66. *l*(−)-Cysteic Acid Decarboxylase

By **Hermann Blaschko**, *From the Physiological Laboratory, Cambridge*

(*Received 23 June 1942*)

The pathway of taurine formation in the animal body is unknown. Friedmann [1903] showed that cystine is easily oxidized *in vitro* to cysteic acid and cysteic acid decarboxylated to taurine by the action of heat [see also White & Fishman, 1936], and he suggested the formation in the living body of taurine from cystine with cysteic acid as an intermediary. The increased output of taurocholic acid in dog's bile under suitable conditions after the administration of either cystine [Bergmann, 1904] or *l*(−)-cysteic acid [Virtue & Doster-Virtue, 1939] gives support to Friedmann's scheme, at least in the dog.

In experiments with tissue preparations from rat's liver an enzyme was found catalysing the oxidation of cystine and cysteine to cysteic acid [Bernheim & Bernheim, 1939; Medes, 1939], but attempts to demonstrate the presence of a decarboxylase for cysteic acid have been unsuccessful [Medes & Floyd, 1942]. In the following, evidence is given of the occurrence in mammalian liver of an enzyme which decarboxylates *l*(−)-cysteic acid.

Material and methods

The author is indebted to Dr A. Neuberger for the samples of *l*(−)-cysteic acid and *dl*-cysteic acid used in these experiments. The dog's livers were in most cases obtained from animals killed by the 'humane killer' and dissected soon after death. The cod's livers came from the Torry Research Station, Aberdeen. They were frozen as soon after death as possible and sent in a thermos flask to Cambridge where they arrived in a semi-frozen condition less than 48 hr. after the dispatch of the material. The human kidney extract was freshly prepared from material removed at operation [case C of paper by Blaschko, 1942]. The organs were weighed, ground in a mortar with sand, and equal amounts of *M*/15 phosphate buffer solution of *p*H 7·4 were added. The enzyme solution used was the supernatant fluid after 10–12 min. centrifuging.

Simple Barcroft-Warburg manometers were used; the flasks were conical with an inner tube and a side bulb. The main flask contained the extract, the side bulb the substrate (neutralized cysteic acid) and the inner tube *N* H_2SO_4. In each case a blank was set up with distilled water in the side bulb; the reading in this flask served for determining the CO_2 present in the extracts. All experiments were carried out at 37·5° and in N_2.

Experimental

When cysteic acid was added to dog's liver extracts there occurred an evolution of gas, starting at a uniform rate, but decreasing later and finally coming to a standstill. The total amount of gas evolved depended on the amount of cysteic acid added. The following observations indicate that the gas formed was CO_2: (1) when the readings had become constant and the extracts were acidified, a further amount of gas was set free from the vessel to which cysteic acid had been added, as compared with the control and, (2) when the inner tube was filled with alkali no development of gas occurred.

A typical experiment is shown in Table 1. The total CO_2 formed was one molecule per molecule of *l*(−)-cysteic acid added. This is the amount to be expected according to the equation:

$$\underset{\text{cysteic acid}}{HSO_3.CH_2.CH(NH_2).COOH} \longrightarrow \underset{\text{taurine}}{H.SO_3.CH_2.CH_2.NH_2} + CO_2.$$

Table 1. *Formation of CO_2 from l(−)-cysteic acid in dog's liver extract*

The contents of the side bulb were tipped into the main compartment at 0 min.; the contents were acidified at 50 min.

Flask no.	1	2
Main flask	1·8 ml. dog's liver extract	1·8 ml. dog's liver extract
Side bulb	0·2 ml. water	0·2 ml. *M*/100 *l*(−)-cysteic acid
Inner tube	0·3 ml. *N* H_2SO_4	0·3 ml. *N* H_2SO_4
μl. CO_2 0–50 min.	6·5	40·5
μl. CO_2 after acidification	13·5	28
μl. total CO_2	20	68·5
μl. CO_2 formed	—	48·5
μl. CO_2 expected	—	45

Distribution and activity. Dog's liver was chosen in the first instance because all observations on the excretion of taurocholic acid had been made on this animal. The choice proved convenient for other reasons. The extracts from dog's liver were most active and there was little CO_2 formation in the absence of substrate. The presence of a relatively large blank in the crude liver extracts of all other animals examined made the determination of the enzymic activity somewhat less accurate. This was especially marked in rat's liver where the activity of the enzyme was relatively high. The enzyme was found in the livers of the guinea-pig, the pig and the rat, but not in those of the cat, the rabbit and the cod. Extracts from the kidneys of the dog, cat and man were inactive.

The enzymic activity of the extracts was measured by determining the μl. CO_2 formed per mg. fresh tissue per hr. This is expressed as 'cysteic acid-$q^{N_2}_{CO_2}$' in Table 2. By multiplying the cysteic acid quotients by 5·58 the amounts of taurine formed can be calculated in mg. per g. fresh weight of tissue. These figures are also given in Table 2. They represent minimum values only, since some active material is lost in the preparation of the extracts.

