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METABOLISM OF [^{14}C]-HISTAMINE IN HEART-LUNG-LIVER PREPARATIONS OF CATS

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The metabolism of injected [^{14}C]-histamine was studied in heart-lung-liver preparations and heart-lung preparations from cats. When the liver was included in the circulation the injected histamine was rapidly eliminated from the blood, some of its metabolites appearing in the blood within 2 min after the injection. More than 70% of the injected histamine was metabolized to 1-methylimidazol-4-ylacetic acid. In the heart-lung preparations [^{14}C]-histamine disappeared much more slowly from the blood and the major metabolite was 4-(2-aminoethyl)-1-methylimidazole (methylhistamine). Less than 4% of the injected ^{14}C was recovered in the form of imidazol-4(or 5)-ylacetic acid. In none of the experiments was there any measurable formation of acetylhistamine.

The inactivation of histamine in liver perfusion experiments has been studied by Dale & Laidlaw (1911), Guggenheim & Loeffler (1916) and Best & McHenry (1930). The latter authors found that the perfused canine liver could inactivate 10 to 60 mg histamine in 4 hr. The mechanism responsible for the disappearance of histamine from the perfusion fluid was not further investigated. It would seem that in man, dog and cat the liver contains little diamine oxidase type of histaminase (Zeller, 1942). However, as first shown by Kobayashi & Schayer (1956), the liver may have other means of inactivating histamine. These authors incubated [^{14}C]-histamine with a homogenate of cat liver and found that the histamine was methylated on the ring nitrogen remote from the side-chain. The methylhistamine formed was to a large extent oxidized to methylimidazolylacetic acid.

The properties of the histamine methylating enzyme in the liver have been elucidated in *in vitro* experiments by Lindahl (1958 and 1960) and by Brown, Tomchick & Axelrod (1959). The latter authors also demonstrated the presence of the enzyme in other tissues, for example, heart and lung.

In the present experiments the capacity of the liver, the heart and the lungs to take up and metabolize [^{14}C]-histamine injected into the blood was studied in heart-lung-liver preparations of cats.

METHODS

Female cats weighing 2.2 to 3.0 kg were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (Nembutal, Abbott) in a dose of 50 mg/kg. The heart-lung-liver preparations and heart-lung preparations were made with the technique described by Örskov (1953). Histamine labelled with ^{14}C in position 2 of the imidazole ring was injected into the portal vein in the heart-lung-liver preparations and into the inferior vena cava in the heart-lung

preparations. The amount injected was 32 μg base with a specific activity of 42.4 μc/mg (purchased from the Radiochemical Centre, Amersham, England). The histamine was dissolved in 5 ml. saline containing glucose 1 mg/ml. The injection time was about 10 sec. Arterial blood was collected from a polythene catheter in the aorta. The arterial blood samples (10 ml. each) were taken 0.5, 2, 10 and 20 min after the injection of histamine. At about 25 min after the injection of histamine trypan blue was injected by the same route in order to demonstrate any major leakage of blood from the heart-lung-liver preparation to the other tissues of the experimental animal. Judged in this way, there was no major leakage. The only place outside the heart, lung and liver where the colour was visible was in some small blood vessels in the diaphragm. At 30 min after the injection of histamine the preparation was bled out. The volume of the collected blood was measured and aliquots taken for isotope dilution assays similar to those made on the preceding samples of arterial blood.

