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THE FORMATION OF [^{14}C]-HISTAMINE *IN VIVO* IN NORMAL RATS AND IN RATS TREATED WITH LIOTHYRONINE

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Female rats treated with liothyronine or its solvent (control rats) were given an intravenous injection of [^{14}C]-L-histidine. The amount of [^{14}C]-histamine in various tissues was measured at 10 min, 60 min and 22 hr after the injection. In control rats the glandular stomach contained large amounts of [^{14}C]-histamine at 10 and 60 min, with a sharp decline at 22 hr. The skin contained small amounts at 10 and 60 min, with a slight rise at 22 hr. In rats treated with liothyronine there was more [^{14}C]-histamine in the stomach at 10 and 60 min, but not at 22 hr after the injection of [^{14}C]-L-histidine. The results support the following conclusions: (1) The glandular stomach in the intact rat forms more histamine than do other tissues; the gastric histamine is in a rapid state of turnover and is likely to contribute substantially to the urinary output of histamine. (2) After treatment with liothyronine the rat has an increased histamine formation in the stomach; this is probably the main cause of the raised urinary histamine excretion in such a rat.

Rats excrete more histamine in the urine when given thyroid hormones (Parratt & West, 1960; Bjurö, Westling & Wetterqvist, 1961). Parratt & West (1960) thought that the increase in urinary histamine was caused by a diminished inactivation of histamine since the activity of histaminase *in vitro* was lower in hyperthyroid rats. However, Bjurö *et al.* (1961) found that the urinary histamine also increased in rats which had been given thyroid hormones and aminoguanidine, an inhibitor of histaminase. Furthermore the catabolism of injected [^{14}C]-histamine was unchanged in rats treated with thyroid hormones. *In vivo* experiments with injected [^{14}C]-L-histidine indicated that the increase in urinary histamine was caused by an increase in formation of histamine in these rats. An increase in formation of histamine *in vitro* in the glandular stomach could also be demonstrated.

The purpose of the present experiments was to examine, *in vivo,* the formation of histamine in various tissues of liothyronine-treated and of control rats.

METHODS

White, female Sprague-Dawley rats with an average body weight of 154 g on the day before operation were given approximately 10 g daily of a semisynthetic food free from histamine (Kahlson, Rosengren & Westling, 1958). Twelve rats were given 0.1 mg of liothyronine once daily by subcutaneous injection. Liothyronine was kindly supplied by Erco, Stockholm, Sweden.

Twelve rats served as controls and were given once daily the liothyronine solvent (Bjurö *et al.*, 1961) by subcutaneous injection. On the eighth day after the beginning of the injections, each of the twenty-four rats received approximately 250 μg of [^{14}C]-L-histidine by injection into a tail vein. The rats were killed at the following times after this injection: after 10 min, 60 min and 22 hr. The whole series of twenty-four rats was thus divided into six groups of four rats, three groups having been given liothyronine and three groups the solvent.

The rats (four liothyronine-treated and four control) that were killed after 22 hr were given food at 4 to 8 hr after the injection. Two liothyronine-treated rats and two controls were kept in metabolism cages during the 22 hr and their urine was collected.

The rats were killed by decapitation. Tissues were removed as quickly as possible for determination of the amount of [^{14}C]-histamine. The tissue specimens were weighed to the nearest 0.1 g and put into beakers containing histamine carrier. Thereafter 20% (w/v) trichloroacetic acid was added to precipitate proteins and extract the histamine. The tissues were ground with sand. After the removal of tissues, the carcasses of the four rats were homogenized; carrier was added to the homogenate and protein precipitated with trichloroacetic acid. Histamine was extracted with butanol and subsequently converted to *p*-iodobenzenesulphonyl chloride-histamine (pipsylhistamine) as described (Kahlson, Rosengren, Westling & White, 1958; Bjurö *et al.*, 1961). The radioactivity of the pipsylhistamine was measured on standard plates at infinite thickness under standardized conditions in a gas-flow counter (background 20 to 22 counts/min). The pipsylhistamine was purified to constant radioactivity by repeated recrystallizations.

