CCXLIII. STUDIES IN THE SULPHUR METABOLISM OF THE DOG.

XI. THE METABOLISM OF METHIONINE AND RELATED SULPHIDES.

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It has long been known that only a fraction of the sulphur in many proteins is present in the form of cystine [Johnson, 1911; Harris, 1923] and it has been known for several years that a part of the remaining sulphur is in the form of methionine. I have already shown that methionine is the principal sulphur-containing amino-acid in caseinogen; this is probably also true in the case of egg-white, for I have isolated, by the same method, 2·5 % of methionine from it. Baernstein [1932], having assumed that proteins contain no methoxyl compounds and no methylthiol compound other than methionine, has published values for the methionine content of a large number of proteins. The validity of his assumptions is doubtful but the values obtained, when taken in conjunction with the cystine contents, enable him to account for approximately 100 % of the sulphur in the proteins used. In most cases the greater part of the sulphur is found to be in the form of methionine. It appears therefore that methionine is generally the principal sulphur-containing constituent of proteins other than scleroproteins and is quantitatively the most important sulphur compound in an ordinary diet. Jackson and Block [1931] and Weichselbaum, Weichselbaum and Stewart [1932] have shown that methionine will supplement a cystine-low diet, but the problem of preparing a diet that is free from either cystine or methionine is still unsolved.

Mueller [1924] showed that, in man, methionine was readily oxidised to sulphate; his experiments were necessarily short and one cannot determine from them the completeness of oxidation of the amino-acid. The present work is an extension of that of Mueller. Dogs have been used and methionine has been compared with cystine on the one hand and with some other sulphides, namely *S*-benzylcysteine, *S*-ethylcysteine and *S*-methylcysteine, on the other.

Methionine is readily oxidised by the dog. The results of three experiments are shown in the figure and are analysed in the table on p. 2042.

The obvious similarity between the metabolic behaviours of methionine and cystine is interesting in view of their widely different chemical structures.

S-Ethylcysteine [Clarke and Inouye, 1931], although closely related chemically to methionine is not oxidised to any appreciable extent by the dog. It is

Substance	Dog	mg. S given	% excreted as sulphate			% excreted as neutral S			% of dose accounted for
			1st day	2nd day	Total	1st day	2nd day	Total	
Methionine	M	320	46	13	59	10	10	20	79
„	P	320	47	19	66	11	9	20	86
„	P	320	58	14	72	9	0	9	81
Cystine	M	320	49	11	60	6	4	10	70
„	P	320	60	11	71	10	3	13	84

These results may be summarised thus.

	% excreted as sulphate in 2 days	% excreted as neutral sulphur in 2 days
Methionine	66	16
Cystine	66	11
Cystine and cysteine [Hele and Pirie, 1931]	70	4

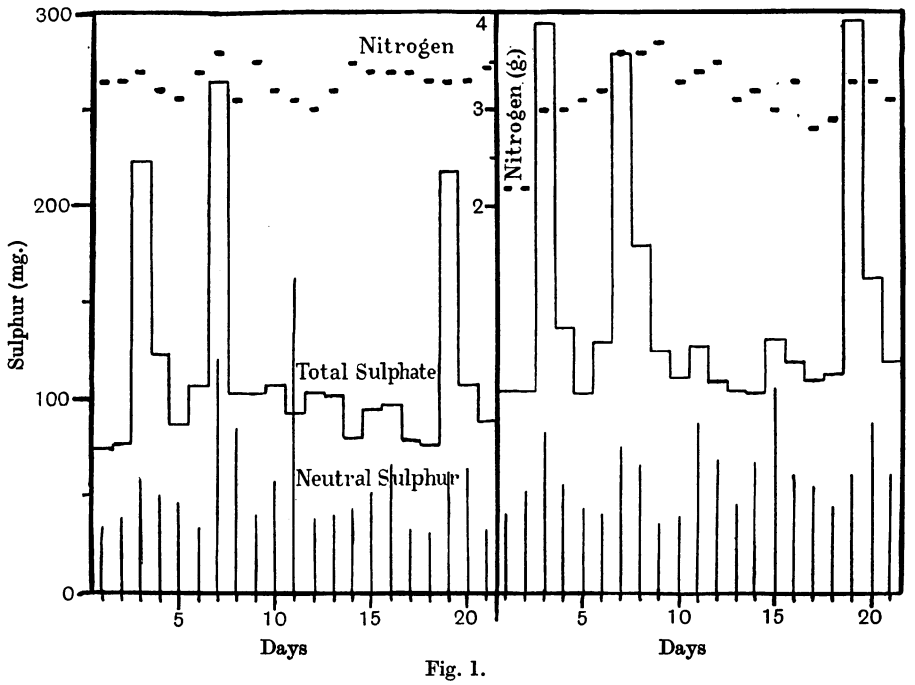

Fig. 1.

