THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Nicholas Eloysius Michels for the degree of Master of Arts.
They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Hal Downey
Chairman

C. P. Fitch

May 26, 1920 Thomas G. Lee

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Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Nicholas Kloysius Michels final oral examination for the degree of Master of Arts . We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 26, 1920

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The Mast Cell in Cold-blooded Animals.

A Thesis Submitted to the

Faculty of the Graduate School of the

University of Minnesota

by

Nicholas A. Michels

In partial fulfillment of the requirements

for the degree of

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THE MAST CELL IN COLD-BLOODED ANIMALS

LITERATURE.

The mast cell was discovered in the year 1877 by Ehrlich. With his pupil Waldeyer he for some time had been actively engaged in determining the effects which various anilin dyes had on the protoplasm of connective tissue cells. On one occaion whilst studying sections of the mesentery of rat, which he had stained with a basic anilin dye, Ehrlich noted peculiar granulated cells along the blood vessels which differed totally from the plasma cell just recently discovered by his pupil Waldeyer. He thereupon called this basophil granulated cell a MAST cell, because he regarded it as a sort of over-nourished connective tissue cell. Since animals were fattened with mast or acorns, he thought the term mast cell to be very appropriate. His terminology seemed to be justifiable for a short time after this Korybutt-Daszkiewicz showed that these mast granules increased in well fed frogs.

Ehrlich and his pupil Westphal thereupon made blood smears of various animals, stained them with basic dyes and came across the selfsame basophilic metachromatic granulated cells. Believing firmly in the theory that a similarity of staining reaction meant a similarity of chemistry, Ehrlich held the blood mast cells to be identical with those he had on a previous occasion found in the connective tissue.

The newly discovered cell led to further investigation. Ballowitz soon showed the fallacy of Ehrlich's notion of the mast granulation. He made a study of a field mouse which had been hibernating all winter and in spite of this long period of emaciation to which the animal had been subjected, found an abundance of mast cells. When this became known, scientists no longer regarded the mast cell granules as stored up food material. The term mast cell, however,

remained, and since Ehrlich and the men of his time did not make a distinction between the histogenous and hematogenous mast cell, the term mast cell for a long time was used indiscriminately both for the mast cells found in the tissues and for those found in the blood.

Ehrlich soon modified his conception of the nature of the mast cell. After Unna had described certain broad stainable areas in the immediate neighborhood of the mast cells, Ehrlich regarded these structures as a proof that the mast cell secrets a substance to the surrounding medium. The mast cell was now considered as being biologically and functionally equivalent to the other granulocytes. The granules were an endogenous progressive differentiation product of the cytoplasm. Ehrlich gave no details as to the origin of the mast cells, but assumed that they, like the other granulocytes, were formed in the bone marrow.

The next great discussion as to the nature of the mast cell was occasioned by the researches of Ranvier. In Anura he came across a peculiar type of cell which he called a clasmatocyte. He used that term because in the immediate neighborhood of the cell he often saw isolated basophilic granules. These he held were given off by the clasmatocyte to the surrounding medium. He described the cells as being sessile, longtitudinally stretched structures whose cytoplasm was densely filled with basophilic metachromatic granules. The cytoplasm was extremely irregular, drawn out in different directions, but did not have any anastomosing processess, differing in this respect from the fixed connective tissue elements. Subsequently Ranvier found the same cells in mammals, but here under inflammatory conditions, brought about by the injection of foreign matter into the body cavity, the clasmato-

cytes of the omentum would round themselves up and become converted into typical leucocytes. Ranvier believing firmly in the theory that similar staining granules were chemically and physiologically equivalent, identified the mammalian clasmatocyte with Ehrlich's mast cell.

Marchand studied the clasmatocytes in mammals, and opposed to the view of Ranvier, he regarded them as typical connective tissue elements. They were not foreign structures, but morphologically equivalent to the adventitial cells of the blood vessels. He agrees with Ranvier however in maintaining that these clasmatocytes have a close relationship to the lymphocytes. They may change their form, become rounded off and metamorphose into typical leucocytes.

