CVII. METABOLISM OF SULPHUR

VII. A QUANTITATIVE STUDY OF THE REPLACEABILITY OF *l*-CYSTINE BY VARIOUS SULPHUR-CONTAINING AMINO-ACIDS IN THE DIET OF THE ALBINO RAT[1]

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In experiments reported previously on the replaceability of *l*-cystine in the diet of albino rats with some partially oxidized derivatives [Bennett, 1937], the compounds fed were *l*-cystine disulphoxide, *l*-cysteinesulphinic acid and S-(guanylthio)-cysteine.2HCl, which gives rise to *l*-cysteinesulphenic acid upon hydrolysis. *l*-Cystine disulphoxide proved capable of replacing *l*-cystine in the diet; *l*-cysteinesulphinic acid produced no growth; *l*-cysteinesulphenic acid resulted in a slight but definite increase in growth. These compounds were fed in sulphur equivalents that were in excess of the minimum amount necessary for maximum growth; therefore the results could not be interpreted quantitatively. In the present series of experiments, cysteine, cystine disulphoxide and methionine were fed, the basal diets being supplemented with smaller graded amounts of these amino-acids, in an attempt to demonstrate quantitative relationships in their ability to promote growth.

Preparation of compounds

The compounds employed were: *l*-cystine (Merck); *l*-cystine disulphoxide (Merck); *l*-cysteine prepared from cysteine hydrochloride [Toennies & Bennett, 1936]; *dl*-methionine (Eastman), 98·0 % purity according to sulphur determination;[2] *l*-methionine, isolated by Dr Toennies from egg albumin, 99·9 % purity, Exp. 2; 97·0 % purity, Exp. 4, according to sulphur determinations.[2] Arachin isolated from peanut meal[3] by the method of Johns & Jones [1916], 0·77 % methionine and 0·92 % cystine according to method of Kassel & Brand [1938].[2]

Experimental

Albino rats, Wistar strain, 25 days old, were used as experimental animals. In Exps. 1–3 they were kept on Dyer & du Vigneaud's [1936] cystine-deficient basal diet containing 6 % casein and 16 % milk vitamin concentrate fed *ad libitum*. In Exp. 4 the basal diet was methionine-deficient; 15 % arachin was used instead of casein as the basal protein and the dextrin was reduced to 24 %; each rat received 100 mg. Harris vitamin B complex daily. Sufficient dry

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[2] I am indebted to Mr Thomas P. Callan for these determinations.

[3] I wish to thank the Planters Peanut Co., Norfolk, Va., for the gift of the peanut meal.

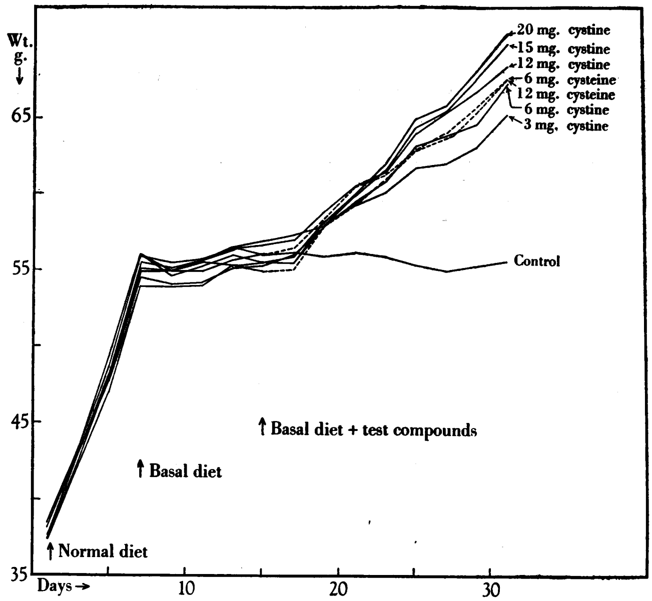

Fig. 1. Determination of the amounts of *l*-cystine and *l*-cysteine just adequate for maximum growth. Du Vigneaud's basal diet was used.