The content of [^{14}C]-histamine in the urine was measured with the same procedure as described above for tissues (Bjurö *et al.*, 1961). In addition the amount of biologically active histamine in the urine was determined according to Angervall, Bjurö & Westling (1961).

The values for [^{14}C]-histamine content are given as counts/min/g of tissue (Table 1) or counts/min in the whole tissue (for example whole stomach or whole skin, see Fig. 1). Background radioactivity was subtracted.

The [^{14}C]-L-histidine was purchased from the Radiochemical Centre, Amersham, England. It was labelled in the 2 position of the imidazole ring to a specific activity of 63.0 μC/mg; 1 μg of [^{14}C]-histamine formed from this [^{14}C]-L-histidine would give approximately 5,500 counts/min when assayed with 40 mg of histamine base under the present conditions. The [^{14}C]-L-histidine originally contained a small amount of [^{14}C]-histamine as an impurity. A butanol extraction of the [^{14}C]-L-histidine removed about 90% of the [^{14}C]-histamine. The remaining [^{14}C]-histamine in the injected [^{14}C]-L-histidine gave approximately 140 counts/min, a small count compared with that from the [^{14}C]-histamine formed from [^{14}C]-L-histidine. Furthermore it would presumably be partially metabolized and excreted; therefore it was disregarded when calculating the results.

RESULTS

Control rats

It is apparent from Table 1 and Fig. 1 that of the tissues examined the glandular stomach contained most [^{14}C]-histamine after an intravenous injection of [^{14}C]-L-histidine. This is particularly so if the [^{14}C]-histamine content per g of tissue is calculated (Table 1). The total amount of [^{14}C]-histamine is also larger in the stomach 10 and 60 min after the injection but at 22 hr the skin contains the larger total amount. It must be noted, however, that the urinary content of [^{14}C]-histamine is high and at 22 hr a large fraction of the [^{14}C]-histamine was actually found in the urine (Table 2).

Table 3 shows a comparison of the content of biologically active histamine, the [^{14}C]-histamine forming capacity *in vitro* and the peak value of [^{14}C]-histamine content in the present study. In addition, an estimation of the turnover rates is

TABLE 1

THE FORMATION OF [^{14}C]-HISTAMINE *IN VITRO* IN LIOTHYRONINE-TREATED AND CONTROL RATS

The rats (twelve liothyronine-treated and twelve control) were killed 10 min, 60 min or 22 hr after being given an intravenous injection of [^{14}C]-L-histidine. The amount of [^{14}C]-histamine is expressed as counts/min/g of tissue. * *P* value for difference from controls, <0·05 ; † <0·01. ‡ Omitted in calculation of mean value

