THE DISPOSITION AND FATE OF

HISTAMINE IN ARTERIES

A THESIS SUBMITTED FOR THE DEGREE OF

Master of Science

by

Michael John Stacey   B.Sc. (Hons.), B.D.S.

Department of Clinical and Experimental Pharmacology

The University of Adelaide

South Australia

March 1984

C O N T E N T S

D E C L A R A T I O N

I declare that this thesis contains no material
which has been accepted for the award of any
other degree or diploma in any university, and
to the best of my knowledge contains no
material previously published by another person,
except where due reference is made in the text.
I give consent to this copy of my thesis, when
deposited in the University Library, being
available for loan and photocopying.

<div align="right">Michael John Stacey</div>

S U M M A R Y

Although histamine is present in mast cells in most species, in many tissues histamine is also contained in cells other than mast cells and its cellular distribution, metabolism, and function is unknown. This thesis is concerned with two questions:

(1) Is histamine present in nerves which innervate small blood vessels? This question relates to the distribution of endogenous histamine and its possible neurotransmitter role.

(2) What is the fate of exogenous histamine in the blood vessel wall? This relates to the mechanisms of uptake and catabolism of exogenous histamine in the smooth muscle cells and nerves of arteries.

Much of the study on endogenous histamine was carried out on the rabbit ear artery (REA). This tissue is more representative of small resistance vessels than the more commonly employed aorta. Furthermore, it is readily denervated and its vascular pharmacology has been extensively studied. In this study it was shown to contain large amounts of histamine. However, neither chronic sympathetic denervation nor chronic afferent denervation caused significant decreases in the histamine content. It was concluded that the major proportion of the endogenous histamine in the artery was not contained in nervous tissue.

The presence and distribution of mast cells was studied in the REA, rabbit aorta, central ear vein and the great auricular nerve. Mast cells were detected in the distal portions of the REA and in the connective tissue around the rabbit ear vein and auricular nerve (distal segments only). Mast cells were not found in the rabbit aorta. Since all the above vessels contained histamine, it is concluded that mast cells may represent a source of histamine in the REA and rabbit ear vein. This could account for the finding that histamine content in the distal region of the REA (10.2 µg/g tissue) is approximately twice that in the proximal region (4.9 µg/g tissue).

The studies on exogenous histamine catabolism required the development of analytical techniques for separating histamine and its catabolites. The results indicated that the rabbit aorta, REA and guinea pig aorta, when

incubated with ^{14}C-histamine, accumulated ^{14}C material against a concentration gradient. The above tissues converted histamine to catabolites, whose distribution suggested that both oxidative deamination and N-methylation were significant pathways of catabolism. This study found no evidence that procedures which disrupt catecholamine uptake and storage in sympathetic nerves affected histamine uptake and metabolism. These procedures comprised the inhibition of neuronal uptake by cocaine in the rabbit aorta, and chronic sympathetic denervation of the REA by removal of the superior cervical ganglion.

The above studies provided no evidence to suggest that the intramural nerves were either a site of storage or catabolism in the REA or aorta. In this respect the results do not support a neurotransmitter role for histamine in the peripheral arteries. However, the results do not exclude the possibility that there may be interaction at the post synaptic level between the distributions of histamine and noradrenaline. This possibility emerges because in the rabbit aorta, corticosterone, an inhibitor of extraneuronal uptake of noradrenaline,also inhibited the uptake and accumulation of ^{14}C-histamine in this tissue.

A I M

The aim of this thesis was to study the disposition and fate of hist-amine in arteries. This study is presented in two parts. Firstly, the localization of endogenous histamine in the vessel wall was investigated by comparing selectively denervated blood vessels with untreated controls. In addition the qualitative distribution of mast cells was studied in three rabbit blood vessels by histochemical techniques. Secondly, the possible role of sympathetic nerves for the uptake and catabolism of exogenous histamine was studied in rabbit blood vessels.

The study arose out of an investigation of the endogenous histamine content and catabolism of exogenous histamine in the rabbit aorta by Dr. Foldes. Most of the candidate's research has been on the rabbit ear artery but some comparative catabolic studies were carried out on the guinea pig aorta. The thesis commences with a brief survey of the disposition and metabolism of histamine in blood vessels (Chapter 1) and of the methods of analysing histamine in biological fluids. Chapters 2 and 3 deal with the effects of denervation on the content of endogenous histamine in the rabbit ear artery. Chapter 4 discusses the comparative distribution of mast cells in the rabbit ear artery, ear vein, great auricular nerve and aorta.

The last chapter (5) describes the comparative catabolism of exo-genous histamine in the rabbit ear artery with that in the rabbit aorta and the aorta of the guinea pig. In both the studies of endogenous and exo-genous histamine, emphasis is placed on the possible neurotransmitter role of histamine.

PUBLICATIONS

Part of the material contained within this thesis has already been published as follows.

FOLDES, A., STACEY, M. and de la LANDE, I.S. (1976):

Evidence for the extraneuronal localisation of histamine in the rabbit aorta.

Proc. Aust. Physiol. Pharmacol. Soc., 7/2:147P.

FOLDES, A., STACEY, M.J. and de la LANDE, I.S. (1978):

Histamine metabolism in aortae of two histamine sensitive species.

(From Proceedings of Aust. Soc. Clin. Exp. Pharmacol. 10th meeting Nov. 1976).

Clin. Exp. Pharmacol. Physiol., 5:239.

STACEY, M.J., FOLDES, A. and de la LANDE, I.S. (1978):

Localisation of histamine in rabbit central ear artery.

(From Proceedings of Aust. Soc. Clin. Exp. Pharmacol. 10th meeting Nov. 1976).

Clin. Exp. Pharmacol. Physiol., 5:240.

FOLDES, A. and STACEY, M.J. (1977):

The metabolism of exogenous histamine by aortic tissues.

Aust. J. Exp. Biol. Med. Sci., 55:441.

These are reproduced for reference within this thesis (see Appendix).

ACKNOWLEDGEMENTS

C H A P T E R 1

REVIEW OF THE METABOLISM OF HISTAMINE
IN VASCULAR TISSUES AND METHODS FOR
THE ASSAY OF HISTAMINE IN BIOLOGICAL FLUIDS

1.1 Synthetic Pathways for Histamine

Two enzymes are capable of converting histidine to histamine in mammalian tissues. The first of these, aromatic amino acid decarboxylase (dopa decarboxylase E.C. 4.1.1.26), has been purified from rabbit and guinea pig kidney tissues. This enzyme was found to have a low affinity for L-histidine and a higher affinity for aromatic amino acids. The enzyme exhibited optimum activity at pH≃9 and its activity towards its weaker substrates was enhanced by the addition of low concentrations of benzene (Lovenberg et al, 1962).

Some tissues were found to contain a second enzyme capable of histamine synthesis from L-histidine. This enzyme had a much greater affinity for L-histidine than dopa decarboxylase. This second enzyme L-histidine apodecarboxylase (HD, E.C. 4.1.1.22) was found in high concentrations in rabbit platelets (Schayer and Kobayashi, 1956) and mast cells (Rothschild and Schayer, 1959). A detailed study of the kinetics of HD from rat stomach and mouse transplantable mast cell tumours showed a hundred fold greater affinity for histidine (Km 5×10^{-4} M) than dopa decarboxylase (Km 5×10^{-2} M). In addition, HD was not inhibited by the addition of methyldopa nor was its activity enhanced by the addition of benzene. Both of the above enzymes require the co-factor pyridoxal phosphate (B_6) to be present for activity (Weissbach et al, 1961).

Unlike catecholamine precursors, histidine is present in all cells, therefore a regulatory mechanism for histamine synthesis must exist via the availability of either the substrate or the enzyme. Several studies (see Schayer, 1963), have reported increases in tissue levels of histidine decarboxylase following exposure to irritants, endotoxin, or infectious agents; similar increases were noted in wound healing or tissues undergoing rapid growth. Schayer (1963) refers to this newly synthesised histamine as induced histamine. These changes are probably due to the denovo synthesis of HD (Schayer and Reilly, 1968). Schayer (1966) regards this inducible pool of the enzyme to be different from the enzyme

associated with mast cells. These increases in tissue HD have also been noted in vascular tissues of rats and mice (Schayer, 1962) after various stimuli; Schayer has also postulated a role for induced histamine regulation of the microcirculation (Schayer, 1965).

The capacity of tissues to form histamine from L-histidine has also been termed the histamine-forming capacity (HFC). The HFC is measured as the amount of $^{14}CO_2$ evolved over a two hour incubation of the tissue with ^{14}C histidine. Hollis has assumed this unit to be a measure of the activity of HD (Hollis and Rosen, 1972). The capacity of blood vessels to form histamine was reported in the rat vena cava (Schayer, 1962), in bovine aorta (Hollis and Rosen, 1972) and later in the rabbit aorta (Hollis and Sloss, 1975) and rat aorta (Yarnal and Hollis, 1976).

1.2 Vascular Synthesis of Histamine

The HFC of aortic tissues has been localised in several studies. In one study, endothelial cells were prepared by washing everted bovine aortic segments with a trypsin solution to release endothelial cells, the activity of this cell suspension had about 15x the HFC of an intact intima-media preparation (Hollis and Rosen, 1972). Similarly, the HFC of rabbit aorta was greater in the intimal cell preparation than intact media-intima layers. The HFC of aortae was shown to be altered under various mechanical stresses and chemical agents, e.g. the effect of feeding rabbits a 0.5% cholesterol diet resulted in a 60% increased HFC in whole thoracic aorta and a small increase in the abdominal aorta (Hollis and Sloss, 1975). The HFC of rabbit thoracic aorta increased 24 hours after the induction of hypertension via carotid sinus ischemia (Bolitho and Hollis, 1975). This elevated HFC did not persist and returned to control values after two weeks. This transient increased HFC is thought to be the result of a shearing stress across the vessel wall as the same elevation in HFC occurred if the aorta was perfused in situ with elevated perfusion pressures (Hollis and Ferrone, 1974). Rabbit aortic HFC was also studied where a

pulsatile perfusion was applied to the aorta in situ. These aortae were perfused with platelet free blood via inflow and outflow cannulae for 1 hour at increasing pulsatile perfusion pressures. These experiments demonstrated an increasing HFC with increases in applied shear stress (De Forrest and Hollis, 1978). This appears to be the first evidence for a possible vascular control mechanism involving the histidine decarboxylase system in larger blood vessels.

A similar mechanism was proposed by Schayer (1962) for the control of the microcirculation as follows. Histamine was thought to be synthesised in or close to endothelial cells in response to changes in the environment of the blood vessel. This amount of newly synthesised histamine was thought to be a small fraction of the total vascular content of histamine. These small amounts of newly synthesised histamine were thought only to have actions within cells or on adjacent cells.

The studies reported above did not measure actual changes in total histamine but rather increased amounts of its synthetic enzyme HD. The relationship between total vascular histamine content and the induction of its synthetic enzyme HD remains unclear. This is illustrated in studies of vascular histamine in hypertensive rats. In one study (Holcslaw and Imhoff, 1978) rats were made hypertensive with deoxycorticosterone implants and Na-Cl administered via the drinking water until their blood pressure was elevated above 160 mm Hg. This treatment resulted in a reduction in the total vascular histamine content of the abdominal aorta and the femoral artery but the levels of HD were not measured. A second study (Yarnal and Hollis, 1976) measured the HFC of rat aorta after hypertension induced by ligation of the abdominal aorta between the origins of the renal arteries. These experiments revealed a peak increase of HFC after 24 hours followed by a lesser but sustained increase (55%) after 8 days. After acknowledging the differences in experimental design it should be recognised that changes in total histamine content may not resemble the levels of HD. Thus, induced HD may only be responsible for the synthesis of a small part of the total pool of histamine.

1.3 Vascular Histamine Contents

Blood vessels are known to contain measurable amounts of histamine (Howland and Spector, 1972, Ryan and Brody, 1972, Maling et al, 1971, Adams and Hudgins, 1976, Foldes et al, 1977). Some general trends are apparent when total histamine contents of blood vessels are examined (Howland and Spector, 1972). In the rat the lowest histamine contents appear in the aorta whilst higher contents were found in the mesenteric and renal arteries,whereas, in the rabbit the veins contained higher contents than their associated arteries in the vessels studied. In addition, there was a regional distribution within the same vessel. The distal segments of the rat aorta and mesenteric artery contained about twice as much histamine as proximal segments of the same vessel. In the dog (Ryan and Brody, 1972) seven pairs of arteries and veins were examined (splenic, brachial, gracilis, femoral, renal, mesenteric and tibial). With the exception of the tibial artery all the veins contained higher concentrations than the corresponding arteries. In contrast to these artero-venous differences for histamine, when the total catecholamine contents are measured they indicate that in the dog the higher levels are found in the arteries with lower levels in the corresponding veins (Mayer et al, 1968, Rolewicz et al, 1970). There is a marked species difference between the aortic histamine contents. In one study the guinea pig aorta contained 43 times as much histamine as the rabbit aorta and 9 times as much as the rat aorta (Maling et al, 1971).

1.4 Mast Cells and Blood Vessels

It was long believed that the HA content of tissues was directly related to the population of mast cells (Riley and West, 1966). This concept was extended by the histological evidence of the microcirculation of the rat where mast cells were seen in close association with blood vessels (Riley, 1953). A later comprehensive study of blood vessels from

various species using histochemical techniques was not able to demonstrate mast cells within the walls of any of the blood vessels studied,with the exception of the bovine aorta (El-Akad and Brody, 1975). This led these authors to postulate that measured vascular histamine must be non-mast cell in origin. The histamine content of two aortic tissues was examined after pretreatment with compound 48/80. Histamine was reduced by 33% in the guinea pig aorta where 48/80 was administered in vivo. A greater (65.7%) reduction was seen in the rat thoracic aorta when 48/80 was administered in vitro (Maling et al, 1971). The observed reduction in the guinea pig tissues was in spite of a reported difficulty in depleting mast cells in vivo in guinea pigs (Mota, 1966). When the morphological evidence is related to the effects of 48/80 it is possible that this agent may be capable of releasing histamine from non-mast cell stores.

1.5 Neuronal Stores of Histamine

The most detailed study of the association between nervous tissue and histamine has been carried out in the central nervous system (see review by Schwartz, 1977). Histamine has been detected in the CNS of various species with a mean cerebral level of 50 ng/g , the highest level being found in the hypothalamus. This amine is unevenly distributed in any given brain region. The synthesis of histamine is thought to occur within the CNS, as histamine does not readily cross the blood brain barrier. Histamine is synthesised via HD and is not thought to be contained within catecholaminergic or serotonergic neurons within the CNS as there is no loss of HD activity on selective denervation of either nerve type. The only example of discrete histaminergic neurones has been found in the mollusc, Apylsia Californica, where all the neurons are found to take up radiolabelled histidine, but only a specific group of neurones convert this to histamine. It is suggested that this enzyme is similar to the vertebrate enzyme (Weinreich and Yu, 1977). Thus the evidence from the CNS suggests that histaminergic neurones may exist but what of the periphery?

The distribution of histamine has been studied in the canine autonomic nervous sytem (Ryan and Brody, 1970). Tritiated histamine was administered to dogs in vivo and the uptake of labelled histamine was measured in various types of nerve bundles. The highest uptake was found in post ganglionic sympathetic nerves with lower concentrations in parasympathetic and motor nerves. When the endogenous histamine contents were measured the highest levels were found in post ganglionic sympathetic nerves (10.1 – 17.3 μg/g), with lower amounts in parasympathetic and motor nerves. Pretreatment with reserpine or compound 48/80 did not reduce the level of histamine in autonomic nerves. At that time these authors considered that histamine may be stored in sympathetic histaminergic fibres (Ryan and Brody, 1970). However, this is not inconsistent with a mast cell store that is inaccessible to degranulation by 48/80, such as those deep within the endonuerium (Olsson Y, 1968). In a later study by Ryan and Brody (1972) the effect of chronic denervation (2-3 weeks) was examined in the dog gracilis muscle. There was a slight increase in the histamine content of the muscle compared with sham operated controls. This result did not support the view that histamine might be exclusively concentrated in nerves but did not discount the view that only a small fraction of tissue histamine may be found in nerves or that there had been a compensatory increase in non-neuronal histamine synthesis. In the same study, the histamine contents of several canine arteries and veins were measured and these values were found to increase or remain unchanged if the adventitia was removed prior to assay. This later finding supported a non-neuronal store of histamine perhaps contained within the intima or medial smooth muscle layer.

1.6 Catabolic degradation of histamine in tissues

The catabolic pathways for histamine in tissues (Figure 1.1) will be discussed including the effects of inhibitors of these processes. Where possible the role of each pathway will be related to its effect on the in-

Figure 1.1   PATHWAYS OF HISTAMINE SYNTHESIS AND CATABOLISM
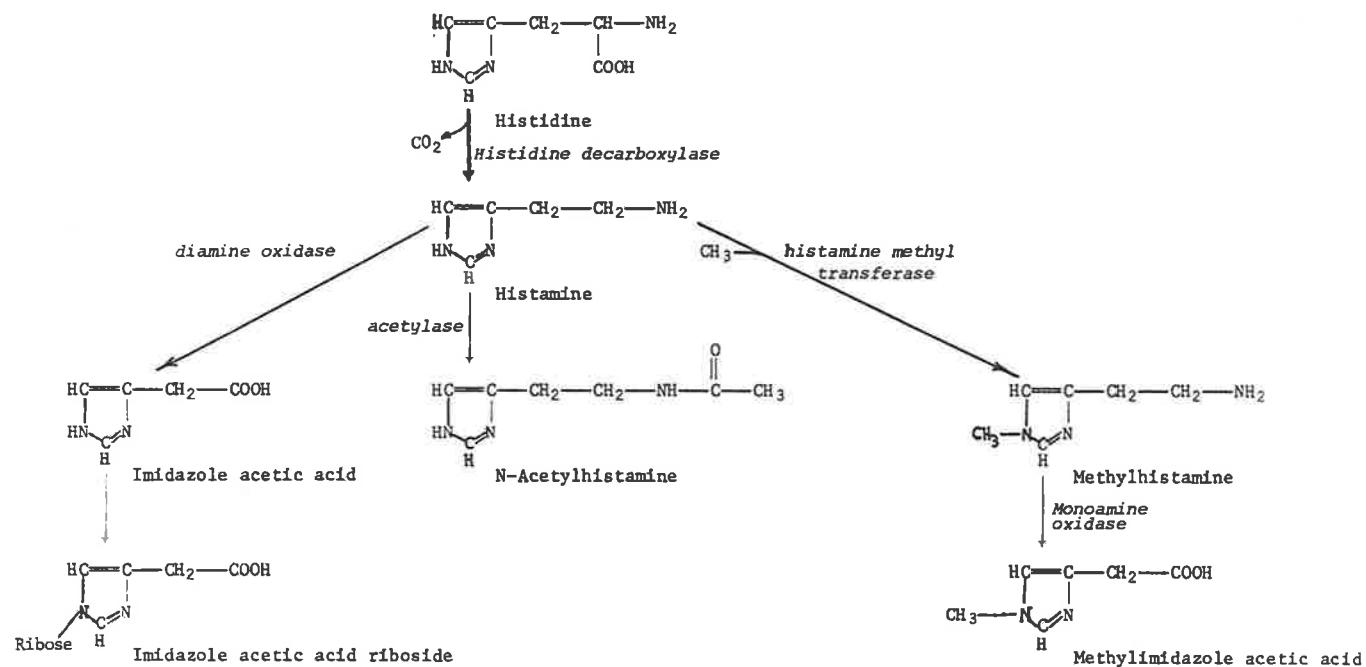
activation of pharmacologic responses in vascular and non-vascular tissues. The catabolism of histamine has been studied for many years. In 1929 Best showed that animal tissues were capable of catabolizing histamine and named the enzyme responsible as histaminase. Subsequently histaminase was considered to be identical to a second enzyme, diamine oxidase (Zeller, 1938). Later studies confirmed that diamine oxidase (Histaminase, pyridoxal dependant amine oxidase EC 1.4.3.6.), could oxidatively deaminate both histamine and diamine substrates, but the enzyme had differing Km and Vmax values for each (Hill and Bardsley, 1975). Several agents are known to inhibit diamine oxidase (DAO) including carbonyl reagents (Zeller, 1942) and aminoguanidine. (Grabbe and Bardsley, 1974). Hydrallazine has been used in this study to inhibit diamine oxidase in isolated blood vessels although its enzyme inhibitory effects had only previously been examined on purified diamine oxidase preparations (Grabbe and Bardsley, 1974).

A second pathway of histamine degradation involves the methylation of the imidazole ring to yield methyl histamine; this product may be converted to methylimidazole acetic acid via the enzyme monoamine oxidase. The above methylation step involves the donation of a methyl group from s-adenosyl methionine in conjunction with histamine methyl transferase (E.C. 2.1.1.8. HMT). Studies on purified extracts of histamine methyl transferase from guinea pig brain have indicated that the enzyme is highly specific for histamine with a pH optima 7.2-7.4 and it is competitively inhibited by its own product (Brown et al, 1959). In the same study histamine methyl transferase activity was found in cardiac tissue of guinea pig, cat, rabbit and mouse but only trace activity in the rat heart. The specificity of this enzyme has rendered it very suitable as a reagent for the radioenzymatic assay of histamine (Snyder et al, 1966). Antimalarial drugs have been shown to be good inhibitors in vitro. Amodiaquin at a concentration of 10^{-3} M was able to completely inhibit the activity of histamine methyl transferase, whilst 10^{-7} M still resulted in 50% reduction of enzyme activity (Cohn, 1965). Guinea pigs and rats were pretreated with amodiaquin which resulted

in inhibition of histamine methyl transferase in all tissues (Cohn, 1966).

