Br. J. Pharmac. (1971), **42**, 375–382.

Techniques for measuring histamine formation in mice

MARGARET A. REILLY AND R. W. SCHAYER

Research Center, Rockland State Hospital, Orangeburg, New York, USA

Summary

1. Formation of ^{14}C-histamine from ^{14}C-L-histidine was studied in mice using various inhibitors of histamine catabolism; these included aminoguanidine, pargyline and methylhistamine, inhibitors of diamine oxidase, monoamine oxidase, and the histamine-methylating enzyme, respectively.

2. Four general approaches were used: inhibiting diamine oxidase and the histamine-methylating enzyme and measuring ^{14}C-histamine in tissues or urine, or inhibiting diamine oxidase and monoamine oxidase and measuring ^{14}C-methylhistamine in tissues or urine. In some tests mice with normal concentrations of histidine decarboxylase were used; in others the enzyme was activated by pretreating mice with Freund's adjuvant.

3. Methylhistamine pretreatment increased ^{14}C-histamine in several tissues of mice but aminoguanidine had no significant effect; it was concluded that endogenously formed histamine is inactivated almost entirely by methylation.

4. There was no evidence of parallelism between the ability of tissues to form histamine and to inactivate endogenous histamine.

5. Effects of Freund's adjuvant on tissue concentrations of ^{14}C-histamine were tested in mice with or without inhibitors of histamine catabolism. Results were essentially parallel in both cases but higher in the former.

6. The method of choice is measurement of ^{14}C-histamine in tissues of mice given aminoguanidine and methylhistamine, followed by ^{14}C-L-histidine.

7. Other approaches listed above may be useful but require improvement, for example, a more specific assay for ^{14}C-methylhistamine and a stronger, longer-lasting inhibitor of histamine-methylation.

Introduction

We have recently shown that the major route of histamine catabolism in mice, methylation of the imidazole ring, can be blocked *in vivo* by large doses of methylhistamine (Reilly & Schayer, 1970). Since oxidation of histamine by the diamine oxidase pathway can be effectively inhibited by aminoguanidine treatment, and since no other major catabolic pathway is known, it now seems possible to study *in vivo* histamine formation in animals rendered incapable of extensive histamine destruction. To date, this has been possible only in female rats owing to their relatively low histamine-methylating ability.

In this paper we test the use of inhibitors of histamine catabolism for measuring *in vivo* histamine formation in mice; presumably the findings would be applicable

to other species which inactivate histamine mainly by methylation, for example, cats, dogs and humans (Schayer, 1959, 1966). Among the possible approaches are (1) inhibiting both diamine oxidase and the histamine-methylating enzyme, injecting ^{14}C-L-histidine, and assaying tissues or urine for ^{14}C-histamine, and (2) inhibiting both diamine oxidase and monoamine oxidase, injecting ^{14}C-L-histidine, and assaying tissues or urine for ^{14}C-methylhistamine ; the latter accumulates in tissues and urine of animals given monoamine oxidase inhibitors (Schayer, 1959, 1966 ; Reilly & Schayer, 1970). In some tests mice with normal tissue histidine decarboxylase activities were used ; in others the enzyme was activated by pretreating the mice with Freund's complete adjuvant.

Methods

Female albino CF-1 mice (19–25 g) from Carworth, Inc., New City, New York, were used. ^{14}C-L-Histidine, specific activity 58 mCi/mmol, purchased from Nuclear-Chicago, Des Plaines, Illinois, was purified before use to remove traces of ^{14}C-histamine (Schayer, 1968). In all experiments assays were made on the pooled tissues or urine of three mice.

Since the rate of histamine formation in stomach is affected by food consumption, mice were fasted for approximately 18 h prior to sacrifice in experiments in which stomach ^{14}C-histamine was assayed. Mice were also fasted during periods of urine collection.

