X. DISTURBANCES IN METABOLISM.

I. VARIATIONS IN PROTEIN METABOLISM AS INDICATED BY SULPHUR EXCRETION.

By JESSIE McCRIE CRAIG AND CHARLES ROBERT HARINGTON.

From the Department of Therapeutics, University of Edinburgh.

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ACCORDING to the generally accepted theory of Folin [1905, 2] protein metabolism is more or less sharply divided into two fractions; the exogenous metabolism, representing the circulating amino-acids which have not yet been built up into tissue-proteins, and the endogenous metabolism representing the breakdown of tissue-proteins. On this theory the former will vary and the latter remain constant with varying intake of protein, unless the intake of protein is reduced to such an extent that a starvation effect is obtained. In support of this theory Folin pointed out that certain of the urinary constituents, *e.g.* creatinine, and the neutral sulphur, remain constant under various dietary conditions, whilst others, *e.g.* urea and inorganic sulphate, vary directly with the amount of nitrogen and sulphur in the diet. He assumed, therefore, that the creatinine and neutral sulphur in the urine indicated the amount of tissue breakdown.

In conditions where the total metabolism, as indicated by the basal metabolic rate, is raised or lowered, it seems of interest to investigate the partition of protein metabolism, with a view to determining whether the increase or decrease in metabolism is due to an alteration in the amount of tissue breakdown, or of exogenous oxidation, or of both.

Assuming the truth of Folin's theory, we undertook the investigation of some cases of disordered metabolism using the partition of sulphur excreted in the urine as an index of the protein metabolism. The cases investigated were principally those of patients suffering from exophthalmic goitre together with a few cases of reduced metabolism, three of fever, and one of starvation. At the outset of the investigation, it was hoped that the absolute amount of neutral sulphur excreted in the day would afford an index of the condition, but this did not turn out to be the case. Not only does the amount of neutral sulphur excreted by different normal individuals show considerable variations, so that there is no standard to which reference can be made [Folin, 1905, 1], but we soon found also that the amount of neutral sulphur excreted bore no obvious

relation to the rate of metabolism. The estimation of the amount of neutral sulphur in one 24-hour specimen of urine gave therefore no useful information, and it was necessary to devise a more systematic means of attacking the problem.

The method adopted was to observe the reaction of the patient to a change in diet. In certain of the cases, the output of urea and creatinine in the urine was also estimated, in order to compare the nitrogen excretion with that of sulphur.

EXPERIMENTAL.

The method employed for the estimation of sulphur was that of Rosenheim and Drummond [1914] as modified by Fiske [1921] with the following alterations:

(1) Such amounts of urine were taken as would afford a convenient amount of benzidine sulphate for titration.

(2) The benzidine sulphate was titrated with $N/40$ sodium hydroxide, using a microburette, to increase the accuracy of the titration.

(3) Instead of the special filtration-tube recommended by Fiske, we found it convenient to employ a small conical porcelain filter-funnel with a perforated bottom, the precipitate and filter-pad being washed into the beaker for titration by means of a jet of hot water.

(4) The addition of acetone to the solution after treatment with benzidine hydrochloride was discontinued, as it was found that there was a danger of precipitation of some of the reagent¹.

All estimations were carried out in the usual way, viz. inorganic sulphates, total sulphates, and total sulphur were separately determined, and ethereal sulphates and neutral sulphur obtained by difference.

Robison [1922] criticises the method for total sulphur on two grounds:

- (a) the inconvenience of Benedict's oxidising reagent through spattering;
- (b) errors introduced through sulphur contained in the gas.

