Br. J. Pharmac. (1971), **43**, 349–358.

Further studies on histamine catabolism *in vivo*

MARGARET A. REILLY AND R. W. SCHAYER

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

Summary

1. Histamine catabolism *in vivo* was studied in mice and rats; tissues from animals killed 2·5 min after intravenous injection of ^{14}C-histamine were assayed for ^{14}C-histamine and total ^{14}C. Aminoguanidine, a diamine oxidase inhibitor, and methylhistamine, an inhibitor of the histamine methylating enzyme, were used to evaluate the roles of these enzymes in individual tissues.

2. Mouse liver and lung appeared to catabolize exogenous histamine rapidly and completely by methylation. *In vivo* histamine methylating activity was also found in mouse muscle, heart, kidney and lymph node, but not in stomach or intestine.

3. In rats ^{14}C-histamine was inactivated more slowly than in mice. Catabolism was most rapid in intestine where both diamine oxidase and methylating activities were found. Liver had only diamine oxidase activity. Heart showed no catabolism but had extraordinary ability to extract ^{14}C-histamine from blood.

4. The *in vivo* evaluation of histamine methylation by individual mouse and rat tissues agrees closely with *in vitro* findings by another laboratory.

5. Aminoguanidine reduced uptake of blood ^{14}C-histamine by some tissues presumably by occupying sites where histamine is normally bound.

6. The marked differences between tissues of mice and rats in destroying ^{14}C-histamine support earlier evidence that there is no apparent relationship between histamine catabolism and function.

7. Urine from mice with both major catabolic pathways blocked showed evidence of an abnormal excretory product of ^{14}C-histamine. Paper chromatograms of urine of male and female mice showed no evidence of a sex difference in catabolism of injected ^{14}C-histamine.

8. A procedure which may permit evaluating contributions of individual tissues to histamine formation *in vivo* is presented.

9. Pretreatment of mice with antihistamines, or with a histamine analogue, betahistine, did not significantly affect the rate of *in vivo* ^{14}C-histamine inactivation.

Introduction

We previously published data on the catabolism of injected ^{14}C-histamine in mice through the two major pathways, methylation, and oxidation catalysed by diamine oxidase, and reported methylhistamine to be the first effective *in vivo* inhibitor of histamine methylation (Reilly & Schayer, 1970). In this paper we have used inhibitors of histamine catabolism in an attempt to evaluate the contributions of individual tissues of rats and mice to *in vivo* formation and destruction of histamine.

Methods

Albino CF-1 mice, female and male (17–21 g) and female CFN rats (approximately 100 g) from Carworth, Inc., New City, New York, were used. ^{14}C-Histamine, specific activity 54 mCi/mmol was purchased from Amersham/Searle, Des Plaines, Illinois.

For intravenous administration of ^{14}C-histamine, rats were anaesthetized with pentobarbitone and injected in the femoral vein. Mice, unanaesthetized, were injected in a tail vein.

TABLE 1. *^{14}C-Histamine and total ^{14}C in tissues of mice 2·5 min after intravenous injection of ^{14}C-histamine. Experiment* 1, *effect of antihistamines chlorpheniramine and pyrilamine. Experiment* 2, *effects of betahistine and of non-isotopic histamine*

