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EXPERIMENTS ON THE INHIBITION OF HISTAMINE FORMATION IN THE RAT

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Experimental work on inhibition of histamine formation *in vivo* originates in two prerequisite advances: the elucidation of the biogenesis of tissue and urinary histamine and the elaboration of procedures to measure the rate of histamine formation. Work in this field derived further impetus from the discovery of significant connexions between changes in the rate of histamine formation and physiological phenomena (for references see Kahlson, 1960; Schayer, 1964; Kahlson & Rosengren, 1965).

Inhibition of histamine formation *in vivo* was first reported by Schayer, Smiley & Davis (1954), who found that injection of cortisone inhibits histamine formation in the skin but not in the stomach of rats, and by Schayer (1956), who showed that prednisolone greatly reduces the histidine decarboxylase activity of particle-free extracts of rat lung. These steroids are relatively weak inhibitors and they do not appreciably reduce whole-body histamine forming capacity (HFC) as reflected in the urinary excretion of histamine (Kahlson, Rosengren & Thunberg, 1963).

Strong inhibition, resulting in lowering whole-body and tissue HFC to 10–20% of normal, was achieved by injecting semicarbazide in rats fed a pyridoxine-deficient diet (Kahlson & Rosengren, 1959a; Kahlson *et al.*, 1963). Although this means of inhibition proved rewarding in terms of new information on the physiology of histamine, these authors emphasized the limitation of the procedure, in that other enzymes in addition to histidine decarboxylase are inhibited. The uncertainty as to specificity can now be overcome by making use of the finding that α-methylhistidine is a strong inhibitor of whole-body HFC as well as of histidine decarboxylase *in vitro* (Kahlson, Rosengren & Svensson, 1962; Kahlson *et al.*, 1963), whereas α-methylDOPA is a weak inhibitor (Ganrot, Rosengren & Rosengren, 1961). These observations have led workers in this laboratory to define histidine decarboxylase as the enzyme responsible for histamine formation *in vivo*; this enzyme is strongly inhibited *in vivo* and *in vitro* by α-methylhistidine, but not by α-methylDOPA. The prefix "specific" is being dropped, and enzymes not complying with the definition, such as DOPA decarboxylase and related enzymes, referred to as "non-specific" histidine decarboxylase, or, by Lovenberg, Weissbach & Udenfriend (1962), as "aromatic L-amino acid decarboxylase," should, owing to their low affinity for histidine, not be classified as histidine decarboxylase.

The object of the present study was not to examine in detail the mechanism of inhibitory action, but merely to find out, using adequate methods, whether compounds alleged

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to be inhibitors, or likely to be endowed with such property, would be useful in experiments where strong inhibition of histamine formation *in vivo* would be helpful in elucidating biological roles for histamine.

METHODS

Animals

Unless otherwise stated, female rats of a strain bred at this institute were used.

Measurement of HFC in vivo

In the course of studies on the biogenesis of histamine Gustafsson, Kahlson & Rosengren (1957) observed that the histamine content of various tissues and the amount of the amine excreted were the same in germ-free reared and ordinary female rats, fed a histamine-free diet. It was concluded that the histamine of tissues and urine was endogenous in origin, and that the urinary excretion of the amine would reflect the rate of histamine formation and changes therein. This assumption has been confirmed many times in this laboratory by an isotopic method involving injection of radioactive histidine, and the simple non-isotopic method has consequently been widely employed to portray the rate of endogenous histamine formation.

In the present study, urine was collected from female rats kept in metabolism cages and fed a semi-synthetic histamine-free diet. The collection vessels contained a few drops of concentrated HCl to prevent formation and inactivation of histamine by bacteria. Serial 24 hr samples of urine were collected before, during and after administration of the drugs. Except where otherwise stated, aminoguanidine sulphate (20 mg/kg) was injected daily to prevent oxidative deamination of histamine (Schayer, Kennedy & Smiley, 1953). Urinary free histamine was assayed on the atropinized guinea-pig ileum after neutralization and dilution. The various tests of identification including cat's blood pressure, histamine antagonists, histaminase, chromatography, were applied as previously described (Gustafsson *et al.*, 1957). The excretion of histamine in the population of female rats studied was in the range 120–220 μg/24 hr, with only small fluctuations in the individual rat. Inhibition of histamine formation, as reflected in the urinary excretion, expressed as per cent, was the same in rats with low or high histamine excretion before inhibition.

