REVIEW ARTICLE

TISSUE MAST CELLS AND TISSUE AMINES

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HISTORICAL INTRODUCTION

THE first description of tissue mast cells was made in 1877 during an investigation of the staining of fresh tissues by the new aniline dyes then being produced by the German chemical industry. Ehrlich[1] observed that some cells in the connective tissue of animals contained granules which changed the colour of toluidine blue and certain other dyes as staining proceeded. The numbers of such cells were greater when chronic inflammation or conditions characterised by increased local nutrition were present. For this reason, Ehrlich considered these cells to be overnourished connective tissue elements and called them "Mast cells" (Mastzellen = well-fed cells). In choosing the name of mast cell, he was also influenced by his finding of a high mast cell content in connective tissue subjected to lymph stasis; as we now know, in the selective lymphatic blockage of elephantiasis, the hypertrophied connective tissue is packed with mast cells and a similar histological picture, though on a smaller scale, is seen in young well-vascularised keloids.

Two years after his first discovery, Ehrlich[2] found similar cells in the blood, but whereas the blood mast cells, or basophils, take origin in the bone marrow and with the other leucocytes enter the peripheral blood, the more common tissue mast cells are born, live and die in the connective tissue. The similarity in the two kinds of cell lies in their content of water-soluble cytoplasmic granules which have a strong affinity for basic dyes, several of which change colour as staining occurs (metachromasia).

Besides discovering and naming the mast cells, Ehrlich also described their morphology, staining properties and distribution, but he then left it to others to elucidate the chemical nature of the granules. But, for the next 60 years, research on this subject remained almost entirely histological and then in 1937 Swedish workers solved one of the riddles of the metachromatic granules of the mast cell. Jorpes and his colleagues in Stockholm had for long been working on the powerful anticoagulant material first isolated from dog liver and hence called heparin. On finding that heparin stained metachromatically with toluidine blue, these workers[3] searched the tissues for metachromatism as a possible clue to the site of formation of heparin. And so it was that Jorpes was able to show that there is a good correlation of the mast cell count of a particular tissue and the amount of heparin that can be extracted from it. At that time mast cells were thought to be perivascular in location so as to produce heparin which pours into the blood stream. But a later study of the movement of the perivascular mast cells revealed that they migrate from the blood

vessels and hence they probably produce a secretion for both blood and tissues. Their function may be less concerned with blood clotting than with the maintenance and repair of the connective tissue.

The simultaneous release of both heparin and histamine was found by Rocha e Silva[4] to occur from the liver of the dog after anaphylactic shock, and it was suggested that perhaps both substances originated from the same cell. Chemical histamine liberators, some of which are fluorescent, were first tried to test this hypothesis. After lethal doses of such substances into rats, the fluorescent material was found to be sharply localised to the mast cells which thereafter broke up and released histamine[5]. Analysis of the tissues then indicated a good correlation of the mast cell content of a particular tissue and the amount of histamine that can be extracted from it. This is particularly evident when pathological tissues rich in mast cells are examined. For example, when the mast cell content of the skin is raised by painting it repeatedly with a carcinogenic hydrocarbon, the histamine content of the skin increases proportionately.

It seems, however, that the production of heparin and histamine are but part of the function of the mast cell, since mast cells are plentiful in lower organisms which lack a blood-vascular system. Even in higher animals, the mast cells appear to be associated with the tissues rather than with the blood vessels. There are other functions which are probably fundamental between mast cells and the connective tissue, and more work along these lines is clearly indicated. Asboe-Hansen[6] has recently described mast cells as unicellular endocrine glands which react as part of the mesenchymal system in time of stress. Such a definition places in a nutshell our present-day views on the importance of these mast cells to the organism in general.

MORPHOLOGY OF TISSUE MAST CELLS

Mast cells vary greatly in size and shape, depending in part upon their age and in part upon the structure and water content of the surrounding tissue. Usually, they measure from 8 to 20 μ in length and appear to be so distended with granules that the nucleus is obscured (as in the rat); in other species, like the hamster, the nucleus is usually visible, being round and about 5 μ in diameter. The cells may be spherical, spindle-shaped, or even stellate in appearance. In certain species, the smaller spindle-shaped cells predominate along the blood vessels while the larger, and probably older, spherical cells are found in extravascular areas. Stellate mast cells are found in the dermis of human skin. Considerable mingling of the several kinds may, however, occur in any one location. Each species has its own particular pattern of distribution for which, as yet, there is no adequate explanation.

The origin of mast cells has yet to be firmly established. They are recognised late in embryonic life but their origin in the embryo is uncertain. For example, tissue mast cells can be identified in the tip of the tail of new-born rats at a time when these cells are virtually absent from the skin (a tissue in which they abound in the adult). Once formed, they rapidly increase in number, size and granulation, and in the adult are

commonly found in the loose reticular adventitia of small blood vessels and in similar tissues which underlie epithelial, serous and synovial membranes. They are especially numerous in loose connective tissue undergoing fibrillogenesis and are without doubt mesenchymal cells. In the adult they may originate from fibroblasts. Dynamic changes occur in adult connective tissue following injury, and fibroplasia is succeeded by the appearance of mast cells. As recognisable collagen begins to take its shape, mast cells appear in the reactive zone. Should fibroplasia be protracted, as in chronic inflammation, the mast cell population further increases. In conditions of chronic lymphatic obstruction in which tissue spaces remain loaded with protein-rich oedema fluid, both mast cell hyperplasia and connective tissue hyperplasia become extreme. The endpoint of this sequence is reached with the formation of a vascular scar tissue in which neither fibroblasts nor mast cells are now present.

Mitotic figures have been observed by Hunt and Hunt in adult mast cells in rat mesentery after small intraperitoneal doses of histamine liberators. These authors also showed that large doses of the liberators disrupted the mast cells in the mesentery but produced mitotic figures in those of the subcutaneous connective tissue. After disintegration, they appeared to be replaced as a result of heteroplastic differentiation of mesenchymal cells or lymphocytes. It would be important to establish beyond question whether differentiated mast cells normally undergo mitosis[8].

It is possible that the granules in the cytoplasm of tissue mast cells are giant mitochondria. This suggestion is in fact supported by their physical behaviour and histochemical properties[9-11]. Since the granules are extremely soluble in water in some species, like the rabbit and in fish, many workers have failed to appreciate the high content of mast cells in certain tissues when watery fixatives are used. The first prerequisite for the histological demonstration of mast cells is therefore adequate fixation. The second determining factor is a suitable basic dye which changes colour as staining proceeds (metachromasia). Even well-fixed mast cell granules usually fail to stain with a commonly used histological technique—the haematoxylin-eosin method.

