J. Physiol. (1959) 149, 34–42

FORMATION AND CATABOLISM OF HISTAMINE IN BRAIN TISSUE *IN VITRO*

By T. WHITE

From the Institute of Physiology, University of Lund, Sweden

(*Received* 23 *March* 1959)

It is known that the brain tissue of various mammals contains histamine (Kwiatkowski, 1943; Harris, Jacobsohn & Kahlson, 1952; Clouet, Gaitonde & Richter, 1957); and it has been reported that brain tissue from cattle or from rats is able to form histamine *in vitro* (Holtz & Westermann, 1956; Schayer, 1956*a*; Kahlson, Rosengren, Westling & White, 1958).

The object of the present investigation was to determine the histamine-forming capacity of brain tissue *in vitro* by an isotope dilution technique which provides a sensitive method permitting the use of low, physiological concentrations of histidine. Other advantages of this method are that any histamine already present in the tissue to be investigated does not interfere, and that the technique also makes possible the measurement of various histamine derivatives. In the course of the experiments it became of interest to investigate the fate of the histamine that was formed. Part of this work has been published in abstract (White, 1958).

METHODS

Brain tissue from cats, dogs and pigs was used. A pig brain was obtained from a slaughter-house, where the animal had been killed by electrocution and bleeding. On the same occasion the pituitary glands and median eminences of five pigs were collected. The dogs were killed by Na pentobarbitone given intravenously. These animals were used for the experiments reported in Table 1.

For the rest of the work cat tissue was used. The cats were of either sex and weighed 2·5–4·5 kg. They were killed by a blow on the neck and exsanguinated. The skull was opened, the brain removed and the pia mater and blood vessels on its surface stripped off.

A specimen of cat cerebral cortex plus some white matter in the gyri was obtained by cutting away the white matter to the level of cortex in the sulci; it is termed 'cortex'. The 'hypothalamic' specimen was taken from an area extending between the optic chiasma and the mammillary bodies and 3 mm to each side of the mid line, including the mammillary bodies and the lowest part of the thalamus, but omitting optic chiasma and pituitary gland. The specimen of 'area postrema' was obtained by cutting out a triangular portion on the dorsal surface of the medulla oblongata by means of two incisions placed just outside the caudal border of the fourth ventricle and parallel to it, and a third, transverse, incision. The specimen includes the caudal part of the floor of the fourth ventricle.

The time interval between the death of the animal and the start of the incubation was about

1 hr. In some experiments the tissue was kept in beakers immersed in iced water during the preparation. The results of these experiments did not differ significantly from those in which the tissues were kept at room temperature.

The tissues were minced with scissors into pieces less than 1 mm in diameter. In each experiment the tissue was first minced, and portions were then taken for incubation. All incubations were performed in a total volume of 4 ml. The minced tissue was suspended in 0·1 M sodium phosphate buffer of pH 7·4 (Gomori, 1955) containing glucose 0·2 g/100 ml., and was then incubated in beakers in a water-bath at 37° C. Unless otherwise stated the incubation time was 3 hr, and the gas phase nitrogen. Blanks were obtained by boiling the incubation mixture for 5 min before the addition of ^{14}C-histidine; all figures for histamine formation are given after subtraction of these blank values.

The method for extracting and measuring the amount of histamine formed from radioactive histidine was that developed by Schayer and others and described in detail by Kahlson *et al.* (1958). At the end of the incubation 66·4 mg of non-radioactive histamine dihydrochloride (as carrier) and 50 mg L-histidine monohydrochloride (as diluent) were added to each sample. Trichloroacetic acid was added to precipitate the protein. The filtrate was saturated with anhydrous Na_2SO_4 and made alkaline with NaOH: it was then extracted twice with butanol and the combined butanol fractions were shaken with an alkaline histidine solution, saturated with anhydrous Na_2SO_4, after which the histidine solution was discarded. (This represents a minor modification of the method described by Kahlson *et al.* (1958); in that, the filtrate was extracted three times with butanol, and the histidine solution was not saturated with Na_2SO_4 and discarded, but was re-extracted with butanol.) The combined butanol fractions were extracted with HCl, and the aqueous phase evaporated to dryness. The residue, consisting mainly of histamine dihydrochloride, was dissolved in water, and picric acid in alcoholic solution was added. Histamine dipicrate crystals were prepared and isolated, and mounted on a plate and their radioactivity was determined at infinite thickness in a gas flow-counter. For further purification the histamine, in the form of dihydrochloride, was made to react with *p*-iodobenzenesulphonyl chloride, and the formed crystals of pipsyl histamine were isolated and examined in the counter in the same way as the histamine dipicrate.

