Br. J. Pharmac. (1968), **34**, 551–563.

Further studies on the histidine-histamine relationship *in vivo*: effects of endotoxin and of histidine decarboxylase inhibitors

MARGARET A. REILLY AND R. W. SCHAYER

Research Center, Rockland State Hospital, Orangeburg, New York, U.S.A.

1. Mice were injected with (^{14}C)-L-histidine, killed at various intervals, and tissues assayed for (^{14}C)-histamine. In some cases free (^{14}C)-L-histidine and total (^{14}C) were also determined.

2. Removal of stomach, the most active histamine-forming tissue, failed to reduce the (^{14}C)-histamine content of any tested tissue; (^{14}C)-histamine concentrations in lung and muscle of gastrectomized mice were higher than in sham-operated controls.

3. In mice pretreated with endotoxin and subsequently injected with (^{14}C)-L-histidine, the (^{14}C)-histamine content of liver, lung and muscle was markedly higher than in controls. The increased concentrations of (^{14}C)-histamine in endotoxin-pretreated mice seemed to reflect a greater rate of formation; they could be attributed neither to changes in tissue concentration of (^{14}C)-L-histidine, to increased uptake from other tissues, nor to impaired ability to inactivate histamine.

4. Results of studies on *in vivo* effectiveness of several histidine decarboxylase inhibitors are reported.

5. The following conclusions are supported by the evidence presented: (*a*) in stressed mice, those tissues which show activation of histidine decarboxylase also show increased ability to form histamine *in vivo*; (*b*) tissue histamine is largely formed locally; (*c*) histidine decarboxylase inhibitors are highly effective in blocking histamine formation in mast cells and in stomach, but do not normally reach the locus of an inducible form of histidine decarboxylase; (*d*) the inducible form of histidine decarboxylase in liver may be located in phagocytic microvascular endothelial cells; (*e*) in conditions favouring near-maximal activation of histidine decarboxylase, the histamine-methylating enzyme of liver and diamine oxidase of intestine showed no inducible characteristics; (*f*) blood histamine concentrations do not accurately reflect changes in tissue histamine formation.

A method for determination of (^{14}C)-histamine and (^{14}C)-L-histidine in the same tissue sample has recently been described (Reilly & Schayer, 1968). Mice were injected with (^{14}C)-L-histidine, killed at various intervals, and tissues assayed; the data enabled estimation of the rate of disappearance of free (^{14}C)-L-histidine from

the tissues, and the rate of appearance of the newly formed (^{14}C)-histamine. A technique was also described for estimating the ability of tissues of the living animal to remove circulating histamine from the blood, and to store or inactivate it. These investigations were the preliminary phase of an attempt to test the significance of *in vitro* findings on histamine metabolism to events in the living animal.

A number of studies (Schayer, 1960a ; Schayer, 1962a) have shown that histidine decarboxylase, the enzyme which catalyses conversion of L-histidine to histamine, has inducible activity. Histidine decarboxylase activation, as shown by *in vitro* assays, can be effected by a number of non-specific " stressor " stimuli, applied to animals either systemically or locally. These findings have been used to develop theories on a microcirculatory function for newly formed histamine (Schayer, 1963a ; Schayer, 1965 ; Schayer, 1968a) and on a unified mechanism of action of the glucocorticoid hormones (Schayer, 1964 ; Schayer, 1967), so clear evidence of stressor-induced activation of histamine formation *in vivo* is required.

In the present studies it is shown that pretreatment of mice with endotoxin, a powerful stimulus for histidine decarboxylase activation in certain tissues (Schayer, 1961), increased the concentrations of (^{14}C)-histamine found in tissues of mice following injection with (^{14}C)-L-histidine. *In vivo* evidence is also reported suggesting that the observed increase in (^{14}C)-histamine in tissues of endotoxin-treated mice is due to increased formation, and not to changes in substrate distribution, reduced ability of tissues to destroy histamine, or to a tendency for certain tissues to accumulate histamine formed elsewhere in the body.

The effects of histidine decarboxylase inhibitors on histamine formation in various tissues of normal mice and of endotoxin-pretreated mice are also reported. Histidine decarboxylase inhibitors have been used to study the physiological functions of histamine. Two powerful *in vitro* inhibitors, the α-hydrazino analogue of histidine (α-HH) and NSD-1055, have been tested by other laboratories for effect on histamine levels in urine and tissues (Levine, Sato & Sjoerdsma, 1965 ; Johnston & Kahlson, 1967). There is little information on the specific site of their inhibitory action *in vivo*.

In this paper a previously unreported histidine decarboxylase inhibitor, *p*-toluenesulphonylhydrazine (PTSH), is introduced and compared with α-HH and NSD-1055 for *in vitro* inhibitory power, and by several *in vivo* procedures.

Methods

Female albino CF-1 mice (18–21 g) and female CFN rats (100–109 g) from Carworth, Inc., New City, New York, were used. (^{14}C)-L-histidine and (^{14}C)-histamine, specific activities 35.0 and 0.90 mc/m-mole respectively, were purchased from Nuclear Chicago. (^{14}C)-L-histidine was purified before injection to remove traces of radioactive histamine (Schayer, 1968b). In all mouse experiments assays were made on the pooled tissues of three animals.