A measure of whole-day histamine formation was also obtained in some cases by injecting subcutaneously 50 μg ^{14}C-histidine (Radiochemical Centre, Amersham, England) and determining the ^{14}C-histamine excreted in the urine on the three following days by an isotope dilution method. (For details of the method see Kahlson *et al.*, 1963). Values obtained during administration of the drugs were compared with those found in a normal control period.

Studies *in vivo* require that the animals become accustomed for at least several days to the metabolism cage, since it has been noticed that urinary histamine values usually decline for a period before a steady base-line is reached. State of feeding is also important, in that it is known that, if rats do not eat, urinary histamine output is reduced (Kahlson *et al.*, 1963). Both of these effects could be mistaken for enzyme inhibition.

Measurement of HFC in vitro

Some compounds were tested for inhibition *in vitro* by observing their effect on the rate of formation of ^{14}C-histamine when incubated with finely cut rat tissues and ^{14}C-histidine. Three separate test tissues were used, abdominal skin, lung and gastric mucosa. Details of the incubation procedure and method for assay of ^{14}C-histamine have been described previously (Kahlson *et al.*, 1963).

In experiments with chloramphenicol, various tissues were investigated for their histidine decarboxylase activity by the *in vitro* method described above, after administration of the substance *in vivo*.

RESULTS

Quercetin

It has been reported that this substance is an effective inhibitor of histidine decarboxylase both *in vitro* and *in vivo* (Smyth, Lambert & Martin, 1964). Among other results, it

was claimed that a dose of 10 mg/kg rat administered intraperitoneally gave 100% inhibition of overall histamine synthesis, as judged by measurement of ^{14}C-histamine in the urine after intravenous injection of ^{14}C-histidine.

In attempts to confirm these results by the methods used in his laboratory, it was found that even in doses higher than those employed by Smyth *et al.* (1964) there was no marked effect on urinary free histamine as assayed on the guinea-pig gut. Doses in the range 5–200 mg/kg were used in 4 rats, single and repeated, and by subcutaneous and intraperitoneal routes. Administration of 1 g/day in the food for 2 days also had no effect on the daily output of free histamine in the urine.

Using the isotopic method, amounts of ^{14}C-histamine which appeared in the urine after injection of ^{14}C-histidine and quercetin at doses of 5 or 10 mg/kg were not significantly different from those obtained in the same rat after injection of ^{14}C-histidine alone.

From these results it was concluded that quercetin was not an effective *in vivo* inhibitor of histidine decarboxylase.

Various antimitotic agents

Since there may be a connexion between histidine decarboxylase activity and certain types of rapid tissue growth (for references see Kahlson, Rosengren & Steinhardt, 1963), it seemed of interest to test some antimitotic agents, developed for cancer therapy, for inhibition of histamine formation.

(a) 1,2-dimethyl-4(p-carboxy-phenylazo)-5-hydroxybenzene (CPA), given in the food at a dose of 90 mg/day for 10 days produced a fall of about 50% in urinary histamine excretion immediately, but the output began to rise again after 1–2 days, despite continued administration of the drug.

(b) 1,2-dichloro-4-benzene-sulphonamido-5-nitrobenzene (DCBN), given in the food at a dose of 7.5 mg/day for 10 days produced no fall in urinary output of free histamine in two rats.

(c) p-di(2-chloroethylamino)-L-phenylalanine (melphalan), given at a dose of 5 mg/kg intraperitoneally in olive oil two times per day for one day produced a reduction of about 50% in urinary free histamine 5 days after administration in both rats used. However, the toxic effects noticed rendered the compound unsuitable for use as an *in vivo* inhibitor of HFC.

(d) NN-di-2-chloroethylaminobenzene (aniline mustard) given at a dose of 50 mg/kg intraperitoneally in olive oil, two times per day for one day, caused no marked change in urinary free histamine output in the two rats employed.

(e) Cyclophosphamide (Endoxan) was administered to 4 rats, 2 with and 2 without aminoguanidine treatment, at a dose of 50 mg/kg intraperitoneally two times per day for one day (in 0.9% w/v NaCl). Maximum effects were from 40–50% reduction of urinary free histamine. When the dose was reduced to 20 mg/kg and given once per day for 9 days there was no change in urinary histamine. *In vitro* experiments failed to demonstrate any effect of cyclophosphamide on rate of histamine formation at a concentration of 10^{-4} M, which corresponds to a dose of 100 mg/kg *in vivo*, assuming even distribution in the animal. It is possible that a reaction occurring *in vivo*, but not under

the *in vitro* conditions used, converts the substance to a relatively weak inhibitor of histidine decarboxylase

(f) Ethyl hydrazide of podophyltic acid (SPI), administered intraperitoneally in 0.9% w/v NaCl in a dose of 10 mg/kg once per day for 3 days and as a single dose of 20 mg/kg was without effect on urinary free histamine excretion in 4 rats.

