LII. THE METABOLISM OF *i*-CYSTINE IN THE RAT.

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THE present work was undertaken in view of the fact that very little information is available regarding the metabolism of *i*-cystine, and because it was felt that a knowledge of the differences in behaviour between *l*- and *i*-cystine in the animal organism might throw light both upon the structure of *i*-cystine and upon the metabolism of this amino-acid.

Neuberg and Mayer [1905] observed the utilisation of *i*-cystine by *Aspergillus niger* and by *Penicillium*. Gortner and Hoffman [1927] observed its utilisation by *A. niger*. Hele and Pirie [1931] have studied the oxidation of *i*-cystine in the dog, and their findings are very similar to those described in this paper for the case of the rat. In their papers on the resolution of *i*-cystine into its *l*- and *d*-isomerides, du Vigneaud and Hollander [1930, 1931] state that they are undertaking the study of the metabolism of *d*-cystine, and their results are awaited with keen interest.

In order to determine to what extent *i*-cystine can replace the naturally occurring amino-acid for purposes of tissue formation, the technique elaborated by Sherman and Woods [1925] was adopted.

I. THE OXIDATION OF *l*- AND *i*-CYSTINE IN THE RAT.

Experimental.

The *l*-cystine used in these experiments was prepared by acid hydrolysis of human hair, and the *i*-cystine by boiling this *l*-cystine with hydrochloric acid, according to the method of Hoffman and Gortner [1922].

Eight adult male rats were taken from the inbred piebald strain used in this laboratory. Their combined weight at the beginning of the experiment was 1900 g., and at the end 2010 g. A daily ration of 15 g. of synthetic diet per rat was fed throughout the experiment. The composition of this diet and the methods employed for collection and analysis of urine were identical with those described in a previous communication [Lawrie, 1931].

On the days when a dose of cystine was to be given, the cystine was thoroughly mixed with the day's ration of synthetic diet, and the mixture was always readily eaten. The influence of this dosage upon the sulphur partition

of the urine is shown in Fig. 1, and the extent to which the ingested cystine had been oxidised to inorganic sulphate is given in Table I.

Fig. 1.

Dosage. On 7th day, 0.7 g. *l*-cystine mixed with diet.
 On 11th day, 0.7 g. *i*-cystine " "
 On 17th day, 1.0 g. *i*-cystine " "
 On 22nd day, 1.0 g. *l*-cystine " "

Table I.

Dose	Extra inorganic sulphate excreted in 24 hours after dose		Extra inorganic sulphate excreted in 48 hours after dose		Extra inorganic sulphate excreted in 96 hours after dose	
	mg. S	% dose	mg. S	% dose	mg. S	% dose
700 mg. of <i>l</i> -cystine	45	49	63	68	—	—
1000 mg. of <i>l</i> -cystine	41	31	57	43	78	59
700 mg. of <i>i</i> -cystine	67	72	—	—	—	—
1000 mg. of <i>i</i> -cystine	79	59	112	84	—	—

Discussion of results.

Reference to Fig. 1 will show that during the 24 hours following each dosage with cystine there is a marked rise in the rate of excretion of inorganic sulphate. A fall in the rate of excretion of this fraction then follows, which, although rapid during the second, and sometimes during the third, period of 24 hours after the dosage, subsequently becomes much slower. The rate of inorganic sulphate excretion then becomes comparatively steady at a higher level than before the dose was given. Since no similar prolongation of the effect due to dosage is seen in the neutral sulphur and ethereal sulphate fractions, and since the animals were slightly gaining in weight throughout the experiment, whilst being restricted to a constant food intake, it is very probable that the tendency of the inorganic sulphate fraction to increase throughout the experiment is due to retention, and slow subsequent excretion, of a part of the sulphur given as cystine.

In order, therefore, to obtain as clear an indication as possible of the rate and extent of the oxidation of any dose, it is necessary to compare the rise in inorganic sulphate excretion immediately following the giving of that dose, with the level of this fraction during those days immediately preceding the dosage which showed a steady rate of excretion of inorganic sulphate. Hence the extra inorganic sulphate excretion following the first dosage is calculated from the average values of the inorganic sulphate fraction for the first 6 days of the experiment. The extra inorganic sulphate produced by the second dose is estimated by comparison with the average of the inorganic sulphate values for the ninth and tenth days of the experiment. Similarly, the values averaged for the estimation of the effect of the third dose were those of the twelfth to the sixteenth days of the experiment; and the effect of the last dose was estimated from the average of the values of the inorganic sulphate fractions on the nineteenth, twentieth and twenty-first days of the experiment. From the above considerations it is clear that the figures for the 24 hours following dosage are likely to give the most reliable indication of the readiness with which the rat oxidises the cystine isomeride in question.

The figures so obtained are given in Table I, and from them it is clear that *i*-cystine is oxidised by the rat at least as readily as *l*-cystine. In fact, the figures suggest a more complete oxidation of the *i*- than of the *l*-isomeride, and this relationship might be explained by the greater solubility of the former [Hoffman and Gortner, 1922].

A transient rise in the rate of excretion of neutral sulphur followed the administration of each dose of cystine. This rise was less marked after dosage with *i*-cystine than after *l*-cystine. This observation again indicates that the former isomeride has been more readily metabolised.