Gastrectomy was performed in mice anaesthetized with pentobarbitone by tying off the oesophagus at the highest point possible, and the small intestine about 1 cm below the pylorus. The stomach was removed by cutting the tissues close to the ligature. Abdominal muscle was closed with sutures and the skin closed with wound clips. Gastrectomized mice were injected intraperitoneally with 0.5 ml. saline in the late afternoon, kept overnight on screens without access to food or water, and used the next morning.

Endotoxin was Difco *E. coli* lipopolysaccharide. The histidine decarboxylase inhibitors, L-2-hydrazino-3-4(5)-imidazolepropionic acid hydrochloride (the α-hydrazino analogue of histidine: α-HH), and *p*-toluenesulphonylhydrazine (PTSH) were provided by the Merck Sharpe and Dohme Research Laboratories; 4-bromo-3-hydroxybenzyl oxamine dihydrogen phosphate (NSD-1055) was donated by Smith and Nephew, Ltd. PTSH can be purchased from K & K Laboratories Inc., Plainview, N.Y.

The method for determining total (^{14}C), (^{14}C)-histamine, and (^{14}C)-L-histidine (free) in the same tissue sample has been described in detail (Reilly & Schayer, 1968). Briefly, tissues of mice injected with (^{14}C)-L-histidine were homogenized in acidic buffer. One aliquot was used to count total (^{14}C); to another, carrier histamine was added and (^{14}C)-histamine then determined by isotope dilution assay. A third aliquot was incubated with a powerful specific bacterial histidine decarboxylase preparation and (^{14}C)-histamine determined by isotope dilution assay. The (^{14}C)-histamine formed during incubation with bacterial histidine decarboxylase provides a measure of the concentration of free (^{14}C)-L-histidine in the tissue. Because the isotope dilution method requires considerable time, in some experiments (^{14}C)-L-histidine assays were omitted.

In experiments in which mice were injected with (^{14}C)-histamine, total (^{14}C) and (^{14}C)-histamine were determined. Histidine decarboxylase activity, *in vitro*, was measured by incubating aliquots of rat stomach extract with tracer amounts of (^{14}C)-L-histidine, plus either inhibitor or saline. Incubates were then transferred to centrifuge tubes (equipped with neoprene stoppers) and the beakers rinsed with 1 ml. water. Then to each tube was added 2 ml. saturated sodium chloride solution, 1.0 ml. of 5 N sodium hydroxide, 12 ml. butanol-chloroform, 3 : 1, and approximately

TABLE 1. *Total (^{14}C) and (^{14}C)-histamine in tissues of gastrectomized and sham-operated mice 10 min after intravenous injection of (^{14}C)-L-histidine*

	(A)		(B)		(A)/(B)		
	(^{14}C)-histamine (d.p.m./g tissue)		Total (^{14}C) (d.p.m./g tissue) ($\times 10^3$ omitted)		(^{14}C)-histamine (d.p.m.) / Total (^{14}C) (10^3 d.p.m.)		
Tissue	Sham	Gastrect.	Sham	Gastrect.	Sham	Gastrect.	
Liver	723	790	2240	3800	0·32	0·21	NS*
	165	546	2660	3290	0·06	0·17	
	367	511	2370	4640	0·16	0·11	
	227	544	2680	3520	0·09	0·16	
Lung	260	1200	764	865	0·34	1·4	$P<0·001$
	331	1330	794	790	0·42	1·7	
	248	2290	718	931	0·35	2·5	
	298	1680	751	829	0·40	2·0	
Muscle	321	1250	655	901	0·49	1·4	$P<0·025$
	246	617	697	788	0·35	0·78	
	408	1300	690	798	0·59	1·6	
	369	698	748	731	0·49	0·96	
Intestine	715	611	967	1090	0·74	0·56	NS
	1070	492	970	1040	1·10	0·47	
	331	702	995	1110	0·33	0·63	
	843	602	1010	1070	0·84	0·56	

* Comparison of the two groups under (A)/(B).

Dose of (^{14}C)-L-histidine 10·9 μc in 0·10 ml. saline per mouse. Single estimations on pooled tissues of three mice.

1.5 g sodium chloride. After mechanical shaking and centrifugation, 10 ml. of the organic (upper) layer was transferred to another tube and washed by shaking with 5 ml. of 0.1 N sodium hydroxide saturated with sodium chloride. After centrifugation, 8 ml. of the organic layer was transferred to another tube and shaken with 5 ml. 0.1 N hydrochloric acid. Four millilitres of the acid layer was transferred to

TABLE 2A. *Total (^{14}C) and (^{14}C)-histamine in tissues of endotoxin-pretreated mice 4 min after intravenous injection of (^{14}C)-L-histidine*

