MAST CELLS IN THE BRAINS OF MICE OF DIFFERENT GENOTYPES: A HISTOLOGICAL STUDY

THESIS

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By

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Histamine is present in the central nervous system and is believed to be derived from neurons (50 percent) and mast cells (50 percent). This experiment was designed to analyze histologically the numbers and distribution of brain-associated mast cells in normal (+/+), mast cell deficient (W/W^V) and heterozygote ($W/+$, $W^V/+$) mice of the $WBB6F_1/J$ strain. Significant variations in the number and distribution of mast cells between the various genotypes were found. Based on the results, a hypothesis is proposed to account for the observed genotypical differences in mast cell numbers and distribution. Based on the total number of mast cells and the content of histamine in a typical mast cell, it is apparent that the mast cell is not a major source of brain histamine, suggesting that another non-neuronal pool of histamine must be present in the brain.

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CHAPTER I

INTRODUCTION

First identified over a century ago by Paul Ehrlich (1878), the mast cell has since been studied extensively in various tissues of the body. Later observations led to the knowledge that the mast cell was involved in the anaphylactic response and that it underwent massive degranulation during the reaction (Webb, 1931, and Selye 1937, 1965). Furthermore, the anticoagulant heparin was discovered to be associated with mast cells, and tissues rich in mast cells contained a considerable content of heparin (Jorpes, 1937). Histamine (Hm), as well, can be liberated from mast cells, as was demonstrated by Riley and West (1953), who found that fluorescent-tagged histamine releasers were concentrated in mast cells which expelled the histamine during degranulation. Mast cell populations have subsequently been correlated with the amount of histamine in tissues (Riley and West, 1953). Of lesser importance to the present study are the findings that mast cells have been shown in some species to secrete serotonin (Benditt, 1955) and hyaluronic acid (Asboe-Hansen, 1954). Immunological studies dealing with antibody receptor binding, mast cell function in immediate hypersensitivity and physiologic factors surrounding release of mediators have

been reported in the literature (see review by Kallos and Waksman, 1964).

Usually located in the proximity of blood vessels or lymphatics, mast cells are elliptical or irregularly shaped cells with an ovoid nucleus and cytoplasmic granules which stain metachromatically with certain basic aniline dyes (Selye, 1965 and Metcalfe, 1981). Mast cells are found mainly in the loose connective tissue around small blood vessels, nerves, and glandular ducts (Compton, 1952). According to Selye (1965) mast cells are concentrated in epithelial linings, skin, trachea and large bronchi, submucosa of digestive tract, salivary glands, peritoneum, thymus, lymph nodes, uterus, urinary bladder, and tongue. Their presence in other tissues is variable among species, but in man, they are present in the heart, pancreas, testes, and associated with some medium sized vessels. In addition, Selye noted that mast cells were not associated with parenchymous tissues unless surrounded by capillaries nor were they concentrated in large numbers in muscle, bone, cartilage, or central nervous system.

The mast cells of the central nervous system constitute the principal focus of the present study which will attempt to localize them as specifically as possible.

Mast Cells In the Central Nervous System

It was long thought that mast cells were associated only with the peripheral nervous system, for they had been found adjacent to peripheral nerves and ganglia (Olsson, 1968 and 1971). Interest in the mast cells of the central nervous system increased when research findings suggested that the endogenous biogenic amines (histamine, serotonin, and dopamine) contained within the cells, have important neuropharmacological functions (Tebcis, 1970 and Monnier et al., 1970). Also, mast cell secretions have been shown to cause histological damage to the brain parenchyma (Ibrahim, 1970). Many studies indicated an absence or paucity of mast cells in the central nervous system (Compton, 1952, Selye, 1965 and Olsson, 1968). Other studies, however, have challenged these reports and found a significant number of mast cells in the brains of various animals. For example, Kelsall and Lewis (1964) discovered mast cells located near blood vessels in the lateral venticles and the thalamus. In the hedgehog, perivascular mast cells were reported in the habenular region of the thalamus (Flood and Kruger, 1970). The area postrema of several mammalian species is also known to contain mast cells in large numbers (Cammermeyer, 1972).

Dropp (1972) studied the brains of the kangaroo rat, albino rat, and gerbil, and found mast cells to be present in all three species, with concentration being highest in the

leptomeninges over the dorsal thalamus. The pia-arachnoid of all three species also contained numerous mast cells. In the brains,the diencephalon contained the highest population of mast cells; the metencephalon the fewest number. When observed, the mast cells were located predominately near blood vessels. In several other species of mammals, mast cells have been consistently found along vessels in the meninges and in the cortex parenchyma. High concentrations occurred in association with the vasculature of the hypothalamus and fewer amounts in the cerebellum (Ronnberg et al., 1973). Dropp's later study elaborated upon these findings, showing that in twenty-nine species of mammals, mast cells were highest in number in the leptomeninges over the dorsal thalamus and cerebrum, in the cerebral cortex, and in nuclei of the dorsal thalamus (Dropp, 1976). Edvinsson et al.(1977) examined a variety of species and discovered that concentrations of mast cells were from highest to lowest as follows: hypothalamus, cerebral cortex, mesencephalon, cerebellum and caudal brainstem. Research using albino rats documents mast cells to be usually associated with blood vessels, most numerous in the leptomeninges of the diencephalon, with high concentrations in the dorsal lateral geniculate body (Ibrahim, 1974 and Mares et al., 1979).

A recent theory that deserves mention is that of Ibrahim and his collegues, who claimed that there are two types of mast cells in the CNS of mammals (Ibrahim, 1974). The Type I

cell is granular and stains metachromatically. The granules mask the nucleus which possesses a central chromatin mass. In the Type II cell, the granules are larger and variable in size: they do not stain metachromatically, and appear to surround rather than mask the nucleus. Type II cells are more often seen near blood vessels, while Type I are mainly in the dorsal thalamus. Further studies have been undertaken by Ibrahim et al., 1979a and Ibrahim et al., 1979b to determine the importance of these findings, and they conclude that the Type II cells are specialized to function solely in the CNS. Due to their high lipid content, they may function in lipid metabolism; thus they have been named neurolipomastocytes. Type II cells have consistently been found along arterioles and venules, particularly at branching points (Ibrahim, 1974 and Sturrock, 1980). At present, there is no evidence that Hm is located in the Type II cell. The validity of the Type I and Type II mast cell classification is questionable, especially since they have never been observed in the mouse. It is preferred in this study to refer to Type I cells as mast cells and refer to the Type II cells as neurolipomastocytes until more conclusive evidence is presented.

Age-related changes in the number and distribution of mast cells have been seen in various species. For example, Kelsall (1966) found that in hamsters, mast cells in the choroid plexus and thalamus increased with age. Dropp (1976) discovered that mast cells in the olfactory peduncles and

thalamus increased with age, but in most other neural structures, the number of mast cells decreased. Ibrahim (1974) noted that the brain of a rat at birth contained Type I mast cells in the leptomeninges, choroid plexus, and olfactory bulbs. Later they appear in the proximity of vessels of the dorsal thalamus, and start to decline in number elsewhere within the central nervous system. This trend holds true similarly for the Type II neurolipomastocyte (Ibrahim, 1974). Recent evidence substantiates the migration of mast cells, but does not differentiate between Type I and Type II cells.

Mast Cells As Producers Of Histamine

The function of mast cells in histamine (Hm) release has long been established (Rocha e Silva et al., 1947). Histamine is synthesized by the decarboxylation of L-histidine by the enzyme histidine decarboxylase. Histamine may be metabolized via methylation by histamine methyltransferase (HMT) or oxidized by diamine oxidase (DAO) to form methylimidazole acetic acid and imidazole acetic acid (Schwartz et al., 1979) (See Figure 1). The principal route of catabolism of histamine in the brain is by methylation, as suggested by the high amount of HMT in the brains of many species (Pollard et al., 1974; Dismukes and Snyder, 1974; and Brown, 1959), as well as by studies in which radioactive histidine was used to monitor Hm synthesis and metabolism in mice (Shayer and Reilly, 1970).

Histamine is released quickly from the mast cell in an immune response or in response to certain mast cell degranulators such as the polyamine Compound 48/80 (Moran, 1962; Green, 1970; Thon and Uvans, 1967; and Johnson and Moran, 1969). Toluidine blue stain has been used to analyze the mechanism of histamine release from mast cells (Smith, 1958; Yamasaki and Komoto, 1971; Johnson and Moran, 1974; and Kruger and Bloom, 1974). Smith (1958) discovered that toluidine blue was an effective histamine releasing agent, and the release of Hm was not necessarily coupled with extensive degranulation of the cell. Electron microscopy later showed that while some degranulation occurred, the formation of vacuoles and pores which permit communication with the extracellular space was also observed (Kruger and Bloom, 1974). In summary, the mechanism of histamine release is thought to occur as follows: the cell generates energy and the granules are displaced to the periphery of the cytoplasm where ionic exchange occurs; the histamine bound to the granules is then exchanged with sodium, potassium, and other extra-cellular cations and released into the microenvironment (Thon and Uvnas, 1967).

After histamine is released from the mast cell, its activity is directed by receptors known as H_1- and H_2- receptors (Schwartz, 1979 and Plaut, 1979). The H_1-receptors are associated with inflammation and its effects: constriction

of smooth muscle, vascular permeability, production of prostaglandins, and pruritis (Review in Metcalfe, 1981).. The H_2-receptors may be anti-inflammatory: They inhibit basophil histamine release, lymphokine release, neutrophil-enzyme release, eosinophil release, and T-cell mediated toxicity (table in Metcalfe, 1981). Cyclic AMP in various tissues of some species has been seen to increase due to stimulation of H_2-receptors, while H_1-receptors may mediate a rise in cyclic GMP (See review in Metcalfe, 1981).

The reported H_1-receptor antagonists include various antihistamines such as mephyramine, and the H_2-receptor antagonists include cimetidine and burimamide (Plaut, 1979).

Neuronal Histamine

Histamine in the central nervous system is thought to be found in two pools. Already discussed was the mast cell store, and recently hypothesized is the neuronal store, which when analyzed biochemically has the charateristics of a neurotransmitter (Schwartz, 1975; Schwartz *et al.*, 1976, 1980; and Green *et al.*, 1980). Evidence for this theory can be summarized as follows: histamine has a nonuniform distribution in the brain, is found through subcellular fractionation procedures to be localized in subcellular synaptosomal fractions (P_2 or the mitochondrial fraction consisting of pinched-off nerve endings), and it can be

synthesized and metabolized by specific enzymes present in the brain (Kuhar and Snyder, 1971, and Green et al., 1978). The Subcellular localization of mast cell histamine is theorized to be in the heavier crude nuclear fraction (P_1 fraction) (Kuhar and Snyder, 1971). Also, the fact that histamine release in hypothalamic slices has been seen to increase following depolarization with potassium and is calcium dependent lends credence to the neurotransmitter hypothesis (Verdiere et al., 1975). Neuronal histamine synthesis (histidine decarboxylase activity) occurs late (third week) in brain development, as does formation of synaptosomes (Aghajanian and Bloom, 1967). In several species, histamine thought to be from mast cells has been observed in large amounts during embryological development and at birth (Kiernan, 1974). It has been proposed that neuronal histamine has a rapid turnover rate, while mast cell histamine turnover is slower by comparison (Green, 1978 and Garbarg, 1980). Histidine decarboxylase activity has been reported to be high in neurons, while it is lower in the mast cell (Baudry et al., 1973). However, in rat peritoneal mast cells there is evidence that mast cells may have a higher histidine decarboxylase activity than was once thought (Bauza and Lagunoff, 1981).