MAUD.

Day	3.	1·2 g. cystine orally	(320 mg. S)
„	7.	1·46 g. cysteine HCl subcut.	(300 mg. S)
„	11.	2·11 g. *S*-benzylcysteine orally	(320 mg. S)
„	15.	0·84 g. *S*-ethylcysteine orally	(179 mg. S)
„	19.	1·49 g. methionine orally	(320 mg. S)

PATSY.

Day	3.	1·2 g. cysteine orally	(320 mg. S)
„	7.	1·49 g. methionine orally	(320 mg. S)
„	11.	1·49 g. *S*-ethylcysteine orally	(320 mg. S)
„	15.	1·09 g. *S*-benzylcysteine orally	(164 mg. S)
„	19.	1·49 g. methionine orally	(320 mg. S)

slightly toxic; both dogs were somewhat lethargic for an hour or two after the dose while one of them (Patsy) vomited a little 11 hours after the larger dose (1·49 g.). The metabolic upset must have been slight since there was no marked effect on the excretion of nitrogen.

No very definite conclusions could be drawn concerning the oxidation of *S*-methylcysteine[1]. Both dogs vomited when given 0·8 g. orally while Maud was made seriously ill by 0·5 g. given subcutaneously, in this case there was also a trace of blood in the urine. Smaller doses than these would contain so little sulphur that it would be difficult to tell whether much had been oxidised or not. There was a definite rise in the total sulphate excretion on both the occasions when the substance was fed, corresponding to 25 % of the dose in the case of Maud and 40 % in the case of Patsy. In each case the urine was contaminated and the effect of the general disturbance on the sulphur metabolism is unknown. It seems to be fairly certain however that *S*-methylcysteine is more readily oxidised than *S*-ethylcysteine but much less readily oxidised than its homologue methionine. It would be interesting to study the behaviour of *homo*methionine and *S*-ethyl*homo*cysteine, *i.e.*

$CH_3SCH_2CH_2CH_2CH(NH_2)COOH$ and $CH_3CH_2SCH_2CH_2CH(NH_2)COOH$.

Differences such as those already mentioned in the metabolism of closely related substances emphasise the need for extreme caution in drawing conclusions, such as those of Sherwin, Shiple and Rose [1927], from the metabolic behaviour of derivatives of cystine or cysteine of unrelated character.

S-Benzylcysteine was prepared according to Suter [1895]. When given by mouth (1·09 g.) it was oxidised to a slight and probably not significant extent by one dog while the other dog did not oxidise a larger dose (2·11 g.) at all. These results contrast with those of Sherwin, Shiple and Rose [1927] who found that the rabbit could oxidise 40 % of the sulphur to sulphate after oral administration of *S*-benzylcysteine. Differences of this sort may easily be due, as Lewis [1924] has pointed out to differences in the intestinal flora of different species. It is noticeable that with *S*-benzylcysteine, as with *S*-ethylcysteine, only about a third of the extra sulphur fed is accounted for in the total sulphur value of the urine. This low recovery is not due to the deficiencies of the method of estimation used [Pirie, 1932, and Appendix to this paper] for control experiments show that 80–90 % of the sulphur in *S*-benzylcysteine or *S*-ethylcysteine added to urine, is converted into sulphate by this method of incineration. There was no change in the excretion of ethereal sulphate after feeding *S*-benzylcysteine. It was noticed that both *S*-benzylcysteine and *S*-ethylcysteine caused a transient diuresis, the volume of urine passed in the course of 24 hours was not increased but in all cases more than usual was passed during the first 12 hours.

Feeding experiments have also been carried out with a number of

[1] The preparation of *S*-methylcysteine has not apparently been described before. It was made by the standard technique with dimethyl sulphate and cysteine hydrochloride. The preparation will be described shortly.

sulphoxides; these will be described fully in a later paper. Preliminary experiments show that methionine sulphoxide is as readily oxidised as methionine whereas the sulphoxides of *S*-ethyl- and *S*-benzylcysteine are completely resistant to oxidation by the dog.

Experimental.

Two bitches, Maud and Patsy, were used. The former has not been used for metabolic work before while the latter is a veteran. The course of the experiments was exactly the same as in the earlier papers of this series [Hele, 1924] and the dogs received the following diets:

	Lean meat g.	Sugar g.	Margarine g.	Milk cc.	Water cc.	Agar g.
Maud (7·3 kg.)	90	60	20	160	100	5
Patsy (7·7 kg.)	70	50	20	160	100	5

There was no alteration in the dogs' weights during the experiments. Agar has recently been added to our standard dog diet since some unpublished work, carried out in collaboration with Hele, has shown that it increases the absorption of some inorganic constituents of the diet.

Total sulphate estimations were carried out by Folin's [1905] method and total sulphur by the method described in the Appendix to this paper. Inorganic sulphate estimations, by the benzidine method, were only made after feeding benzylcysteine; they showed that there had been no change in the excretion of ethereal sulphate.