The extraction of methylhistamine is described by Rothschild & Schayer (1958). The principle of this method is based upon the finding that chloroform is an excellent solvent for the separation of methylhistamine from histamine. According to these authors, methylhistamine is almost wholly recovered from strongly alkaline aqueous solution, saturated with Na_2SO_4, by three chloroform extractions, whereas only 5–6% of ^{14}C-histamine is extracted by this procedure. Thus chloroform extraction, followed by recrystallization of the picrate, eliminates significant contamination with histamine. In the present experiments the incubation mixture, after addition of 71 mg non-radioactive methylhistamine as carrier, was treated as described for histamine determination, but extracted three times with chloroform instead of butanol. The combined chloroform fractions, after addition of HCl in alcoholic solution, were evaporated, the residue consisting mainly of methylhistamine dihydrochloride. Methylhistamine dipicrate was prepared and counted.

The radioactive measurements were made under infinite-thickness conditions in a gas flow-counter. The crystals were deposited on plates of equal size, so that the exposed surface was equal for all samples. The crystal layer was made sufficiently thick for the radiation originating in its deeper levels to be completely absorbed in the sample and not detected. Under these conditions the observed count is proportional to the specific activity of the sample, i.e. to the ratio of radioactive to inactive molecules in it. Once the necessary standardizations have been performed, the radioactivity of any substance in the incubation mixture can be calculated from the known amount of inactive carrier added to it. The technique, as used here, was standardized by subjecting a known amount of ^{14}C-histamine to dilution with the standard amount of non-radioactive carrier and counting at infinite thickness in the form of dipicrate and pipsyl derivative.

In the experiments in which one sample was analysed for several metabolites of histamine the following procedure was employed. After the incubation, the sample was acidified to pH 2 with

HCl, homogenized and divided into portions. After the addition of the inert carriers the portions were treated as described for blood by Lindell & Schayer (1958). The following metabolites were studied: Methylhistamine (1-methyl-4(β-aminoethyl)-imidazole), methylimidazoleacetic acid (1-methyl-imidazole-4-acetic acid), and imidazoleacetic acid (imidazole-4(5)-acetic acid).

The criterion of purity was constant radioactivity after repeated recrystallizations with different charcoal adsorbents. Three to six recrystallizations were needed to obtain constant radioactivity. After each recrystallization at least 1000 counts were taken of each sample. It is not claimed that the extraction procedures are absolutely specific for the respective substances.

Substances. Two different preparations of ^{14}C-histidine, labelled in the 2-position in the imidazole ring, have been used. One was kindly provided by Dr Schayer and gave $9{\cdot}8 \times 10^6$ counts/min/mg base at zero thickness measured in the flow-counter. One microgram of ^{14}C-histamine, derived from this ^{14}C-histidine, after addition of carrier histamine and precipitation as dipicrate in the usual manner, gives 650 counts/min measured at infinite thickness in the flow-counter. This preparation of ^{14}C-histidine HCl was used for the experiments concerning histamine formation, except Expts. 4, 6, and 7 in Table 1 and the experiments on histamine binding.