Other evidence for the Hm neurotransmitter proposal includes use of pharmacological agents such as reserpine,

Compound 48/80 for mast cells, and α-fluoromethylhistidine. Reserpine, which stimulates release of certain biogenic amines from synaptic vesicle storage sites in nerve endings and prevents their reuptake into the neurons, is currently being investigated with regard to histamine (Schwartz, 1976 and Pollard et al., 1973). Reserpine has been biochemically shown to alter release and synthesis of Hm from neuronal stores if it is presumed that neuronal Hm has a more rapid turnover rate. It apparently has no effect on mast cell Hm if Hm has a slower turnover rate (Pollard et al., 1973). The mast cell degranulator, Compound 48/80, has been shown to release what is thought to be mast cell Hm on the basis of Hm turnover rate, and leaves presumed neuronal concentrations unaltered (Green, 1970 and Erjavec, 1982). Alpha-fluoromethylhistidine was recently discovered to be an irreversible inhibitor of Hm synthesis and will be a significant tool in future studies of Hm function, since the two cellular pools of histamine have different turnover rates (Garbarg, 1980). The fact that histamine increases synthesis of the nucleotide cyclic AMP may lead to a further understanding of the neuronal function of histamine. Cyclic AMP in brain neurons is thought to be a "second messenger" at synapses and may take part in neurotransmission mechanisms (Bloom, 1975). Studies such as these have contributed to the overall knowledge that histamine may reside in neurons as well as mast cells of the brain.

Histamine And Cerebral Circulation

Of special significance to the present research is the belief that histamine has a role in the blood circulation of the brain. Many authors have proposed this over the last twenty years, and its implications are still being investigated. Evidence for this theory is supported by the fact that histamine is located in mast cells which are frequetnly associated with blood vessels (Ibrahim, 1974 and Edvinsson et al., 1977), and it has been detected in the vascular smooth muscle of the brain (El-Ackad and Brody, 1974). Furthermore, the cerebral arteries are known to contain histamine receptors (Jarrott et al., 1979 and Gross, 1981). Reports based on physiological studies have indicated that histamine causes vasodilation of the vessels of the brain (Schayer, 1962 and 1974; Beck, 1965; and Tobia et al., 1969).

Nevertheless, intravascular administration of histamine may have varying and inconsistent results, perhaps because histamine does not cross the blood-brain barrier (Oldendorf, 1971). Either an increase or no change in cerebral blood flow was observed by numerous authors (see review by Gross, 1981). Intracarotid infusion of histamine with the blood-brain-barrier intact had no effect on cerebral blood flow, but when the barrier was broken, blood flow increased proportional to the dose of histamine infused (Gross et al., 1981a). Also intra-arterial infusion may possibly cause stimulation of the H_1- and H_2-receptors. It has been proposed that H_2-

receptors are located primarily in the outer layers of cerebral arteriolar smooth muscle, while both H_1- and H_2-receptors are found within the inner layers of the vessel (Gross, 1981a)

It is believed that when histamine is secreted upon the exterior of pial vessels by neurons of the central nervous system, it is the H_2-receptors that cause the relaxation of the vessel (Wall and Kuchinsky, 1979). This has been further confirmed by Gross and his collegues, who discovered that impromidine, an H_2-receptor agonist, causes dilation of surface arterioles in the brain (Gross et al., 1981b). The H_1-receptor antagonists have been shown to dilate cerebral arteries in the rabbit, while H_2-antagonists have no effect (Shibata et al., 1980). This suggests that H_1-receptors may cause vasoconstriction. Compound 48/80 also has been shown to cause constriction in pial arteries and arterioles, implying that histamine or some other component of the perivascular mast cell, that is released upon degranulation, is a vasoconstrictor (Rosenblum, 1973). But since histamine is widely known for its vasodilation properties, this seems unlikely, and more research is required to learn more about this phenomena.

The cerebral microvasculature has recently been examined specifically with regard to the function of histamine in the brain. Cerebral microvessels serve to maintain homeostasis by providing a nutritive blood supply to the individual

brain cells (Schayer, 1974). These vessels are usually smaller than 100 μm and have been found to contain high amounts of histamine, however histidine decarboxylase activity is negligible. This implies that the mast cells may serve as the source of the histamine (Jarrott et al., 1979). Also, homogenates of whole brain contain less Hm than those of the microvessels (Jarrott et al., 1979). Further, light and electron microscopic examination of isolated microvessels revealed granules in the adventitia which are presumed to be mast cell granules, and biochemical analysis of the vessels has detected the presence of histamine (Head et al., 1980).

Another possible function of histamine in association with cerebral vasculature is its effect upon the permeability of vessels. Facts already mentioned about histamine, such as location of mast cells near vessels, the presence of histamine in blood vessels, and receptors for histamine in the cerebral vessels suggest a histaminergic role in permeability. Many of the positive results concerning this question came from Gross and his workers, who injected histamine intra-arterially (Gross, 1982 and Gross et al., 1981b and 1982). The pertinent findings from that study are as follows: Initially, there seems to be an increase in microvascular permeability, especially in the thalamus, hippocampus, and cortex. This response is mediated by H_2-receptors and the effect is blocked by an H_2-antagonist, metiamide (Gross et al., 1982). Vascular transfer is promoted, as witnessed

by an increase in the number of pinocytotic endothelial vesicles (Gross et al., 1982).

A further role of histamine in the cerebral vascular system may be found in the nerves surrounding pial vessels. The H_1-receptors are thought to be involved in vasoconstriction of the extracranial vessels, as opposed to the H_2-receptors which cause vasodilation (Edvinsson, 1975). Although at present, no methods exist to identify histamergic neurons, it is believed that histamine may act as an inhibitor at the noradrenergic neurovascular synapse (Edvinsson et al., 1976, and Gross, 1982). Histamine releases noradrenalin from the sympathetic nerve endings in cerebral arteries of the cat, which in turn causes constriction of the vessels (Marco et al., 1980). Studies have shown that there is histamine-sensitive adenylate cyclase in brain microvessels, suggesting the involvement of cyclic AMP in vasoconstriction by histamine (Baca and Palmer, 1978, and Karnushina et al., 1980).

Due to the effects of histamine on the cerebrovascular system, it has been implicated in a number of vascular dysfunctions. Inflammation, for example, involves the action of histamine on blood vessels, which can be "proinflammatory" or "anti-inflammatory" depending on which receptors are activated (Plaut, 1979). Increases in blood flow due to histamine-induced vasocilation have been implicated in migraine headaches, though the total mechanism by which histamine causes

headaches is still being investigated (Krabbe and Olesen, 1980). Further research should be undertaken to learn of the possible influences of histamine on cerebrovascular disorders such as stroke, hypertension, and trauma (Gross, 1982). Other effects of histamine in the brain which may not involve the circulatory system are being uncovered; these include temperature regulation, feeding and drinking behavior, emesis, and the behavioral processes such as aggression, motor activity, sleep patterns, and stress (See review by Schwartz et al., 1979).

Mast Cell Deficient Mice In Experimentation

Another route that may lead to conclusive proof that histamine is a functional neurotransmitter in the brain, as well as determine the contribution of mast cells to the histamine content and function within the brain would be to analyze mice thought to be "normal" in the number of mast cells they possess, and mice thought to be deficient in mast cells. Specifically, allophenic mice with the genotypes +/+ (non -anemic mast cell normal) and W/W^V (anemic mast cell deficient), can be compared to determine amount of neuronal and mast cell histamine (Kitamura and Hatanake, 1978). Research using these mice, as well as heterozygotes W/+ and $W^V/+$, has recently begun to appear in the literature because of the potential information to be gained. Kitamura and

Hatanake (1978) discovered that W/W^V mice are mast cell deficient because they possess by comparison only one percent of the total number of mast cells within the skins of +/+ mice, and an absence of cells were found in other tissues examined, such as the brain, mesentery, spleen, thymus, heart, lung, liver, and kidney. In addition, bone marrow transplantation from the +/+ to the W/W^V mice caused an increase in the number of mast cells in the skin, mesentery, and other tissues (Kitamura et al., 1979). This finding may clarify the origin of mast cells by suggesting a clonal origin of mast cell clusters from one precursor cell line (Kitamura et al., 1979).

It has been demonstrated that in mast cell deficient mice, the activity of histidine decarboxylase derived from whole animal analysis is not drastically lower than that of mast cell normal mice, yet total Hm concentrations are less, due to the lack of mast cells (Watanabe et al., 1980). This suggests that histamine synthesis occurs somewhere else besides mast cells. However, histidine decarboxylase activity in mast cell deficient mouse whole embryos was shown to be low in concurrance with small amounts of histamine and low mast cell numbers (Watanabe et al., 1981). In +/+ mice, this was just the opposite, suggesting that in whole embryos, histamine was probably derived predominantly from mast cells (Watanabe et al., 1981). Yamatodani and associates (1982) analyzed the histamine content in W/W^V mice and found that

the whole bodies contained 5 - 10 percent of the histamine content of the +/+ mice. In a careful study of the distribution of histamine in tissues they discovered that the brains and stomachs of W/W^V mice contained roughly 34 - 45 percent of the normal content of histamine of +/+ mice, suggesting a relatively large non-mast cell pool of histamine in these tissues. In descending order of magnitude the distribution of non-mast cell histamine in other tissues surveyed is liver, spleen, and kidneys (8 - 15 percent), and skin, lungs, and heart with even lesser amounts.

In the brains of W/W^V mice, no mast cells have been identified by histological techniques (Kitamura et al., 1979, and Grzanna and Shultz, 1982). However, when concentrations of histamine in various brain regions were measured by biochemical assay, there was a decrease in cerebral cortex, thalamus, hypothalamus, and midbrain, but there was no decrease in the pons, medulla, and cerebellum compared to the +/+ controls (Grzanna and Shultz, 1982). This may mean that in the forebrain regions, the presence of mast cells contributes more histamine than in the hindbrain, and that the hindbrain may be the primary site of neuronal histamine. Yamatodani's group (1982) measured whole brain Hm concentrations in +/+ and W/W^V mice and concluded that the non-mast cell source of histamine makes up half of the concentration of brain Hm, while mast cell histamine comprises the other half. How much of brain histamine is mast cell derived and