We ourselves found no such difficulty in the use of Benedict's reagent. It was found advisable however to use spirit-lamp oxidation in place of coal-gas as recommended by Robison, his results being confirmed by blank experiments of our own. With the method as finally adopted, using a solution made up of pure sodium sulphate and pure cystine, it was found that it could be analysed in duplicate to within 1 % of the theoretical content. At first we did only three or four estimations on each case, but the results were so remarkable, that, in order to exclude the possibility that the variations we observed were due to inaccuracies in the 24-hour specimens, we decided to carry the experiment over a longer period. We finally adopted a method of more rigid control as follows. After a preliminary period of a few days on ordinary ward diet, the patient was transferred to a constant rich protein

¹ A private communication of one of us with Fiske has revealed that the above observation was due to the fact that we employed absolute acetone whereas he used 95 %. The additional concentration which we used appears to account for the precipitation of the benzidine hydrochloride.

diet of known sulphur content, whose composition is shown in Table I; the patient was then put on a diet, equal in calorific value to the rich protein diet, but in which the main sulphur-containing proteins were replaced by arrowroot and cream. This diet continued for a week, after which the rich protein diet was resumed for a further week, daily estimations being carried out over the whole period.

Table I. *Composition of diet for controlled experiments.*

A. *Rich protein diet.*

Food	Intake in g. per diem	Carbohydrate	Protein	Fat	Content in sulphur (g.)
"Laitproto"	100	—	82.86	0.78	0.68
Egg-albumin	50	—	50	—	0.80
Milk	560	28	18.48	22.4	0.018
Bread	175	92.75	15.75	2.8	0.035
Butter	75	—	—	62.5	—
Potatoes	90	18	3	0.7	—
Vegetables	150	4.5	2.25	—	—
	Total	143.25	172.34	88.98	1.533
	Calories	573	689.36	860.8	—

Total calories = 2063.18.

In addition 4 g. of cystine were given daily, bringing the total intake of sulphur to 2.593 g. in the day.

B. *Low protein diet.*

For "Laitproto" was substituted 50 g. arrowroot = 168.65 calories.

" egg-albumin " 6 oz. cream = 334.8 "

The cystine was omitted.

The other items of the diet A remained unchanged.

Sulphur content of low protein diet = 0.053 g. per diem.

As seen from Table I, the daily intake of sulphur during rich protein diet was 2.59 g., and on low protein diet was 0.053 g. The laitproto and egg-albumin were analysed by ourselves, by the method of fusion with sodium peroxide. The figures for the sulphur content of the bread and milk, which contain a relatively very small amount, were from standard text-book sources. In all cases, the possibility of interference with the results by the administration of drugs was carefully excluded. To raise the sulphur content of the rich protein diet and make the change more marked from the low protein diet, 4 g. of pure cystine (analysed) were added to the daily intake of food.

EXPLANATION OF TABLES.

The results of the experiments are shown in tables as follows:

Table II *a* shows the complete results of all analyses in one case of exophthalmic goitre under rigid dietary control as described and the averages for a second case; also the results of a normal worked out in exactly the same manner; the results in this case have been averaged.

Table II *b* shows the results in seven other cases of exophthalmic goitre. In Case 3, the total duration of experiment was 13 days, and in Case 4, 17 days, but the results in the different periods have been averaged.

Cases 5-9 are the earlier ones, in which we had a few observations only.

Table III contains the results of two cases of reduced metabolism. Case 10 was a definite myxoedema. Case 11 was a patient suffering from mitral stenosis, with enlarged thyroid, and facies suggesting acromegaly; B.M.R. slightly reduced.

Table IV shows the results of four cases (12-15) of fever, and one case (16), of starvation. Case 12 is the same case as Case 2 in Table II *a*, the patient having an acute infection at the end of the last period there shown.

Case 16 was a destitute woman, brought in in a state of starvation, who was observed during her recovery.

In order to save space, the figures for excretion of ethereal sulphates are omitted from the tables.

Table II *a*.

Case 1. Female. Aet. 30. Exophthalmic goitre.