The other preparation of ^{14}C-histidine was purchased from the Radiochemical Centre, Amersham, Bucks, and had a higher specific radioactivity. It gave $5{\cdot}4 \times 10^7$ counts/min/mg base at zero thickness measured in the flow-counter, and 1 μg of ^{14}C-histamine derived from this ^{14}C-histidine, after addition of carrier histamine and precipitation as dipicrate in the usual manner, gives 3600 counts/min measured at infinite thickness in the flow-counter. This preparation of ^{14}C-histidine was used in the form of free base for Expts. 4, 6 and 7 in Table 1 and for the experiments concerning histamine binding and methylhistamine formation.

^{14}C-histamine labelled in the 2-position of the imidazole ring was purchased from the Radiochemical Centre, Amersham, Bucks. It gave $4{\cdot}2 \times 10^7$ counts/min/mg base at zero thickness and 2000 counts/min/μg base measured in the form of the dipicrate at infinite thickness, measured in the flow-counter. All figures for added radioactive histamine and histidine refer to the base.

Hydrazine sulphate (Merck, Darmstadt) and semi-carbazide HCl (British Drug Houses) were dissolved in buffer solution and added to the incubation mixture to give the final stated concentration. Pyridoxal-5-phosphate (Roche Products Ltd.) was added to the buffer solution in a concentration of 60 μg/ml.

RESULTS

Histamine formation

From preliminary experiments it was evident that the amount of histamine formed was dependent upon the concentration of ^{14}C-histidine. Therefore in subsequent experiments, including those presented in Table 1, all incubations were carried out with 40 μg ^{14}C-histidine/4 ml. incubation mixture. The cat's brain contains 9 μg free histidine/g tissue (Tallan, Moore & Stein, 1954).

As can be seen in Table 1, in which the results are expressed per gram of tissue, the highest rate of histamine formation was found in hypothalamic tissue. In the other regions histamine was formed to a marked extent only by the cerebral cortex and the area postrema. In pooled tissue from five pigs the histamine formation was virtually nil in the anterior pituitary, 12 ng/g in the posterior pituitary, and 214 ng/g in the median eminence. In the medulla oblongata tissue from three cats it was 3–5 ng/g and in one cat pituitary, nil.

It was found that small amounts of tissue with low histamine-forming capacity gave variable values. In these and subsequent experiments 2 g cortex has been used in most of the experiments; but the amounts of hypo-

thalamus had to be smaller, as each animal provides only about 0·7 g of this tissue. In one experiment portions of 0·25, 0·50, 1·00, and 2·00 g of cortex were incubated with 40 μg ^{14}C-histidine. In the first portion there was no detectable formation of ^{14}C-histamine; the second portion gave 11 counts/min and the last two 17 and 15 counts/min respectively. In another similar experiment 0·25 g of cortex failed to form histamine, whereas 2·00 g gave 10 counts/min.

The time course of the ^{14}C-histamine formation from ^{14}C-histidine has not been studied in detail. But in one experiment with portions of 2 g of cortex it was found that the ^{14}C-histamine formed after 1 hr gave 14 counts/min and after 3 hr, 32 counts/min (mean of duplicates). A similar time course has been described for histamine formation by free mast cells (Schayer, 1956*b*).

TABLE 1. Histamine formed in 3 hr by brain tissue in N_2 from 40 μg ^{14}C-histidine (expressed as ng/g tissue)

Species	Cortex (2·00 g)	Cerebellum (1·40–2·00 g)	Hypothalamus (0·25–0·75 g)	Area postrema (0·25 g)
Cat	14	$\leqslant$2	191	—
Cat	11	$\leqslant$2	141*	—
Cat	11	$\leqslant$2	134*	—
Cat	6	2	333	—
Pig†	17	0	—	—
Dog	10	—	355	50
Dog	14	—	1116	23

* Pooled tissue from 2 cats; † pooled tissue from 5 pigs.

The influence of oxygen on the reaction was studied by comparing the histamine formation in oxygen with that in nitrogen. The ^{14}C-histamine formed in oxygen gave, with 0·20 g hypothalamus 102 counts/min and with 2·00 g cortex 75 counts/min; the corresponding figures for nitrogen were 130 and 87 counts/min.