4-bromo-3-hydroxy benzyloxyamine (*NSD 1055*) has been reported to be an inhibitor of histidine decarboxylase *in vitro* (Reid & Shepherd, 1963) and *in vivo* (Levine, Sato & Sjoerdsma, 1965). In the present study, urinary histamine excretion was followed over a period of about one month in 3 rats and during this time the drug was administered in 0.9% w/v NaCl on various occasions and in different doses. In one rat a dose of 100 mg/kg intraperitoneally was followed 6 days later by 100 mg/kg subcutaneously twice a day for 3 days, and after a further 12 days a single dose of 200 mg/kg (intraperitoneal) was given. On no occasion was there any reduction in urinary histamine excretion as judged by bioassay. In another 2 rats a dose of 100 mg/kg (in one case intraperitoneal and in the other subcutaneous) per day for 6 successive days failed to have any effect on urinary free histamine. In all 3 rats aminoguanidine had been administered daily, so in these last 2 rats this treatment was discontinued and, after a steady baseline had again been obtained, 200 mg/kg was injected intraperitoneally on 2 successive days, again without effect.

In another rat, an intraperitoneal dose of 200 mg/kg was given together with a subcutaneous injection of ^{14}C-histidine, and the radio-activity of the urine due to ^{14}C-histamine on the 3 days following was compared with that of a control period when no drug was given. Although no difference in non-isotopic histamine was seen in the bioassay, there was a 54% reduction in output of isotopic histamine when NSD 1055 was administered (see Table 1).

TABLE 1

EFFECT OF NSD1055 and α HH ON THE URINARY EXCRETION OF ^{14}C-HISTAMINE AFTER INJECTION OF ^{14}C-HISTIDINE

Compounds were administered in the doses indicated in the table at the beginning of the 24 hr collection period on the first day of the inhibitor test period.

Figures without brackets are C^{14} histamine in counts/min/total daily urine volume; figures in parentheses are the corresponding bioassay values in μg free histamine/24 hr (i.p. = intraperitoneal; s.c. = subcutaneous)

	Inhibitor test period			Control period		
Dosage (mg/kg)	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Rat M26 NSD1055 200 i.p.	62 (91)	34 (110)	23 (91)	130 (111)	26 (111)	17 (100)
Rat M25, α HH 200 i.p.	29 (79)	25 (83)	13 (79)	110 (66)	20 (79)	36 (86)
Rat M29, α HH 400 s.c.	18 (89)	19 (127)	10 (140)	94 (100)	17 (110)	13 (115)
Rat M30, α HH 400 i.p.	13 (109)	9 (114)	8 (120)	98 (120)	15 (144)	12 (120)

The compound was tested *in vitro* by adding it in an amount equivalent to an *in vivo* dose of 200 mg/kg (assuming even distribution) to the incubation mixture described earlier, using abdominal skin, lung and gastric mucosa as test tissues. The results, shown in Table 2, indicate a very strong inhibition, 98%.

TABLE 2

INHIBITION OF HISTAMINE FORMATION *IN VITRO* BY αHH AND NSD1055

The isotopic method used is briefly described in the text

	HFC, ng histamine formed/g tissue/3 hr		
	No addition	+αHH $0{\cdot}5\times10^{-3}$ M	+NSD1055 $0{\cdot}5\times10^{-3}$ M
Abdominal skin	50	2	1
Lung	1,220	144	7
Gastric mucosa	910	14	11

α-Hydrazino analogue of histidine (*αHH*) has been reported to be an inhibitor of histidine decarboxylase both *in vitro* and *in vivo* (Levine et al., 1965). In 3 rats various doses were tried: a single intraperitoneal injection of 100 mg/kg and 200 mg/kg/day for 3 days in one rat had no effect on urinary histamine output as judged by bioassay. In another 2 rats 100 mg/kg was injected intraperitoneally every day for 6 days and no reduction was seen. After aminoguanidine administration was discontinued, doses of 200 mg/kg (intraperitoneal) were given for 2 days, again without effect. In a further 3 rats, excretion of ^{14}C-histamine after injection of ^{14}C-histidine was measured with and without administration of the drug, doses of 400 mg/kg (intraperitoneal), 400 mg/kg (subcutaneous) and 200 mg/kg (intraperitoneal) being used. The results, shown in Table 1, indicate inhibitions of 87%, 81% and 75% respectively, although the bioassay of non-isotopic urinary histamine showed no inhibition. For 2 of these rats the bioassay figures were compared with those obtained by a fluorometric method (Shore, Burkhalter & Cohn, 1959, as modified by Green & Erickson, 1964, and further modified in this laboratory, Thunberg, unpublished).