Jolly then took up the problem to determine the exact relationship between the two types of cells. In Batrachia, Amphibia characterized by a very large quota of mast cells, he found mast cells and clasmatocytes lying close to one another, but with no anastomosing processes between them. He came to the conclusion that the "clasmatocyte" which Ranvier found in Amphibia was identical with the mast cell described by Ehrlich, but the clasmatocytes of the mammals were totally different structures. They had nothing to do with the mast cell. Pardi subsequently came to a similar conclusion. In the large omentum of mouse the two types of cells may be seen with all their differentiating characteristics.

G. Schwarz studied the question in the omentum of mammals. He claims that Ranvier was not justified in maintaining the clasmatocyte to be a distinct type of cell, since some of the Ranvierian clasmatocytes were nothing else but mast cells, others were macrophages. Dominici held the Ranvierian clasmatocytes to be identical with macrophages. Schreiber and Neumann held them to be identical with mast cells.

Maximow finally succeeded in distinguishing the two types of cells. Ranvier was right as far as the Amphibia were concerned, for here his clasmatocyte was nothing else but the mast cell of Ehrlich. But his contention as to the identity of the clasmatocyte and mast cell in mammals was false, for the clasmatocytes of the mammals had nothing to do with the mast cells. They were totally different structures. They were lymphocytes which either had originated from the blood vessels or which had preexisted in the connective tissue from embryonic times, and in later life had come to rest in the connective tissue. Hence, Maximow called them resting wandering cells.

Pappenheim then attacked the position of Maximow. He maintained that not all the mast granulated clasmatocytes of the connective tissue can be placed under the term leucocytoid wandering cells. " Maximow regards only the resting fixed cell of the connective tissue as a histogenous mast cell, while I regard also the mast granulated wandering cell of the connective tissue as a mobilized histogenous wandering cell". Pappenheim thereupon distinguished two types of histogenous wandering cells 1) sessile and 2) leucocytoid.

Maximow is usually regarded as being the first to have made a distinction between the two types of mast cells. Pappenheim, however, claims that he and Michaelis were the first to have pointed out the differences between the two types. This priority is conceeded by Maximow. Pappenheim based his view on the fact that the mast leucocytes had a polymorphic nucleus, whilst the histogenous type of mast cell had a spherical shaped nucleus and a more uniform and finer granulation. Michaelis separated the two types on the basis of a different " Mästung" or eating power which he claimed

to have observed in the two types of mast cells.

Altho Maximow conceeds that he was not the first to have made the distinction, it is evident from his writings that he more than anybody else succeeded in actually proving the distinction. Maximow regards the rabbit as the most suitable animal to study the question, for here alongside of the connective tissue mast cells one often sees mast leucocytes which have emigrated out of the blood stream into the tissues. The bone marrow of any animal will also show the two types of mast cells in their respective ontogenetic developmental stages side by side.

Maximow's researches have been so conclusive that today nearly all hematologists have accepted, at least in mammals, the presence of two distinct types of mast cells, viz. those of the tissues and those of the blood. A further subdivision of the histogenous type into sessile and wandering as advocated by Pappenheim is denied by Maximow. Türk, Helly, Levaditi and Zimmerman however, even today, will not admit a distinction between the two types. Zimmerman claims to have found intermediate stages between the two types in guinea pig. Weidenreich goes a step farther and shows that in mammals (not however in other vertebrates) there are two types of mast leucocytes, viz., the human type, and the guinea pig type.

The Histogenous Mast Cell.

It is extremely difficult to give the early literature on the tissue mast cell. The reason for this is that many authors have failed to distinguish between the two types of mast cells, or if they actually did make the distinction they failed to indicate of which type they were speaking. A common criterion for the mast cell accepted by all from the very beginning is the presence of basophilic granules which stain metachromatic with basic anilin dyes.

The granulation is usually spoken of as being coarse or fine, of varying size, shape and distribution in the cell body. An important item that helped to confuse matters was the solubility of the mast granules. This varied in the two types of mast cells; the granules of the mast leucocytes being less soluble than those of the tissue mast cell.

