Observations on Cysteic Acid Decarboxylase

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Evidence has been given (Blaschko, 1942) of the occurrence in the liver of some mammals (e.g. the dog and the rat) of an enzyme which decarboxylates *L*-cysteic acid. It was shown that prolonged dialysis led to the inactivation of the extracts. Since extracts inactivated in this way could not be reactivated by adding a boiled extract, it seemed possible that the dialysis had removed a water-soluble factor. The enzymic activity of livers from aneurin-deficient rats was therefore studied. These experiments are described below.

Medes & Floyd (1942) have reported experiments according to which a cysteic acid decarboxylase occurs in the rat's kidney. They determined the amino nitrogen and the amino-acid carboxyl groups and found a decrease in the latter and no change in the former on incubation of rat's kidney brei with cysteic acid under anaerobic conditions. Using the manometric technique, the enzyme was not found in the kidney of the dog, the cat or man (Blaschko, 1942), and it seemed therefore of interest to examine the enzymic activity of rat's kidney, using the manometric technique.

L-Cysteic acid is the only known substrate of the enzyme. In what follows, experiments with homocysteic acid are described from which it can be seen that this substance, although not a substrate, has some affinity to the enzyme.

MATERIALS AND METHODS

The preparation of the liver extracts and the plan of the manometric experiments have been previously described (Blaschko, 1942). It was found convenient to use the period from the 3 min. reading to the 15 min. reading as a measure of enzymic activity, since the rate of formation of CO_2 during this interval was uniform.

The *DL*-homocysteic acid was prepared according to Butz & Du Vigneaud (1932) from homocystine for which the author is indebted to Mr L. J. Goldsworthy of the Dyson Perrins Laboratory. All the animals used in the feeding experiments were kept in the Dunn Nutritional Laboratory; the author wishes to thank Dr L. J. Harris for the facilities offered and Mr A. Ward for looking after the animals.

RESULTS

Effect of diet on enzymic activity. When the liver extract from aneurin-deficient rats was compared with extracts from litter-mates kept on a normal

diet, it was found that the liver of the deficient animal gave a preparation of greatly reduced decarboxylase activity. A typical experiment is shown in Fig. 1.

Fig. 1. *L*-Cysteic acid decarboxylase activities of the liver extracts from an aneurin-deficient rat and from its normal litter-mate. Total volume: 2.0 ml.; 1.6 ml. liver extract + 0.4 ml. *m*/100 *L*-cysteic acid. The arrow denotes 1 mol. of CO_2 per mol. of cysteic acid.

The low activity of the extracts was not due to the absence of either aneurin or cocarboxylase. This was shown in an experiment in which liver extracts were prepared from two deficient animals; the extracts were pooled and the enzymic activity was tested: (a) without any addition, (b) after adding aneurin to make an initial concentration of $50 \mu\text{g.}/\text{ml.}$, (c) after adding cocarboxylase to make an initial concentration of $50 \mu\text{g.}/\text{ml.}$ It was found that in all three cases the enzymic activity was the same.

The dietary lack of aneurin was only indirectly responsible for the low decarboxylase activity of the extracts. This was shown in experiments in which the total food intake of the animals was restricted. Of two rats one was given a diet deficient in aneurin; the amount of food taken was measured each day and on the following day a second rat received the same amount of food plus a daily dose of $12 \mu\text{g.}$ of aneurin. This procedure is similar to that used by Lu & Needham (1941). The two animals were killed when symptoms of polyneuritis were fully developed in the first rat or when its growth curve began to decline. Five such pairs of rats were examined; the results are given in Table 1A. The

result may be compared with the figures of Table 1B, which gives the activities for normal animals of approximately the same age. In the experiments shown in Table 1B the amount of liver extract used was always 1.6 ml.; in the experiments of Table 1A the amounts of extract available were somewhat smaller and the total volume of fluid in the main manometer flask was brought up to 1.6 ml. by adding M/15 sodium phosphate buffer of pH 7.4. In a control experiment it was established that the dilution of the extracts did not modify the enzymic activity.

In the one experiment in which a difference between blank and experimental flask was found, extract corresponding to 1600 mg. of fresh weight of kidney formed 5 μ l. CO₂ in 35 min. If this figure lies outside the range of experimental error, which seems doubtful, it would correspond to a 'cysteic acid quotient' of 0.006. This is one-twentieth of the average activity previously reported for rat's liver. The data cannot be compared with those of Medes & Floyd (1942), as their paper contains no information as to the amounts of tissue used, but it seems likely that the enzyme responsible for the

Table 1A. *Cysteic acid decarboxylase activity of aneurin-deficient rats compared with that of rats receiving diet equal in amount but containing aneurin*

Exp. no.	Rat wt. (g.)	Liver wt. (g.)	Aneurin in diet	Volume of extract (ml.)	CO ₂ formed 3-15 min. (μ l.)	CO ₂ formed calc. for 1.6 ml. extract (μ l.)
1a	97	3.3	Absent	1.0	14.0	22.5
1b	102	2.2	Present	1.0	6.5	10.5
2a	86	2.7	Absent	1.3	24.5	30.0
2b	94	2.8	Present	1.3	19.0	23.5
3a	94	2.9	Absent	1.1	21.5	31.5
3b	122	2.7	Present	1.1	8.5	12.5
4a	83	3.1	Absent	1.5	29.5	31.5
4b	93	2.7	Present	1.5	19.5	21.0
5a	88	2.5	Absent	1.1	12.5	18.0
5b	97	2.3	Present	1.1	5.5	8.0

Table 1B. *Cysteic acid decarboxylase activity of normal rats*

(In all experiments 1.6 ml. of liver extract were used.)