Later studies have confirmed the inhibitory effects of amodiaquin on histamine methylation both in vivo and in vitro (Schayer and Reilly, 1974, 1975). The actions of amodiaquin may be more complex than simple enzymic inhibition as suggested by studies in the guinea pig ileum (Fontaine, 1980). In the later study amodiaquin (10^{-7} M) was seen to potentiate contractile responses of the ileum to agents including histamine, acetycholine and barium chloride. This result would not have been expected on the basis of a simple enzymic inhibition of histamine methyl transferase. The only effects of amodiaquin (10^{-5} M) tested in this study have been those relating to its ability to inhibit histamine methylation. The concentration used in these studies should be enough to provide 100% inhibition of the enzyme although this has not been tested directly.

The possibility that there may be a sex difference in the activity of histamine methyl transferase has been investigated. The determination of urinary excretion of histamine and methyl histamine after administration of labelled histamine in gonadectomized male and intact female guinea pigs revealed no sex differences. The effect of gonadectomy did not influence the histamine and methyl histamine output (Jonassen and Setterqvist, 1975). There is a sex difference in the urinary excretion of histamine and methyl histamine in rats but this can be explained by greater amounts of methylating enzyme in the male rat kidney (Snyder and Axelrod, 1965). Thus sex differences are probably related to the distribution of the enzyme rather than hormonal effects.

Acetylation appears to be a minor pathway of inactivation and this activity is very low or lacking except in the flora of the intestine (Maslinski, 1975). Acetylhistamine is thought to be devoid of pharmacological activity. The presence of a deacetylating enzyme has been reported in rats, mice and guinea pigs and some of its properties characterized (Endo et al, 1976). The significance of this is also unknown. Other pathways for histamine catabolism include Riboside conjugation and methylation of imidazole acetic acid by monoamine oxidase (MAO).

The contractile responses to histamine on the isolated guinea pig ileum were shown to be potentiated by inhibitors of DAO (Arunlakshana et al, 1954). These authors found a close parallel between the degree of inhibition of DAO activity and the potentiation observed. The tissues which displayed the greatest inhibition of DAO activity showed the greatest potentiation. Compounds that were not inactivated by histaminase, e.g. Ach, did not have responses potentiated by inhibitors of DAO activity. These authors were not able to localize DAO activity but the value of their study was that it was the first demonstration that the magnitude of the contractile response of the ileum was influenced by catabolic degradation of histamine.

Comparable studies on the role of histamine catabolism in blood vessels were not reported until later (Kalsner and Nickerson, 1968). Segments of rabbit aorta were contracted with various agonists and allowed to come to steady state contraction; at this point the aqueous bath media was replaced by oil thus trapping a fixed amount of agonist in the extracellular plus cellular compartments. These authors suggested that the rate of relaxation of the tissues was directly related to the catabolism of the agonist. Using the above oil immersion technique, strips of rabbit aorta were contracted by histamine at a concentration of 1 μg/ml. The degradative enzyme DAO was inhibited by iproniazid and semicarbazide (1 x $10^{-6}\mu$g/ml). This resulted in a two-fold increase in the time required for the tissue to relax by 30% of maximal contracted length. These authors considered that although DAO played an important role in inactivation in aortic tissues at least one other pathway was operating, possibly N-methylation.

The study of histamine catabolism in aortic strips was continued to include the effect of steroid hormones (Kalsner, 1970). The effects of 17-β-estradiol on the contractile dose-response curves to histamine indicated that the steroid shifted the response to the left producing a two-fold potentiation but with no increase in maximal response. The effects of the steroid persisted after washout for about 60-120 minutes. In the same study when diamine oxidase was inhibited by iproniazid the potentiation produced by 17-β-estradiol was increased compared to the effect of 17-β-

estradiol alone. This suggested that 17-β-estradiol was inhibiting a pathway that normally competes with oxidative deamination. The addition of methylation inhibitor amodiaquin (3×10^{-6} g/ml) or quinadine (3×10^{-5} g/ml) increased the responses to histamine with a lesser potentiation at higher histamine concentrations. When 17-β-estradiol was added to aortic strips already contracted to histamine in the presence of amodiaquin no further increase resulted. In addition the same resulted when amodiaquin was added to strips already contracted with histamine with 17-β-estradiol present. The contractile responses to methyl histamine were not increased by 17-β-estradiol nor inhibitors of histamine methyl transferase. This suggested that 17-β-estradiol may be an inhibitor of methylation of histamine.

The oil immersion technique was used to further study the role of the two catabolic pathways in termination of the action of histamine. The aortae were contracted to histamine and when pretreated with diamine oxidase inhibitors took twice as long, when compared with controls, to relax by 50%. With both pathways inhibited the aortae took six times as long to relax by 11% of maximal contraction. The relaxation rate of strips contracted to histamine was not altered significantly by pretreatment with 17-β-estradiol although relaxation was increased by the addition of diamine oxidase inhibitors. The authors concluded that diamine oxidase and histamine methylation each accounted for about 50% of the inactivation of histamine and that in the presence of diamine oxidase inhibition 17-β-estradiol produced a significant decrease in the rate of histamine methylation. The lack of effect of 17-β-estradiol on the relaxation of aortae could not be explained. It was concluded that 17-β-estradiol enhanced responses to vascular histamine by inhibiting methylation but perhaps as an alternative the steroid prevents access of the amine to histamine methylation.

Further studies in the rabbit aorta suggested that extraneuronal mechanisms exist for catecholamines in this tissue (Kalsner, 1975). In addition it was suggested that the extraneuronal uptake of histamine and serotonin was probably identical to that for catecholamines.

## 1.7 Analytical Methods for Histamine in Biological Fluids

Prior to the introduction of a sensitive chemical assay for histamine, specimens were assayed biologically, e.g. the isolated guinea pig ileum (Barsoum and Gaddum, 1935). The biological assay still remains a useful check for chemical methods since the specificity of the assay can be readily determined with histamine H-1 receptor antagonists (Ash and Schild, 1966).

The first chemical assay methods involved the formation of derivatives which were measured colourmetrically (Rosenthal and Tabor, 1948, McIntire et al, 1950, Lowry et al, 1955) but these lacked specificy and sensitivity. Subsequently, a sensitive fluorimetric assay was reported by Shore et al (1959), this method has also been modified for the assay of spermine and spermidine (Shaw, 1972). In the original method histamine was extracted into an organic phase of n-butanol, the organic phase was washed with NaOH to remove precursor amino acid histidine, histamine is then returned to the aqueous phase in dilute acid with a reported recovery of 90-100%. Histamine has no native fluorescent properties, the assay requires its condensation with orthophthaldialdehyde (OPT) under alkaline conditions to yield a fluorescent product which is stable in acid solution. The fluorescent product is quantitated spectrofluorimetrically (350/400 mµ). The mechanism depicted in Figure 1.2 was described by Shore et al (1959). This mechanism is now thought to include two molecules of OPT (Figure 1.3) to give a polycyclic compound (Rönnberg et al, 1977). Other endogenous compounds were tested for the fluorescence with OPT, those without significant fluorescence included norepinephrine, serotonin, urocranic acid, carnosine, anserine, spermine, spermidine and ammonia in concentrations less than 4 mg/ml (Shore et al, 1959).

For most peripheral tissues studies there was close agreement between biological and fluorimetric assays for tissue histamine (Shore et al, 1959). It soon became apparent that for brain tissues solvent extraction followed by fluorimetric assay resulted in much higher histamine contents than the
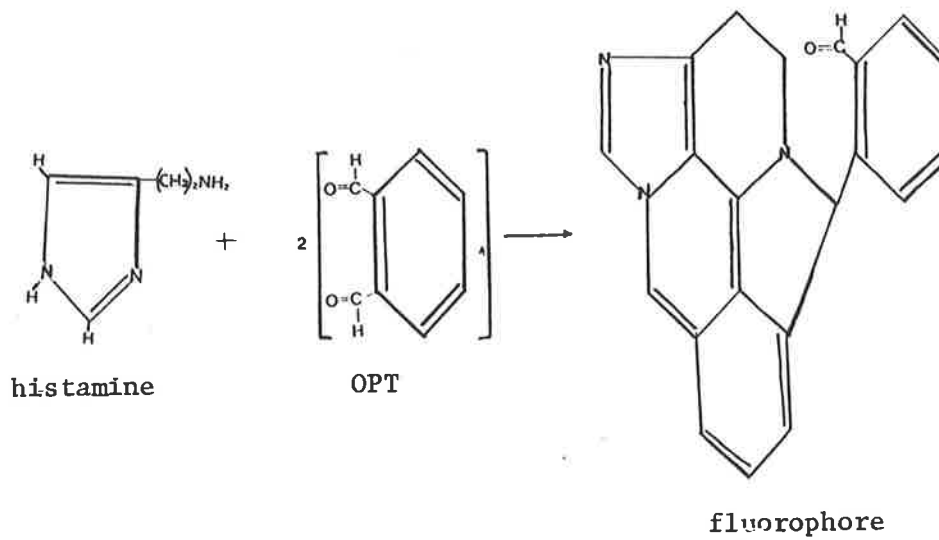
Chemical Mechanisms for the
Production of OPT-Histamine Fluorophore

Figure 1.2

OPT   histamine   fluorophore
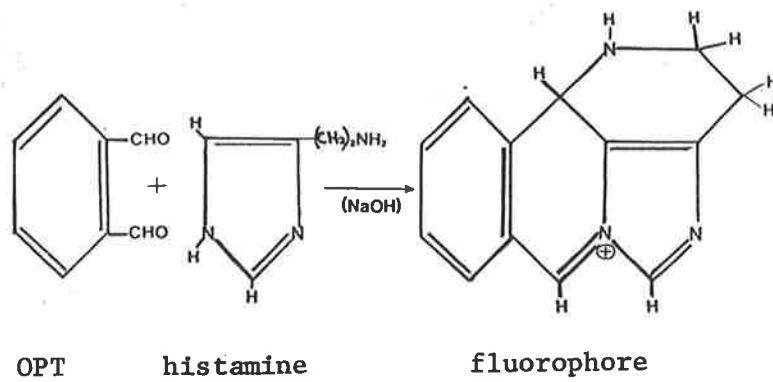
(from Shore et al, 1959).

Figure 1.3

histamine   OPT

fluorophore

(from Rönnberg et al, 1977)

biological assay (Carlini and Green, 1963, Kremzer and Pfeiffer, 1966). Spermidine was identified as the major contaminant which is extracted with histamine during n-butanol extraction (Michaelson, 1967) which reacts with OPT to form a fluorphore with similar spectral properties to that of the histamine OPT condensate. Very high levels of spermidine are required to interfere with the fluorimetric assay of histamine since the fluorescence per mole of histamine OPT fluorophore is thirty times that of the spermidine-OPT fluorophore. These high levels are found in brain, for example the histamine/spermidine ratio in the guinea pig mid brain is 500:1 (Michaelson, 1967). Histamine has been separated from spermidine by a modified solvent extraction technique (Anton and Sayre, 1969). Other methods have employed some form of ion exchange chromatography. The published column chromatographic procedures, e.g. Michaelson (1967), clearly demonstrate a large amount of OPT fluorescent material that does not co-chromatograph with histamine, and thus indicates the necessity of a purification step prior to fluorimetric assay. A weakly acidic cation exchanger was selected for use in the studies to be reported in this thesis from a range of alternatives which will be discussed.

Strongly acidic cation exchange resins have been used to separate noradrenaline, dopamine, 5HT, and histamine individually using Dowex 50W-X4 200 -400 mesh (Atack and Magnusson, 1970). The use of Dowex 50 yields a histamine recovery of 85% (Atack, 1977) which is comparable to recoveries obtained in the studies reported in this thesis. The use of strongly acidic cation exchangers appears to provide a complete separation of histamine from spermidine and therefore a very suitable method for brain tissue extracts. One disadvantage appears to be the high acid concentrations required to elute the amine fractions from the column, this may have presented difficulties in neutralization prior to fluorescence assay.

Intermediate acidic cation exchangers ($R-PO_3^=$ (Na^+)$_2$) provide a good separation for histamine from other OPT reactive material. The functional phosphoric acid groups were initially employed on a cellulose support, cell-ex P, but this resin suffered the disadvantage of a low exchange capacity

(Kremzner and Pfeiffer, 1966, Medina and Shore, 1966).

The reproducibility of the elution pattern and an increase in ion exchange capacity was achieved by using resins with a polystyrene support for phosphoric acid groups (Bio Rex 64 (Bio Rad Labs), Duolite ES-63). The additional ion exchange capacity of these resins was about three times the capacity of Cellex P and reduced the possibility of overloading. Histamine and spermidine were eluted from the resin with weaker acid concentrations than for Dowex 50 (Michaelson and Coffman, 1969). Unfortunately, these resins are no longer readily available.

Weakly acidic cation exchangers ($RCOO^- Na^+$) have been used in the separation of histamine from other OPT reactive material both in the studies in this thesis and in other laboratories. The major advantage of separating histamine from other OPT reactive materials on weakly acid cation exchangers, e.g. Amberlite CG-50 is that the eluant acids are weaker than those used for more acidic resins and this was much more conveniently adapted to the automated fluorimetric assay used in this study. In addition stronger acids are more likely to leach out fluorescent materials from the ion exchange resin itself, which could result in higher blank values. The disadvantage of these weaker exchangers is that they are sensitive to changes in ionic strength of both samples and eluant acids. The elution profile of this type of column tends to be more variable than that reported for stronger cation exchangers (Atack, 1977). The properties of the present chromatographic procedure will be presented later in the experimental section of this thesis (Chapter 2).

In addition to improvements in the column chromatographic procedures above, the reaction conditions for the production of the OPT-histamine fluorophore have been studied. The original fluorimetric method of Shore (1959) employed 3M HCl to stabilize the histamine-OPT fluorphore. The intensity of the fluorophore is sensitive to changes in pH (Kremzer and Wilson, 1961) which is an inherent source of error with manual methods. The manual method was later modified by Anton and Sayre (1969) who favoured the use of 2M citric acid because fluorescence remained constant over a wide range of pH. In addition variations in ambient temperature influence the maximal development of the fluorophore (Shaw, 1972); at least 20° C is req-

uired for histamine and 25°C for spermidine for incubation times of 4.0 and
6.5 minutes respectively. The sensitivity of the fluorimetric assay can be
increased by a reduction in the blank values. One laboratory has succeeded
in reducing blank values by using reagents prepared with millipore ⓡwater
and careful attention to all sources of blank fluorescence, e.g. plastic-
isers from plastic bottles, etc. Reduction of blank values has allowed as
little as 20 pg histamine/ml plasma to be differentiated from the blanks.
Multiple assays of a plasma pool (mean 280 pg histamine/ml) yielded a co-
efficient of variation of only 6.9% (Lorenz et al, 1978). The OPT assay has
been successfully automated thereby increasing speed and the ability to hold
pH and temperature constant. Most techniques employ an automated solvent
extraction procedure with or without dialysis depending on the protein con-
tent of the sample (Ruff et al, 1967, Ruff et al, 1968, Siraganian, 1975,
Evans et al, 1973). The original manual procedure of Shore (1959) has been
automated using the Technicon ⓡ autoanalyser for the assay of histamine in
this laboratory (Foldes et al, 1977). The above automated technique omits
the solvent extraction step and samples are assayed after column chromato-
graphic purification. Other methods are available for the assay of hist-
amine and these are briefly reviewed below.

High performance liquid chromatography (HPLC):

HPLC has been applied to the rapid measurement and separation of hist-
amine and spermidine in extracts of rat gastric mucosa (Onda et al, 1978).
Chromatographic separation of putrescine, histamine, hexanediamine and
spermidine were possible, but cadaverine was not well separated from hist-
amine. The coefficient of variation for replicate samples was less than 5%.
These amines were detected by a continuous flow fluorimetric method, after
separation by HPLC using hexanediamine as an internal standard. The sens-
itivity of the assay permitted the measurement of 200 p moles of histamine
applied to the column.

The radioenzymatic assay of histamine:

A double isotopic radioenzymatic assay was introduced (Snyder et al, 1966) in an attempt to measure brain histamine levels free of interferences known to cause elevated levels (Michaelson and Coffman, 1969, Carlini and Green, 1963) with the fluorimetric assay (Shore et al, 1959). Tissues for histamine assay were homogenised and then heated to destroy endogenous S-adenosylmethiamine (S-AME) and to free bound histamine. Then supernatants of homogenates were incubated with ^{14}C S-AME, ^{3}H histamine and purified preparation of HNMT (Brown et al, 1959). The tissue histamine and ^{3}H histamine were converted to the ^{14}C methyl metabolite of histamine (both ^{14}C methyl histamine and ^{14}C, ^{3}H methyl histamine. The amount of ^{14}C methyl radioactivity present in the products is constant, whereas the amount of ^{3}H present in the product is dependent on the amount of endogenous histamine in the tissue sample. The ratio $^{14}C/^{3}H$ bears a direct relationship to the amount of tissue histamine present in homogenates. The recovery of authentic histamine added to tissue homogenates was between 90-100%. The sensitivity of the above method was enhanced by other workers (Miller et al, 1970, Beaven and Jacobsen, 1971).

A single isotopic radioenzymatic method using ^{14}C S-AME was introduced (Taylor and Snyder, 1972) for the assay of brain histamine contents, which gave a reported sensitivity of 10 pg of tissue histamine that could be assayed reliably. In the same study microassays for histidine decarboxylase, histamine methyl transferase and histidine were also reported. Some of the difficulties with the original radioenzymatic method of Snyder et al (1966) were later overcome with further modifications (Beaven et al, 1972). These modifications enhanced the sensitivity of this assay to 0.1 ng of histamine. The measurement of plasma histamine levels in normal subjects has presented difficulty by the radioenzymatic method (Horakova et al, 1977). In a recent study the sensitivity of the radioenzymatic assay was reported to be increased by the use of thin layer chromatography (t.l.c.) to purify the labelled methyl histamine formed by the incubation (Dent et al, 1979). In a preliminary study of plasma histamine this gave a value of 0.8 ng/ml,

which was almost twice the blank value.

Overall the radioenzymatic assay has the advantage of good sensitivity without the problem of interfering substances found in the fluorimetric assay (Michaelson and Coffman, 1966, Carlini and Green, 1963 and Shore et al 1959). Some of the disadvantages of the radioenzymatic assay include the cost of radioactive tracer compounds and the laborious purification of the enzyme reagent.

C H A P T E R   2

THE EFFECT OF SURGICAL AND CHEMICAL
SYMPATHECTOMY ON THE HISTAMINE
CONTENT OF THE RABBIT EAR ARTERY

2.1 Introduction

As mentioned in the introductory survey there have been a number of attempts to assign a neurotransmitter role to histamine in peripheral organs. One requirement of a putative neurotransmitter is that it is contained within the nerves from which it is released. In an attempt to localize histamine in canine arteries and veins to sympathetic nerve terminals Ryan and Brody (1972) examined intact vessels and those from which the adventitia had been removed. The removal of the adventitial layer would remove sympathetic nerves from these blood vessels as they are located in the adventitia near the advential-medial junction (Waterson and Smale, 1967). These anatomically denervated vessels were found to contain more histamine indicating that this amine was not localized to the adventitia. However, this conclusion was of necessity, applied only to large vessels of the corductive type. However in the same study the canine gracilis muscle was chronically denervated and the histamine content of the muscle was found to increase compared with contralateral controls. The latter study did not indicate whether these were changes in the histamine content of the vessels within the muscle. In a previous study carried out in the rabbit aorta by the candidate, in collaboration with Dr. Foldes, chemical sympathectomy did not result in a decrease of the endogenous histamine content of the vessel (Foldes et al, 1976).

The present study was carried out on a smaller muscular type of artery namely the rabbit ear artery. The selection of this vessel was based on the following considerations (a) that it has a dense sympathetic innervation (Beven et al, 1972) and (b) unlike the aorta it is easily sympathetically denervated by removal of the superior cervical ganglion (de la Lande and Rand, 1965). Hence, it offered the advantages

(i)   that it was a vessel more representative of arterioles controlling peripheral resistance than the conducting vessels

(ii) if histamine was present in sympathetic nerves the relatively dense innervation meant that its presence may be easier to quantify as a decrease

in content following denervation

(iii) if histamine was present in separate nerves relaying through the gang-
lion, their presence should also be demonstrable by a decrease in histamine
content following denervation. In the last respect the ability to surgical-
ly denervate represented an advantage over chemical sympathectomy since
there is no evidence to suggest that the agent used to produce chemical symp-
athectomy of nerves (6OHDA) in the rabbit aorta was effective in non-cat-
echolaminergic neurones.

2.2 Methods

Semi-lop eared rabbits of either sex weighing 2.0-2.8 kg were used
in the following experiments. Animals were housed in controlled temperature
and lighting environments at the central animal house of The University of
Adelaide.

