1960

Review of recent studies of the mast cell

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A REVIEW OF RECENT STUDIES OF THE MAST CELLS

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

March 30, 1960

Omaha, Nebraska

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I. INTRODUCTION

The mast cell was discovered by Paul Ehrlich in 1877. Since the cells were large and filled with granules and are more numerous in healthy connective tissue, he gave them the name "Mastzellen", which means well-fed cell or fodder cell, or cells concerned with fattening or nutrition. In English, the name has no functional implication and seems somewhat inappropriate. Ehrlich so named the cells because he thought they were involved in nutrition of the connective tissue.

Since its discovery the mast cell has been studied quite extensively by histological techniques. Its most outstanding characteristic was it affinity for basic dyes, and of these, toluidine blue seems to have been the one used most for staining the mast cell. The early studies apparently were pursued more as a pure histological science than for any practical reason. Results were quite variable. Present day investigators explain this on the basis on variations in technique, for as Ehrlich pointed out, the mast cell granules are soluble in water, and if the tissue bearing mast cells are left in the water too long, the mast cell morphology and content is changed.

The result of the histological studies was to firmly establish the mast cell as a distinct entity, to describe it

adequately as a large irregular cell filled with granules which stain metachromatically (i.e. alter the color of the dye during the staining process) with basic dyes, and with a nucleus which was often invisible by this technique. The location of the mast cell was verified as occurring mainly in the loose connective tissue of the body such as the adventitia of large vessels, about capillaries, and in the serous membranes covering the various organs. The studies halted there for a number of years.

The discovery of the anticoagulant, heparin, in the liver of the dog was ultimately shown to be precipitated by and to stain metachromatically with toluidine blue. This lead to a search for heparin in the tissues by finding the location of the metachromatically staining material there. Since mast cells are numerous in the dog's liver and since the mast cell granules stain metachromatically with toluidine blue, attention was turned to the mast cell again. Further studies lead to the conclusion that mast cells contain heparin and that they release the heparin into the blood when a dog is injected with peptone causing peptone shock. Following confirmation of this finding, interest in the mast cell began to wane, and from the late 1930's to the late 1940's it was the subject of few research projects.

The third wave of interest in the mast cell began when

it was noted that mast cells increase markedly in the skin of mice before the development of cancer as a result of the application of tar and its derivatives, and when Riley and his colleagues in Scotland postulated that mast cells might be the source of tissue histamine. One factor leading to Riley's postulation was the similarity of the triple response of the skin to the urtication which develops when lesions of urticaria pigmentosa are irritated.

Most of the work regarding the mast cell as a possible source of histamine, and a participant in acute inflammatory reactions has been done since 1950. The purpose of this thesis is to review a broad sample of the information which has been gleaned from these studies, and to draw from it some conclusions relating to mast cell physiology and function in the human.

II. METHODS OF STUDY

Histological

The histological methods were used to study the mast cell first. The fixing and staining techniques are quite important because aqueous solutions such as formaldehyde have the ability to alter mast cell morphology and lead to striking differences in the results of such studies. If tissue is improperly handled, the mast cells can be de-granulated and the granules dissolved so that counts of

mast cells in such tissue may be grossly in error. It has been well established that the fixatives with a low water content such as concentrated alcohols are the better fixatives, and by using them it is possible to preserve cell structure and entity of the granules to the nighest degree.

The basic aniline dyes have proven most valuable in staining mast cells. The granules stain metachromatically, altering the shade of toluidine blue to a reddish blue or purple.

Techniques which require keeping the tissue in fixatives and stains the shortest possible period of time yield the best results.

Biochemical

The desire to learn what the granules of the mast cell are composed of required the development of other techniques than the purely histological ones employed at first. As a result, attempts to extract mast cell constituents from tissue were made. This was done by physical methods involving breakdown of the tissue by mincing, followed by proteo lytic digestion with trypsin and chymotrypsin. The supernatant fluid was then assayed for heparin or histamine content by its influence on well controlled physiological reactions. For heparin, this was prevention of blood clotting and reversal of this action by protamine. For histamine, the effect of the

supernate on blood pressure of the cat or its effect on an isolated segment of guinea pig ileum was used to evaluate presence and content.

Experimental

Since 1950 the use of the experimental method has been popular in studying mast cells. Most of the work has been done with rats, mice and dogs. The designs have been quite simple involving the intravenous, intraperitoneal or local injection of a hormone, foreign protein, antibiotic, or pure chemical substance. Local injections were sometimes made into air pouches and the result studied with phase contrast microscopy or by fixing and staining biopsies of the pouch wall. More often the animals were sacrificed at a given time following the injection and a particular tissue or tissues removed and studied. The mesentery of the rat has often been used to learn the effect of a given substance on the mast cells there, both in vitro and in vivo.

Another type of experiment tested the effect of X-ray irradiation on mast cell counts of subcutaneous tissue and mesentery in the rat. The response of skin mast cells of white mice to carcinogens has also been studied.

One interesting experiment involved the use of chemicals in vitro to prevent the action of known degranulating substances.

Culturing of neoplastic mast cells in vitro has been accomplished, and is a valuable aid in studying the metabolism of mast cells. The source of the cells has been mastocytomas from dogs. Culturing was successful both in large and small preparations.

Isolation of Mast Cells

Padawer described in 1955 a method of isolating mast cells from other cellular elements of peritoneal fluid of rats. This is a useful technique because if provides high concentrations of mast cells which react normally in vitro to the usual agents used in studying physiology of the cells. The principle involved is the layering of the cell suspension possessing a relatively low density over a high density medium. The high density medium consists of sucrose, gelatin, sodium chloride and distilled water. Following centrifugation at about 700 rpm for five minutes, the mast cells are found in the upper layer and may be isolated by drawing the lower layers off with a drawn out medicine dropper. The only distortion of cells by this method is some decrease in size due to the osmotic action of the sucrose solution which causes partial dehydration of the cell. Spreads of these cells have been shown to react to 48/80 as do rat mast cells in vivo. This technique makes possible more accurate analyses of mast cells than is possible using extractions of tissue mast cells.

III. RESULTS

Histological Studies

Mast cells have a polymorphous or oval form, and in some tissues the cellular outline is somewhat irregular. In the rat the polymorphous form is seen about vessels in the connective tissue while the oval form is found in the mesentery. Free cells in the peritoneal fluid are round. The nuclei are round and tend to resemble those of histiocytes or plasma cells.

Under the electron microscope, the granules are round, oval or irregular. The irregular forms are probably due to artefactual changes. No fine structure has been noted within the granules. No characteristic mitochondrial elements have been seen in the cell. There is occasionally a thin dense line at the perimeter of the cell which suggests a cell membrane.

The diameter of free cells from peritoneal fluid is on the average 13.4 micra. The volume as determined by packing a known number of cells or by estimations from the size of tissue cells is 1.32 plus or minus 0.2 X 10^{-6} cu. mm. per cell. When isolated from the peritoneal fluid, the cells have a specific gravity between 1.088 and 1.100.[45]

The mast cells stain best with basic dyes and the granules seem to absorb most of the stain. The nucleus and the intergranular material both stain poorly with

basic dyes but stain well with acidic dyes. Since the nuclei of the cells resemble those of histiocytes or plasma cells, it is almost impossible to distinguish mast cells in the usual hematoxylin-eosin preparations.