Recent advances in the complex cytochemistry of mast cells indicate that they contain, in addition to the amines discussed later, albumen, glycoprotein, polysaccharides, and phospholipids. Mast cells may be the site of active metabolic processes since enzymes such as acid and alkaline phosphatases, lipase and cytochrome oxidase can be detected in homogenates of cell concentrates. Heparin, usually localised in the granules, is a polymer consisting of disaccharide units each containing glycuronic acid and an amino sugar, glucosamine. It resembles another naturally occurring polymer of great physiological interest, namely hyaluronic acid. Whereas the amino sugar in hyaluronic acid is acetylated, that in heparin is sulphated, and it is the sulphate moiety which confers on the heparin molecule its anticoagulant property. The more sulphate groups which are incorporated in the molecule, the greater is the anticoagulant activity and also the intensity of metachromasia. Natural

heparin is probably a mixture of the di- and tri-sulphuric acid esters of the polymer. Heparin monosulphate has been found in mast cells and is probably the agent responsible for the positive test given by the cells after treatment with periodic acid and Schiff's reagent. It may be that the mast cells secrete a heparin-like substance into the tissues where it is desulphated and then acetylated into hyaluronic acid. In this way, it is possible that the mast cells produce the precursors of the mucinous inter-fibrillary cement.

DISTRIBUTION OF TISSUE MAST CELLS

The distribution of tissue mast cells in normal and pathological tissues of various species has been described in detail by Michels[12] and more recently by Asboe-Hansen[6] and Arvy[13]. In general, mast cells are present in large numbers in the subcutaneous connective tissue, lung pleura, mesentery, scrotum, uterus and thymus of mammals. They occur predominantly in the loose tissue around small blood vessels of warm-blooded vertebrates but are virtually absent from the central nervous system, though many are present in the sheaths of peripheral nerves. Parenchymatous organs are poor in mast cells but many can be found in the connective tissue of their capsules. Noteworthy exceptions to this are the widespread distribution of tissue mast cells throughout the parenchyma of the liver of the dog, and in the lung of the cat and ox. The rabbit is exceptional among laboratory animals in having few tissue mast cells but many blood mast cells.

The skin and ears of most mammals are rich in tissue mast cells, but whereas most of the large densely-staining cells are located in the outer-most layers of the skin of many mammals, the reverse is true for the rat. In this latter species, the innermost layers of the skin contain the numerous large cells.

In such low forms of life as the sponges, starfishes, sea-urchins and molluscs, there are cells in the mesenchyme with basophilic metachromatic granules in their cytoplasm—by definition, mast cells. At the level of the crustaceans, mast cells can readily be demonstrated around the arterioles of crayfish. In the salmon, they are present in the gut-wall but not in the blood. In contrast, mast cells are common in the tissues and in the blood of reptiles and amphibia.

The mast cell content of the skin of the mouse can be increased by painting-on a carcinogenic hydrocarbon. Administration of oestrogens likewise may increase the mast cell population of tissue areas such as the fringe of the uterus in the female or the scrotum in the male. Such procedures also allow a study of the components of tissue mast cells. The common mast cell lesion in man is the skin disease of childhood, urticaria pigmentosa, in which focal collections of mast cells are found in the dermis, and may also occur in the liver and spleen[14]. Occasionally urticaria pigmentosa presents as a solitary tumour-like nodule, very rarely as a generalised mastocytoma. Such tumours are relatively common in dog where lymph nodes in the skin are usually involved. In chronic myeloid

leukaemia, blood mast cells are greatly increased in number but tissue mast cells are unaltered.

PHARMACOLOGY OF TISSUE MAST CELLS

Heparin

Swedish workers have long been interested in anticoagulants and credit goes to Jorpes and his colleagues for having traced the source of tissue heparin. For example, Holmgren and Wilander[15] analysed ox liver, an organ rich in both mast cells and sulphuric acid esters, and found a strong anticoagulant substance which gave a pronounced metachromatic staining reaction. Much less anticoagulant activity was found in sheep liver which is sparsely supplied with mast cells and contains only traces of sulphuric acid esters. The anticoagulant activity of ox lung was likewise greater than that of pig lung (see Table I). Since heparin appeared to be the only naturally occurring mucopolysaccharide with strong anticoagulant action, Jorpes determined the activity of such tissues in terms of a standard heparin prepared from dog liver.

TABLE I

HEPARIN CONTENT (I.U./G.) OF TISSUES OF DIFFERENT SPECIES COMPARED WITH RELATIVE MAST-CELL CONTENT AND HISTAMINE CONTENT (μG./G.)

Tissue	Species	Heparin*	Mast cells	Histamine
Liver	Sheep	10	+	11
	Ox	32	+ +	25
Lung	Pig	21	+ +	28
	Ox	34	+ + +	40

* Based on results of Holmgren and Wilander. [15]

The metachromatic reaction, once said to be specific for sulphated mucopolysaccharides, may occur with a number of acid polyelectrolytes which do not contain sulphate groups. Cartilage stains metachromatically but it has no anticoagulant activity and no mast cells. The theory that mast cells produce heparin[16] is based on the metachromasia of the granules, the fact that heparin may be extracted from the tissues rich in mast cells, and a proportionality between the mast cell count and heparin content of a tissue (Table II). Recently, mast cell granules have been isolated from mouse connective tissue and shown to possess powerful anticoagulant properties[17].

TABLE II

HEPARIN CONTENT (I.U./G.) OF TISSUES COMPARED WITH RELATIVE MAST-CELL CONTENT AND HISTAMINE CONTENT (μG./G.)

Tissue	Heparin	Mast cells	Histamine
Rat liver	0	0	1
Pig aorta	0	0	1
Ox-liver parenchyma	4	+	5
Ox aorta	4	+	10
Rat subcutaneous tissue	6	+ +	16
Ox inferior vena cava	11	+ +	20
Ox-liver capsule	54	+ + +	40

Heparin is released into the bloodstream of the dog in peptone shock, in anaphylactic shock, and in the shock which follows the injection of chemical histamine-liberators. The blood becomes incoagulable and crystalline heparin has been recovered from the blood. Lymph in the thoracic duct also contains much heparin derived from the liver mast cells which disrupt under such treatment. In other species, like the rabbit and the guinea pig, little or no anticoagulant activity can be demonstrated in the blood when similar shock is produced[18], and the same is true for the rat which possesses an enormous population of perivascular mast cells in its connective tissues. The reason for these species differences is not exactly known. In the rat, the released heparin may not be allowed to leave the tissue, since Riley, Shepherd, West and Stroud[19] were able to show that complete disruption of tissue mast cells (and loss of histamine) produces a loss of only 50 per cent of the heparin in the tissue. By histological methods these authors were able to show that some of the released metachromatic material had been disposed of locally by macrophages and fibroblasts[20], whilst some had adhered to adjacent connective tissue fibrils and cells. The results suggest that the function of heparin may be concerned rather with events in the tissues than with the coagulability of the circulating blood.