Surgical sympathectomy

Rabbits were atropinised (0.25 mg/kg) prior to anaesthesia with i.v.
sodium pentobarbitone injected into the marginal ear vein (Murdoch, 1969).
The level of surgical anaesthesia was determined by increased effort of
breathing (protracted ventilation) as other signs proved unreliable. The
ventral surface of the neck was shaved and further surgical procedures were
carried out under aseptic conditions. A midline incision was made and the
superior cervical ganglion was removed in the manner described by de la
Lande and Rand (1965). The position of the superior cervical ganglion show-
ed considerable variability being sometimes a few centimetres from the bi-
furcation of the common carotid artery. Animals were allowed to recover
and seven days after the operation were killled by cervical dislocation and
bled from cervical vessels.

Chemical sympathectomy

Each of the two rabbits were treated with a total of 300 mg/kg of 6-

hydroxy dopamine (6OHDA) in divided doses (Finch, Haeusler and Thoenen, 1973). Rabbits were injected with 6OHDA into the marginal ear vein in two doses of 50 mg/kg on the first day and seven days later they were again given two doses of 100 mg/kg, on the eighth day the rabbits were killed as described above. 6OHDA was prepared for intravenous injection by dissolving in 0.001 M HCl bubbled with N_2 immediately prior to injection.

The efficiency of both forms of sympathectomy was checked by the fluorescence method of Waterson and Smale (1967) which is a minor modification of the method of Falck and Owman (1965). Denervation was indicated by a lack of monoamine specific fluorescence (Figure 2.1a and b) at the medial adventitial border where the sympathetic nerves are known to be located (Waterson and Smale, 1967). Apart from these more objective methods of assessing sympathectomy the denervated ear was observed for the first few days following sympathectomy to determine whether it was warmer than the control ear, and the eyes were examined to assess whether they were more dilated on the sympathectomised side. Both of these organs are also innervated by the superior cervical ganglion.

Histamine assay

Extraction:

Segments of REA were dissected free from excess connective tissue and the lumen was gently cleared of blood with gassed Krebs solution. The tissues were sectioned into required lengths and blotted on filter paper then weighed. Histamine was extracted from tissues with two successive 1 ml aliquots of 0.03 mM EDTA in 0.1 M HCl for a total of about 36 hours at $4^{o}C$. The remaining pooled extract was adjusted to a pH of 6.5.

Amberlite chromatography:

A small amount of ^{14}C HA was added to a 1 ml aliquot of the acid extract to provide a calculated recovery in subsequent column chromatography. The resin was prepared by washes with 2 M HCl, water, 2 M NaOH, water,
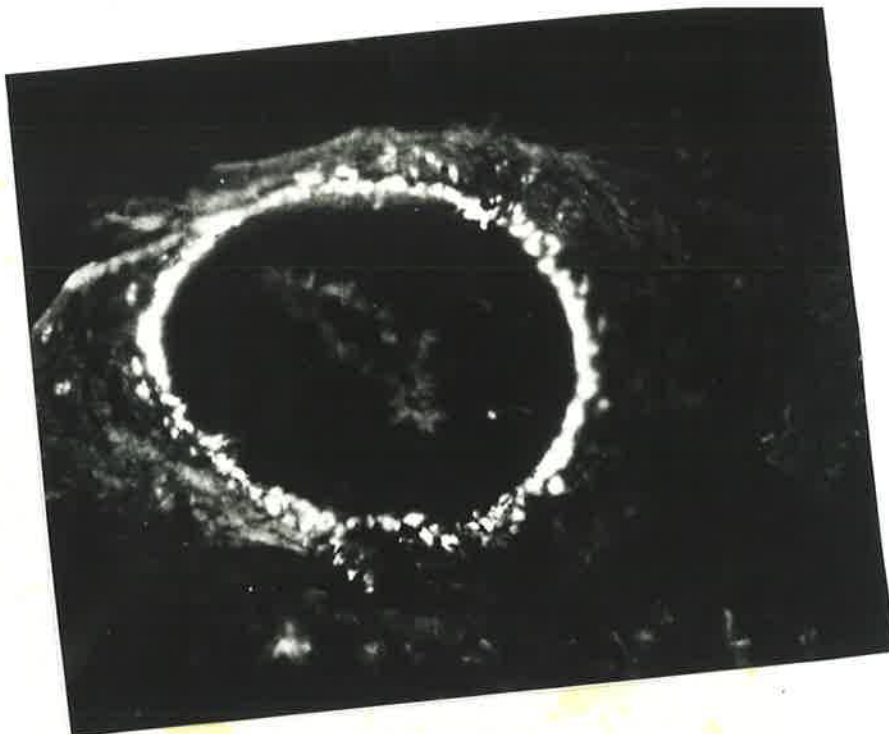
Figure 2.1 (a)

Photomicrograph of an untreated section of REA indicating monoamine fluorescence at the medial adventitial border. (x100)
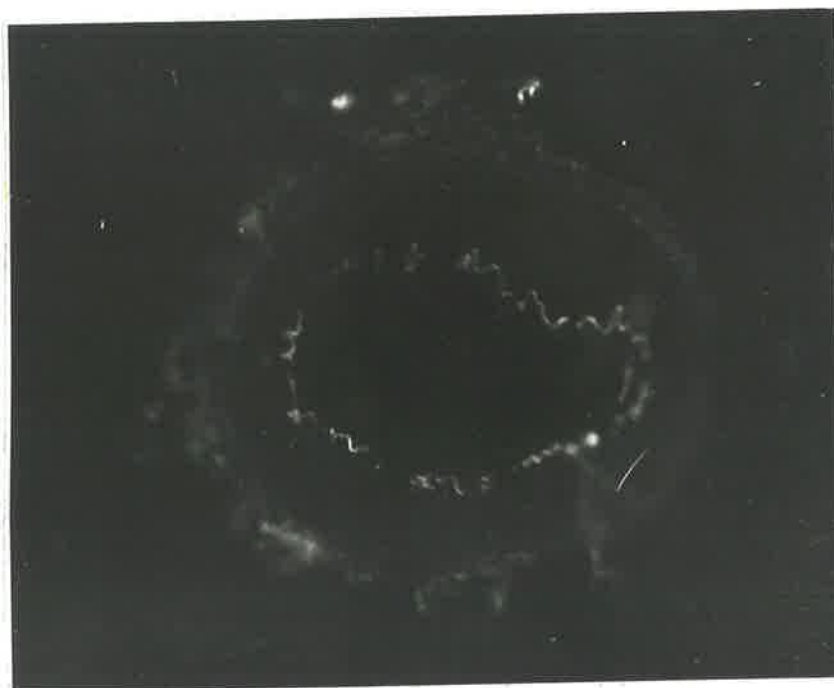

Figure 2.1 (b)

Photomicrograph of REA after surgical sympathectomy showing absence of monoamine fluorescence at medial adventitial border. (x100)

and equilibriated with 0.01 M $(Na^+)_2PO_4^=$ buffer pH 6.5. The column (1 x 10 cm) was packed and equilibriated with pH 6.5 buffer. The one ml aliquot of sample was applied to the column and non-binding species were washed from the column with 0.01 M $(Na^+)_2PO_4^=$ buffer delivered at a constant rate with a Technicon proportioning pump (0.45 ml/min) for 100 mins collected in 4.5 ml fractions. Histamine and other bound species were eluted from the column with 0.1 M HCl at a constant flow rate (0.45 ml/min) and collected in 4.5 ml fractions. All fractions were assayed fluorimetrically and an aliquot was taken for scintillation counting. It should be noted that the Amberlite CG-50 is a weakly acidic cation exchanger, its advantages and disadvantages being discussed on page 14 of this thesis.

Automated fluorimetric assay:

Histamine was assayed by an automated adaption of the condensation (OPT) fluorimetric method (Shore et al, 1959) to the Technicon autoanalyser by Foldes et al (1977). The flow diagram is given in Figure 2.2. The fluorescence intensity was measured with a Technicon spectrofluorimeter where the output was modified to quantitate on a Rikadenki multirange recorder. Histamine standards were prepared in 0.1 M HCl and these were assayed in duplicate in the range 5-50 ng/ml every 40 samples. The fluorimetric assay was linear up to at least 100 ng/ml (Figure 2.3).

Scintillation spectrophotometry:

Usually one ml of each fraction was taken for counting and added to 15 mls of 0.025% POPOP, 0.825% PPO, in 2/3 toluene 1/3 triton X-100 mixture. ^{14}C radioactivity was measured on a Packard Tri-carb scintillation spectrophotometer. The counting efficiency was determined by the addition of a ^{14}C toluene standard.

Statistical analysis:

Where two groups were compared the homogeneity of the variances was calculated first, then if these were not found to be different these

Figure 2.2   Automated Fluorimetric Analyser
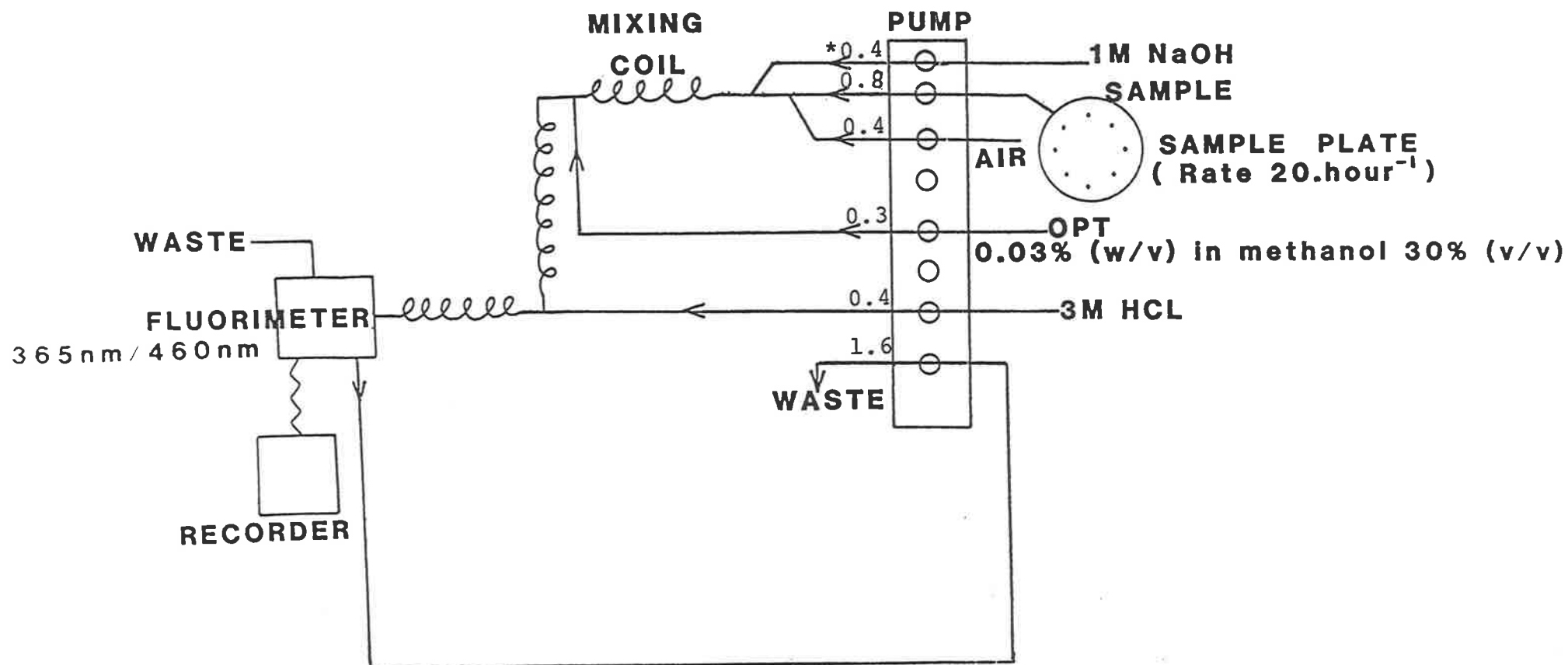
Flow diagram for the Technicon autoanalyser for the automated assay of histamine (adapted from Foldes et al, 1977).
* Numbers refer to flow rates in ml min^{-1}.

Figure 2.3   Curve of Fluorescence Intensity
              vs. Histamine Concentration of Standards
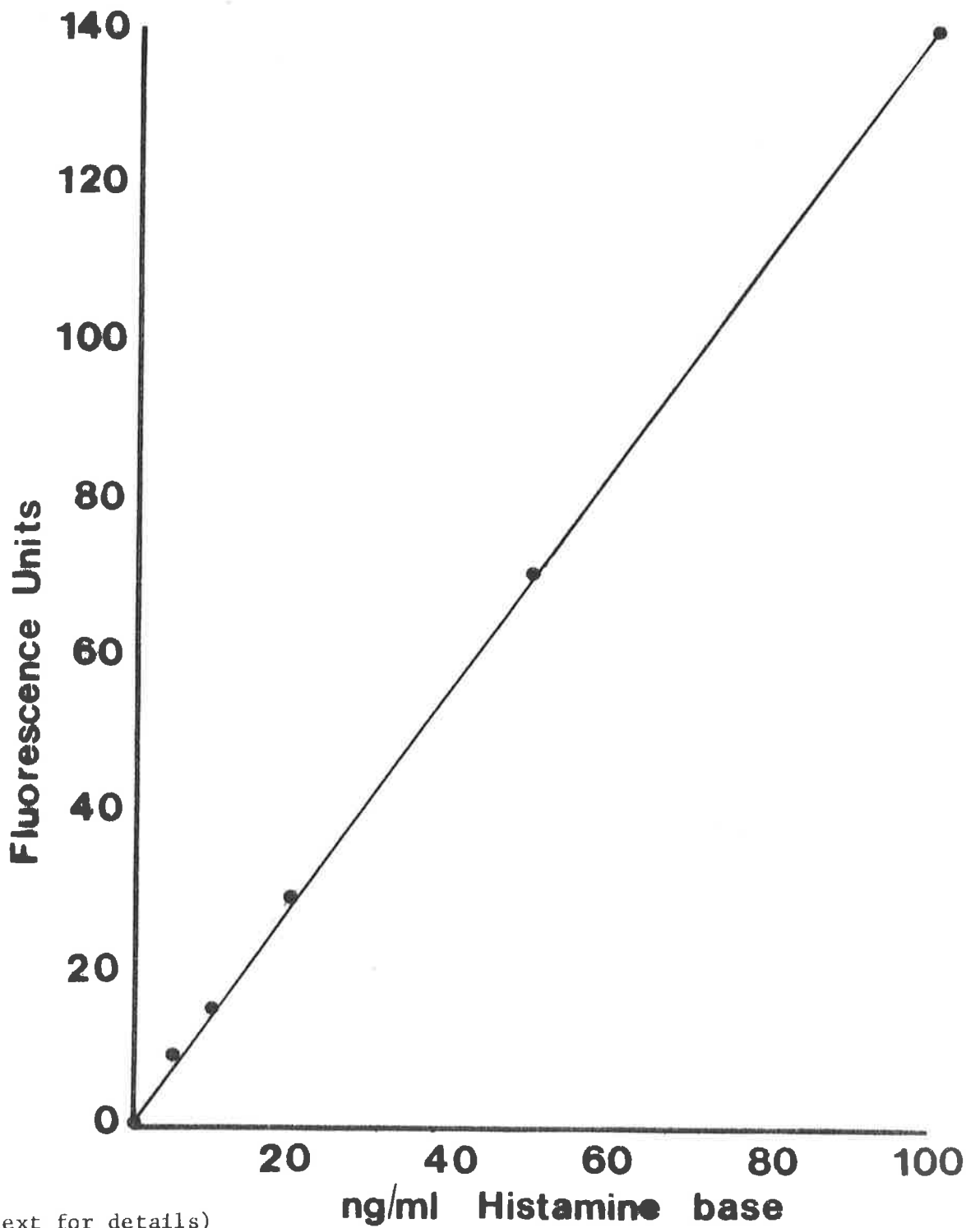
(see text for details)

groups were compared by paired or unpaired t-tests. A probability level of less than 0.05 was considered to be significant.

Biological assay:

Acid eluates containing OPT reactivity were neutralized and bioassayed on an isolated segment of guinea pig ileum. The contractile responses produced by neutralized eluates were quantitated by a bracketed assay with histamine standards. Only an approximate quantitation for histamine was possible as contractions occurred on the non-linear part of the dose-response curve. The specificity of contraction was confirmed by the addition of histamine H-1 and H-2 blockers (mepyramine maleate and burimamide) both at a concentration of 1 μg/ml bath concentration prior to the addition of neutralized eluants.

Sensitivity and specificity of automated assay:

The properties of the automated assay have been examined by Foldes et al (1977). Briefly, the assay is sensitive to concentrations of histamine standards down to 2 ng/ml, with no appreciable carryover of samples.

Notes on Methods

Specificity and efficiency of column separations:

It has been suggested that the column chromatographic step separates a histamine containing fraction from acid extracts of rabbit aorta (Foldes et al, 1977). similar evidence is presented that this procedure separates a histamine containing fraction from acid extracts of REA. The elution profile for Amberlite chromatography of REA acid extract is shown in Figure 2.4.

(1)   A peak of OPT fluorescence co-chromatographed with ^{14}C histamine added to the applied sample. Due to the stability of histamine it is unlikely to have undergone any chemical conversion on the column.

(2)   Neutralised fractions of acid eluates that co-chromatographed with ^{14}C histamine were bioassayed on the guinea pig ileum. The contractile

Figure 2.4   Profile of ^{14}C Elution from Amberlite CG-50
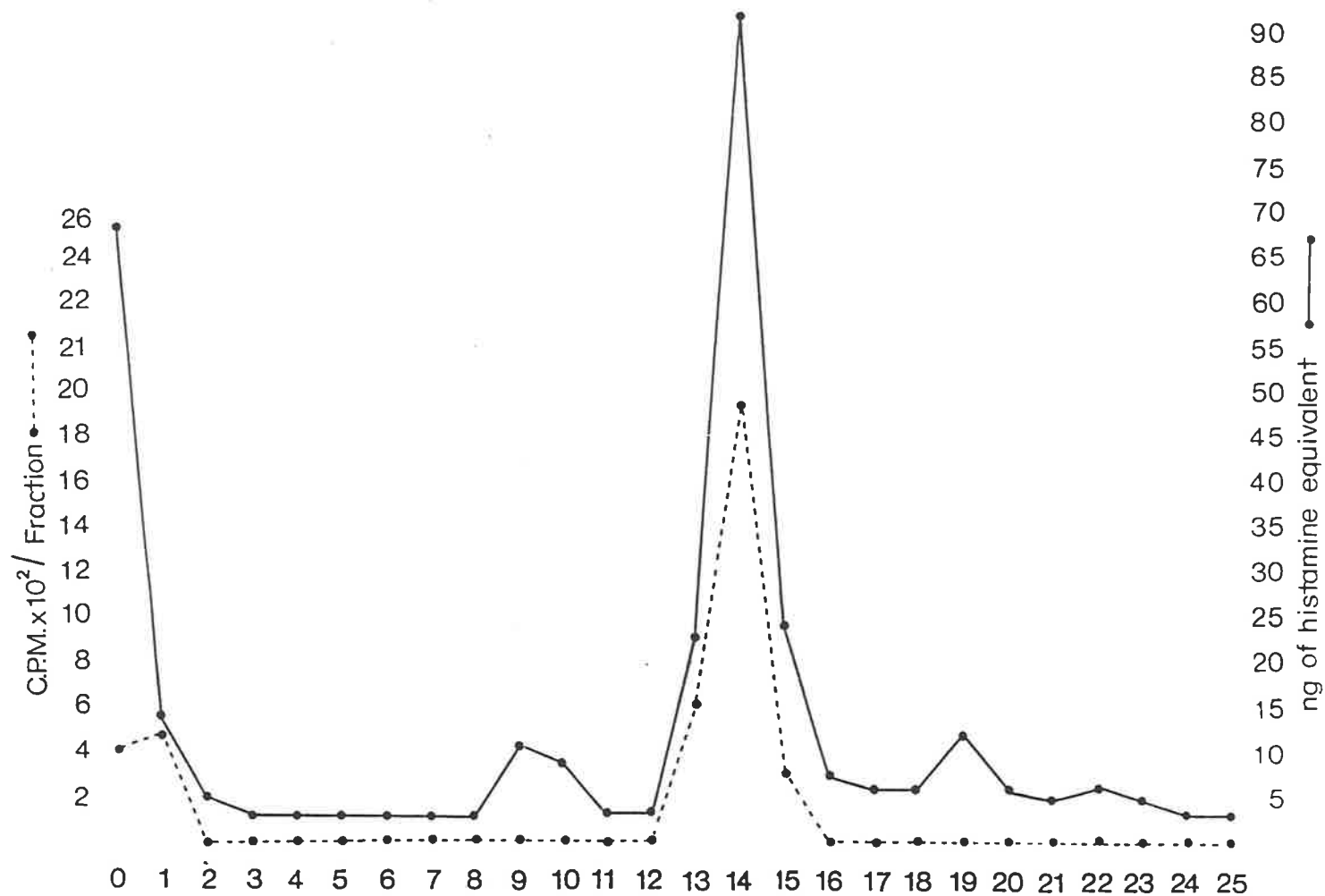
Sample on column at 0. Numbers shown refer to successive 5 minute collections of eluate (i.e. 4.5 ml fractions). Fractions 1-9 are washes with phosphate buffer (0.01 M), then 10-26 représent acid (0.1 M HCl) eluants. Authentic ^{14}C histamine (.---.), sample OPT fluorescence (._____.).

activity of these fractions showed approximate equivalence with hist-
amine contents measured by OPT fluorescence. These contractions were
greatly reduced by the combination of H-1 and H-2 blockers, mepyramine
maleate and burimamide, at a concentration of 1 μg/ml.

