and to a slight extent on the first day after with the carbohydrate diet, and on the first and second on the fat diet. This excess forms only 27 % of the nitrogen ingested with the carbohydrate diet: thus there is well-marked retention. But the retention is even more definite when the sulphur moiety is considered, as in the two days there is an excretion of only 0.094 g. total sulphur, i.e. 19.5 % of the sulphur ingested. When the fat diet is considered the results are different, as in the case of the nitrogen 58 % of that ingested is excreted and 48 % of the sulphur. In each instance the bulk of the sulphur excreted is in the form of inorganic sulphates.

As regards the other question, as to whether the carbohydrate-rich or fat-rich diet affects the distribution of the sulphur, there is no doubt about the fact that in this experiment when the outputs on the two diets are compared, although the total nitrogen output is slightly lower on the carbohydrate diet, the output of total sulphur is somewhat higher. As regards the partition of the sulphur it is of interest to note that on both diets about half of the sulphur excreted is in the form of neutral sulphur. Taking the mean of the three pre-days, it is found on the carbohydrate diet that 44.8 % is in the form of inorganic sulphates, 7.2 % as ethereal sulphates and 48.0 % as neutral sulphur. On the fat diet 43.6 % is present as inorganic sulphates, 9.6 % as ethereal sulphates, and 46.8 % as neutral sulphur. There is a slight

increase both in absolute and percentage amount in the output of ethereal sulphates on the fat diet.

Experiment II. Egg albumin.

50 g. of finely ground egg albumin containing 6.14 g. of nitrogen and 0.671 g. of total sulphur (with 0.041 g. in the oxidised form) were superimposed on the two standard diets as in the previous experiment. Unfortunately the fat diet could not be carried on until the old level was reached as the dog after the twelfth day refused to eat the food. The results are found in Tables III and IV.

The extra-elimination of the nitrogen and the sulphur is shown in Tables III *A* and IV *A*.

In the present experiment there is quite a marked difference between the output of the extra nitrogen and sulphur on the carbohydrate and fat diets. It required in the case of the carbohydrate-rich diet three days for the excretion of the excess nitrogen, 41.5 % of that ingested being turned out in this time, whereas the excretion of the sulphur continued quite definitely for seven days, 49.5 % of that ingested being excreted. With the fat diet this disparity of excretion was not found although the excretion of the nitrogen was continued over five days, with the result that 56.7 % of that ingested was turned out. In the case of the sulphur this also continued for five days, 47.4 % of that ingested being excreted.

Here then again, just as in the case of the caseinogen, the conditions for the retention of nitrogen on a carbohydrate diet are better than in the case of a fat diet, a difference of 31 % with the caseinogen and over 15 % with the egg albumin.

As in the previous experiment, the great bulk of the sulphur excreted is in the form of inorganic sulphates.

In this experiment not only is the total nitrogen output on the carbohydrate diet quite definitely lower but so is also the total sulphur output. The ratio of the output of sulphur to nitrogen in spite of these differences is practically identical in both experiments. It is therefore simply a clear case of complete reduction of the total protein catabolism.

When the form in which the sulphur is excreted is considered it will be noted that although on both diets the output is practically distributed between the inorganic sulphates and the neutral sulphur, in the case of the carbohydrate diet the greater part is in the form of neutral sulphur, whereas on the fat diet the greater part is present as inorganic sulphates.

TABLE III. (*Carbohydrate diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.				Per cent. of T. sulphur			Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
vii	570	amph.	1009	2.35	0.126	0.051	0.010	0.065	40.5	7.9	51.6	T.S. : T.N. 1 : 18.6
viii	610	"	1008	2.33	0.133	0.056	0.012	0.065	42.1	9.0	48.9	1 : 17.5
ix	500	"	1009	2.33	0.130	0.057	0.010	0.063	43.8	7.7	48.5	1 : 17.9
x	550	"	1014	4.37	0.273	0.188	0.018	0.067	68.9	6.6	24.5	1 : 16.0
xi	560	acid	1010	2.74	0.214	0.123	0.018	0.073	57.5	8.4	34.1	1 : 12.8
xii	670	amph.	1008	2.45	0.171	0.088	0.015	0.068	51.5	8.8	39.8	1 : 14.3
xiii	530	"	1011	2.24	0.150	0.067	0.012	0.071	44.7	8.0	47.3	1 : 14.9
xiv	590	"	1007	2.32	0.150	0.069	0.014	0.067	46.0	9.3	44.7	1 : 15.5
xv	590	"	1008	2.27	0.144	0.066	0.012	0.066	45.8	8.3	45.8	1 : 15.8
xvi	610	acid	1011	2.37	0.140	0.064	0.013	0.063	45.7	9.3	45.0	1 : 17.1
xvii	710	amph.	1006	2.37	0.130	0.055	0.013	0.062	42.3	10.0	47.7	1 : 18.2

