CCXXII. METABOLISM OF SULPHUR

X. THE REPLACEABILITY OF *dl*-METHIONINE IN THE DIET OF ALBINO RATS WITH ITS PARTIALLY OXIDIZED DERIVATIVE, *dl*-METHIONINE SULPHOXIDE¹

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IN view of the fact that methionine has been found necessary for growth its derivatives become of biological importance. An oxidation product, *dl*-methionine sulphoxide, has been prepared [Toennies, 1938; Toennies & Kolb, 1939], which should be of interest as a possible intermediate oxide of sulphur in metabolism. The roles of several oxides of *l*-cystine in the intermediary metabolism of cystine have been previously investigated [Bennett, 1937; 1939]. Growth curves were obtained on albino rats fed *l*-cystine disulphoxide, *l*-cysteinesulphinic acid and *S*-(guanythio)-cystine.2HCl, which hydrolyses to *l*-cysteinesulphenic acid. *l*-cystine disulphoxide proved capable of replacing *l*-cystine in the diet, but only one molecule of cystine is apparently available from three of the disulphoxide. The *l*-cysteinesulphinic acid produced no growth, while *l*-cysteinesulphenic acid gave rise to a slight but definite increase in growth. The present experiment was undertaken to find out whether *dl*-methionine sulphoxide could replace the growth-promoting properties of *dl*-methionine in the diet of the albino rat.

Preparation of compounds

The compounds employed were: *dl*-methionine (Eastman), 98.0 % purity according to S determination; *dl*-methionine sulphoxide [Toennies & Kolb, 1939], 99.8–99.9 % purity (0.05 % cystine); arachin isolated from peanut meal² by the method of Johns & Jones [1916]. Acetone was used as the drying agent instead of alcohol and ether. The arachin used in the present experiment was analysed for methionine by the method of Kassel & Brand [1938].³ The volatile iodide determinations gave a higher figure for methionine than the homocysteine titration, the values according to the former being 0.93 % and to the latter 0.53 % of the undried protein; the cystine values were 0.47 % if the higher methionine value be accepted, and 0.81 % if the lower be taken.⁴ Organic drying agents such as acetone and alcohol-ether, do not seem to be the cause

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² I wish to thank the Planters Peanut Co. for the peanut meal.

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⁴ Eight determinations of methionine, done on three other dried preparations, gave an average of 0.79 % $\pm$ 0.12 by the volatile iodide determinations and 0.47 % $\pm$ 0.04 by the homocysteine titration. At present the cause of this variation found in arachin is not known.

of the discrepancy since substantially the same results were obtained on acetone-, alcohol-ether- and air-dried samples. The arachin used when dried to constant weight at 100° lost approximately 7.5 %, and had 0.86 % ash. The value of 0.77 % methionine given for arachin in a previous paper [Bennett, 1939] was based on the volatile iodide figure, the homocysteine titration being 0.38 %.

Animal experiments

Albino rats, Wistar strain, were used as experimental animals. They were kept on a methionine-deficient basal diet, a modification of the cystine-deficient diet of Dyer & du Vigneaud [1936], 16 % milk vitamin concentrate, 15 % arachin as the basal protein and 24 % dextrin; each rat received 100 mg. of Harris vitamin B complex daily. Sufficient dry mixture was made at the beginning of the experiment to last through the entire period. From this, fresh basal diet was prepared every 3 days. All food was kept in the refrigerator. The special compounds were fed individually in small pieces of butter, the control animals receiving the same amount of butter. These butter mixtures were made once a week, keeping all instruments on ice during the mixing, and were kept in individual covered glass dishes in the refrigerator at 0°, the methionine sulphoxide at -10°. The animals were maintained on a normal diet for 8 days, at the end of which the basal diet was supplemented with the special compounds for a period of 19 days. The rats were weighed every other day and the average weight of the group plotted. The approximate amount of the basal diet consumed per rat each day was determined by weighing daily the basal food given each group and the residual food and dividing by the number of animals in the group (Table I).