Benzene is reported to enhance the activity of histidine decarboxylase from rabbit kidney and to inhibit strongly the enzymic action of histidine decarboxylase obtained from rat stomach and from mast cells (Waton, 1956*a*, *b*; Schayer, 1957; Rothschild & Schayer, 1958). In the present experiments the addition of one drop (approx. 20 mg) of benzene A.R. to the incubation mixture of cortex had no effect.

In three experiments pyridoxal-5-phosphate was added to the buffer solution in a concentration of 60 μg/ml. Pyridoxal-5-phosphate is a co-enzyme of several amino acid decarboxylases, including histidine decarboxylase (Blaschko, 1957; Rothschild & Schayer, 1958). No difference was observed in the formation of ^{14}C-histamine between samples of cortex and of hypothalamus incubated with and without pyridoxal-5-phosphate.

Hydrazine and semicarbazide have been reported to be inhibitors of histidine decarboxylase (Waton, 1956*a*; Schayer & Kobayashi, 1956). Histamine formation was abolished in three experiments on three cats in which the

^{14}C-histamine formation was measured with and without these inhibitors, using 2 g of cortex in each case. In the first experiment the histamine formed in the control gave 15 counts/min; with semicarbazide in a concentration of $0{\cdot}6 \times 10^{-2}$ M it was zero. In the second experiment the control yielded 10 counts/min, but with $0{\cdot}6 \times 10^{-3}$ M semicarbazide it was zero. In the third experiment, with 10^{-4} M hydrazine, the counts were 9/min without and zero with the inhibitor.

Histamine binding. In many tissues part of the newly formed histamine is firmly bound, so that it cannot be easily washed away. The amount of binding seems to vary from one tissue to another (Schayer, Davis & Smiley, 1955; Schayer, 1956*b*; Kahlson, Rosengren & White, 1959). The present experiments show that, in brain, histamine is loosely bound. Thus, in one experiment on 0·24 g hypothalamus, when the incubation with ^{14}C-histidine was stopped the sample was diluted with buffer solution to 8 ml. and centrifuged for 5 min at 4000 rev/min. After decanting off the supernatant fluid, the tissue was washed and re-centrifuged twice. The sediment and combined supernatants were then analysed separately for ^{14}C-histamine and a control sample was analysed for the ^{14}C-histamine content of the total, uncentrifuged, incubation mixture. The results were: total, 240 counts/min; supernatant, 204 counts/min; sediment, 63 counts/min. In a second similar experiment on 2·00 g cortex the results were: total, 93 counts/min; supernatant, 59 counts/min; sediment, 32 counts/min. Thus in both experiments the greater part of the ^{14}C-histamine formed was found in the supernatant fluid.

Histamine catabolism in brain tissue

The figures for histamine formation given in the previous section should be regarded as minimum values, as it is possible that formation and destruction of histamine proceed simultaneously. To investigate this point brain tissue was incubated with added ^{14}C-histamine under conditions identical with those of the histamine-formation experiments, and the metabolites of histamine were assayed by isotope dilution methods. It was found that under the conditions of the experiment methylhistamine was the major metabolic product of histamine. As is shown in Table 2, the formation of methylhistamine did not appear to be oxygen-dependent, but the formation of methylimidazoleacetic acid was favoured by the presence of oxygen. Imidazoleacetic acid was not formed in significant amounts.

Formation of methylhistamine from histidine

In the previous sections it has been shown that histamine can be formed from labelled histidine in brain tissue and that methylhistamine can be formed from added histamine. There is therefore a possibility that part of the histamine formed from added labelled histidine is transformed into methylhistamine.

If so, it should be possible to demonstrate the presence of ^{14}C-methylhistamine after incubation of brain tissue with ^{14}C-histidine. This deduction was tested in two experiments and the results are presented in Table 3. It will be seen that ^{14}C-methylhistamine was in fact produced in substantial amounts.