Diet	Day	Creatinine	Sulphur as g. SO ₃ in 24 hours			% of total sulphur as		Remarks
		g. in 24 hours	Inorganic	Neutral	Total	Inorganic	Neutral	
R.P.D. (a)	1	0.58	1.14	0.10	1.27	89.8	7.8	B.M.R. + 58 %
"	2	0.54	1.95	0.08	2.13	91.5	3.8	
"	3	0.54	4.01	0.24	4.30	93.2	5.6	
"	4	0.54	3.32	0.17	3.55	93.5	4.8	
"	5	0.65	3.35	0.30	3.78	88.6	7.9	
"	6	0.54	4.96	0.25	5.45	83.6	4.6	
"	7	0.54	4.65	0.37	5.13	90.6	7.2	
L.P.D.	1	—	—	—	Lost	—	—	B.M.R. + 48 %
"	2	0.44	1.07	0.18	1.32	81.0	13.6	
"	3	0.54	1.31	0.14	1.53	87.3	9.3	
"	4	0.44	0.97	0.09	1.17	82.8	7.8	
"	5	0.51	0.97	0.11	1.22	79.5	9.0	
"	6	0.36	0.73	0.06	0.96	76.2	6.2	
"	7	0.45	1.00	0.13	1.16	86.2	11.2	
R.P.D. (b)	1	0.48	1.53	0.12	1.72	88.9	7.0	B.M.R. + 15 %
"	2	0.55	4.70	0.46	5.20	90.4	8.8	
"	3	0.52	5.58	0.45	6.18	90.3	7.3	
"	4	0.45	5.25	0.40	5.78	90.8	6.9	
"	5	0.50	5.70	0.47	6.34	89.9	7.4	
<i>Averages.</i>								
R.P.D. (a)		0.56	4.06	0.27	4.44	91.5	6.1	
L.P.D.		0.46	1.00	0.11	1.21	82.6	9.1	
R.P.D. (b)		0.51	5.31	0.45	5.87	90.4	7.7	

Case 2. Female. Aet. 27. Exophthalmic goitre.

<i>Averages.</i>							
O.D. 4 days' av.	0.95	1.64	0.50	2.22	74.0	22.5	
R.P.D. (a) 6 days' av.	0.96	4.74	0.56	5.45	87.0	10.3	
L.P.D. 7 days' av.	0.75	1.55	0.19	1.87	83.0	10.2	
R.P.D. (b) 5 days' av.	0.67	4.61	0.54	5.29	87.0	10.2	

Normal Case. Male. Aet. 14. Convalescent from acute rheumatism.

<i>Averages.</i>							
O.D. 4 days' av.	1.14	2.36	0.49	3.06	77.0	16.0	
R.P.D. 5 days' av.	1.56	5.27	0.58	6.04	87.0	9.5	
L.P.D. 5 days' av.	1.50	2.25	0.54	2.97	76.0	18.2	

ANALYSIS OF RESULTS.

On examination of the results from the cases of exophthalmic goitre, the most obvious point is the striking reduction of the neutral sulphur on low protein diet. The inorganic sulphate is also reduced, but parallel with, and not to a greater extent than the neutral sulphur, so that the percentages of the total sulphur, represented in the two forms, tend to remain the same on both diets, or the percentage of total sulphur as neutral sulphur may even be lower on

Table II b.