Tissue and treatment	1 ^{14}C-Histamine (d.p.m./g)	2 Total ^{14}C (d.p.m./g)	3 ^{14}C-Histamine (% of total ^{14}C)
Liver			
A. Control 1	984± 16	145,000± 5,360	0·68±0·033
B. Chlorphenir.	1,010± 35	98,500± 4,450*	1·03±0·045*
C. Pyrilamine	942± 34	134,000± 4,380	0·70±0·017
D. Control 2	973± 82	179,000±11,900	0·54±0·036
E. β-histine	1,230± 115	179,000± 7,490	0·68±0·046
F. Histamine	5,070±1,080‡	161,000± 4,090	3·12±0·578†
Intestine			
A. Control 1	3,370± 501	93,500± 3,200	3·59±0·49
B. Chlorphenir.	3,060± 356	85,100± 3,710	3·56±0·30
C. Pyrilamine	2,590± 258	86,800± 3,920	2·99±0·24
D. Control 2	3,480± 311	112,000± 2,840	3·14±0·33
E. β-histine	3,250± 324	112,000± 5,520	2·90±0·15
F. Histamine	9,130±1,570‡	113,000± 2,270	8·03±1·26†
Muscle			
A. Control 1	1,220± 45	19,900± 1,280	6·25±0·59
B. Chlorphenir.	1,370± 40§	21,900± 470	6·29±0·30
C. Pyrilamine	1,180± 75	20,400± 542	5·81±0·45
D. Control 2	1,750± 297	23,500± 1,380	7·51±1·31
E. β-histine	1,640± 36	25,900± 953	6·36±0·33
F. Histamine	11,500±1,310*	26,400± 2,560	43·3 ±2·26*
Lung			
A. Control 1	3,820± 426	55,600± 3,900	6·89±0·66
B. Chlorphenir.	3,830± 87	50,800± 865	7·55±0·24
C. Pyrilamine	2,580± 207§	58,600± 3,740	4·41±0·25‡
D. Control 2	3,040± 474	51,800± 2,010	5·81±0·74
E. β-histine	3,100± 495	51,000± 6,090	5·98±0·36
F. Histamine	17,200±1,390*	42,800± 2,070§	40·3 ±2·95*
Blood			
A. Control 1	11,500±2,630	40,300± 3,300	27·7 ±3·73
B. Chlorphenir.	11,100± 562	40,500± 829	27·3 ±1·51
C. Pyrilamine	11,500±1,370	41,300± 1,730	27·7 ±2·16
D. Control 2	9,860± 488	45,900± 969	21·5 ±1·41
E. β-histine	9,450± 882	46,600± 2,600	20·2 ±0·989
F. Histamine	21,600± 937*	49,100± 2,100	44·2 ±1·34*

Expt. 1, saline, chlorpheniramine 200 μg, or pyrilamine 200 μg, injected intraperitoneally; Expt. 2, saline, betahistine 200 μg, or histamine dihydrochloride, 200 μg base, injected subcutaneously; approximately 20 min later all mice were injected intravenously with ^{14}C-histamine, 0·5 μCi. Values are means ±S.E.M. of four assays per group. * Differs from controls, $P<0·001$; † $P<0·01$; ‡ $P<0·025$; § $P<0·05$; others NS.

For tissue analysis, the pooled tissues of three mice, or of two rats, were homogenized in cold 0·4 M perchloric acid. Aliquots were assayed for total ^{14}C by direct count, and for ^{14}C-histamine by isotope dilution as benzenesulphonylhistamine as previously described (Reilly & Schayer, 1968; Schayer, 1968).

For examination of urine by paper chromatography, mice were injected intraperitoneally with various inhibitors, and approximately 20 min later injected subcutaneously with ^{14}C-histamine, 2·0 μCi. The combined urine was collected by gentle squeezing of the mice hourly for 3 hours.

Aminoguanidine sulphate, chlorpheniramine maleate and pyrilamine maleate were purchased from K & K Laboratories, Inc., and 1-methyl-4 (β-aminoethyl) imidazole (referred to in this paper as methylhistamine) from the Regis Chemical Co. Betahistine beta-histamine analogue with a weak histamine-like pharmacological action (Hunt & Fosbinder, 1942) was kindly provided by Unimed, Inc., Morristown, New Jersey; it is β-(2-pyridyl) ethylmethylamine hydrochloride.

Results

Effect of antihistamines, betahistine and non-isotopic histamine on catabolism of ^{14}C-histamine in female mice, in vivo

Cohn & Wynn (1968) reported that a wide variety of antihistamines competed for the active sites of histamine-catabolizing enzymes and inhibited partially purified histamine N-methyl transferase and diamine oxidase. In experiment 1, two relatively specific antihistamines, chlorpheniramine and pyrilamine, were tested; in experiment 2, betahistine, a histamine analogue, and non-isotopic histamine were tested (Table 1). Chlorpheniramine reduced total ^{14}C of lung thereby increasing ^{14}C-histamine (% of total ^{14}C), while pyrilamine reduced ^{14}C-histamine in lung; otherwise there were no significant effects. Betahistine had no discernible influence on ^{14}C-histamine metabolism, but in mice pretreated with non-isotopic histamine. ^{14}C-histamine concentrations were strongly increased in all tissues.