	(A) (^{14}C)-histamine (d.p.m./g tissue)		(B) Total (^{14}C) (d.p.m./g tissue) ($\times 10^3$ omitted)		(A/B) (^{14}C)-histamine (d.p.m.) / Total (^{14}C) (10^3 d.p.m.)		
Tissue	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin	
Blood	202	224	331	516	0·61	0·43	NS*
	80	286	251	514	0·32	0·56	
	139	208	513	513	0·27	0·41	
Liver	696	4210	4930	2120	0·14	2·0	$P<0·01$
	452	5800	3930	4550	0·12	1·3	
	764	3900	5230	3400	0·15	1·2	
Lung	2040	9260	758	1050	2·7	8·8	$P<0·01$
	926	11400	608	1290	1·5	8·8	
	546	5850	833	1080	0·66	5·4	
Muscle	472	1070	426	201	1·1	5·3	$P<0·05$
	1000	1560	414	190	2·4	8·2	
	804	1200	289	266	2·8	4·5	
Intestine	726	1390	192	450	3·8	3·1	NS
	1020	2170	281	421	3·6	5·2	
	472	1430	279	228	1·7	6·3	
Stomach	16900	9500	827	559	20·5	17·0	NS
	17600	14000	809	781	21·7	17·9	
	19600	33700	680	721	28·8	46·7	

* Comparison of the two groups under (A)/(B).

Mice pretreated with saline or with endotoxin, 100 μg i.p.; 4 hr later all injected with (^{14})-L-histidine, 10·9 μc i.v., and killed 4 min later. Single estimations on pooled tissues of three mice.

TABLE 2B. *(^{14}C)-Histamine and (^{14}C)-L-histidine in liver and lung of normal and endotoxin-pretreated mice 4 min after intravenous injection of (^{14}C)-L-histidine*

	(A) (^{14}C)-histamine (d.p.m./g tissue)		(B) (^{14}C)-L-histidine (d.p.m./g tissue) ($\times 10^3$ omitted)		(A)/(B) (^{14}C)-histamine (d.p.m.) / (^{14}C)-L-histidine (10^3 d.p.m.)	
Tissue	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin
Liver	696	4210	3320	2030	0·21	2·1
	452	5800	3120	3390	0·15	1·7
	764	3900	3940	2650	0·19	1·5
Lung	2040	9260	630	878	3·2	10·6
	926	11400	536	1060	1·7	10·8
	546	5850	584	771	0·9	7·6

Same experiment as in Table 2a showing results of free (^{14}C)-L-histidine assays.

TABLE 2C. *Total (^{14}C)-histamine in liver and stomach of normal and endotoxin-pretreated mice 4 min after intravenous injection of (^{14}C)-L-histidine*

	(^{14}C)-Histamine, d.p.m. in entire tissue					
	Control			Endotoxin		
Liver	2510	1680	2770	17900	24500	17500
Stomach	10300	11800	12700	5800	8400	22200

Same experiment as in Table 2a; data recalculated on whole tissue basis.

a counting vial, evaporated to dryness in a stream of warm air, and the residue dissolved in 0.5 ml. of water. A water-miscible phosphor was added and the (^{14}C) counted and corrected for blank and background.

Counting was done on either a Packard Tri-Carb liquid scintillation spectrometer (background about 20 c.p.m.) or on a Beckman DPM-100 liquid scintillation system (background about 12 c.p.m.). A minimum of 4,000 counts was obtained on all samples and background. The calculations are the same as in Reilly & Schayer (1968).

Results

Experiment 1 ; effect of gastrectomy on distribution of (^{14}C)-histamine in mice injected with (^{14}C)-L-histidine

Reilly & Schayer (1968) found that after injection of (^{14}C)-L-histidine into mice, all tested tissues contained (^{14}C)-histamine, but stomach contained most, by far ; this observation was valid for intervals ranging from 4 to 390 min after injection. It was therefore necessary to determine whether (^{14}C)-histamine found in other tissues could have been formed in stomach and distributed through the blood.

In Experiment 1 gastrectomized mice, or sham-operated mice, were injected with (^{14}C)-L-histidine, killed 10 min later, and tissues assayed for total (^{14}C) and (^{14}C)-histamine. Experimental details and results are shown in Table 1. Gastrectomy did not materially reduce the (^{14}C)-histamine content of any tested tissue ; in lung and muscle the concentration is significantly increased.

Experiment 2 ; effect of endotoxin-pretreatment on distribution of (^{14}C)-histamine in mice injected with (^{14}C)-L-histidine

In early studies (Schayer, 1960a, 1962a), it was found that a few minutes after injection of (^{14}C)-L-histidine into endotoxin-pretreated mice, liver and intestine contained an abnormally large amount of (^{14}C)-histamine. These studies, however, did not conclusively demonstrate increased histamine formation *in vivo,* because several alternative explanations exist: (*a*) endotoxin may have increased the tissue concentration of the precursor, (^{14}C)-L-histidine ; (*b*) the additional (^{14}C)-histamine may have come from stomach, normally the most active histamine-producing tissue ; (*c*) endotoxin may have impaired the ability of the tissues to destroy histamine.

Experiment 2 was designed to extend these earlier studies. Normal mice, or endotoxin-pretreated mice, were injected with (^{14}C)-L-histidine, killed 4 min later, and tissues assayed for (^{14}C)-histamine, total (^{14}C), and in some cases (^{14}C)-L-histidine. Experiment details and results are shown in Tables 2a, 2b and 2c. Endotoxin-pretreatment increased (^{14}C)-histamine content of liver, lung, muscle and intestine but had less effect on total (^{14}C) and (^{14}C)-L-histidine levels. The significance of Tables 2b and 2c will be clarified in the discussion.