Histidine decarboxylase was measured by incubating aliquots of tissue extracts with tracer amounts of ^{14}C-L-histidine, and assaying the ^{14}C-histamine formed by an isotope dilution method as benzenesulfonylhistamine (BSH) (Schayer, 1968) ; units of enzyme activity are expressed as c.p.m./100 mg BSH. For *in vivo* experiments, ^{14}C-histamine was determined as BSH by isotope dilution, and ^{14}C-methylhistamine by measuring chloroform-extractable ^{14}C (Snyder, Axelrod & Bauer, 1964) ; ^{14}C-L-histidine (free) was decarboxylated quantitatively by a bacterial enzyme and the resulting ^{14}C-histamine determined as BSH. Counting was done with a Beckman DPM-100 liquid scintillation system, background about 12 c.p.m. A minimum of 4,000 counts was obtained for all samples and background. Full details of our methods have been published (Reilly & Schayer, 1968a, 1970).

Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) was used as an activator of histidine decarboxylase because it maintains activity at high levels for several days and is not excessively toxic (Schayer, 1962, 1967). Methylhistamine was purchased from the Regis Chemical Co. (Chicago, I11.), and aminoguanidine sulphate from K & K Laboratories, Inc. (Plainview, N.Y.). We are indebted to the Merck Institute for Therapeutic Research (Rahway, N.J.) for cortisol acetate, to Abbott Laboratories (N. Chicago, I11.) for pargyline (Eutonyl) and to Hoffman-LaRoche, Inc. (Nutley, N.J.) for 1-isobutyl-2-isonicotinyl-hydrazine (IBINH).

Results

Experiments 1 and 2: effect of methylhistamine and pargyline on histamine formation

If methylhistamine and pargyline, inhibitors of histamine methylation and monoamine oxidase, respectively, are used to aid studies on histamine formation *in vivo*, they must not affect the conversion of histidine to histamine. Another commonly

used inhibitor of histamine catabolism, aminoguanidine, has no effect on mammalian histidine decarboxylase (Schayer, unpublished results; Levine & Watts, 1966; Radwan & West, 1968). Methylhistamine and pargyline were first tested on histidine decarboxylase activity of liver of mice injected 3 days earlier with Freund's adjuvant. Homogenates were incubated in triplicate with methylhistamine, 200 μg per ml, pargyline hydrochloride, 10 μg per ml, or with buffer. The drug concentration was that estimated to be in tissues 20–30 min after injection of an effective dose. Mean histidine decarboxylase activities (units ± standard error of

TABLE 1. *Effect of aminoguanidine and methylhistamine (MeH) on concentrations of ^{14}C-histamine in tissues of mice 10 min after intravenous injection of ^{14}C-L-histidine*