Table 2. *l(−)-Cysteic acid decarboxylase activities of various liver extracts*

Animal	No. of experiments	Cysteic acid-$q^{N_2}_{CO_2}$	mg. taurine formed per hr. per g. tissue
Guinea-pig	2	0·045; 0·0525	0·25; 0·29
Pig	1	0·089	0·50
Rat	3	0·12 (0·073–0·180)	0·67 (0·41–1·00)
Dog	6	0·22 (0·11–0·33)	1·23 (0·61–1·84)

Stereospecificity. From *dl*-cysteic acid only half a molecule of CO_2 per molecule of substrate was formed. Since the total amount of CO_2 formed from the laevorotatory stereoisomeride was one molecule per molecule of substrate we may conclude that *d*(+)-cysteic acid was not attacked by the enzyme.

Inhibitors. The enzyme was inhibited by cyanide. With dog's liver extract the inhibition was complete in *M*/1000 HCN. The inhibition was of the reversible type. This was shown by the following procedure. Six manometers were used for the three parallel experiments of Table 3. The first two manometer flasks contained extract only, for the determination of its enzymic activity; to the other four flasks cyanide was added, but in flasks nos. 5 and 6 alkali and a filter paper were placed in the inner tubes in order to remove the HCN from the atmosphere over the extracts. Before the reaction was started the alkali and the filter paper were removed. Table 3 shows how the manometer flasks were set up. The flasks were attached to the manometers and filled with N_2; they were left standing at room temperature for 90 min. and then shaken in the thermostat at 37·5° for another 20 min. Filter paper and alkali were replaced by 0·3 ml. *N* H_2SO_4 in the inner tubes of flasks nos. 5 and 6. The rates of decarboxylation were then determined. The results are given in Table 3.

Table 3. *Inhibition by HCN* (M/*1000*)

Flask no. ...	1	2	3	4	5	6
Main flask:						
1·6 ml. dog's liver extract	+	+	+	+	+	+
0·2 ml. water	+	+	−	−	−	−
0·2 ml. *M*/50 HCN	−	−	+	+	+	+
Side bulb:						
0·2 ml. water	+	−	+	−	+	−
0·2 ml. *M*/50 *l*(−)-cysteic acid	−	+	−	+	−	+
Inner tube:						
0·3 ml. *N* H_2SO_4	+	+	+	+	−	−
0·3 ml. *N* KOH and filter paper	−	−	−	−	+	+
μl. CO_2 formed in 20 min.		48·5		0		43·5
Inhibition %		−		100 (with HCN)		10 (after HCN removed)

This experiment shows that during the incubation with alkali most of the HCN was removed from the enzyme with the result that the cyanide inhibition was almost completely reversed.

The enzyme was not inhibited by octyl alcohol.

Effect of dialysis. When dialysed against distilled water for 18 hr. at 0–2°, the extracts lost much of their activity. Addition of boiled extract did not increase the activity of the dialysed extracts.

Discussion

The experiments reported show that CO_2 is formed from *l*(−)-cysteic acid under anaerobic conditions. The fact that one molecule of CO_2 is formed per molecule of substrate can only mean that the reaction catalysed is the decarboxylation of *l*(−)-cysteic acid.

The presence of the enzyme in dog's liver would furnish a simple explanation of the observation that in this animal cysteic acid is excreted as taurocholic acid in the bile [Virtue & Doster-Virtue, 1939]. The enzyme has not been found in the livers of all animals which excrete taurocholic acid, e.g. the cat [Iwato & Watanabe, 1935] and the cod [Hammarsten, 1904]. Other pathways of taurine formation may exist [Medes & Floyd, 1942], and the possibility of formation of taurocholic acid without the intermediary appearance of free taurine has been discussed [Blum, 1904]. The failure of Medes & Floyd [1942] to find the enzyme in rat's liver is probably explained by the fact that they tested for decarboxylase activity in the insoluble residue only, thereby losing the active fraction in the supernatant fluid during repeated washings. Another difference was that their experiments were carried out in air and ours in N_2.

The decarboxylase for cysteic acid shows many similarities with dopa decarboxylase, an enzyme which occurs in the liver and kidney of many mammals and decarboxylates dihydroxyphenylalanine [Holtz, Heise & Lüdtke, 1938; Blaschko, 1939; 1942]. Both enzymes are reversibly inhibited by cyanide, they are not inhibited by octyl alcohol and they are stereospecific for the laevorotatory stereoisomeride. The two enzymes, however, must be different, as shown by their different distributions; cysteic acid decarboxylase does not occur in the human kidney. Their similarities indicate that the *l*-amino acid decarboxylases of vertebrates form a well-defined group with common properties.

Summary

Liver extracts of the dog, rat, pig and guinea-pig, but not of the cat, rabbit and cod, contain an enzyme, *l*(−)-cysteic acid decarboxylase, which forms CO_2 anaerobically from *l*(−)-cysteic acid, one molecule of CO_2 being formed from one molecule of *l*(−)-cysteic acid. The enzyme is stereospecific for the laevorotatory stereoisomeride, it is reversibly inhibited by cyanide, but insensitive to octyl alcohol. It is different from *l*(−)-dopa decarboxylase with which it shares common properties.

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