Experiment 3 ; effect of endotoxin on rate of inactivation of histamine by mouse tissues in vivo

Liver and lung of endotoxin-pretreated mice contain abnormally large amounts of (^{14}C)-histamine 4 min after injection of (^{14}C)-L-histidine (Tables 2a and 2b), so the additional (^{14}C)-histamine might be attributable to endotoxic impairment of

histamine destruction. In Experiment 3 normal mice, or endotoxin-pretreated mice, were injected intravenously with (^{14}C)-histamine, killed 4 min later, and tissues assayed for total (^{14}C) and (^{14}C)-histamine. This experiment is based on evidence (Schayer, 1953 ; Reilly and Schayer, 1968) which suggests that within a few minutes after intravenous injection of (^{14}C)-histamine, the ratio of total (^{14}C) to (^{14}C)-histamine in a tissue provides a rough measure of the *in vivo* histamine-inactivating power of that tissue. Experimental details and results are shown in Table 3. In liver of normal mice, 4 min after injection of labelled histamine, 97.7% of the total (^{14}C) was due to inactivation products of (^{14}C)-histamine ; in endotoxin-pretreated mice the value was 96.9%. In lung, as in liver, there was no apparent difference between normal and endotoxin-treated mice. In intestine, it is possible that endotoxin-pretreatment reduced the (^{14}C)-histamine inactivation rate ; however, additional work would be required to establish a real difference.

Experiment 4 ; comparison of in vitro *inhibition of histidine decarboxylase activity by PTSH, α-HH and NSD-1055*

The three histidine decarboxylase inhibitors PTSH, α-HH and NSD-1055 were tested for *in vitro* effectiveness. Results are shown in Table 4. All drugs were strong inhibitors, but NSD-1055 was more effective in lower concentrations.

Experiment 5 : effect of PTSH, α-HH and NSD-1055 on (^{14}C)-histamine in skin of mice injected with (^{14}C)-L-histidine

In mice injected subcutaneously with (^{14}C)-L-histidine, (^{14}C)-histamine can be detected for many days at the site of the injection (Schayer, 1961, and unpublished).

TABLE 3. *Effect of endotoxin-pretreatment on rate of inactivation of injected (^{14}C)-histamine in mice*

	(A) (^{14}C)-Histamine (d.p.m./g tissue)		(B) Total (^{14}C) (d.p.m./g tissue)		(B–A)/(B) (^{14}C)-Histamine-metabolites (% of total ^{14}C)	
Tissue	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin
Liver	1110	1660	44500	49000	97·5	96·6
	1170	1240	44700	40000	97·4	96·9
	812	1040	48500	35600	98·3	97·1
Lung	4300	7780	13700	21400	68·6	63·6
	3910	6540	29000	25300	86·5	74·2
	7130	3800	23100	21900	69·2	82·7
Intestine	2950	6780	34200	45000	91·4	85·0
	3150	5760	41300	47200	92·4	87·8
	3240	2980	42300	45500	92·3	93·5

Mice pretreated with saline or endotoxin, 100 μg i.p.; 4 hr later all injected with (^{14}C)-histamine, 0·25 μc i.v., and killed 4 min later. Single estimations on pooled tissues of three mice.

TABLE 4. *Inhibition of rat stomach histidine decarboxylase* in vitro *by PTSH, α-HH and NSD-1055*

	Percent inhibition at various concentrations		
Inhibitor	2×10^{-6}M	5×10^{-7}M	$1{\cdot}25\times10^{-7}$M
PTSH	97	86	45
α-HH	98	87	42
NSD-1055	100	89	69

Presumably (^{14}C)-L-histidine enters mast cells, some is decarboxylated, and the (^{14}C)-histamine retained in bound form (Schayer, 1963b). The effect of PTSH, α-HH and NSD-1055 on this process was tested.

Groups of mice were given saline or one of the inhibitors, and injected subcutaneously with (^{14}C)-L-histidine. Four days later the abdomen was shaved, the mice were killed, washed thoroughly to remove urinary (^{14}C), and uniform segments of abdominal skin taken for assay. Experimental details and results are shown in Table 2. PTSH and α-HH markedly reduced the (^{14}C) histamine found in abdominal skin ; NSD-1055 was less effective.

Experiment 6 ; effect of PTSH, α-HH and NSD-1055 on (^{14}C)-histamine in stomach of mice injected with (^{14}C)-L-histidine

In mice injected intravenously with (^{14}C)-L-histidine, (^{14}C)-histamine can be detected in tissues at the earliest interval tested—4 min ; under these conditions stomach contains much more (^{14}C)-histamine than any other tissue (Reilly & Schayer, 1968). It is presumed that the greater part of this (^{14}C)-histamine is formed by the highly active histidine decarboxylase of stomach.

In Experiment 6 the effect of histidine decarboxylase inhibitors on the apparent formation of histamine in mouse stomach was tested.

Groups of mice pretreated with saline or with one of the inhibitors were injected intravenously with (^{14}C)-L-histidine and killed 5 min later, and the stomach was assayed for (^{14}C)-histamine. Experimental details and results are shown in Table 3. PTSH and α-HH markedly reduced the (^{14}C)-histamine found in stomach ; NSD-1055 was less effective.