Tissue and treatment	I ^{14}C-Histamine (d.p.m./g)	II Total ^{14}C (d.p.m./g) ($\times 10^{-3}$)	III D.p.m. ^{14}C-histamine per 10^3 d.p.m. total ^{14}C	
Blood				
A. Control	263±18	792±32	0·33±0·02	(A–B, NS)
B. Aminoguan.	358±39	770±66	0·49±0·08	(A–C, $P<0·001$)
C. MeH	456±17	770±46	0·60±0·03	(A–D, $P<0·01$)
D. Aminoguan. & MeH.	526±50	760±34	0·70±0·09	(B–D, NS) (C–D, NS)
Liver				
A. Control	1,000±87	6,210±292	0·16±0·01	(A–B, NS)
B. Aminoguan.	1,180±185	6,260±198	0·19±0·03	(A–C, $P<0·001$)
C. MeH	1,590±108	6,400±277	0·25±0·01	(A–D, $P<0·001$)
D. Aminoguan. & MeH.	1,940±42	6,050±120	0·32±0·01	(B–D, $P<0·01$) (C–D, $P<0·01$)
Intestine				
A. Control	1,710±235	1,010±93	1·78±0·32	(All NS)
B. Aminoguan.	1,610±212	906±99	1·82±0·21	
C. MeH	2,110±229	995±126	2·16±0·16	
D. Aminoguan. & MeH.	2,280±152	1,300±100	1·81±0·21	
Heart				
A. Control	273±34	1,080±62	0·25±0·01	(A–B, NS)
B. Aminoguan.	285±23	1,110±59	0·26±0·03	(A–C, $P<0·01$)
C. MeH	1,520±194	1,240±82	1·25±0·19	(A–D, $P<0·025$)
D. Aminoguan. & MeH.	3,350±1,040	1,270±67	2·54±0·67	(B–D, $P<0·025$) (ED, NS)
Lymph nodes				
A. Control	400±46	1,190±96	0·35±0·06	(A–D, $P<0·1$)
B. Aminoguan.	568±97	1,250±120	0·46±0·07	(all others
C. MeH	740±71	1,520±46	0·49±0·04	NS)
D. Aminoguan. & MeH.	855±154	1,400±118	0·62±0·01	
Stomach				
A. Control	47,800±4,720	2,440±91	19·9±2·6	(A–C, $P<0·1$)
B. Aminoguan.	50,400±1,980	2,330±115	23·0±1·9	(A–D, $P<0·1$)
C. MeH	70,000±6,930	2,600±132	26·9±2·3	(all others
D. Aminoguan. & MeH.	70,900±4,400	2,630±88	27.2±2·2	NS)
Muscle				
A. Control	738±54	1,040±66	0·72±0·05	(All NS)
B. Aminoguan.	805±112	1,000±51	0·81±0·02	
C. MeH	774±43	1,070±39	0·73±0·05	
D. Aminoguan. & MeH.	856±72	1,090±27	0·79±0·08	
Kidney				
A. Control	1,120±88	1,880±25	0·60±0·05	(A–B, NS)
B. Aminoguan.	1,390±163	2,120±127	0·65±0·05	(A–C, $P<0·001$)
C. MeH	2.390±250	2,300±140	1·03±0·06	(A–D, $P<0·01$)
D. Aminoguan. & MeH.	2,870±405	2,380±102	1·20±0·15	(B–D, $P<0·01$) (C–D, NS)

Expt. 3: Saline, aminoguanidine 200 μg, methylhistamine 6 mg, or aminoguanidine, 200 μg, plus methylhistamine, 6 mg i.p. Twenty minutes later each mouse was injected intravenously with ^{14}C-L-histidine, 19 μCi, killed 10 min later, and pooled tissues of three mice were assayed. Values are means ± standard error of mean (S.E.M.) of five assays per group (four assays for blood A and C, liver B and D, heart A and B and lymph node A). Statistical analysis is for data in column III.

the mean) were for controls $154 \pm 3{\cdot}4$, for methylhistamine samples 189 ± 14, and for pargyline samples $169 \pm 8{\cdot}9$; differences between control and test groups are not significant.

Methylhistamine and pargyline were next tested for effect on histamine formation *in vivo*. Stomach was selected for assays because of its high histamine-forming capacity (Reilly & Schayer, 1968a, b). Mice were injected intraperitoneally with saline, methylhistamine 6 mg, or pargyline hydrochloride, 200 μg. Approximately 20 min later each mouse was injected intravenously with 1·9 μCi ^{14}C-L-histidine, sacrificed 5 min later, and stomachs assayed for ^{14}C-histamine and total ^{14}C. Results of five assays per group, expressed as d.p.m. ^{14}C-histamine/g stomach, were for controls $3{,}610 \pm 161$, for the methylhistamine group $3{,}830 \pm 399$, and for the pargyline group $3{,}630 \pm 255$; results expressed as d.p.m. ^{14}C-Histamine/10^3 d.p.m. total ^{14}C, were for controls $21 \pm 0{\cdot}9$, for the methylhistamine group $24 \pm 2{\cdot}1$, and for the pargyline group $22 \pm 2{\cdot}0$; differences between control and test groups are not significant.

Experiment 3: effect of aminoguanidine and methylhistamine on ^{14}C-histamine in tissues of mice injected with ^{14}C-L-histidine

This experiment tests the ability of two inhibitors, separately and in combination, to increase ^{14}C-histamine concentrations in tissues of normal mice. The data will also provide information on catabolic pathways for endogenous ^{14}C-histamine in the various tissues. Mice were injected with saline, aminoguanidine or methylhistamine. Approximately 20 min later each mouse was injected with ^{14}C-L-histidine, sacrificed 10 min later, and tissues assayed. Table 1 shows that aminoguanidine had no significant effect on the ^{14}C-histamine concentrations (column I) in any tissue, that methylhistamine produced a definite increase in ^{14}C-histamine in all tested tissues except intestine, and that ^{14}C-histamine concentrations were highest when both inhibitors were given. The drugs had relatively little effect on total ^{14}C (column II).