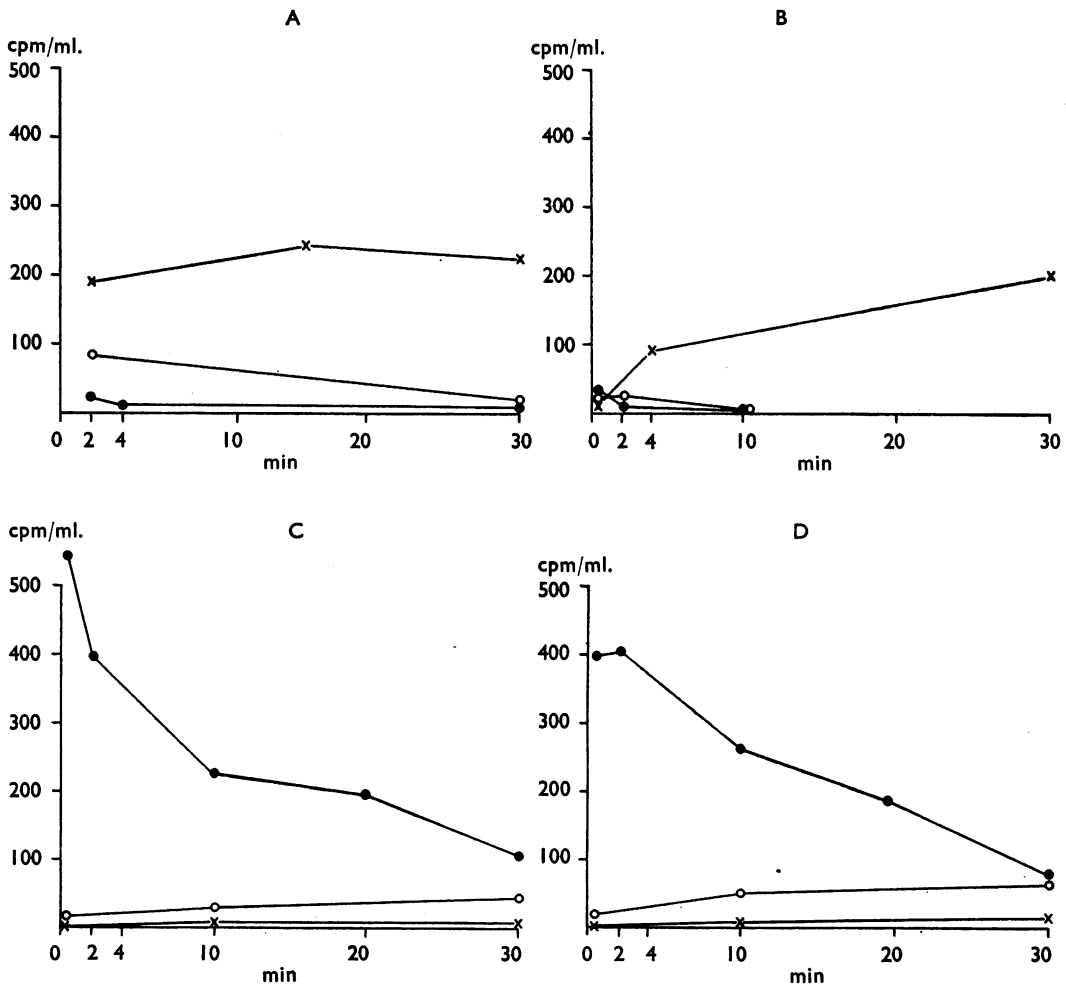

Fig. 1. The concentrations of labelled histamine (●—●), methylhistamine (○—○) and methylimidazoleacetic acid (X—X) in arterial blood at various times after the injection of 32 μg [^{14}C]-histamine into the portal vein of heart-lung-liver preparations from cats (Expts. A and B) or into the inferior caval vein in heart-lung preparations (Expts. C and D).

Isotope dilution assays for [^{14}C]*-histamine and some of its known metabolites in blood*

Each 10 ml. blood sample was divided into 2 ml. aliquots, to which the respective carrier was added immediately. The carrier was non-radioactive histamine, 4-(2-aminoethyl)-1-methylimidazole (methylhistamine), 1-methylimidazol-4-ylacetic acid (methylimidazoleacetic acid), imidazol-4(or 5)-ylacetic acid (imidazoleacetic acid), *or* acetylhistamine, all in amounts equivalent to 206 mg of the corresponding picrate. Each compound was then extracted and its radioactivity measured as described by Lindell & Schayer (1958).

Isotope dilution assays for [^{14}C]*-histamine and its metabolites in the tissues at the end of the experiment*

At the end of each experiment the heart, lungs and liver were weighed and then minced with scissors. Aliquots of 2 g were taken, to which carrier was added. The samples were then ground with sand in mortars, trichloroacetic acid being added. The extraction and measurement of radioactivity of respective compound was as mentioned above.