Tissue	Time between injection and death	Control		Liothyronine-treated	
		Values	Mean	Values	Mean
Glandular stomach	10 min	470, 380, 190, 160	300	1,370, 770, 760, 470	843*
	60 min	890, 580, 570, 520	640	2,000, 1,550, 1,540, 870	1,490
	22 hr	140, 93, 89, 86	102	130, 120, 100, 67	104
Skin	10 min	7·4, 7·2, 6·8, 6·4	7·0	10·7, 9·3, 6·8, 6·8	8·4
	60 min	10·2, 8·1, 7·0, 5·9	7·8	12·3, 8·2, 7·3, 7·0	8·7
	22 hr	12·1, 10·0, 7·8, 5·4	8·8	18·0, 16·7, 15·1, 13·2	15·8†
Small intestine	10 min	10·7, 9·5, 8·2, 8·2	9·2	8·2, 7·5, 5·0, 4·6	6·3*
	60 min	15·8, 13·1, 11·3	13·4	12·1, 10·2, 8·6, 7·0	9·5
	22 hr	22·6, 18·3, 13·1, 11·8	16·5	16·4, 15.9, 15·3, 5·5	13·3
Kidneys	10 min	16·8, 14·2, 12·7	14·6	11·0, 10·5, 7·5, 4·9	8·5*
	60 min	7·3, 5·7, 4·8, 3·5	5·3	8·5, 5·4, 5·3, 4·8	6·0
	22 hr	0, 0	0	0, 0	0
Lungs	10 min	14·2, 13·8	14·0	10·8, 8·2, 7·3, 6·8	8·3
	60 min	13·8, 9·2, 7·7, 6·5	9·3	13·0, 8·5, 7·3, 6·2	8·8
	22 hr	5·4, 5·4	5·4	6·2, 4·6	5·4
Liver	10 min	(27·8‡), 9·6, 9·5, 8·7	9·3	12·9, 11·9, 8·6, 8·2	10·4
	60 min	9·7, 6·7, 5·7, 4·6	6·7	11·4, 5·9, 5·1, 5·0	6·9
	22 hr	1·5, 1·4, 1·3, 1·0	1·3	1·8, 1·8, 1·5, 1·0	1·5
Heart	10 min	—	—	—	—
	60 min	—	—	—	—
	22 hr	4·7, 3·5	4·1	4·4, 3·6	4·0
Spleen	10 min	—	—	—	—
	60 min	—	—	—	—
	22 hr	10·0, 2·8	6·4	6·9, 4·3	5·6

given. These were obtained in two ways, by relating the content of biologically active histamine to the histamine-forming capacity and by the decrease in [^{14}C]-histamine content from 60 min to 22 hr after the injection of [^{14}C]-L-histidine. The exact calculation of turnover rate of histamine in various rat tissues would require a considerable amount of [^{14}C]-L-histidine and a large number of estimations. The present observations appear however, to justify some conclusions about the turnover rate in some of the tissues investigated. It is possible to divide the tissues examined into two groups: (1) tissues with an initial rapid increase in the content

TABLE 2

THE CONTENT OF [^{14}C]-HISTAMINE IN THE CARCASS AND THE URINARY EXCRETION OF [^{14}C]-HISTAMINE AND OF BIOLOGICALLY ACTIVE HISTAMINE IN RATS KILLED 22 HR AFTER THE INJECTION OF [^{14}C]-L-HISTIDINE

Rats	[^{14}C]-Histamine in carcass (counts/min)	Urinary content at 22 hr of	
		[^{14}C]-Histamine (counts/min)	Free histamine base (μg)
Liothyronine-treated	505	1,175	98
	505	890	69
Control	545	570	35
	590	480	30