It was initially expected that column separation of histamine from ex-
tracts of REA would be very similar to separations from extracts of rabbit
aorta performed in this laboratory by Foldes et al (1977). The efficiency of
^{14}C histamine recovered from column chromatography of aortic extracts had
been 94-101%. In these studies separation of histamine from REA extracts
were rather different from aorta.   Most of the radioactivity applied
to the column was recovered in   thé fractions collected with an average
recovery of 90.5% (n=10) with a range of 82-102%. In most cases some of the
^{14}C histamine was not bound to the resin and appeared in the first few
washes. The acid eluted peak of OPT fluorescence contained an average of 73%
(range 49-96%; n=10) of the applied label. The method of estimating ^{14}C
histamine recoveries had been modified throughout the course of these exper-
iments so that the following approach was used to correct for efficiencies
of the analytical technique. In all cases paired observations had been
analysed simultaneously to minimise analytical variation. The variation in
recoveries for both paired and unpaired observations were included in the
tissue variation of histamine contents per gram.

2.3 Results

(a) Endogenous histamine contents of untreated segments of REA

Untreated segments of rabbit ear artery contained 7.0 $\pm$ 0.7 µg of endogenous histamine/g tissue (n=18 REA in 9 rabbits). When the endogenous histamine content/g of REA segments from the left ear were compared with those from the right ear there was a small but insignificant difference (Table 2.1). Hence in subsequent experiments we were able to treat one artery and consider the other side as a paired control.

Table 2.1   Endogenous content (µg/g) of the REA from left and right ears

left REA	6.2 $\pm$ 1.0	(n=5)	n.s.
right REA	6.9 $\pm$ 1.2	(n=5)	

Expressed as mean $\pm$ S.E.M., n = number of animals, compared by paired t-test, n.s. = non significant at p = 0.05.

(b) The effects of 60HDA on the histamine content of REA

For the two rabbits treated with the sympatholytic agent 60HDA this resulted in small reduction in the histamine content/g (Table 2.2). However this was not significant at the level of p = 0.05. If more animals had been studied this difference may have become significant but it appears to be a small effect on total histamine content/g of the vessel.

Table 2.2   The effect of 6-Hydroxydopamine (60HDA) on the endogenous histamine content (µg/g) of REA

control	7.0 $\pm$ 0.70	(n=9)	n.s.
60HDA pretreated	6.0 $\pm$ 1.1	(n=2)	

Expressed as mean $\pm$ S.E.M., n = number of animals, compared by unpaired t-test, n.s. = non significant at p = 0.05.

(c) <u>The effects of surgical sympathectomy on histamine content of REA</u>

Surgically sympathectomised rabbits showed physical signs of denervation and histochemical studies of REA confirmed this. Paired segments of REA from sympathectomised ears and contralateral controls showed a reduced histamine content/g with sympathectomy. This reduction was small (Table 2.3) although not significant in these experiments, with greater numbers of animals this may have resulted in a small but significant difference.

Table 2.3   <u>The effect of surgical denervation</u>
<u>on the endogenous level of histamine</u>
<u>(μg/g) in the REA</u>

control REA   8.6 + 1.5   (n=6)   n.s.

paired denervated REA   6.7 + 0.9   (n=6)

Expressed as mean + S.E.M., n = number of animals, compared by paired t-test, n.s. = non significant at p = 0.05.

2.4 <u>Discussion</u>

These experiments in the REA are supported by a similar result in the rabbit aorta where treatment with 60HDA did not result in a decreased histamine content of the vessel (Foldes et al, 1976). The results of surgical denervation in this study indicate that there may be a small decrease in histamine content but this is not comparable to the results obtained with noradrenaline after sympathectomy of the REA (Head et al, 1977). The present study indicates that histamine is not exclusively localised within post ganglionic sympathetic nerves and the integrity of these nerves is not required to maintain most of the total vascular stores of histamine. A possible alternative that cannot be excluded with the present information is that any decrease in histamine content may be masked by an increase in histamine synthesis in the denervated vessel. Further studies using ^{14}C histidine incorporation would be indicated to detect increases in newly synthesised histamine as a result of denervation. The results in Table 2.3 are in agreement with the results of Ryan and Brody (1972) who did not detect a decrease in

tissue histamine levels after chronic denervation of the dog gracilis muscle.

2.5 Summary

The selective denervation of post ganglionic sympathetic fibres inner-vating the REA did not result in a large decrease in the total histamine content of the REA. These experiments suggest that histamine stores are not dependent on an intact sympathetic innervation of the REA and that sympath-etic fibres are not a site for the localization of histamine in the REA.

C H A P T E R 3

THE EFFECT OF SECTIONING THE GREAT AURICULAR NERVE ON THE HISTAMINE AND CATECHOLAMINE CONTENT OF THE CENTRAL EAR ARTERY OF THE RABBIT

3.1 Introduction

In the preceding section, the possible presence of histamine in sympa-
thetic nerves in REA was examined by comparing histamine contents of ear
arteries from innervated and sympathetically denervated arteries. In the
present study the possibility that the histamine present in the artery may
be located in afference sensory and efferent vasodilator nerves was studied. A
study by Feldberg (1926) describing the innervation of the rabbit ear it was
suggested that most of the vasodilator and sensory fibres are carried in the
great auricular nerve (auricularis anterior). This nerve runs in close
proximity to the REA and presumably innervates it, although this has not
been confirmed directly.

Histamine is capable of dilating the REA via H-2 receptors (Ercan and
Turker, 1975, Glover et al, 1973). The vasodilator responses of the REA
have been reported to be two-fold more sensitive when histamine is applied
extraluminally (Galeno et al, 1979). This perhaps indicates that extralum-
inal factors may be more responsible for vasodilation than circulating
amine. The possibility that histamine may be localised in or maintained by
vasodilator or sensory nerves (in the great auricular nerve) will be exam-
ined in this chapter. In the present study the great auricular nerve was
sectioned at the base of the ear and then the histamine contents of the de-
nervated artery and the innervated artery were compared.

3.2 Methods

Great auricular nerve denervation:

Semi lop-eared rabbits of either sex, weighing 1.4-3.2 kg were used in
the following experiments. Animals were atropinised (0.25 mg/kg) then anae-
sthetised with sodium pentobarbitone. An incision was made obliquely to the
midline at the base of the ear to be denervated. The connective tissue was
blunt dissected to reveal the great auricular nerve which lies deep to the
auricular vein. The nerve was dissected free of connective tissue then a

section 2-3 cm long was taken, weighed (22-62 mg) and extracted (0.1 M HCl 0.03 mM E.D.T.A.). The incision was closed with black silk sutures and the animal was allowed to recover. The animal was killed twelve days later with a blow to the head and exsanguinated from cervical vessels. Sections of rabbit ear artery were taken as follows. The vessel was dissected from its exit from the cranial vault distally to approx. 2 cm past the bifurcation of the great auricular vein on the dorsal surface.

The lumen was cleaned with gassed Krebs solution, then the artery was divided into equal proximal and distal segments (Figure 3.1). The tissues were blotted, weighed and extracted (0.1 M HCl 0.03 mM E.D.T.A.) for approximately 18 hours then transferred to a second aliquot for approximately 24 hours, the extracts were then combined and assayed for histamine and total catecholamines.

Total catecholamines:

The total catecholamine content of the tissues was measured by an automated modification of the trihydroxyindole method using the Technicon autoanalyser (Head et al, 1977). Although this method measures both noradrenaline and adrenaline earlier studies by de la Lande and Head (1967) concluded that the REA contained mostly noradrenaline.

Histamine:

The tissue extract was neutralised with small volumes of NaOH ($<$ 50 μL) to a pH of 6.5. Histamine, 5.0×10^{-9} Ci (45 p. moles) ^{14}C, was added to 1 ml of neutralised extract. The histamine content of the extract was determined by the method of Foldes et al (1977) as described in the methods section. The recovery of ^{14}C histamine was calculated and tissue contents were corrected accordingly.

The efficiency of column chromatography:

The recovery of ^{14}C histamine applied to column chromatographs during this series of experiments was found to average 70.1% (S.E.M. = 1.11 n=24),

≈ 2cm

Vein

G.A.N.

distal R.E.A. segment

proximal R.E.A. segment

Adipose Tissue

Cranial exit
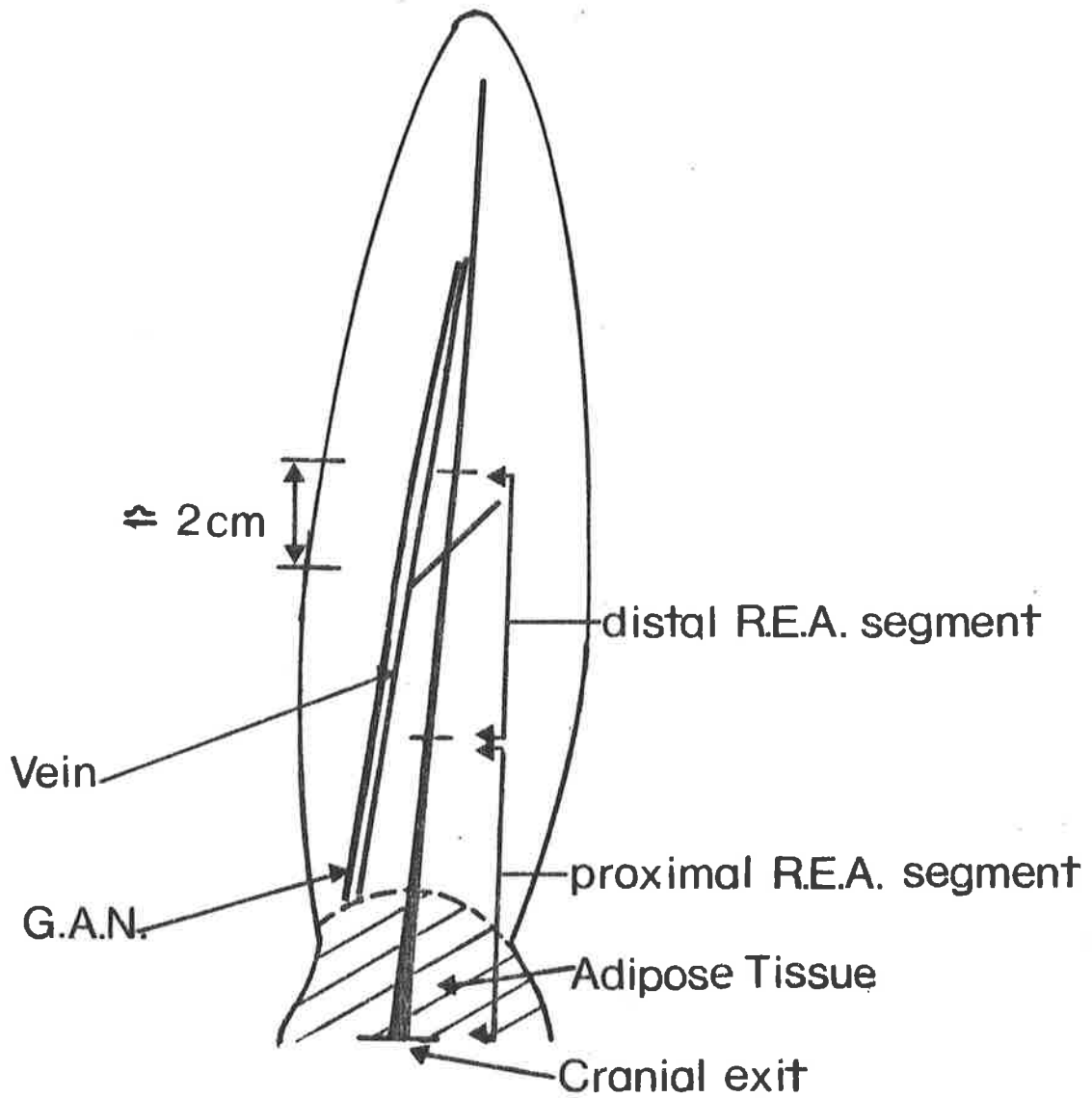
Figure 3.1

The structures shown above are on the medial surface of the left ear. The
relative positions of the great auricular nerve (GAN), the central vein and
the central ear artery (REA) are shown. Proximal and distal segments of
REA referred to in these experiments were excised from the areas indicated
above. It should be noted that part of the proximal REA segments runs
under a pad of adipose tissue and the GAN travels through it.

this was a little less than recoveries obtained for identical studies in the rabbit aorta. The reason for this is unclear.

Statistical procedures:

Where groups were compared with parametric tests of significance their sample variances were tested for homogeneity, where the samples had significantly different variances these were converted to the logarithmic transforms ($\log_{10}x$) then retested for homogeneity of variance. If log. transformation failed to equalise the variances, then non-parametric tests were used (Mann Whitney U-test). Where the variances were found to be equal the means were compared using t-test statistics. The means were assumed to be normally distributed. Data were considered to be significantly different where $p < 0.05$.

3.3 Results

(a) The effects of denervation on endogenous histamine content of REA

In a preliminary study, unilateral denervation was performed on four rabbits by sectioning the great auricular nerve on one side and treating the other ear as a paired control. Whole segments of ear artery were removed without division into proximal and distal segments. The histamine content for controls had a mean of 11.0 µg/g (S.E.M. ± 0.56) which did not differ significantly from the content of denervated tissues mean 11.3 µg/g (S.E.M. ± 1.42). Therefore, denervation did not alter the histamine contents/gm of complete segments of artery.

A second group of rabbits was unilaterally denervated in an attempt to study the regional distribution of endogenous histamine in proximal and distal segments. Any regional differences in innervation of the artery may have resulted in a difference with denervation that was obscured within studies of complete segments (Table 3.1).

Table 3.1   The regional distribution of
                   histamine contents in REA in
                   control and denervated vessels

	Proximal		Distal	
control	4.9 ± 0.7	(n=7)	*10.2 ± 1.0	(n=7)
denervated	5.3 ± 0.7	(n=7)	*12.6 ± 3.0	(n=7)

Expressed as mean ± S.E.M., n = number of animals, compared by Mann-Whitney U-test, n.s. = non significant at p = 0.05, * p < 0.05 when distal segments are compared with proximal segments.

Therefore the effect of sectioning the great auricular nerve was not to alter the histamine content of the vessel significantly. However, histamine clearly shows a regional distribution in both control and treated segments with distal segments containing about twice as much histamine as paired proximal segments (Table 3.1).

It is interesting to note that the average value between proximal and distal histamine contents is close to 7 µg/g which is the value obtained for whole (proximal and distal) segments of REA in the earlier experiments.

(b)   The effect of denervation on the total catecholamine content of REA

The catecholamine content of control and denervated segments of REA was measured in the above segments taken for histamine analysis. In the preliminary series the mean content of histamine in control segments was 1.95 µg/g (S.E.M. ± 0.03 n=4) which was identical with that of denervated segments 1.95 µg/g (S.E.M. ± 0.01 n=4).

In the second group of rabbits the catecholamine content of proximal and distal segments was compared in both treated and control segments (Table 3.2).

Table 3.2   Catecholamine contents (g/g) of REA
                   after sectioning of the GAN

	Proximal		Distal	
control	1.42 ± 0.06	(n=6)	1.68 ± 0.10	(n=6)
denervated	1.68 ± 0.06	(n=6)	1.53 ± 0.08	(n=6)
	n.s.		n.s.	

Expressed as mean ± S.E.M., n = number of animals, compared by paired t-tests, n.s. = non significant at p = 0.05.

In all cases the variances were not found to be significantly different and the groups were compared by paired t-tests. The catecholamine content of the vessel was not seen to vary between proximal and distal segments unlike the differences noted for the histamine content.

(c) Amines in the great auricular nerve

The histamine and catecholamine content of the great auricular nerve was also examined (Table 3.3). Segments of nerve were obtained from the operative sites as described in methods.

Table 3.3   Amine contents of great auricular nerve

Histamine (µg/g) tissue		Noradrenaline (µg/g)	
5.1 ± 0.47	(n=9)	2.23 ± 0.23	(n=4)

Expressed as mean ± S.E.M., n = number of animals.

The histamine content of the great auricular nerve bundle appears to be slightly less than found in the REA but substantially more than detected in rabbit aorta. The origin of this histamine will be discussed later (Chapter 4).

3.4 Discussion

The ubiquitous occurence of histamine in nerves is well documented (Ryan and Brody, 1970) and the great auricular nerve of the rabbit is no exception. These studies indicate significant quantities of histamine in nerve bundles. The discrete localization of histamine within peripheral nerves is unknown. The great auricular nerve of the rabbit is known to contain sensory and dilator fibres which distribute to a large area of the ear including much of the central ear artery (Feldberg, 1926). It is suggested

on the basis of the present results that these vasodilator fibres are not a site for the localization of vascular histamine.

Feldberg (1926) suggested that the great auricular nerve contained post ganglionic fibres from the stellate and the superior cervical ganglion and that stimulation of the auricular nerve resulted in constriction of the distal vasculature of the ear. Hence, if the great auricular nerve was innervating the distal segment of the rabbit ear artery sectioning this nerve would have resulted in a decreased noradrenaline content of the vessel. The absence of such a decrease implies therefore, that sympathetic nerves in the great auricular nerve may be innervating smaller or more distal vasculature, e.g. more distal arteries, arterioles, arterio-venous anastomosis or small veins. The lack of a decrease in noradrenaline content of both proximal and distal segments of REA after sectioning of the great auricular nerve is consistent with Feldberg's (1926) suggestion that the fibres from the superior cervical ganglion are carried to the proximal part of the vessel via the posterior facial nerve. The study by de la Lande and Rand (1965) indicates that vasoconstrictor fibres from the great auricular nerve probably overlap in the more distal regions of the rabbit ear artery.

With reference to the lack of difference of catecholamine contents between untreated proximal and distal segments, Griffith et al (1982) found a significant decrease in distal segments. Such a decrease was not observed in unpublished studies from the present laboratory (de la Lande and Head, unpublished results). It is possible that Griffith et al may have used more distal segments than those used here. It should be noted that wide differences have been reported in NA contents of REA from different laboratories (Bevan, 1972).

The regional distribution of histamine observed in the REA is consistent with studies in other species; in the rat the aorta and mesenteric artery showed a two-fold greater histamine content in distal segments than in proximal segments (Howland and Spector, 1972). The reason for this unequal distribution is not clear but an increased number of connective tissue mast cells being included in tissue segments on excision cannot be ruled out. An

alternative to this explanation is a greater non-mast cell histamine content in distal segments. The development of more specific assays for heparin may provide an approach to the quantitation of mast cell contents of vascular and neuronal tissues (Jacques, 1975).

3.5 Summary

The histamine content of the REA shows a regional distribution with the proximal segment possessing about 50% of the content of the distal segment.

Sectioning the great auricular nerve did not alter the amount of histamine present in proximal or distal segments of the REA.

The catecholamine content of the REA did not show a regional distribution, i.e. the proximal and distal segments possessed equal content of catecholamines.

The great auricular nerve contains both histamine and catecholamines.

CHAPTER 4

THE MAST CELLS IN THE NEUROVASCULAR
TISSUES OF THE RABBIT EAR

4.1 Introduction

The tissue mast cell is of interest as it is known to synthesize and store large amounts of histamine, serotonin, bradykinin and highly sulphated acid mucopolysaccarides (which is mostly heparin) (Jaques, 1975). This cell, whose function was originally thought to be a product of overfeeding (Erhlich, 1879), is now considered by some to have a more chemical function of binding extracellular amines in the fashion of an ion exchange resin (Padawar, 1974). The capacity of cells to take up and store amines in this way is not exclusive to mast cells, the high affinity of sympathetic nerves for noradrenaline and the somewhat lower affinity of the smooth muscle for catecholamines (uptake 2) is well documented. It appears valuable to study the distribution of the mast cell in the neurovascular tissues of the rabbit to investigate the presence of a mast cell compartment in these pharmacologically important tissues. Several authors have suggested that mast cells are rare in rabbit tissues, although there are larger numbers of mast cells in the skin of the ear, paws and the nose (Hunt et al, 1961).

El-Akad and Brody (1976) did not detect any mast cells in the blood vessels of a large number of species, although these authors did not report any studies with rabbit blood vessels. When rabbit nerves were studied (Torp, 1961) the histamine contents were reported to be directly related to the mast cell numbers. The following study examines the mast cell distribution of the rabbit ear artery, rabbit ear veins and the great auricular nerve.

4.2 Methods

General:

Sections of rabbit ear artery, vein and great auricular nerve were taken from semi-lop eared rabbits of either sex. The animals were killed by a blow to the head and bled from cervical vessels. The skin over the ear was rapidly trimmed away to dissect the above structures free from the bulk of

adherent connective tissue. The large vessels were gently washed with saline free of blood to avoid traumatic degranulation of the mast cells. Rats were killed with a blow to the head then pieces of tongue were removed. Rat tongue is known to be rich in mast cells (Riley, 1953) and thus provided a control tissue in these studies.

Fixation:

Tissues from four rabbits were fixed in 0.5% (0.025 M) cyanuric chloride in anhydrous methanol containing 1% (0.1 M) N-methylmorpholine for 18-24 hours (Goland et al, 1967). The tissues were then washed with two changes of methanol over two hours, cleared in cedar wood oil for two days, vacuum embedded in paraffin wax (mp 55°C) and sections were cut at 7μ. Tissues from two rabbits were cooled to the temperature of liquid nitrogen in isopropane and freeze dried at -35°C and 9×10^{-3} Torr for three days the temperature was raised to -15°C for a further day then equilibrated with room temperature on the fifth day. The dried tissues were vacuum embedded and 7μ sections were cut. Pieces of rat tongue were fixed in an identical manner to rabbit tissues. Sections were subsequently stained with either Alcian blue or Toluidine blue.

