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The Effect of Organic Solvents on Mammalian Histidine Decarboxylase

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Mammalian histidine decarboxylase has been described by Werle and his colleagues (Werle, 1940, 1942; Werle & Heitzer, 1938; Werle & Herrmann, 1937; Werle & Krautzun, 1938; Werle & Koch, 1949) and by Holtz (1941; see also Holtz & Credner, 1944). This enzyme is present in certain mammalian tissues and may be responsible for the formation of histamine from histidine absorbed into the circulation from the gut. Its distribution and possible significance in tissue metabolism have been discussed by Waton (1956).

While testing for potentiators of the histidine-decarboxylase activity of rabbit-kidney tissue, Waton (1956) found that chloroform and benzene considerably increased histamine production from histidine. Enzyme potentiation by an organic solvent has been described for the choline acetylase of brain tissue. Stedman & Stedman (1939), Mann, Quastel & Tennenbaum (1939) and Feldberg (1945) reported that formation of acetylcholine by ox and rat brain was greatest when the incubation medium was 100% ether or chloroform. In the present case, the potentiation of histidine-decarboxylase activity was produced in an aqueous medium containing only a trace of organic solvent. Experiments were therefore performed to elucidate the mechanism of this action.

METHODS

Source of enzyme

Rabbit-kidney histidine decarboxylase was used. The rabbit kidneys were removed and dropped into 0.9% NaCl. They were freed from all extraneous tissue, cut into small pieces and minced in a Latapie mincer. Tissue extracts were

prepared by grinding the minced tissue for 10 min. in a mortar with 1 g. of silver sand and 10 ml. of 0.9% NaCl/g. of tissue. The mixture was centrifuged at 2500 g for 10 min. and the supernatant fluid used for some of the experiments.

In other experiments an extract of rabbit kidney was prepared by homogenizing the minced tissue with 0.25 M sucrose solution in a Potter-Elvehjem homogenizer for 10 min. at 0°. The homogenate was successively centrifuged at 2500, 37000 and 90000 g for 10 min., 45 min. and 1 hr., respectively. The minced tissue and a measured volume of each supernatant fluid were incubated in the presence and absence of benzene. The final supernatant would be free from all particulate cell matter and would contain only substances in colloidal suspension or in solution (Schneider & Hogeboom, 1950).

In a control experiment it was shown that the production of histamine from histidine by rabbit kidney was unaffected by changing from Tyrode's solution and buffer mixture to 0.25 M sucrose-buffer mixture.

Method of incubation

The minced tissue (0.4 g.) and 6 ml. of Tyrode's solution, or 4 ml. of tissue extract and 2 ml. of Tyrode's solution, were placed in a 25 ml. conical flask together with 3 ml. of 0.2 M Na_2HPO_4 solution to buffer at pH 8.2 (glass electrode), and 1 ml. of neutralized histidine solution (15 mg./ml.). The mixture was incubated at 38° for 3 hr. with mechanical agitation.

To show the effect of CHCl_3 and benzene, one drop (about 20 mg.) of one of these substances was added (a) before incubation, (b) after incubation, (c) before incubation, no histidine in the incubation mixture, and (d) before incubation, no enzyme preparation present. Any difference between (a) and (b) would show a potentiating or inhibitory effect. A positive value for (c) would represent either free tissue histamine or histamine liberated by the solvent from bound histamine, and a positive value for (d) would represent histamine produced by an interaction of the reagents.

Duplicate experiments were carried out on each enzyme preparation, and the experiment was repeated with the tissue of another animal.

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Estimation of histamine present after incubation

N-HCl was added dropwise from a micro-burette into the reaction mixture at the end of the incubation until the pH was between 5.5 and 5.9. The mixture was boiled for 1 min. to coagulate proteins and prevent further enzyme action. After cooling, the contents of the flasks were filtered through a Whatman no. 1 paper.

The histamine content of the filtrate was assayed *in vitro* on guinea-pig ileum suspended in 2 ml. of atropinized Tyrode's solution at 34°. The presence of histamine was confirmed by the use of mepyramine (Reuse, 1947).