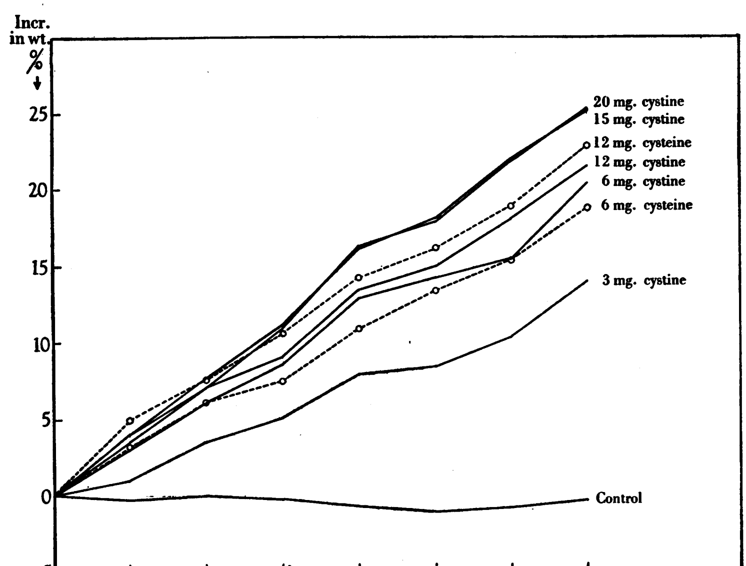

Fig. 1*a*. Fig. 1 recalculated, expressing the increase in weight in percentage of the weight at the beginning of the special diets.

mixture was made at the beginning of each experiment to last through the entire period. From this, fresh basal diets were 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. On account of the extreme lability of the sulphur compounds, especially the cysteine, great care was taken to prevent decomposition. 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 more labile 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 2 weeks. The rats were weighed every other day and the average weight of the group plotted.

Exp. 1. *To determine the amount of cystine just adequate for maximum growth.* In the first experiment, 96 rats from 11 litters, 25 days old, were divided into 8 comparable groups of 12 animals each, 6 males and 6 females. After 8 days on the cystine-deficient diet, cystine was fed in addition to the basal diet in 20, 15, 12, 6 and 3 mg. portions daily to five groups respectively. Two additional groups received 12 and 6 mg. of *l*-cysteine; one group was continued unsupplemented (Figs. 1, 1*a*). In Fig. 1*a*, the increase in weight is expressed in percentage of the weight at the beginning of the special diets. This allows the curves to start from a single point and brings out the true grouping which is slightly changed from that of the other graph. This experiment showed that 15 mg. of cystine added daily in excess of the basal diet produced maximum growth. It also brought out that the upper portion of the range was less suited to quantitative measurements than the lower in which slight differences in intake might produce greater spread of the curves. Therefore the scale of reference for the succeeding experiments was reduced to 12, 6, 3 and 1·5 mg. of cystine. The maximum amount of cystine added to these figures, due to the daily consumption of 5–10 g. of du Vigneaud's basal diet, would not exceed 1·8 mg. of cystine. These amounts of cystine are below the level of those used in most growth experiments reported in the literature. For instance, du Vigneaud, in his studies, fed the animals 20 mg. of cystine, or its sulphur equivalent, daily in addition to the cystine in the basal diet [Loring *et al.* 1933; Dyer & du Vigneaud, 1935; 1936]. A computation of the amounts of cystine ingested daily by the rats used by Womack *et al.* [1937] in their study of the growth-promoting potencies of cystine and methionine, show (their Tables II and III) that on an average approximately 12–16 mg. of cystine were consumed daily by each rat.