Summary.

Methionine is oxidised by the dog to the same extent as cystine.

S-ethylcysteine and *S*-benzylcysteine are not appreciably oxidised.

S-methylcysteine is oxidised to some extent but is too toxic to allow an accurate estimate of its oxidation to be made.

I am grateful to Sir F. G. Hopkins for his interest in this work. The expenses of the research were largely covered by a grant to Dr Hele from the Royal Society.

APPENDIX.

Determination of total sulphur in dog's urine.

The estimation of total sulphur in urine is much the most laborious part of the work entailed in studying sulphur metabolism. We have hitherto used Folin's [1905] method; this involves fusion with sodium peroxide and it is the fusion that is most troublesome. The subsequent precipitation of barium sulphate and weighing is, we think, unavoidable with dog urine. Those methods which employ a titration (*e.g.* using benzidine [Fiske, 1921] or barium chromate [Morgulis and Hemphill, 1932]) involve the preliminary removal of phosphate from the urine. I have found that the published methods of doing this, although no doubt admirable when used with human urine, remove part of the neutral sulphur from dog's urine.

The incineration technique now adopted is based on that of Lematte, Boinot and Kahane [1927].

The oxidising agent consists of 3 volumes of concentrated nitric acid and 1 volume of 60 % perchloric acid, 3 volumes of this mixture are then mixed with 1 volume of the same mixture saturated with copper nitrate. The reagent is perfectly stable.

5 cc. of filtered urine and 5 cc. of the acid mixture are heated in a 6 × 1 in. monax test-tube which is two-thirds immersed in an air-bath at about 200°. The temperature is allowed to rise slowly and after 2–3 hours the contents of the tube are dry. It is removed from the bath, allowed to cool for a minute, and 1 cc. of the acid mixture added. After a further 2–3 hours in the bath at a temperature up to 280° the contents will be black or a dirty green, they should not still be blue. The tube is now held in crucible tongs while being heated all over for a few seconds in a large, but not very hot, Bunsen flame. If the heating in the air-bath has been adequate there will only be a few flashes, due to residual perchlorate, in the upper parts of the tube. When cool 2 cc. of *N* hydrochloric acid are added and the tube is replaced in the air-bath while latter is cooling. Boiling water is added till the tube is two-thirds full followed by 5 cc. of the 5 % barium chloride solution. The barium sulphate may be filtered off and weighed in the usual way after 2–4 hours. As a rule the quantity of barium sulphate weighed is of the order of 20 mg., the crucibles must therefore be packed carefully. I have found no difficulty in getting duplicates consistently agreeing to within 0·3 mg.; since the incineration and precipitation are carried out in the same vessel loss can only occur during the filtration. The same Gooch crucible can safely be used 4–5 times without re-packing with these small amounts of sulphate.

The air-bath used consists of a heavy iron saucepan with an inch of sand in it, an unglazed earthenware plate which is 1 inch less in diameter than the pot is supported on legs over the sand. The tubes and a thermometer pass through holes in an asbestos sheet cover and rest on the plate. The whole is heated by a large burner. Twelve tubes can conveniently be heated simultaneously.

This method has been checked on a number of different dog urines against my own estimations and those of Hele with the peroxide method. The differences were within the experimental error.

When used with materials having a low salt content, amino-acid fractions from a protein for example, the incineration can be carried through in an hour and, if very accurate results are not required, the estimation may be finished by the benzidine method.

REFERENCES.

Baernstein (1932). *J. Biol. Chem.* **97**, 669.
Clarke and Inouye (1931). *J. Biol. Chem.* **94**, 548.
Fiske (1921). *J. Biol. Chem.* **47**, 59.
Folin (1905). *J. Biol. Chem.* **1**, 131.
Harris (1923). *Proc. Roy. Soc. Lond.* B **94**, 426.
Hele (1924). *Biochem. J.* **18**, 586.
—— and Pirie (1931). *Biochem. J.* **25**, 1095.
Jackson and Block (1931). *Science*, **74**, 414.
Johnson (1911). *J. Biol. Chem.* **9**, 437.
Lematte, Boinot and Kahane (1927). *J. Pharm. Chim.* **5**, 325.
Lewis (1924). *Physiol. Rev.* **4**, 408.
Morgulis and Hemphill (1932). *J. Biol. Chem.* **96**, 576.
Mueller (1924). *J. Biol. Chem.* **58**, 373.
Pirie (1932). *Biochem. J.* **26**, 1270.
Sherwin, Shiple and Rose (1927). *J. Biol. Chem.* **73**, 667.
Suter (1895). *Z. physiol. Chem.* **20**, 562.
Weichselbaum, Weichselbaum and Stewart (1932). *Nature*, **129**, 795.