Case	Diet	Period	Crea- tinine g. in 24 hrs.	Sulphur as g. SO ₃ in 24 hrs.			% of total sulphur as		Remarks
				In-			In-		
				organic	Neutral	Total	organic	Neutral	
III	O.D.	7 days' av.	0.81	1.34	0.68	2.27	59.0	30.0	Female. Aet. 29. B.M.R. + 75 %
	L.P.D.	3 "	0.75	0.92	0.51	1.63	56.5	31.3	
	O.D.	3 "	1.03	1.71	0.86	2.90	59.0	29.6	
IV	O.D.	7 "	1.50	2.96	1.03	4.30	69.0	24.0	Male. Aet. 19. Exoph. goitre
	L.P.D.	4 "	1.20	2.42	0.98	3.73	65.0	26.3	
	O.D.	6 "	1.51	3.35	1.22	4.96	67.5	24.6	
V	O.D.	3 "	—	1.72	0.36	2.28	75.4	15.8	Female. Aet. 30. B.M.R. + 24 %
	L.P.D.	1 day	—	1.29	0.18	1.95	66.1	9.2	
VI	O.D.	1 "	0.84	0.85	0.18	1.02	80.7	17.5	Female. Aet. 30. Last observation was made after treatment with iodine for two weeks
	L.P.D.	1 "	0.33	0.56	0.11	0.76	73.5	13.8	
	O.D.	1 "	0.49	0.95	0.18	1.19	80.6	14.6	
	O.D.	1 "	0.79	0.88	0.24	2.23	84.4	10.9	
VII	O.D.	1 "	0.66	1.40	0.67	2.22	63.1	30.2	Female. Aet. 27
	L.P.D.	1 "	0.61	1.22	0.29	1.62	75.3	17.9	
	O.D.	3 days' av.	1.00	1.28	0.47	2.05	62.5	23.0	
VIII	O.D.	1 day	0.65	1.05	0.37	1.47	71.4	25.2	Male. Aet. 33. B.M.R. + 37 %
	L.P.D.	1 "	0.90	1.59	0.27	1.89	84.1	14.3	
	O.D.	1 "	0.45	1.29	0.31	1.72	75.0	18.0	
IX	O.D.	4 days' av.	0.70	1.39	0.48	1.94	71.6	24.8	Female. Aet. 31. B.M.R. + 71 %. Between L.P.D. and 2nd O.D. Pt. menstruated
	L.P.D.	5 "	0.58	0.91	0.37	1.36	66.8	27.2	
	O.D.	6 "	0.91	1.81	0.99	3.08	58.7	32.2	

the low protein diet than on the rich. In the normal, on the other hand, it will be seen from the case recorded (and from Folin's work on the subject) the inorganic sulphate falls markedly on the change from rich to low protein diet, and the neutral sulphur remains unchanged. Hence the percentage of total sulphur as neutral sulphur rises.

The output of creatinine does not show such marked variation in the exophthalmic cases as does the neutral sulphur.

In the cases embodied in Table III an entirely different picture is presented. The neutral sulphur on low protein diet tends to increase, while the inorganic sulphate decreases very considerably. The creatinine remains fairly constant throughout.

Table III. *Reduced metabolism.*

Case	Diet	Day	Crea- tinine g. per diem	Sulphur in g. SO ₃ per diem			% of total sulphur as		Remarks
				In-			In-		
				organic	Neutral	Total	organic	Neutral	
X	O.D.	1	0.55	2.86	0.29	3.23	89.0	9.0	Female. Aet. 17. B.M.R. - 40 %. Myxoedema
	"	2	0.77	3.69	0.48	4.35	84.8	11.0	
	L.P.D.	3	0.85	2.04	0.49	2.65	77.0	18.5	
	"	4	0.84	1.02	0.51	1.75	58.2	29.2	
	"	5	0.91	0.91	0.66	1.82	50.0	36.3	
	O.D.	6	0.82	0.77	0.47	1.42	54.2	33.1	
	"	7	0.86	1.73	0.26	2.13	81.2	12.2	
XI	O.D.	7 days' av.	0.99	1.40	0.44	1.98	70.7	22.2	Mitral stenosis; enlarged thyroid. Facies suggest- ing acromegaly, B.M.R. reduced
	L.P.D.	4 "	0.83	0.93	0.65	1.80	51.7	36.2	
	O.D.	6 "	0.85	1.27	0.59	2.09	60.7	28.2	

In Table IV we have a case of starvation (No. 16) which reacts as one would expect to improved diet, the output of neutral sulphur decreasing with the return to normal conditions. The four cases of fever show a marked increase in the output of neutral sulphur during the febrile condition.

The inorganic sulphate and creatinine are also increased. In Case 12, note the preliminary fall in neutral sulphur immediately before the rise of temperature, and compare this with the steady rise in neutral sulphur in Case 15.

DISCUSSION.