Exp. 2. *To determine the quantitative metabolic relationships of* l*-cystine,* l*-cysteine,* l*-cystine disulphoxide, and* l*-methionine, as expressed by growth curves.* In the second experiment, 88 rats from 12 litters, 25 days old, were divided into 11 comparable groups of 8 animals each, 4 males and 4 females. After 8 days on the cystine-deficient diet, the various groups, with the exception of the control group, were supplemented daily. Four groups received 12, 6, 3 and 1·5 mg. of cystine respectively. The other six groups were fed daily 3·0 and 12·1 mg. of *l*-cysteine; 3·7 and 14·9 mg. of *l*-methionine; and 3·4 and 13·6 mg. of cystine disulphoxide, respectively; amounts containing the sulphur equivalents of 3 and 12 mg. of cystine. Fig. 2 gives the customary type of growth curve; Fig. 2*a* shows the increase in weight expressed in percentage of the weight at the beginning of the special diets. As shown, all the curves representing 12 mg. equivalents (letter *D*) fall in a group as do those corresponding to 3 mg. (letter *B*). In both groups disulphoxide curves fall lower as the experiment progresses.

Exp. 3. *Repetition of Exp. 2 with* dl-*methionine in place of the* l-*form.* In the third experiment, 110 rats from 13 litters, 25 days old, were divided into 11

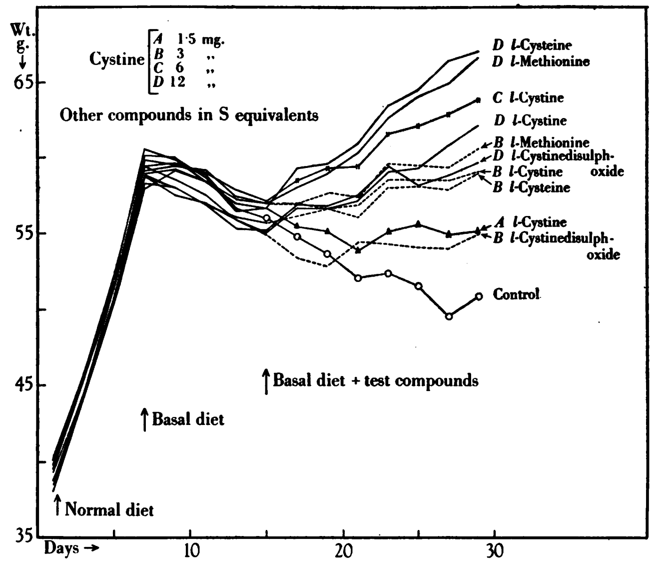

Fig. 2. Growth curves expressing the quantitative metabolic relationships of *l*-cystine, *l*-cysteine *l*-cystine disulphoxide and *l*-methionine. Du Vigneaud's basal diet was used.

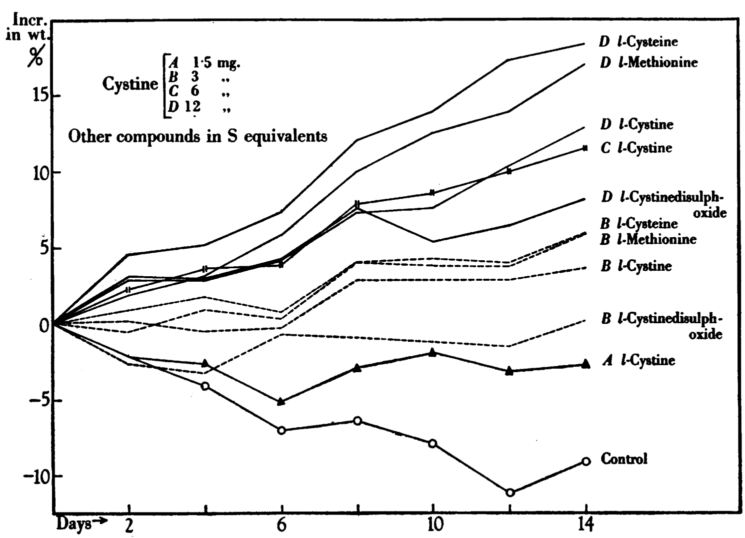

Fig. 2*a*. Fig. 2 recalculated, expressing the increase in weight in percentage of the weight at the beginning of the special diets.