The steps in the transformation of histidine to methylhistamine were then examined in more detail by studying the action of semicarbazide upon this

TABLE 2. Histamine metabolism in cat brain tissue; 3 hr at 37° C; 2 μg ^{14}C-histamine added to each sample. The figures denote the percentage of added histamine recovered as histamine and metabolites

		Percentage recovered as				
Gas phase	Tissue	^{14}C-histamine	^{14}C-methyl-histamine	^{14}C-methyl-imidazole-acetic acid	^{14}C-imidazole acetic acid	Total recovery
N_2	C*	32	70	$\leqslant 1$	0	103
N_2	C	31	71	$\leqslant 2$	0	105
O_2	C	23	66	13	$\leqslant 1$	102
O_2	C	14	60	11	0	85
N_2	H	74	20	$\leqslant 1$	0	95
N_2	H	73	19	$\leqslant 1$	0	94
O_2	H	82	20	5	0	106
O_2	H	64	20	4	$\leqslant 1$	90

* C, cortex 2·00 g; H, hypothalamus 0·25 g.

TABLE 3. Formation of ^{14}C-methylhistamine and ^{14}C-histamine from 40 μg ^{14}C-histidine in 3 hr at 37° C in N_2; histamine and methylhistamine were determined on samples incubated separately. Expts. 1 and 5 were made on one cat, and Expts. 2, 3 and 4 on another

Expt.	Tissue	^{14}C-histamine (ng)	^{14}C-methylhistamine (ng)
1	C_1*	5	31
2	C_1	15	32
3	C_2	0	14
4	H	45	27
5	H	79	25

* C_1, cortex 2·00 g; C_2, cortex 0·25 g; H, hypothalamus 0·25 g.

TABLE 4. The influence of semicarbazide upon the formation in cat brain tissue of methylhistamine from histidine and histamine, and of histamine from hisitidine. Incubation 3 hr at 37° C in N_2

Expt.	Tissue	Concn. of semi-carbazide (M)	^{14}C-histamine added (μg)	^{14}C-histidine added (μg)	^{14}C-histamine formed (ng)	^{14}C-methyl-histamine formed (ng)
1	C*	0	1	—	—	844
1	C	10^{-3}	1	—	—	853
1	H_1	0	1	—	—	262
1	H_1	10^{-3}	1	—	—	234
2	C	0	—	40	21	—
2	C	10^{-3}	—	40	0	—
2	H_2	0	—	40	45	—
2	H_2	10^{-3}	—	40	0	—
3	C	0	—	40	—	30
3	C	10^{-3}	—	40	—	14
3	H_2	0	—	40	—	19
3	H_2	10^{-3}	—	40	—	6

* C, cortex 2·00 g; H_1, hypothalamus 0·20 g; H_2, hypothalamus 0·25 g.

reaction. Semicarbazide was added to the incubation mixture to a concentration of 10^{-3}M and the formation was followed of (*a*) methylhistamine from histamine, (*b*) histamine from histidine, and (*c*) methylhistamine from histidine. As is shown in Table 4, semicarbazide did not prevent the formation of methylhistamine from added histamine, but as in the experiments already described it did prevent the formation of histamine from added histidine, and also reduced substantially the formation of methylhistamine from added histidine.

DISCUSSION

In the discussions of the possible functions of 5-hydroxytryptamine and of the catechol amines in various parts of the brain, importance has been assigned to the similarities in distribution between the enzymes capable of forming them from amino acids, the substances themselves and the enzymes capable of metabolizing them (Brodie, Pletscher & Shore, 1955; Vogt, 1954; Bogdanski, Weissbach & Udenfriend, 1957).

In the present study it has been found that the regions of the brain rich in histamine are also highly active in forming histamine. It would seem, however, that at least in the cerebral cortex and the hypothalamus the capacity to metabolize histamine does not parallel the capacity to form histamine.

The significance of the formation of methylhistamine is not clear. Ring-methylation might be a means of inactivating histamine. Methylhistamine is practically inactive in comparison with histamine on some biological test objects, such as the bronchi and isolated ileum of the guinea-pig and the cat's blood pressure (Lee & Jones, 1949; Westling, 1957). It is of interest to note in this connexion that adrenaline and noradrenaline can be methylated on a hydroxyl group attached to the ring and that this reaction is catalysed by cerebral tissues among others (Axelrod, Witkop & LaBrosse, 1958).