Metabolism of [^{14}C]*-histamine* in vitro

Lung and heart tissue from cats anaesthetized and bled out was minced with scissors and 2 g aliquots suspended in 2 ml. 0.1 M phosphate buffer (*p*H 7.4). This suspension was incubated with 0.18 μg [^{14}C]-histamine at 37° C for 2 hr. For comparison similar incubations were also carried out with feline blood instead of minced tissue. The amount of radioactive methylhistamine formed was determined as described above.

Of the labelled histamine used, 1 μg gives 2,040 counts per min above background (cpm) under our standardized conditions of assay.

RESULTS

The amount of [^{14}C]-histamine injected in each experiment would give about 65,000 cpm under our conditions of assay. As may be seen in Fig. 1, in experiments A and B the arterial blood taken 0.5 to 2 min after the intraportal injection contained very little [^{14}C]-histamine (20 cpm or less per ml.). Already 2 min after the injection arterial blood contained some [^{14}C]-methylhistamine and a fair amount of [^{14}C]-methylimidazoleacetic acid. Thus the injected histamine disappeared very rapidly from the blood, where some of its metabolites appeared within 2 min. In the course

TABLE 1

THE DISTRIBUTION OF [^{14}C]-HISTAMINE AND SOME OF ITS METABOLITES IN THE BLOOD AND TISSUES OF TWO HEART-LUNG-LIVER PREPARATIONS

Samples taken 30 min after the injection of 32 μg [^{14}C]-histamine into the portal vein

Experiment A	Blood cpm	Heart (20 g) cpm	Lungs (10 g) cpm	Liver (86 g) cpm	Total cpm
Histamine	500	280	100	700	1,580
Methylhistamine	2,000	200	100	1,400	3,700
Methylimidazole-acetic acid	30,000	3,600	1,900	30,800	66,300
Imidazoleacetic acid	750	0	30	420	1,200
	33,250	4,080	2,130	33,320	72,780
Experiment B	**Blood cpm**	**Heart (15 g) cpm**	**Lungs (20 g) cpm**	**Liver (80 g) cpm**	**Total cpm**
Histamine	0	160	40	160	360
Methylhistamine	0	390	0	0	390
Methylimidazole-acetic acid	24,000	1,950	2,300	20,000	48,250
Imidazoleacetic acid	150	0	20	240	410
	24,150	2,500	2,360	20,400	49,410

of the experiment the concentration of labelled histamine and methylhistamine in the blood continued to fall and the concentration of labelled methylimidazoleacetic acid rose. There was never any measurable quantity of radioactive imidazoleacetic acid (free and conjugated) or acetylhistamine in the blood 30 min after the injection of [^{14}C]-histamine. The amounts of radioactive histamine, methylhistamine, methylimidazoleacetic acid and imidazoleacetic acid (free and conjugated) in the tissues at the end of the experiment are shown in Table 1. By far the greatest part of the ^{14}C was in the form of methylimidazoleacetic acid. [^{14}C]-Acetylhistamine was not present in measurable amounts.

In these heart-lung-liver preparations the injected histamine was very rapidly removed from the blood and metabolized. In order to see to what extent this was due to the liver, similar experiments were performed in heart-lung preparations, where the arterial blood was returned to the heart via a polythene catheter tied into the inferior vena cava well above the liver, which was thus excluded from the circulation. The results of 2 experiments in heart-lung preparations are shown in

TABLE 2

THE DISTRIBUTION OF [^{14}C]-HISTAMINE AND SOME OF ITS METABOLITES IN THE BLOOD AND TISSUES OF TWO HEART-LUNG PREPARATIONS

Readings made 30 min after the injection of 32 μg [^{14}C]-histamine into the inferior vena cava