comparable groups of 10 animals each, 5 males and 5 females. The various groups were treated as in Exp. 2; the compounds and amounts fed were the same, with the exception of methionine. The results are depicted in Fig. 3. The curves representing the 12 mg. equivalents fall in a group as before and about the same growth is attained in all cases, indicating that with this intake all the compounds are present in sufficiently large amounts to insure maximum growth. In the group of curves representing the 3 mg. intake, however, a spreading occurs. The cystine disulphoxide curve, as before, is the lowest, dropping below that for 1·5 mg. cystine, nearer the probable location of a 1 mg. cystine curve. The 3 mg. *dl*-methionine curve falls slightly above the 1·5 mg. cystine curve.

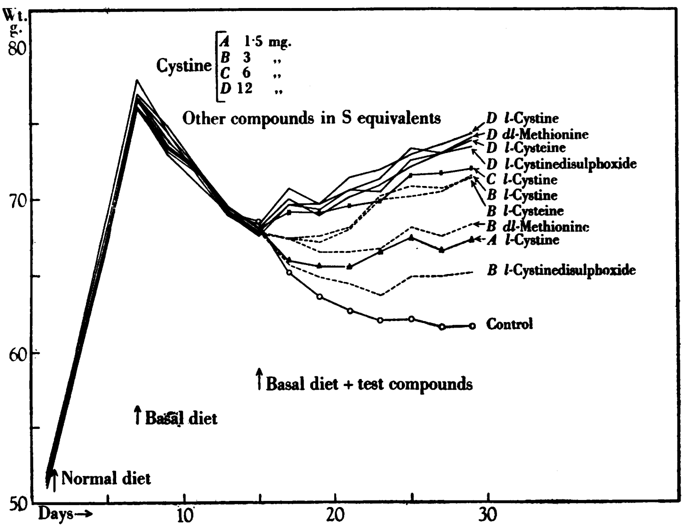

Fig. 3. Growth curves expressing the quantitative metabolic relationships of *l*-cystine, *l*-cysteine, *l*-cystine disulphoxide and *dl*-methionine. Du Vigneaud's basal diet was used.

Exp. 4. *To determine whether* dl-*methionine is as readily utilized for growth as the* l-*form*. A comparison of the growth curves of rats receiving the smaller amount of *l*-methionine in Exp. 2, and *dl*-methionine in Exp. 3, suggests that the *dl*-methionine may not be exactly the equivalent of the *l*-form. Since these curves are from separate experiments, it was decided to test this more adequately by feeding the *l*- and *dl*-forms of methionine in one experiment (4). The casein used in the basal diet provided 1·9 mg. of methionine for each g. of basal food. In an attempt to reduce this basal intake, arachin was substituted for casein since it is said to contain 0·54 % methionine [Baernstein, 1932] amounting to about 0·8 mg. of methionine per g. of basal food. The arachin isolated for the present experiment, however, showed 0·77 % methionine by Kassel & Brand's [1938] modification of Baernstein's [1932] method or 1·2 mg. of methionine per g. of basal food. Twenty-five male rats, 23 days old, from 5 litters were divided into 5 groups, one rat from each litter in each group. After 8 days on the methionine-deficient diet, the various groups, with the exception of the control, were supplemented daily. Groups B and C received 6 and 12 mg. of *l*-methionine, respectively, and D and E received the same amounts of *dl*-methionine daily. The approximate amount of the basal diet consumed per rat per 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). This experiment was conducted on a methionine-deficient diet, with arachin as the basal protein. Fig. 4 gives the curves obtained.