Catabolism of ^{14}C-histamine in female mice; effects of aminoguanidine and methylhistamine

Mice were pretreated with aminoguanidine, methylhistamine, or both, and then injected with ^{14}C-histamine; experimental procedures and findings are shown in Table 2. (To reduce the number of assays, heart, lymph node and intestine, for which Saline *vs* Aminoguanidine data had been published (Reilly & Schayer, 1970) were examined only for effects of methylhistamine in the presence of aminoguanidine; liver, which had been fully studied, was omitted.) Aminoguanidine had relatively minor effects in tissues other than intestine while methylhistamine produced a strong rise in ^{14}C-histamine concentrations in all. Methylhistamine also increased total ^{14}C in kidney. With both inhibitors, ^{14}C-histamine levels did not rise above 70% of total ^{14}C.

Catabolism of ^{14}C-histamine in female rats; effects of aminoguanidine and methylhistamine

In rats the major pathway of histamine catabolism involves diamine oxidase; studies of urinary excretory products of ^{14}C-histamine show that methylation does

TABLE 2. *^{14}C-Histamine and total ^{14}C in tissues of mice 2·5 min after intravenous injection of ^{14}C-histamine; effects of aminoguanidine and methylhistamine (MeH)*

Tissue and treatment	1 ^{14}C-Histamine (d.p.m./g)	2 Total ^{14}C (d.p.m./g)	3 ^{14}C-Histamine (% of total ^{14}C)*	4 ^{14}C-Histamine (% of blood ^{14}C-histamine)	P†
Blood					
A. Saline	19,100± 1,240	98,200± 4,160	19·5±1·36	—	
B. Aminoguanidine	41,400± 3,010	102,000± 1,660	40·8±3·09	—	
C. MeH	58,300± 1,870	108,000± 3,230	54·3±2·04	—	
D. Aminoguan. & MeH	75,900± 7,670	125,000± 7,720	60·3±3·46	—	
Lung					
A. Saline	6,840± 163	166,000± 3,710	4·1±0·17	37± 3·6	A–B, NS
B. Aminoguanidine	12,500± 968	149,000± 9,140	8·4±0·47	31± 2·5	A–C, $P<0·001$
C. MeH	83,600± 5,720	151,000± 8,190	55·2±1·48	143± 7·2	B–D, $P<0·001$
D. Aminoguan. & MeH	86,900± 5,250	155,000± 4,800	55·9±3·18	117± 7·7	C–D, $P<0·05$
Muscle					
A. Saline	5,690± 481	57,600± 2,280	9·8±0·48	32± 3·8	A–B, NS
B. Aminoguanidine	10,100± 884	56,800± 3,170	17·6±0·72	25± 2·7	A–C, $P<0·001$
C. MeH	36,300± 1,380	56,800± 3,210	64·6±3·31	63± 2·9	B–D, $P<0·001$
D. Aminoguan. & MeH	39,000± 3,630	59,900± 4,650	65·0±2·36	52± 4·7	C–D, $P<0·1$
Kidney					
A. Saline	44,900± 5,160	304,000±13,000	14·6±1·18	241±23·3	A–B, $P<0·05$
B. Aminoguanidine	72,300± 1,820	285,000± 6,750	25·5±1·01	177± 9·6	A–C, $P<0·001$
C. MeH	368,000±41,800	558,000±49,900	65·5±2·70	627±58·2	B–D, $P<0·001$
D. Aminoguan. & MeH	413,000±31,600	584,000±55,900	71·1±1·44	549±18·6	C–D, NS
Stomach					
A. Saline	8,570± 689	46,300± 2,190	18·5±1·09	49± 3·1	A–B, $P<0·01$
B. Aminoguanidine	13,200± 889	42,900± 1,980	31·0±2·09	32± 2·1	A–C, NS
C. MeH	27,800± 2,030	45,300± 3,100	61·8±3·48	48± 2·9	B–D, $P<0·05$
D. Aminoguan. & MeH	30,400± 2,160	45,100± 2,370	67·1±2·03	41± 2·8	C–D, NS
Heart					
B. Aminoguanidine	37,200± 4,750	340,000±20,200	10·8±1·05	89± 8·4	
D. Aminoguan. & MeH	225,000±10,800	332,000±18,600	67·8±0·60	304±23·9	B–D, $P<0·001$
Lymph node					
B. Aminoguanidine	17,500± 1,510	104,000± 3,620	16·8±1·50	42± 0·7	
D. Aminoguan. & MeH	62,300± 3,210	98,200± 3,720	63·6±2·89	85± 7·5	B–D, $P<0·001$
Intestine					
B. Aminoguanidine	50,200± 3,330	146,000± 5,000	34·3±1·85	123± 7·9	
D. Aminoguan. & MeH	93,600± 2,810	142,000± 3,300	65·9±1·00	127± 8·4	B–D, NS