Table I. *Daily basal food consumption per rat in g. averaged over 2-day period*

Days	Group A	Group B	Group C
	Unsupplemented		
1-2	5.8	4.7	4.7
2-4	6.7	5.9	5.9
4-6	7.9	7.1	7.1
6-8	7.5	6.9	6.9
	Daily supplement		
	Unsupplemented control	<i>dl</i> -methionine mg. 6.00	<i>dl</i> -methionine sulphoxide, mg. 6.64
8-10	5.8	5.3	5.4
10-12	5.3	4.9	5.5
12-14	6.5	5.8	6.2
14-16	5.5	5.3	5.9
16-18	5.5	6.2	6.1
18-20	5.5	6.0	6.2
20-22	5.7	6.3	7.0
22-24	6.0	7.1	6.7
24-26	5.9	6.9	6.8
27	6.5	7.6	6.8

In the experiment reported, 9 male rats from two litters born on the same day, were divided into three comparable groups of three animals each, 25 days old. After 8 days on the methionine-deficient diet, group B was supplemented with 6 mg. *dl*-methionine daily for 19 additional days, group C with 6.64 mg.

dl-methionine sulphoxide, the S equivalent of 6 mg. *dl*-methionine, and group A was unsupplemented. Fig. 1 shows the results obtained; the curves for groups B and C practically coincide.

Fig. 1. Growth curves expressing the quantitative metabolic relationship of *dl*-methionine and *dl*-methionine sulphoxide. The casein of du Vigneaud's basal diet was replaced by arachin. A, control; B, *dl*-methionine; C, *dl*-methionine sulphoxide.

DISCUSSION

The sulphoxide of methionine, according to Toennies & Kolb [1939], has certain properties, such as increased solubility, in common with the corresponding cystine derivative, cystine disulphoxide, because of the state of oxidation of the sulphur. Yet these authors state that the oxygen potential of the methionine sulphoxide, to judge from its behaviour *in vitro* with iodide and cysteine, is much lower than that of the cystine derivative. There is an "absence of any tendency of the methionine sulphoxide to form sulphenic or sulphinic acids by hydrolytic cleavage which is so pronounced in the case of cystine disulphoxide and characteristic of the —S—S— linkage". However, in the present experiment, the *dl*-methionine sulphoxide proved capable of replacing *dl*-methionine; identical growth curves were obtained with both compounds. It would seem from these results that *dl*-methionine sulphoxide could possibly play an intermediate role in the metabolism of *dl*-methionine.

An arachin basal diet seems to produce growth in the control groups. Experiments reported by White & Beach [1937] and Baernstein [1938] show an upward trend of their control curves. Baernstein states that 9 rats fed a 20% arachin diet showed very poor growth and lost weight at first but soon regained their original weight. "A slow growth ensued which averaged 0.5 g. per day." In the present experiment the rats in the control group lost weight at first, but in 22 days they had come back to their original weight and continued to gain weight until the end of the experiment. In spite of the growth attained by the controls, the animals on the supplemented diet consumed a greater amount of methionine, since their daily consumption of the basal diet was practically the same. Group A ate an average of 5.8 g. per rat each day, group B, 6.1 g. and group C, 6.2 g. Because of the discrepancies in the methionine values of the arachin (see preparation of compounds), it is difficult to state the exact amount of methionine in the basal diet. The homocysteine titration figures agree quite well with the original analysis by Baernstein [1932]. Some unknown impurity may be the

cause of the high volatile iodide figures. If the homocysteine titrations are correct, the basal diet would have 0.08 % methionine and 0.12 % cystine; if the volatile iodide figures are correct, the basal diet would contain 0.14 % methionine and 0.07 % cystine.

SUMMARY

Albino rats showed identical growth curves when *dl*-methionine and *dl*-methionine sulphoxide, respectively, were added to a methionine-deficient diet. Therefore under the conditions of the experiment reported, *dl*-methionine sulphoxide is able to replace *dl*-methionine in the diet of the albino rat.

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