The compound was tested *in vitro* as described for NSD 1055. The results, shown in Table 2, indicate an inhibition of 88–98%.

Having seen that NSD 1055 and α-HH inhibited the decarboxylation of injected ^{14}C-histidine more effectively in experiments in which these compounds were injected concurrently with, or shortly after, the injection of ^{14}C-histidine, it was decided to investigate whether repeated injections of these inhibitors over 2 days would inhibit the decarboxylation of intracellular histidine. In 2 rats α-HH was injected subcutaneously, twice 100 mg/kg on the first day and a further four times 50 mg/kg on the second day. On the second day only, a significant reduction in urinary histamine excretion occurred, 20% and 28% inhibition respectively as compared with histamine excretion before inhibition. In 4 rats, 2 of which were the ones previously tested for the effect of α-HH, the compound NSD was administered for 2 days, three times 100 mg/kg subcutaneously on the first day, three times 200 mg/kg intraperitoneally on the second day. As with α-HH, reduction in urinary histamine was insignificant during the first day, but on the second day 35%, 44%, 50% and 63% inhibition occurred as expressed in the urinary histamine. It thus appears that even under the influence of successive doses of these compounds the inhibition of whole-body HFC is slight or moderate. Obviously, with the procedures adopted in the experiments with α-HH, formation of histamine from exogenous histidine is much more strongly inhibited than is the decarboxylation of endogenous histidine. Consequently, results with exogenous histidine, by the procedures employed, do not parallel inhibition of intracellular histamine formation.

Daily output of urinary non-isotopic histamine was followed by bioassay in 8 rats treated with chloramphenicol as indicated in Table 3. This table gives estimated inhibitory effects, comparing the minimum post-treatment value with the pre-treatment level of urinary histamine in each individual rat. It can be seen that, except in one case, the inhibitory effects are very small.

TABLE 3

INHIBITORY EFFECT OF CHLORAMPHENICOL ON URINARY HISTAMINE AS MEASURED BY BIOASSAY AND BY EXCRETION OF ^{14}C-HISTAMINE AFTER INJECTION OF ^{14}C-HISTIDINE

The figures for inhibitory effects as measured by the bioassay and isotopic method cannot be directly compared in each rat, since both were not necessarily calculated for the same days. The inhibitory effect is expressed as per cent of non-isotopic histamine (bioassay) excreted and of ^{14}C-histamine excreted as compared with the pre-treatment values

Rat	Dosage (mg)	Treatment No. of days	Inhibitory effect % Bioassay	Inhibitory effect % Isotopic Method
1	32 in food/day	1	15	10
2	32 in food/day	3	0	20
3	32 in food/day	3	30	10
4	32 in food/day	4	20	
5	16 s.c. twice/day	1	30	0
6	16 s.c. twice/day	3	50	20
7	16 s.c. twice/day	3	30	30
8	16 s.c. twice/day	4	37	

Assays of isotopic histamine excretion were made in 6 of the rats detailed in Table 3, where inhibitory effects, as judged by comparison with a pre-treatment control period are shown. The effects of chloramphenicol as measured by this method were very small.

The tissues of 2 rats which had been given chloramphenicol for 4 days, one 32 mg in the food and the other 16 mg twice per day subcutaneously, were incubated to determine HFC. On comparison with values obtained from control rats it was found that the HFC of gastric mucosa only was reduced to 50% and 20% respectively. However, a more detailed investigation aimed at confirming these results failed to do so, and in 18 Sprague Dawley rats treated in the same way with the drug none showed any significant difference from the controls.