The solubility of the granules in water was recognized by Ehrlich when he first studied mast cells. Through the years, fixatives contining a high per cent of water have been used occasionally with the result that much disagreement arose concerning the nature of the cytoplasm of the fixed-stained cell, the cell counts in tissue, and the staining of the surrounding tissue. Probably the more reliable results have been attained by workers using non-aqueous solutions.

The locations of mast cells in body tissues has been well worked out histologically in animals and in man. It is interesting that there is much variation between species as to the morphology of the cells, and the tissues in which they are found in highest concentration. For example mast cells are present in the dog's liver parenchyma in large numbers, while in the ox they are sparse in liver parenchyma and plentiful in the liver capsule. In the rat, mast cells are seen in highest concentration in the foot pads, about the ano-genital orifices and in the snout. They are also present in significant numbers in the subcutaneous tissue and mesentery. Free mast cells are found in the rat's peritoneal fluid.

Mast cells in humans are seen in largest numbers in skin, synovium, pleura, pericardium, ciliary body, gastro-intestinal tract including the pylorus of the stomach, and in and near blood vessels. No mast cells are seen in dense scar tissue, Achilles tendon or ligamentum nuchae, various other ligaments, and cartilage. They frequently occur in the connective tissue of the female breast, uterus, the urinary bladder, tongue and heart. In the skin the mast cells tend to accumulate around the hair follicles, glands, small blood vessels, and between fat cells. In the gut they are situated chiefly in the villi, around the necks of the glands of Lieberkühn and throughout the submucosa of the entire tract.

Mast cells are not seen in the parenchyma of the central nervous system and are rare in and around autonomic ganglia. They are present in the meninges, and about the vessels of the choroid plexuses and the pituitary stalk.[31]

Hence from the foregoing, one may generalize that mast cells are most numerous in the loose connective tissue, but are absent in cartilage, tendon, ligament, scar and such dense well-organized connective tissue.

The characteristic location of the mast cells in the loose connective tissue has been established. They tend to be most numerous near the capillaries and become sparse at a distance from the capillaries. The increased numbers

near capillaries has lead to the hypothesis that mast cells arise from mesenchymal cells near capillary walls.

Mast cells are present in the walls of larger vessels also, but they are most plentiful in the adventitia and are rarely seen in the inner layers of the vessel wall.

Mast cell distribution in a few pathological states has been studied. The one which received attention first was urticaria pigmentosa. In this disease mast cells proliferate in the dermis about blood vessels and sometimes the other structures such as hair follicles and glands. The cutaneous lesions appear as yellow-brown to violet macules, papules or nodules, which urticate when irritated as by rubbing. Dermographism is present in the skin between infiltrated areas. Two cases of urticaria pigmentosa have been reported in which there was associated proliferation of mast cells in liver, spleen, and bone marrow as well as in the dermis. In one case the patient had disseminated xanthomata, hepatomegaly to six centimeters below the right costal margin, basophilia in the peripheral blood of three to nine percent, hypergammaglobulinemia, prothrombin time four times normal, but no evidence of leukemia.[2,27]

Mast cell counts in patients dying from atherosclerosis and its complications have been compared with those in patients dying from other causes, and with counts in surgically removed

tissues. Pollak reported a study recently which was based on counts on tissue from about sixty patients. He found mast cells in the wall of all the larger vessels, both arteries and veins. Mast cells were present regardless of intimal alterations. His sections showed increased mast cells about atheromatous coronary arteries. The cells were located in the muscularis about the artery. The highest counts in Pollak's series were in patients who had milky plasma at autopsy.[26]

In another study Cairns and Constantinides reported that senile atherosclerotic patients had lower mast cell counts about vessels than non-atherosclerotic seniles and adults. Atherosclerotic females had lower counts than non-atherosclerotic females, and non-atherosclerotic males had lower counts than non-atherosclerotic females. It is only fair to say that a small number of patients were used in both these groups and that the differences between the groups was not striking.[6]

Paterson and others reported no correlation between the number of myocardial mast cells and the degree of coronary sclerosis as evaluated by vessel morphology, plaque thickness, lipid content and concentration, and calcium content and concentration. He did report a correlation between mast cell count and complications of atherosclerosis in that mast cells are decreased where thrombosis, infarction, and acute coronary

insufficiency had occurred.[24] One investigator has reported a rise in mast cell counts up to middle life and then a decisive fall thereafter. This was especially true of veins.[31(p16)]

Smyth and Gum state that study of diseased tissue from patients with lupus erythematosus, scleroderma and periarteritis nodosa reveals increased numbers of mast cells.[36]

Finally there have been several reports that mast cells are increased at the periphery of growing neoplasms. It is said there is a halo about these growths which is basophilic and that it is larger in slowly growing tumors than in those which grow rapidly. The mast cells in this area are assumed to be the source of the material which causes this halo.

Lascano studied the mast cell content within tumors of man.[13] He found them markedly decreased in number compared with normal tissue. This was true of all malignant tumors except basal cell carcinoma. For example, almost none are present in carcinomas of esophagus, stomach, colon, lung, squamous cell carcinoma of the breast, cancer of the cervix and gallbladder and lymphosarcoma. They are usually fewer in benign tumors also with the exception of the pigmented nevus. They are present in considerable numbers in angiomas and fibromas, and in the skin lesions of neurofibromatosis.[31]

Mastocytomas are mast cell tumors which have been found

in dogs, horses, cows, mice, and humans. They are found in dog's more commonly than in other animals. They consist almost entirely of mast cells, and have a low to moderate grade malignancy. Eventually widespread mastocytosis leads to the death of the organism. These tumors have been valuable in research dealing with mast cell physiology.

Biochemical and Experimental Studies.

A wide variety of substances have been shown to cause degranulation of mast ells. These substances vary from simple inorganic chemicals to highly complex proteins. First let us consider some of the results which have been recorded from the action of the simpler substances on the mast cells.

The simplest substance which has been shown to cause degranulation of mast cells is water. This can be observed under the microscope if one stains a mesenteric spread supravitally with toluidine blue. The granules swell as does the cell, and soon the granules are extruded from the cell as the cell seems to burst. Or the cell may remain intact and the metachormatic material diffuse from the cell forming a halo around it. Injection of water into the peritoneum of the rat results in degranulation of the mast cells in the mesentery, and subsequent assays for histamine in the mesentery show it to be lowered. Thus there is little doubt that water is

important in the degranulation process.

A recent study was carried out to determine the effect of tissue hydration on degranulation. It was found that dropping sterile water on living tissue in a cheek pouch preparation caused degranulation, swelling and disruption of the mast cells. Physiological saline and Tyrode's solution caused degranulation also. Two percent saline caused shrinkage of the cells. The addition of hyaluronidase to saline or Tyrode's solution caused rapid degranulation, but addition of hyaluronic acid had no appreciable effect. Traumatization of tissue in this preparation lead to degranulation, disruption of the cell and clumping of granules within intact cells.[40]

Another chemical which has been shown to bring about a sudden and explosive degranulation is sodium hydroxide.[31] This can be demonstrated either by dropping a solution of N/10 sodium hydroxide directly on a fresh spread containing mast cells or by placing wire electrodes and a piece of litmus paper on a similar spread covered with normal saline and closing the circuit. The result is sudden and explosive degranulation with rupture of the cell. When the circuit is closed, the mast cells near the cathode become tense, round up, and explode. This process spreads to the center of the field and stops. If the current is reversed, the mast cells on the other half of the field undergo the same process.