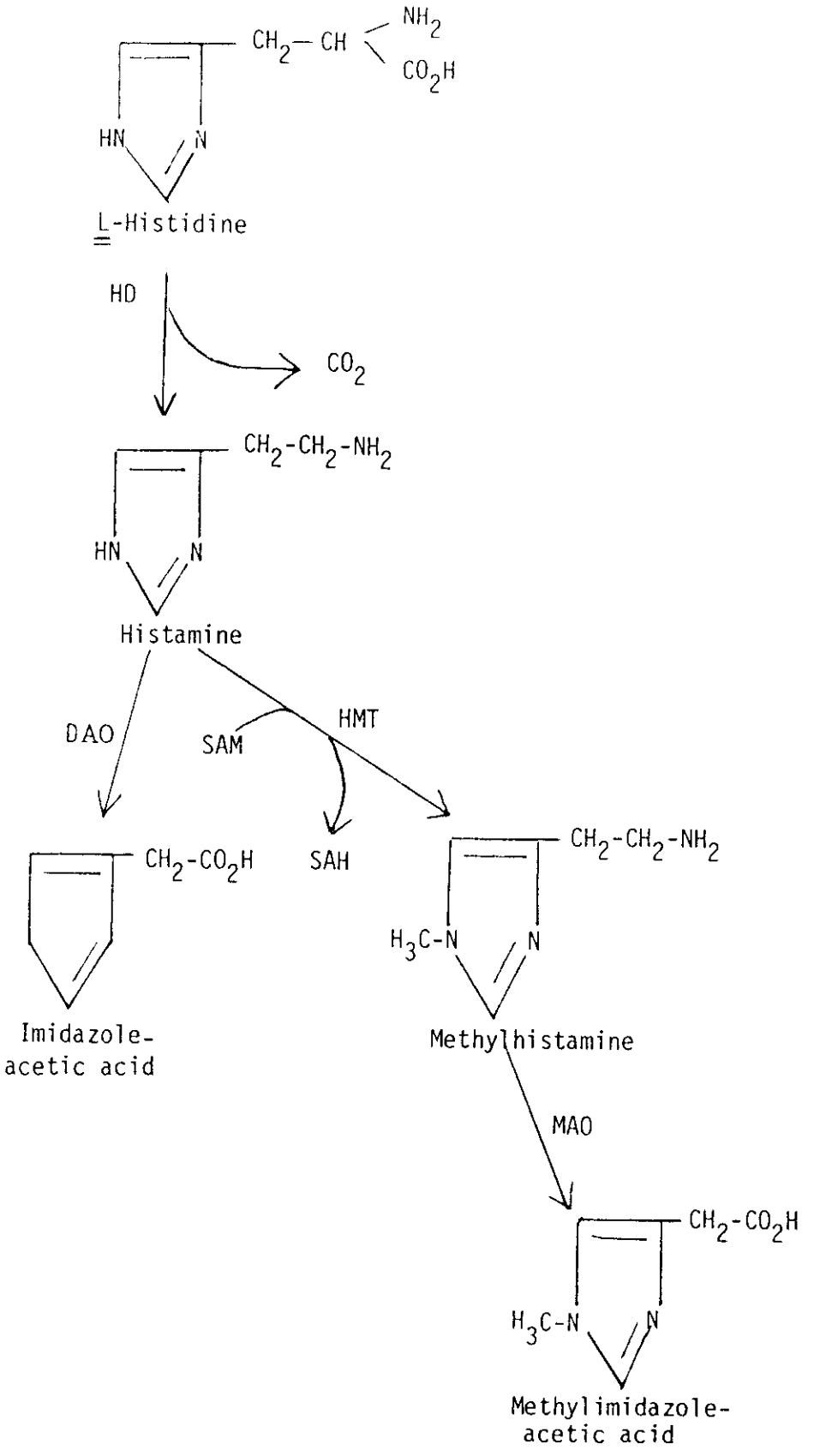
how much is neuronal is a question yet to be fully resolved. Though research continues in this area, the fact that histamine is found in brains of mice lacking mast cells, and that respective amounts vary among brain regions serves as the impetus for continued study on the function and localization of the various pools of histamine in the central nervous system.

Present Study

A histological study of the brains of the above mentioned congenic strains of mice as to the number and location of brain mast cells would be of significance. Coupled with correlative studies in histamine research that would clearly delimit the areas of mast cell populations in the genetically controlled mice, it should broaden our understanding of the role of histamine in the brain. At the present time, no such studies have been made that would offer a clearer picture of the location and distribution of the brain mast cell, or contribute further information concerning the other pool of histamine in the brain which is presumably from a neuronal source. A quantitation of the number of mast cells and description of their location in relation to various brain structures, particularly the blood vessels, will be conducted at the light microscope level. A thorough analysis of the regional distribution of mast cells in the four strains of mice will hopefully offer a base of information

for regional brain studies. For example, if the number of mast cells seen does not appear significant enough to contribute greatly to brain histamine, yet biochemical experimentation finds high concentrations of the amine, it may lead to the theory that much of it is neuronal. Thus, the present study of brains of mice considered 'normal' in their mast cell numbers, heterozygotes, and the mast cell deficient should prove pertinent to an understanding of the two pools of histamine in the central nervous system.

Figure 1. Synthesis and metabolism of histamine. Abbreviations: HD=histidine decarboxylase; DAO=diamine oxidase (histaminase); HMT=histamine N-methyltransferase; SAM=S-adenosylmethionine; SAH=S-adenosylhomocysteine; MAO=monoamine oxidase.


L-Histidine
HD
CO_2
Histamine
DAO
SAM
HMT
SAH
Imidazole-
acetic acid
Methylhistamine
MAO
Methylimidazole-
acetic acid

CHAPTER II

MATERIALS AND METHODS

Animals

Animals used in this experiment were bred in our animal facilities from parental stocks ($Wbb6F_1/J$-W/+ and $WBB6F_1/J$-$W^V/+$) obtained from The Jackson Laboratory (Bar Harbor, ME). The mast cell deficient (W/W^V) and mast cell normal (+/+) were distinguished from each other and from the heterozygotes (W/+ and $W^V/+$) by their coat color. All animals were maintained on a 12 hours light-dark cycle (lights on from 7AM-7PM) and the temperature remained constant at 24^oC. From the time of weaning, the animals shared small cages with their siblings of like sex and were watered and fed food pellets (Ralston-Purina Lab Chow). For this study, five male animals of each genotype were used, as well as five normal albino (random bred Swiss) mice obtained from Timco Breeding Labs, Houston, TX) were also included. The mice ranged in age from three to five months at the time of sacrifice.

Dissection And Tissue Preparation

Sacrifice of the animals was performed over a series of consecutive days at approximately 9AM. Cervical dislocation without prior anesthetizaiton which could affect brain chemistry proved the most efficient method. Whole brains,

excluding the olfactory bulbs, were carefully dissected away from skull attachments and placed individually in vials containing 10 percent neutral buffered formalin. Brains were allowed to fix for 48 hours after sacrifice at room temperature before being processed overnight in the Fisher Histomatic™ tissue processor (Model 166). The following morning, brains were embedded in paraffin using the Tissue Tek II embedding center (Lab-Tek Products, Naperville, IL.).

Staining And Sectioning

The American Optical microtome (Buffalo, N.Y.) was used to section the brains into serial coronal sections approximately 7 µm thick. The first of every ten sections was mounted on a glass slide for staining. This method did not allow counting of the same mast cell twice, as mast cells are about 10 - 15 µm in diameter (Kiernan, 1976). The slides were dried for 10 minutes in a slide heater and placed in numerical order in slide boxes. The number of slides per brain averaged 90. The procedure for staining mast cells was as follows: slides were deparaffinized in two changes of xylene for three minutes each, placed in three changes of 100 percent ethyl alcohol for two minutes each, stained in 0.5 percent aqueous toluidine blue 0 (Fisher Scientific, Fair Lawn, N.J.) for two minutes, differentiated in two changes of 50 percent ethanol (15-second agitations each), dehydrated with two changes of 100 percent ethanol (ten dips

each), and cleared with two changes of xylene (three minutes each). This procedure was modified from the technique of Sheehan and Hrapchack, 1980. Slides were then mounted with Permount (Fisher Scientific) and coverslipped. Toluidine blue was the stain of choice because it stains cytoplasmic granules of mast cells metachromatically (Yamasaki and Komoto, 1971). The proposed mechanism of staining metachromatically is as follows: toluidine blue, a cationic dye, binds to heparin (a sulfated glycosaminoglycan) and causes granules to enlarge, scatter and become metachromatic (Yamasaki and Komoto, 1971, and Kruger and Bloom, 1974). Toluidine blue also is thought to release histamine perhaps by displacing it from its attachement to the granules (Johnson and Moran, 1974; Yamasaki and Komoto, 1971; and Kruger and Bloom, 1974).

Quantitation Of Mast Cells

Stained slides were examined in consecutive order from the anterior to the posterior regions of the brain using a Zeiss microscope. A quantitation of metachromatic granulated mast cells in each section was made, and the specific anatomical location of the mast cells was determined. After all stained sections were thoroughly investigated, photomicrographs were taken of sections illustrating examples of the mast cells in various locations using the Zeiss Photomicroscope II. Also, graphs for each genotype, illustrating the hippocampal region broken down into five sections, was made

to plot averages of mast cells for each fifth.

Statistics

One way analysis of variance test (ANOVA) were performed on mast cell totals per brain in all genotypes, as well as on mast cell totals along the meninges overlying the thalamus. A p value of 0.05 or less was considered significant, and when this was the case, subsequent t-tests (1-tailed or 2-tailed) were performed using the Bonferonni procedure (Kirk, 1968). This procedure is valid when one has an a priori expectation of the results. It was predicted that +/+, W/+, and W^V/+ mice would be significantly different from W/W^V mice, due to literature reports cited in the Introduction stating the absence of mast cells in the W/W^V mice. A one-tailed t-test was applicable due to this absence of mast cells. In addition, +/+ mice were expected to be similar to normal outbred mice, since they are thought to be mast cell normal. Lastly, since W/+ and W^V/+ mice are heterozygotes, it was thought that they might differ from the +/+ mice. Since it was not known how the genotypes would differ, , a two-tailed t-test was indicated. No presumptions could be made concerning differences between W/+ and W^V/+ mice, since no information was known about mast cell numbers of these two genotypes. The last statistical analysis performed focused on the distribution of mast cells along the hippocampus, specifically in the meninges

Using the graphs drawn of quantitation of mast cells in each fifth of the hippocampus (which overlies most of the thalamus), two way analysis of variance tests (ANOVA) were applied to analyze the variation in mast cell numbers in the anterior 3/5 of the hippocampus versus the posterior two-fifths. One test compared +/+, W/+, and W^V/+ mice, and a second test compared +/+ and normal outbred mice.

CHAPTER III

RESULTS

TOTAL MAST CELL NUMBERS

Quantitation of mast cells in serial sections of the brains of the +/+, W/+, W^V/+, and normal outbred albino mice uncovered some interesting results. Table I shows mast cell totals for each mouse as well as the calculated mean ($\overline{X}$) standard deviation (σ) and variance (σ^2) of each genotype.

TABLE I

Total Mast Cells

Mouse #	Normal Outbred	+/+	W/+	W^V/+	W/W^V
1	269	87	550	8	0
2	82	34	180	10	0
3	102	144	584	5	0
4	227	42	708	17	0
5	154	203	*506	1	0
	$\overline{X}$ = 167	$\overline{X}$ = 102	$\overline{X}$ = 506	$\overline{X}$ = 8	$\overline{X}$ = 0
	σ = 80	σ = 71	σ = 197	σ = 6	σ = 0
	σ^2=6400	σ^2=5041	σ^2=38809	σ^2= 36	σ^2=0

* The mean of the above four mice was used for statistical purposes to represent the fifth mouse brain, which was not available for use in the experiment.

The numbers of mast cells varied within each genotype (except the W/W^V) as well as between different genotypes. The W/+ genotype contained the highest number of mast cells, followed by the normal outbred mice and then the +/+ mice. Having very few mast cells were the $W^V/+$ mice. A one-way ANOVA (analysis of variance) test comparing the genotypes proved to significant, with $p<<0.05$ ($F=7.722$, and $df=4,23$). Subsequent t-test (to determine which genotypes differed from each other) using the Bonferonni procedure indicated that the W/+ mice has significnatly more mast cells than the +/+ ($p<0.005$) and W/W^V ($p<0.005$). There was not significant difference between +/+ and the normal outbred mice, and between +/+ and $W^V/+$. However, a difference did occur between $W^V/+$ and W/W^V genotype, with p 0.01. This can be clarified as follows: $(W/+)>(+/+)=(W^V/+)>(W/W^V)$, and (+/+=Normal outbred.