DISCUSSION

The female rat is particularly suitable for studies of inhibition of histamine formation, as in this animal histidine decarboxylase, to the best of present knowledge, is the enzyme largely, or solely, instrumental in the formation of histamine *in vivo*. This view is based on the observation that in the rat α-methylDOPA, which is known to inhibit amino acid decarboxylases other than histidine decarboxylase, does not appreciably inhibit histamine formation, as judged by urinary excretion of the amine (Kahlson *et al.*, 1963). In the guinea-pig, by contrast, a reduction in urinary histamine occurs after α-methylDOPA administration, presumably owing to inhibition of DOPA decarboxylase in the kidney (Schayer & Sestokas, 1965).

Among the various drugs investigated in the present study, none was more effective than the non-toxic α-methylhistidine ; none produced inhibition of whole-body and tissue HFC to the extent of 80–90% as previously seen with semicarbazide superimposed on the

pyridoxine deficient diet. There is evidence to suggest that, when the intracellular histamine content falls, a feed-back accelerates histidine decarboxylase activity (Kahlson *et al.*, 1963 ; Kahlson, Rosengren, Svahn & Thunberg, 1964). A coupling between end-product and enzyme would make it difficult or impossible to inhibit histamine formation completely *in vivo*. This should be borne in mind when contemplating inhibition of histamine formation as a means to explore physiological functions of histamine. Thus foetal development in the rat could be prevented (Kahlson & Rosengren, 1959b) and wound healing retarded (Kahlson, Nilsson, Rosengren & Zederfeldt, 1960) when histamine formation was inhibited by about 85% but not 50%. Parietal cells, however, gave normal secretory responses even when mucosal histamine content had been reduced to very small fractions of their initial values (Kahlson *et al.*, 1964).

As mentioned earlier, the two methods used for measuring whole-body HFC, the non-isotopic and the isotopic, as far as experience in this laboratory goes, have given consistent results. This applies particularly to circumstances in which whole-body HFC is elevated. In the present report, however, substantial inhibition was noted with two compounds, NSD 1055 and the α-hydrazino analogue of histidine, with the isotopic method, whereas the non-isotopic method failed to reveal inhibition on the first day of treatment, this being in complete contrast to the isotopic method. On the second day of treatment, with repeated injections of inhibitor, a slight inhibition was obtained with α-HH, and a moderate inhibition with NSD 1055. As judged by the first day of urinary excretion a single injection of inhibitor is more effective in inhibiting the decarboxylation of injected ^{14}C-histidine than are repeated injections in inhibiting decarboxylation of histidine in cells. Not only may the duration of action of a single injection be brief, but the cessation of inhibition may be followed by a phase of " overshoot " in the rate of histamine formation of the kind described earlier (Kahlson *et al.*, 1963), thus concealing an inhibition which might actually have occurred. Alternatively, these two inhibitors might for unknown reasons interfere more easily with the conversion of injected histidine than with the substrate normally contained in the cells.

Studies with the object of assessing the potency of an *in vivo* inhibitor of histidine decarboxylase should be so conducted as to ensure that the decarboxylation of intracellular histidine is determined. This object can be achieved with the non-isotopic method. The most effective means of *in vivo* inhibition available at present has been discovered using this method. The cause of the discrepancy in results with NSD 1055 and α-HH obtained with the non-isotopic and the isotopic methods is not clear. The fact that these two inhibitors, under the experimental conditions employed, are much more effective in inhibiting the decarboxylation of injected histidine than intracellular histidine, raises the question as to the usefulness of the isotopic method in determination of histamine formation *in vivo*. The issue appears complex ; more work is required to elucidate the circumstances which result in the discrepancy between the two methods. The issue appears the more complex as the discrepancy between the methods has so far been found only with these two particular inhibitors.

In experiments employing inhibitors of histidine decarboxylase with the object of learning the role for histamine, it appears a prerequisite to prove that the intracellular histamine formation, and not merely the histamine formation from injected histidine, is being inhibited.

It appears disturbing that so much of the work published in this field could not be confirmed in the present investigation. Similarly, wide discrepancies exist in published records regarding the rate of histamine formation and changes therein in normal states and tissues. These inconsistencies are likely to become settled by agreement as to what enzyme(s) is to be measured and by what methods.

SUMMARY

1. A number of compounds have been examined for their inhibitory effect on histamine formation in rats *in vivo* and *in vitro* using non-isotopic and isotopic methods.

2. The compounds CPA (1,2-dimethyl-4(p-carboxy-phenylazo)-5-hydroxybenzene), melphalan and cyclophosphamide produced a maximum inhibition of about 50% *in vivo*.

3. Quercetin, alleged to be a potent inhibitor of histamine formation, was found to be ineffective in the present experiments.