Table I. *Daily basal food consumption per rat in g. Averaged over 2-day period. Exp. 4*

Days	Unsupplemented				
	Group A	Group B	Group C	Group D	Group E
1–2	4·1	4·5	5·2	4·7	4·6
2–4	4·9	5·4	5·7	5·4	5·4
4–6	4·7	5·2	4·9	5·5	5·0
6–8	4·4	5·9	5·9	5·3	5·2

		Daily supplement			
	Unsupplemented Control	*l*-Methionine, mg. 6	*l*-Methionine, mg. 12	*dl*-Methionine, mg. 6	*dl*-Methionine, mg. 12
8–10	4·5	6·4	6·2	5·4	5·8
10–12	4·7	6·8	5·4	5·2	6·2
12–14	5·0	6·2	6·3	5·5	6·6
14–16	4·8	5·8	5·7	6·0	6·6
16–18	4·7	6·2	6·2	6·1	7·3
18–20	4·4	6·3	6·3	5·9	7·4
20–22	4·8	6·6	6·8	6·4	7·8

Table II. *Relation of growth to food consumption. Averaged per rat over a 14-day period of feeding. Exp. 4*

Group	Daily supplement	Increase in weight g.	Food consumption g.	Gain per g. of food consumed g.
B	6 mg. *l*-methionine	11·2	88·6	0·126
C	12 mg. *l*-methionine	13·1	85·8	0·152
D	6 mg. *dl*-methionine	8·1	81·0	0·100
E	12 mg. *dl*-methionine	12·4	95·4	0·129

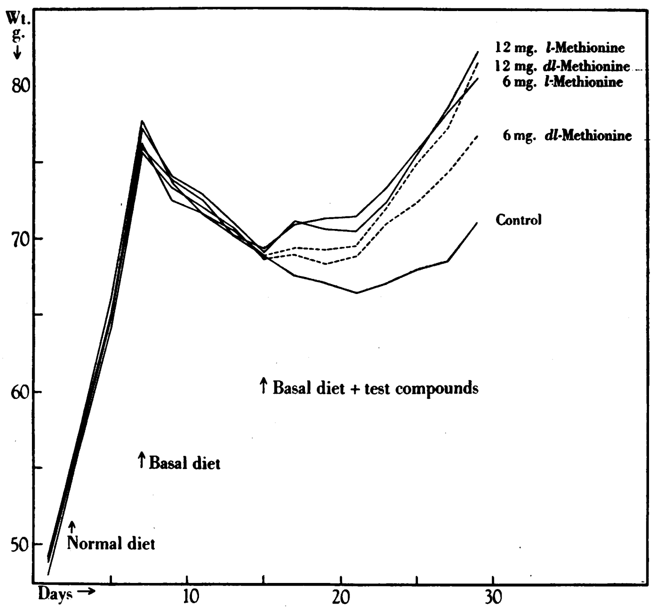

Fig. 4. A comparison of the growth-promoting properties of *l*-methionine and *dl*-methionine. The casein of du Vigneaud's basal diet was replaced by arachin.

Discussion

There has been some question as to whether the cystine-cysteine relationship in metabolism is solely an oxidation-reduction relationship or whether hydrolytic cleavage of cystine also plays a part. In the former case, one molecule of cystine should be the equivalent of two molecules of cysteine; in the latter case, a one to two relationship would not hold. Shinohara & Kilpatrick [1934], working in acid solution without addition of heavy metals, reported that through hydrolysis and subsequent dismutation three molecules of cystine gave rise to five of cysteine and one of cysteic acid. This was in agreement with earlier work of Vickery & Leavenworth [1930] who worked with heavy metals in solution, whereby cysteine was removed from the mixture as rapidly as it was formed. Lavine [1937], also with heavy metals present, found that two molecules of cystine gave rise to three molecules of cysteine and one of sulphinic acid. In Exps. 2 and 3 the clear-cut separation of the curves into two groups representing those rats which received the equivalents of 12 mg. cystine and those which received the equivalents of 3 mg. indicates (Figs. 2, 2*a*, 3) that, under these conditions, two molecules of cysteine are fully the equivalent of one of cystine.