Experiment C	Blood cpm	Heart (20 g) cpm	Lungs (21 g) cpm	Total cpm
Histamine	10,000	10,800	5,000	25,800
Methylhistamine	5,700	15,000	1,140	21,840
Methylimidazole-acetic acid	400	750	4,000	5,150
Imidazoleacetic acid	1,000	500	500	2,000
	17,100	27,050	10,640	54,790
Experiment D	**Blood cpm**	**Heart (15 g) cpm**	**Lungs (25 g) cpm**	**Total cpm**
Histamine	8,500	1,800	2,100	12,400
Methylhistamine	7,500	10,800	7,700	26,000
Methylimidazole-acetic acid	1,500	750	4,000	6,250
Imidazoleacetic acid	1,000	500	500	2,000
	18,500	13,850	14,300	46,650

the lower part of Fig. 1. It may be seen that the radioactive histamine disappeared much more slowly from the blood in these experiments. After 30 min there was still some unchanged histamine in the circulating blood. During the experiments the concentration of [^{14}C]-methylhistamine rose slowly. Very little [^{14}C]-methylimidazoleacetic acid appeared in the blood. Again there was little or no radioactive imidazoleacetic acid or acetylhistamine in the blood at the end of the experiment. The amounts of labelled histamine or histamine metabolites remaining in the tissues at the end of the experiments are given in Table 2. The blood and the tissues contained considerable quantities of unchanged histamine. Methylhistamine was the most important metabolite; it occurred especially in the heart.

To find out if the methylation of histamine occurred in the blood, the heart or the lungs [^{14}C]-histamine was incubated with blood or heart or lung tissue and the

TABLE 3

METABOLISM OF [^{14}C]-HISTAMINE IN BLOOD, HEART AND LUNG TISSUE *IN VITRO*

[^{14}C]-Histamine was incubated with 2 g minced tissue or 2 ml. blood, suspended in 2 ml. phosphate buffer for 2 hr at 37° C

	Blood	Heart	Lung
Amount of [^{14}C]-histamine added (cpm)	375	375	375
Amount of [^{14}C]-methyl-histamine formed (cpm)	10	300	250

[^{14}C]-methylhistamine formed measured. The results of one such experiment are summarized in Table 3. It may be seen that both heart and lung tissue could methylate histamine *in vitro*. Under the same conditions there was little or no methylation in the blood sample.

DISCUSSION

In the present experiments it was found that injected histamine disappeared very rapidly from the circulating blood in heart-lung-liver preparations of cats. The amount of histamine injected corresponded to about 10 μg/kg. It was given intravenously in less than 10 sec, and yet arterial blood collected 0.5 to 2 min after the injection did not contain more than about 0.01 μg per ml. On the other hand, in the heart-lung preparations the injected histamine disappeared much more slowly from the blood. Steggerda, Essex & Mann (1935) found that heart-lung preparations of dogs did not inactivate measurable amounts of histamine when a dose of 20 to 50 mg was given. The rate of inactivation seen in our heart-lung preparations seems too slow to be of any physiological significance.

According to Brown, Tomchick & Axelrod (1959) the heart and the lungs of cats are as efficient as the liver in methylating histamine *in vitro*. The great difference in our experiments between heart-lung-liver preparations and heart-lung preparations as regards the metabolism of histamine is thus not likely to be due to differences in concentration of the methylating enzyme. To some extent the difference can be explained by the greater amount of tissue in the heart-lung-liver preparations. It should be noted, however, that in the heart-lung-liver preparations the main metabolite was methylimidazoleacetic acid, whereas in the heart-lung preparations it was methylhistamine. It is thus possible that in the absence of the liver the limiting factor was the lack of the enzyme responsible for the oxidation of methylhistamine.

It has been suggested that amine oxidase should be important for the inactivation of unchanged histamine *in vivo* (Zeller, Stern & Blanksma, 1956). In the present experiments, however, less than 4% of the injected histamine was converted to imidazoleacetic acid, the end product of the oxidation of histamine by amine oxidase or diamine oxidase (Zeller, 1951, and Zeller, Stern & Blanksma, 1956). Thus amine oxidase, which is present in the liver in great amounts (Blaschko, 1951), seems to play a minor role in the metabolism of histamine. On the other hand, the present experiments support earlier observations, according to which amine oxidase or a similar catalyst takes a part in the further oxidation of methylhistamine (see Lindell, Nilsson, Roos & Westling, 1960).

There was no evidence of acetylation of the injected histamine. This is in agreement with Livingston & Code (1955), who did not find any increase in the excretion of conjugated histamine in urine during an intraportal infusion of histamine in dogs.

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