The color change of the litmus paper indicates that the degranulation coincides with the generation of NaOH at the cathode and its spread across the field, and this explains why the results of either method are identical.

The simplest of all amines, ammonia, will cause degranulation. The result is not as explosive as with sodium hydroxide but the end result is the same.

Of the more complex chemical compounds which have a dramatic effect on the mast cell, the compound 48/80 has been used most and seems to be the most effective degranulating substance known. It is used as the standard for comparison. This substance is a condensation product of p-methoxyphenethylmethylamine and formaldehyde. Solutions of 48/80 have an alkaline pH. It is very valuable as an experimental tool because of high potency and low toxicity, and because it seem equally effective in all species.

When 48/80 is injected into rats intraperitoneally, it results in the characteristic signs of histamine release--- shock, erythema, cyanosis and edema of the muzzle, paws and about the anogenital orifices. If the tissues are removed from the animal and studied microscopically, the mast cells are seen in a state of disruption with granules strewn about in the connective tissue. If this tissue is assayed for histamine, the yield is less than that of tissue from controls.

In an experiment where 48/80 was given over a thirty day period, the initial results were as described above, but subsequent injections caused little to no observable reaction. This seemed to indicate depletion of tissue histamine and sections of tissues at this stage indicated a marked decrease in the number of mast cells present. The tissues returned to normal histological appearance rather quickly when injections ceased. During the course of the injections there was no observable recovery in numbers of mast cells in in the omentum and mesentery of the rats. One can summarize the action of 48/80 by saying that this substance has been proven as a potent histamine liberator which causes decrease in number of mast cells and histamine content in tissue following injection, and this situation will persist over a long period of time if sublethal injections of 48/80 are given daily.

Stilbamidine, propamidine, pentamidine, d-tubocurarine chloride, and peptone are other chemicals which have an effect on mast cells similar to 48/80. They are more toxic and hence have less value in research. In animals killed with a slow injection of a lethal dose of stilbamidine, the mast cells are shattered and granules are released. The only evidence of the existence of the cell may be a remnant of frothy cytoplasm. D-tubocurarine, which is used primarily as a muscle relaxant

during surgical procedures, is less effective than 48/80, but definitely does act as a histamine liberator.

The foreign proteins which act on mast cells include bacterial toxins, special sera, ovomucoid, and anaphylatoxin. Mota reported a study in which rats were treated with one ml of horse serum subcutaneously, the test animals receiving one ml of Hemophilus pertussis phase I vaccine containing twenty billion organisms in addition by intraperitoneal injection. Ten days later, all animals were challenged with one ml of horse serum intravenously. The plasma of all animals was assayed for histamine and the tissue of the tongue, snout, and lips was fixed with perfusion through the carotid artery of a solution of ethanol, formaldehyde and acetic acid. Tissues of the test animals contained thirty-two to eighty-five percent disrupted mast cells while none were seen in control animals. The plasma histamine of test animals was 2.0 to 0.8 micrograms per ml while that of animals not receiving H. pertussis vaccine contained only 0.03 micrograms per ml. This represents twenty-seven to sixty-seven times more plasma histamine in the test animals. The over all result here is that the H. pertussis vaccine acted in some was to sensitize the mast cells to the horse serum so that challenge with a second dose caused a significant increase in mast cell disruption and histamine release.

Carter studied the reactions of mast cells of mice which

had been sensitized to horse serum.[7] The mice were sensitized by three injections on the ventral surface and studies were made on tissues from the dorsal surface where an air pouch was made. The challenging dose of horse serum was injected into the air pouch rather than intravenously. The result was that there was widespread degranulation and dispersion of granules in the tissues of the sensitized mice whereas only a few of the non-sensitized controls shed granules. This author states that fibroblasts ingested many of the shed granules and that within one to four hours, metachromatic cells re-appeared. Three to five days later, granulated cells were present but had cytoplasmic processes which suggested fibroblastic origin. The author believed the material released from the mast cell increased the fibroblastic response to injury. He also believed that the granules had 5-hydroxytryptamine and chymotryptic activity which may increase the inflammatory response. This study shows that mast cells are effectively degranulated by a foreign serum when injected into a sensitized animal.

Equally impressive are the results of the studies of Benditt and his group on the mechanism of acute vascular reactions to injury.[3] The experiment involved the use of ovomucoid to sensitize rats. Following sensitization, the rats were challenged with an intravenous dose of ovomucoid.

This is a proteinaceous material which is extracted from the white of eggs. The gross effects are swelling of the snout, muzzle, and forepaws, and dripping of fluid from the nose. These sites coincide with the sites of greatest concentration of mast cells in the rat. Microsections of tissue from these areas reveals degranulation of mast cells. Extractions of this tissue after one injection reveal a large reduction in the amount of histamine present. However, maximal edema in the same animal develops after a second injection of the ovomucoid. Thereafter, the amount of edema with subsequent injections decreases until it is nil. Benditt suggests that possibly a factor other than histamine is partially responsible for the development of edema. The rat seems to be quite sensitive to serotonin and he suggests this may be an important factor in edema production.

Benditt also assayed the amount of serotonin in the mast cells of the rat by concentrating cells from peritoneal fluid. The preparation was not pure mast cells. Some mesothelial, mononuclear, and polymorphonuclear cells were present. Assay for serotonin by its action on rat colon and by chromatography revealed 0.7 micrograms of serotonin per cu.mm. of mast cells. At the same time he assayed the cells for histamine and heparin and found ten and thirty micrograms per cu.mm. present. He stated the amont of serotonin present paralleled the content of mast cells but not the content of other cells in the preparation.

The work of Bhattacharya and Lewis in England indicates the rat mast cell has significant amount of serotonin.[5] He perfused the hind limb with a solution of 48/80 and showed in his preparations that the greatest amount of serotonin came from the skin of the feet. Here also one finds the largest number of subcutaneous mast cells. He found very little serotonin present in perfusate of the skeletal muscle. He perfused the hind limbs of the cat, rabbit, and dog and found no serotinin present. Morphine was placed in the perfusate and it released more serotonin than histamine. He also noted that maximal release of serotonin precedes that of histamine.

The next foreign protein to be discussed is anaphylatoxin. This substance was first prepared by Bordet in 1913 to produce anaphylactic shock in the guinea pig. The material is prepared by incubating a mixture of five parts of species specific serum with one part of semisolid agar for two hours at 37°C. The mixture is then centrifuged and the supernatant liquid is injected intravenously. When injected into rats, this produces acceleration of heart rate and respiration, but not anaphylactic shock. If the animals are killed three to five minutes later and the tissues inspected, the more mature mast cells seem swollen and in the process of vacuolation and degranulation. The younger mast cells near the capillaries are relatively unaffected. In summary, anaphylatoxin did not

cause marked degranulation of mast cells in any part of the rat, but did cause swelling and vacuolation of the more mature cells as if they were in the process of degranulating.

Irradiation

The interest in the effect of radiation on mast cells has arisen partly because it is known that they contain heparin and humans have died from massive hemorrhage after recieving heavy doses of irradiation. This was first noted in World War II when the atom bomb was dropped on Japan. Then several years later a nuclear device accidentally detonated at White Sands Proving Ground and all three persons present were killed. Blood was drawn from one of these individuals at the time of his death and it failed to clot until a heparin antagonist was added. Therefore, it has been suggested that radiation may cause release of heparin from mast cells causing hyperheparinemia and death from hemorrhage.