Experiments 4 and 5: urinary ^{14}C-histamine as indicator of in vivo *histamine formation in mice*

Since in experiment 3 aminoguanidine, given alone, had little if any effect on ^{14}C-histamine concentrations, in experiments 4–7, to reduce the number of samples, all mice were given aminoguanidine and the effects of other factors were tested.

TABLE 2. *Effect of Freund's adjuvant and methylhistamine on ^{14}C-histamine and total ^{14}C in urine of mice injected with ^{14}C-L-histidine*

Treatment	I ^{14}C-histamine (d.p.m. in urine)	II Total ^{14}C (d.p.m. in urine ($\times 10^{-3}$)	III D.p.m. ^{14}C-histamine per 10^3 d.p.m. total ^{14}C	
A. Saline	10,700±1,750	2,780±83	3·80±0·53	(A–B, $P<0{\cdot}01$)
B. Methylhistamine	17,200±1,470	2,830±225	6·08±0·28	(A–C, $P<0{\cdot}05$)
C. Freund	10,400±1,830	1,950±283	5·30±0·17	(B–D, $P<0{\cdot}01$)
D. Freund and methylhistamine	21,900±4,790	1,940±406	11·50±1·30	(C–D, $P<0{\cdot}01$)

Expt. 5: Freund's complete adjuvant 0·25 ml, or saline, was injected intraperitoneally on day −3. Day 0 all mice were injected intraperitoneally with aminoguanidine, 200 μg, and with either saline, or methylhistamine at 0 and at 2 h (6 mg first dose; 3 mg second). ^{14}C-L-Histidine, 5 μCi, was injected subcutaneously at 30 minutes. Urine of three mice per cage was collected for 19 hours. Values are means±S.E.M. of four assays per group. Statistical analysis is for data in column III.

Mice were pretreated with aminoguanidine, 200 μg i.p. (controls), or with aminoguanidine plus methylhistamine, 6 mg i.p. After 20 min each mouse was injected subcutaneously with ^{14}C-L-histidine, 0·5 μCi, and placed in a urine collection cage, three mice per cage. A second injection of inhibitors, one-half of the initial dose, was given 100 min after ^{14}C-L-histidine. Urine was collected after 19 h and assayed. Results of five assays per group, expressed as ^{14}C-histamine in the entire urine sample were for controls 112 ± 18, and for the methylhistamine group 228 ± 43; results expressed as d.p.m. ^{14}C-Histamine/10^3 d.p.m. total ^{14}C, were for controls $8{\cdot}9 \pm 0{\cdot}9$, and for the methylhistamine group $20 \pm 3{\cdot}6$. Inhibition of histamine methylation caused a significant increase in urinary ^{14}C-histamine ($P < {\cdot}025$).

In another experiment of this type, the effect of Freund's adjuvant was also tested (Table 2); it produced significant increases in urinary ^{14}C-histamine in both saline treated and methylhistamine treated mice.

Experiment 6: urinary ^{14}C-methylhistamine as indicator of in vivo *histamine formation*

In mice given aminoguanidine plus pargyline, a monoamine oxidase inhibitor, injection with ^{14}C-histamine leads to accumulation of ^{14}C-methylhistamine in the tissues (Reilly & Schayer, 1970). The purpose of our experiment was to test the feasibility of using urinary ^{14}C-methylhistamine concentrations to measure rates of *in vivo* histamine formation. The effect of pargyline and Freund's adjuvant were tested (Table 3). Both substances increased ^{14}C-methylhistamine; highest concentrations were found in urine of mice receiving both.