Mast Cells Of The Meninges Overlying The Thalamus

A striking difference between the genotypes concerned location of the mast cells in the brain. Table II illustrates this by comparing the normal outbred and the four gentoypes, +/+, W/+, $W^V/+$, and W/W^V. Shown are totals of mast cells in the meninges overlying the thalamus, as well as totals in other brain areas. The means and standard deviations for the totals are given.

TABLE II

Distribution Of Mast Cells In The Brain

	Mouse #	Number In Meninges Overlying Thalamus	Other Brain Areas
Normal Outbred	1	249	20 (lateral thalamus)
	2	78	4 (lateral thalamus)
	3	102	None
	4	212	15 (lateral thalamus)
	5	141	3 (meningial arteries around brain) and 11 (lateral thalamus)
		$\bar{X}$ = 156	$\bar{X}$ = 10.6
		σ = 72	σ = 8.3
		σ^2=5184	σ^2= 55.0
	Mouse #		
+/+	1	84	3 (lateral thalamus)
	2	34	None
	3	124	19 (mammillary bodies) and 1 (lateral thalamus)
	4	37	5 (lateral thalamus)
	5	151	52 (lateral thalamus)
		$\bar{X}$ = 86	$\bar{X}$ = 16
		σ = 52	σ = 21.3
		σ^2=2704	σ^2=455

NOTE: $\bar{X}$ = mean, σ=standard deviation, σ^2= variance

	Mouse #	Number in Thalamic Meninges (pia)	Other Brain Areas
W/+	1	81	469 (meningeal arteries around brain)
	2	25	155 (meningeal arteries around brain)
	3	21	563 (meningeal arteries around brain)
	4	78	630 (meningeal arteries around brain)
	5	*51.25	N/A
		$\bar{X}$ = 51.25	$\bar{X}$ = 454.25
		σ = 33	σ = 210.15
		σ^2=1089	σ^2=44161
		*mean of mice 1-4	
$W^V/+$	1	2	6 (meningeal arteries)
	2	10	None
	3	0	5 (meningeal arteries)
	4	10	7 (meningeal arteries)
	5	0	1 (meningeal arteries)
		$\bar{X}$ = 4.4	$\bar{X}$ = 4.75
		= 5.18	= 2.63
		= 26.8	= 6.92
W/W^V	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
		$\bar{X}$ = 0	0

Figure 2 illustrates that area of the brain where the majority of the mast cells were found. Specifically, the pia overlying the dorsal thalamus was the focal area of mast cell populations, except in W/+ where the majority surrounded pial arteries of the cerebrum. Mast cells primarily were clustered or lined up in the adventitia of the arterioles in the pia overlying the thalamus (see Figures 4 and 6 for examples). The normal outbred mice brains showed mast cells localized in this region (Figure 3 and 4), with the remainder found within the dorsal lateral thalamus associated with arterioles and capillaries (Figure 3). Figure 4 also shows the granules of the mast cells which stained metachromatically with toluidine blue. The +/+ mice, supposedly "mast cell normal" also contained most of their mast cells around the thalamus. Figure 5 shows mast cell clusters along thalamic meninges (pia) at the most medial part of the dentate gyrus, and Figure 6 shows their affinity for the adventitia of arterioles along the pia between the dentate gyrus and the lateral thalamus. Also seen is a mast cell near a capillary in the lateral thalamus parenchyma.

The W/+ genotype was alone in its high distribution of mast cells in meninges (pia) surrounding the exterior of the brain (See Table II). The W/+ mice contained many more mast cells than any of the other genotypes examined, most of which were localized in the pia over the cortex, but some of which rested in the pia over the thalamus. Figure 7 illustrates

the large clusters of mast cells associated with arterioles in the pia overlying the cortex, and Figure 8 shows an even larger population around vessels of the anterior longitudinal fissure. The $W^V/+$ genotype had variable but few numbers of mast cells placed along the meninges overlying the thalamus and the pial arterioles outside the cortex (Table II). The W/W^V genotype had no mast cells in the brain at all (See Table II and Figures 2, 9, and 10).

Statistics performed on the mast cell counts in the meninges overlying the thalamus paralleled those done on the total mast cell numbers in whole brains. A one-way analysis of variance (ANOVA) test proved to be significant, with $p<<0.005$ ($F=13.7$, and $df=4,23$). Subsequent t-tests using the Bonferonni procedure gave these results: W/+, +/+, and normal outbred mice did not differ significantly in their distribution of mast cell numbers, in the meninges overlying the thalamus, though they did differ significantly from $W^V/+$ and W/W^V ($p<0.005$). W/W^V and $W^V/+$ did not show statistically significant differences. To summarize W/+ = +/+ = Normal outbred $>W^V/+ = W/W^V$.

Distribution Of Mast Cells Along The Meninges Overlying The Thalamus

Due to the presence of mast cells in the pia overlying the dorsal thalamus of the mice genotypes studied, it was necessary to determine if a pattern existed in the distribution

of the mast cells along this region. Since the mast cells were primarily located adjacent to the dentate gyrus of the hippocampal region, the hippocampus was divided up into five parts and mast cells were tallied for each division in each mouse. A graph was made plotting averages for each genotype to give a clear picture of exactly where mast cells exist (see graphs on subsequent pages-Figs. 11 - 14). Since the graphs showed that the anterior three-fifths of the brain differed greatly from the posterior or caudal two-fifths of the brain, a comparison was drawn using these two divisions. A two way ANOVA of anterior versus posterior of the $WBB6F_1/J$ showed significant differences between the anterior three-fifths and posterior two-fifths, significant differences between genotypes and significant in interaction ($F=3.524$, degrees of freedom $=2,24$, $p<0.05$). A separate two way ANOVA comparing anterior and posterior of +/+ and normal outbred mice was not significant ($F=3,524$, $df=2,24$, $p<0.05$).

Figure 2. A coronal section of the brain of a W/W^V mouse. It illustrates the hippocampal formation (HPC), dentate gyrus (DG), thalamus (TH), and lateral geniculate nucleus (LG). These are primary locations for mast cell populations within the brain parenchyma of the other genotypes, and the outbred white mice. Also labelled are the third venticle (V), the choroidal fissure (CF) and the corpus callosum (CC). Stain: toluidine blue (25X).


V
CF

Figure 3. A coronal section of the brain of a normal outbred mouse. It demonstrates the position of mast cells (arrows) surrounding vessels at the tip of the dentate gyrus (DG) as well as mast cells in the dorsal thalamus (arrowhead). Mast cells can be recognized in the light microscope by their metachromasia, and they appear darker in photomicrographs than other types of cells in the area. Arterioles are indicated by the letter A. Stain: toluidine blue (100X).


A
A

Figure 4. A coronal section of the brain of a normal outbred mouse. This section not only shows localization of mast cells (arrows) around arterioles (A) in the choroidal fissure (CF) between the dentate gyrus (DG) and the thalamus (TH), but it also shows the intracellularly arranged mast cell granules which stain metachromatically with toluidine blue (250X).


F
A

Figure 5. A coronal section of the brain of a +/+ mouse. The micrograph demonstrates the position of mast cells (arrows) in association with the pial vessels of the choroidal fissure (CF), as well as the medial tip of the dentate gyrus (DG). Stain: toluidine blue (100X).

Figure 6. A coronal section of the brain of a +/+ mouse. This illustration demonstrates the affinity of mast cells (arrow) for the adventitia of an arteriole in the pia of the choroidal fissure (CF). Also seen is a mast cell associated with a capillary in the lateral thalamus (arrowhead). Stain: toluidine blue (100X).


CF

Figure 7. A coronal section of the brain of a W/+ mouse. The micrograph shows an example of mast cells associated with a pial arteriole (A). The pia is beneath the cerebral cortex (CC). Stain: toluidine blue (250X).

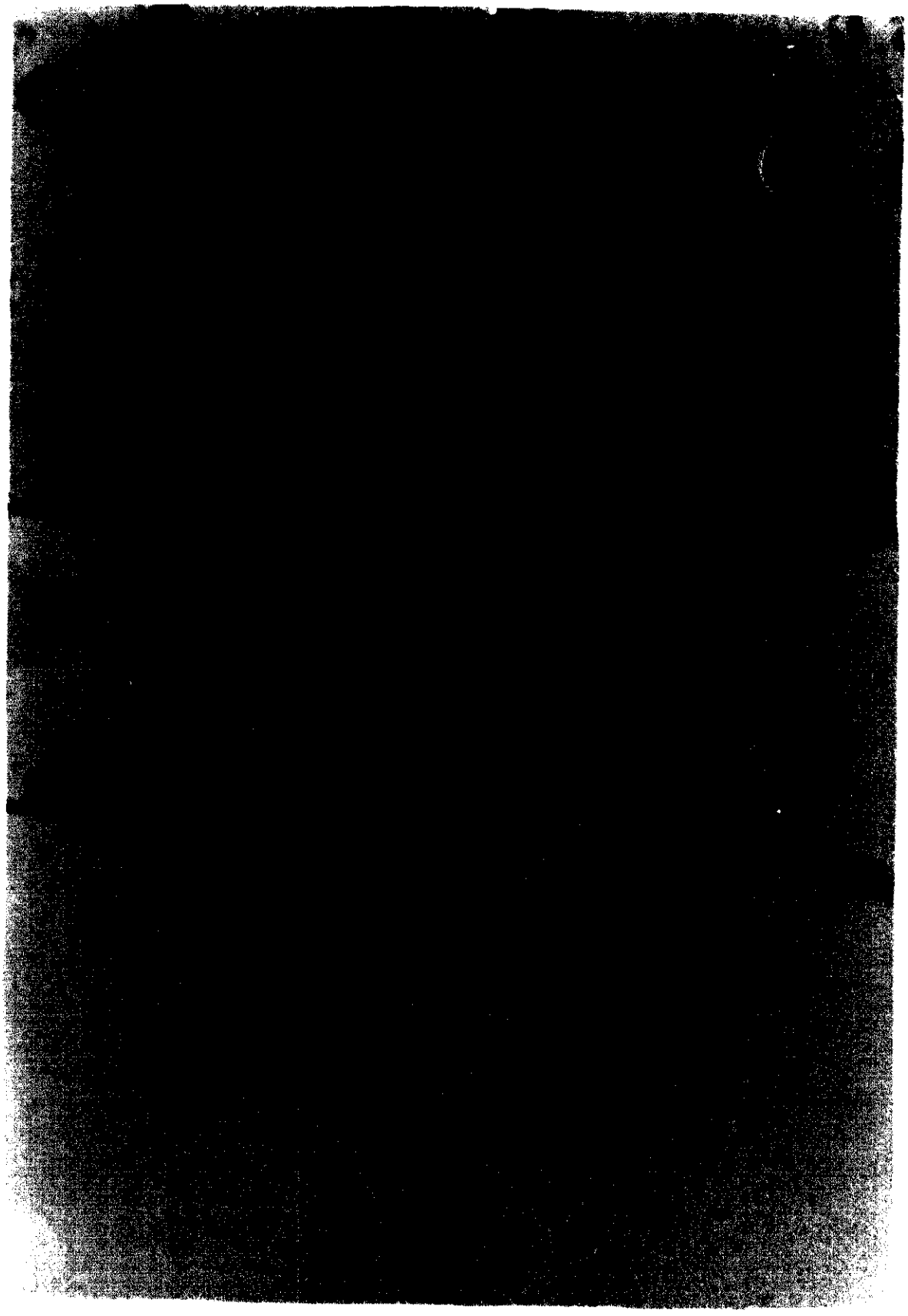


Figure 8. A coronal section of the brain of a W/+ mouse. This is a high magnification of the anterior longitudinal fissure which shows a large cluster of mast cells (arrows) in the pia. An arteriole is labelled (A). Stain: toluidine blue (250X).