Very early in our literature we have indications as to the solubility of mast granules. Thus Michaelis and Arnold criticizing the light areas (Hofen) which Ehrlich and Unna saw around the mast cells, said they were pure artefacts due to the fact that the granules had dissolved in water. Weidenreich was unable to demonstrate the structures in the mast leucocytes of mammals and guinea pig, but with poor fixation and watery stains was able to demonstrate them in the mast leucocytes of Amphibia. In these animals the mast cytoplasm was extremely vulnerable and had a tendency to liquify. A similar condition was found to obtain in the mast cell of pathological human blood.

Von Wolff was the first to show that mast granules could be preserved with a 50% solution of alcohol. Maximow, however, did most to develope the proper technique for the study of mast cells. The isolated trail of mast granules found in the connective tissue by Ballowitz, Loewenthal, Schwenter-Trachsler, Zimmerman and Pappenheim, he held to be due to poor histological methods. With absolute alcohol fixation these structures do not appear. In a recent paper Maximow pleads for a total elimination of the dry smear method so prevalent amongst clinicians. An accurate representation of cytological details can be obtained only with wet preparations. The gross mistakes made by Pappenheim and his students on the nature of the mast cell he attributes to the fact that these men made dry smears,

a fact which of itself was sufficient to destroy most of the mast myelocytes, and should perchance some of them have escaped this "barbaric" method, they would surely have disappeared in toto with the subsequent May-Giemsa treatment.

The histogenous mast cell was studied in detail in different animals by Ehrlich, Waldeyer, Westphal, Ranvier, Jolly, Kanthack and Hardy, Gulland, Loewenthal, Pappenheim, Maximow, Weidenreich, Downey and Ringoen.

Practically all admit that in the adult animal they are exclusively tissue cells, having no genetic relationship with the mast leucocytes of the blood. The only common property possessed by both types of mast cells is a basophilic granulation which stains metachromatic with basic dyes. In all other respects the two types show a varied morphology. Maximow especially has shown that in all animals the tissue mast granulation varies in size, form, water solubility and tinctorial properties from that of the mast cells of the blood. The mast leucocyte is usually much smaller and has more the character of a lymphocyte. There is also a decided difference in the structure of the nucleus, that of the tissue mast cell being spherical, that of the mast leucocyte being lobulated. Other differences cannot be given, for from a comparative study of the blood of different animals we note that both the histogenous and hematogenous mast cells present decided differences in the different animals as to granules, shape and size of nucleus, number present and distribution in the organism.

The histogenous mast cell is found in the connective tissue of all mammals and vertebrates. Pappenheim even holds them to be very abundant in fish. They are extremely abundant in the rat, a large percentage of them being normally present in the peritoneal cavity of that animal. The peritoneal fluid of other animals, how-

ever does not contain tissue mast cells. As a rule the tissue mast cell is a very characteristic type of cell, having a morphology totally different from that of the mast leucocyte. Usually they are large irregular cells with an abundant quota of weak basophilic cytoplasm. The nucleus is usually compact and homogeneous in character. Its shape is at all times spherical or oval, never lobulated or horse-shoe shaped. Invariably the nucleus has a dense coarse chromatin network, which in some animals (horned toad) reaches the stage of pycnosis. The granules are basophilic and metachromatic in staining reaction; their size varies in the different animals. Generally they are finer and more uniform than those of the blood mast cell. Other morphological criteria cannot be given, since each animal has its own specific type of tissue mast cell. This is evident from a comparison of the human type of tissue mast cell with that of the rabbit. In the latter animal they are very sparse and were overlooked for a long time. Thus Westphal, Ranvier, G. Schwarz denied their presence in rabbit. Schreiber and Neumann identified the Ranvierian clasmatocyte of the rabbit with the mast cell. Maximow finally gave the reasons why they were overlooked in the rabbit, 1) the rabbit has very few histogenous mast cells, 2) the granules are extremely fine and extremely soluble in water. With proper fixation he found them to be abundantly present in the subcutaneous tissue and in the adventitial coats of the blood vessels. The general connective tissue, especially that of the bone marrow, however, contained very few histogenous mast cells. To compensate for the sparsity of the histogenous mast cell, the rabbit has very many blood mast cells which may readily migrate out into the tissues. Jolly on the other hand, found that the dog had very many connective tissue mast cells, but no blood mast cells. Pappen-

heim and Jolly maintain that the rat has many connective tissue mast cells but few blood mast cells. In some animals therefore, there seems to be a compensatory relation between the two types of mast cells in the sense that one type may act as a substitute for the other. Animals having few blood mast cells (dog, rat) have many connective tissue mast cells and vice versa animals having few histogenous mast cells (rabbit) have many blood mast cells.