Experiment 7: tissue ^{14}C-methylhistamine as indicator of in vivo *histamine formation*

Mice were pretreated with aminoguanidine or with aminoguanidine plus IBINH; the latter is a monoamine oxidase inhibitor. After 20 min each mouse was injected intravenously with ^{14}C-L-histidine, 10 μCi, sacrificed 5 min later and livers assayed (three assays per group). ^{14}C-Methylhistamine concentrations expressed as d.p.m./g liver, were for controls $1{,}520 \pm 38$, and for test mice $1{,}660 \pm 101$; expressed as d.p.m. ^{14}C-Methylhistamine/10^3 d.p.m. total ^{14}C, values were $0{\cdot}47 \pm 0{\cdot}035$ and $0{\cdot}48 \pm 0{\cdot}025$, respectively. There is no significant difference between groups.

TABLE 3. *Effect of Freund's adjuvant and pargyline on ^{14}C-methylhistamine and total ^{14} in urine of mice injected with ^{14}C-L-histidine*

Treatment	I ^{14}C-Methylhistamine (d.p.m. in urine)	II Total ^{14}C (d.p.m. in urine) ($\times 10^{-3}$)	III D.p.m. ^{14}C-methylhistamine per 10^3 d.p.m. total ^{14}C	
A. Saline	$7{,}240 \pm 708$	$3{,}670 \pm 296$	$1{\cdot}96 \pm 0{\cdot}04$	(A–B, $P < 0{\cdot}001$)
B. Pargyline	$13{,}200 \pm 2{,}560$	$3{,}380 \pm 393$	$3{\cdot}81 \pm 0{\cdot}30$	(A–C, $P < 0{\cdot}01$)
C. Freund	$12{,}000 \pm 1{,}620$	$3{,}250 \pm 192$	$3{\cdot}67 \pm 0{\cdot}32$	(B–D, $P < 0{\cdot}001$)
D. Freund and pargyline	$31{,}200 \pm 4{,}700$	$3{,}440 \pm 568$	$9{\cdot}18 \pm 0{\cdot}30$	(C–D, $P < 0{\cdot}001$)

Expt. 6: Freund's complete adjuvant, 0·25 ml, or saline, was injected intraperitoneally on day −3. Day 0 all mice were injected intraperitoneally with aminoguanidine, 200 μg, and with either saline or pargyline at 0 and again at 3 h (200 μg first dose; 100 μg second). ^{14}C-L-Histidine, 5 μCi, was injected subcutaneously at 30 minutes. Urine from three mice per cage was collected for 19 hours. Values are means $\pm$ S.E.M. of four assays per group. Statistical analysis is for data in column III.

Experiments 8 and 9: effect of Freund's adjuvant on formation of ^{14}C-histamine in vivo

Two tests were made on effects of Freund's adjuvant, an activator of histidine decarboxylase in certain tissues; in the first no inhibitors of histamine catabolism were used; in the second, both major routes of histamine catabolism were inhibited by pretreatment with aminoguanidine plus methylhistamine (Table 4). In both experiments, test mice had abnormally high concentrations of ^{14}C-histamine in lymph node, spleen and liver, but not in thymus. The ratio of ^{14}C-L-histidine to total ^{14}C was reasonably constant for the different groups of any tested tissue.

Discussion

Since methylhistamine and pargyline, inhibitors of histamine methylation and of monoamine oxidase, respectively, do not inhibit histidine decarboxylase (experiments 1 and 2) they may be used in studies of histamine formation *in vivo*.

Experiments 4–7 will not be discussed in detail; approaches used in 4 and 5 are promising but for improvement require a long-acting inhibitor of histamine methylation. Inhibitory effects of methylhistamine are largely lost within 5 h (unpublished results). For experiments 6 and 7 a more specific assay for ^{14}C-methylhistamine is required for the chloroform-extraction method undoubtedly also measures some of the huge pool of total ^{14}C.