Saline, aminoguanidine sulphate, 200 μg base, methylhistamine, 6 mg base, or aminoguanidine plus methylhistamine, injected intraperitoneally approximately 20 min before injection of ^{14}C-histamine, 1·0 μCi. Values are means ± S.E.M. of five assays per group (except lung, group A, three assays and blood group A, four assays). Statistical analyses: Column 1, for all tissues A–B, A–C and B–D, $P<0·01$; C–D, NS. Column 2, all group differences for all tissues NS (exceptions, kidney A–C and B–D, $P<0·01$, and blood B–D, $P<0·025$). Column 3, for all tissues A–B, A–C and B–D, $P<0·001$; C–D NS (exception, blood B–D, $P<0·01$). * Individual values calculated, then mean and S.E.M. determined; therefore values of column 3 may not be exactly equal to 1/2. † Refers to data of column 4.

TABLE 3. *^{14}C-Histamine and total ^{14}C in tissues of rats 2·5 min after intravenous injection of ^{14}C-histamine; effects of aminoguanidine and methylhistamine (MeH)*

Tissue and treatment	1 ^{14}C-Histamine (d.p.m./g)	2 Total ^{14}C (d.p.m./g)	3 ^{14}C-Histamine (% of total ^{14}C)*	4 ^{14}C-Histamine (% of blood ^{14}C-histamine)	*P*†
Blood					
A. Saline	12,100± 933	34,800± 1,130	34·7±1·62		
B. Aminoguanidine	20,600± 1,140	31,300± 1,340	65·6±1·52		
C. MeH	19,200± 1,510	39,600± 1,440	48·1±2·36		
D. Aminoguan. & MeH	26,800± 1,240	36,800± 620	72·6±2·77		
Liver					
A. Saline	23,300± 1,580	155,000± 3,530	15·0±1·02	196± 16·8	A–B, $P<0·1$
B. Aminoguanidine	50,300± 3,830	85,200± 3,070	58·7±2·83	245± 17·5	A–C, $P<0·05$
C. MeH	47,800± 3,370	113,000± 5,650	43·1±4·64	251± 10·7	B–D, NS
D. Aminoguan. & MeH	62,900± 2,560	94,800± 3,050	66·4±2·00	236± 5·2	C–D, NS
Intestine					
A. Saline	4,470± 274	143,000± 5,130	3·1±0·20	38± 3·9	A–B, $P<0·001$
B. Aminoguanidine	14,000± 770	112,000±12,900	12·8±0·89	68± 1·6	A–C, $P<0·001$
C. MeH	23,100± 3,700	165,000± 4,180	13·9±1·98	126± 13·8	B–D, $P<0·001$
D. Aminoguan. & MeH	49,300± 5,610	145,000± 6,960	34·0±3·28	182± 16·3	C–D, $P<0·05$
Heart					
A. Saline	136,000±14,500	189,000±20,200	71·6±0·75	1120±111	A–B, $P<0·1$
B. Aminoguanidine	178,000±16,900	236,000±20,300	75·4±1·70	864± 63	A–C, $P<0·1$
C. MeH	163,000± 6,670	232,000±15,000	70·8±2·26	849± 51	B–D, NS
D. Aminoguan. & MeH	193,000± 3,320	243,000± 7,520	79·7±1·64	720± 39	C–D, $P<0·1$
Lymph node					
A. Saline	16,700± 654	74,700± 654	22·3±0·92	140± 11·1	
Spleen					
A. Saline	14,900± 610	49,000± 1,080	30·4±0·98	125± 6·6	
Lung					
A. Saline	22,600± 1,590	93,600± 5,010	24·6±2·50	193± 25·7	
Kidney					
A. Saline	130,000± 7,210	372,000±27,500	35·6±2·82	1100±113	
Muscle					
A. Saline	6,780± 510	14,600± 921	46·4±0·68	57± 6·4	