TABLE IV. (*Fat diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.				Per cent. of T. sulphur			Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
v	490	alk.	1014	2.67	0.163	0.088	0.011	0.064	54.0	6.7	39.3	T.S. : T.N. 1 : 16.4
vi	680	"	1009	2.52	0.145	0.069	0.011	0.065	47.6	7.6	44.8	1 : 17.4
vii	740	"	1007	2.60	0.136	0.063	0.010	0.063	46.3	7.4	46.3	1 : 19.1
viii	1050	amph.	1007	4.20	0.275	0.174	0.014	0.087	63.3	5.1	31.6	1 : 15.3
ix	690	alk.	1010	3.08	0.262	0.161	0.015	0.086	61.5	5.7	32.8	1 : 11.8
x	960	amph.	1006	2.90	0.181	0.094	0.011	0.076	51.9	6.1	42.0	1 : 16.0
xi	1100	"	1007	3.25	0.176	0.093	0.010	0.073	52.8	5.7	41.5	1 : 18.5
xii	1000	"	1005	3.05	0.164	0.080	0.012	0.072	48.8	4.3	43.9	1 : 18.6

TABLE III A. (*Carbohydrate diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	2.03	0.143	0.133	0.007	0.003	1 : 14.2
First "	0.40	0.084	0.068	0.007	0.009	1 : 4.8
Second "	0.11	0.041	0.033	0.004	0.004	1 : 2.7
Third "	—	0.020	0.012	0.001	0.007	—
Fourth "	—	0.020	0.014	0.003	0.003	—
Fifth "	—	0.014	0.011	0.001	0.002	—
Sixth "	—	0.010	0.009	0.002	—0.001	—
Total	2.54	0.332	0.280	0.025	0.027	1 : 7.6

 TABLE IV A. (*Fat diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	1.60	0.127	0.101	0.003	0.023	1 : 12.6
First "	0.48	0.114	0.088	0.004	0.022	1 : 4.2
Second "	0.30	0.033	0.021	—	0.012	1 : 9.1
Third "	0.65	0.028	0.020	—0.001	0.009	1 : 23.2
Fourth "	0.45	0.016	0.007	0.001	0.008	1 : 28.1
Total	3.48	0.318	0.237	0.007	0.074	1 : 10.9

Experiment III. Gelatin.

As in both of the previous experiments the extra output of total nitrogen was higher on the fat than on the carbohydrate diet, i.e. more perfect retention having taken place on the latter diet, it was of some interest to see whether when an "imperfect" protein like gelatin was employed a similar disparity would occur. In this experiment 40 g. of gelatin containing 5.86 g. of nitrogen and 0.541 g. of sulphur (0.109 in an oxidised form) were dissolved in water and superimposed on the standard diets (Tables V and VI).

The extra-elimination of the nitrogen and the sulphur is shown in Tables V A and VI A.

As in the other experiments there is again a greater output of the super-imposed nitrogen in the case of the fat diet; on the carbohydrate diet 78.3 % of the gelatin nitrogen ingested reappeared in the urine in the course of three days, and on the fat diet within the same period 88.7 % reappeared. In the case of the sulphur the result was very similar, on the carbohydrate diet 24.7 % and on the fat diet 30.3 % was excreted, in both instances the extra output, mainly in the form of inorganic sulphates, was confined to the fed-day.