Several studies on the effect of irradiation have been done. One of the better of these was carried out by Petterson.[25] He found that X-ray irradiation caused observable effects on mast cells of the rat when doses of 620 to 1320 r were given to the animal as total body radiation. No further change was noted with doses above 1320 r. In the range stated he noted a fifty percent decrease in mast cell counts of the skin, but was unable to demonstrate hyperheparinemia. His results are in

accordance with those of others in that no one has been able to elicit hyperheparinemia with high doses of irradiation.

Carcinogen

The effect of carcinogen has aroused interest in mast cells for two reasons. The first is the markedly increased number of cells in the skin when a material such as methyl cholanthrene is applied to mice. This occurs prior to the development of cancer. Secondly, if the skin in this pre-cancerous state is assayed for histamine, that treated with the carcinogen has ninety-five micrograms of histamine per gram of tissue while the untreated skin has only forty-five micrograms per gram of tissue.[28] This raises the question of the role of the mast cell in carcinogenesis. No experimental work has been done which sheds any real light on this problem.

Hormones

The effect of the adrenal cortical hormones on the mast cell has been of particular interest because of the correlation between mast cell activity and the inflammatory process. Several well controlled studies have been completed on this, and results have been similar.[1,14] They show that adrenal steroids do not affect the mast cells except in relatively high doses. Then they cause vacuolation of cytoplasm, clumping of granules, and gradual degranulation with clearing of the cytoplasm. This degranulation occurs slowly and is

apparently of a different type than that discussed above, for the number of mast cells degranulated in anaphylaxis is increased in adrenalectomized rats.[18] This tends to indicate a protective action of the adrenal hormones against degranulation rather than a potentiating one.

Thyroid stimulating hormone seems to have a significant action on mast cells. Following thyroidectomy the cells increase in number, size and granule content. Giving thyrotrophin gives the same results but the changes are more marked.[14]

Groups of rats and guinea pigs fed thyroxine showed a decrease in size and granulation, especially of cells distant from vessels. Cells near the vessels appear normal.[14]

The effect of estrogen on mast cells has been studied in relation to counts in the mouse vagina. Westin has noted a significant decrease in mast cells here following administration of estrogen to castrated mice.[43,44] The numbers of cells per cross section of the vagina decreased from 103 to 39. Progesterone seemed to counteract this action of extrogen, for when the two hormones were given simultaneously, the mast cells decreased to 59 per cross section.

Smith attempted to study the influence of the pituitary as a whole on mast cells. Following hypophysectomy he noted only a transient increase in mast cells of the skin.[35] There was no change in the mast cells of the mesentery.

Vitamin C

Since this vitamin is so important in the metabolism of connective tissue its action on mast cells is of particular interest. When animals are rendered scorbutic, there is a marked degranulation and vacuolization of the cytoplasm. These changes tend to be progressive as the deficiency is maintained. There is noted an associated depolymerization of the ground substance. The changes here are histologically similar to those brought about by high doses of cortisone.

Antibiotics

Norton and others studied the effects of some of the commonly used antibiotics on the rat mast cells with regard to their ability to cause release of histamine.[20] They found that polymixin was about eighty percent as effective as 48/80 in releasing histamine. Aureomycin had a slight effect, while bacitracin and penicillin had no significant action. Tetracycline and sulfanilamide had no effect at all. Polymyxin appeared to exert its effect through changing the osmotic balance of the mast cell. In this same experiment Marezine (cyclizine hydrochloride) demonstrated ability to inhibit fragmentation and degranulation of the cells in serum concentration of over five micrograms per ml.

Colchicine

The effect of colchicine on young male and female rats

was observed following subcutaneous injection of the alkaloid in normal saline.[22] Damaged cells in one to ninety-five per cent of the total cells present were seen at the end of one hour. Animals killed at later periods revealed mast cell damage so extensive it was impossible to make counts. Large numbers of anuclear mast cell fragments were seen and innumerable macrophages engorged with mast cell material were present. The recognizable cells seemed to be extruding the nucleus. The authors summarized the effect of colchicine as fragmentation of the cell without nuclear damage.

Chemical Inhibitors of Degranulation

The inhibition of mast cell degranulation has also been studied.[12] The compound 48/80 was used as the liberator of histamine in the studies and it caused a marked extrusion of granules in vitro down to concentrations of 1:500,000.

Low temperature and pH below 7 prevented degranulation. No degranulation occurred at temperatures below 25°C. Degranulation occurred only in a neutral to alkaline medium up to pH 9.27.

The chemical inhibitors p-chloromercuribenzoate, o-isodosobenzoate and iodoacetate block sulfhydryl groups and inhibit action of 48/80. Arsenite, 2,4,dinitrophenol and urethane block phosphorylative mechanisms and inhibit granule extrusion. Uranil nitrate, which supposedly inhibits cell membrane enzymes

prevents degranulation. Finally cyanide, which deactivates cytochrome oxidase prevents degranulation. Glutathione reverses the action of -SH blocking agents but ATP does not reverse inhibition of phosphorylation.

Antihistamines

The effect of antihistamines in the mast cells is of particular interest because of the recent information indicating mast cells are a primary source of tissue histamine. This has not been studied extensively, but a few investigations have been made.

Norton used an antihistaminic substance in his study of of the effect of antibiotics on mast cells.[20] He used N-benzhydryl-N'-methyl piperazine monohydrochloride (cyclizine hydrochloride---brand name Marezine). This substance is an antinauseant, antiemetic, and antihistaminic drug. He found that it tended to inhibit fragmentation and degranulation of mast cells in serum contrations over five micrograms per ml. Antihistan, N-P-methoxybenzyl-N'-N'-dimethyl-N-2-pyridl-ethelenediamine acid maleate prevented degranulation of mast cells in hamsters in response to challenge with anti-hamster serum from the rabbit.[41] This was noted only if the antihistan was injected into the cheek pouch five minutes prior to the intraperitoneal injection of the serum. When the antihistan was injected intraperitoneally one half hour before injecting

the serum, rupture of mast cells was observed. The author also noted antihistan caused deeper staining of mast cells with toluidine blue so that individual granules could not be easily distinguished.

Mast Cell Cultures

MacDougall succeeded in culturing neoplastic mast cells from mastocytomas in dogs.[15] The culturing was done in cockerel plasma, autologous dog serum, horse serum and embryonic extract.

The mast cells grew well in all the culture media and no advantage from using the autologous serum was noted. Chick heart fibroblasts and mast cells were grown together with no inhibition of either. He found that adding mast cells to fibroblast cultures appeared to favor formation of reticular fibers. Fiber formation could also be induced by adding extract from mast cell tumors. The author stressed the significance of the apparent ability of mast cells to stimulate fiber formation.

Interrelationship with Neoplasms

The relationship of mast cells to neoplastic growths has been studied experimentally in an attempt to determine whether they increase or decrease the rate of growth. There is general agreement that there is an increased number of mast cells around slowly growing tumors associated with metachromasia of the ground substance. As the rate of tumor

increases, the metachromatic halo decreases in size and may be nearly ablent in tumors which grow very rapidly.

In the growth process, tumors release a polypeptide which causes iodine trapping. Scott showed that this polypeptide causes decrease in the number of mesenteric cells when administered to rats in amounts that cause iodide trapping.[33] They also found that having a sarcoma which equalled seven per cent of body weight reduced the number of mast cells in the mesentery. Disruption of mast cells with a degranulating substance prior to implantation of a tumor lead to a decreased rate of growth. Disruption of mast cells before and/or during the period of growth, or administration of histamine during the growing period lead to an increased rate of growth.