Effect of organic solvents on histidine-decarboxylase activity

To determine whether the increased production of histamine from histidine was an effect peculiar to CHCl_3 alone, several other organic solvents were tested.

Reaction mixtures were incubated with (a) one drop of the organic solvent added before incubation, (b) one drop of organic solvent added after incubation and (c) no addition of organic solvent. If the solvent potentiated or inhibited the decarboxylation of histidine, this would be seen as a difference between (a) and (b). If the substance affected the gut, this would be seen as a difference between (b) and (c).

Since rabbit kidney contains no detectable histamine, and because the effect of CHCl_3 and benzene could not be accounted for by a liberation of preformed histamine, sample (c) as used in the previous experiment was omitted; as the observed effect had been found not to be accounted for by an interaction of reagents, (d) was also omitted.

Partition of histamine between water and benzene

To determine whether the increased production of histamine could be accounted for by a mass-action effect due to

the greater solubility of histamine in the benzene present, the distribution of histamine between a saline tissue extract and benzene was determined.

Two tubes were taken containing: (1) 1 ml. of histamine solution (10 $\mu\text{g./ml.}$), 5 ml. of Tyrode's solution, 1 ml. of 0.2M- Na_2HPO_4 solution and 2 ml. of benzene (A.R.); (2) 1 ml. of histamine solution, 5 ml. of Tyrode's extract of rabbit kidney (1 g. of tissue/5 ml.), 1 ml. of 0.2M- Na_2HPO_4 solution and 2 ml. of benzene. The tubes were shaken for 1 hr. at 18° and then centrifuged; 3 ml. of the aqueous layer was removed, N-HCl added dropwise to adjust to pH 5.5-5.9 and the mixture brought to the boil, allowed to simmer for 1 min. to remove any benzene, cooled and filtered, and the histamine content of the filtrate determined. A volume (1 ml.) of the benzene layer was removed and 1 ml. of N-HCl added. The mixture was evaporated to dryness in a water bath under reduced pressure, and the residue taken up in 2 ml. of water, adjusted to pH 5.5-5.9 with solid NaHCO_3 , brought to the boil, cooled, filtered and assayed for histamine.

A third sample (3) consisting of 1 ml. of histamine solution (10 $\mu\text{g./ml.}$), 5 ml. of extract and 1 ml. of Na_2HPO_4 solution was adjusted to pH 5.5-5.9, brought to the boil, cooled, filtered and assayed for histamine. All tests were done in duplicate.

RESULTS

Table 1 gives the results of four experiments with chloroform, eight with benzene and five in which the effects of chloroform and benzene were compared on the same tissue. Benzene increased the rate of decarboxylation of histidine 4- to 12-fold and in one case 40-fold. It was found to have at least 2-3 times the activity of chloroform.

Table 1. *Histamine production from minced rabbit kidney in the presence of chloroform or benzene*

Minced rabbit kidney (0.4 g.) was incubated for 3 hr. at 38° in 10 ml. of Tyrode's solution containing 15 mg. of histidine and chloroform or benzene (final concn. 2.0 mg./ml.). Values are averages of duplicate incubations.

Expt. no.	Histamine formed ($\mu\text{g./g.}$ of wet tissue)		b/a (c)	Histamine formed ($\mu\text{g./g.}$ of wet tissue)		e/d (f)	f/c
	Chloroform added after incubation	Chloroform present during incubation		Benzene added after incubation	Benzene present during incubation		
	(a)	(b)		(d)	(e)		
1	6.6	19.7	3.0	—	—	—	—
2	10.8	34.4	3.2	—	—	—	—
3	1.5	6.8	4.3	—	—	—	—
4	3.2	11.0	3.5	—	—	—	—
5	—	—	—	1.8	12.6	7.0	—
6	—	—	—	1.2	15.4	12.5	—
7	—	—	—	1.25	6.7	4.7	—
8	—	—	—	31.2	114.0	3.8	—
9	—	—	—	3.0	27.0	9.1	—
10	—	—	—	4.5	24.2	5.3	—
11	—	—	—	3.2	88.6	6.7	—
12	—	—	—	3.3	14.8	4.5	—
13	2.2	9.0	4.1	2.2	27.6	12.5	3.1
14	13.5	36.4	2.7	13.5	99.6	7.4	2.7
15	7.2	24.9	3.4	7.2	56.9	7.9	2.3
16	0.25	0.99	4.0	0.25	10.0	40.0	10.0
17	2.2	5.5	2.5	2.2	6.2	6.2	2.5