1% Alcian blue 8GX at pH 1.1:

Paraffin sections were taken to water then stained with 1% Alcian blue in 0.1 MHCl (pH 1.1) by the method of Pearse (1968) and counterstained with 0.1% nuclear fast red, dehydrated in alcohol, cleared in Xylene and mounted in PIX.

Toluidine blue at pH 3.0:

Paraffin sections were taken to water then stained with 1% Toluidine blue in acetic acid at pH 3.0 by the uranyl nitrate metachromatic method of Hughsdon (1949), then the sections were dehydrated in ethanol, cleared in Xylene and mounted in PIX.

4.3 Results

In general mast cells were identified by their metachromatic staining
of cytoplasmic granules with Toluidine blue. The granules did not appear
as densely packed as in rat control tissues. Mast cells were identified by
specificity of their staining reaction with Alcian blue at pH 1.1 for poly-
sulphated acid mucopolysaccharides. Although there was some variability in
staining of the granules within the same section, many cells had an identical
appearance to those from rat tissues.

(a) Rabbit ear artery

In general there were few mast cells associated with this blood vessel.
A study of several hundred sections of artery taken from the proximal seg-
ment only revealed occasional mast cells. Sections of the artery taken ½
to 1/3 the way up the ear distally showed more frequent mast cells in the
adventitia of the ear artery. The connective tissue adjacent to the adven-
titia of distal segments showed occasional mast cells near to the endothel-
ial cells of capillaries which occurred in groups of 1-2 along the vessel
viewed in serial sections. Where a small branch of the great auricular
nerve had been included in the section mast cells were seen in the connect-
ive tissue of the nerve bundles (Figure 4.1).

(b) The great auricular nerve and ear veins

Due to difficulty in sectioning the isolated ear veins these were best
sectioned with the great auricular nerve. The veins of proximal sections
were not associated with any mast cells (including the adjacent connective

Figure 4.1 (a)

The photomicrograph shows a 7 μm section of rat tongue (x125). Mast cells
are dense metachromatically stained cells. This tissue was included to act
as a control for stains and procedures outlined in methods. (Fixation;
cyanuric chloride, stain; Toluidine Blue).

Figure 4.1 (b)

The photomicrograph shows a 7 μm section of rat tongue (x125). Mast cells
contain densely packed blue granules. (Fixation; cyanuric chloride, stain;
Alcian Blue/nuclear fast red).

Figure 4.1 (c)
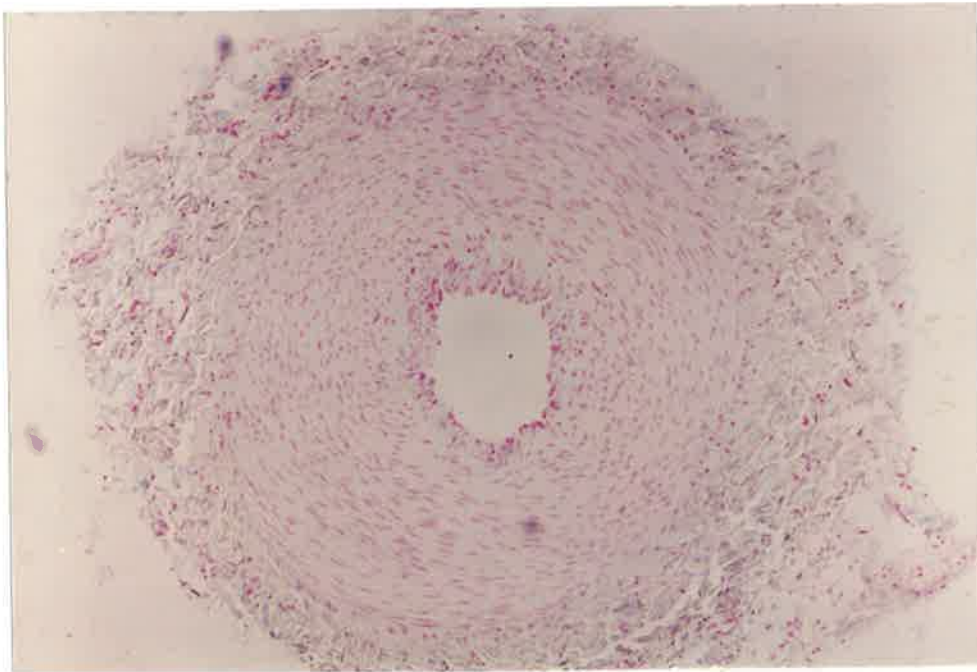
The photomicrograph shows a 7 μm transverse section of a proximal segment of a rabbit ear artery (x100).   This typical section did not contain mast cells.   (Fixation; cyanuric chloride, stain; Alcian Blue/nuclear fast red).

Figure 4.1 (d)
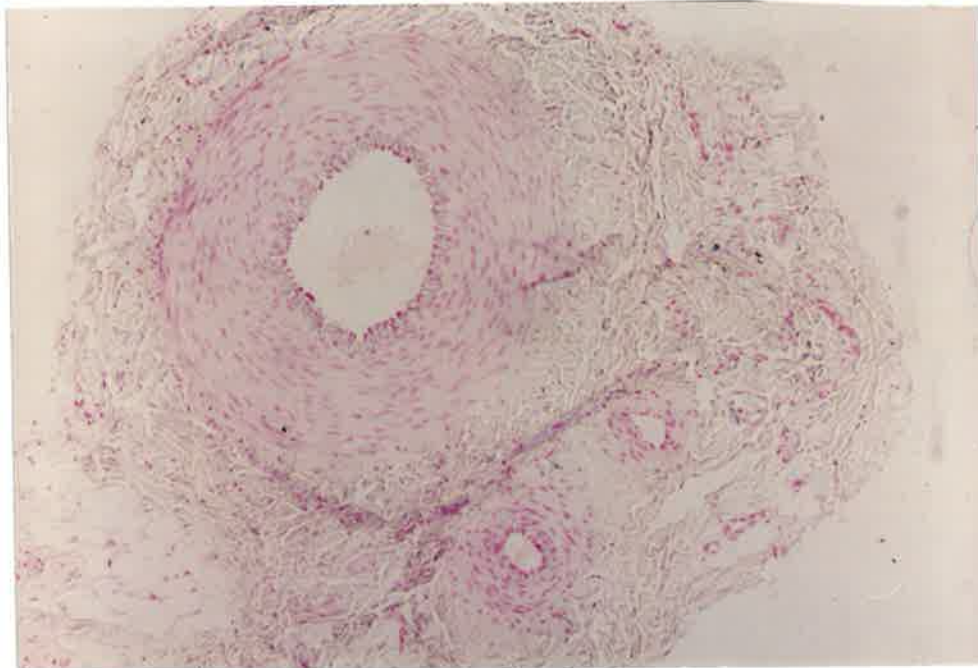
The photomicrograph shows a 7 μm transverse of a distal segment from a rabbit ear artery (x100).   A few mast cells are seen in the connective tissue.   (Fixation; cyanuric chloride, stain; Alcian Blue/nuclear fast red).

Figure 4.2 (a)
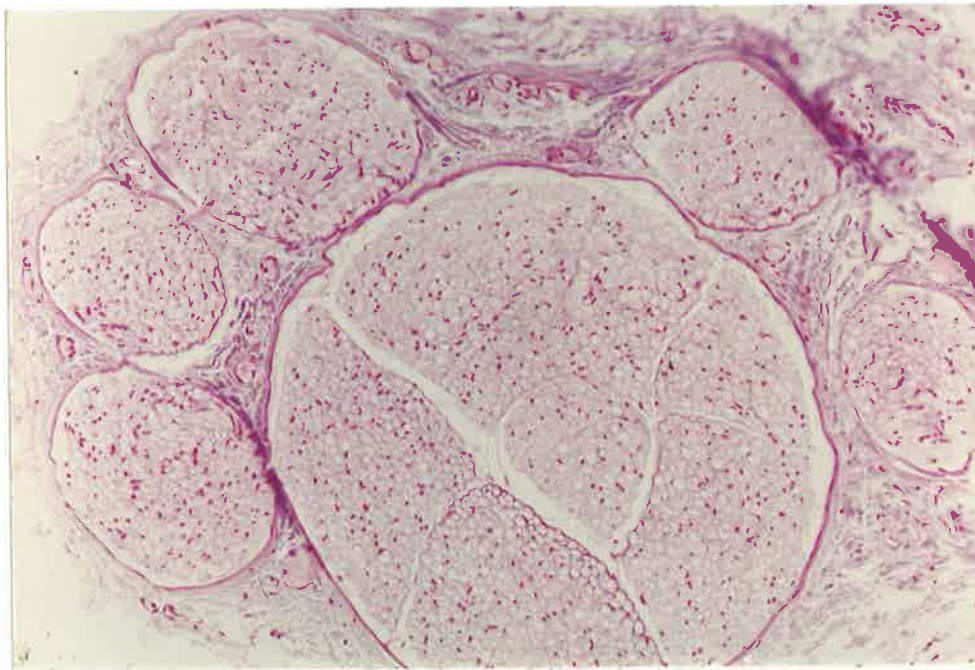
The photomicrograph shows a 7 µm transverse section of a proximal segment of the great auricular nerve (x125). This typical section does not show any mast cells. (Fixation; cyanuric chloride, stain; Alcian Blue/nuclear fast red).

Figure 4.2 (b)
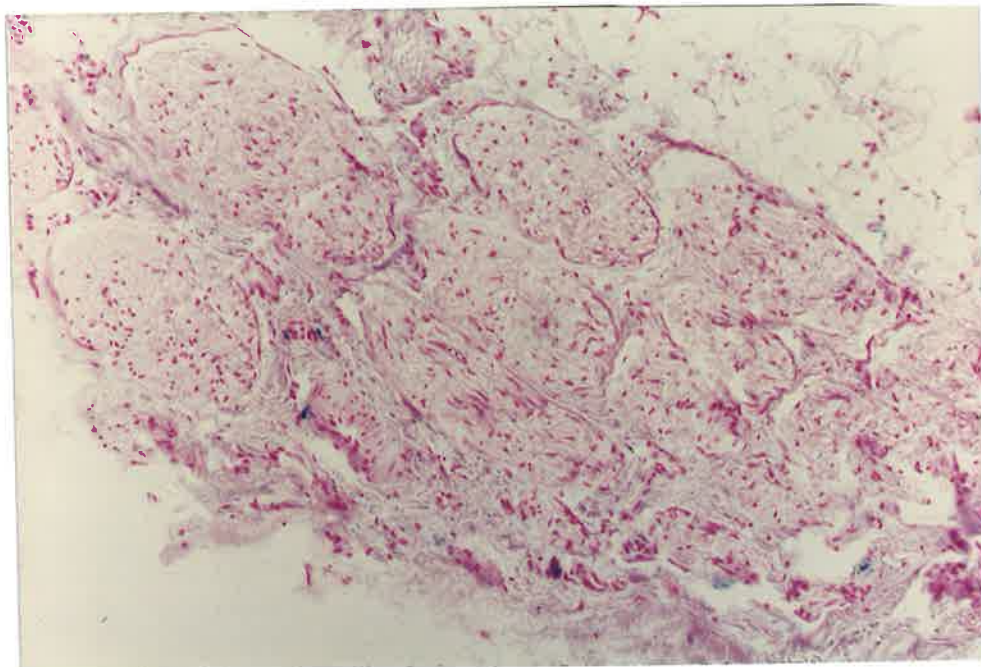
This photomicrograph shows a 7 µm transverse section from a distal segment of the great auricular nerve (x125). A few mast cells are distributed throughout the connective tissue surrounding the nerve bundles. (Fixation; cyanuric chloride, stain; Alcian Blue/nuclear fast red).

Figure 4.2 (c)
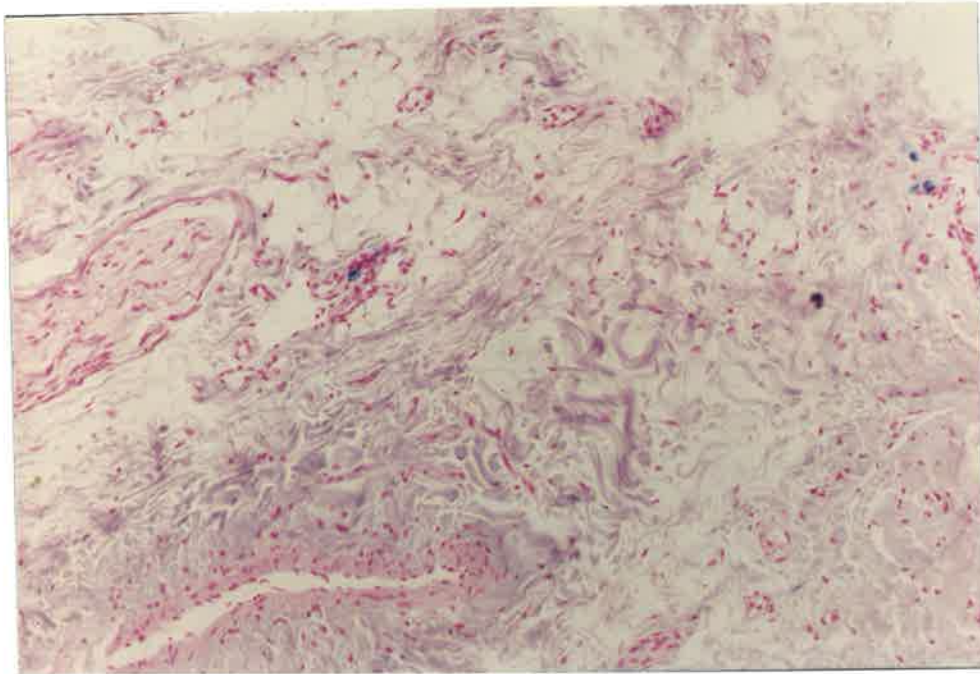
This photomicrograph shows a 7 m section of the distal segment of a great auricular vein (x125). A few mast cells are seen within the connective tissue of the adventitia. (Fixation; cyanuric chloride, stain; Alcian Blue/ nuclear fast red).

Figure 4.3 (a)
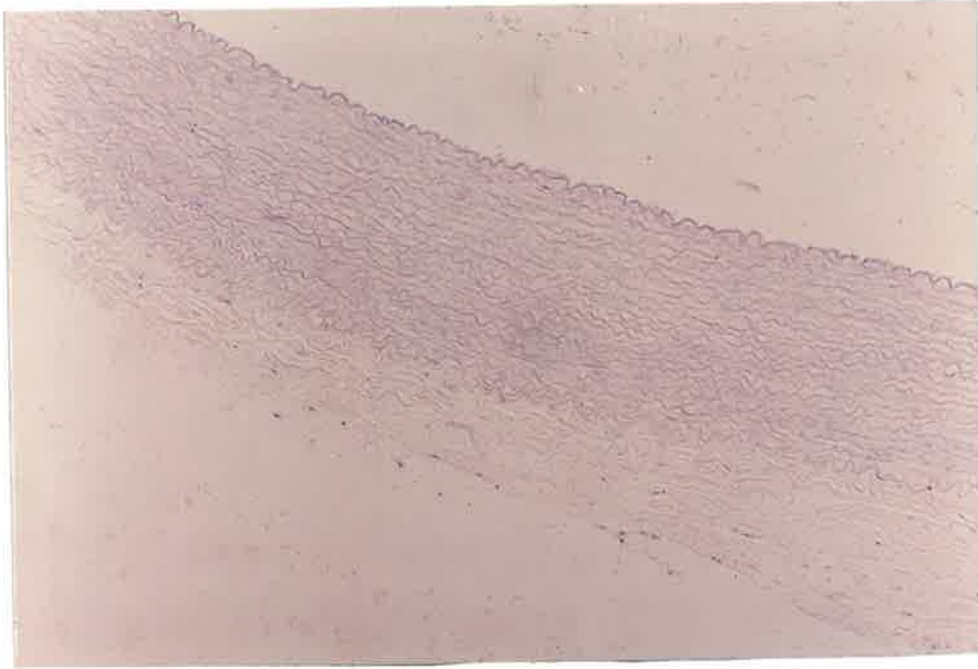
The photomicrograph shows a 7 μm transverse section of a rabbit aorta
(x 125). No mast cells are evident in this section as was the case for all
sections of aorta examined using any of the techniques outlined in methods.
(Fixation; cyanuric chloride, stain; Alcian Blue/nuclear fast red).

Figure 4.3 (b)
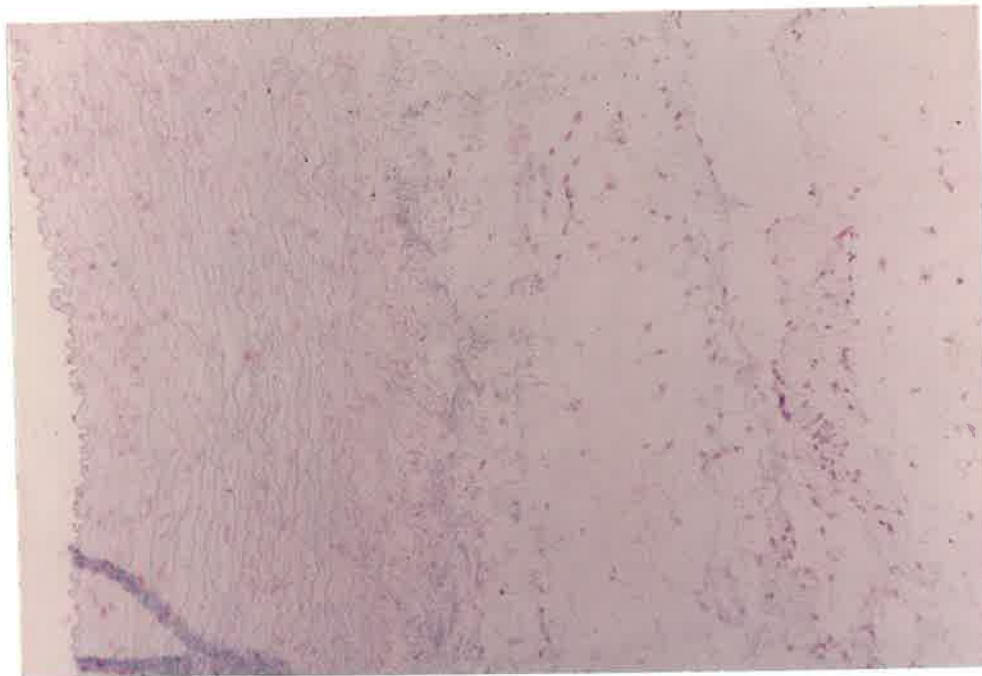
The photomicrograph shows a 7 μm transverse section of a rabbit aorta
(x 125). This section typically contained no mast cells. (Fixation; freeze
dried, stain; Alcian Blue/nuclear fast red).

tissue). Distal sections of the vein often had one or two mast cells close
to the adventitia, some were seen quite clearly in the adventitia.
Sections of the great auricular nerve showed the same general trend as the
artery and veins in the distribution of the mast cells. In proximal
sections, which contain one or two distinct nerve bundles, no mast cells were
seen; distally in the same nerve, where there were now many small separate
branches, there were 4-6 mast cells per section. Most of these mast cells
were seen in close association with small vessels. (Figure 4.2).

(c) Rabbit aorta

Over three hundred sections of rabbit aorta were examined and no pos-
itive mast cells were identified using both staining techniques (Figure 4.3).

4.4 Discussion

Although mast cells have been studied in a variety of blood vessels
from many species, few have been as systematic as that of El-Ackad and Brody
(1975) although they did not include rabbit vessels in that study. The
tissues of the rabbit have in general been described to be poor in mast
cells (Michels, 1938, Riley and West, 1956). Blood basophils in rabbit are
suggested to play a functional role more characteristic of mast cells
in other species. The cutaneous tissue of the rabbit paw, nose and ear
are some areas in the rabbit where greater number of mast cells have
been found (Hunt et al, 1961). The lack of mast cells in the aorta of the
rabbit is supported by a similar finding by Hunt and co-workers (1961) using
different fixation and staining methods. The marginal ear vein has been
examined for mast cells and the effect of experimentally induced thrombosis
has been studied (Petterson and Hjelmman, 1964). There were a few mast
cells observed in control sections and these were seen to increase with
thrombosis. In addition a number of unidentified cells appeared to contain met-

achromatic material in thrombosed veins. The origin of the non-mast cell metachromasia is of interest because other workers have described an appearance of metachromasia in non-mast cells of rabbit tissues treated with compound 48/80, and it is possible that these are the released products of mast cells. The ability of endothelial cells and fibroblasts to take up heparin after injection has been reported in rat tissues and may explain the observations above.

The present study in the rabbit supports the view of El-Akad and Brody (1975) that there are few mast cells in association with major blood vessels and in the smaller arteries very few mast cells were located in the adventitial layer. In addition, the presence of mast cells in the walls of veins in the rabbit ear is indicated by this study.