Mast cells are present in chemically induced fibrosarcoma of rats. The mast cells are scattered diffusely among the malignant fibroblasts. The mast cells are always numerous at the periphery of tumors, irrespective of their internal mast cell count. Splenic tumors and luteomas in inbred mice have been seen which contain numerous mast cells.[31] They were found mainly about the periphery of the tumor and associated with the connective tissue stroma. The splenic tumors grew very slowly.

The increase of mast cells in the skin in response to application of carcinogen has already been mentioned.[28] This

is in contrast to the decrease in skin mast cells following irradiation in doses of 620 to 1320 r noted above. No studies have been reported on mast cell changes during induction of tumors by irradiation.

Mast Cell Constituents

Until recently it was possible to determine mast cell contents and amounts present only by extraction of tissues. Extraction of heparin and histamine and correlation with mast cell counts have been accomplished by several investigators. Jorpes found no mast cells and no heparin in rat liver. In ox liver capsule he found abundant mast cells and 540 to 830 mg heparin per kg. of tissue. Riley found positive correlation of mast cell number and extractable histamine. Liver capsule of the ox has abundant mast cells and 40 micrograms of histamine per gram of tissue. Ox pleura contains more mast cells and has 200 micrograms of histamine per gram of tissue.[31]

Assay of mastocytomas from dogs and other animals has revealed exceptionally high values for both histamine and heparin. One dog mastocytoma contained 1,290 micrograms of histamine and 110 i.u. of heparin. The highest heparin content of normal tissue known is 50 i.u. in ox liver capsule. By extraction of histamine from tissue, Riley has estimated the amount of histamine in one mast cell of ox liver capsule. He calculated this at 25 micromicrograms of histamine base.[31]

Benditt has analyzed isolated suspensions of rat mast cells obtained by differential centrifugation. He found 11 micrograms of histamine and 0.6 micrograms of serotonin per cu. mm. of cells, or 1.1 and 0.06 per cent of the cell by volume. He estimated that heparin composes 3 to 4 percent of the mast cell. He also found a hydrolytic enzyme in mast cells which has substrate requirements similar to those of chymotrypsin. This enzyme may compose 1 percent of the cell.

Benditt found that administration of reserpine lowers the concentration of serotonin in peritoneal mast cells by about 50 per cent with no detectable change in cell numbers, morphology, or histamine content. He believes at least the pyridoxal-5-phosphate part of the 5-hydroxytryptophane decarboxylase system is present in the extragranular substance of the cell.

Dynamics of Histamine Release

Using transilluminated mesentery of the rat, Smith observed the action of 48/80, stilbamidine, protamine sulfate, and toluidine blue on mast cells. Prior to treatment the mast cells are round or spindle shaped and densely packed with dark granules. "After application of the test solutions the granules lose their dark appearance and become almost invisible. First one granule and then another reacts until all have become involved and the cell is barely discernible."

During this the cells swell to 1 1/3 times original size. "When most or all of the granules are scarcely visible, metachromatic staining of the faded granules begins. At first a few are stained purple, then more and more until all are so stained." The cells now resemble ones fixed with alcohol and stained with toluidine blue. From these observations, the author believes that histamine is normally bound to granules and is osmotically inactive, and that when it is freed it becomes active and causes water to enter the cell causing swelling. He suggests the histamine liberators have a stronger affinity for heparin than histamine and eo displace it.[34]

IV. DISCUSSION

Origin of Mast Cells

The origin of the mast cells is still unknown. When they were first discovered, it became more or less the accepted hypothesis that they were identical with the blood basophiles except that they had diffused through the capillary wall and had taken up a perivascular habitat in the connective tissue. However the fact that in myelogenous leukemia the mast cells unaffected tends to contradict this hypothesis.

The presently accepted theory that they arise from mesenchymal cells near the capillary wall may be correct. The evidence indicates that they are functionally related to the reticuloendothelial system and to the vascular system, as shown in their participation in anaphylaxis and edema formation. Also, the notion that the cells near the capillary wall are younger cells seems justified since these cells do not react like those more distant from the capillary in some cases. The difference in morphology and granulation also supports this idea.

Some workers have suggested that mast cells may arise from other cells such as macrophages or fibroblasts which have ingested mast cell granules. There is little if any reliable evidence to support this. But regardless of the origin of these cells, it is obvious they are omnipresent in loose connective tissue and it is more important to learn their function.

Mechanism of the Mast Cell Response

From the results presented above,it is obvious that the mast cells respond to a tremendous number of stimuli. This causes one to wonder whether the cells are reacting to the stimuli directly or whether they are transmitted to the mast cell through some mediator apparatus. For example how can a complex compound like 48/80 and stroking of the skin with a blunt instrument elicit the same response. The experiments presented offer abundant evidence that the response to these two stimuli is the same or very similar. Can this be explained on the basis of tissue hydration? Are subtle pH changes a significant factor? Can the answer lie in changes in the ionic nature of the ground substance and the cell membrane? Or is there some unknown specific mast cell "activator" in the ground substance which acts on the cell in a specific way? It is impossible to answer these questions, but one could find evidence to argue in favor of any one of them if he chose.

Riley's data with regard to NaOH indicates that pH and electrochemical forces are important, as does Junqueira's data with regard to inhibition of degranulation. However this does not seem to explain mast cell response in anaphylaxis, nor its response to trauma. If histamine is bound to heparin in ionic linkage, what displaces it when tissue is traumatized? Perhaps it is something quite simple such as sodium ion.

Even more confusing however is the fact that some substances cause release of histamine almost exclusively, others cause mostly release of serotonin, and others cause release of both when injected into the rat. This indicates that the mast cell response is very complex and cannot be explained on a simple basis such as displacement of histamine from its assumed bond with heparin. With this in mind, it seem just as well to dispense with this and speculate about another topic.

Function of Mast Cell Components

The function of the mast cell will be understood when it is accurately analyzed and the functions of its components are known. The first of these mast cell components to be discovered was heparin. This is best known as an anticoagulant, but it has other capabilities including clearing the plasma of lipids. Whether either of these actions is important with regard to mast cell function is unknown. It may be possible that the anticoagulant action of heparin is important in inflammatory reactions.

Histamine is probably one of the most important substances in the human body. It is believed to be one cause of headache. It is said to cause dilatation of the coronary arteries. It apparently causes activation of the phagocytic properties of endothelial cells, and may play a part in depolymerizing the

ground substance in inflammatory states. The fact that most of the drugs, or at least many drugs which are antihistaminics are also antinauseants and antiemetics suggests that histamine may be capable of producing nausea and vomiting. This may explain why they are present in almost all diseases.

The function of serotonin is probably quite important in man also. Judging from animal experiments it increases capillary permeability. It is also supposed to activate smooth muscle. Perhaps it causes constriction of smooth muscle of vessel walls, with tightening of sphincters and increased intracapillary pressure.

The action of chymotrypsin, if it is present in the mast cell, is undoubtedly proteolytic. The mast cell may provide some of the proteolytic enzyme necessary to lyse fibrin and other proteins in the healing stage of the inflammatory reaction.