Table 2. *Histamine production from rabbit kidney in the presence of various organic solvents*

Minced rabbit kidney (0.4 g.) was incubated for 3 hr. at 38° in 10 ml. of modified Tyrode's solution containing 15 mg. of histidine and organic solvent (final concn. 2.0 mg./ml.). Values are averages of duplicate determinations.

Solvent	Histamine formed ($\mu\text{g./g. of wet tissue}$)			<i>b/a</i> for solvent (c)	<i>b/a</i> for CHCl_3 (d)	<i>b/a</i> for benzene (d)	<i>c/d</i>
	Control	Solvent added after incubation (a)	Solvent present during incubation (b)				
Pyridine	13.6	13.6	109.0	8.0	2.7	7.4	1.10
Toluene	2.2	1.8	19.7	9.0	4.1	12.5	0.72
Chlorobenzene	7.2	6.9	36.8	5.1	3.5	7.9	0.65
Light petroleum (b.p. 80–100°)	2.2	2.4	13.4	6.1	4.1	12.5	0.48
cycloHexane	13.6	12.7	37.4	2.8	2.7	7.4	0.37
Bromoform	9.9	9.7	36.0	3.7	4.3	—	—
Nitrobenzene	7.2	6.3	17.9	2.5	3.5	7.9	0.32
Aniline	7.2	7.1	15.6	2.2	3.5	7.9	0.28
Diethyl ether	2.2	1.9	6.3	2.9	4.1	12.5	0.23
Carbon tetrachloride	9.9	9.9	28.9	2.9	4.3	—	—
Acetone	2.2	2.2	3.4	1.5	4.1	12.5	0.12
cycloHexanone	2.1	2.1	2.2	1.0	2.5	6.2	0.15
Tetrachloroethane	2.1	1.5	1.5	1.0	2.5	6.2	0.15
Pentan-1-ol	13.6	12.7	15.0	1.1	2.7	7.4	0.15
n-Butanol	8.5	8.7	8.8	1.0	3.1	7.1	0.13
Tetralin	7.2	7.9	6.3	0.87	3.5	7.9	0.11
Ethyl acetate	13.6	13.6	2.3	0.17	2.7	7.4	0.02
Methyl iodide	2.1	2.3	<0.4	—	2.5	6.2	—
Quinoline	2.1	<0.5	Nil	—	2.5	6.2	—
Ethyl malonate	13.6	13.6	No assay	—	2.7	7.4	—

Fig. 1. Relation between the histamine formed by rabbit kidney and the concentration of benzene or pyridine present. Minced rabbit kidney (0.4 g.) was incubated for 3 hr. at 38° in 10 ml. of modified Tyrode's solution containing 15 mg. of histidine and varying concentrations of benzene (○) or pyridine (●).

Among the organic solvents tested (Table 2) benzene and pyridine appeared to be most active, and toluene, chlorobenzene, light petroleum (b.p. 80–100°) and cyclohexane showed activity greater than, or as great as, chloroform.

The effects of various concentrations of benzene and pyridine (Fig. 1) were obtained by shaking the

solvents with Tyrode's solution at 38° (benzene 1.25 and 0.25 mg./ml., pyridine 3.8 mg./ml.) and using a measured volume of this in place of an equivalent volume of Tyrode's solution for the incubation mixtures. In another experiment, the histamine formed was more than doubled when the concentration of benzene was only 0.025 mg./ml. These results showed that benzene produced an increase in histamine formation in concentrations as low as 0.025 mg./ml. and had a maximum effect at 0.38–0.81 mg./ml. At a concentration around 0.81 mg./ml. water is fully saturated with benzene. Thus benzene has its maximum effect at full saturation. The effect of pyridine was not seen in concentrations lower than 0.1 mg./ml., but above this the potentiation increased to a maximum at a concentration of 3.8 mg./ml. Above this concentration the effect became less, but a slight potentiation still occurred at concentrations as high as 15.5 mg./ml.