TABLE 4. *Effect of Freund's adjuvant on histamine formation in mice. ^{14}C-Histamine and total ^{14}C in tissues of mice 4 min after intravenous injection of ^{14}C-L-histidine*

Tissue and treatment	^{14}C-Histamine (d.p.m./g tissue) Expt. 8	Expt. 9	Total ^{14}C (d.p.m./g tissue) ($\times 10^{-3}$) Expt. 8	Expt. 9	^{14}C-Histamine (d.p.m./10^3 d.p.m. total ^{14}C) Expt. 8	Expt. 9
Lymph node						
Control	113±36	423±43	593±119	64±43	0·22±0·09	0·44±0·04
Freund	867±85 ($P<0·001$)	2,120±270 ($P<0·001$)	626±12 (NS)	910±31 (NS)	1·38±0·11 ($P<0·001$)	2·35±0·34 ($P<0·01$)
Spleen						
Control	250±53	582±42	457±14	545±26	0·54±0·11	1·07±0·07
Freund	3,480±445 ($P<0·001$)	3,860±267 ($P<0·001$)	642±21 ($P<0·001$)	689±33 ($P<0·025$)	5·38±0·60 ($P<0·001$)	5·63±0·47 ($P<0·001$)
Thymus						
Control	448±119	729±47	394±6	430±13	1·14±0·29	1·69±0·08
Freund	497±151 (NS)	884±95 (NS)	465±22 ($P<0·025$)	406±20 (NS)	1·09±0·35 (NS)	2·17±0·19 (NS)
Liver						
Control	323±38	1,030±128	3,210±53	4,700±182	0·10±0·01	0·22±0·02
Freund	967±152 ($P<0·01$)	3,340±259 ($P<0·001$)	3,320±175 (NS)	4,460±263 (NS)	0·29±0·03 ($P<0·01$)	0·76±0·09 ($P<0·01$)
Blood						
Control	Not	374±16	—	749±23	—	0·50±0·01
Freund	Assayed	514±35 ($P<0·025$)	—	760±50 (NS)	—	0·68±0·05 ($P<0·025$)

Expt. 8. Freund's complete adjuvant 0·25 ml, or saline, injected intraperitoneally 3 days before intravenous injection of ^{14}C-L-histidine, 10 μCi. No inhibitors used.

Expt. 9. Same, except all mice were injected intraperitoneally with aminoguanidine, 200 μg, and methylhistamine 6 mg, 20–30 min before ^{14}C-L-histidine. Values are means±S.E.M. of four assays per group. All mice were pretreated with inhibitors of histamine catabolism. In expt. 9 free ^{14}C-L-histidine was assayed in some tissues; its concentration, expressed as % of total ^{14}C was for lymph node, control 77±1·9 and Freund 78±3·3; for spleen, control 77±3·5 and Freund 77±2·6; for liver, control 80±0·9 and Freund 86±6·2. There is no significant difference between groups for any tissue.

Experiment 3 (Table 1), in which ^{14}C-histamine is measured by a specific method, shows methylhistamine to be a valuable aid in short-term studies on histamine formation; methylhistamine increased ^{14}C-histamine levels in all tissues (compare A–C and B–D, column I).

Assuming that effects of aminoguanidine and methylhistamine are due to inhibition of diamine oxidase and histamine-methylation, respectively, the following conclusions on catabolism of endogenous histamine in the mouse are suggested:

(1) Diamine oxidase probably has little if any role in local catabolism; the small observed effects of aminoguanidine could arise from endogenous ^{14}C-histamine entering the blood, where, being equivalent to intravenously injected histamine, it is catalysed in part by diamine oxidase (Reilly & Schayer, 1970).

(2) Since intestine shows strong diamine oxidase activity *in vitro*, and also when tested with ^{14}C-histamine given by mouth or by injection, the main function of intestinal diamine oxidase is probably destruction of diamines of dietary or bacterial origin.

(3) If histamine-forming ability of the tissues is assumed to relate to values of group D, column I (maximum protection of newly formed histamine from destruction) stomach is highest, heart, intestine and kidney are intermediate, while muscle, lymph node and liver are low. However, in ability to inactivate endogenous histamine, as adjudged by the effect of methylhistamine on ^{14}C-histamine concentrations, stomach is low or negative (negative results were obtained in a repeat test on stomach), intestine is negative, kidney moderate, heart highly active, muscle negative, lymph node low and liver active. Thus there is no discernible parallelism between the *in vivo* rates of histamine formation and destruction in mouse tissues. We had previously shown a lack of parallelism in histamine formation and catabolism of intravenously injected ^{14}C-histamine (Reilly & Schayer, 1970). Brain data, reported elsewhere (Schayer & Reilly, 1970) show a relatively low rate of histamine formation, but active catabolism by methylation.