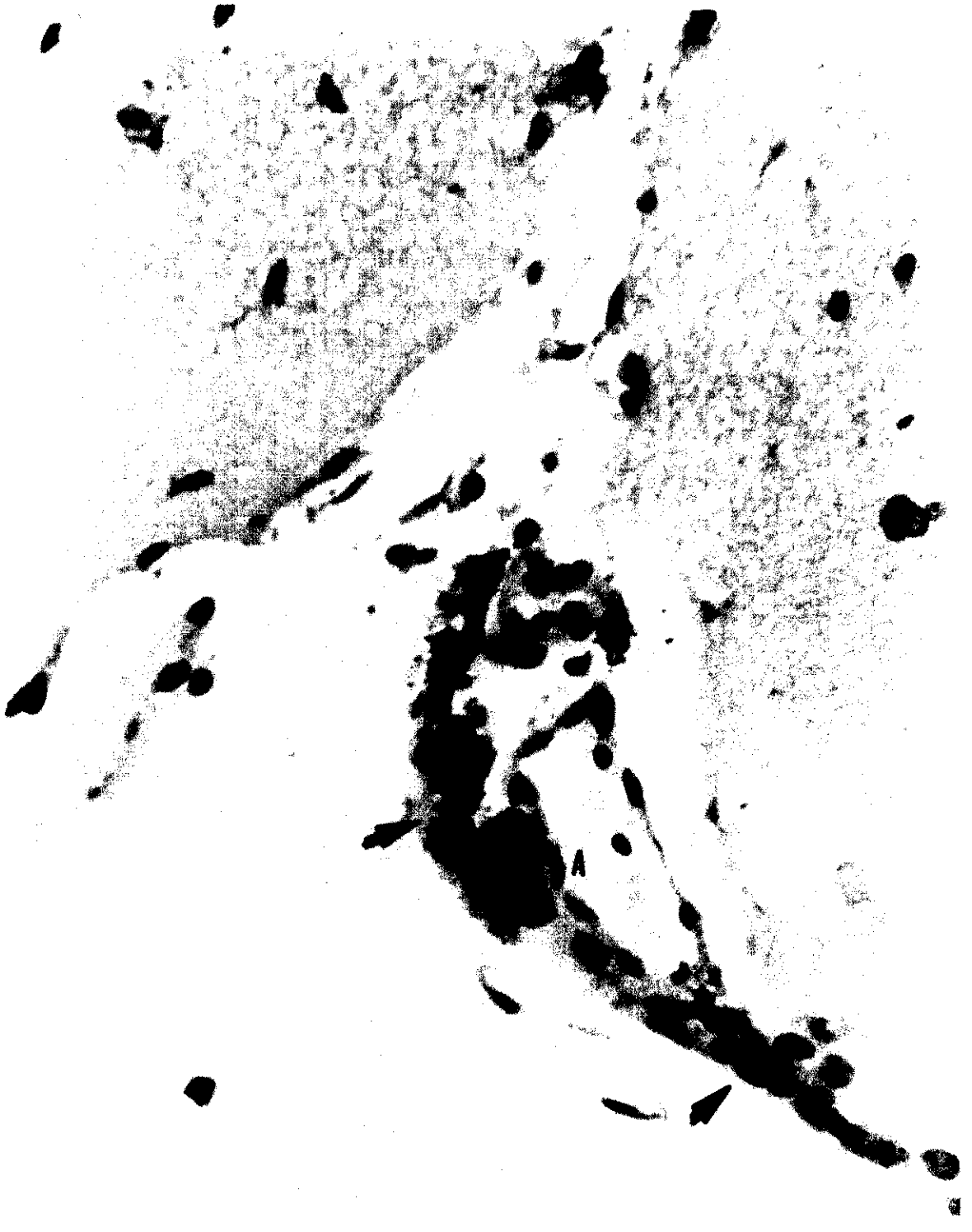

A

Figure 9. A coronal section of the brain of a W/W^V mouse. The illustration shows the absence of mast cells within the choroidal fissure (CF) and lateral thalamus (TH). Stain: toluidine blue (100X).

Figure 10. A coronal section of the brain of a W/W^V mouse. The plate shows the absence of mast cells in the pia overlying the cerebral cortex (CC). Stain: toluidine blue (100X).

Figure 11. Graph of five sections of hippocampus versus the number of mast cells found in this area in the normal outbred stain. The posterior two-fifths of the brain (last two sections of figure) has higher counts of mast cells than the anterior three-fifths (left three sections of figure). Lines running through each bar represent standard error. Numbers below bars represent mean ± standard error for each one-fifth.

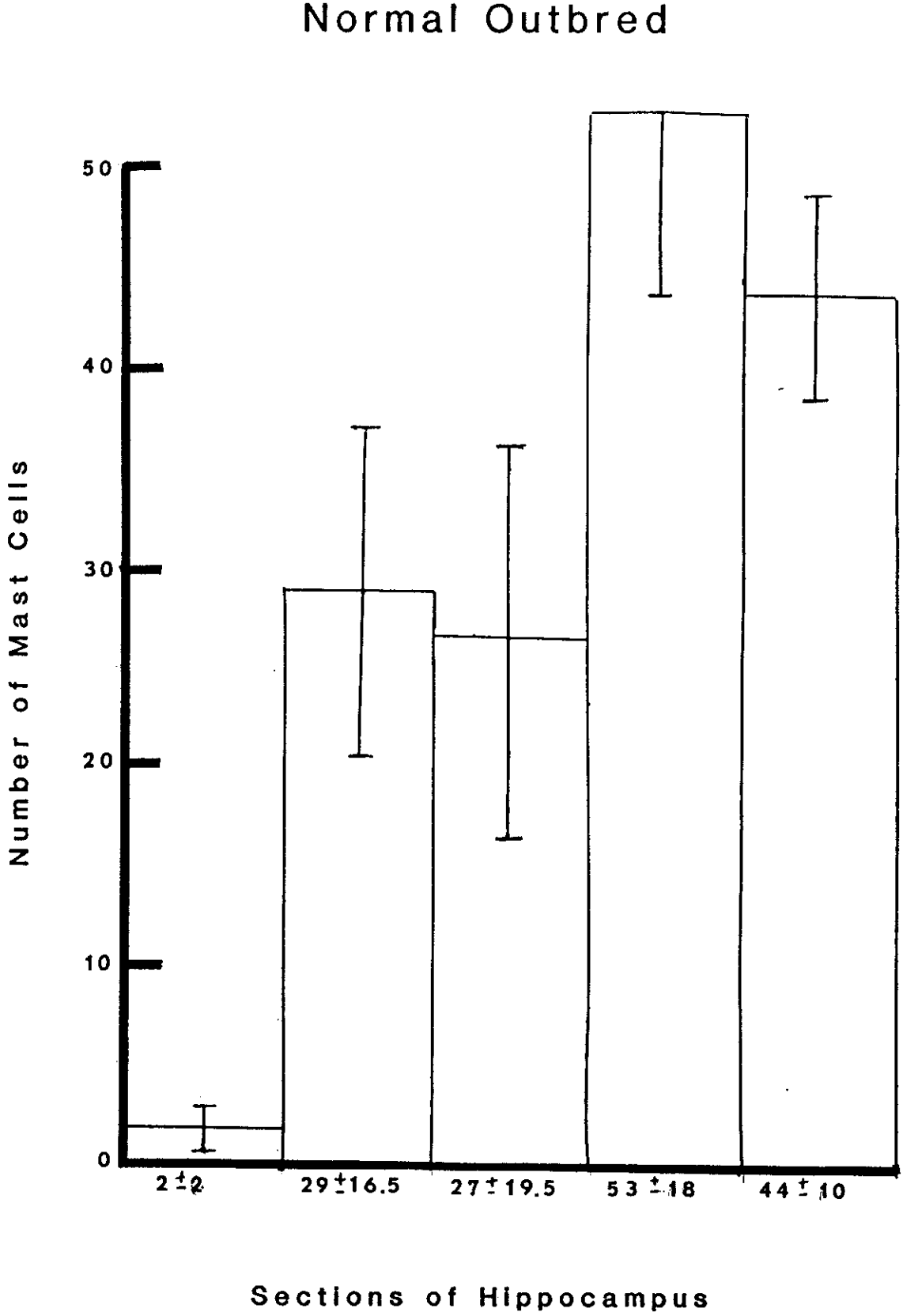

Normal Outbred
Number of Mast Cells
50
40
30
20
10
0
2 ± 2
29 ± 16.5
27 ± 19.5
53 ± 18
44 ± 10
Sections of Hippocampus

Figure 12. Graph of five sections of hippocampus versus number of mast cells in this area in the +/+ genotype. Shown here are larger populations of mast cells in the posterior two-fifths of the hippocampal formation (right two sections on figure). The most rostral section of the brain has fewer mast cells in comparison with the caudal section. Numbers below bars represent mean ± standard error for each one-fifth.