The results in Table 3 show that in the presence of chloroform and benzene there is an approximately linear relation between the histamine formed and the time of incubation up to 3 hr. They also show that the effect of both chloroform and benzene is noticeable within 30 min.

When the maximum time of incubation was increased (Table 3, Expt. 2), it was noticeable that the histamine content reached a maximum at about

4-6 hr., after which histamine formation ceased. The slow destruction of histamine after this time was shown, in a further experiment, to be equal to the normal destruction of histamine in Tyrode's solution at a similar pH. However, after 16-24 hr. there was a rise in the production of histamine in the controls which became very marked after 40 hr. This sudden rise could have been due to histamine formation by bacteria.

The results in Table 4 showed that not only was benzene an effective potentiator of histidine decarboxylase present in minced tissue, but that it was equally effective when the enzyme preparation was a tissue extract subjected to centrifuging at 90 000 g. Thus benzene does not produce an effect

on the membrane of any cell or cell body, but acts on the enzyme when it is in solution.

Oxytetracycline, in concentrations considered to be bacteriostatic (0.2 and 0.4 mg./ml.), did not potentiate the formation of histamine and it is therefore unlikely that the effects of the solvents were due to bacteriostasis. Similarly, in doses which did not inhibit the gut during subsequent assay (0.0005 mg./ml.), the anionic detergent lauryl sulphate (Teepol XL, British Drug Houses Ltd.) and the cationic detergent cetyl trimethylammonium bromide [Cetrimide, Imperial Chemicals (Pharmaceuticals) Ltd.] produced no potentiation of histamine formation.

It was thought that the action of benzene might be due to some change which would persist after the benzene was removed, and the following experiments were carried out to test this possibility.

Into each of six 25 ml. conical flasks were pipetted 3 ml. of tissue extract, 2 ml. of 0.2M-Na₂HPO₄ solution and 2 ml. of Tyrode's solution. In addition, one drop (22 mg.) of benzene was introduced into three of the mixtures (*C*, *D* and *F*). The flasks were stoppered and the mixtures incubated for 2.5 hr. at 38°. Two of the samples containing benzene (*C* and *D*) and two to which no benzene had been added (*A* and *B*) were freeze-dried (20 hr.). Each resulting residue was dissolved in 7 ml. of water to which 2 ml. of Tyrode's solution and 1 ml. (15 mg.) of histidine solution were added. One drop (22 mg.) of benzene was added to *B* and *D*, in one of which (*D*) benzene had been present during the preliminary incubation. No benzene was added to *A* or *C*. The mixtures were incubated at 38° for 3 hr. and the histamine contents then determined.

To the two original incubation mixtures not subjected to freeze-drying (*E* and *F*) were added 2 ml. of Tyrode's solution and 1 ml. (15 mg.) of histidine solution, and the mixtures maintained at 38° for 3 hr. before determining the histamine contents. The results are shown in Table 5. Rabbit-kidney extract that had been treated with benzene and then freeze-dried (*C*) showed about the same activity as extracts which had not been treated with

Table 3. *Relation between the histamine formed by rabbit kidney in the presence of chloroform or benzene and the time of incubation*

Minced rabbit kidney (0.4 g.) was incubated at 38° for varying times in 10 ml. of modified Tyrode's solution containing 15 mg. of histidine and chloroform or benzene (final concn. 2.0 mg./ml.). Values are averages of duplicate incubations.