Saline, aminoguanidine sulphate, 1 mg base, methylhistamine, 10 mg base, or aminoguanidine plus methylhistamine injected intraperitoneally approximately 20 min before injection of ^{14}C-histamine, 2·0 μCi. Values are means ± S.E.M. of five assays per group (except intestine, group C, four assays). Statistical analyses: Column 3, A–B for blood, liver and intestine $P<0·001$, heart NS; A–C for liver and intestine $P<0·001$, blood $P<0·01$, heart NS; B–D for intestine $P<0·001$, others NS; C–D for blood $P<0·001$, liver and intestine $P<0·01$, heart $P<0·025$. * Individual values calculated, then mean and S.E.M. determined; therefore values of column 3 may not be exactly equal to 1/2. † Refers to data of column 4.

occur to a small extent in the female and somewhat more in the male (Schayer, 1959, 1966). Rats were pretreated with aminoguanidine, methylhistamine, or both, and then injected with ^{14}C-histamine; experimental procedures and findings are shown in Table 3. The results differ from those obtained in mice and can be better interpreted after a discussion of the mouse data.

Paper chromatography of urine of mice injected with ^{14}C-histamine; effects of aminoguanidine and methylhistamine

Blocking of major catabolic routes for a substance may tend to increase its catabolism through a normally minor pathway. To test this possibility the effect of aminoguanidine, 200 μg, plus methylhistamine, 6 mg, on excretory products of ^{14}C-histamine by female mice was tested (Fig. 1). In urine from normal mice, chromatogram A, the usual three peaks occur; peaks 1 and 2 contain imidazoleacetic acid, free and conjugated, and methylimidazoleacetic acid; peak 3 includes histamine and methylhistamine, but no other known metabolite in significant quantities. In urine from mice given inhibitors, chromatogram B, peaks 1 and 2 are markedly suppressed and virtually all ^{14}C falls in peak 3. When urine from mice given inhibitors is developed in a solvent mixture known to separate histamine and methylhistamine (Snyder, Axelrod & Bauer, 1964) three peaks are obtained (chromatogram C). These chromatograms provide evidence that at least one minor histamine catabolite may be formed in larger quantities if normal catabolic channels are blocked. The substance has not yet been identified.

To test for a sex difference in histamine catabolism by mice, similar experiments were made with male mice; all chromatographic patterns so closely matched those obtained with females that no further work on this matter was done.

Discussion

To interpret the results of the *in vivo* experiments, the same working assumptions stated previously (Reilly & Schayer, 1970) are used. Briefly, they are (a) a low ratio of ^{14}C-histamine to total ^{14}C in a tissue may indicate the presence of one or more

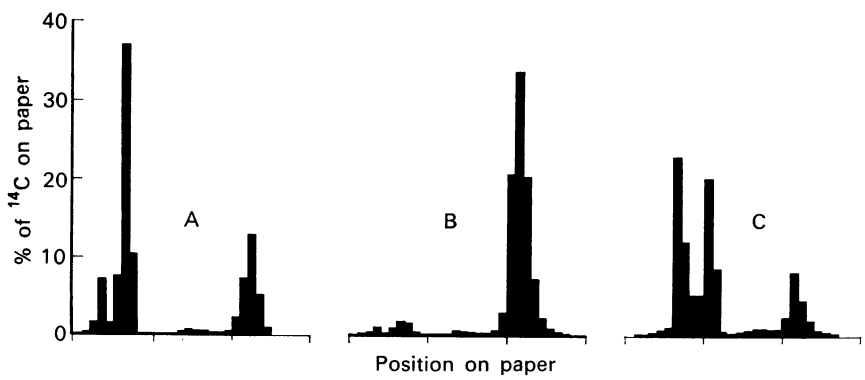

FIG. 1. Paper chromatograms of urine collected for 3 h after injection of mice with ^{14}C histamine. (A) normal mice, no inhibitors of histamine catabolism (solvent 1); (B) mice given aminoguanadine and methylhistamine (solvent 1); (C) mice given aminoguanidine and methylhistamine (solvent 2). (Solvent 1 is butanol, 80 parts, ethanol, 10 parts and concentrated ammonium hydroxide, 30 parts. Solvent 2 is ethyl acetate, butanol, acetic acid and water in equal parts).

histamine catabolizing enzymes; (b) a marked increase of ^{14}C-histamine in a tissue, resulting from aminoguanidine or methylhistamine pretreatment, may indicate the presence of diamine oxidase or the histamine-methylating enzyme, respectively.