A significant function of heparin may be chylomicron dissolution. For example, when heparin is injected into dogs, the passage of neutral fat through the capillary walls is facilitated. Submucous and subserous layers of the digestive tract of mammals are generally well supplied with mast cells and these may be predominantly involved in fat metabolism and in the depositing of fat in the gut vessels. Constantinides[21] attempted to relate the species difference in mast cell numbers and susceptibility to experimental atherosclerosis. The rabbit with few tissue mast cells is particularly susceptible to atherosclerosis brought about by cholesterol feeding, while the rat with numerous mast cells is refractory.

Histamine

In their classical paper on chemical histamine liberators, MacIntosh and Paton[22] showed that histamine can be released from tissues by many basic substances such as diamines, diamidines, or diguanidines. Since all these substances bear at least some structural resemblance to histamine itself, the authors suggested that histamine is displaced by these substances from its normal location in the tissues. It is now known that alkali or even tap water are also good histamine releasers, and it is probable that histamine is held, preformed in a loose complex in the granules of mast cells, and it is necessary to disturb only one component of the complex to permit the histamine to escape.

Histamine most probably is derived from the amino acid histidine by simple decarboxylation. A study of the distribution of histidine decarboxylase shows that the enzyme may be concentrated in the tissue mast cells. Radioactive histidine, for example, is decarboxylated to form radioactive histamine, and this is concentrated in the regions of the mast cells. On the other hand, radioactive histamine is not taken up by mast cells[23].

Histamine is generally recognised as a strong stimulant of the parietal cells of the gastric mucosa and of the smooth muscle in the gut wall, yet in certain species it is difficult to identify more than a few mast cells in this region. Histamine is also a strong vasodilator substance capable of increasing the permeability of capillary walls and of causing stasis in these vessels, and mast cells reside in these locations.

Rocha e Silva[4] was one of the first to show that a simultaneous release of heparin and histamine occurs from the liver of the dog after anaphylactic shock, and it thus seemed logical that both substances may originate from the same cell. To test this possibility, certain chemical substances capable of releasing histamine were first studied. When a rat was killed quickly by an intravenous dose of a fluorescent liberator, the fluorescence was at first sharply localised to the mast cells, especially those within the loose

TABLE III

THE EFFECT OF HISTAMINE LIBERATORS ON THE HISTAMINE CONTENT (μG./G.) AND RELATIVE MAST-CELL CONTENT OF SOME RAT TISSUES

Tissue	Control		Stilbamidine		Tubocurarine	
	Histamine	Mast cells	Histamine	Mast cells	Histamine	Mast cells
Omentum	20	+ +	10	0 to +	2	0
Subcutaneous tissues	19	+ +	10	0 to+	3	0
Mesentery	8	+	6	0 to +	4	+

TABLE IV

COMPARISON OF THE MAST-CELL COUNT (PER H.P. FIELD) AND HISTAMINE CONTENT (μG./G.) IN THE SKIN OF VARIOUS SPECIES

	Guinea pig	Rabbit	Man	Dog	Cat	Rat	Mouse	Hamster
Mast cells	3	2	7	40	26	30	40	50
Histamine	2	2	7	8	14	22	38	54

TABLE V

COMPARISON OF THE RELATIVE MAST-CELL CONTENT AND HISTAMINE CONTENT (μG./G.) OF SOME TISSUES OF RAT AND HAMSTER

Tissue	Rat		Hamster	
	Mast cells	Histamine	Mast cells	Histamine
Ears	+ + +	58	+ + + +	112
Bristle region	+ + +	62	+ + + +	94
Abdominal skin	+ +	35	+ + +	54
Cheek pouch or area ..	+ +	43	+ +	24
Feet skin	+ +	40	+	12
Stomach	+	30	+	19
Spleen	0	2	+	13

tissues of the peritoneum[24]. Thereafter the mast cells broke up and the histamine content of the tissue fell[5]. Other histamine liberators such as tubocurarine produced greater disruption of mast cells, and a greater release of histamine (Table III). Subsequent work showed that, as with heparin, there is a good correlation of the histamine content of a particular tissue and the number of mast cells which it contains. For example, the

abdominal skin of the hamster contains much histamine and many mast cells whereas that of the guinea pig or rabbit is deficient in both (Table IV). Even within one particular species there is an association between the numbers of mast cells of various areas of skin and the corresponding histamine content (Table V). As mast cells form and increase in number so the histamine content of that tissue rises (Table VI). Foetal tissues, for example, contain few mast cells and little histamine whereas similar tissues in the adult contain many cells and much histamine. Graham and her colleagues[25] obtained similar results with a chemical, instead of a biological, method of assay for histamine.

TABLE VI

HISTAMINE CONTENT (μG./G.) AND RELATIVE MAST-CELL CONTENT OF SOME TISSUES OF YOUNG AND ADULT ANIMALS

Tissue	Species	Young		Adult	
		Histamine	Mast cells	Histamine	Mast cells
Skin	Cat	2	0	20	+ +
Lung	Cow	7	+	25	+ +
Lung	Man	6	+	25	+ +
Liver	Cow	2	0	25	+ +

TABLE VII

HISTAMINE CONTENT (μG./G.) OF SEVERAL MAST-CELL TUMOURS IN DOGS COMPARED WITH THEIR HEPARIN CONTENT (I.U./G.)

Dog	Age (year)	Sex	Site	Histamine (control 9)	Heparin (control 11)
Terrier	12	M	Flank	179	211
Boxer	5	M	Flank	295	110
Labrador	12	M	Thigh	550	174
Labrador	10	F	Leg	700	130
Terrier	10	M	Flank	765	61

When pathological tissues rich in mast cells were examined, they were found to be rich in heparin as well as in histamine[26]. In Table VII are recorded some values for dog mast-cell tumours which generally are localised to skin sites. These were obtained at post-mortem examination, usually within a few minutes of the death of the animal. On occasions, the primary tumour has been removed and the animal allowed to recover. But within two months, it has recurred at another site and then the animal has been killed. Such examples provide additional data on the relationship between histamine, heparin and mast cells. It will be seen from Table VIII that there is a wider variation in the heparin values than in the histamine values, and tumours containing weakly-staining mast cells usually contain less material with anticoagulant properties than those with mast cells which stain intensely with toluidine blue. If metastases are found in other areas, these too may be filled with mast cells. Four such instances are shown in Table IX. Lymph nodes are usually involved but not the kidneys. In such instances, one can predict from the histamine values alone where metastases have occurred, and these can be confirmed later by histological examination.

In man, the lesions of urticaria pigmentosa have been shown to contain much more histamine and heparin than the adjacent skin. Light stroking of one of these lesions rapidly produces a reactive weal limited to the lesion itself, most probably as a result of the released histamine. Mast cells are known to be increased in such examples of urticaria pigmentosa.

TABLE VIII

HISTAMINE CONTENT (μG./G.) OF MAST-CELL TUMOURS IN DOGS COMPARED WITH THE HEPARIN CONTENT (I.U./G.)