Maximow found the histogenous mast cells to be ubiquitous connective tissue elements in guinea pig, hedge hog (Erinaceus),rat and mouse. They are very sparse in the bone marrow of guinea pig, yet so totally different from the mast leucocytes that it is impossible to confuse the two. Maximow also investigated various tissues of dog and cat. Here they are present in the subcutaneous and intermuscular tissue, in the omentum and mesentery and lymph nodes. They are to be found in large masses around the hair follicles and in the mucosa of the digestive tract. The bone marrow parenchyme of mouse, dog, and cat contain no connective tissue mast cells.

In the mucosa of the digestive tract and, especially around the hair follicles of rat, Maximow came across a peculiar type of connective tissue mast cell. Its morphology was so totally different from that of the ordinary type of tissue mast cell, that he was inclined to regard it as a special type of mast cell. He gave the problem to his pupil Samsonow for further investigation. Samsonow studied in detail the different wandering cells in the mucosa of the digestive tract of various mammals. He worked on the mouse, rat, rabbit, guinea pig, hedge hog, dog, cat, pig, horse, stear. He found the tissue mast cells to be distributed thruout the digestive tract. They were especially abundant in the caecum. They were so totally different from the ordinary tissue mast cell that Samsonow likewise regarded them as specific structures for the

digestive tract. The granules were not monobasophil as is usually the case, but were decidedly amphophil. The basophilic portion of the granules dissolved readily in water, whereas the acid substance remained insoluble in water. A condition therefore analogous to what Kollmann found in the leucocytes of reptiles and birds. Samsonow maintains that these specific mast cells had the power of ameboid motion, since many of them were seen forging their way thru the epithelial cells. They also had the power of homoplastic regeneration, since mitotic figures were seen. Samsonow was inclined to believe that some of these mast cells originated from ordinary lymphocytes (heteroplastically). He subjected some of the animals to various treatments. Mechanical irritation of the digestive tract had no apparent effect on the mast cells. After calomel was fed to the animals the number of mast cells increased tremendously, their cytoplasm becoming completely filled with basophilic granules. After a period of fasting, the mast cells increased in number, took on a spherical shape and their cytoplasm became filled with granules. Various food stuffs had no effect on the mast cells. Thus egg albumin caused a tremendous increase of eosinophils (which had totally dissapeared during the fasting period) but resulted in no increase of mast cells. Samsonow concluded from this that the mast cells had nothing to do with the process of digestion, but the eosinophils evidently had an important function to perform in that process.

Samsonow's discoveries probably throw some light on the observations made by R.Heidenhain and Erdely. Sectioning portions of the digestive tract of dog and staining them with triacid, Heidenhain found peculiar " red granulated" cells. He did not regard them as eosinophils, since the granules did not stain with eosin. Erdely found the same type of red granulated cell in the rat. The degree of their accumulation was dependent on nutritive conditions.

Weidenreich regards these " red granulated" structures as variations of mast cells.

Downey did considerable work on the origin of the histogenous mast cells. He showed them to be present in large numbers in the subcutaneous and intermuscular tissue and lymph nodes of adult animals. The animals selected were cat and guinea pig. In the cat's body wall large masses of them were found in the upper layer of the cutis around the hair follicles. A few of them presented developmental stages. In guinea pig he found them to be very numerous in the adventitial sheaths of the arteries of the intermuscular tissue. The abdominal wall of this animal had many mast cells of the clasmatocyte type with many intermediate stages of their development. Downey pointed out that the presence of diverse types of histogenous mast cells in one and the same animal was due to the fact that mast cells may take their origin from different types of cells, from non-granular lymphocytes, clasmatocytes, fibroblasts, adventitial cells and plasma cells. All of these cells were closely related and fundamentally belonged to the same cell line and consequently any of them could act as an independent parent cell for the mast cell.

