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THE DIFFERENCE IN THE METABOLISM OF INJECTED [^{14}C]HISTAMINE IN MALE AND FEMALE RATS

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[^{14}C]Histamine was injected subcutaneously in rats. The urine was collected for 24 hr. and analysed for [^{14}C]histamine and its various metabolites. It was found that male rats excreted less unchanged [^{14}C]histamine than female ones, the difference between the sexes existing also when the animals were treated with aminoguanidine. The cause of the sexual difference appeared to be that the male rats had a larger capacity to methylate histamine.

Leitch, Debley, and Haley (1956) observed that male rats excreted about 10 times less histamine in the urine than female ones. Similar results were obtained by Gustafsson, Kahlson, and Rosengren (1957), who found that males excreted much less free histamine than females.

The urinary excretion of histamine, particularly in rats, has been used as an indicator of liberation or production of histamine in the body (see, for example, Wilson, 1954; Schayer, Davis, and Smiley, 1955; Leitch *et al.*, 1956). It appeared therefore of interest to study more closely the cause of the difference in histamine excretion between the sexes. The smaller urinary histamine excretion in male rats could be due to many causes. *A priori*, it seems most likely that it is due either to a lower rate of production or to a more efficient metabolic transformation of histamine. The latter possibility was examined in the experiments described below.

METHODS

White rats, 4 to 8 months old, were used. They were of the same inbred stock as the animals used by Gustafsson *et al.* (1957) and Kahlson, Rosengren, and Westling (1958). The rats were given a synthetic, histamine-free diet and were kept in metabolism cages as described by Kahlson *et al.* (1958). Urine was collected for 24 hr. in a vessel containing hydrochloric acid so as to bring the *p*H of the collected urine to below 2.

Histamine labelled with ^{14}C in the 2-position of the imidazole ring (The Radiochemical Centre, Amersham, England) was injected subcutaneously in a dose of 0.03 to 0.16 mg./kg. body weight. Aminoguanidine sulphate (Eastman Organic Chemicals, Rochester, U.S.A.) was injected subcutaneously in a dose of 20 mg./kg. twice daily.

Two rats were injected with [^{14}C]histamine twice, with an interval of three weeks; at one of the injections the rats were also treated with aminoguanidine.

The urine was assayed for total ^{14}C content with the procedure described by Schayer and Cooper (1956). The urinary content of [^{14}C]histamine and its various radioactive metabolites was measured with the isotope dilution techniques developed in recent years by Schayer and his colleagues. The following metabolites of [^{14}C]histamine were studied: methylhistamine {4-(2-aminoethyl)-1-methyl-imidazole}, methylimidazoleacetic acid (1-methylimidazol-4-ylacetic acid), imidazoleacetic acid (imidazol-4 (or 5)-ylacetic acid), and acetylhistamine. Histamine and the various metabolites were used as carriers in amounts equivalent to 206 or 412 mg. of the respective picrates. Imidazoleacetic acid was determined after hydrolysis of the urine in 1 N-HCl in a sealed glass tube at 160° for 5 hr. The values given for imidazoleacetic acid are therefore considered to include also conjugates (1-ribosylimidazol-4-ylacetic acid). The methods used were the same as described by Lindell and Schayer (1958). The estimations of methylimidazoleacetic acid and imidazoleacetic acid were made in duplicates, which agreed well.

^{14}C radioactivity was measured at infinite thickness under standardized conditions in a flow counter with a background of 21 to 25 counts/min.

RESULTS

Table I gives the amount of [^{14}C]histamine excreted, and it may be seen that the male rats excreted considerably less unmetabolized [^{14}C] histamine than the females. If the rats were given aminoguanidine sulphate, in a dosage which inhibits the action of histaminase (diamine oxidase)

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almost completely (Schayer, Kennedy, and Smiley, 1953), a larger proportion of the injected [^{14}C]histamine was excreted unchanged but the differences between the sexes persisted.

TABLE I

URINARY EXCRETION OF UNCHANGED [^{14}C]HISTAMINE DURING THE 24 HR. FOLLOWING A SUBCUTANEOUS INJECTION IN MALE AND FEMALE RATS AND AFTER INJECTION SUBCUTANEOUSLY WITH AMINOGUANIDINE SULPHATE

Two experiments were made on Rat No. 2 and 6. + = 20 mg./kg. of aminoguanidine was injected twice daily.

	Males					Females				
Rat No. ..	1	2	2	3	4	5	6	6	7	8
Body weight (g.)	250	320	315	415	250	230	210	225	185	265
Aminoguanidine treatment ..			+	+	+			+	+	+
[^{14}C]Histamine injected (mg./kg.) ..	0·15	0·16	0·16	0·03	0·15	0·15	0·15	0·16	0·05	0·05
[^{14}C]Histamine excreted (% of injected amount)	<2	1	3	2	<5	10	12	33	36	33

Table II gives the results of experiments in which some metabolites of [^{14}C]histamine were measured, and it may be seen that the relative amount of imidazoleacetic acid was about the same in males and females. In animals not given aminoguanidine, the total urinary imidazoleacetic acid accounted for slightly more than 50% of the injected [^{14}C]. With aminoguanidine treatment, this value decreased to 7 to 9%.