Despite the obvious difficulties involved, interpretation of the data of Table 1 must be attempted since an understanding of the catabolism of endogenous histamine has been the ultimate goal of virtually all past work on enzymatic destruction of histamine *in vitro* and *in vivo*. Further, there appears to be no other available approach to this problem; assay of tissues or urine for non-isotopic histamine and its catabolites is complicated by the presence of histamine formed in the intestine by bacteria or from the diet, and because certain histamine catabolites, imidazoleacetic acid and its conjugation products, can be formed from L-histidine by a pathway not involving histamine.

In experiments 8 and 9 (Table 4) inhibitors of histamine catabolism are shown to be useful in measuring enhanced histamine formation induced by an irritant stimulus, Freund's adjuvant. Although experiments 8 and 9 were done at different times and are therefore not strictly comparable, values of ^{14}C-histamine were higher in all tissues of mice receiving inhibitors.

In both experiments ^{14}C-histamine concentrations in mice treated with Freund's adjuvant are abnormally high in liver, spleen and lymph node but not in thymus; blood values are slightly elevated in test mice. *In vitro* data (Schayer, 1962, 1967) show marked activation of histidine decarboxylase in spleen and lymph node of mice treated with Freund's adjuvant, but almost no effect in thymus.

These findings support our earlier conclusions (Reilly & Schayer, 1968a, b) that tissue histamine is mainly formed locally from L-histidine, that the isotope dilution assay for histidine decarboxylase is a reliable indicator of *in vivo* histamine formation, and that blood histamine concentrations do not adequately reflect changes in histamine production.

R.W.S. is supported by U.S. Public Health Service Grant AM 10155. We are indebted to Mrs. Christine Sokolski and Mrs. Mary Castro for excellent technical assistance. This work was supported in part by General Research Support Grant FR 05651. Statistical analyses were made by the Information Sciences Division (Supported by Grant MH 14934). Rockland State Hospital is an institution of the New York State Department of Mental Hygiene.

REFERENCES

LEVINE, R. J. & WATTS, D. E. (1966). A sensitive and specific assay for histidine decarboxylase. activity. *Biochem. Pharmac.*, **15**, 841–949.

RADWAN, A. G. & WEST, G. B. (1968). The effect of aminoguanidine, histamine, chlorpromazine and antibacterial agents on histidine decarboxylase in the stomach of the rat. *Br. J. Pharmac. Chemother.*, **33**, 177–183.

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

REILLY, M. A. & SCHAYER, R. W. (1968b). Further studies on the histidine–histamine relationship *in vivo*. Effects of endotoxin and of histidine decarboxylase inhibitors. *Br. J. Pharmac.*, **34**, 551–563.

REILLY, M. A. & SCHAYER, R. W. (1970). *In vivo* studies on histamine catabolism and its inhibition *Br. J. Pharmac.*, **38**, 478–489.

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

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

SCHAYER, R. W. (1966). Catabolism of histamine in vivo. *Handbook of Experimental Pharmacology*, ed. Rocha e Silva, M., Vol. **18**, part 1, pp. 672–683. Berlin: Springer.

SCHAYER, R. W. (1967). Histamine and stress responses of lymphoid tissues. *Endocrinology*, **81**, 1357–1361.

SCHAYER, R. W. (1968). 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. A. (1970). *In vivo* formation and catabolism of [^{14}C] histamine in mouse brain. *J. Neurochem.*, **17**, 1649–1655.

SNYDER, S. H., AXLEROD, J. & BAUER, H. (1964). The fate of C^{14}-histamine in animal tissues. *J. Pharmac. exp. Ther.*, **144**, 373–379.

(*Received November* 20, 1970)