Duration (hr.)	Histamine formed (μg./g. of wet tissue)		
	Control	Chloroform present during incubation	Benzene present during incubation
Expt. 1			
0.5	0.6	2.8	7.8
1	1.8	4.7	13.0
1.5	2.2	7.4	16.4
2	3.2	8.5	20.4
2.5	4.4	10.0	24.9
3	5.1	12.5	29.8
Expt. 2			
0.75	4.5	25.7	48.2
1.5	22.0	46.9	94.1
3	33.0	68.4	137.0
7	38.6	60.1	160.0
11.5	36.8	57.3	116.3
24	62.0	51.3	132.0
30	82.0	39.6	98.2
48	182.0	29.8	92.5

Table 4. *Histamine production in the presence of benzene, from extracts of rabbit kidney that had been subjected to centrifuging at varying gravitational forces*

Extracts of rabbit kidney incubated for 3 hr. at 38° in 10 ml. of modified Tyrode's solution containing 15 mg. of histidine and benzene (final concn. 2.0 mg./ml.).

Presence of benzene	Histamine formed (μg./g. of original wet tissue)			
	Expt. 1		Expt. 2	
	-	+	-	+
Minced tissue	7.0	31.3	3.1	17.5
Extract after centrifuging at 2500 g	6.4	27.6	4.8	16.5
Extract after centrifuging at 17 000 g	6.0	37.9	3.2	16.9
Extract after centrifuging at 90 000 g	6.2	31.2	3.5	16.2

Table 5. *Histamine production from extracts of rabbit kidney in the presence of benzene before and after freeze-drying of the tissue extract*

Extracts of rabbit kidney, before and after freeze-drying, were incubated for 3 hr. at 38° in 10 ml. of modified Tyrode's solution containing 15 mg. of histidine and benzene (final concn. 2.0 mg./ml.). Values are averages of duplicate incubations.

Sample	Benzene added before freeze-drying	Benzene added after freeze-drying	Histamine formed ($\mu\text{g./g.}$ of original wet tissue)	
			Expt. 1	Expt. 2
A	—	—	1.4	0.7
B	—	+	5.2	9.2
C	+	—	1.0	0.8
D	+	+	5.9	10.5
E	—	No freeze-drying	1.1	0.8
F	+	No freeze-drying	10.8	16.7

benzene at all (A or E), but freeze-dried extracts could still be activated, provided the benzene was present during incubation (B or D).

No histamine was found to pass into the benzene layer when benzene was shaken with either saline or a tissue extract containing added histamine. Thus it is unlikely that the benzene present in incubation mixtures removes histamine as soon as it is formed, so allowing more to be formed.

DISCUSSION

The observed effects have been shown to be due to an action of the organic solvent on the enzyme during incubation. They were not due to an action of the solvents on the ileum used for histamine assay. They probably are not due to inhibition of histaminase, since chloroform and benzene (2 mg./ml.) do not inhibit this enzyme in hog's kidney (Watson, 1956). There is evidence that the active substance was histamine and that it was not formed by the liberation of preformed histamine. The effects were not due to a bacteriostatic action since they were observed within 1 hr.; a secondary formation of histamine, which was probably due to bacteria, occurred after 24 hr. The bacteriostatic agent oxytetracycline had no effect on histamine formation.

Several organic solvents were effective (Table 2) and activity did not appear to be associated with their physical properties. The most active compounds contained aromatic rings. The presence of a second phase was unnecessary since the effect was

observed in concentrations that dissolved completely in water, and with pyridine, which is freely miscible with water. When there was a separate phase of benzene, no histamine could be detected in it.

It is suggested that the effect is due to a change in the physical state of the enzyme, such as a break-up of micelles, so that active groups that had previously been buried inside the micelle became accessible and free to react with histidine. Whatever may be the true explanation, the change in the enzyme is reversible, since benzene lost its effect after it was removed by freeze-drying before the incubation (Table 5).

SUMMARY

1. It has been shown that the action of rabbit-kidney histidine decarboxylase is considerably increased by the addition of very small quantities of several organic solvents. Benzene was found to be one of the most active substances, increasing the yield of histamine from histidine 5- to 10-fold. A submaximal effect was produced by concentrations of benzene as low as 0.025 mg./ml.

2. The effect is due to benzene in solution acting on the enzyme in solution. Evidence is presented that it does not depend on the presence of two liquid phases, nor on the suppression of bacteria, nor on a release of the enzyme from mitochondria or microsomes. It is probably not due to the inhibition of histaminase.

3. A possible explanation of the observed effect is discussed.

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