Exp. no.	Rat wt. (g.)	Liver wt. (g.)	CO ₂ formed 3-15 min. (μ l.)
6	220	9.0	50
7	226	9.4	45
8	233	10.2	59
9	220	9.2	40
10	218	10.4	40
11	225	9.4	57

It can be seen that in all experiments of Table 1A the enzymic activity was low as compared with Table 1B. Previously (Blaschko, 1942) the enzymic activity was expressed as the 'cysteic acid quotient', i.e. as the μ l. CO₂ formed/mg. fresh tissue/hr. The average figure for this quotient in the normal controls is 0.15, for the aneurin-deficient animals it is 0.083, and for the partially starved animals it is 0.046. The figures for the animals with restricted food supply are lower than those of the aneurin-deficient animals, but in some of the preliminary experiments the activity of extracts from deficient animals was equally low.

Enzymic activity of the normal rat's kidney. Extracts of normal kidney, unlike liver, when incubated with *l*-cysteic acid did not show a measurable formation of CO₂ in excess of the CO₂ formed in the blanks.

loss in carboxyl groups was not present in our extracts.

Experiments with dl-homocysteic acid. Homocysteic acid, used in a concentration of 2×10^{-3} M, did not give rise to any formation of CO₂; it was tested with extracts of both dog's and rat's liver. But it seems that the homocysteic acid has an affinity to the enzyme: if it was added to an extract of dog's liver together with an equimolecular concentration of *l*-cysteic acid, it reduced the rate of decarboxylation of the latter substance almost to one-half. This is shown in the following experiment:

Total volume: 2.0 ml.; 0.8 ml. dog liver extract. 38°; N₂. Initial concentration of:

<i>l</i> -Cysteic acid	2×10^{-3} M	2×10^{-3} M
<i>dl</i> -Homocysteic acid	0	4×10^{-3} M
μ l. CO ₂ formed in 30 min.	54.5	29

DISCUSSION

The evidence given above makes it unlikely that the low enzymic activity of the extracts from aneurin-deficient animals is explained by a co-enzyme-like role of aneurin or cocarboxylase. It appears more probable that the lack of the vitamin exerts its effect indirectly, by reducing the food intake of the animals. This interpretation is similar to that given by Lu & Needham (1941) of their observations on the reduction of the adenylic deaminase activity in the breast muscle of aneurin-deficient pigeons. These observations are not without a more general interest for the understanding of metabolic changes during starvation: it is well known that the reduction of the weight and composition of different organs in starvation is not uniform and it seems as if there is also a sequence in the loss of activity of different enzymic systems. A general survey of the order in which different enzymes of importance for metabolism are reduced in activity during starvation has never been made.

The difference between our own results on the rat's kidney and those of Medes & Floyd (1942) has not been fully investigated. In the manometric experiment no reliable proof of the existence of a cysteic acid decarboxylase in the kidney was obtained, but it is possible that the difference in the methods employed may be responsible for our negative results.

SUMMARY

1. Extracts from the liver of rats deficient in aneurin showed marked decrease of cysteic acid decarboxylase activity. No increase in activity occurred after adding aneurin or cocarboxylase *in vitro*.

2. Feeding experiments suggest that the reduction of enzymic activity is due to restricted food intake and that the connexion with the aneurin deficiency is an indirect one.

3. No certain evidence of the presence of *l*-cysteic acid decarboxylase in extracts of rat's kidney has been obtained.

4. *dl*-Homocysteic acid was not decarboxylated by enzyme preparations from the dog or the rat, but it reduced the rate of decarboxylation of *l*-cysteic acid.

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Experiments (in collaboration with Dr Ruth Duthie) on enzyme preparations from dog's liver have recently shown that (a) removal of a precipitate formed at pH 4.5 does not lead to impairment of the enzymic activity, and (b) active acetone-dried preparations of the enzyme can be obtained.

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The Effect of Unsaturated Fatty Acids on *Lactobacillus helveticus* and other Gram-positive Micro-organisms

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Bauernfeind, Sotier & Boruff (1942) reported that certain fatty acids stimulated the growth of *Lactobacillus helveticus* in the presence of suboptimal amounts of riboflavin. Strong & Carpenter (1942) confirmed this effect with a number of fatty acids, but with linoleic acid they observed that, whereas

lower concentrations stimulated the production of lactic acid, higher concentrations had an inhibitory action. Both groups of workers made their tests with the original medium of Snell & Strong (1939), which has been used for the microbiological assay of riboflavin. It is evident, therefore, that the pre-