Experiment 7 ; effect of PTSH, α-HH and NSD-1055 on urinary excretion of (^{14}C)-histamine by rats injected with (^{14}C)-L-histidine

In female rats, histamine is inactivated principally through the action of diamine oxidase. If female rats are pretreated with aminoguanidine, an effective *in vivo* inhibitor of diamine oxidase, injected (^{14}C)-histamine is excreted largely unchanged. In all other tested species, including mice, histamine methylation is a major route of inactivation. Because effective *in vivo* inhibitors of histamine methylation are not known, meaningful studies on whole body histamine must be done in female rats (Schayer, 1959).

In Experiment 7, the effect of histidine decarboxylase inhibitors on whole body histamine formation was tested.

Groups of rats pretreated with saline, or with one of the inhibitors, were injected subcutaneously with (^{14}C)-L-histidine ; urine was collected overnight and assayed for (^{14}C)-histamine.

Experimental details and results are shown in Table 4. PTSH and α-HH caused only a moderate reduction of urinary (^{14}C)-histamine excretion ; NSD-1055 was even less effective.

Experiment 8; effect of α-HH on (^{14}C)-histamine in tissues of endotoxin-pretreated mice injected with (^{14}C)-L-histidine

In endotoxin-pretreated mice injected with (^{14}C)-L-histidine, the concentrations of (^{14}C)-histamine in some tissues are greater than normal (Table 2a). Experiment 8 was designed to test the effect of a histidine decarboxylase inhibitor on the (^{14}C)-histamine concentrations in tissues of endotoxin-pretreated mice. Presumably the results might show whether the inducible form of histidine decarboxylase, during a period of marked activation, was reached by the inhibitor. Only α-HH was tested; it is more potent than NSD-1055 *in vivo* (Tables 5 and 6) and more thoroughly tested than PTSH. Experimental details and results are shown in Table 8. (^{14}C)-Histamine concentrations were subnormal in all tested tissues of α-HH treated mice.

Experiment 9; effect of α-HH on (^{14}C)-histamine in liver of gastrectomized mice injected with (^{14}C)-L-histidine

The data of Table 8 show that α-HH reduced (^{14}C)-histamine levels in tissues of endotoxin-pretreated mice, so the effect of α-HH on histamine production in mice without endotoxin-pretreatment was tested. This experiment was intended to evaluate the effect of α-HH on the basal level of the non-mast cell, inducible histidine decarboxylase, which may be present in every tissue; the experiment was designed to reduce errors from three potential sources.

First, histamine production is usually very high in stomach of normal mice and is markedly reduced by α-HH (Table 6). If α-HH reduced (^{14}C)-histamine concentrations in other tissues, one could not be certain that the effect was local, or was secondary to the reduction in stomach histamine formation. Gastrectomized mice were therefore used.

TABLE 5. *Effect of histidine decarboxylase inhibitors on (^{14}C)-histamine in skin of mice injected with (^{14}C)-L-histidine*

Treatment	(^{14}C)-Histamine, d.p.m./g skin			
Saline (control)	27500	28100	31100	32400
PTSH	3210	4190	4470	4560
α-HH	2040	2770	2770	2790
NSD-1055	11500	14700	18800	21700

Saline or inhibitor, 5 μM/20 g body weight, i.p. at 0 time; (^{14}C)-L-histidine, 5 μc, injected under abdominal skin at 0·5 hr; saline or inhibitor, 2·5 μM/20 g body weight at 2 hr. Mice killed 4 days later. Single estimations on pooled abdominal skins from three mice.

TABLE 6. *Effect of histidine decarboxylase inhibitors on (^{14}C)-histamine in stomach of mice injected with (^{14}C)-L-histidine*

Treatment	(^{14}C)-Histamine, d.p.m./g stomach		
Saline (control)	5380	6810	6920
PTSH	337	694	1030
α-HH	694	971	1030
NSD-1055	3190	3330	3620

Mice fasted overnight. Saline or inhibitor, 6 μM/20 g body weight i.p. at 0 time; (^{14}C)-L-histidine, 2·0 μc, injected i.v. at 0·5 hr. Mice killed 5 min later. Single estimations on pooled stomachs of three mice.

Second, only liver was assayed. It was selected for several reasons: (*a*) it is a large tissue which appears to destroy blood-borne histamine efficiently (Table 3); it therefore seems probable that liver is the tissue least likely to retain (^{14}C)-histamine formed elsewhere in the body; (*b*) (^{14}C)-histamine levels in liver were not significantly affected by gastrectomy (Table 1); (*c*) mouse liver contains very few mast

TABLE 7. *Effect of histidine decarboxylase inhibitors on (^{14}C)-histamine in urine of female rats injected with (^{14}C)-L-histidine*

Treatment	(^{14}C)-Histamine, d.p.m. in total urine		
Saline (control)	2560	2960	3150
PTSH	1000	1270	1610
α-HH	1370	1440	1740
NSD-1055	2210	2480	2650

First injection, 0 time, saline or inhibitor, 25 μM/100 g body weight combined with aminoguanidine, 1 mg/100 g body weight; second injection, 40 min, (^{14}C)-L-histidine 3·0 μc s.c.; third injection, 2 hr, equivalent to one-half of dose used in first injection. Single estimations on pooled urines from two rats.