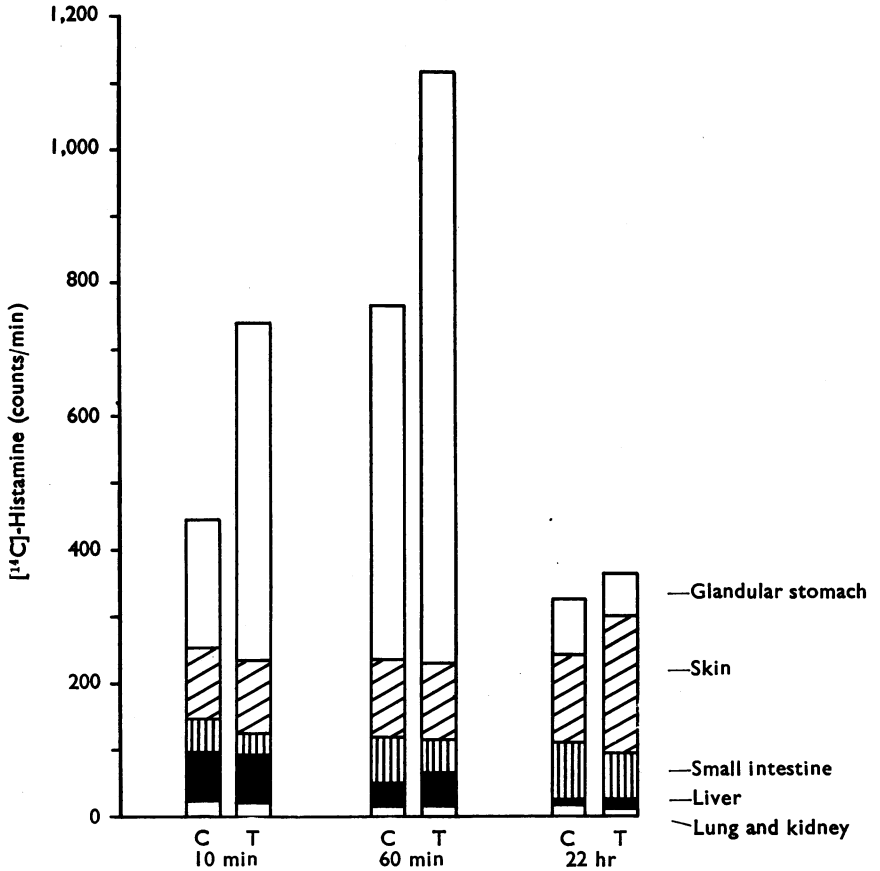

Fig. 1. The content of [^{14}C]-histamine in whole tissues in liothyronine-treated and control rats. T = liothyronine-treated rats ; C = control rats. The rats were killed 10 min, 60 min and 22 hr after being given an intravenous injection of [^{14}C]-L-histidine. The amount of [^{14}C]-histamine is expressed as counts/min in the whole tissue. The tissues represented by different areas of the columns are shown on the right.

of [^{14}C]-histamine and then a rapid decrease (glandular stomach, liver and kidney); and (2) tissues in which the amount of [^{14}C]-histamine steadily increases up to 22 hr (skin, small intestine). The lung occupies an intermediate position, with an initial increase and a rather slow decrease in the [^{14}C]-histamine content at 22 hr.

Rats treated with liothyronine

The larger urinary excretion of [^{14}C]-histamine in the urine of liothyronine-treated rats (Table 2) confirmed previous results (Bjurö *et al.*, 1961). A correspondingly higher tissue [^{14}C]-histamine level could only be demonstrated in the glandular stomach and in the skin. At 10 and 60 min after the injection of [^{14}C]-L-histidine the rats given liothyronine had from two- to three-times more [^{14}C]-histamine in the glandular stomach than had the controls (Table 1, Fig. 1). This difference was

TABLE 3

APPROXIMATE ESTIMATION OF TURNOVER RATE OF HISTAMINE IN VARIOUS RAT TISSUES

The values of biologically active histamine content and of histamine-forming capacity are unpublished results from this laboratory. Histamine-forming capacity means histidine decarboxylase activity expressed as counts/min of [^{14}C]-histamine formed by 1 g of tissue during 3 hr incubation with [^{14}C]-L-histidine *in vitro*. Peak [^{14}C]-histamine content is expressed as counts/min of [^{14}C]-histamine in 1 g of tissue after an intravenous injection of [^{14}C]-L-histidine (this paper, Table 1)

Tissue	Free histamine base content (μg/g)	Histamine-forming capacity (counts/min/g)	Peak [^{14}C]-histamine content (counts/min/g)	Turnover rate
Glandular stomach	17	2,000	640	Rapid
Kidneys	1	Not measurable	22	Rapid
Liver	1	25	14	Rapid
Lungs	2	800	18	Intermediate
Skin	30	30	9	Slow
Small intestine	10	—	17	Slow

statistically significant ($P<0.05$). At 22 hr, however, liothyronine-treated and control rats did not differ in the amount of [^{14}C]-histamine in the glandular stomach. By contrast liothyronine-treated and control rats did not differ in the [^{14}C]-histamine content of the skin at 10 and 60 min after the injection of [^{14}C]-L-histidine.