Recurrences occurred about 2 months after each primary tumour was removed

Dog				Primary		Recurrence	
				Histamine	Heparin	Histamine	Heparin
Terrier..	..	..	..	500	147	790	54
Spaniel	..	..	..	520	110	650	357
Scottie	..	..	..	661	21	277	21
Terrier..	..	..	..	1290	434	629	590

TABLE IX

HISTAMINE CONTENT (μG./G.) OF MAST-CELL TUMOURS AND OTHER TISSUES FROM VARIOUS DOGS COMPARED WITH THEIR HEPARIN CONTENT (I.U./G.)

Tissue	Control		Spaniel		Terrier		Scottie		Mastiff	
	Hista-mine	Heparin	Hista-mine	Heparin	Hista-mine	Heparin	Hista-mine	Heparin	Hista-mine	Heparin
Skin tumour..	—	—	650	357	629	590	277	21	219	368
Lymph node	4	9	600	293	600	290	139	24	234	78
Spleen ..	5	7	70	80	194	312	6	5	5	8
Liver	25	20	60	50	40	62	22	21	24	34
Lung	25	13	30	29	140	40	24	19	30	10
Normal skin..	9	11	10	8	12	11	8	8	44	3
Kidney ..	1	6	1	5	1	4	1	9	1	4

Present evidence suggests that histamine is located in the mast cell granule. West[27] assayed mouse mast cell granules, obtained by Köksal's method, and found more histamine in the granules than in any other fraction. Similar results have been obtained for other species. From the results of their experiments using skin preparations, Riley and West[28] are convinced that the bulk of the extractable histamine is located in the tissue mast cells. Simple dissection of the components of skin and ears of several species show a similar regional distribution for mast cells and heparin on the one hand, and histamine on the other.

Since these two substances carry opposite charges, it might be assumed that they co-exist in the granules in the form of a salt—the heparinate of histamine. But this simple explanation is not entirely satisfactory. For example, Sanyal and West[29] obtained a complex formation between heparin and histamine in which the proportions of each substance (about 20 times as much heparin as histamine) were similar to those found in extracts of tissues rich in mast cells. The staining properties and chromatographic behaviour of the complex resembled those of the naturally-occurring mast cell granules, but the histamine was not releasable by the powerful chemical histamine liberators. Further work to elucidate the linkages involved is thus necessary.

The release of histamine can occur with little or no visible alteration to the mast cells. For example, West[30] has reported up to a 50 per cent release in the rat after injections of weak histamine liberators with only traces of degranulation of the cells. This reversible change most probably portrays the physiological release of histamine. Higher concentrations of powerful histamine liberators which include water[31] cause irreversible damage to the mast cells, followed in the recovery phase, by the appearance of new cells from the connective tissue precursors in the adventitia of small blood vessels, the milk spots of the omentum, and the septa of fat cells[32]. Such histamine liberators release large amounts of histamine from most tissues, the chief exception being the gut[33].

This is the story in the rat but in other species the release of much histamine may be achieved only with difficulty. Maximally tolerated doses of most histamine liberators release only about 50 per cent of the tissue histamine in the hamster and mouse and correspondingly tissue mast cells degranulate but do not disrupt[34,35]. Such a result is illustrated in Figure 1 where the effect of histamine liberators on tissue mast cells in the hamster and rat are compared; it will be seen that the mast cells in the hamster are resistant to the action of the liberators whilst those in the rat completely disrupt and disappear. In a similar way, most of the liberators do not affect tissue mast cells in the guinea pig, though anaphylactic shock in this species is usually accompanied by mast cell disruption.

The histamine-releasing power of the antibiotic, polymyxin B, has been described by Bushby and Green[36]. Although it releases maximal amounts of histamine from many tissues of the rat, accompanied by complete disruption of the mast cells, it fails to release much 5-hydroxytryptamine from similar areas[37]. Reserpine treatment on the other hand releases 5-HT but has little effect on the tissue histamine or mast cells. Bhattacharya and Lewis[38] were the first workers to show that the histamine liberator, compound 48/80, also releases 5-HT from perfused tissues of the rat and subsequent work proved that this release can occur in the whole animal. There seems clear evidence, however, that the release of one amine is not dependent upon the release of the other and most probably the major amount of each amine does not originate from the same mast cell.

Hyaluronic Acid

Hyaluronic acid is a high polymer composed of equimolecular quantities of acetyl glucosamine and glycuronic acid residues joined by beta-glycosidic links. As stated earlier, its chemical structure differs from that of heparin in being sulphate-free, in acetylation of the amino sugar, and in the type of glycosidic linkage. It is one of the best characterised of the extracellular substances of the connective tissue, being a component of the ground substance which partly supports capillary walls and acts as a barrier to the free passage of materials to and from the capillaries, tissue cells and environment.

In 1954, Asboe-Hansen[6] reported that a parallelism exists between the mast cell content and the hyaluronic acid content of many normal and
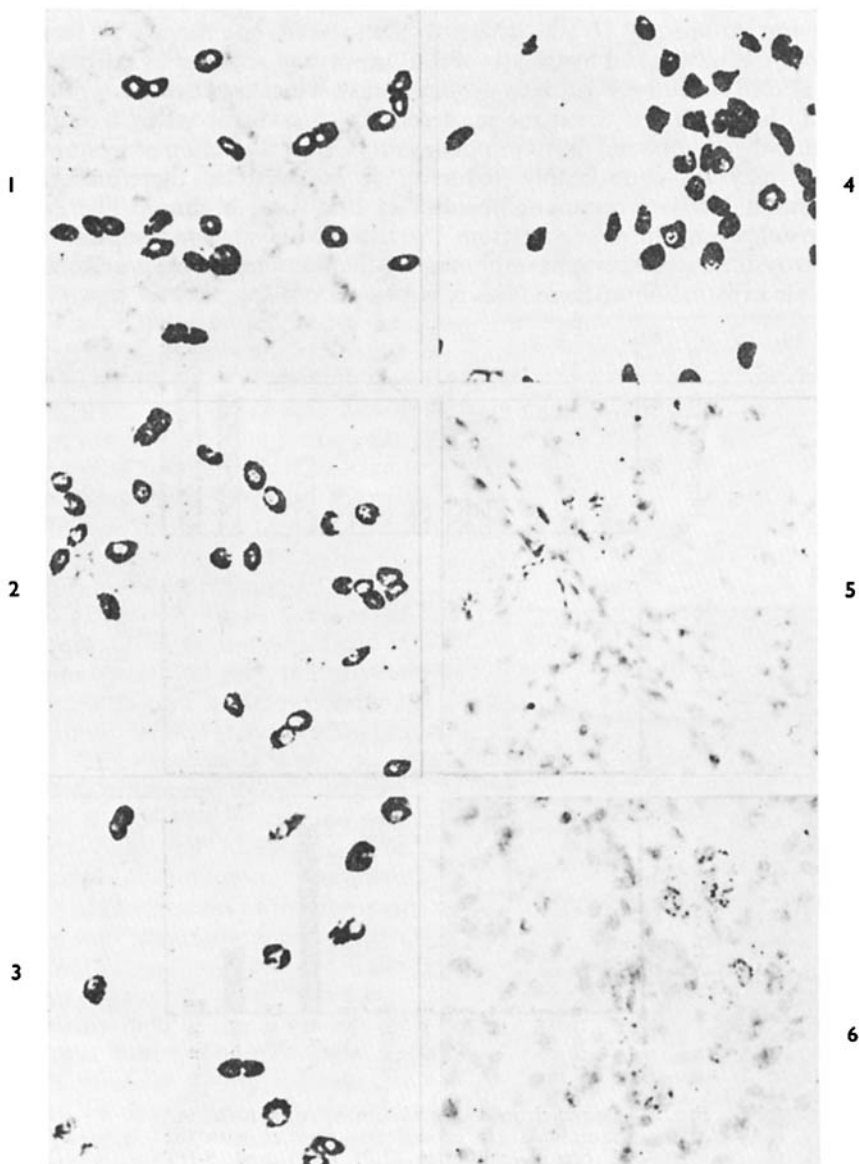
FIG. 1. Mast cells in the subcutaneous connective tissue of the hamster (1 — 3) and of the rat (4 — 6): toluidine blue, × 205; (1) and (4), normal animals: (2) and (5), after treatment with doses of compound 48/80: (3) and (6) after treatment with polymyxin B. Note that the tissue mast cells in the hamster are resistant to the action of the histamine liberators, whilst those in the rat are not.