TABLE V. (*Carbohydrate diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.			Per cent. of T. sulphur			T.S. : T.N.	Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
iv	930	amph.	1008	2.03	0.141	0.056	0.015	0.070	39.7	10.6	49.6	1 : 14.4
v	820	"	1009	2.04	0.137	0.051	0.017	0.069	37.2	12.4	50.4	1 : 14.9
vi	1050	"	1007	2.16	0.141	0.058	0.016	0.067	41.1	11.3	47.5	1 : 15.3
vii	1000	"	1009	5.94	0.274	0.170	0.016	0.088	62.0	5.9	32.1	1 : 21.7
viii	670	"	1010	2.60	0.118	0.039	0.013	0.066	33.1	11.0	55.9	1 : 22.0
ix	950	"	1007	2.29	0.126	0.043	0.016	0.067	34.1	12.7	53.2	1 : 18.2
x	730	"	1008	2.08	0.144	0.060	0.017	0.067	41.7	11.8	46.5	1 : 14.4
xi	790	"	1008	2.18	0.153	0.071	0.015	0.067	46.4	9.8	43.8	1 : 14.3

TABLE VI. (*Fat diet.*)

Day	Amount in cc.	Reaction	Specific gravity	T.N. in g.	S. in g.			Per cent. of T. sulphur			T.S. : T.N.	Remarks
					T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
ii	800	amph.	1009	2.32	0.145	0.062	0.013	0.070	42.8	9.0	48.3	1 : 16.0
iii	700	"	1010	2.29	0.157	0.070	0.016	0.071	44.6	10.2	45.2	1 : 14.5
iv	1100	"	1005	2.54	0.162	0.071	0.016	0.075	43.8	9.9	46.3	1 : 15.7
v	1360	acid	1010	6.68	0.319	0.213	0.018	0.088	66.8	5.6	27.6	1 : 20.9
vi	850	amph.	1007	3.06	0.140	0.057	0.015	0.068	40.7	10.7	48.6	1 : 21.9
vii	1000	"	1006	2.60	0.149	0.063	0.015	0.071	42.3	10.1	47.7	1 : 17.4
viii	1000	acid	1006	2.23	0.135	0.051	0.014	0.070	37.8	10.4	51.9	1 : 16.5

TABLE V A. (*Carbohydrate diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	3.86	0.134	0.115	—	0.019	1 : 28.8
First „	0.52	—	—	—	—	—
Second „	0.21	—	—	—	—	—
Total	4.59	0.134	0.115	—	0.019	1 : 34.3

 TABLE VI A. (*Fat diet.*)

Day	T.N. in g.	S. in g.				T.S. : T.N.
		T.S.	Inorganic sulphate	Ethereal sulphate	Neutral sulphur	
Fed day	4.30	0.164	0.145	0.003	0.016	1 : 26.2
First „	0.68	—	—	—	—	—
Second „	0.22	—	—	—	—	—
Total	5.20	0.164	0.145	0.003	0.016	1 : 31.7

It is of interest to note that the output of extra total nitrogen after the gelatin feeding is very definitely higher than is the case with the more "perfect" proteins caseinogen and egg albumin. It is difficult to say why the retention of sulphur is more marked than in the other experiments. It is of course possible that the standard diet, on which the animal was mainly living, was poor in sulphur and that there was a real demand for this substance. It must not be forgotten that in all probability the demand for sulphur by an animal like the dog is relatively greater than in the case of man, due to the fact that there is always a very steady loss of sulphur-rich material like hair.

These results certainly do not agree with many previous experiments, which however were carried out on human beings, where it has been practically uniformly found that if the output of sulphur did not actually exceed the total nitrogen, it at the very least ran parallel with it.

As regards the output of nitrogen and sulphur on the standard diet in this experiment, just as in Exp. II on the carbohydrate diet, the output of both nitrogen and sulphur is lower than on the fat diet. With respect to the partition of the sulphur on both diets, neutral sulphur is the most predominant constituent, although in both ethereal sulphates are excreted in greater amount than in either of the other two experiments.

These experiments not only bear out the statement first put forward by Falta and since confirmed by others that the rate of the excretion of the catabolic products of protein depends on the nature of the protein ingested,

but they allow of the further amplification that the rate at which these products are excreted depends not only on the nature of the protein but also on the nature of the diet on which the protein has been superimposed.

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SUMMARY.

1. In all three experiments the retention of superimposed nitrogen is greater on a carbohydrate than on a fat diet.
2. In two experiments out of the three the same holds good for sulphur.
3. The amount of extra nitrogen and sulphur excreted varies with the protein used.
4. The greater part of the extra sulphur excreted is in the form of inorganic sulphates.
5. The partition of sulphur on the carbohydrate and fat diets is very similar—it is for the most part excreted in the form of inorganic sulphates and neutral sulphur in almost equal amount.
6. There is no evidence that the protein retained after superimposition is poor in sulphur. Indeed with the exception of one experiment sulphur is definitely retained in larger amount than nitrogen.

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