It is well known that there is a correlation between the histamine content and the number of mast cells in many tissues (Riley & West, 1953), and it has been shown by Schayer (1956*b*) that free mast cells are able to decarboxylate histidine with ease. Yet mast cells are reported to be practically absent from brain (Riley, 1956). Therefore the differences observed in the capacity to form histamine cannot be due to differences in mast-cell content, and a more specific action of neural tissue in forming histamine is indicated. The lack of correlation in general between histamine-binding power and mast-cell content has been pointed out by Schayer (1956*a*).

Decarboxylation of histidine and formation of methylhistamine both occur during incubation of brain tissue with histidine. One possible pathway is: histidine → histamine → methylhistamine. Another is: histidine → methylhistidine → methylhistamine. The experiments reported here would accord with either possibility. There is, however, no evidence in favour of the second pathway; and it is likely that at least part of the methylhistamine is formed

from histamine, because methylhistamine can easily be formed from added histamine, and because most of the newly formed histamine appears to be only loosely bound to the tissue particles and would thus act like added histamine. Both ring-N methylhistidines are excreted in the urine of man, cat, and rat, but cat brain contains little or none (Tabor, 1954; Tallan *et al.* 1954; Wolf, Wu & Heck, 1956).

Formation of methylhistamine in brain tissue is largely independent of oxygen, in contrast to mouse liver where ring-methylation of histamine is known to require oxygen (Lindahl, 1958).

SUMMARY

1. Minced brain tissue from cat, pig, and dog was incubated with ^{14}C-histidine and the ^{14}C-histamine formed was determined by an isotope dilution technique. The formation of histamine was greatest in the hypothalamus.

2. The reaction was inhibited by semicarbazide and by hydrazine. It was not influenced by pyridoxal-5-phosphate or by benzene.

3. When cat brain tissue was incubated with ^{14}C-histamine the principal metabolite found was ^{14}C-methylhistamine (1-methyl-4(β-aminoethyl)-imidazole), formed by ring-methylation.

I am grateful to Dr R. W. Schayer, who during a stay at this Institute introduced me to his methods. The investigation was supported by a grant from the Medical Faculty, University of Lund, Sweden.

REFERENCES

AXELROD, J., WITKOP, B. & LABROSSE, E. H. (1958). O-methylation of epinephrine and norepinephrine *in vivo* and *in vitro*. *Abstr. IV. internat. Congr. Biochem.* 106.

BLASCHKO, H. (1957). Metabolism and storage of biogenic amines. *Experientia*, **13**, 9–12.

BOGDANSKI, D. F., WEISSBACH, H. & UDENFRIEND, S. (1957). The distribution of serotonin, 5-hydroxytryptophan decarboxylase and monoamine oxidase in brain. *J. Neurochem.* **1**, 272–278.

BRODIE, B. B., PLETSCHER, A. & SHORE, P. A. (1955). Evidence that serotonin has a role in brain function. *Science*, **122**, 968.

CLOUET, D. H., GAITONDE, M. K. & RICHTER, D. (1957). The free histidine, histamine, and arginine content of the rat brain. *J. Neurochem.* **1**, 228–233.

GOMORI, G. (1955). Preparation of buffers for use in enzyme studies. In *Methods in Enzymology*, ed. COLOWICK, S. & KAPLAN, N. O. **1**, p. 143. New York: Academic Press Inc.

HARRIS, G. W., JACOBSOHN, D. & KAHLSON, G. (1952). The occurrence of histamine in cerebral regions related to the hypophysis. *Ciba Foundation Colloquia on Endocrinology*, **4**, 186–194.

HOLTZ, P. & WESTERMANN, E. (1956). Über die Dopadecarboxylase und Histidindecarboxylase des Nervengewebes. *Arch. exp. Path. Pharmak.* **227**, 538–546.