TABLE II

URINARY EXCRETION OF [^{14}C]HISTAMINE AND SOME OF ITS METABOLITES DURING THE 24 HR. FOLLOWING A SUBCUTANEOUS INJECTION OF [^{14}C]HISTAMINE

The values are expressed as % of the amount of ^{14}C injected and were obtained in the same experiments reported in Table I. For explanation of +, see Table I.

	Males			Female	
Rat No...	2	2	3	6	6
Aminoguanidine treatment ..		+	+		+
Histamine	1	3	2	12	33
Imidazoleacetic acid	51	9	7	51	7
Methylhistamine	16	41	34	4	17
Methylimidazoleacetic acid ..	7	17	22	6	14
Acetylhistamine	2	3	4	3	3
Total ..	77%	73%	69%	76%	74%

In the male rats methylation of [^{14}C]histamine, with consequent excretion of methylhistamine and its oxidation product, methylimidazoleacetic acid, was more important than in the females. In males given aminoguanidine, methylation of histamine (indicated by excretion of methylhistamine and methylimidazoleacetic acid) accounted for 56 and 58% of the injected [^{14}C].

Acetylation seemed to be of little importance in the metabolism of injected [^{14}C]histamine. Acetylhistamine accounted for only a few per cent. of the injected ^{14}C both in males and females, without or with aminoguanidine treatment.

Nearly all of the injected [^{14}C]histamine (87 to 104%) appeared in the urine during the 24 hr. collection period. All of the injected or excreted ^{14}C could, however, not be accounted for in terms of the radioactive compounds measured, which when added together constituted 69 to 76% of the injected ^{14}C. This result is similar to those obtained in men by Schayer and Cooper (1956) and in women by Lindell, Nilsson, Schayer, and Westling (unpublished observations). The failure to account for all [^{14}C] may have several explanations, but the possibility obviously exists that unknown metabolites may be present.

DISCUSSION

Under the prevailing experimental conditions male rats metabolized injected [^{14}C]histamine more efficiently than female ones. The greater efficiency was not connected with histaminase (diamine oxidase), since the expected product of the action of this enzyme, imidazoleacetic acid, occurred in equal amounts in males and females. Moreover, the difference between the sexes persisted under treatment with aminoguanidine, a powerful inhibitor of diamine oxidase (Schuler, 1952; Schayer *et al.*, 1953). The difference between the sexes in the metabolism of injected [^{14}C]histamine appears largely to be due to a greater capacity of the male rat to methylate histamine.

The observation of a more efficient histamine degradation in male rats gives a satisfactory explanation for the smaller excretion of free histamine in this sex (Leitch *et al.*, 1956; Gustafsson *et al.*, 1957), provided that histamine liberated in the body undergoes essentially the same metabolism as injected [^{14}C]histamine. This assumption receives some support from a comparison between the proportion of injected [^{14}C]histamine excreted unchanged and the amount of free urinary histamine excreted by the rats when they were not injected with [^{14}C]histamine. The 24 hr. excretion of free histamine (expressed as histamine base and determined by bioassay) in these rats was very roughly in the males 1 μg. (3 μg. with aminoguanidine) and in the females 20 μg. (80 μg. with aminoguanidine). The parallelism between these estimates and the proportion of injected [^{14}C]histamine excreted unchanged in

males and females, without and with aminoguanidine, makes it tempting to assume that the total daily liberation of histamine in male and female rats is about the same and that the fraction of this histamine escaping into the urine is determined by the capacity of the metabolizing enzymes. Direct evidence on this point is, however, at present lacking.

The finding of imidazoleacetic acid as the most important metabolite confirms the conclusion of Schayer (1953), that histaminase (diamine oxidase) is the most important histamine-metabolizing enzyme in rats. Earlier reports (Schayer *et al.*, 1953; Schayer *et al.*, 1955) indicated that, after inhibition of the histaminase in rats by aminoguanidine, the greater part of liberated or injected histamine came out unchanged in the urine. In the present experiments, even in female rats, not more than about one third of injected [^{14}C]histamine was excreted unchanged. The different results are likely to be due to the fact that, in the earlier studies which were made principally with paper chromatography, [^{14}C]methylhistamine gave spuriously high values for [^{14}C]histamine, a finding which has been discussed by Schayer and Cooper (1956).

It appears that the female rat is more suitable than the male for studies with the urinary excretion of histamine as an indicator of production or liberation of histamine in the body. Particularly so if aminoguanidine is given and precautions taken to diminish the contribution to the urinary excretion by histamine in the food and by histamine produced by intestinal bacteria (see Schayer *et al.*, 1955, and Kahlson *et al.*, 1958).

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