The data of Table 1 concern the effects of drugs on the rate of destruction of ^{14}C-histamine; no attempt was made to identify the catabolic pathways involved. As chlorpheniramine and pyrilamine had no common effect, there is no evidence that antihistamines, as a group, significantly influenced the rate of histamine catabolism. Betahistine had no detectable effect; it inhibits pea seedling diamine oxidase (Werle & Palm, 1953). As expected, pretreatment of mice with non-isotopic histamine caused a pronounced increase in ^{14}C-histamine concentrations of all tissues; presumably the non-isotopic histamine occupied enzyme sites so that ^{14}C-histamine was excluded to some extent. Nevertheless, ^{14}C-histamine destruction was still relatively rapid in liver and intestine.

Evidence on the contribution of various tissues to catabolism of exogenous histamine in mice is found in Table 2. Aminoguanidine increased ^{14}C-histamine concentrations (groups A–B and C–D, columns 1 and 3), but concentrations relative to blood ^{14}C-histamine (column 4) were all reduced by aminoguanidine (significant for lung C–D, $P<0{\cdot}5$; kidney A–B, $P<0{\cdot}05$; stomach A–B, $P<0{\cdot}01$). Aminoguanidine may occupy histamine binding sites in tissues and reduce uptake of histamine from blood; further evidence on this point will be given later. Diamine oxidase does not seem to participate significantly in histamine catabolism in mouse lung, muscle, kidney or stomach. Our earlier evidence indicated the presence of diamine oxidase in intestine, heart and lymph node (Reilly & Schayer, 1970).

The contribution of the histamine-methylating enzyme is adjudged by the effect of methylhistamine (groups A–C and D–B, Table 2). Methylhistamine pretreatment increased ^{14}C-histamine concentrations, but had little effect on total ^{14}C, except in kidney; the additional kidney ^{14}C can be entirely attributed to ^{14}C-histamine. In column 4, an increase from A–C, or B–D, is considered evidence that histamine methylation normally occurs in the tissue, and that its inhibition increases tissue ^{14}C-histamine relative to that in blood. An alternative possibility, that methylhistamine might increase ^{14}C-histamine concentrations of tissue by enhancing uptake of blood ^{14}C-histamine, is unsatisfactory; blood concentrations were increased, not decreased. Other possible explanations of the data seem even less plausible. By the foregoing procedure, we infer that *in vivo* methylating activity occurs in all mouse tissues listed in Table 2 except stomach and intestine. Brown, Tomchick & Axelrod (1959) found *in vitro* histamine methylating activity in mouse kidney, lung, liver, stomach, heart and muscle; intestine was not reported. Only for stomach is there a discrepancy between the *in vivo* and *in vitro* findings. Stomach forms histamine at a rate much greater than any other mouse tissue ; if catabolic enzymes were present, they would be exposed to high concentrations of newly formed endogenous histamine, and might not be detectable by testing changes in catabolism of injected ^{14}C-histamine. Obviously relative rates of histamine methylation obtained by the *in vivo* and *in vitro* methods are not comparable, for in the latter, S-adenosylmethionine, coenzyme, was added to incubates.

Histamine methylation by lung appears to be rapid and extensive. If lung contains 30–35% blood virtually all lung ^{14}C-histamine (columns 1 and 4) could be in the blood perfusing it. The same conclusion can be drawn from lung and blood

data of Table 1 (controls 1 and 2). Liver seems to be even more effective (Table 1, controls 1 and 2). Assuming liver to contain 15% blood, its ^{14}C-histamine is much less than that calculated from its blood content. Evidently mouse liver cells avidly extract histamine from the blood perfusing its sinusoids and methylate it quickly. From this data it seems doubtful that exogenous histamine can remain unchanged in liver cells for a significant time.*

Data for rat tissues (Table 3) show liver ^{14}C-histamine to be increased by aminoguanidine and by methylhistamine. Total ^{14}C is abnormally low in liver of rats treated with aminoguanidine (A–B and A–D, $P<0{\cdot}001$); as a similar phenomenon has been observed only for mouse intestine, a tissue with *in vivo* diamine oxidase activity (Reilly & Schayer, 1970), the drop in total ^{14}C in rat liver may be evidence for diamine oxidase activity. In column 4 A–B tends to confirm the presence of diamine oxidase activity, but B–D shows no evidence for histamine methylation. A–C is anomalous and might be due to weak inhibition of diamine oxidase by methylhistamine; this postulate receives some support from mouse data (Table 2) for in column 3, the A–B difference is greater than that of C–D.