As to the human connective tissue mast cell we have very little information. H.Rable finds them to be very abundant in the subcutaneous tissue. They are varied structures, some of them being round, others irregular and angular, others spindle shaped. The granules varied in size but the largest of them seldom measured more than 1 μ. Meirowsky found that the exposure of human skin to the action of Finsen light rays was accompanied by an increase of nucleolar material. This gradually would work its way out into the cytoplasm and there break up into fine non-metachromatic granules and finally become converted into typical pigment granules.

Meirowsky concludes from this that the mast granules are of nucleolar origin and that the mast cell is simply an intermediate stage in the development of pigment cells.

Downey worked over a large amount of pathological human material and found the mast cells to be of two types with apparent intergrades between them. 1) Lymphocytic type; 2) larger and more irregular structures (es. abundant in epithelioma). The granulation was about the same in both types, altho exceptions to this rule were found to exist. Thus in the mucous membrane of turbinate bone he found mast cells with extremely large black granules. Many of the cells having these granules were evidently undergoing a process of degeneration, since a pycnotic fragmenting nucleus was found in many of the cells. Downey therefore regarded these large black granules as products of degeneration, rather than phagocytosed material, as the first impression would incline one to believe.

Unna maintains that all granulomas are characterized by large accumulations of mast cells. In the immediate neighborhood of fusocellular sarcomas however, they are very sparse, inside of them they are entirely lacking. In a peculiar type of sarcoma described by Grosz as lymphogranuolomatosis he finds abundant masses of mast cells in the central portion of the infiltration. Some of the mast cells had two nuclei, an indication of amitotic division.

Timphus investigating the mast cells in human lymphoid tissue, found them to be rather large structures possessing a varied morphology. Some of them were round, others longtitudinal stretched elements. The different shapes assumed by the mast cells was due to the surrounding medium in which they happened to be located. The nucleus was large, always round or oval, never lobulated. Quite frequently the nucleus was peripheral in position. The mast gran-

ules were generally of the same size. In some cells they were more dense around the nucleus and had a tendency to become finer toward the peripheral portion of the cell. Mitotic figures were not seen.

According to Timphus the percentage of mast cells in the spleen and in the digestive tract was subject to great variations. In the spleen they predominated in the trabeculae and in the neighboring parenchyme; in the digestive tract they were very numerous in the submucosa and in the muscularis mucosae. Mast cells were extremely abundant in the connective tissue parts of the lymph nodes, but in the follicles they were very sparse.

Timphus found evidence for the migration of a mast cell into a blood vessel. He quotes Hirschmann as having observed a similar phenomenon. For Hirschmann found a mast cell so situated in the wall of a blood capillary that a portion of the cell was lying in the lumen of the blood vessel, the other portion was distinctly outside of the endothelial cells. At the point where the mast cell made its way thru the blood vessel one could observe a constriction of the mast cell. Hirschmann saw in this cell a direct proof that mast cells may emigrate out of the blood vessels.

Timphus maintains that, since mast cells often migrate into the lymph sinuses, and since he himself on one occasion saw a mast cell immigrate into a blood vessel, and Hirschmann on the other hand found evidence for the emigration of mast cells, we no longer can apodictically maintain a distinct separation of the histogenous and hematogenous mast cells. He claims that his discoveries call for a renewed discussion as to whether after all the mast cells in the tissues and those in the blood are really two distinct entities or as to whether we have only one mast cell, which functions equally well in the tissues and in the blood.

Regeneration

Maximow has shown that the mast cells are very labile structures. They are the first to be devoured by phagocytes and leucocytes during inflammatory conditions. In scar tissue mast cells are entirely lacking. Weidenreich maintains the mast cell cytoplasm to be very vulnerable. Since many of them dissolve in the normal organism (Schwenter-Trachsler), a means of regeneration is essential to maintain the supply necessary to the life of the animal. This may be accomplished in two ways, homoplastically and heteroplastically.