pathological tissues. He has in fact proposed that mast cells under varying hormonal influences secrete hyaluronic acid, perhaps by way of a heparin precursor. Hyaluronidase is said to be the specific enzyme for the depolymerisation and hydrolysis of hyaluronic acid. When hyaluronidase is injected subcutaneously into the feet of rats, a localised oedema develops in an hour. But if the tissue mast cells are first disrupted by treatment with either compound 48/80 or polymyxin B, then the action of hyaluronidase may be considerably reduced. It is possible, therefore, that hyaluronidase is a histamine liberator or that some histamine liberators also release hyaluronic acid from the tissues. Hyaluronidase does not destroy the metachromasia exhibited by the granules of mast cells, so a simple explanation of these facts is not as yet possible.
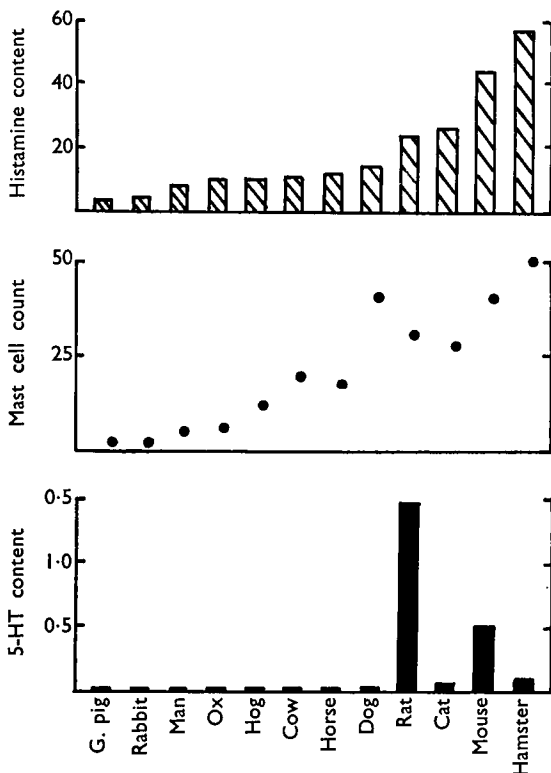

FIG. 2. Comparison of the histamine content (μg./g.) of the abdominal skin of different species with the mast cell count (cells per H.P. field) and 5-HT content (μg./g.)

5-Hydroxytryptamine (5-HT)

This amine is formed from the amino acid, tryptophan, which after hydroxylation is decarboxylated. The distribution of 5-hydroxytryptophan decarboxylase has been well studied and results suggest that the enzyme is not concentrated in mast cell-rich tissues. However, Benditt

and others[39] have reported the isolation of 5-HT from peritoneal washings in the rat; such washings are rich in mast cells and histamine. Extensive studies by Parratt and West[40] indicated that 5-HT is not concentrated in tissue mast cells of the guinea pig, rabbit, man, ox, hog, cow, horse, dog cat or hamster, though it may be associated with mast cells in the rat or mouse (Fig. 2). It is only in the rat and mouse that much 5-HT is contained in the skin. Later work[41] showed that, in 10 out of 17 specified regions of rat skin, there was a relation between the 5-HT and histamine contents and the mast cell population, but in the other 7 regions there was relatively much less 5-HT (Table X). In fact, it is easy to release 5-HT from rat tissues leaving mast cells and tissue histamine little affected, and conversely easy to release histamine from rat tissues and disrupt mast cells leaving tissue 5-HT almost unchanged[37]. The effect on the tissue amines is illustrated in Figure 3. Thus it seems most unlikely that tissue mast cells even in the rat contain any considerable quantity of 5-HT.

In other species, it is also possible to obtain results opposing the view that 5-HT is associated with mast cells and histamine. For example, pathological specimens of mast cell lesions of the dog, cat, cow and man have failed to give a 5-HT reaction when the tissue histamine content has been exceptionally high[42]. In Table XI, the results of assays on two dog mastcell tumours clearly indicate this lack of association between mast cells and 5-HT, and the same is true for the cat (Table XII). It is of particular interest that the

FIG. 3. Changes in the 5-HT ($\bigcirc$--- $\bigcirc$) and histamine ($\bullet$—$\bullet$) contents of the abdominal skin of the rat after injections of (A) 5 doses of polymyxin B, (B) 7 doses of compound 48/80, (C) a single dose of reserpine (at the arrows). All values are expressed as percentages of the control levels before injection. Note the effects of polymyxin are almost the reverse of that of reserpine.