Role of the Mast Cell in Human Disease

Inflammatory Reactions

Among the stimuli to which the mast cells react are a number which lead to inflammatory reactions. Some of these are radiation, bacterial toxins, foreign proteins, chemicals, and trauma. Many of these stimuli form a part of the normal enviornment of all living things. However in excessive quantities, they cause a threat to life. It seems that the result of the mast cell response is to increase host defenses.

Let us look at a few examples of how this might be true.

When bacteria are implanted in the dermis or subcutaneous tissue either through a normal opening in the skin or a traumatic one, an inflammatory response ensues. This inflammatory response is due to the reaction of the tissue to the metabolites of the bacteria. The studies on the mast cell indicate that it reacts to the type of substances which such invaders produce. Therefore, although this has not been reported per se, except that there are no mast cells in areas of acute inflammation, one might assume that in the infected area, mast cell degranulation occurs. The result of this is the release of heparin, histamine and the other substances within the mast cell. These substances then play some part in causing the capillary stasis, which tends to localize the infection and protect the rest of the organism from attack. The depolymerization of the ground substance which occurs may also help fight the infection by promoting rapid diffusion of antibodies, toxins and leukocytes from the vascular system to and away from the infected area. The studies of mast cell cultures with fibroblasts may be evidence that mast cell constituents then help with the repairing processes following infection. This type of activity of the mast cell makes it a participant in response to minor trauma to the organism. And as stated above, the histamine from the mast cell may help make the individual aware of his injury.

Anaphylaxis and Allergy

The response of the mast cells of the rat when ovomucoid is injected indicates that mast cells of the rat play an important part in anaphylactic reactions and suggests the same may be true in man. Anaphylactic reactions in general seem to be a type of faulty host response in that there is a major response to a substance which is not lethal in itself, but the response may lead to death. This seems to be mediated through a type of antigen-antibody reaction. The result of the anaphylactic reaction is smooth muscle activation associated with capillary dilatation and stagnation of blood, and sometimes respiratory difficulty also. The result of this may be hypovolemic shock or asphyxiation, either leading to death. This reaction of the host is as though a major onslaught against life were being made and the response is commensurate. An example of this in humans is death resulting from injections of penicillin into sensitive people. It would be valuable to know if the mast cells play any part in causing this reaction in man in that such knowledge should lead to more effective therapy. That mast cells may play a part in anaphylactic reactions is almost certain when one considers that these cells contain histamine and possibly serotonin.

A very dramatic clinical entity which may be in part

a result of a hypersensitivity response of the mast cells of man is the Waterhouse-Friderichson syndrome. In the past this has been considered a result of adrenal failure. Since adrenal steroids have been available for therapeutic use, they have been included in the treatment of this syndrome. A recent review of cases so treated compared with cases before the advent of steroids indicates the survival rate is almost the same. Also, the syndrome does not develop immediately in an individual but follows after a period of time which would allow the antigen to be present in the body for some days. This could sensitize the tissues of the body to this antigen and then when the infection becomes more widespread the higher production of antigen could trigger a widespread connective tissue response with mast cell degranulation, release of histamine and other constituents, a tremendous capillary dilatation and hypovolemic shock resulting in death. Since Mota has shown that more mast cells are degranulated when anaphylaxis is induced in adrenalectomized rats, adrenal failure may play a part here even though results to date may not clearly indicate it. If the above is true then high doses of antihistaminics should be given to a patient who might develop this syndrome, for in two references listed above evidence is given that they may prevent mast cell response if given in adequate dosage. Also it has been shown that promethazine decreases capillary

capillary permeability and endothelial phagocytic activity. Hence antihistaminics could be valuable in the treatment of the W-F syndrome in two different ways.

Although allergy is a less dramatic than the anaphylactic type of hypersensitivity reactions, it is of greater medical importance because it is so much more common. There have been no studies on humans to prove or disprove it, but from the results of animal studies one can easily see that mast cells may play a key role in the allergic response. The fact that one can give horse serum to a man in small doses when he cannot tolerate a large dose suggests that the larger dose results in a massive sudden release of histamine and other substances which could come from mast cells. If a person were to be constantly bombarded with small doses of an allergen such as ragweed pollen, then a rather constant amount of mast cell constituents could be released into the tissues resulting in the clinical picture of allergy. Studies indicate that it is usual for only part of the mast cells to react at one time, and they also show that mast cells quickly regenerate released histamine. Thus it is possible for mast cells to mediate a chronic reactive state. Research in this area would be valuable to determine whether or not mast cells are an important factor. Evidence to date suggests they are, even on the general principle that highest cell counts are found in chronically

inflammed tissue. Information in this area might prove valuable in the treatment of allergy, dermatitis, asthma, and some of the idiopathic dermatoses.

Hypertension

The finding of serotonin in the rat mast cell is worthy of a bit of speculation. If this is pieced together with some other information, one can do some interesting thinking about other diseases of mankind. The first bit of information is that reserpine causes release of serotonin and depletion of tissue stores of it. The second is that lysergic acid diethyl amide is an antagonist of serotonin and has marked psychological action when given to humans. Third is that reserpine is effective in lowering blood pressure in essential hypertension. Fourth is that most antihistaminics also have anticholinergic properties. Fifth is that the action of the various tranquilizers indicates thare are two mechanism of action and that the phenothiazines and reserpine act by two different mechanisms. Sixth is that morphine releases more serotonin than histamine, that it is noted for its cholinergic side effects and that it is a very potent analgesic and hence has potent psychological effects on man also. From all of this, it appears that there is some relationship between histamine, serotonin, and the functioning of the autonomic and central nervous systems. Histamine and serotonin may a significant part in mediation of

cholinergic effects, and therefore in the genesis of such diseases as peptic ulcer, hypertension, and ulcerative colitis. There are physicians who feel sure that the above diseases are due to a state of mind, particularly depression or from the repression of hostility, and it seems that the facts support this. In this connection, one should include coronary heart disease also, as there is now some evidence that the mono-amine oxidase inhibitors relieve anginal pain, as well as depression. The beneficial action of reserpine in hypertension may be due to both a central and peripheral action which leads to tissue depletion of serotonin and lowering of blood pressure. If human mast cells produce or play a part in the production of serotonin, then reserpine may act directly on the mast cell to cause release of serotonin as fast as it is formed or even to inhibit production of it. The central action of reserpine is believed to reduce the tension of the autonomic nervous system, and indeed in some cases it produces irreversible depression. Therefore, one wonders if hypertension is partially produced by the action of neuro-humoral substances on the mast cells with resultant excessive production of serotonin. If the mast cell can be affected by such neuro-humoral substances, then perhaps a mechanism for an emotional state to bring on an acute attack of peptic ulcer, or neurodermatitis is present here.

Connective Tissue Metabolism and Collagen Diseases

The function of the mast cell with relation to the connective tissue has not been ascertained entirely, and especially with regard to metabolism. Studies on animals have shown that the mast cells contain heparin and histamine in all species and that serotonin is present in the mast cells of the rat and mouse. The function of heparin in the connective tissue has bot been defined. Some suggestions have been made above. One of the more promising ideas of its overall primary function is that the heparin produced in the mast cells is released from them and metabolized to form hyaluronic acid. It is fairly certain that in most animals it does not act as an anticoagulant. Heparin may play some role in preventing formation of fibrin in the early stages of tissue injury, which would be most likely to occur if fibrin diffused into the tissue spaces and apparently it does. The result of fibrin formation initially would be to decrease the effectiveness of the inflammatory reaction, and delay repair of injury. In summary, it appears at this time that the heparin of mast cells is not involved with anticoagulation of the blood but is involved in the metabolism of the loose connective tissue.