Homoplastic regeneration. Maximow holds this to be the only method for the adult animal. During embryonic life and in young animals, however, tissue mast cells are differentiated from non-granular cells. In embryonic bone marrow and in a post foetal heterotopic histogenesis of myeloid tissue of rabbit's kidney he found mast myelocytes in division. In the process of regeneration which followed the placing of a foreign body under the skin of Axolotl, he saw mitotic figures in several of the tissue mast cells. Sabrazes and Lafon found mitotic figures in the mast cells of horse. Samsonow came across similar structures in the digestive tract of mammals.

Heteroplastic regeneration. A large portion of the tissue mast cells undoubtedly take their origin from non-granular cells, or from cells containing a primitive, non-specific granulation. Samsonow in the digestive tract of mammals derives them from lymphocytes. Downey and Weidenreich in lymph node of cat derive them from small and large sized lymphocytes and plasma cells. Downey maintains that in guinea pig and cat clasmatocytes and lymphocytes differentiate into mast cells with participation of nucleus. In the guinea pig the metachromatic substance elaborated in the nucleus is passed out into the cytoplasm in the form of distinct granules. In cat

the metachromatic substance is likewise elaborated in the nucleus but is passed out into the cytoplasm in the form of a solution. Jolly and Pappenheim maintain that lymphocytes may give rise to mast cells. Heller gives evidence for the fact that emigrated lymphocytes may differentiate into mast cells in the walls of the blood vessels. Ziegler maintains the mast cells to be nothing else but polymorphic lymphocytes and clasmatocytes with metachromatic granules. Schridde's characteristic plasmamast cell with a "Radkern" comes from a plasma cell which has developed mast granules. Krompecher, Marschalko, Weishaupt, Downey and Pappenheim, all hold that plasma cells may give rise to tissue mast cells.

Sabrazes and Lafon maintain that in the horse the histogenous mast cells take their origin from various lymphocytic elements, from large mononuclears, clasmatocytes, hemic macrophages, pigment-leucocytes, and plasma cells. Whether the fusiform and branching mast cell should be traced back to the fibroblast, could not be ascertained beyond doubt. They suggested, however, that the branching type of mast cell possibly represented a metamorphosed connective tissue cell. The two authors find evidence for a direct and indirect transformation of muscle fibers into connective tissue mast cells. The direct method consisted in the elaboration of mast granules in the sarcoplasm of the muscle fiber. They give three drawings of such cells. The indirect method consisted in the fact that fragments of muscle fibers found especially abundant around the border of mast cell islands in the loose connective tissue, would gradually liberate themselves entirely from the muscular tissue and then differentiate into typical mast cells.

Sabrazes and Lafon stand alone in maintaining a direct transformation of muscle fibers into mast cells. It is not very likely

that isolated muscle fragments could differentiate into mast cells. Most probably therefore, they were describing a direct transformation of the intermuscular connective tissue cells into histogenous mast cells.

Maximow conceeds a heteroplastic development of tissue mast cells from indifferent lymphoid cells during embryonic life, but denies it for the adult organism. He maintains that recent observations of normal and pathological tissues do not justify us in maintaining that mast cells may be differentiated from lymphocytes or polyblasts. He admits the fact that polyblasts may develope metachromatic granules after they have phagocytosed certain types of cocci. But these in his opinion are not specific mast granules, but merely temporary structures due to the phagocytosed material. Whilst studying sectioned material of rabbit, guinea pig and cat Maximow found certain cells which seemed to indicate that the nucleus was actually engaged in the elaboration of mast granules. Many of the nuclei had large metachromatic particles which at times seemed to adhere to the nuclear membrane. Fearing these structures to be identical with the diffusion pictures of Ehrlich and Lazarus caused by a partial solution of the granules, Maximow failed to interpret the cells as evidences of a heteroplastic differentiation of mast cells from non-granular cells. Downey working over the problem in cat and guinea pig showed that the structures seen by Maximow were true mast cells in the process of differentiating their granules with participation of the nucleus. He showed conclusively that they were not diffusion pictures by subjecting some of the cells to watery fixation and watery stains, the mast granules dissolved and the metachromatic substance diffused back into the nucleus. But on no occasion did the substance accumulate in the form of granules,

as is the case when the nucleus elaborates the metachromatic substance. Furthermore, the metachromatic substance was usually located in distinct vacuoles; but this would certainly not have been the case if the metachromatic material were merely dissolved substance of the cytoplasm which had diffused back into the nucleus.