histamine content of the spleen of the cat exceeded 1 mg./g. of fresh tissue and the mast cell count was correspondingly high. In this species, it is possible that the primary tumour originates in the spleen, and not in the skin as in the dog. In guinea pigs, tissue mast cells are partly disrupted during the anaphylactic reaction and histamine is found in the
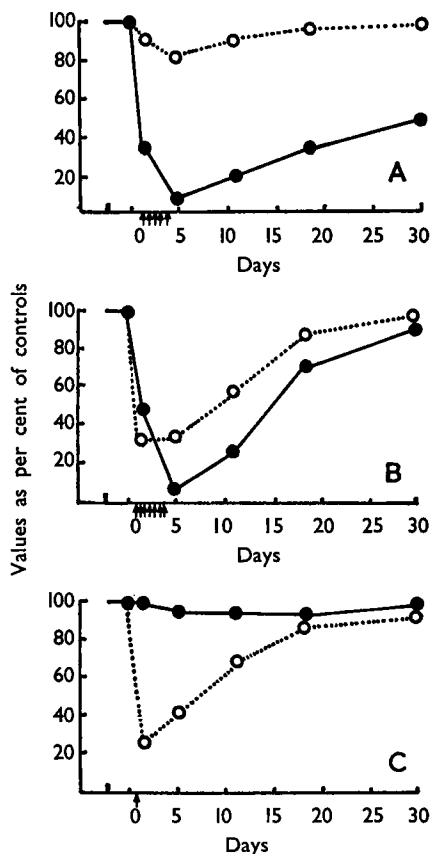
TABLE X

HISTAMINE CONTENT (μG./G.) OF VARIOUS REGIONS OF SKIN OF THE RAT COMPARED WITH THE RELATIVE MAST-CELL CONTENT AND THE 5-HT CONTENT (μG./G.)

Region	Mast cells	Histamine	5-HT	Ratio Histamine/5-HT
Lips and nose	++++	72	4·36	17
Submental	+++	54	2·60	21
Throat	+'+	48	4·20	11
Eyelids	+++	48	2·20	22
Cheek	++	43	2·20	19
Ear base	++	41	3·20	13
Groin	++	38	1·80	21
Nipples	++	33	2·47	13
Abdomen	++	30	2·60	12
Anus	++	24	2·10	11
Scrotum	+++	63	1·40	45
Bristle area	+++	62	2·00	31
Hindlegs, dorsal skin ..	+++	52	1·31	40
Forelegs, dorsal skin ..	+++	48	1·32	36
Ear margin	+++	48	1·40	34
Tail margin	+	19	0·43	44
Tail base	+	19	0·36	53

TABLE XI

MAST-CELL COUNTS (PER H.P. FIELD) OF SOME TISSUES OF DOGS WITH MAST-CELL TUMOURS COMPARED WITH THE HISTAMINE AND 5-HT CONTENTS (μG./G.)

Tissue	11 year Spaniel ♀			6 year Spaniel ♂			Control dog		
	Mast cells	Hista-mine	5-HT	Mast cells	Hista-mine	5-HT	Mast cells	Hista-mine	5-HT
Inguinal skin tumour	201	678	0·03	112	800	0·03	5	10	0·03
Lymph node ..	223	788	0·03	58	200	0·03	2	4	0·03
Spleen	141	495	1·00	27	150	1·16	2	7	2·30
Liver	38	245	0·08	14	100	0·22	10	24	0·27

TABLE XII

MAST-CELL COUNTS (PER H.P. FIELD) OF TISSUES OF A CAT WITH A MAST-CELL TUMOUR COMPARED WITH THE HISTAMINE AND 5-HT CONTENTS (μG./G.)

Tissue	3 year castrated ♂			Control cat		
	Mast cells	Histamine	5-HT	Mast cells	Histamine	5-HT
Inguinal skin tumour ..	42	80	0·03	4	24	0·08
Lymph node	33	285	0·03	2	11	0·06
Spleen	81	1230	1·20	1	2	4·25
Liver	56	14	0·08	8	1	0·28

TABLE XIII

COMPARISON OF THE EFFECTS OF ANAPHYLAXIS AND HISTAMINE LIBERATORS IN VARIOUS SPECIES. ACTIONS RECORDED ARE THE DISRUPTION OF TISSUE MAST-CELLS, AND THE RELEASE OF HISTAMINE OR OF 5-HT FROM THE TARGET ORGANS

Species	Anaphylaxis			Histamine liberators		
	Mast cells	Histamine	5-HT	Mast cells	Histamine	5-HT
Dog	Disruption	Release	0	Disruption	Release	Slight
Guinea pig	Disruption	Release	Slight	Slight	Slight	0
Rat	Slight	Slight	Slight	Disruption	Release	Release
Mouse	Slight	Slight	Slight	Slight	Slight	Slight
Hamster	0	0	0	Slight	Slight	Slight
Rabbit	Slight	Release	Release	Slight	Release	Release

blood, but only traces of 5-HT are released[43]. In dogs, tissue mast cells are partly disrupted during anaphylaxis and histamine is released (with heparin) from the liver into the thoracic duct and hepatic vein, but 5-HT release is not seen[44] despite the fact that a relatively high 5-HT amount is known to be present in the liver, the target organ in that species. Histamine liberators likewise can release histamine from dog liver and disrupt mast cells without affecting the tissue 5-HT levels. Some of these results are tabulated in Table XIII and illustrate how mast cells in different species react in different ways to different agents or procedures.

5-HT is known to be present in the blood platelets, the spleen, and cells of the enterochromaffin system (particularly in the gut). It is thought to be released (along with histamine) from the platelets during injury. It generally exerts a vasoconstrictor action on arterioles and also an effect on capillary permeability. Two of the consistent symptoms of human argentaffinoma (tumours of the enterochromaffin tissue) are diarrhoea and oedema with flushing attacks, and it is now certain that 5-HT stimulates intestinal movement in most species and may be a local hormone within the gut wall. 5-HT is a potent stimulator of peristalsis in guinea pigs, if placed inside the lumen of the gut. It does not appear to be associated in this region with tissue mast cells but with argentaffin cells, and when argentaffin cells are scarce, as in the rat, there are generally argyrophil cells, which stain with silver salts but not with chromate.

When dextran or eggwhite is injected into rats, oedema of the extremities develops on the first injection. This anaphylactoid reaction is the result of a release of 5-HT (with some histamine) from such areas of skin. The reaction is prevented by depletion of skin 5-HT by previous treatment with reserpine, but it is not affected by depletion of only histamine[45]. The skin of other species does not contain 5-HT and injections of dextran or eggwhite do not produce local oedema on the first injection[46].

DIFFICULTIES CONCERNING THE RELATION OF TISSUE
AMINES TO TISSUE MAST CELLS

Evidence is now so convincing that heparin and the bulk of the histamine in a tissue are normally contained in the mast cells that interest turns to possible exceptions to the rule. One such unusual location for histamine is in the mucosa of the pyloric portion of hog stomach. Here some cell other than the mast cell must be binding the histamine since mast cells are scarce in the inner layers of the mucosa where the histamine content is high. An obvious choice for the binding component is the mucin of the mucous cells, lining the lower two-thirds of the pyloric glands[47]. Indeed, gastric mucin can be shown in vitro to bind histamine. Like the granular material of the mast cells, this mucin also stains metachromatically with toluidine blue. Metachromasia with toluidine blue, however, is not in itself an indication of the ability of the tissue to bind histamine, since cartilage, for example, binds very little histamine. There is little evidence, as yet, to suggest a functional relation between mast cells and the gastric mucin.