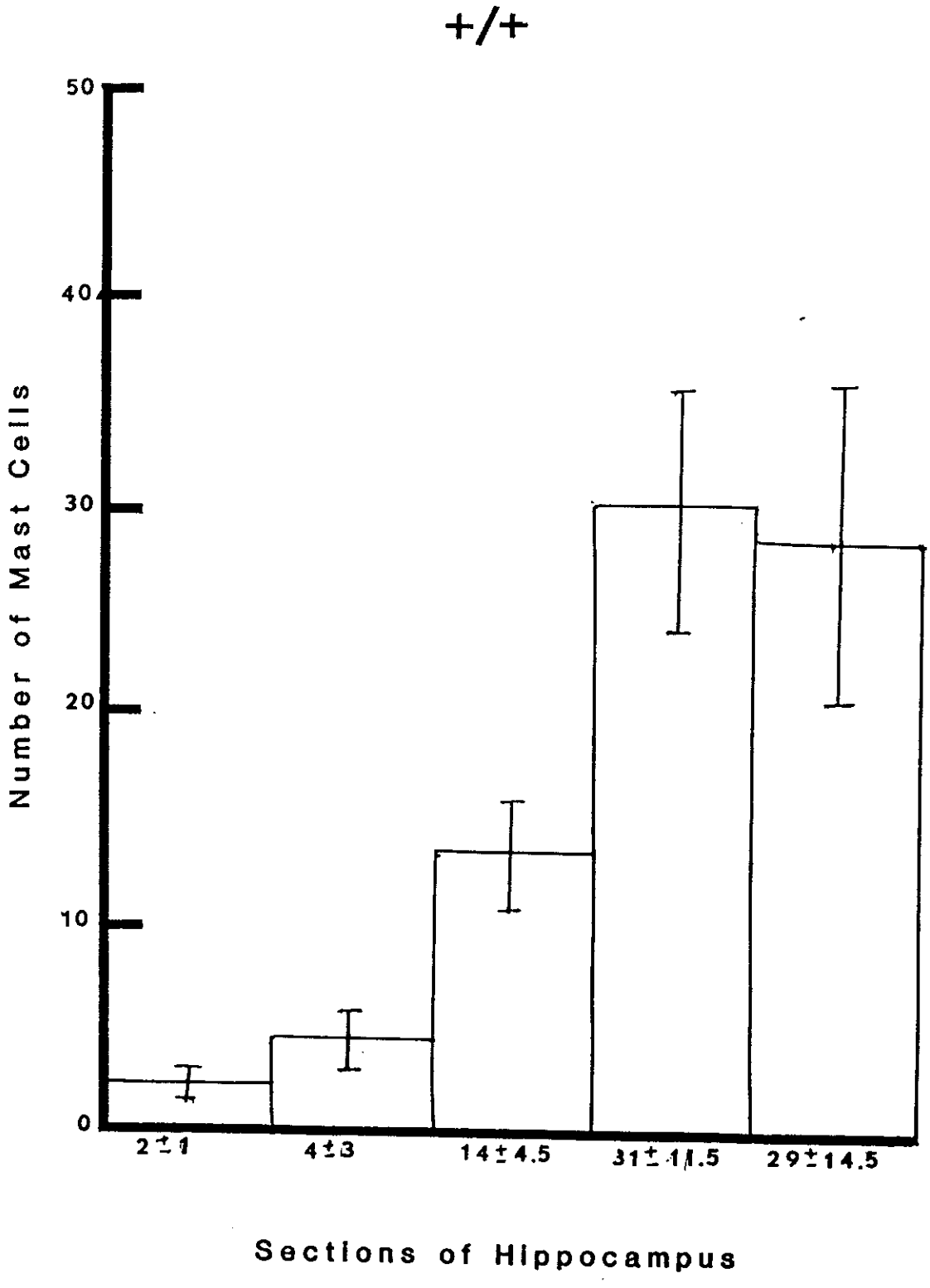

+/+
50
40
30
20
10
0
Number of Mast Cells
2±1
4±3
14±4.5
31±11.5
29±14.5
Sections of Hippocampus

Figure 13. Graph of five sections of hippocampus versus number of mast cells in this area in the W/+ genotype. The left three sections (anterior three-fifths) of hippocampi are free of mast cells, and the posterior two-fifths contain all of the mast cells of this region. The last section (right) has the highest population of mast clels. Numbers below bars represent mean ± standard error for each one-fifth.

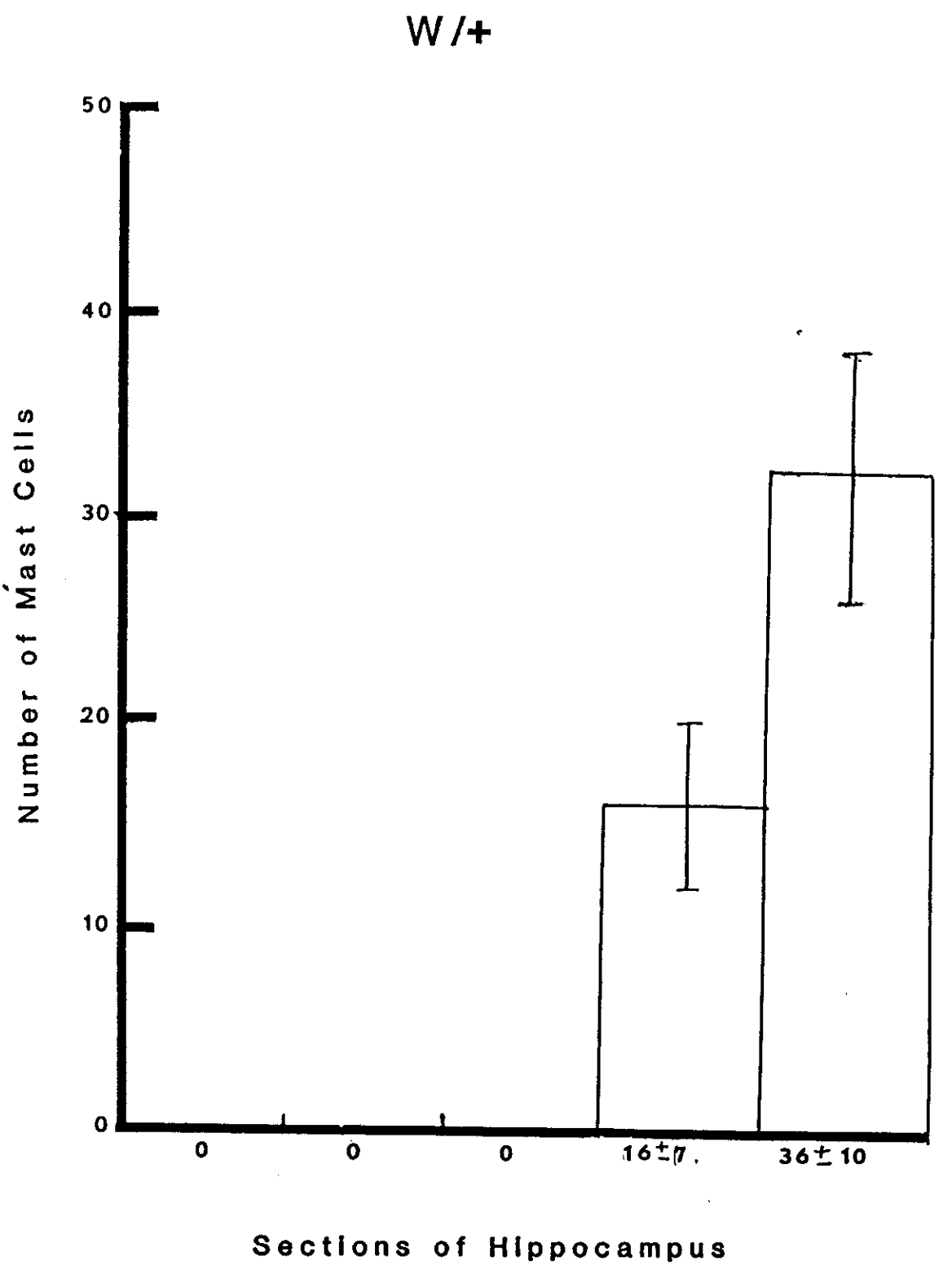
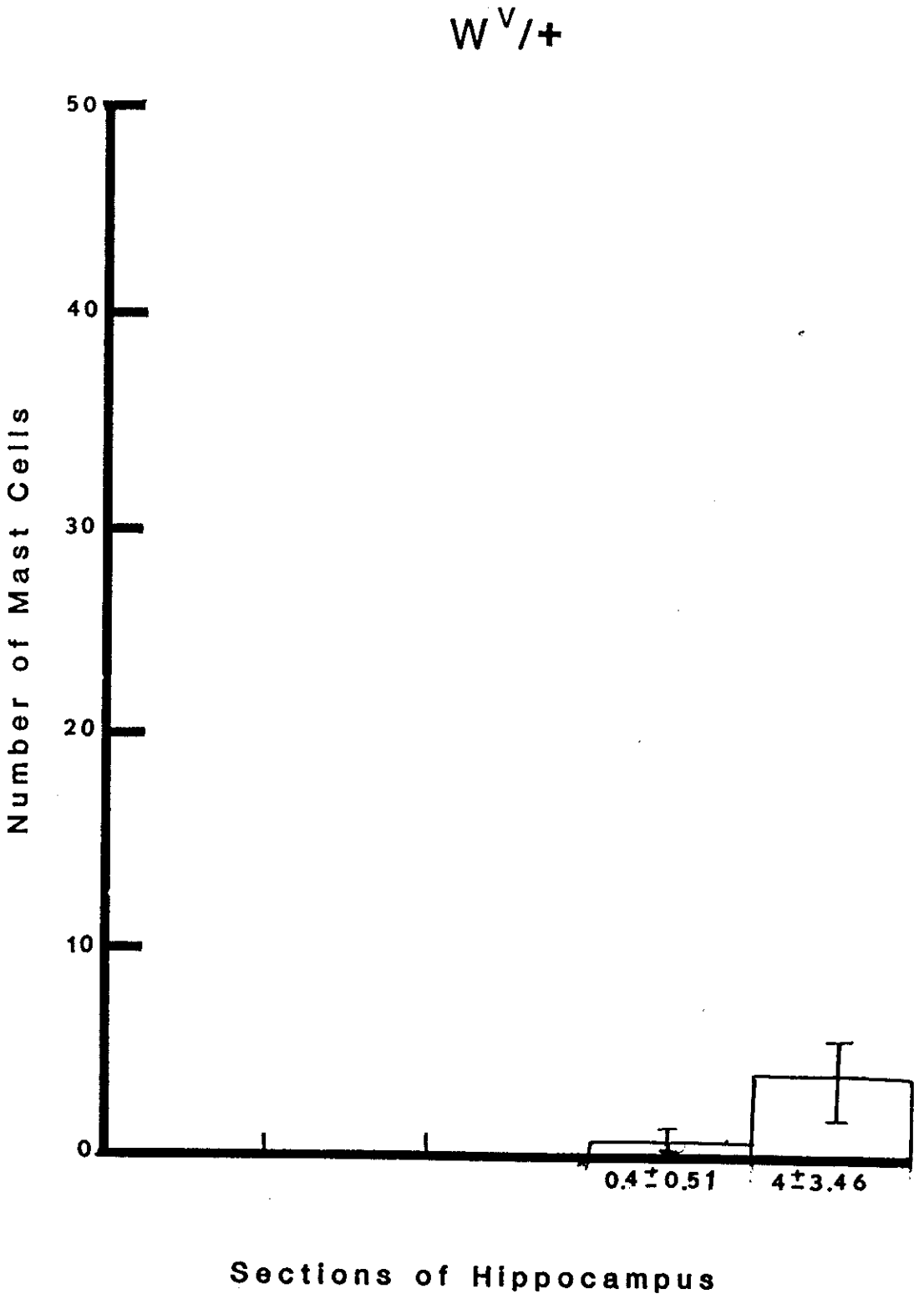

W/+
50
40
30
20
10
0
Number of Mast Cells
0
0
0
16±7.
36±10
Sections of Hippocampus

Figure 14. Graph of five sections of hippocampus versus number of mast cells in this area in the W^{V}+ genotype. This genotype has the least amount of mast cells of those plotted, and these are found in the caudal two-fifths of the hippocampal formation (right two sections of figure). The last section contains the majority of mast cells. Numbers below bars represent mean ± standard error for each one-fifth.


W^V/+
50
40
30
20
10
0
Number of Mast Cells
0.4±0.51
4±3.46
Sections of Hippocampus

CHAPTER IV

DISCUSSION

The purpose of this project was to investigate the presence and distribution of mast cells in the brains of a strain of inbred mice ($WBB6F_1/J$) as well as random outbred Swiss mice. A histological study of this type may indicate the significance of mast cells as a pool of histamine in the central nervous system. The findings not only revealed the numbers and locations of brain mast cells in all genotypes studied, but they also showed that significant differences exist between the genotypes. As a consequence, this study revealed a detailed histological picture of the brain-associated mast cells on which to base further studies. These may include biochemical and physiological studies on the potential role of mast cell histamine in the CNS.