Although the actions of serotonin have not been worked out entirely, the fact that fibrosis of the endocardium of the right side of the heart occurs in association argentaffinoma of the small intestine suggests that it may stimulate fibrotic

changes in connective tissue. If such were the case then serotonin could be an important factor in the genesis of arteriosclerosis. Serotonin and histamine may indirectly affect connective tissue metabolism by perpetuating a chronic inflammatory state in some diseases. Perhaps this is part of the reason for the difficulty in obtaining healing in a peptic ulcer.

The effect of scurvy on the mast cell raises the question of the effect of mast cells on wound healing. They are said to be present in the area in larger numbers than in normal tissue. Perhaps they stimulate fibrillogenesis there.

Studies have been carried out to determine whether or not mast cells are important in atherosclerosis and arteriosclerosis. The first condition was investigated because heparin has the ability to clear lipemic plasma. The second is a type of fibrotic change in the vessels. So far no correlation has been found between mast cell count and these diseases which can be considered of any real importance.

As stated above, some workers have noted an increase in mast cells in association with the collagen diseases. The histological changes in these diseases suggest a defect of some sort in the metabolism of the connective tissue. This is undoubtedly related to the connective tissue cells. The question here is whether the mast cells play a part in the

production of the diseases or whether they are just affected along with everything else. Either is possible as far as one can see at this time, and the later seems more likely.

Hormones and Mast Cells

Since the various hormones control body metabolism in general, it is not surprising to find that certain of them have definite effects on the mast cells. Of those which have been studied, TSH seems to have the most definite and reproducible effect. Perhaps this action of TSH on the mast cells is partly responsible for the development of myxedema in thyroid disease.

The action of the corticosteroids is as would be expected from their general ability to decrease inflammatory reactions. As seen above, the mast cells seem to stimulate and add to inflammatory responses. Therefore it is a problem of some importance to learn whether or not mast cells are important in the chronic inflammatory reactions of the rheumatic and collagen diseases, and whether the beneficial effects of cortisone is through suppression of mast cell metabolism.

The effect of the other hormones mentioned in part III is not striking. The action of estrogen on the mast cells may be of importance in maintaining pregnancy for histamine stimulates the smooth muscle of the uterus. Thus, suppression of mast cells during pregnancy could protect the pregnancy by maintaining low levels of histamine in the body.

Colchicine and the Mast Cells

Colchicine is a substance which has mystified physicians for years. It is very effective in relieving the pain of an attack of gout, and so far no one has been able to demonstrate a mechanism of action. After the drug is given there is a lapse of 12 to 24 hours before the patient is relieved. As noted above, this substance causes marked degranulation of mast cells in animals. If mast cells are actively involved in the attack of gout and are adding to or producing in total the inflammatory response, then one would expect the attack to be aborted by a substance which arrests mast cell activity. If the mechanism of action of the substance were by causing breakdown of the cell and release of its products, one would expect a lapse of several hours before the patient is relieved. So it is possible that colchicine relieves gouty attacks by its action on the mast cells, and this hypothesis fits well with the observed clinical effects of the drug.

Neoplasms and Mast Cells

The increase in the number of mast cells surrounding neoplastic growths has lead to speculation concerning the effect of mast cells on neoplastic processes. If one views the entire life span of a population, he finds that the incidence of neoplastic growths increases with age. This has been explained by some physicians with the statement that the

bodies defenses against neoplasms decreases with age. No one has stated exactly what these defenses are, but for purposes of discussion let us assume they exist. If so, then in early life mast cells would have relatively less importance than in later life. The ability of the mast cells to cause inflammatory reaction with production of edema and depolymerization of the ground substance undoubtedly facilitates the spread of cancer both by lymphatic and hematogenous routes. The histological finding that they are present in large numbers at the periphery of neoplasm, the fact that a polypeptide has been isolated which causes degranulation of mast cells, and the finding that injection of histamine causes increase in the rate of sarcoma growth in mice all suggest that the mast cells do not defend the organism against cancer, but through their non-specific inflammatory response make it more susceptible to this disease.

The importance of mast cell neoplasm is of minimal importance in clinical medicine. The two cases of widespread mastocytosis in humans suggests a type of neoplastic growth of the mast cells, or perhaps just a hyperplasia. The location of the accumulations of mast cells and the associated changes in relation to the vascular and reticuloendothelial system which have been found in these cases may be taken as evidence that mast cells are an integral part of these systems in origin and function.

V. SUMMARY

The purpose of this thesis has been to review the recent studies on the mast cells and in so far as possible correlate the findings with diseases of man. An attempt has been made to explain the pathologic physiology of certain diseases on the basis of the findings in the animal studies. This has been done with the realization that one cannot generalize from animals to man indiscriminately, but that such thinking leads to some idea of how human diseases may develop.

The action of numerous substances on mast cells has been presented. On the basis of this, some suggestions regarding therapeutic approaches to specific human diseases have been made.

The conditions of the human organism in which mast cells appear to be significant participants are the inflammatory reaction, the hypersensitivity reactions in general, the collagen diseases, neoplasia, certain diseases with a psychosomatic component, and possibly gout.

The material presented suggests several interesting problems for research. These include the action of meningococcal toxin on the mast cells in regard to the Waterhouse-Friderichson syndrome; the function of mast cells in allergy; the effect of 48/80 on wound healing; the relationship of mast cells to

the collagen diseases; and the effect of changing mast cell metabolism on the progress of malignant neoplasms.

VI. CONCLUSIONS

From the results of studies on the mast cells it can be concluded at this time that:

1. the mast cells of the rat, ox, pig, dog, and other animals tested have significant quantities of histamine and heparin.

2. that the mast cells of the rat have significant quantity of serotonin.

3. that the above substances and therefore the mast cells play an important part in control of edema and have a marked effect on capillary permeability.

4. that the mast cells have an important function in the hypersensitivity response of animals and therefore possibly of humans also.

5. that mast cell metabolism is effected by thyroid stimulating hormone, cortisone and hydrocortisone, thyroxine, estrogen, progesterone, and possibly other pituitary substances.

6. that mast cell metabolism is to some extent dependent on a adequate supply of vitamin C.

7. that mast cells promote fibrillogenesis and possibly other phases of connective tissue metabolism are affected by them.

8. that mast cells probably promote the spread of malignancies.

BIBLIOGRAPHY

1. Asboe-Hansen, G. and Zachariae, L., "Mast Cell Changes Induced by Hydrocortisone Acetate in Experimental Skin Papillomas in Mice", Acta Pathologica and Microbiologica Scandanavica, 37(2): 145, 1955.

2. Asboe-Hansen, G., and Kaalund-Jorgensen, O., "Systemic Mast Cell Disease Involving Skin, Liver, Bone Marrow, and Blood with Disseminated Xanthomata", Acta Haematologica, 16:273, 1956

3. Benditt, Earl P., Bader, S., and Lam, K.B., "Studies of the Mechanism of Acute Vascular Reactions to Injury", A.M.A. Archives of Pathology, 60(1):104, July 1955.

4. Benditt, Earl P., Wong, R.L., Arase, M., and Roeper, E., "5-Hydroxytryptamine in Mast Cells", Proceedings of the Society for Experimental Biology and Medicine, 90(1):303 October 1955.