In the rat intestine, a similar difficulty arises. Even those mast cells which normally reside in this region are more resistant to the action of the now traditional histamine liberators than are, say, mast cells in the skin. Tissue mast cells in other species may be likewise resistant to the damaging action of these liberators. It seems likely that an extensive investigation into the chemical structure and properties of the heparin of different laboratory species might provide some clue to this problem.

Eosinophils for long were thought to be the chief source of histamine in the body[48]. Recently the relationship of eosinophils to histamine has been found to be less close. Thus the highest amounts of histamine have been found in tissues when the predominant cell has been the mast cell, and there is proportionately less histamine when eosinophils appear in increasing numbers. This occasional association of eosinophils with mast cells may itself indicate the true function of the eosinophils in the histamine problem. In general, eosinophils are found in mast cell lesions when the mast cells are in the process of disruption. For example, eosinophils appear in lesions of urticaria pigmentosa in man when these have been subjected to mechanical irritation and they can be found in mast cell tumours when dogs have been treated with histamine liberators. Further, there is apparently developed in the lungs of anaphylactically shocked guinea pigs some factor that draws eosinophils to the site[49]. This factor is certainly not 5-HT since guinea pig lung contains only traces of this amine, but it might be histamine. It is therefore of particular interest that the isolated granules of eosinophils have been shown to possess anti-histamine properties[50]. Perhaps the eosinophil is concerned more with detoxification and disposal of histamine than with its elaboration.

Objections to the theory that mast cells secrete hyaluronic acid are based on the facts that hyaluronidase does not destroy the metachromasia of mast cell granules and that there is a poor correlation of the mast cell counts and the hyaluronic acid content of certain tissues. Thus mast cell tumours and organ capsules exceptionally rich in mast cells contain little ground substance and conversely embryonic tissue rich in ground substance has few mast cells.

Mention has been made already of the fact that no parallelism exists in many species between the mast cell count and 5-HT content of the skin and other tissues. Even within one species such as the rat, 5-HT may be concentrated in areas where mast cells are few in number. Treatment with drugs (like polymyxin B and reserpine) likewise illustrates how disruption of mast cells and 5-HT release may be independent of each other. Feldberg and Smith[51] have already shown that large doses of 5-HT can release histamine from rat tissues and damage mast cells.

In a study of the physiological phenomena influencing the mast cell population in rats, Parratt and West[40] found that the histamine content of the skin is considerably raised at birth yet there is no corresponding increase in mast cell numbers. At weaning time, both the histamine and 5-HT concentrations in the skin are doubled (Fig. 4) but again the mast cell population is unaltered. Similarly during lactation, the histamine and 5-HT contents of certain tissues are raised with no increase in mast cells.

When thyroxine is administered daily to rats for 14 days or more, the skin 5-HT level is raised but this increase is associated with only a slight increase in the histamine content and mast cell population. Thyroxine-treated animals are very sensitive to histamine liberators (for example, dextran), but even the lethal action of these liberators is associated only with slight swelling and degranulation of the mast cells. No adequate explanation of these results is at once apparent.
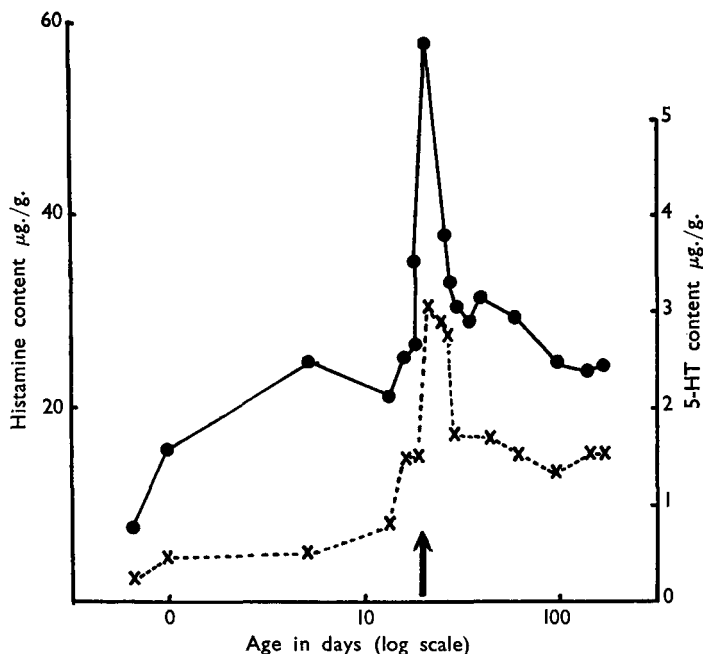

FIG. 4.   Influence of age on the histamine content (●——● μg./g.) and 5-HT content (×---× μg./g.) of the abdominal skin of the rat. Note the abrupt increase in both amines at weaning time (indicated by the arrow), although the number of tissue mast cells is not increased at this time period.

BIOLOGICAL SIGNIFICANCE OF TISSUE MAST CELLS

The tremendous number of mast cells especially along blood vessels is sufficient cytological evidence to credit an important role to these cells. From Ehrlich's time onwards, histologists have suggested that the function of the mast cell is concerned in some way with connective tissue and particularly with the formation of its fibrils. Formation of the first mast cells in the embryo is preceded by a general metachromatism of the tissues, probably produced by the connective tissues themselves. Thereafter the tissue mast cells undergo cyclic changes, disappearing in areas of acute tissue injury and reappearing when connective tissue fibrils begin to be laid down and the ground substance shrinks. This behaviour on the part of the mast cell suggests that they alternately store and release substances, one

of which may be the mucopolysaccharide of the ground substance. Heparin, which may be the sulphated precursor of hyaluronic acid, has been used by Morrione[52] to precipitate fibrils from sollubilised collagen.

The activation of the reticulo-endothelial system, which follows the release of amines from mast cells and which is manifest by an increased phagocytic capacity of the endothelial cells, is only part of a more wide-spread mobilisation of the entire loose mesenchyme. This mobilisation begins around the small blood vessels and extends away from them into the tissues. The changes may thus be due to a flooding of the tissues with protein-rich oedema fluid, formed as a result of the increased vascular permeability[53]. The general appearance of these active cells closely resembles that seen in the inflammatory process.

Mast cell production appears to be under some hormonal control though the precise mechanism has yet to be elucidated. Pituitary thyrotropin is said to increase their numbers whereas ACTH and cortisone generally produce a decrease. Different dosages and routes of administration may account for the apparently contradictory effects obtained by certain workers. Oestrogens have been shown by Arvy[54] to increase the numbers of tissue mast cells particularly in the thymus, gut and sub-cutaneous connective tissue of mice. Although the number of mast cells increases in hypothyroidism and becomes normal in hypothyroid subjects treated with thyroxine, the true role of the thyroid gland in the control of mast cells and in histamine metabolism in particular is not clear. Early experiments showed that removal of the thyroid protected guinea pigs against the effects of anaphylactic shock whereas large doses of thyroxine had the opposite effect. Thyroid hormone, however, markedly influences the sensitivity of tissues of the rat both to extrinsic histamine and to released histamine whether liberated by primary release (compound 48/80, polymyxin B, eggwhite or dextran) or by anaphylaxis. Thyroid does not increase the amine release but appears to decrease the ability of the animal to destroy the released amine. Dysfunction of the thyroid gland may ameliorate certain allergic reactions in man and it would be interesting to know how far these changes are due to an increase in the sensitivity of the tissues.