DISCUSSION

The present observations may be commented upon from two points of view: first, the normal turnover rate of histamine in rat tissues and, second, the changes caused by liothyronine. As to the first point the present results suggest that the glandular stomach occupies a cardinal position in histamine metabolism in the rat; this is reflected in a high rate of formation and rapid turnover.

The liver and kidneys also contain [^{14}C]-histamine in a rapid state of turnover although in much smaller amounts than in the stomach. The absence, *in vitro*, of rapid histamine formation in these tissues may indicate that the [^{14}C]-histamine found was not formed in these organs.. The [^{14}C]-histamine in the kidney might be undergoing excretion into the urine. Telford & West (1961a), from their *in vitro* studies, have suggested that the liver is an important producer of histamine in the rat. The present observations suggest that the liver, in spite of its weight, is not particularly dominant in this respect. We believe that the presence, or absence, of a small histamine-forming capacity *in vitro* is not very helpful in deciding whether histamine is actually formed or not in a certain tissue. *In vivo* studies of [^{14}C]-histamine formation from [^{14}C]-L-histidine suffer from the drawback that it is difficult to exclude transfer of formed histamine from one tissue to another. At present, a combination of the *in vitro* and *in vivo* techniques appears to provide the most conclusive results.

The skin would seem to rank, next to the glandular stomach, as the second most important organ in histamine metabolism in the rat; however, the relatively slow build up of histamine in the skin suggests that under the prevailing conditions skin histamine does not contribute much to the total turnover of histamine in the rat. In fact, the present observations suggest that the greater part of the urinary hista-

mine stems from the gastric mucosa, which appears to form histamine rapidly but not to store it for a long time. Kahlson, Rosengren & Thunberg (1963) inhibited histamine formation by keeping rats on a pyridoxine deficient diet and treating them with semicarbazide. This treatment inhibits histamine formation by inhibiting the enzyme histidine decarboxylase. Information about the lifetime of the amine in various tissues could be obtained by following the decrease of biologically active histamine. The results of Kahlson *et al.* (1963b) showed that gastric histamine in the rat has a rapid turnover rate, cutaneous histamine having a slow one and the lungs and intestines occupying intermediate positions. Thus these results accord with our findings.

Previous observations (Bjurö *et al.*, 1961) suggested that the increased amount of biologically active histamine in the urine of rats treated with thyroid hormones was caused by an increased formation of histamine in the animal as a whole. Preliminary *in vitro* studies revealed an increased rate of formation in the glandular stomach but not in the skin. The present study confirmed these findings and provided direct evidence that the liothyronine-treated rat forms more histamine (from [^{14}C]-L-histidine) in the glandular stomach. In the skin there was a larger accumulation of [^{14}C]-histamine at 22 hr in liothyronine-treated rats. This is somewhat surprising because there was no demonstrable increase in histidine decarboxylase activtity of the skin in rats given liothyronine (Bjurö *et al.*, 1961). The observation of [^{14}C]-histamine accumulation in the skin accords, however, with findings of increased skin histamine concentrations in hyperthyroid states in rat and in man (Gotzl & Dragstedt, 1940; Feldberg & Loeser, 1954).

Several observations, apart from those reported here, indicate that important changes in histamine formation occur in the rat's stomach: histamine formation *in vitro* increase in the stomach of the rat in adrenocortical insufficiency (Schayer, 1956; Bjurö, 1963), in the stomach of chilled rats (Telford & West, 1961b) and as a response to treatment with adrenocortical steroids (Telford & West, 1960). It thus seems likely that the thyroid and adrenocortical hormones alter the urinary histamine excretion (Bjurö, Westling & Wetterqvist, 1962, 1964) through their effect on the rate of histamine formation in the glandular stomach. Primary changes in feeding or gastric secretion may of course elicit the changes in histamine metabolism in the glandular stomach (Kahlson, Rosengren, Svahn & Thunberg, 1963).

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