TABLE 8. *Effect of α-HH on (^{14}C)-histamine in tissues of endotoxin-pretreated mice injected with (^{14}C)-L-histidine*

	(A) (^{14}C)-Histamine (d.p.m./g tissue)		(B) Total (^{14}C) (d.p.m./g tissue) (× 10^3 omitted)		(A)/(B) (^{14}C)-Histamine (d.p.m.) / Total (^{14}C) (10^3 d.p.m.)		
Tissue	Control	α-HH	Control	α-HH	Control	α-HH	
Liver	2950	491	4150	1960	0·71	0·25	$P<0·025$*
	3300	489	2150	2980	1·5	0·16	
	4810	564	3800	2080	1·3	0·27	
Lung	4650	350	686	618	6·8	0·57	$P<0·01$
	4280	261	844	1130	5·1	0·23	
	3850	180	1110	957	3·5	0·19	
Spleen	6330	769	704	923	9·0	0·83	$P<0·05$
	7020	556	1170	1000	6·0	0·56	
	4650	278	1400	1500	3·3	0·19	
Stomach	19100	14600	882	1150	21·7	12·7	NS
	13400	5960	982	790	13·6	7·5	
	9620	4890	1070	973	9·0	5·0	

* Comparison of the two groups under (A)/(B).

Mice, all pretreated with endotoxin, 100 μg i.p. at 0 time. At 3·5 hr half given saline i.p. and half given α-HH, 1 mg/20 g body weight i.p. At 4 hr all injected with (^{14}C)-L-histidine, 10·9 μc i.v., and killed 4 min later. Single estimations on pooled tissues of three mice.

TABLE 9. *Total (^{14}C) and (^{14}C)-histamine in liver of gastrectomized mice 25 min after intravenous injection of (^{14}C)-L-histidine*

	(A) (^{14}C)-Histamine (d.p.m./g tissue)		(B) Total (^{14}C) (d.p.m./g tissue) (× 10^3 omitted)		(A)/(B) (^{14}C)-histamine (d.p.m.) / Total (^{14}C) (10^3 d.p.m.)		
Tissue	Control	α-HH	Control	α-HH	Control	α-HH	
Liver	159	180	916	659	0·17	0·27	NS*
	250	219	526	1025	0·48	0·21	
	311	191	1150	1150	0·21	0·17	
	378	163	1590	1580	0·24	0·10	

* Comparison of the two groups under (A)/(B).

Mice gastrectomized; the next day half received saline, and half α-HH, 1 mg/20 g body weight i.p. at 0 time. At 0·5 hr all injected with (^{14}C)-L-histidine, 10·9 μc i.v. and killed 25 min later. Single estimations on pooled livers of three mice.

cells; (*d*) puromycin treatment reduces mouse liver histidine decarboxylase activity to approximately one-fourth the normal level (Schayer & Reilly, 1968); this finding suggests that the basal level of activity in liver may be largely attributable to the inducible form of the enzyme.

Third, the time of killing the mice was selected as 25 min after injection of (^{14}C)-L-histidine. At this interval levels of free (^{14}C)-L-histidine and total (^{14}C) have fallen well below their peak levels while the (^{14}C)-histamine content remains essentially constant for a prolonged period (Table 2 in Reilly & Schayer, 1968). Accordingly, use of the 25 min interval reduces the possibility that radioactive contamination might cause a significant error in the (^{14}C)-histamine determinations.

Experimental details and results are shown in Table 9. There is no significant effect of α-HH.

Discussion

Experiment 1

Under the conditions of this experiment, liver of gastrectomized mice contained somewhat more (^{14}C)-histamine than liver of sham-operated mice (Table 1); however, there was also more total (^{14}C) in the former, and the ratio (A)/(B) was essentially the same for both groups. Because in tissues of mice a few minutes after injection of (^{14}C)-L-histidine, total (^{14}C) is roughly proportional to free (^{14}C)-L-histidine (Reilly & Schayer, 1968), these results suggest that removal of stomach had no important effect on histamine formation in liver.

In contrast, lung and muscle of gastrectomized mice showed a definite increase in (^{14}C)-histamine, relative to controls, which cannot be attributed to a higher level of substrate (Table 1); these increased levels may be reasonably attributed to the increased histidine decarboxylase activity induced in lung and muscle by any non-specific stressor which causes catecholamine release (Schayer, 1960b). Liver histidine decarboxylase activity is affected little, if any, by catecholamine release or injection (Schayer, 1962b). In intestine, gastrectomy produced no significant effect (Table 1); histidine decarboxylase activity of intestine has not been tested for effects of catecholamine release or injection.

Although stomach has an extraordinarily high potency in forming histamine *in vivo* and *in vitro*, its removal failed to produce a significant decrease in (^{14}C)-histamine level of any tissue tested. It seems therefore justified to question whether stomach, or any other exogenous source, contributes a major portion of the histamine found in tissues. Rather, the data suggest that much tissue histamine is formed locally.