Mast cells appear to be involved in physiological processes by the formation and release of certain agents. The presence of heparin in these cells is now well established but its release may not be their primary function. Mast cells also release histamine and possibly 5-HT—two substances which increase capillary permeability. The function of these two amines might therefore be inter-related. But when a large number of species are examined, there appears to be no relation, except in the rat and mouse, between the histamine level and mast cell content on the one hand and the 5-HT level on the other. The rat and the mouse have some 5-HT in areas rich in mast cells and it is possible therefore that in these two species 5-HT may act as a defence agent and perhaps take over part of the function of histamine. In periods of stress, for example at weaning, lactation, and in cold months, the levels of 5-HT are raised and so also are those of histamine. It is significant perhaps that similar changes in

the histamine level occur in other species when the skin is subjected to injury.

At least one action of 5-HT in the skin of the rat is the marked effect of increasing capillary permeability and in this species it is much more active than histamine[55].   Of the species studied so far by Sparrow and Wilhelm[56], only in the rat is 5-HT more active than histamine in increasing capillary permeability, and only in the rat (and to a minor extent, in the mouse) is 5-HT present in the skin in substantial amounts.   It is unlikely that these two factors are coincidental.   Neither can it be by chance that the only species developing the full anaphylactoid reaction is the one which contains much 5-HT in its skin.   It may be that this ability of 5-HT to increase capillary permeability in minute doses is related to a defence function.

When histamine liberators are given to rats, oedema develops in the extremities and capillary changes occur leading to accumulation of the circulating colloidal dye.   These changes show a characteristic distribution and occur in skin regions such as those of the feet and face.   These regions are generally rich in histamine but there are at least three other areas which do not exhibit the full phenomena and yet have much histamine.   These areas are the ear margin, the dorsal skin of the foot, and the scrotum.   They contain relatively less 5-HT than do other areas with similar histamine concentrations and this fact alone may account for the lack of blueing of such regions, as manifested by oedema and exudation of circulating dye.

It was noted earlier that rat and mouse mast cells may contain a part of the tissue 5-HT.   One of the reasons why the cells of these two species bind 5-HT as well as histamine is probably to be found in the type of heparin, and a biochemical study of this problem is urgently needed. Rat mast cells appear to contain slight 5-hydroxytryptophan decarboxylase activity as well as histidine decarboxylase activity so that both amines could be ready-made within the cell.   This result explains why the tissue amines recover their normal levels only slowly after depletion by chemical liberators.   If the intact mast cell is the only prerequisite for amine formation, then after a few weeks when the mast cell population is normal again the amines will be manufactured.   Recovery of the levels of the tissue amines is dependent not only on the binding by heparin of exogenous amines but also on the presence of active decarboxylases.

Lastly, the question arises why human mast cells do not concentrate 5-HT.   It may be too toxic for the sensory organs in the skin for it to remain there.   It is known that the 5-HT concentration in wasp venom exceeds the pain-producing threshold concentration of 5-HT when applied to human skin as at the base of a blister, and a similar comment may be made about the stings of nettles[42].

The function of histamine and 5-HT in the tissues remains an enigma, and any attack on this problem is bound to be an indirect one.   Already the new tools are available to enable each amine to be released separately. Many facts suggest that the function of histamine is concerned with the defence of the body, since its release is followed by a temporary mobilisation of the loose mesenchyme.   It is remarkable that in every species

studied (except the rat) histamine is concentrated in the outer layers of the skin—cell layers which come into contact with the outside world. In the rat, 5-HT is concentrated in these outer layers of the skin and probably takes over part of the defence function of histamine in this species.

CONCLUSIONS

Although mast cells have been the subject of numerous publications since they were first clearly described by Ehrlich some 80 years ago, it is only recently that they have come to interest the pharmacologist. Modern work in Scandinavia showed one functional activity of mast cells in producing heparin, and this seemed to provide an obvious and teleo-logically satisfactory explanation of the perivascular location of these cells. Mast cells are perivascular in location because they produce heparin which they pour into the blood-stream. Yet it was a study of this very relation of mast cells to blood vessels which led to the discovery in this country of a completely different function of the cells, that of pro-ducing histamine. This was achieved using simple extraction and assay procedures, specific histamine antagonists to check the specificity of the responses, and various chemical substances which selectively disrupt mast cells and release histamine from tissues. These histamine liberators have not only paved the way for locating the site of storage of histamine but they have also enabled experiments to be carried out on animals whose tissues have been made deficient in histamine. In this way, a fresh attack has been made on the function of mast cells, so that they now appear to be less concerned with blood clotting (as was at first thought) than with the maintenance and repair of the connective tissues. Besides, in certain regions of the body mast cells may also contain 5-hydroxytryptamine, a substance which like histamine increases capillary permeability. Whatever other materials remain to be discovered in mast cells, it is certain that they are the site of active metabolic processes, since several enzymes can be detected in homogenates of cell concentrates.

Tissue mast cells seem to undergo cyclic changes, disappearing in areas of acute tissue injury and reappearing when connective tissue fibrils begin to be laid down. This behaviour suggests that mast cells alternately form, store and release certain substances. When such a release occurs, the tissues are flooded with a protein-rich oedema fluid, formed as a result of the increased vascular permeability by histamine and possibly by 5-HT also. This oedema fluid not only assists in the removal of foreign materials but also provides the tissues with some of the materials ready for the job of repair. Thus I believe that mast cells act as part of the defence mechanism of the body whereby they react quickly to any kind of cell injury. However, this cannot be the full story as animals whose mast cells have been disrupted by potent histamine liberators show few abnormal reactions when subjected to stress or injury. More work along this line is clearly indicated.

Finally, it seems that the tissue mast cells and the amines they produce or store are under hormonal influence. Over-activity of some of the

endocrine glands not only increases the mast cell population and the hista-mine and 5-HT contents of particular tissues but it also renders the animals hypersensitive to various agents, such as egg white, dextran, histamine liberators, adrenaline and particular antibiotics. The reaction in such hypersensitive animals provides the opportunity to evaluate experimentally the possible mechanism of particular allergies in man and further work in this direction is now taking place. The new association between hista-mine (and possibly 5-HT) and the heparin-containing mast cells of the tissues should become increasingly important in the future in assigning the various functional roles these substances play in a range of physio-logical and pathological reactions.

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