5. Bhattacharya, B.K., and Lewis, G.P., " The Release of 5-Hydroxytryptamine by Histamine Liberators", British Journal of Pharmacology and Chemotherapy, 11:202, 1956.

6. Cairns,A., and Constantinides, P., "Mast Cells in Human Atherosclerosis", Science 120(3105):31, 1954

7. Carter, P.B., Higginbotham, R.D., and Dougherty, T.F., "The Local Response of Tissue Mast Cells to Antigen in Sensitized Mice", Journal of Immunology, 79(3):259, 1957.

8. Cass, R., Head, J.B., and Riley, J.F., "Heparin and Histamine in Mast Cell Tumors from Dogs," Nature 174(4424):318, 1954.

9. Cavallero,C., and Braccini, C., "Effect of Cortisone on Mast Cells of the Rat", Proceedings of the Society for Experimental Biology and Medicine, 78(1):141, 1951.

10. Cornbleet, T., "Negative Test for Heparin in Mast Cells of Urticaria Pigmentosa", A.M.A. Archives of Dermatology and Syphilology 70(3):347, 1954.

11. Hunt, T.E., and Hunt, E.A., "Mitotic Activity of Mast Cells", Proceedings of the Society for Experimental Biology and Medicine, 94(1):165, 1957.

12. Junqueira, L.C.U. and Beiguelman, B., "Studies on the In-Vitro Action of Compound 48/80 , a Formaldehyde Condensation Product, on Rat Mast Cells", Texas Reports on Biology and Medicine, 13(1):69, 1955.

13. Lascano, E.F., "Mast Cells in Human Tumors", Cancer, 11(6):1110, 1958

14. Larsen, G., "The Mast Cell in the Uveal Tract of the Eye and Changes Induced by Hormones and Avitaminosis", American Journal of Ophthalmology 47(1):Part 2, Jan 1959.

15. MacDougall, J.D.B., "Experimental Studies on Neoplastic Mast Cells Cultivated in Vitro", Verhandlungen des Anatomischen Gesellschaft des Anatomischen Anzeiger, 101(6):322, 1954.

16. Mills, J., Strickland, B.A., and Paterson, J.C., "Validity of Tissue Mast Cell Counts in Post-Mortem Tissues", A.M.A. Archives of Pathology 66(3):330, 1958.

17. Moore, R. D., "Mast Cells of the Human Umbilibal Cord", American Journal of Pathology 32(6):1179, 1956.

18. Mota, I., "Mast Cells and Histamine in Rat Anaphylaxis: the Effect of Haemophilus pertussis", Nature 182(4641): 1021, 1958.

19. Norton, S., "Quantitative Determination of Mast Cell Fragmentation by Compound 48/80", British Journal of Pharmacology and Chemotherapy 9:494, 1954.

20. Norton, S. and DeBeer, E.J., "Effects of Some Antibiotics on Rat Mast Cells in Vitro", Archives Internationales de Pharmacodynamie et de Therapie 102(3):352, 1955.

21. Padawer, J. and Gordon, A.S., "Isolation of Mast Cells from Other Cellular Elements of Rat Peritoneal Fluid", Prodeedings of the Society for Experimental Biology and Medicine, 88(1):29, 1955.

22. Padawer, J. and Gordon A.S., "Effects of Colchicine on Mast Cells of the Rat", Proceedings of the Society for Experimental Biology and Medicine 88(4):522, 1955.

23. Parratt, J.R., and West, G.B., "5-Hydroxytryptamine and the Anaphylactoid Reaction in the Rat", Journal of Physiology 139:27, 1957.

24. Paterson, J.C., and Mills, J., "Myocardial Mast Cells in Coronary Sclerosis", A.M.A. Archives of Pathology 66-3:335, 1958

25. Petterson, T., "The Effect of X-ray Total Body Irradiation on the Mast Cell Count in the Skin", Acta Pathologica et Microbiologica Scandinavica, Supplementum 102, 1954.

26. Pollak, O.J. "Mast Cells in the Circulatory System of Man", Circulation 16(6):1084, 1957.

27. Reilly, E.B., Shintani, J., and Goodman, J., "Systemic Mast Cell Disease with Urticaria Pigmentosa", A.M.A. Archives of Dermatology 71:561, 1955.

28. Riley, J.F. and West, G.B., "Histamine in Tissue Mast Cells", Journal of Physiology 120:528, 1953.

29. Riley, J.F., "Pharmacology and Functions of Mast Cells", Pharmacological Reviews 7:267, 1955.

30. Riley, J.F. and West, G.B., "Histamine Liberation in Rat and Mouse", Archives Internationales de Pharmacodynamie et de Therapie 102(3):304, 1955.

31. Riley, J.F. The Mast Cells, E.S. Livingstone Ltd., 1959.

32. Rondanelli, E.G., "Biochemistry of Specific Granules in Mastleucoblasts, Mastleucocytes and Mast Cells in Normal and Pathological Conditions: A Cytochemical Evaluation", Revue Belge de Pathologie et de Medicine Experimentale 24(4):329, 1955.

33. Scott, K.G., Scheline, R.R., and Stone, R.S., "Mast Cells and Sarcoma Growth in the Rat", Cancer Research 18(8):927, 1958.

34. Smith, D.E., "Dynamics of Release of Histamine from Tissue Mast Cells", Science 128(3317):207, 1958.

35. Smith, D.E. and Lewis, Y.S., "Influence of Hypophysis and Adrenal Cortex upon Tisue Mast Cells of the Rat", Proceedings of the Society for Experimental Biology and Medicine 87(3):515, 1954.

36. Smyth,C. and Gum,O. "Mast Cells in Connective Tissue Diseases", Arthritis and Rheumatism 1(2):178, 1958

37. Urbach, F.D., Bell, W.N. and Jacobson, C., "Release of Heparinoid Material into the Circulation Following Direct Stimulation of Urticaria Pigmentosa Lesions", Journal of Investigative Dermatology 25(4):211, 1955.

38. Watson, W.C., "Role of Tissue Mast Cells in Experimental Atherosclerosis", British Journal of Experimental Pathology 39(5):540, 1958.

39. Wegelius, O. and Hjelmann, G., "Vital Staining of Mast Cells and Fibrocytes", Acta Pathologica et Microbiologica Scandinavica 36(4):304, 1955.

40. Wegelius, O. and Asboe-Hansen, G., "Mast Cells and Tissue Water", Experimental Cell Research 11:437, 1956.

41. Wegelius, O., Hjelmann, G., and Wasatjerna, C., "Morphological Changes Caused by Anti-Hamster Serum in Mast Cells of the Hamster", Acta Pathologica et Microbiologica Scandanavica 36(4):304, 1955.

42. Weiser, R.S., "Mechanism of Immunologic Tissue Injury", Journal of Immunology 28(6):475, 1957.

43. Westin, B., "The Influence of Some Ovarian Hormones on the Occurrence of Mast Cells in the Mouse Uterus", Acta Pathologica et Microbiologica Scandanavica 36(4):337, 1955.

44. Westin, B., and Odeblad, E., "The Acute Influence of Some Ovarian Hormones on the Occurrence of Mast Cells in the Mouse Vagina", Acta Pathologica et Microbiologica Scandanavica 39:81, 1956.

45. Benditt, E.P., "Morphology, Chemistry and Function of Mast Cells", Annals of the New York Academy of Sciences, vol. 73, art. 1, pp204-211, 1958.