KAHLSON, G., ROSENGREN, E., WESTLING, H. & WHITE, T. (1958). The site of increased formation of histamine in the pregnant rat. *J. Physiol.* **144**, 337–348.

KAHLSON, G., ROSENGREN, E. & WHITE, T. (1959). Formation of histamine by the foetus in the rat and man. *J. Physiol.* **145**, 30–31*P*.

KWIATKOWSKI, H. (1943). Histamine in nervous tissue. *J. Physiol.* **102**, 32–41.

LEE, H. M. & JONES, R. G. (1949). The histamine activity of some β-aminoethyl heterocyclic nitrogen compounds. *J. Pharmacol.* **95**, 71–78.

LINDAHL, K. M. (1958). Methylation of histamine by mouse liver *in vitro*. *Acta physiol. scand.* **43**, 254–261.

LINDELL, S.-E. & SCHAYER, R. W. (1958). The renal removal of injected [^{14}C] histamine from the blood in dogs. *Brit. J. Pharmacol.* **13**, 44–51.

RILEY, J. F. (1956). Cited by SCHAYER, R. W. (1956). Formation and binding of histamine by rat tissues *in vitro*. *Amer. J. Physiol.* **187**, 63–65.

RILEY, J. F. & WEST, G. B. (1953). The presence of histamine in tissue mast cells. *J. Physiol.* **120**, 528–537.

ROTHSCHILD, Z. & SCHAYER, R. W. (1958). Synthesis and metabolism of a histamine metabolite, 1-methyl-4(β-aminoethyl)-imidazole. *Biochim. biophys. acta*, **30**, 23–27.

ROTHSCHILD, A. M. & SCHAYER, R. W. (1958). Histidine decarboxylase from rat peritoneal fluid mast cells. *Fed. Proc.* **17**, 136.

SCHAYER, R. W. (1956*a*). Formation and binding of histamine by rat tissues *in vitro*. *Amer. J. Physiol.* **187**, 63–65.

SCHAYER, R. W. (1956*b*). Formation and binding of histamine by free mast cells of rat peritoneal fluid. *Amer. J. Physiol.* **186**, 199–202.

SCHAYER, R. W. (1957). Histidine decarboxylase of rat stomach and other mammalian tissues. *Amer. J. Physiol.* **189**, 533–536.

SCHAYER, R. W., DAVIS, K. J. & SMILEY, R. L. (1955). Binding of histamine *in vitro* and its inhibition by cortisone. *Amer. J. Physiol.* **182**, 54–56.

SCHAYER, R. W. & KOBAYASHI, Y. (1956). Histidine decarboxylase and histamine binding in rabbit platelets. *Proc. Soc. exp. Biol., N.Y.*, **92**, 653–655.

TABOR, H. (1954). Metabolic studies on histidine, histamine, and related imidazoles. *Pharmacol. Rev.* **6**, 299–342.

TALLAN, H. H., MOORE, S. & STEIN, W. H. (1954). Studies on the free amino acids and related compounds in the tissues of the cat. *J. biol. Chem.* **211**, 927–939.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol.* **123**, 451–481.

WATON, N. G. (1956*a*). Studies on mammalian histidine decarboxylase. *Brit. J. Pharmacol.* **11**, 119–127.

WATON, N. G. (1956*b*). The effect of organic solvents on mammalian histidine decarboxylase. *Biochem. J.* **64**, 318–322.

WESTLING, H. (1957). Observations on the action of histamine and related substances on the bronchial resistance in the guinea-pig. *Acta physiol. scand.* **40**, 75–82.

WHITE, T. (1958). Formation of histamine by brain tissue of the cat *in vitro*. *J. Physiol.* **143**, 62*P*.

WOLF, G., WU, P. L. & HECK, W. W. (1956). The metabolism of α-C^{14}-histidine in the intact rat. II. Radioactive excretion products in urine. *J. biol. Chem.* **222**, 159–169.