Rat intestine data indicate the presence of both diamine oxidase and the histamine-methylating enzyme, mainly the latter. In group D, column 4, only 34% of the total ^{14}C is histamine; this low value may reflect an abnormally large uptake of a circulating ^{14}C-histamine metabolite(s) by rat intestine. Rat heart data are consistent with the absence of both histamine-catabolizing enzymes. The lower values of ^{14}C-histamine in groups B, C and D, relative to A, suggest that both aminoguanidine and methylhistamine reduce the normally large uptake of circulating histamine.

Of these three rat tissues, *in vitro* tests for histamine methylation by Brown *et al.* (1959) showed intestine to be positive, liver negative, and heart to contain a trace. As with mouse tissues, there is substantial agreement between the two types of test.

For several rat tissues only group A was assayed. As the values did not indicate potent histamine-catabolizing ability, no further assays were made.

The marked differences between mice and rats in catabolism of histamine, Tables 2 and 3, contrast with similarities in some other histamine-related phenomena. For example, both species are relatively insensitive to the effects of injected histamine, both can undergo large changes in histidine decarboxylase activity, in both, mast cells contain 5-hydroxytryptamine as well as histamine, and in both, 5-hydroxytryptamine released from mast cells produces a histamine-like action.

The species difference in histamine catabolism relates not only to the major pathway, a fact long known (Schayer, 1959), but also to participation of individual tissues. For example, liver is far more potent in mice than in rats in histamine inactivation. Heart, which in mice is one of the most active histamine-destroying tissues, seems devoid of such activity in rats; such observations suggest a lack of an evident relationship of histamine catabolism to histamine function. However, in rats uptake of ^{14}C-histamine by heart is remarkable, the concentration in controls

* The tissue blood volumes were derived by consulting the papers by Friedman (1955, 1959, 1960), Kaliss & Pressman (1950), and Wish, Furth & Storey (1950). To obtain conservative estimates we selected the lower values and then reduced them by one-third to correct for blood lost after decapitation. Corrections for blood volume of tissues other than lung and liver are negligible for interpreting the data on histamine catabolism.

being 11 times that of blood under our conditions; possibly rat heart may ' inactivate ' excess histamine by loose binding and release it at a later time.

Intestine is the most active histamine-catabolizing tissue of those tested in rats. Yet in this predominantly non-methylating species intestine does methylate while in mice, a predominantly histamine-methylating species, intestine shows no methylation but destroys injected histamine through the oxidative pathway.

Lung of rats contained considerable injected ^{14}C-histamine even after correction for blood content and was not examined further. We had previously concluded that mouse lung did not destroy histamine rapidly (Reilly & Schayer, 1970) but at that time were not aware of its high blood content; our present view is that mouse lung destroys injected histamine rapidly and perhaps completely.

Aminoguanidine appears to have at least two effects on catabolism of blood-borne histamine; first, by inhibiting diamine oxidase, it tends to increase histamine concentrations in tissues containing this enzyme, and second, by occupying histamine receptors it tends to reduce the concentration of histamine in tissue. Both effects tend to increase the blood concentration, the former by transfer of unchanged histamine from tissue to blood, and the latter by reducing removal of blood histamine by tissue.

After considerable experience with *in vivo* studies on histamine catabolism and formation in mice, we feel it may be possible to estimate the contribution of individual tissues to formation of histamine *in vivo* by comparing the concentrations of ^{14}C-histamine in tissue (% of blood ^{14}C-histamine) in (a) animals administered ^{14}C-histamine, with (b) those administered ^{14}C-L-histidine. The process is illustrated in Table 4. Some data are from this paper, and some from previous ones, but all are from experiments done under standardized conditions. Values in the first column indicate how each tissue handles blood-borne ^{14}C-histamine. Values in the second column depend on two factors. First is the ability of the tissue to extract ^{14}C-histamine from blood and catabolize it; second is the ability to decarboxylate circulating ^{14}C-L-histidine. The difference between values in the two columns should provide a rough measure of the ability of each tissue to form histamine. For example, in mice injected with ^{14}C-L-histidine, stomach contains about 370 times as much ^{14}C-histamine as can be accounted for by uptake from blood; clearly, stomach is forming histamine *in vivo*. Brain takes up no blood histamine (Schayer