The observation of mast cells in the adventitia of ear veins may be peculiar to regions where the subcutaneous tissue is richer in mast cells and may reflect a property of the surrounding connective tissue rather than that of the blood vessels in the rabbit. The regional distribution of mast cells in the rabbit vessels studied suggests that the connective tissue rather than the blood vessel increases in mast cell content as the vessels are traced distally up the rabbit ear. This has implications in the assay for endogenous histamine contents of rabbit vessels where, in attempting to isolate more distal vessels, a greater number of mast cells may be included in the tissues excised. An apparent increase in the tissue histamine content of distal vessels may reflect an increased presence of mast cells. When the histamine contents of rat mesenteric artery in the proximal and distal segments are examined the distal segments are found to contain twice as much histamine as the proximal segments of the same vessel (Howland and Spector, 1972). Studies with the histamine content of proximal and distal segments of the rabbit ear artery show a two-fold increase in histamine content in the distal segment which may be attributed to the increased presence of mast cells.

4.5 Summary

Distal segments of the rabbit ear artery contained increased numbers of mast cells compared with the proximal region of this vessel. Mast cells in the ear artery tended to be located in the loose connective tissue some distance away from the adventitia. There appeared to be some relationship between mast cells and the capillaries of the loose connective tissue in some sections. Distal segments of the great auricular nerve contain increased numbers of mast cells. No mast cells were detected in the rabbit aorta.

CHAPTER   5

THE ACCUMULATION AND CATABOLISM
OF HISTAMINE BY VASCULAR TISSUES

ACKNOWLEDGEMENT

The following comparative study was carried out
in conjunction with Dr. A. Foldes. Most of the
studies on the rabbit aorta were carried out by
Dr. Foldes, while the present author carried
out the studies on the guinea pig aorta and the
rabbit ear artery. Methods were developed con-
jointly. The joint study is presented in this
chapter as the detail of the rabbit aorta study
is essential to enable comparative conclusions
to be drawn.

5.1 Introduction

Evidence that the rabbit aorta accumulates exogenous histamine was pre-
sented by Adams and Hudgins (1976). A later study in this laboratory
(Foldes et al, 1977) confirmed this finding. In addition various agents
were tested for their effect on the histamine uptake process in rabbit aorta.
When cocaine, deoxycorticosterone acetate, normetanephrine, and phenoxyben-
zamine were tested, all compounds, with the exception of cocaine, resulted
in a reduction in the tissue uptake of ^{14}C histamine from the medium. This
suggested that histamine was not transported into sympathetic nerves via a
cocaine sensitive mechanism, with the qualification that the distribution of
catabolites was not investigated. This study did however indicate some
similarity with extraneuronal uptake mechanisms for catecholamines. In view
of the importance of histamine in blood vessels (Schayer, 1965) and the role
of catabolism in terminating the action of histamine and other biogenic
amines (Kalsner, 1970, 1975) the effects of various pharmacological agents
on the catabolism of exogenously applied histamine was tested in the rabbit
thoracic aorta.

Two other vascular tissues were included, namely the rabbit ear artery
(REA) and the guinea pig thoracic aorta (GPA). The REA was included because
it represents a different type of vessel, a small muscular artery in which
the muscular media is more pronounced. Furthermore, unlike the aorta it is
readily sympathetically denervated by surgical means (de la Lande and Rand,
1965). The GPA was studied partly to ascertain whether species differences
exist for the catabolism of histamine in blood vessels. It is also known
that the guinea pig aorta is more sensitive to histamine than the rabbit aorta
(Maling et al, 1972). Hence, the present study also offered the possibility
of studying whether the differences in uptake and catabolism are factors in-
volved in the difference in pharmacological responsiveness of the vessels
from the two species.

Factors which may reduce the concentration of histamine and its recept-

ors and thus modify the pharmacological response include:

(1) diffusion of the agonist from the biophase

(2) uptake into binding sites or cells

(3) enzymic inactivation

The third factor will be examined in some detail in the present study.

A major requirement for this study was a reliable separation technique for the radioactive catabolites of histamine. Several methods (Schayer, 1959, Snyder, Axelrod and Bauer, 1965, Schwartzman, 1973) having yielded unsatisfactory separation in our hands, a paper chromatographic technique (Cabut and Haegermark, 1966) was adapted to cellulose thin layer chromatography plates. The adapted method was shown to yield good separation of histamine, methyl histamine, deaminated acid catabolites and N-acetyl-histamine.

5.2 Materials and Methods

Semi-lop eared rabbits of either sex or guinea pigs of either sex were killed by cervical dislocation. Rabbit ear artery and aortic tissues were excised and incubated in the presence of ^3H or ^{14}C histamine and the drugs under test. The accumulated ^3H or ^{14}C label was extracted and quantitated as previously described (Foldes et al, 1977). Studies with the accumulation of ^3H or C^{14} label in the rabbit ear artery differed slightly from the rabbit aorta in that tissues were washed for 30 seconds, rather than 60 secs after the incubation step.

Calculation of extracellular space equivalents in guinea pig aortae:

An approximation of the extracellular space equivalent of the GPA was obtained using ^{14}C sorbitol at an equivalent bath concentration of unlabelled histamine, as used in the labelled histamine incubation experiments. ^{14}C sorbitol was extracted from the tissues after a 60 second wash after the incubation step. This produced an estimation of the labelled amine retained by the extracellular space equivalent during this step. This assumed equal

losses of labelled amine and sorbitol from the extracellular space.

Chemical and surgical sympathectomy of rabbits:

A unilateral superior cervical ganglionectomy was performed, as described earlier, this allowed the contralateral REA to act as a paired control. The animals were killed 7 days later by cervical dislocation. Two rabbits were treated with 300 mg/kg of 6-hydroxydopamine (6OHDA) in divided doses (Finch, Haesler and Thoenen, 1973).

Efficiency of sympathectomy:

In order to maximise the use of rabbit tissues for histamine uptake studies, physical signs were used as an index of post operative success. All animals showed increased constriction of the pupil on the operative side compared to the contralateral eye. Most animals showed a detectable difference in ear temperatures (Feldberg, 1926). The effect of 6OHDA was confirmed by catecholamine content of the rabbit's uterus (personal communication, Dr. J.A. Kennedy).

Acid extracts of identically treated tissues (3 to 5 per experiment) were pooled and cold carrier (histamine (H) and the catabolites   methyl histamine (M), imidazole acetic acid (IAA) and N-acetylhistamine (N) were added (25 µg of each)). The pooled samples were snap frozen, lyophylized and extracted with small volumes of ethanol, then of water. The combined extracts were concentrated under nitrogen and applied to cellulose thin layer chromatography plates.

Incubation fluids were pooled, lyophylized, extracted and plated similarly to the tissue extracts, with the addition of a low speed centrifugation step prior to plating of the supernatant.

Thin layer chromatography:

After application of samples and standards (H, M, IAA, N and also a mixture of all four) the cellulose plates were run in ter -butanol: formic acid: pyridine (25% v/v): (70:15:15) until the solvent front reached the

top of the plate and the plates were then air dried.

Visualization of imidazole compounds:

In initial experiments, the entire plate, including unknowns and standards, was visualized by spraying. Subsequently, it was found preferable to spray the standards only. The positions of the unknowns were then compared to those of the standards under ultraviolet light. Histamine and methyl histamine standards were first visualized on the plates by spraying lightly with 0.1% (w/v) ninhydrin in acetone, drying the plates and heating at 80° C until the characteristic purple colour appeared. The position of each spot was marked and the plates cooled to room temperature. Next, imidazole acetic acid and N-acetylhistamine were visualized using Pauli's reagent. The plates were lightly sprayed with freshly prepared 1:1 mixture of 5% (w/v) sodium nitrite solution and 1% (w/v) sulphanilic acid solution in HC (2 mole.1^{-1}). This reagent was followed by 15% (w/v) sodium carbonate, which caused the immediate development of a yellow-orange colour indicating the positions of IAA and N. Histamine also reacted under these conditions. Another histamine catabolite, methylimidazole acetic acid, could not be visualized using either ninhydrin or Pauli's reagent, the two spray reagents used in this study, hence, methylimidazole acetic acid was not localized or reported on in this study. (Using ammoniacal silver nitrate (Snyder et al, 1964), methyl-imidazole acetic acid was subsequently seen to co-migrate with IAA in this system, hence, the reference to an "acid" or "deaminated catabolite" fraction A, rather than IAA, in the tissue extracts).

Quantitation of the metabolites:

For each unknown extract, the region of the cellulose t.l.c. plate corresponding to each standard imidazole compound was carefully scraped into a scintillation vial, extracted by vigorous shaking in 1 ml of distilled water or ethanol and triton/toluene scintillant was added.

Histamine and the labelled catabolites were quantitated in each incubation fluid and tissue extract by liquid scintillation spectrophotometry and

the effects of various drug treatments on the distribution of the catabolites were determined. The counts were corrected for quenching of radioactivity where necessary. Overall recovery of label from the tissue extracts varied between 40% and 85% and from the incubation fluids between 70% and 90% in different experiments, but recovery of label from simultaneously chromatographed control and treated tissue extracts was constant. The results are expressed as percentage of total ^{14}C label recovered from the plate in the regions corresponding to histamine and to each of the catabolites, irrespective of the overall recovery of applied label. Crossover of ^{14}C histamine into the regions of the catabolites varied between 0% and 2%.

Estimate of recoveries of each catabolite:

Since no convenient chemical assay method was available for the assay of each catabolite in the low concentration ranges, the recovery of each of the catabolites from the thin layer plate was estimated using labelled catabolites. The labelled catabolites were isolated by the above thin layer chromatography system acid extracts in the ter-butanol: formic acid: pyridine solvent following incubation of large (>100 mg) segments of rabbit aorta with undiluted stock ^{14}C histamine (in the absence of cold carrier histamine). The isolated fractions were re-chromatographed as before and were then taken up in ethanol, their radioactivity was determined and the catabolites stored at 0°. To determine recovery of each catabolite, four segments of rabbit artery were incubated in the presence of 200 ng of histamine (including 0.05 μCi ^{14}C histamine) and extracted in HCl/EDTA as before. Each extract was separated into two halves, one half to act as control and the other half, following the addition of ^{14}C histamine or of a ^{14}C histamine catabolite (2000 c.p.m.) to be tested for recovery. Each extract was then lyophylized and chromatographed as usual and recoveries of each labelled species individually estimated.

Percent recoveries of ^{14}C-H, ^{14}C-M, ^{14}C-A and ^{14}C-N were, respectively, 38.3 (range 33.8-42.9), 33.9 (range 26.7-41.5), 38.4 (range 34.6-42.2) and 42.0 (range 37.4-46.7) in four experiments. None of these recoveries were

different from each other.

Materials:

Histamine dihydrochloride, Koch-Light Laboratories, Bucks, England; Ring-2- ^{14}C histamine dihydrochloride (Spec. Act 59 mCi/mmol) Amersham, England; Histamine catabolites methylhistamine (M), imidazole acetic acid (IAA) and N acetylhistamine (N) Calbiochem, California, U.S.A.; Cellulose thin layer chromatography plates with fluorescent indicator, E. Merck A.G. Darmstadt, Germany; Amadiaquin, Parke Davis and Co., N.S.W., Australia; ^{14}C Sorbitol (200 mCi/mmol) Amersham, England; 6-hydroxydopamine (6OHDA) was a gift from Sigma Chemical Company, St. Louis, Mo. U.S.A.

Analysis of data:

The values of total labelled amine uptake into the REA and the guinea pig aorta were first tested for homogeneity of variance (Bartletts test) then paired or unpaired t-test statistics were employed.

As standard errors of means of percentages cannot be calculated directly, percentage results were converted by means of the arcsin transformation to a quantity $\emptyset$ for statistical treatment, where $\emptyset = \sin -1 \sqrt{\%}$ and are expressed as equivalent mean % with upper and lower limits.

Notes on Methods

Separation of standards:

The Rf's for the various standards at room temperature on cellulose chromatography plates in ter-butanol: formic acid: 25% (v/v) aqueous pyridine (70:15:15) were as follows:

histamine:0.20; methylhistamine:0.31; imidazole acetic acid:0.49; N-acetyl histamine:0.72.

These individual Rf values did not change appreciably when a mixture of all four substances was applied to the plate and proved constant over at least 40 determinations.

Effect of ambient temperature:

The migration of the imidazole compounds was found to be temperature dependent, faster migration occurring at higher ambient temperatures. This faster migration resulted in a decreased resolution of the component, and, conversely, slower migration with a concomitant slight improvement in resolution was noted at lower temperatures.

Separation of unknowns:

Incubation fluids and tissue extracts were resolved into four components (H, M, A and N) each of which co-chromatographed with the corresponding standard, as seen following staining with ninhydrin/Pauli reagent or under UV light.

The chromatographic separation method presented here provides a convenient assay method for the study of histamine catabolism in tissues. The method compares favourably with published chromatographic separations (Schayer, 1959; Snyder et al, 1964; Schwartzman, 1973) in its reproducibility and in the extent of separation achieved.

The results are presented as percentages of the total label applied to each t.l.c. plate recovered in the region of each catabolite. This overcomes the difficulty of variable total radioactivity recovered from the differing amounts initially present in treated and untreated tissue extracts and incubation fluids. It was found that losses during the preparation procedure affected histamine and each of the catabolites equally. Extraction of the lyophylized samples in ethanol and in water was intended to minimize differential losses, and the results obtained using labelled catabolites show that losses of each of the catabolites are comparable throughout the entire assay procedure. However, even in the event of non-uniform losses of the catabolites, comparisons between controls and drug treatments would still be valid, since samples are treated identically and percentage recoveries of label from the thin layer plate are comparable between control and treated extracts chromatographed simultaneously.

5.3 Results

(a) Studies in the rabbit aorta

Catabolism of ^{14}C histamine by rabbit aorta:

Table 5.1 shows the relative proportions of ^{14}C histamine and ^{14}C histamine catabolites in both rabbit aorta extracts and incubation fluids, in the absence of any pharmacological agents. In the untreated tissue oxidative deamination appears to be the predominant pathway of catabolism, as acid catabolites comprise the largest fraction.

The effects of various drugs on the catabolism of ^{14}C histamine in rabbit aorta:

The effects of 3 groups of agents were investigated: inhibitors of histamine accumulation, inhibitors of histamine catabolism and drugs which interact with the catabolism or uptake of catecholamines.

The effects of the histamine N-methyl transferase (HNMT) inhibitor amodiaquin and of the diamine oxidase (DAO) inhibitor hydrallazine (Grabbe and Bardsley, 1974) are shown in Table 5.1 The inhibition of methyl histamine formation and of oxidative deamination, respectively, by these agents provides confirmation of the specificity of the chromatographic separation procedure as well as complementing the effects of these drugs on total histamine uptake into the rabbit aorta (Foldes et al, 1977) .   The effect of hydrallazine was concentration dependent, acid catabolite formation being progressively inhibited at increasing hydrallazine concentrations from 1 to 100 µg/ml.

An Inhibitor of amine uptake, namely, the steroid corticosterone was seen to   alter   the pattern of histamine catabolism in the rabbit aorta (Table 5.2), as did the monoamine oxidase inhibitors iproniazid and tranylcypromine.

The mast cell degranulating agent compound 48/80 caused a small increase in unchanged amine levels. Ouabain, a general transport antagon-

Table 5.1

Distribution of ^{14}C histamine and its catabolites in the rabbit aorta

Treatment	^{b} n	^{a} Histamine and catabolites (% of total ^{14}C)			
		H	M	A	N
Control					
Control tissue	21	24.0 22.0 20.1	20.4 18.3 16.4	56.9 55.0 53.1	3.1 2.3 1.7
Control incubation fluid	10	91.8 90.4 88.9	5.7 4.8 3.9	3.8 3.2 2.7	1.6 1.1 0.7
Effect of enzyme inhibitors					
Amodiaquin (10 µg/ml)	5	41.8 *36.7 31.7	9.5 *8.8 7.2	59.2 53.0 46.8	1.7 1.5 1.5
Hydrallazine (10 µg/ml)	5	59.0 *51.9 44.7	45.5 *35.9 26.9	9.0 *6.8 4.8	4.5 2.7 1.3

^{a} The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total ^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min.$^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetylhistamine.

^{b} Three or more tissues were included from each animal, where n refers to the number of animals in each treatment group.

* $p < 0.05$ compared with control values, unpaired t-test.

Table 5.2

Effect of drugs on the distribution of ^{14}C histamine and its catabolites in the rabbit aorta

Treatment	[b] n	[a] Histamine and catabolites (% of total ^{14}C)			
		H	N	A	N
Iproniazid (10 µg/ml)	5	35.7 28.1 21.1	38.1 * 33.8 29.7	41.4 * 35.3 29.3	1.3 0.8 0.5
Tranylcypromine (10 µg/ml)	5	43.3 * 39.3 35.4	51.2 * 46.3 41.4	12.9 * 10.1 7.6	2.3 1.7 1.1
DOCA (10 µg/ml)	7	31.9 27.0 22.3	18.0 14.5 11.2	61.4 56.6 51.9	0.5 0.2 0.1
Corticosterone (10 µg/ml)	4	20.8 18.8 16.8	11.5 9.8 8.2	74.1 * 70.8 67.4	0.6 0.2 0.1
Cocaine (10 µg/ml)	5	47.9 * 41.5 35.3	22.2 16.3 11.3	41.9 * 37.0 32.3	3.6 3.1 2.7
Compound 48/80 (10 µg/ml)	4	46.3 * 38.2 30.4	17.8 14.0 10.6	53.4 43.0 32.1	N.D. 2.6 N.D.
Ouabain (10 µg/ml)	5	44.6 * 38.2 31.9	23.2 17.7 12.8	46.6 * 39.4 32.4	3.2 2.6 2.1
Phenoxybenzamine (10 µg/ml)	5	38.0 31.7 25.8	13.3 11.3 9.5	59.2 52.7 46.1	4.0 3.3 2.7

[a] The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total ^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min.$^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetyl-histamine, N.D. = range not determined.

[b] Three or more tissues were included from each animal, where n refers to the number of animals in each treatment group.

* $p < 0.05$ compared with control tissue values in table 5.1, unpaired t-test.

Table 5.3

Distribution of ^{14}C histamine and its catabolites
in the separated adventitial and medial layers of rabbit aorta

Treatment	^{b} n	^{a} Histamine and catabolites (% of total ^{14}C)			
		H	N	A	N
Intact aorta	21	24.0 22.0 20.1	20.4 18.3 16.4	56.9 55.0 53.1	3.1 2.3 1.7
Adventitia	5	67.9 * 65.5 63.1	13.0 10.9 9.0	23.4 * 19.4 15.7	4.1 2.9 1.9
Media	5	28.7 24.6 20.8	19.4 14.9 10.9	57.5 58.6 56.3	1.6 1.9 1.3

^{a} The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total
^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min.
$^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetylhistamine.

^{b} Three or more tissues were included from each animal, where n refers to
the number of animals in each treatment group.

* p < 0.05 compared with control values of intact aorta, unpaired t-test.

ist which had been shown partially to inhibit histamine uptake (Foldes et al, 1977 a), also partially inhibited the catabolism of histamine (Table 5.2).

Since the neuronal catecholamine uptake inhibitor, cocaine, also altered the histamine catabolite pattern (Table 5.2), the distribution of histamine catabolites in the separated adventitial and medial layers of the aorta were next studied. The pattern of catabolism was very different in each separated layer (Table 5.3). The smooth muscular medial layer was seen to be the region where the bulk of the histamine catabolism in the aorta occurred, while most of the label present in the nerve-terminal-containing adventitial layer was in the form of unchanged histamine. The result, however, is difficult to interpret in view of the much decreased total label recovered from the separated layers (Foldes, Stacey and de la Lande, 1976).

Catabolite distribution in the incubation fluid of the rabbit aorta:

The proportions of histamine and of the various catabolites in the incubation fluids bathing the tissues were also determined in the presence and absence of various agents. In control incubation fluid over 90% of the recovered label was unchanged histamine (90.1 $\pm$ 1.5%, n=10), with M 4.9 $\pm$ 1.1% (n=10) acid catabolites 3.5 $\pm$ 0.6% (n=10) AND N 2.1 $\pm$ 0.4% (n=7) (Table 5.1). None of the drugs or treatments tested appeared to change this distribution greatly, with the exception of tranylcypromine (99.3% H, 0.3% M, 0.1% A, 0.3% N) which depressed all catabolites in the incubation fluid, with the greatest effect being on the amount of acid catabolites released.

No significant changes were noted in the distribution of label in the wash fluid or in the tissues with increased washing times from 0 to 6 minutes in absence of drugs.

(b) Studies in the guinea pig aorta

The accumulation of ^{14}C in the guinea pig aorta:

The guinea pig aorta is known to be more sensitive to the contractile effects of histamine than the rabbit aorta (Maling, Fleisch and Saul, 1971). The accumulation of ^{14}C histamine and catabolites over 30 minutes was found

to be similar for both rabbit and guinea pig aortae. The effects of enzyme inhibitors were also examined (Table 5.4). The effects of the N-methyltransferase inhibitor, amodiaquin, did not significantly effect the uptake of ^{14}C histamine equivalent into the rabbit aorta, but it significantly decreased the uptake of ^{14}C in the guinea pig aorta. Whereas hydrallazine, a DAO inhibitor, significantly decreased ^{14}C uptake in both species. An approximation of the amount of ^{14}C histamine remaining in the extracellular space after a 60 second wash was made using ^{14}C Sorbitol as a marker. The assumption was made that ^{14}C Sorbitol and ^{14}C histamine efflux from the extracellular space at an equal rate during the 60 second wash step. It is reasonable to assume that radiolabelled histamine and unlabelled histamine behave in an equivalent manner. The extracellular space equivalent was studied in six aortic segments from two guinea pigs with a mean value of 0.25 mls/g tissue ($\pm$ 0.02 S.E.M.). Therefore, approximately 50 ng/g of the measured histamine uptake into guinea pig aorta can be considered as accumulated within the extracellular space.