4. The isotopic but not the non-isotopic method, detected an inhibition, after a single injection, of 54% using NSD and of 80–90% using α-HH, *in vivo* ; on repeated injections of these inhibitors a moderate degree of inhibition was noted with the non-isotopic method.

5. *In vitro* the compounds NSD 1055 and α-HH were strong inhibitors, reducing histamine formation by 80–100%.

6. Chloramphenicol, the effects of which were taken earlier as evidence of exogenous biogenesis of tissue histamine, was found in the present study not to reduce significantly the rate of whole-body histamine formation.

7. Possible explanations of the discrepancies observed with the isotopic and the non-isotopic method are discussed, and it is emphasized that in experiments aiming at studying the consequences of inhibition of histamine formation, evidence must be provided that the decarboxylation of intracellular physiological histidine has been inhibited.

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REFERENCES

GANROT, P., ROSENGREN, A. M. & ROSENGREN, E. (1961). On the presence of different histidine decarboxylating enzymes in mammalian tissues. *Experientia*, **17**, 263–264.

GREEN, H. & ERICKSON, R. W. (1964). Effect of some drugs upon rat brain histamine content. *Int. J. Neuropharmac.*, **3**., 315–320.

GUSTAFSSON, B., KAHLSON, G. & ROSENGREN, E. (1957). Biogenesis of histamine studied by its distribution and urinary excretion in germ free reared and not germ free rats fed a histamine free diet. *Acta physiol. scand.*, **41**, 217–228.

KAHLSON, G. (1960). A place for histamine in normal physiology. *Lancet*, **1**, 67–71.

KAHLSON, G. & ROSENGREN, E. (1959a). Inhibition of histamine formation and some of its consequences. *J. Physiol.*, **149**, 66–67P.

KAHLSON, G. & ROSENGREN, E. (1959b). Prevention of foetal development by enzyme inhibition. *Nature, Lond.*, **184**, 1238–1239.

KAHLSON, G. & ROSENGREN, E. (1965). Histamine. *A. Rev. Pharmac.*, **5**, 305–321.

KAHLSON, G., NILSSON, K., ROSENGREN, E. & ZEDERFELDT, B. (1960). Wound healing as dependent on rate of histamine formation. *Lancet*, **2**, 230–234.

KAHLSON, G., ROSENGREN, E. & STEINHARDT, C. (1963). Histamine-forming capacity of multiplying cells. *J. Physiol.*, **169**, 487–498.

KAHLSON, G., ROSENGREN, E. & SVENSSON, S. E. (1962). Inhibition of histamine formation *in vivo*. *Nature, Lond.*, **194**, 876.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1963). Observations on the inhibition of histamine formation. *J. Physiol.*, **169**, 467–486.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol.*, **174**, 400–416.

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 hydroxy benzyloxyamine. *Biochem. Pharmac.*, **14**, 139–149.

LOVENBERG, W., WEISSBACH, H. & UDENFRIEND, S. (1962). Aromatic L-amino acid decarboxylase. *J. biol. Chem.*, **237**, 89–93.

REID, J. D. & SHEPHERD, D. M. (1963). Inhibition of histidine decarboxylases. *Life Sci. Oxford*, **2**, 5–8.

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

SCHAYER, R. W. (1964). Histamine and autonomous responses of the microcirculation; relationship to glucocorticoid action. *Ann. N.Y. Acad. Sci.*, **116**, 891–898.

SCHAYER, R. W. & SESTOKAS, E. (1965). Histidine decarboxylating enzymes of guinea pig liver and kidney. *Fedn. Proc. Fedn. Am. Socs. exp. Biol.*, **24**, 578.

SCHAYER, R. W., KENNEDY, J. & SMILEY, R. L. (1953). Studies on histamine-metabolizing enzymes in intact animals. II. *J. biol. Chem.*, **205**, 739–748.

SCHAYER, R. W., SMILEY, R. L. & DAVIS, K. J. (1954). Inhibition by cortisone of the binding of new histamine in rat tissues. *Proc. Soc. exp. Biol. Med.*, **87**, 590–592.

SHORE, P. A., BURKHALTER, A. & COHN, V. H. (1959). A method for the fluorometric assay of histamine in tissues. *J. Pharmac. exp. Ther.*, **127**, 182–186.

SMYTH, R. D., LAMBERT, R. & MARTIN, G. J. (1964). Quercetin inhibition of specific histidine decarboxylase. *Proc. Soc. exp. Biol. Med.*, **116**, 593–596.