TABLE 4. *Tissue ^{14}C-histamine, expressed as % blood ^{14}C-histamine, in mice injected intravenously with ^{14}C-histamine or ^{14}C-L-histidine*

Tissue	Tissue ^{14}C-histamine (% of blood ^{14}C-histamine)			
	Exogenous; ^{14}C-histamine injected*		Endogenous; ^{14}C-L-histidine injected*	
Stomach	38	(1)†	18,200	(5)
Brain	0	(2)	578	(2)
Liver	low	(3)	380	(5)
Lung	low	(3)	475	(6)
Intestine	80	(4)	650	(5)
Muscle	32	(1)	281	(5)
Lymph node	28	(4)	152	(5)
Heart	55	(4)	104	(5)
Kidney	197	(1)	426	(5)

* Mice killed 2·5 min after injection of ^{14}C-histamine, 1·0 μCi, or 10 min after ^{14}C-L-histidine approximately 19 μCi. † Data are from several comparable experiments; numbers in parentheses identify the source: (1) Table 2 of this paper; (2) Schayer & Reilly, 1970; (3) Table 1 of this paper; (4) Table 2 of Reilly & Schayer, 1970; (5) Table 1 of Reilly & Schayer, 1971; (6) Table 2 of Reilly & Schayer, 1968.

& Reilly, 1970) and liver and lung retain very little in unchanged form (Table 1); therefore, these tissues probably make virtually all of their histamine. For intestine, muscle and lymph node, the relatively high values in the second column suggest that these tissues make most of their histamine. Only for heart and kidney are the two values fairly close. Obviously the two types of experiment cannot be made equivalent; one involves a single injection of histamine before being killed 2·5 min later; the other involves histamine newly formed from its precursor during a 10 min period. Hence, only large differences can be interpreted. Heart and kidney extract considerable histamine from the blood (see Table 2, total ^{14}C values). *In vivo* histamine formation by heart and kidney cannot be proved by the present data, but as both tissues show a low but definite histidine decarboxylase activity, it may occur.

To our knowledge, the attempts in this paper to evaluate under fully *in vivo* conditions (a) the presence of catabolic enzymes in individual tissues through injection of inhibitors, and (b) participation of individual tissues in formation of a substance from its precursor, are novel. If these approaches are valid, they may be of more general applicability and of considerable value.

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. In part, this work was supported by General Research Support Grant RR 05615. 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

BROWN, D. D., TOMCHICK, R. & AXELROD, J. (1959). The distribution and properties of a histamine-methylating enzyme. *J. biol. Chem.*, **234**, 2948–2950.

COHN, V. C. & WYNN, W. (1968). Inhibition of histamine metabolism by antihistamines. *Fedn Proc.*, **27**, 243.

FRIEDMAN, J. J. (1955). Organ plasma volume of normal unanesthetized mice. *Proc. Soc. exp. Biol., N.Y.*, **88**, 323–325.

FRIEDMAN, J. J. (1959). Circulating and tissue hematocrits of normal unanesthetized mice. *Am. J. Physiol.*, **196**, 420–422.

FRIEDMAN, J. J. (1960). Alterations in tissue blood volume induced by tourniquet application. *Am. J. Physiol.*, **198**, 886–890.

HUNT, W. H. & FOSBINDER, R. J. (1942). A study of some beta-2, and 4, pyridylalkylamines. *J. Pharmac. exp. Ther.*, **75**, 299–307.

KALISS, N. & PRESSMAN, D. (1950). Plasma and blood volumes of mouse organs, as determined with radioactive iodoproteins. *Proc. Soc. exp. Biol., N.Y.*, **75**, 16–20.

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

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

REILLY, M. A. & SCHAYER, R. W. (1971). Techniques for measuring histamine formation in mice. *Br. J. Pharmac.*, **42**, 375–382.

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

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

WERLE, E. & PALM, D. (1953). Pyridylalkylamines and diamine oxidase. *Biochem. Z.*, **323**, 424–427.

WISH, L., FURTH, J. & STOREY, R. H. (1950). Direct determinations of plasma, cell, and organ-blood volumes in normal and hypervolemic mice. *Proc. Soc. exp. Biol., N.Y.*, **74**, 644–648.

(*Received June* 8, 1971)