Total Mast Cell Numbers

The evidence that the total numbers of mast cells proved to be rather small in every genotype is critical for further research. It has been speculated that mast cells account for 50 percent of brain histamine; the other 50 percent being of non-mast cell origin (Garbarg, et al., 1976). Beaven (1978) found that individual rat mast cells contained 10-20 pg of histamine. Yamatodani et. al. (1982) found histamine

content in the brain of W/W^V mice and +/+ mice to be 24 ng/g and 53 ng/g respectively. Table 1 in the Results section shows the mean number of mast cells in the +/+ mice to be 102, and assuming there are 15 pg of Hm per mast cell, mast cells would contribute only 1.53 ng of Hm, which is musch less than the 50 percent contribution once suspected of mast cells in the CNS. Also, Orr and Pace (In Preparation) recently found insignificant differences in Hm concentration within the brains of the +/+, $W^V/+$ and W/W^V mice. The W/+ had a higher concentration of Hm which concurs with the present data showing that the highest number of mast cells in the brains are associated with this genotype. Nevertheless, even in this case, mast cells would contribute less than 50 percent to the total pool of brain Hm. This experiment showed that the total numbers of mast cells differed within and among the genotypes. Variations within each genotype may be significant to the extent that in future biochemical experiments which measure cerebral Hm, each animal would have to act as its own control, rather than be compared to other animals. Reiterating the statistical difference between genotypes regarding the total mast cell counts in the brain; W/+ proved to be signifcantly different form all other types, +/+ was not statistically different from $W^V/+$, and $W^V/+$ was unlike W/W^V. Also, +/+ and normal albino mice were very similar. Since W/+ and $W^V/+$ have been overlooked in other brain histamine studies, it was interesting to learn of their

dissimilarlity to the +/+ and the W/W^V mice. The fact that the +/+ and normal white Swiss mice were alike may validate the hypothesis that the +/+ mice are "mast cell normal", though overall the mast cell counts were higher in the normal outbred white mice. Though the +/+ and $W^V/+$ were not statistically different, there was a large difference in the mast cell totals, an indication that the $W^V/+$ is somewhat less than "normal", in its number of mast cells, yet, it was found to be different from the W/W^V mice, which in this study, as in others, lacked mast cells (Grzanna and Schultz, 1982). Evidence here also correlates with other studies which showed that +/+ and W/W^V mice were significantly different in their number of mast cells (Yamatodani et al., 1982). Thus, this information concerning mast cells totals in the genotypes studied should serve as a basis for future determinations of brain histamine distribution.

Association Of Mast Cells With Vasculature

The primary location of the mast cells in the brain sections observed was in association with blood vessels, more specifically arterioles, capillaries, and to a lesser extent, venules. This knowledge is in accord with authors such as Selye (1975) and Compton (1952). The Introduction also discussed the posssible ramifications of histamine

production at blood vessel sites, so it will not be repeated here. It is sufficient to note that most of the mast cells were located in close proximity to the brain vasculature, many lying in the adventitia of the arterioles. This knowledge suggests that perhaps mast cell histamine, besides regulating vasodynamic mechanisms, may manifest some effect upon the blood per se that circulates throughout the central nervous system vasculature. Further research will address this hypothesis.

Mast cells were found in association with blood vessels and the number of mast cells varied at each location. For example, in the W/+ mice the majority of mast cells were in association with the pia surrounding the brain cortex, while the +/+ and Albino Swiss mice displayed mast cells primarily in the pia of the choroidal fissure between the dorsal thalamus and the hippocampal formation. These locations were cited by authors such as Dropp (1976) and Ibrahim (1974) who found mast cells there as well as in the dorsal thalamus. Summarizing the results of mast cell totals in the pia overlying the thalamus: W/+ = +/+ = Normal Outbred > W^V/+ = W/W^V. Though W/+ had the highest number of mast cells overall, its mast cell numbers in the meninges overlying the thalamus was found to be comparable to that of the +/+ and normal white mice. The W^V/+, with a mean of only eight mast cells in varying locations, was significantly different from all other genotypes except W/W^V.

Based on these results, a hypothesis can be formed concerning the genotypical influence on numbers and locations of mast cells. The + locus is probably necessary for the "normal" number of mast cells for the species, while presence of the W locus may influence mast cell populations in regard to their location and increased numbers. The W^V locus seems to deter the presence of mast cells. This hypothesis seems logical because although the W/+ genotype was found to be similar to the +/+ in its distribution of mast cells in the choroidal fissure, it showed a much higher mast cell total due to its mast cell populations in the pia overlying the cortex. The W^V/+ mice on the other hand, had low mast cell numbers presumably due to the presence of the W^V locus, but not a total absence of mast cells, due to the presence of the + locus. All of these presumptions are interesting possiblities to help account for the genotypical differences, but it will require more research for their confirmation. In looking at the distribution of mast cells along the pia encasing the thalamus, it was found that highest quantities of mast cells occurred along the posterior two-fifths of the hippocampus. In fact, in the heterozygotes, the anterior three—fifths of the hippocampi were free of mast cells. To summarize results obtained from comparison of genotypes based on this distribution: Normal outbred = +/+ > W/+ > W^V/+. Although genotypical differences were found, all WBB6F_1/J mice (except W/W^V)

has more mast cells in the posterior thalamic meninges than in the anterior thalamuc meninges (see Figs. 12 - 14). The same was true of normal outbred mice (Fig. 11), which were very similar to the +/+ mice. It can be suggested that the reason for the more caudal concentration of mast cells is due partly to the vasculation of the posterior brain. This leads us to a discussion of the cerebral blood supply.

Cerebral Blood Supply

Due to the fact that mast cells in the W/+ genotype were seen in association with the pial vessels overlying the cortex, it was necessary to examine the blood supply to this area. According to Craigie's atlas, branches of the internal carotid artery supply the cerebral hemispheres (Zeman and Innes, 1963). The largest of these is the middle cerebral which branches and courses laterally and dorsally over the brain surface. It is the primary blood supply to the cerebral cortex and the pia overlying it. Lesser contributions of blood vessels to the exterior of the cortex arise from the anterior and posterior cerebral arteries. The latter vessel arises from the basilar artery and is actually attached to the internal carotid by a connection called the posterior communicating artery. The anterior cerebral artery courses anteriorly to supply the foremost part of the brain, including the olfactory bulbs and medial hemisphere surface. The posterior cerebral artery extends caudally to the

occipital region of the brain (Zeman and Innes, 1963). Figure 15 shows a simplified sketch of these major vessels. Since no mast cells were found adjacent to or within the cerebelli of the animals studied; vessels supplying that structure will not be covered here. The venous supply can best be understood by referring to Figure 16.

The W/+ genotype localized the majority of the mast cells in the vicinity of the branches off the middle cerebral artery, and to a lesser extent, along branches off the anterior cerebral artery. Only a small number of cells may be accounted for along branches of the posterior cerebral artery. The mast cells are often found in association with the microvessels instead of the direct branches off the main arteries. Association of mast cells with veins would be primarily with branches off the superior cerebral vein and superior sagital sinus. (See Fig. 16). It is not known why there is an accumulation of mast cells in the pia overlying the cortex, but in reviewing the possible functions of mast cells and the effect of Hm upon blood vessels (vasodilation , homeostatic maintenance and permeability), it can be deduced that additional mast cells may be needed in this genotype to maintine adequate cerebral blood circulation. Part of the explanation for the large number of mast cells may also be due to the fact that mast cells in the CNS migrate from the exterior to the interior of the brain in very young rats (Persinger, 1981). Perhaps the mast cells

of the W/+ mice simply undergo a slower rate of migration into the brain than do the other genotypes. The implications of this phenomenon are not known, but the brains of W/+ mice should be researched more extensively due to their uniqueness in numbers and locations of mast cells.

Another area of the brain's blood supply that must be examined is in the region of the hippocampal formation and the thalamus. Another primary location of mast cells is in the vicinity of microvessels in the pia overlying the dorsal thalamus of the W/+, +/+ and normal outbred Swiss mice. The mast cell rich pia lies at the junction between the dentate gyrus of the hippocampal formation and the thalamus. By examining the circulatory patterns in the vicinity of the hippocampus, it is possible to denote the vessels which are surrounded by mast cells. The arterial supply to the hippocampal formation is shown in Figure 17. The posterior cerebral artery branches into the longitudinal hippocampal artery,which divides into the internal and external transverse hippocampal arteries that supply the dentate gyrus (Coyle, 1976, 1978). Originating from the internal carotid, the choroidal artery lies in the choroidal fissure and supplies blood to the thalamus. Branches of this artery are thought to be the vessels around which mast cells are found in this study.

Since a few mast cells were seen in the dorsal-lateral thalamus, in the vicinity of the lateral geniculate nucleus,

it is important to understand the arterial supply to the thalamus. As very few investigations have been initiated to thoroughly examine the cerebral blood supply of the rat or mouse, speculation is necessary about the thalamus. Coyle (1975) showed that the posterior cerebral artery runs medially and caudally in the thalamus, and branches to form the posterior medial choroidal arteries, which supply the choroid plexus of the third venticle, and the posterior lateral choroidal artery which loops around the thalamus to join the anterior choroidal artery. Distal to the loop formation, the anterior chorodial is termed the common choroidal. Thalamo-perforating arteries branch deep into the thalamus. Mast cells seen in the thalamus in the present study were in the parenchyma, yet some were associated with capillaries and arterioles. These small blood vessels were probably branches off the anterior choroidal or the thalamoperforating arteries.

Venous drainage in the rat and mouse has not been well described. The hippocampus is drained by the deep and external transverse veins, and the thalamostriate veins drain the thalamus. A small number of mast cells may be associated with the thalamostriate veins, but the venous drainage of the thalamus is poorly described at present. However, research directed toward identification of the venous system of the mouse brain would be an asset to the localization of mast cells in the vasculature.

Lateral Geniculate Body Of Thalamus

The function of the lateral geniculate body is for vision. The dorsal nucleus carries visual impulses to the cortex, and the central nucleus receives the impulses for pupil reflexes (Zeman and Innes, 1963). Most mast cells of the thalamus were found within this nucleus. In addition, Mares, et al. (1979) found increased numbers of mast cells in the dorsal lateral geniculate bodies of rat brains after exposure to darkness. Consequently further experimentation needs to be performed to investigate mast cell function in the thalamus.

CHAPTER V

SUMMARY

The purpose of this experiment was to determine the number and location of mast cells in the mouse brain. Of particular interest was the variation in the parameters in the offspirng obtained by mating $WBB6F_1/J$ - W/+ and - $W^V/+$ mice, since one of the obtained genotypes (W/W^V) has been shown to be mast cell deficient (Kitamura et al., 1979). Mast cells were visualized and quantitated in serial cross-sections stained with toluidine blue. Significant variations between genotpyes in the number and location of brain-associated mast cells were found. (Tables 1 and 2, Figures 2 - 10). On the basis of results obtained, I propose that the presence, absence, or combination of certain gene alleles might determine the number and particular location of mast cells in this strain of mice. The results also suggest that the mast cells are a relatively small and variable contribution to the total amount of histamine in the brain, implicating the neuronal pool of Hm as the largest contributor to brain Hm. Finally, the results of this investigation offer researchers a detailed picture of mast cells in the CNS on which to base future studies on the significnace and role of neuronal and mast cell Hm.

Figure 15. Diagram of the blood supply on the ventral aspect of the rat or mouse brain. Branches of the internal carotid and basilar arteries supply the cerebral hemispheres. Mast cells are located near branches of the middle cerebral, and to a lesser extent, the anterior and posterior cerebral arteries. Modified from Zeman and Innes, 1963.