The catabolism of ^{14}C histamine in the guinea pig aorta:

Table 5.5 shows the amount of labelled histamine and its catabolites present in the tissues after 30 minutes incubation with labelled histamine. It will be seen that the catabolism of histamine in the guinea pig aorta appears to proceed with N-methylation being of equal importance to oxidative deamination, which contrasts with the rabbit aorta, where oxidative deamination appears to predominate (Tables 5.1 and 5.5). In the rabbit aorta amodiaquin only decreased the proportions of methyl histamine formed. However, amodiaquin decreased the proportions of methyl histamine and the deaminated catabolites in the guinea pig vessel. Hydrallazine decreased the proportion of unchanged histamine. These findings suggest species differences in the compartmentalization of the accumulated histamine or of their catabolic enzymes.

Table 5.4

Effect of enzyme inhibitors on content of ^{14}C
in the thoracic aortae of the guinea pig and the rabbit

	Treatment	[b] n	^{14}C Accumulation ng.g^{-1}.30 min.$^{-1}$	% inhibition
GUINEA PIG	Control (+ Ethanol)	9	417.03 ± 30.56	35% inhibition
	Amodiaquin (10 μg/ml)	9	* 271.3 ± 23.4	
GUINEA PIG	Control	4	314.2 ± 8.6	38% inhibition
	Hydrallazine (10 μg/ml)	4	* 194.6 ± 31.3	
[c] RABBIT	Control (+ Ethanol)	5	459.1 ± 33.1	
	Amodiaquin (10 μg/ml)	5	426.0 ± 13.1	
[c] RABBIT	Control	5	561.7 ± 47.2	35% inhibition
	Hydrallazine (10 μg/ml)	5	* 364.3 ± 37.7	

[a] The ^{14}C accumulation is expressed as ng.gm^{-1} tissue (mean $\pm$ S.E.M.) histamine equivalent following incubation with ^{14}C histamine 200 ng.ml^{-1}. 30 min.$^{-1}$.

[b] Three or more tissues were included from each animal, where n refers to the number of animals in each treatment group.

[c] Data from Foldes et al (1977).

* $p < 0.05$ compared with control values for each treatment group, unpaired t-test.

Table 5.5

Distribution of ^{14}C histamine and its catabolites
in the guinea pig aorta

Treatment	^{b} n	^{a} Histamine and catabolites (% of total ^{14}C)			
		H	M	A	N
Control	11	21.8 20.1 18.5	39.5 † 37.1 34.8	39.1 † 36.3 33.7	6.2 4.6 3.2
Amodiaquin (10 µg/ml)	5	81.1 * 78.4 75.4	10.6 * 9.5 8.5	9.6 * 8.5 7.4	4.8 3.9 3.1
Hydrallazine (10 µg/ml)	5	56.0 * 48.4 40.8	53.1 45.5 38.0	5.0 * 4.3 3.6	2.0 1.8 1.6

^{a} The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total ^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min. $^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetyl-histamine.

^{b} Three or more tissues were included from each animal, where n refers to the number of animals in each treatment group.

* $p < 0.05$ compared with control values, unpaired t-test.

† indicates that the percentage in the control guinea pig aorta is significantly different ($p < 0.05$, unpaired t-test) from the percentage in the control rabbit aorta (Table 5.1).

Table 5.6

Guinea pig aorta; distribution of ^{14}C histamine
and its catabolites in the incubating medium

Treatment	[b] n	[a] Histamine and catabolites (% of total ^{14}C)			
		H	M	A	N
Control	6	89.2 87.4 85.6	4.9 3.9 2.9	5.1 4.6 4.2	2.1 1.7 1.4
Amodiaquin (10 µg/ml)	6	92.0 91.1 90.1	3.9 2.9 2.9	3.7 3.3 2.9	2.2 1.9 1.7
Control	5	87.0 85.7 84.3	7.5 6.7 6.0	5.7 5.1 4.6	2.3 1.9 1.6
Hydrallazine (10 µg/ml)	5	87.9 87.6 87.4	7.3 7.0 6.7	3.6 3.4 3.2	N.D. 2.0 N.D.

[a]   The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total
^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min.
$^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetyl-
histamine, N.D. = range not determined.

[b]   Three or more tissues were included from each animal, where n refers to
the number of animals in each treatment group.

No significant differences (at $p = 0.05$) were found between control and treat-
ment groups.

The proportion of histamine and catabolites present in the incubation media are shown (Table 5.6). The incubation fluids all contained a large proportion of unchanged histamine, the balance being catabolites of histamine. The changes in the arithmetic means of treatment groups compared with controls are in agreement with much larger changes seen in the tissue itself (Tables 5.5 and 5.6). However, it must be emphasised that these data do not indicate the amounts of catabolites formed, only their relative percentages under the various conditions.

The amount of N-acetyl histamine measured in the incubation fluids probably is an artifact of the thin layer chromatography system with these high salt samples. This was also observed for incubates of rabbit ear artery and rabbit aorta.

The amounts of methyl and acid histamine catabolites found in the incubation fluids suggest that these catabolites were equally lost from the tissue during the 30 minute incubation. Significant differences between catabolites in incubation fluids of control and treatment groups were not expected, as the mean concentrations of the catabolites were close to the resolving power of the separation method.

(c) Studies in the rabbit ear artery

The effect of sympathectomy on histamine accumulation in rabbit ear artery:

It has been demonstrated that sympathectomy produced a small but insignificant decrease in the endogenous histamine content of the rabbit ear artery (Chapter 2). The capacity of sympathectomized rabbit ear artery to accumulate endogenous histamine also appeared to be less than control values but this again was not significant (Table 5.7).

The uptake of ^{14}C histamine into control segments of rabbit ear artery was of the same order of magnitude as both the rabbit and the guinea pig aortae. It should be noted however that a 30 second wash step was employed in rabbit ear artery experiments compared with 60 seconds for aortic studies.

Table 5.7

$$\underline{\text{Effect of sympathetic denervation on the content}}$$
$$\underline{\text{of } {}^{14}\text{C in the rabbit ear artery}}$$

Treatment	[b] n	[a] ${}^{14}\text{C Accumulation}$ $\text{ng.g}^{-1}.30 \text{ min.}^{-1}$
Control	6	$621.4 \text{ ng/gm} \pm 38.4$
Sympathectomised	6	$535.9 \text{ ng/gm} \pm 24.4$

[a] The ${}^{14}\text{C}$ accumulation is expressed as ng.gm^{-1} following incubation with ${}^{14}\text{C}$ histamine 200 $\text{ng.ml}^{-1}.30 \text{ min.}^{-1}$.

[b] Two tissues were pooled from either control or treated rabbit ears, n refers to the number of animals.

Table 5.8

Distribution of ^{14}C histamine and its catabolites in control and sympathectomised rabbit ear artery

Treatment	[b] n	[a] Histamine and catabolites (% of total ^{14}C)			
		H	M	A	N
Control	5	29.7 27.0 24.5	51.5 48.8 46.1	23.4 21.2 19.2	2.8 2.6 2.3
Sympathectomised	5	37.2 31.4 26.7	47.3 43.7 40.2	24.4 22.0 19.7	2.6 2.4 2.1

[a] The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total ^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min. $^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetyl-histamine.

[b] Two tissues were pooled from either control or treated rabbit ears, n refers to the number of animals.

The mean values for histamine and catabolites did not differ significantly (at p = 0.05) between control and sympathectomised arteries.

Table 5.9

Rabbit ear artery; distribution of ^{14}C histamine and its catabolites in the incubating medium

Treatment	b n	a Histamine and catabolites (% of total ^{14}C)			
		H	M	A	N
Control	6	87.1 84.7 82.2	8.3 7.5 6.7	7.1 5.7 4.5	2.2 1.6 1.2
Sympathectomised	6	88.0 85.1 81.9	8.8 7.6 6.5	6.9 5.5 4.2	2.4 1.9 1.4

a The distribution is expressed as a percentage (mean $\pm$ S.E.M.) of the total ^{14}C in the tissues after incubating with ^{14}C histamine 200 ng.ml^{-1}.30 min. $^{-1}$. H = histamine, M = methyl histamine, A = acid fraction, N = N-acetyl-histamine.

b Two tissues were pooled from either control or treated rabbit ears, n refers to the number of animals.

The mean values for histamine and catabolites in medium did not differ significantly (at p=0.05) between control and sympathectomised arteries.

The catabolism of histamine in the rabbit ear artery:

Estimates of the relative proportions of catabolites in the rabbit ear artery suggested that methylation of histamine is the predominant pathway in contrast to the aorta where oxidative deamination appears to be the major degradative mechanism (Tables 5.1 and 5.8). In addition, surgical sympathectomy did not alter the pattern of catabolic degradation of histamine (Table 5.8).

Histamine catabolites in rabbit ear artery incubation fluids:

The catabolites of histamine analysed in incubation fluids for rabbit ear artery reflected their proportions found in the tissues. Once again the proportion of N-acetyl histamine appeared to be unusually large. This suggested that this was probably an artifact of the thin layer chromatography system. Sympathectomy did not alter the proportion of histamine catabolites found in the incubation fluids (Table 5.9).

5.4   Discussion

The results from the guinea pig aorta and rabbit ear artery will be in-
terpreted in terms of the more extensive results obtained with the rabbit
aorta by Dr. Foldes.

Rabbit aorta

The earlier study by Foldes et al (1977) has indicated that there was
about a two fold accumulation of ^3H material in the rabbit aorta, above that
accounted for by its presence in the extracellular space when the aorta was
incubated for 30 minutes with ^3H-histamine (0.02 µCi, 200 ng/ml). It was
suggested that at least part represented uptake by a mechanism similar to
that of extraneuronal uptake of catecholamines since inhibitors of the lat-
ter process (corticosteroids and normetanephrine) decreased the ^3H accumul-
ated by between 20% to 50%.

The present study shows that only 22% of the ^3H was present as un-
changed ^3H histamine, the major percentage (55%) being present as acid cat-
abolites. The latter are immidazole acetic acid (IAA) and its   methyl
derivative (MIAA) which are presumably formed by diamine oxidase, since hydrall-
azine a known inhibitor of DAO (Grabbe and Bardsley, 1974) virtually elim-
inated the acid catabolite fraction. In addition hydrallazine also in-
creased the percentage of unchanged histamine in the tissue from 22% to 52%.
It must be concluded that diamine oxidase is an important factor in deter-
mining the accumulation of exogenous histamine by the rabbit aorta. N-
methyltransferase seems less important since inhibition of this enzyme by
amodiaquin (Cohn, 1965) while decreasing the percentage of methyl histamine
in the tissue from 18 to 9% produced a smaller increase in the ^3H histamine
content of the tissue (to 37%). The fact that the percentage of methyl
histamine in the tissue (18%) is less than that of the acid catabolites
(55%) does not in itself constitute an argument that N-methyltransferase
plays a quantitatively less important role than diamine oxidase in histamine
catabolism. This is because the absolute quantities of catabolites in the

incubation medium were not obtained, only their relative proportions. The fact that the percentage of methyl histamine in the medium exceeds that of the acid catabolites, i.e. the opposite of their distribution in the tissue, suggests that, following formation the N-methyl catabolite may efflux from the tissue more rapidly than the acid catabolites. Since we have no measure of the tissue to medium ratios of catabolites, this possibility cannot be assessed. Hence the importance to be placed on the catabolite data is that it indicates the extent to which catabolism influences the histamine content of the tissue. Corticosteroids and phenoxybenzamine failed to alter the distribution of ^3H histamine and its catabolites with the possible exception of an increase in the percentage of acid catabolites produced by corticosterone but not by Deoxycorticosterone acetate. This suggests that the retention and subsequent catabolism of histamine occurs after the histamine has been taken up into the steroid sensitive compartment. The failure of cocaine to effect either the accumulation of ^3H by the tissue (Foldes et al, 1977) or the distribution of ^3H histamine and its catabolites in the tissue, argues against a role for the sympathetic nerves in the disposition and catabolism of histamine in the rabbit aorta.

The above results were confirmed in an independent study in the rabbit aorta of Adams and Hudgins (1976). There was an approximate agreement with the quantitative uptake of exogenous histamine into rabbit aorta and the predominant histamine catabolite in the tissue was found to be imidazole acetic acid. The only disagreement between the two studies stems from the report in the Discussion of Adams and Hudgins (1976) that the catabolites in the incubation medium were distributed in the same relative proportions as in the tissue. This was not the case in the present study where acid catabolites (IAA + MIAA) were about three fold the amount of methyl histamine in the tissue but were represented in about equal proportions in the incubation medium. In addition, the proportion of methyl histamine found in the tissue was only 4% compared with 18% in the present study. The only drugs examined by Adams and Hudgins (1976) were aminoguanidine, compound 48/80, and reserp-

ine. The uptake of ^{14}C by rabbit aorta exposed to ^{14}C histamine (2 μM 0.15 μCi/ml) was slightly decreased by aminoguanidine and slightly increased by 48/80; reserpine was without effect. The effect of compound 48/80 to increase the uptake of ^{14}C histamine into the rabbit aorta was not apparent in the study of Foldes et al (1977). In Adams and Hudgins (1976) experiments the diamine oxidase inhibitor aminguanidine sharply decreased the formation of acid catabolites in a similar fashion to the inhibition by hydrallazine in the present study. Therefore Adams and Hudgins (1976) concluded that oxidative deamination was the primary catabolic pathway for histamine in the rabbit aorta. As indicated above, differing proportions of MH and acid catabolites (IAA + MIAA) in the tissue and medium do not enable us to exclude an important role for methylation in this tissue. This permits only a more limited conclusion, that oxidative deamination is an important catabolic pathway.

It should be noted that Kalsner and Nickerson (1969) and Kalsner (1970), on the basis of pharmacological studies with rabbit aorta, concluded that methylation and oxidative deamination were about equally important histamine inactivating mechanisms. Hence the pharmacologic data is in closer agreement with the present studies. Adams and Hudgins (1976) also studied the efflux of ^{14}C material from rabbit aorta after ^{14}C histamine was washed from the tissue in the presence of compound 48/80. They concluded that ^{14}C histamine was not taken up and accumulated in mast cells in this tissue. This is compatible with the results of histological studies by the present author, which did not reveal the presence of mast cells in the aorta (Chapter 4, this thesis).
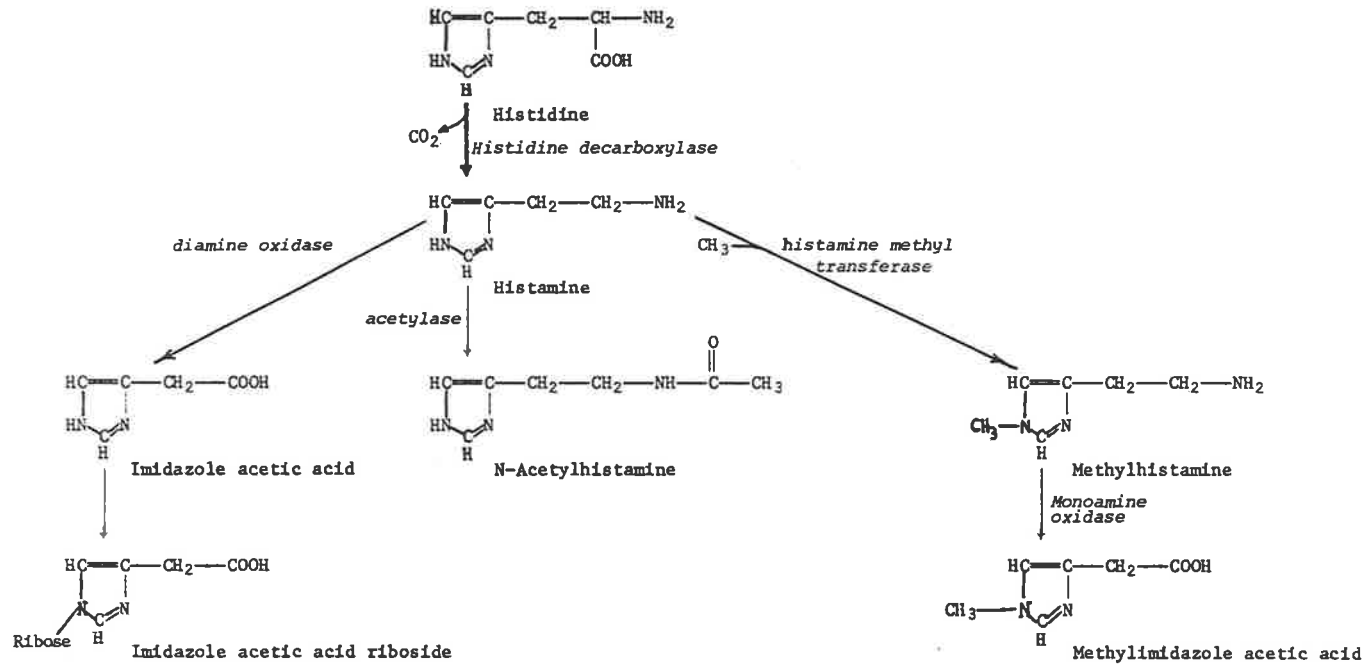
Guinea pig aorta:

The guinea pig aorta was seen to accumulate ^{14}C histamine and catabolites in approximately the same quantity as observed in the rabbit thoracic aorta. The histamine methyltransferase inhibitor amodiaquin and the diamine oxidase inhibitor hydrallazine were found to inhibit the accumulation of ^{14}C histamine in the guinea pig aorta. These results suggest that the histamine

uptake processes in the guinea pig aorta were linked to the catabolic degradation of this amine. The distribution of ^{14}C histamine and acid catabolites in the guinea pig aorta and incubation media suggest that methylated and oxidative catabolites are found in nearly equal proportions. The reduction of oxidative deaminated catabolites caused by hydrallazine in the above tissue could be the result of simple enzymic inhibition of diamine oxidase, or the inhibition of access of substrate to the enzyme. In contrast the effect of amodiaquin in the same tissue cannot be accounted for by a simple enzymic inhibition of histamine methyl transferase. The distribution of ^{14}C histamine and its catabolites in amodiaquin treated guinea pig aorta showed a marked decrease in the proportions of both methyl histamine and acid catabolites. There are at least two explanations for the reduction of the acid metabolite fraction in amodiaquin treated guinea pig aorta. Firstly, amodiaquin may be inhibiting diamine oxidase or access of the substrate to this enzymic compartment. The results in this study do not support this suggestion, as amodiaquin did not decrease the large acid metabolite fraction in the rabbit aorta. The second alternative depends on a large amount of the acid fraction in the guinea pig aorta being in the form of methyl immidazole acetic acid. It should be noted that the relative proportions of immidazole acetic acid and methyl immidazole acetic acid (acid fraction) were not determined separately in these studies. Reference to Figure 5.10 indicates that methyl immidazole acetic acid is formed from methyl histamine via monoamine oxidase. Monoamine oxidase has been detected in both neuronal and extraneuronal compartments in at least one vascular tissue (Head et al, 1977). The second explanation for reduced amounts of oxidative catabolites in amodiaquin treated guinea pig aorta is as follows. When the guinea pig aorta is incubated with ^{14}C histamine in the presence of amodiaquin the tissue contained reduced amounts of methyl histamine therefore less substrate was available for conversion to methyl immidazole acetic acid. It is difficult to confirm the latter suggestion without specific data on the proportion of methyl immidazole acetic acid.

The finding that methylation is an important pathway of degradation in

PATHWAYS OF HISTAMINE SYNTHESIS AND CATABOLISM

Figure 5.10

the guinea pig aorta is consistent with studies in the guinea pig heart. In isolated preparations of the guinea pig heart ^{14}C histamine and catabolites were found to accumulate in the tissue from a media concentration of 100 ng. ml^{-1}. This cardiac tissue was found to contain only about 4% unchanged ^{14}C histamine and about 50% of the ^{14}C content was in the form of methyl histamine (Mannaioni and Moroni, 1973).

Rabbit ear artery:

The results of these studies indicate that the rabbit ear artery was able to accumulate ^{14}C histamine and catabolites to a similar degree as the thoracic aortae of the guinea pig and the rabbit. The distribution of ^{14}C histamine and its catabolites in both the tissue and the incubation media suggests that methylation is the predominant pathway of catabolism. The comparison between the rabbit thoracic aorta and the rabbit ear artery suggests the increasing importance of histamine methylation with decreasing arterial diameter. However, further studies would be required to confirm this suggestion. These results indicate that sympathetic nerves are not a significant site of storage and accumulation in the rabbit ear artery.

In conclusion this thesis does not support a neuronal role for histamine in blood vessels. This view is consistent with the findings of other workers using different tissues and species.

5.5 Summary

This study presents a convenient thin layer chromatographic technique that is semi quantitative for radiolabelled histamine and its catabolites. Species differences between aortae of the guinea pig and the rabbit indicate that whilst oxidative deamination is prominent in the rabbit, methylation probably plays an equal role in the guinea pig. At least in the rabbit, methylation may be of increasing importance in smaller arteries when compared with larger conducting arteries. The sympathetic innervation of the REA does not appear to be a major site for the uptake and catabolism of ^{14}C histamine.