Experiment 2

In endotoxin-pretreated mice (Table 2a) levels of newly formed (^{14}C)-histamine are definitely increased in liver and lung, relative to controls, and slightly increased in muscle and intestine. The values of (A)/(B), used to estimate *in vivo* histamine forming power of tissues, show the same pattern. Data for blood and stomach show no distinct effect of endotoxin.

In Table 2b free (^{14}C)-L-histidine in liver and lung was assayed and the ratio (^{14}C)-histamine to (^{14}C)-L-histidine calculated. The higher ratios for endotoxin-pretreated mice are similar to the data of Table 2a.

These *in vivo* data agree with comparable *in vitro* findings ; histidine decarboxylase activity of lung and liver is strongly increased by endotoxin-pretreatment while muscle shows a smaller increase (Schayer, 1960 ; Schayer, 1962a). Intestine has been inadequately tested, and stomach histidine decarboxylase activity is altered erratically by endotoxin (Schayer, unpublished).

Blood has no significant histidine decarboxylase activity, so its (^{14}C)-histamine is probably formed elsewhere. From the data of Table 2a it is apparent that blood assays may be worthless for evaluating changes in histamine formation rates *in vivo*.

In Table 2c data for liver and stomach from Table 2a are restated in terms of (^{14}C)-histamine and total (^{14}C) in the entire tissue (actually three pooled tissues). The purpose of this recalculation is to show that in endotoxin-pretreated mice, liver contains considerably more (^{14}C)-histamine than does stomach, a fact which supports local formation.

Experiment 3

The possibility was tested that the increased (^{14}C)-histamine levels in liver and lung of endotoxin-pretreated mice (Table 2b) might be due to reduced ability to destroy histamine. The data of Table 3 are from mice injected with (^{14}C)-histamine ; differences between columns (B) and (A) represent inactivation products of (^{14}C)-histamine, and the final column provides a measure of the extent of inactivation. As found previously (Reilly & Schayer, 1968) liver has the greatest apparent *in vivo* histamine-inactivating power, intestine is next most active, and lung least. There is no significant difference between control and endotoxin groups for any of the tissues. Hence it is improbable that endotoxic impairment of histamine-inactivating processes contributed significantly to the effect shown in Table 2b.

From the above discussion another tentative conclusion may be reached. Because in conditions which induce activity of histidine decarboxylase (endotoxin is the strongest stimulus known) there is no evidence of increased histamine destruction, the data show no indication that the histamine-methylating enzyme of liver, or the histamine-inactivating enzyme of intestine, may have inducible characteristics. According to K. S. Kim (personal communication) diamine oxidase is the principal histamine-inactivating enzyme in mouse intestine.

Experiment 4

NSD-1055, at the lowest concentrations tested, was a more effective *in vitro* inhibitor of rat stomach histidine decarboxylase than either PTSH or α-HH (Table 1). There seems to be no important difference between the latter two drugs.

Experiment 5

In mice given PTSH or α-HH, plus (^{14}C)-L-histidine, the amount of (^{14}C)-histamine found in skin 4 days later was drastically reduced relative to control values (Table 5). NSD-1055 had a much weaker effect. This test is believed to measure, primarily, *in vivo* inhibition of mast cell histidine decarboxylase activity. Mouse skin contains

large numbers of mast cells, cells known to contain histidine decarboxylase and to bind the histamine formed within them for prolonged periods (Schayer, 1963b).

Experiment 6

Injection of mice with PTSH or α-HH strongly reduced the amount of (^{14}C)-histamine found in stomach 5 min after intravenous injection of (^{14}C)-L-histidine (Table 6). NSD-1055 had a much weaker effect. This test is believed to measure, primarily, *in vivo* inhibition of stomach histidine decarboxylase activity. Hakanson & Owman (1967) have recently shown that histamine formation in rat and mouse stomach may occur to a large extent in mucosal enterochromaffin-like cells. The chief activity of stomach histidine decarboxylase is probably concerned with gastric secretion (Kahlson & Rosengren, 1968).

Experiment 7

In rats given PTSH or α-HH, plus (^{14}C)-L-histidine, the amount of (^{14}C)-histamine excreted in the urine during a 22 hr period was only moderately reduced relative to control values (Table 7). NSD-1055 had an even weaker effect. This test is believed to provide a rough measure of total body histamine formation. Although data from rats may not be strictly comparable with those from mice, this relatively small inhibitory effect (compare data of Table 7 with data of Tables 5 and 6) is compatible with the existence of histidine decarboxylase-containing cells which are poorly permeable to the drugs tested. Johnston & Kahlson (1967) found α-HH to be a strong inhibitor of (^{14}C)-histamine excretion by rats given (^{14}C)-L-histidine. The doses used were high (200–400 mg/kg), however, and their experimental design differed from ours. It seems probable that large doses of any hydrazine derivative, even without contact with the histidine decarboxylase holoenzyme, can reduce histamine formation by trapping the loosely held coenzyme, pyridoxal phosphate.

The weak action of NSD-1055 in all *in vivo* tests may be due to steric factors ; the bromine atom may retard entry of the molecule into cells.

PTSH seems to be roughly as effective as α-HH in all tests. It is commercially available in large quantities, so its use may permit extension of comparable studies to larger animals.