II. A COMPARISON OF THE VALUES OF *l*- AND *i*-CYSTINE AS SUPPLEMENTS FOR A DIET DEFICIENT IN CYSTINE.

As a basal diet in which cystine deficiency is the factor limiting growth rate, the diet described by Sherman and Woods [1925] was used with the following slight modifications. The salt mixture was replaced by that used in this laboratory, and in some of the later experiments the percentage of cod-liver oil was raised to 1.65. The dried milk powder was supplied by Messrs Glaxo, Ltd.

With the exception of 6 albino rats supplied by the above firm, and referred to in this paper as "litter 2," all the animals used in this experiment were bred in this laboratory, and were of the same strain as those used in Part I of this work.

Fig. 2. Growth curves of rats fed upon a basal diet deficient in cystine, marked (b); and upon the same diet supplemented with 5 g. of *i*-cystine per 10,000 g. of dry diet, marked (i); and supplemented with the same proportion of *l*-cystine, marked (l).

In a preliminary experiment, the growth rates of rats fed upon the basal diet were compared with those of rats receiving additions to their diet of 1, 2.5, 5, and 10 g. per 10,000 g. of dry diet, of *l*- and *i*-cystine respectively. The results obtained confirmed the findings of Sherman and Woods that addition of *l*-cystine to the basal diet caused an increase in growth rate over a period of 6 weeks which is proportional to the percentage of cystine added, until 5 g. of cystine per 10,000 g. of dry diet have been added. They also indicated that

in order to obtain a rate of growth equal to that shown by rats fed upon the basal diet *plus l*-cystine in proportions up to 5 g. per 10,000 g. of dry diet, approximately twice the percentage of *i*-cystine must be added to the diets of control rats receiving the basal diet.

The diet was therefore suitable for a comparison between *l*- and *i*-cystine; and in the remaining experiments litter-mates, of the same sex, and started upon experiment at the same time, were divided into the following three groups. Group 1 received the basal diet. Group 2 received the same diet *plus* 5 g. of *l*-cystine per 10,000 g. of dry diet, whilst group 3 received the same basal diet supplemented with 5 g. per 10,000 g. dry diet of *i*-cystine. These diets were all fed *ad lib.* for a period of 6 weeks.

The differences in growth rate produced by feeding these three diets to litter mates are shown graphically in Fig. 2. Table II shows the growth rates calculated in grams per week; the increase in growth rate shown by each animal over its litter-mate fed upon the basal diet; and the ratio (expressed as a percentage) of this increase, for each animal of group 3 to the corresponding increase shown by its litter-mate of the same sex in group 2.

Table II. *Growth rates of rats over a period of 6 weeks, expressed as g. per week.*

Litter no. and sex	Diet			B - A	C - A	$\frac{B-A}{C-A} \times 100$
	Cystine-low basal diet Column A	Cystine-low basal diet + 5/10,000 of <i>i</i> -cystine Column B	Cystine-low basal diet + 5/10,000 of <i>l</i> -cystine Column C			
1 ♀	9.0	—	—	—	—	—
2 ♀	(1) 5.8 } Av.	(3) 8.2 } Av.	(5) 10.2 } Av.	—	—	—
	(2) 6.7 } 6.3	(4) 8.2 } 8.2	(6) 11.0 } 10.6	1.9	4.3	44
3 ♀	5.9	—	—	—	—	—
4 ♂	—	(3) 13.3 } Av.	(1) 18.5	—	—	—
		(2) 16.7 } 15.0				
5 ♀	(1) 9.2	(3) 10.8	(2) 14.4	1.6	5.2	31
5 ♂	(1) 8.7	(3) 11.2	(2) 16.3	2.5	7.6	33
6 ♀	(1) 6.8	(3) 8.8	(2) 10.8	2.0	4.0	50
7 ♀	(1) 9.0	(3) 10.2	(2) 11.5	1.2	2.5	48
8 ♂	(1) 7.0 } Av.	(3) 14.5 } Av.	(5) 17.7 } Av.	—	—	—
	(2) 10.2 } 8.6	(4) 16.7 } 15.6	(6) 21.2 } 19.5	7.0	10.9	64
						Av. 45

Note. The figures in brackets serve to distinguish litter-mates of the same sex in Fig. 2.

Discussion of results.

The results given in Fig. 2 and Table II show clearly that *i*-cystine is able to supplement a diet deficient in cystine. They also show equally definitely that it is not so efficient for this purpose as the normally occurring *l*-cystine. Under these circumstances one may conclude that the rat is capable of resolving *i*-cystine, and using the *l*-isomeride, for tissue building, whilst the *d*-cystine is excreted, probably after complete oxidation of its sulphur. That the difficulty

experienced by the rat in utilising *i*-cystine for purposes of tissue building arises subsequent to its absorption from the alimentary canal is rendered very probable by the results given in Part I of this paper.

Previous workers have described a persistence of a type of fur characteristic of young rats and the occurrence of symmetrical areas of baldness in rats fed upon diets deficient in cystine. These effects have been noticed in the present experiment in some of the rats of groups 1 and 3.

Cystine and tryptophan appear to be markedly different in respect to the relationships which they show between their optical activity and nutritional values; for Berg and Potgieter [1932] have very recently shown that the feeding of *dl*-tryptophan is as efficient in promoting growth in rats fed upon a diet deficient in tryptophan, as is the feeding of the same quantity of the naturally occurring *l*-tryptophan.

SUMMARY.

The rat can oxidise *i*-cystine as rapidly and completely as *l*-cystine.

The value of *i*-cystine for tissue building purposes in the rat lies between one-third and two-thirds of that of *l*-cystine.

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