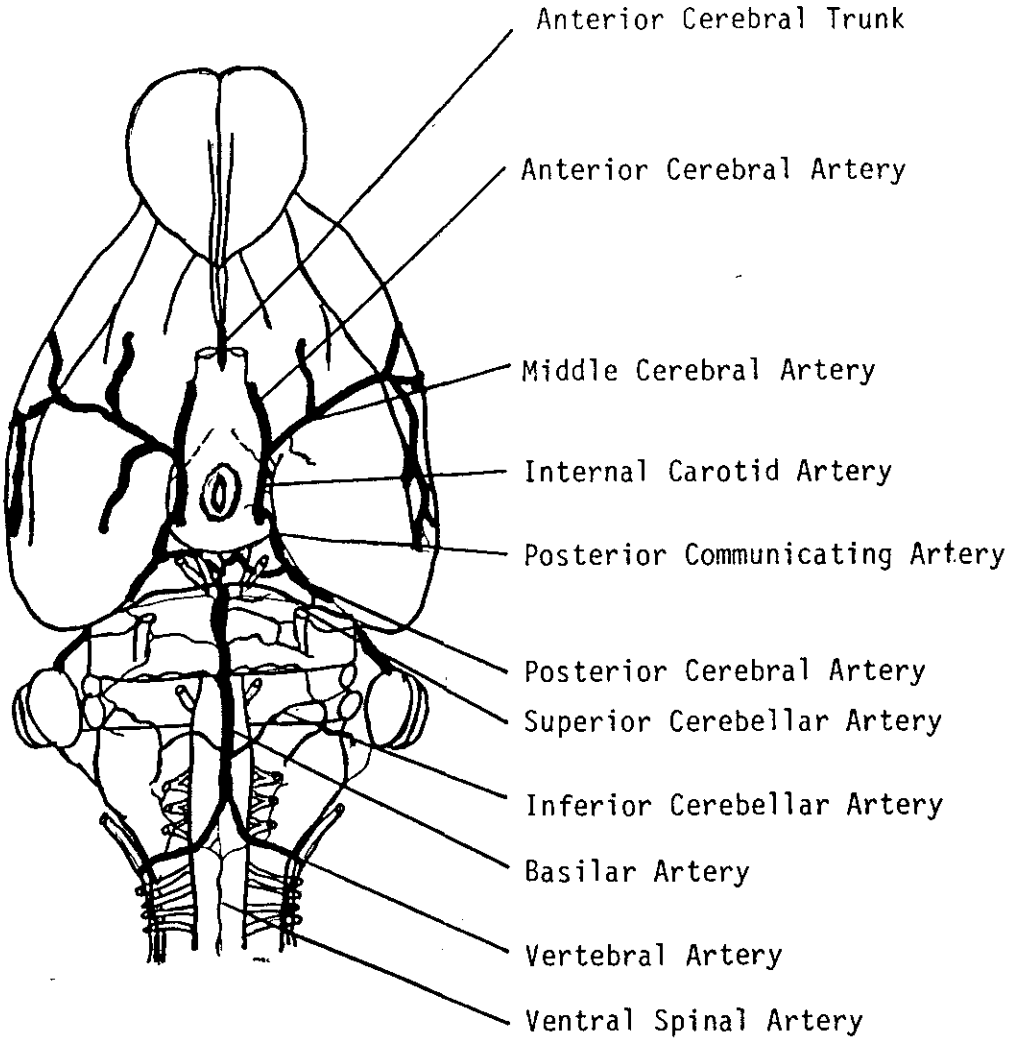

Anterior Cerebral Trunk
Anterior Cerebral Artery
Middle Cerebral Artery
Internal Carotid Artery
Posterior Communicating Artery
Posterior Cerebral Artery
Superior Cerebellar Artery
Inferior Cerebellar Artery
Basilar Artery
Vertebral Artery
Ventral Spinal Artery

Figure 16. Dorsal aspect of the rat or mouse brain showing the venous supply of the cerebral hemispheres. Mast cells would be found near microvessels off the superior cerebral vein, the superior sagittal sinus, and in smaller numbers derived from other veins shown here. Modified from Zeman and Innes, 1963.

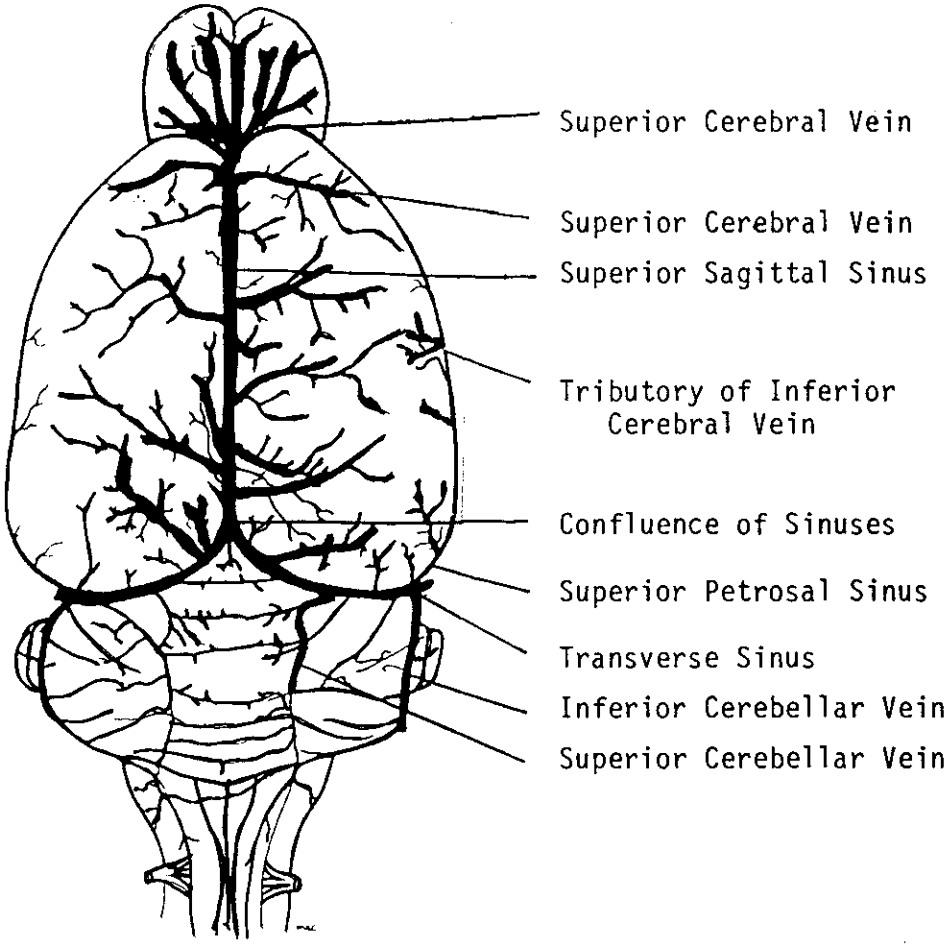
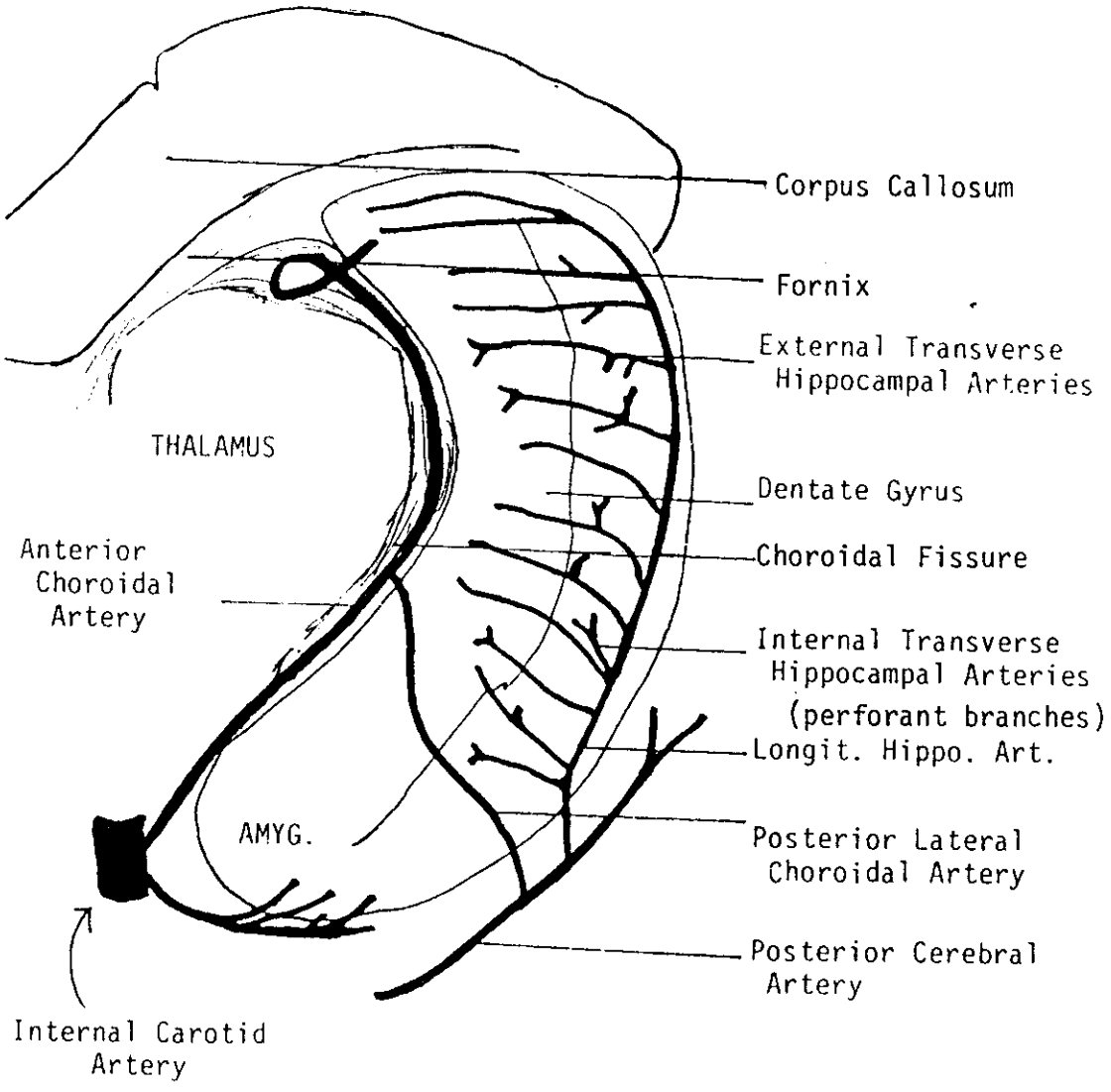

Superior Cerebral Vein
Superior Cerebral Vein
Superior Sagittal Sinus
Tributory of Inferior Cerebral Vein
Confluence of Sinuses
Superior Petrosal Sinus
Transverse Sinus
Inferior Cerebellar Vein
Superior Cerebellar Vein

Figure 17. Lateral view of the hippocampal formation showing the arterial supply to the hippocampus, as well as the dentate gyrus and thalamus. Modified from Coyle, 1978.


Corpus Callosum
Fornix
External Transverse
Hippocampal Arteries
THALAMUS
Dentate Gyrus
Anterior
Choroidal
Artery
Choroidal Fissure
Internal Transverse
Hippocampal Arteries
(perforant branches)
Longit. Hippo. Art.
AMYG.
Posterior Lateral
Choroidal Artery
Posterior Cerebral
Artery
Internal Carotid
Artery

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