Experiment 8

In endotoxin-pretreated mice, α-HH sharply reduced (^{14}C)-histamine content of liver, lung and spleen (Table 8), tissues which phagocytize endotoxin and subsequently undergo pronounced activation of histidine decarboxylase. Apparently α-HH reached the site of the activated histidine decarboxylase in liver and possibly also in lung and spleen ; however, the latter are small tissues, so it remains a possibility that they reflect events in liver. α-HH reduced (^{14}C)-histamine in stomach of endotoxin-pretreated mice (Table 8), but this effect was smaller than found in stomach of normal mice (Table 6) and was not statistically significant.

Experiment 9

This experiment was designed to test the ability of a histidine decarboxylase inhibitor, α-HH, to reach the locus of the histidine decarboxylase existing in the basal or non-induced state. The data of Table 9 show that the (^{14}C)-histamine

levels in α-HH treated mice were not significantly different from control levels. From these data, and those of Table 8, a tentative conclusion on the locus of inducible histidine decarboxylase in liver is suggested. Endotoxin-pretreatment probably permits α-HH to reach the site of liver histidine decarboxylase by increasing permeability of cells which contain the enzyme. Endotoxin is phagocytized by endothelial cells lining the sinusoids and subsequently activates histidine decarboxylase. Because histamine alters permeability of microvascular endothelial cells, the data could be readily explained if histidine decarboxylase were located in sinusoidal endothelial cells. No alternate interpretation of comparable simplicity is known to us.

R. W. S. is supported by U.S. Public Health Service Grant AM-10155. We are grateful to Mrs. C. Sokolski and Miss S. Vijino for excellent technical assistance. In part this work was supported by Clinical Research Center Grant MH-07292 and General Research Support Grant FR-05561. Statistical analyses were made by the Information Sciences Division (supported by Grants FR-00268 and MH-02740).

REFERENCES

HAKANSON, R. & OWMAN, C. (1967). Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffin-like cells of gastric mucosa. *Life Sci., Oxford*, **6**, 759–766.

JOHNSTON, M. & KAHLSON, G. (1967). Experiments on the inhibition of histamine formation in the rat. *Br. J. Pharmac. Chemother.*, **30**, 274–282.

KAHLSON, G. & ROSENGREN, E. (1968). New approaches to the physiology of histamine. *Physiol. Rev.*, **48**, 155–196.

LEVINE, R. J., SATO, T. L. & SJOERDSMA, A. (1965). Inhibition of histamine synthesis in the rat by α-hydrazino analog of histidine and 4-bromo-3-hydroxybenzyloxyamine. *Biochem. Pharmac.*, **14**, 139–149.

REILLY, M. A. & SCHAYER, R. W. (1968). Studies on the histidine–histamine relationship *in vivo*. *Br. J. Pharmac. Chemother.*, **32**, 567–574.

SCHAYER, R. W. (1953). Studies on histamine–metabolizing enzymes in intact animals. *J. biol. Chem.*, **203**, 787–793.

SCHAYER, R. W. (1959). Catabolism of physiological quantities of histamine *in vivo*. *Physiol. Rev.*, **39**, 116–126.

SCHAYER, R. W. (1960a). Relationship of induced histidine decarboxylase activity and histamine synthesis to shock from stress and from endotoxin. *Am. J. Physiol.*, **198**, 1187–1192.

SCHAYER, R. W. (1960b). Relationship of stress-induced histidine decarboxylase to circulatory homeostasis and shock. *Science, N.Y.*, **131**, 226–227.

SCHAYER, R. W. (1962a). Evidence that induced histamine is an intrinsic regulator of the microcirculatory system. *Am. J. Physiol.*, **202**, 66–72.

SCHAYER, R. W. (1962b). Role of induced histamine in tourniquet shock in mice. *Am. J. Physiol.*, **203**, 412–416.

SCHAYER, R. W. (1963a). Induced synthesis of histamine, microcirculatory regulation and the mechanism of action of the adrenal glucocorticoid hormones. *Prog. Allergy*, **7**, 187–212.

SCHAYER, R. W. (1963b). Histidine decarboxylase in mast cells. *Ann. N.Y. Acad. Sci.*, **103**, 164–178.

SCHAYER, R. W. (1964). A unified theory of glucocorticoid action. *Perspect. Biol. Med.*, **8**, 71–84.

SCHAYER, R. W. (1965). Histamine and circulatory homeostasis. *Fedn Proc.*, **24**, No. 6, 1295–1297.

SCHAYER, R. W. (1967). A unified theory of glucocorticoid action. II. *Perspect. Biol. Med.*, **10**, 409–418.

SCHAYER, R. W. (1968a). Histamine and a possible unity of autonomous microcirculatory dilator responses. *Med. Coll. Virginia Q.*, in the Press.

SCHAYER, R. W. (1968b). Determination of histidine decarboxylase activity. *Methods of Biochemical Analysis*, ed. Glick, D., vol. 16, pp. 273–291. New York: Interscience.

SCHAYER, R. W. & REILLY, M. (1968). Suppression of inflammation and histidine decarboxylase by protein synthesis inhibitors. *Am. J. Physiol.*, **215**, 472–476.

(*Received May* 24, 1968)