As has been mentioned already, Folin found that in normal individuals the creatinine and neutral sulphur remained constant under low protein diet, whereas the inorganic sulphate and the urea were very considerably decreased. We have ourselves worked out three cases, two normal, the other a patient with an enlarged thyroid but normal metabolism, and in these cases we obtained results similar to those of Folin.

In addition, as will be seen from the results, in the normal case in Table II *a*, the output of inorganic sulphate during low protein diet was many times greater than the intake. It is therefore obvious that inorganic sulphate in the normal may originate from endogenous sources (this may also be deduced from Folin's own results). We would, however, agree with Folin that, in the normal, the neutral sulphur remains absolutely unchanged, even on severe changes of diet, short of a starvation effect. From this there seems to be no doubt that the neutral sulphur, in the normal person, represents at least a constant part of the metabolism. It also appears that there is a minimum excretion of inorganic sulphate which may represent a constant part of the endogenous metabolism, and may be derived from the same source as the neutral sulphur. It remains to put forward some explanation of the results in pathological cases.

If the theory of Folin with regard to the origin of the neutral sulphur is correct, the reduction in the output of this constituent during low protein diet in the cases of exophthalmic goitre would seem to indicate that under these conditions the amount of tissue breakdown diminished, but the clinical condition of the patient, the relative invariability of the creatinine output and the basal metabolic rate all seem to show no diminution in the rate of the metabolism as a whole. What then can be the significance of the reduction in neutral sulphur? We have here to deal with a condition widely differing from the normal. In exophthalmic goitre we have reason to believe that the oxidative processes of the body are accelerated, this acceleration being under the control of the thyroid gland, and independent of dietary factors. If, therefore, we reduce the intake of protein appreciably, it follows that tissue-proteins will be oxidised to a greater extent to make up the deficiency of exogenous protein. If this involved a quantitative increase in the tissue-breakdown only, we should expect to find an increase in the output of neutral

sulphur; in actual fact we find the reverse. We suggest the following explanation. Under the abnormally high oxidation potential which obtains in the body in this disease, some of the sulphur-containing products of tissue-breakdown which are normally excreted in the unoxidised form may be further oxidised and excreted in the form of inorganic sulphate. With a liberal protein diet, there would not necessarily be the demand for this further oxidation, but, if the exogenous supply is cut off, the body is faced with a demand for more

Table IV. *Starvation and fever.*

Case	Day	Crea- tinine g. in 24 hrs.	Sulphur in g. SO ₃ per diem			% of total sulphur as		Temp.	Remarks
			In- organic	Neutral	Total	In- organic	Neutral		
XII	1	0.79	3.21	0.20	3.73	86.1	5.4	Normal	Case II in Table II a.
	2	0.63	1.35	0.25	1.69	79.8	14.8	100.0°	Exophthalmic
	3	0.73	2.75	0.63	3.39	81.1	18.6	100.4°	goitre. Observation
	4	0.86	1.93	0.74	2.78	69.4	26.6	100.0°	made during attack
	5	0.91	1.79	0.53	2.57	69.6	20.6	100.0°	of tonsillitis.
	6	0.61	2.14	0.60	2.83	75.6	21.2	101.0°	Ordinary ward diet
	7	1.03	2.51	0.67	3.43	73.2	19.5	99.5°	throughout
	8	0.69	1.84	0.56	2.46	74.9	22.8	N.	
	9	0.82	1.46	0.64	2.33	62.7	27.5	"	
	10	0.78	1.68	0.41	2.23	75.3	18.4	"	
	11	0.53	0.85	0.39	1.36	62.5	28.7	"	
	12	0.66	1.16	0.32	1.62	71.6	19.7	"	
XIII	9 days' av.	1.48	2.35	0.33	2.89	81.4	11.4	101.8° to 104.4°	Case of lymphadenoma. Ordinary ward diet throughout
	7 days' av.	0.91	1.33	0.23	1.75	76.0	13.1	N.	
XIV	After 7 days' fever	0.84	1.45	0.29	1.74	83.2	15.1	Max. 103.6°	Case of influenza
	After 10 days' norm. temp.	0.57	0.86	0.14	1.03	83.5	13.6	—	
XV	1	1.76	2.11	0.75	3.24	65.1	23.1	N.	Male. Aet. 24. Normal. Acute infection
	2	1.71	2.30	0.77	3.31	68.4	22.6	N.	occurred during experiment
	3	1.77	1.66	1.11	3.31	50.1	33.5	101.0°	
XVI	On admission	0.95	0.80	0.29	1.38	58.4	21.4	N.	Case of starvation.
	After 7 days	0.98	0.88	0.26	1.30	67.7	20.0	"	Kept on ordinary
	After 14 days	1.08	1.12	0.28	1.60	70.4	17.5	"	ward diet through-
	After 21 days	0.64	0.66	0.12	0.83	79.5	14.5	"	out