A P P E N D I X

Foldes, Stacey and De La Lande. (1976)   Evidence for the extra neuronal localisation of histamine in the rabbit aorta. *Proc of the Aust Physiol and Pharmacol Soc* , 7, 147 P

Foldes, A, Stacey, M.J. & de la Lande, I.S.(1978). Histamine metaboloism in aortae of two histamine sensitive species. *Clinical and Experimental Pharmacology & Physiology, 5*(3), 239

Stacey, M.J., Foldes, A & de la Lande, I.S. (1978). Localization of histamine in rabbit central ear artery. *Clinical and Experimental Pharmacology & Physiology, 5*(3), 239-296.

Foldes, A., & Stacey, M. J. (1977). The metabolism of exogenous histamine by aortic tissues**.** *Australian Journal Of Experimental Biology And Medical Science, 55*, 441.

BIBLIOGRAPHY

ADAMS, M.D. and HUDGINS, P.M. (1976):

Characteristics of histamine disposition in vascular smooth muscle.

Pharmacology, 14:330.

ANTON, A.H. and SAYRE, D.F. (1969):

A modified fluorimetric procedure for tissue histamine and its distrib-

ution in various animals.

J. Pharmacol. Exp. Ther., 166:285.

ARUNLAKSHANA, O., MONGAR, J.L. and SCHILD, H.O. (1954):

Potentiation of pharmacological effects of histamine by histaminase in-

hibitors.

J. Physiol., 123:32.

ASH, A.S.F. and SCHILD, H.O. (1966):

Receptors mediating some actions of histamine.

Br. J. Pharmac. Chemother., 27:427.

ATACK, C. (1977):

Measurement of biogenic amines using cation exchange chromatography and

fluorimetric assay.

Acta Physiol. Scand. Supp. 451.

ATACK, C.V. and MAGNUSSON, T. (1970):

Individual elution of noradrenaline (together with adrenaline), dopamine,

5-hydroxytryptamine and histamine from a single, strong action exchange

column, by means of mineral acid-organic solvent mixtures.

J. Pharm. Pharmac., 22:625.

BARSOUM, G.S. and GADDUM, J.H. (1935):

The pharmacological estimation of adenosine and histamine in blood.

J. Physiol., (Lond). 85:1

BEAVEN, M.A. and JACOBSEN, S. (1971):

A new assay for histaminase activity measurement of tritiated water from

β (side chain label) $-^3$H-histamine.

J. Pharmacol. Exp. Therap., 176:52.

BEAVEN, M.A., JACOBSEN, S. and HORÁKOVÁ, Z. (1972):

Modification of the enzymatic isotopic assay of histamine and its

application to measurement of histamine in tissues, serum and urine.

Clin. Chim. Acta, 37:91.

BEVAN, J.A., BEVAN, R.D., PURDY, R.E., ROBINSON, C.P., SU, C. and WATERSON,

J.G. (1972):

Comparison of adrenergic mechanisms in an elastic and a muscular artery

of the rabbit.

Circulation Res., 30:541.

BEST, C.H. (1929):

The disappearance of histamine from autolysing lung tissue.

J. Physiol., 67:256.

BOLITHO, G.A. and HOLLIS, T.M. (1975):

Rat aortic intima-media histamine synthesis in experimental neurogenic

hypertension.

Proc. Soc. Expt. Biol. Med., 148:1159.

BROWN, D.D., TOMCHICK, R. and AXELROD, R. (1959):

The distribution and properties of a histamine methylating enzyme.

J. Biol. Chem., 234:2948.

CABUT, M. and HAEGERMARK, O. (1966):

Uptake, storage and release of histamine by rat peritoneal mast cells in

vitro.

Acta Physiol. Scand., 68:206.

CARLINI, E.A. and GREEN, J.P. (1963):

The subcellular distribution of histamine, slow reacting substance and

5-hydroxytryptamine in the brain of the rat.

Br. J. Pharmac. Chemother., 20:264.

COHN, C.V. (1965):

Inhibition of histamine methyltransferase by antimalarial drugs.

Biochem. Pharmac., 14:1686.

COHN, V.H., KIM, K.S. and RICHENS, E. (1966):

Inhibition of histamine metabolism (methylation) by antimalarial drugs.

Federation Proceedings, 25:691 (abstract number 2837).

CRABBE, M.J.C. and BARDSLEY, W.G. (1974):

Monoamine oxidase inhibitors and other drugs as inhibitors of diamine

oxidase from human placenta and pig kidney.

Biochem, Pharmac., 23:2983.

DE FORREST, J.M. and HOLLIS, T.M. (1978):

Shear stress and aortic histamine synthesis.

Am. J. Physiol., 234:H701.

DE LA LANDE, I.S. and HEAD, R.J. (1967):

The catecholamines in the central artery of the rabbit ear.

Aust. J. Exp. Biol. Med. Sci., 45:707.

DE LA LANDE, I.S. and RAND, M.J. (1965):

A simple isolated nerve-blood vessel preparation.

Aust. J. Exp. Biol. Med. Sci., 43:639.

DENT, C., NILAM, F. and SMITH, I.R. (1979):

Application of thin layer chromatography to histamine radioenzymatic

assay.

Agents and Actions, 9/1:34.

EL-ACKAD, T.M. and BRODY, M.J. (1975):

Evidence for non-mast cell histamine in the vascular wall.

Blood Vessels, 12:181.

ENDO, Y. AND OGURA, Y. (1976):

Purification and properties of N-acetylhistamine deacetylase.

Proceedings of Jap. Pharm. Soc., March, 1976, Jap. J. Pharm. 26 supp.

:76P.

ERCAN, Z.S. and TÜRKER, R.K. (1975):

Histamine receptors in the vasculature of the rabbit ear.

N aunyn- Schmiedeberg's Arch. Pharmacol., 291:23.

ERHLICH, P. (1879):

Beiträge zur kenntmiss der granulirten bindegewebszellen und der

eosinophilen leukocythen.

Arch. Anat. Physiol., 3:166.

EVANS, D.P., LEWIS, J.A. and THOMSON, D.S. (1973):

An automated fluorimetric assay for the rapid determination of histamine

in biological fluids.

Life Sciences, 12:327.

FALCK, B. and OWMAN, C. (1965):

A detailed methodological description of the fluorescent method for the

cellular demonstration of biogenic amines.

Acta Univ. Lund., 11: No. 7.

FELDBERG, W. (1926):

The peripheral innervation of the vessels of the external ear of the

rabbit.

J. Physiol. (Lond). 61:518.

FINCH, L., HAUESLER, G. and THOENEN, H. (1973):

A comparison of the effects of chemical sympathectomy by 6-hydroxy-

dopamine in the newborn and adult rats.

Br. J. Pharmacol., 47:249.

FOLDES, A., HEAD, R.J. and DE LA LANDE, I.S. (1977):

Endogenous and exogenous histamine in rabbit thoracic aorta.

Aust. J. Exp. Biol. Med. Sci., 55:89.

FOLDES, A., STACEY, M.J. and DE LA LANDE, I.S. (1976):

Evidence for the extraneuronal localization of histamine in the rabbit

aorta.

Proceedings of the Aust. Physiol. Pharmacol. Soc., 7:147P.

FONTAINE, J., FAMAEY, J.P. and REUSE, J. (1980):

A study of the effects of amodiaquine on the guinea-pig isolated ileum.

Naunyn-Schmiedeberg's Arch. Pharmacol., 313:165.

GALENO, T.M., KNUEPFER, M.M. and BRODY, M.J. (1979):

Vasodilator response to histamine: dependence up the site of adminis-

tration.

European J. Pharmacol., 56:257.

GLOVER, W.E., CARROLL, P.R. and LATT, N. (1973):

Histamine receptors in human temporal and rabbit ear arteries:

In International Symposium on histamine H_2-receptor antagonists.

p. 163, (published S.K. & F. U.K.).

GOLAND, P. GRAND, N.G. and KATELE, K.Y. (1967):

Cyanuric chloride and n-methyl morpholine in methanol as a fixative for

polysaccharides.

Stain Technology, 42:41.

GRIFFITH, S.G., CROWE, R., LINCOLN, J., HAVEN, A.J. and BURNSTOCK, G. (1982):

Regional differences in the density of perivascular nerves and varico-

sities, noradrenaline content and responses to nerve stimulation in the

rabbit ear artery.

Blood Vessels, 19:41.

HEAD, R.J., STITZEL, R.E., DE LA LANDE, I.S. and JOHNSON, S.M. (1977):

Effect of chronic denervation on the activities of monoamine oxidase

and catechol-o-methyl transferase and on the contents of noradrenaline

and adenosine triphosphate in the rabbit ear artery.

Blood Vessels, 14:229.

HILL, C.M. and BARDSLEY, W.G. (1975):

Histamine and related compounds as substrates of diamine oxidase (hist-

aminase).

Biochem. Pharmacol., 24:253.

HOLCSLAW, T.L. and IMHOFF, L. (1978):

Alterations in levels of vascular histamine in the DOCA-hypertensive rat.

Clin. Exp. Pharmacol. Physiol., 5:405.

HOLLIS, T.M. and ROSEN, L.A. (1972):

Histidine decarboxylase activity of bovine aortic endothelium and

intima-media.

Proc. Soc. Expt. Biol. Med., 141:978.

HOLLIS, T.M. and FERRONE, R.A. (1974):

Effects of shearing stress on aortic histamine synthesis.

Exp. and Molecular Pathol., 20:1.

HOLLIS, T.M. and SLOSS, R.J. (1975):

Aortic histamine synthesis following short term cholesterol feeding.

Artherosclerosis, 21:125.

HORÁKOVÁ, Z., KEISER, H.R. ,and BEAVEN, M.A. (1977):

Blood and urine levels in normal and pathological states as measured by

a radiochemical assay.

Clin. Chim. Acta, 79:447.

HOWLAND, R.D. and SPECTOR, S. (1972):

Disposition of histamine in mammalian blood vessels.

J. Pharmacol. Exp. Ther., 182:239.

HUGHESDON, P.E. (1949):

Two uses of uranyl nitrate. I. Permanent metathromatic staining of mucin.

J. of the Royal Microscopical Society, 69:1.

HUNT, T.E., HUNT, E.A. and CURRY, D. (1961):

Basophil leucocytes and mast cells in the rabbit before and after

intravenous injection of compound 48/80.

Anat. Rec., 140:123.

JACQUES, L.B. (1975):

The mast cells in the light of new knowledge of heparin and sulphated

mucopolysaccharides.

Gen. Pharmac., 6:235.

JONASSEN, F. and WETTERQUIST, H. (1975):

Effects of female sex hormones on histamine metabolism in guinea-pigs.

Agents and Actions, 5/4:302.

KALSNER, S. (1970):

Potentiation of responses to histamine in aortic strips by steroids.

Can. J. Physiol. Pharmacol., 48:443.

KALSNER, S. (1975):

Role of extraneuronal mechanisms in the termination of contractile

responses to amines in vascular tissue.

Br. J. Pharmac., 53:267.

KALSNER, S. and NICKERSON, M. (1968):

A method for the study of mechanisms of drug disposition in smooth

muscle.

Can. J. Physiol. Pharmacol., 46:719.

KREMZNER, L.T. and PFEIFFER, C.C. (1966):

Identification of substances interfering with the fluorimetric determin-

ation of brain histamine.

Biochem. Pharmacol., 15:197.

KREMZNER, L.T. and WILSON, I.B. (1961):

A procedure for the determination of histamine.

Biochim. Biophys. Acta, 50:364.

LORENZ, W., SCHÖNING, B., SCHWARZ, B. and NEUGEBAUER, E. (1978):

Increase in sensitivity of the fluorimetric plasma histamine determin-

ation: Routine assay in the femtomol range.

Naunyn-Schmiedeberg's Arch. Pharmacol. Supp., 302:634

LOVENBERG, W., WEISBACH, H. and UDENFRIEND, S. (1962):

Aromatic-L-amino acid decarboxylase.

J. Biol. Chem., 237:89.

LOWRY, O.H., GRAHAM, H.T., HARRIS, F.B., PRIEBOT, M.K., MARKS, A.R. and

BREGMAN, R.U. (1954):

The chemical measurement of histamine in blood plasma and cells.

J. Pharmacol. Exp. Ther., 112:116.

McINTIRE, F.C., WHITE, F.B. and SPROULL, M. (1950):

The determination of histamine with 2,4,dinitrofluorobenzene.

Arch. Biochem., 29:376.

MALING, H.M., FLEISCH, J.H. and SAUL, W.F. (1971):

Species differences in aortic responses to vasoactive amines: The

effects of compound 48/80, cocaine, reserpine and 6-hydroxydopamine.

J. Pharmacol. Exp. Ther., 176:672.

MANNAIONI, P.F. and MORONI, F. (1973):

Uptake, disposition and metabolism of histamine in isolated heart

preparations.

Br. J. Pharmacol., 49:457.

MÁSLINSKI, C. (1975):

Histamine and its metabolism in mammals Part II Catabolism of histamine

and histamine liberation.

Agents and Actions, 5/3:193.

MAYER, H.E., ABBOUD, F.M., BALLARD, D.R. and ECKSTEIN, J.W. (1968):

Catecholamines in arteries and veins of the foreleg of the dog.

Circ. Res., 23:653.

MEDINA, M. and SHORE, P.A. (1966):

Increased sensitivity in a specific fluorimetric method for brain hist-

amine.

Biochem. Pharmacol., 15:1627.

MICHAELSON, I.A. (1967):

Spermidine: A contaminant in the n-butanol extraction of brain in the

fluorimetric assay of histamine.

European J. Pharmacol., 1:378.

MICHAELSON, I.A. and COFFMAN, P.Z. (1969):

An improved Ion-exchange purification procedure for the fluorimetric

assay of histamine.

Anal. Biochem., 27:257.

MICHELS, N.A. (1938):

The mast cells. Handbook of haematology Sect. IV. Edited by Downey,

O.O. Hoeber Inc., p. 235.

Reprinted in Ann. N.Y. Acad. Sci., 103:Art 1 (1963).

MILLER, R.L., McCORD, C., SANDA, M., BOURNE, H.R. and MELMON, K.L. (1970):
Application of the enzymatic double isotope dilution assay for the
study of histamine in plasma.
J. Pharmacol. Exp. Therap., 175:228.

MOTA, I. (1966):

Release of histamine from mast cells. In Handbook of experimental

pharmacology. Vol. XVIII, Part I : 569 (Springer-Verlag, New York).

MURDOCK, H.R. (1969):

Anesthesia in the rabbit.

Federation Proc., 28/4:1510.

OLSSON, Y. (1968):

Mast cells in the nervous system.

Int. Rev. Cytol., 24:27.

ONDA, M. et al (1978):

Determination of tissue histamine and spermidine by means of high per-

formance liquid chromatography.

Hiroshima J. Med. Sci., 27:93.

PADAWER, J. (1974):

The ins and outs of mast cell function.

Am. J. Anat., 141:299.

PEARSE, A.G. (1968):

Histochemistry: Theoretical and applied.

Third edition, Vol. 1, p. 672 (Ed. J.A. Churchill Ltd., London).

PETTERSSON, T. and HJELMMAN, G. (1964):

The effect of experimental venous thrombosis on the mast cells and

fibrocytes in the vascular wall of the rabbit.

Acta Med. Scand. Supp., 412:265.

RILEY, J.F. (1953):

Relationship of tissue mast cells to blood vessels in the rat.

J. Pathol. Bacteriol., 65:461.

RILEY, J.F. and WEST, G.B. (1956):

Skin histamine - its location in tissue mast cells.

Arch. Der., 74:741.

RILEY, J.R. and WEST, G.B. (1966):

The occurence of histamine in mast cells. In Handbook of experimental

pharmacology. Vol. XVIII, Part I : 116-135 (Springer, Berlin).
Ed. Rocha and Silva.

ROLEWICZ, T.F., GISSLEN, J.L. and ZIMMERMAN, B.G. (1970):
Uptake of norepinephrine-^{3}H by cutaneous arteries and veins of the dog.
Am. J. Physiol., 219:62.

RÖNNBERG, A.L., CEDERLUND, B. and HAKANSON, R. (1977):
Reaction of histamine with o-phthaldialdehyde: preparation and identi-
fication of the fluorophore.
Manuscript submitted for publication, see Atack (1977).

ROSENTHAL, S.M. and TABOR, H. (1948):
An improved colorimetric method for the estimation of histamine.
J.Pharmacol.Exp. Ther., 92:425.

ROTHSCHILD, A.M. and SCHAYER, R.W. (1959):
Histidine decarboxylase from rat peritoneal fluid mast cells.
Biochim. Biophys. Acta, 34:392.

RUFF, F., SAINDELLE, A., DUTRIPON, E. and PARROT, J. (1967):
Continuous automatic fluorimetric evaluation of total blood histamine.
Nature, 214:279.

RUFF, F., SAINDELLE, A., DUTRIPON, E. and PARROT, J. (1968):
Automatisation du dosage fluorometrique de l'histamine.
Automation in Analytical Chemistry, 2:217.

RYAN, M.J. and BRODY, M.J. (1970):
Distribution of histamine in the canine automatic nervous system.
J. Pharmacol. Exp. Ther., 174:123.

RYAN, M.J. and BRODY, M.J. (1972):

Neurogenic and vascular stores of histamine in the dog.

J. Pharmacol. Exp. Ther., 181:83.

SCHAYER, R.W. (1959):

Catabolism of physiological quantities of histamine in vivo.

Physiol. Rev., 39:116.

SCHAYER, R.W. (1962):

Evidence that induced histamine is an intrinsic regulator of the micro-

circulatory system.

Am. J. Physiol., 202:66.

SCHAYER, R.W. (1963):

Induced synthesis of histamine, microcirculatory regulation and the

mechanism of action of the adrenal glucocorticoid hormones.

Progr. Allergy, 7:187.

SCHAYER, R.W. (1965):

Histamine and circulatory homeostasis.

Federation Proceedings, 24:1295.

SCHAYER, R.W. (1966):

Enzymatic formation of histamine from histidine. In Handbook of

experimental pharmacology, Vol. 18:688 (Springer, Berlin) Ed. Rocha

and Silva.

SCHAYER, R.W. and KOBAYASHI, Y. (1956):

Histidine decarboxylase and histamine binding in rabbit platelets.

Proc. Soc. Exp. Biol. Med., 92:653.

SCHAYER, R.W. and REILLY, M.A. (1968):

Suppression of inflammation and histidine decarboxylase by protein

synthesis inhibitors.

Am. J. Physiol., 215:472.

SCHAYER, R.W. and REILLY, M.A. (1974):

Histamine catabolism in guinea pigs, rats and mice.

European J. Pharmacol., 25:101.

SCHAYER, R.W. and REILLY, M.A. (1975):

Comparison of in vitro and in vivo histamine methylation by tissues.

Agents and Actions, Vol. 5/2:119.

SCHWARTZ, J.C. (1977):

Histaminergic mechanisms in brain.

Ann. Rev. Pharmacol. Toxicol., 17:325.

SCHWARTZMAN, R.M. (1973):

Quantitative thin layer chromatography of histamine and its metabolites.

J. of Chromatography, 86:263.

SHAW, G.G. (1972):

Observations on the fluorimetric assay of histamine, spermidine and

spermine in brain.

European J. Pharmacol., 20:389.

SHORE, P.A., BURKHALTER, A. and COHN, V.H. (1959):

A method for the fluorimetric assay of histamine in tissues.

J. Pharmacol. Exp. Ther., 127:182.

SIRAGANIAN, R.P. (1975):

Refinements in the automated fluorimetric histamine analysis system.

J. Immunological Methods, 7:283.

SNYDER, S.H. and AXELROD, J. (1965):

Sex differences and hormonal control of histamine methyl transferase
activity.

Biochim. Biophys. Acta., 111:416.

SNYDER, S.H., AXELROD, J. and BAUER, H. (1964):

The fate of ^{14}C histamine in animal tissues.

J. Pharmacol. Exp. Ther., 144:373.

SNYDER, S.H., BALDESSARINI, R.J. and AXELROD, J. (1966):

A sensitive and specific enzymatic isotopic assay for tissue histamine.

J. Pharmacol. Exp. Ther., 153:544.

TAYLOR, K.M. and SNYDER, S.H. (1972):

Isotopic microassay of histamine, histidine, histidine decarboxylase
and histamine methyl transferase in brain tissue.

J. Neurochem., 19:1343.

TORP, A. (1961):

Histamine and mast cells in nerves.

Med. Exp., 4:180.

WATERSON, J.G. and SMALE, D.E. (1967):

Location of noradrenergic structures in the central artery of the
rabbit ear.

Aust. J. Exp. Biol. Med. Sci., 45:301.

WEINREICH, D. and YU, Y.T. (1977):

The characterization of histidine decarboxylase and its distribution in

nerves, ganglia and in single neuronal cell bodies from the CNS of

Aplysia Californica.

J. of Neurochemistry, 28:361.

WEISSBACH, H., LOVENBERG, W. and UNDENFRIEND, S. (1961):

Characteristics of mammalian histidine decarboxylating enzymes.

Biochim. Biophys. Acta, 50:177.

YARNAL, J.R. and HOLLIS, T.M. (1976):

Rat aortic histamine synthesis during short-term hypertension.

Blood Vessels, 13:70.

ZELLER, E.A. (1938):

Über den enzymatischen abbau von histamin und diaminen (Zur spezifität

der histaminase).

Naturwissenschaften, 26:282.

ZELLER, E.A. (1942):

Diamin-oxydase.

Advanc. Enzymol., 2:93.