Whether oxidation of disulphoxide sulphur ever occurs *in vivo* is uncertain. Evidence from the present experiments shows that the formation of cystine from its disulphoxide can occur, although one molecule of cystine disulphoxide did not give rise to the same amount of growth as did one molecule of cystine. Possibly a series of reactions takes place in the body similar to that found in test-tube experiments by Lavine [1936], whereby three molecules of cystine disulphoxide, through hydrolysis and dismutation, give rise to four molecules of cysteinesulphinic acid and one molecule of cystine.

The results of Exp. 4 substantiate those of Exps. 2 and 3; the *dl*-methionine when fed in submaximal amounts did not produce quite as much growth as the *l*-form. Since both the *l*- and the *dl*-forms are oxidized to sulphate [Stekol, 1935], it might seem reasonable to suppose that they have equal growth-promoting properties. However, it is known that the readiness with which a compound is oxidized and its availability for growth do not run parallel in some compounds. For instance, du Vigneaud *et al.* [1934] found that *S*-methylcysteine is readily oxidized, but that feeding this compound does not bring about growth of rats on a cystine-deficient diet.

Considering the relation of growth to food consumption (Exp. 4, Table II) the gain in weight for each g. of food consumed is practically the same with an intake of 6 mg. *l*-methionine (group B) and 12 mg. *dl*-methionine (group E), 0·126 and 0·129 g. respectively. The lowest relative gain was attained with the 6 mg. *dl*-methionine supplement, or 0·10 g. for each g. of food ingested. An intake of 12 mg. *l*-methionine definitely increased the utilization of food for growth; these animals show 0·15 g. increase in weight for each g. of food consumed. Kik [1938] similarly reported a better utilization of food when a casein ration was supplemented by methionine than when supplemented by cystine. Animals on the 12 mg. *dl*-methionine, after several days, grew at approximately the same rate as those on 12 mg. *l*-methionine; it is attained, however, only by increasing intake, 85·8 g. of food for those on 12 mg. *l*-methionine compared with 95·4 g. for those receiving 12 mg. *dl*-methionine. Growth curves reported by Jackson & Block [1938] demonstrate this same tendency. Their rats receiving the *d*-methionine, increase their daily food consumption to a greater extent than those receiving the *l*-methionine, while gaining approximately the same number of g. in weight. This tendency can be seen by a rough estimation of g. gained and daily food consumption given in Fig. 1 of their paper.

From Exp. 4 some idea of the absolute amounts of a mixture of cystine and methionine which will produce normal growth in albino rats may be obtained. Calculating the amounts of methionine and cystine consumed in the basal diet in addition to those fed as supplement, Group C had a daily intake of 19·3 mg. *l*-methionine and 8·5 mg. *l*-cystine; group E, 12 mg. *dl*-methionine, 8·2 mg. *l*-methionine, and 9·5 mg. *l*-cystine; group B, 13·6 mg. *l*-methionine and 8·8 mg. *l*-cystine. These three groups undergo practically the same increase in weight during the experimental period. The last group D, which received a daily intake of 6 mg. *dl*-methionine, 7·0 mg. *l*-methionine and 8·1 mg. *l*-cystine, grew somewhat less; therefore, in this case, we conclude that methionine and cystine were present in suboptimal amounts.

Summary

Under the conditions of the experiments reported, the following quantitative metabolic relationships are indicated as expressed by growth curves:

One molecule of *l*-cystine is available from three molecules of *l*-cystine disulphoxide.

One molecule of *l*-cystine is equivalent to two molecules of *l*-cysteine.

One molecule of *l*-cystine is equivalent to two molecules of *l*-methionine.

dl-Methionine is somewhat less readily utilized for growth than is the *l*-form.

l-Methionine definitely increases the utilization of food for growth.

The absolute amounts of methionine and cystine, which permit normal growth in young albino rats on an otherwise adequate diet under the conditions of the last experiment, are estimated.

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