protein material which must come from endogenous sources. We think that our results show that the first response to this demand is the further oxidation above referred to, shown by the reduced output of neutral sulphur in the low protein diet. There is probably a minimum consisting of substances which cannot under any circumstances be oxidised in the body, and below which the neutral sulphur cannot be reduced. When the demand for oxidisable protein is such that this limit is reached, then increased tissue-breakdown must ensue. We believe, then, that the decrease in neutral sulphur output observed represents the first physiological response of the body to a reduction of the exogenous protein below its minimum requirements in this disease; contrast this with the fact that, in the normal, under the same dietary conditions, the output of neutral sulphur remains constant because, as we take it,

the demand for oxidisable protein is not so great under a normal rate of oxidation. The results in fever and in the case of starvation also illustrate this point. In these cases there is no reason to assume the existence of a specifically higher rate of oxidation, but during the periods of starvation and of fever there is increased tissue-breakdown, and we find correspondingly a higher output of neutral sulphur than is normal for the individual concerned. The preliminary fall in neutral sulphur at the onset of fever in the exophthalmic Case 12 gives rise to an interesting hypothesis. It appears as if, at the onset of fever, the rate of oxidation in the body is suddenly raised, and this rise is followed by a fall below the previous level.

In myxoedema, it is more difficult to explain the results obtained. Possibly the rate of oxidation, always slow in this condition, is influenced more profoundly than in the normal person by changes in diet. On removal of the stimulating influence of a full protein diet, the rate of oxidation would be depressed and the portion of sulphur originating from tissue-breakdown and perhaps even part of that from the food which is normally oxidised, will, in this case, tend to be excreted as neutral sulphur.

If this theory in regard to exophthalmic goitre is correct, it is an interesting deduction that a low protein diet is contra-indicated in this condition; as the rate of oxidation is being kept up by the hyper-functioning of the thyroid, the removal of oxidisable protein taken with the food must lead to an increased demand for such substances from the tissues, and hence to an increased tissue-breakdown, although the first effect is to call into play the reserve physiological mechanism suggested above. We have never observed any ill-effects from giving a liberal protein diet, and, in fact, the basal metabolic rate tends to be lower on the rich protein diet.

SUMMARY.

1. Determinations have been made of the sulphur excretion under various dietary conditions in cases of disordered metabolism.

2. From the results obtained it is deduced that the increase in metabolism in exophthalmic goitre is due primarily to the acceleration of the exogenous oxidative processes, the increase in tissue-breakdown being secondary to this effect.

In conclusion we wish to express our thanks for assistance and advice to Prof. J. C. Meakins, in whose laboratory the work was carried out, and to the Carnegie Post-graduate Research Fund, for the grant of a scholarship to one of us (J. M. C.).

REFERENCES.

- Fiske (1921). *J. Biol. Chem.* 47, 59.
Folin (1905, 1). *Amer. J. Physiol.* 13, 97.
— (1905, 2). *Amer. J. Physiol.* 13, 117.
Robison (1922). *Biochem. J.* 16, 134.
Rosenheim and Drummond (1914). *Biochem J.* 8, 143.