All mammals, vertebrates and fish (Pappenheim) have connective tissue mast cells which in adult life are regenerated by homoplastic and heteroplastic means. The cells present a varied morphology in the different animals, due to the fact that each animal has its own specific type of tissue mast cell. A further subdivision of the histogenous type of mast cell is regarded as unjustifiable by most authors. In all adult mammals the tissue mast cell is an independent type of cell, having no genetic relationship with the mast leucocytes of the blood. In bone marrow of rat, however, Maximow claims to find doubtful intergrades between the young connective tissue mast cells and the mast myelocytes. Whether we have a common stem cell for both types in the embryo is still an open question. Some are enclined to believe that there is such a common mother cell.(Dantschakoff, Maximow, Weidenreich). Maximow has shown that in some animals (rabbit) the mast leucocytes are the first to appear, the tissue mast cells appearing much later. Whether these arise from wandering cells or from mast leucocytes could not be determined. Maximow conjectures that perhaps in this case we have for the embryonic life a common basophil metachromatic granulated cell functioning as the precursor of both the histogenous and hematogenous mast cell. On the other hand, Maximow finds that in other animals (rat) the histogenous type of mast cell is the first to make its appearance. The few mast myelocytes seen in the adult organism are still lacking in the new born rat. In a third group of animals (guinea pig) Maximow found that the two types arise immediately

as two totally independent types of cells. Weidenreich maintains that during embryonic life the mast cell is an ubiquitous structure. This condition is still persistant in Amphibia, reptiles, and birds, for in these animals the mast cells of the tissue and those of the blood are identical structures. In adult mammals, however, we have a decided change in the differentiation product of the original embryonic type of mast cell. The mast cells in the tissues remain exclusively tissue mast cells; those given off to the blood stream are transformed into mast leucocytes and thruout life are normally restricted to the blood stream.

MAST LEUCOCYTES

Mast leucocytes are to be found in the blood of all animals, tho not in equal abundance. Thus the rat has very few mast leucocytes, whereas the rabbit has very many. They are very sparse in the human blood, the percentage varying from one half to one percent. Pappenheim regards a mast leucocyte count of over 1% as a sign of a pathological condition. He maintains that a natural pathological mast leucocytosis in human blood is unknown, even in skin diseases. Experimental mast leucocytoses however have been reported by Schmauch in cat (pyrodin poisoning), by Levaditi in rabbit (hemialbumose and staphylotoxis), by Schlecht in guinea pig (repeated intraperitoneal injections of sheep and horse serum).

The nature and origin of the mast leucocyte has been the subject of extensive experimental research both in this country and abroad for the last few years. The main reason for this was undoubtedly the fact that many regarded the mast cell as a degenerative type of cell. That such a notion is still prevalent is plainly evident from a perusal of a recent paper on the " Hemic Basophil" written by G. Graham.

Pappenheim is the author and chief defender of the theory that the mast cell is neither morphologically nor biologically equivalent to the eosinophils or special cells. Pappenheim's final views as to the nature of the mast cell are to be found in a recent work of his entitled " Morphologische Haematologie", a work published after his death. Speaking of mast cells in general, he says we must distinguish two types, viz., those of the tissues and those of the blood. Aside from the basophilic metachromatic granulation found in both types, they have nothing in common. The mast cell of the blood is not a true granulocyte. It is not a specific type of cell. Its granules are not a progressive differentiation product of the cell's functional paraplasm, as is the case with the other granulocytes, but represents a physiological mucoid deterioration of the functional indifferent spongioplasm. In short, the mast cells are simply various types of lymphoid cells, i.e.,lymphocytes and monocytes which have undergone a mucolipoid physiological degenerative metamorphosis of their cytoplasm. The indifferent spongioplasm degenerates perhaps under participation of nuclear derivatives. The exact reason for this is still unknown, but perhaps the phenomenon is connected with certain vegetative processes that accompany cell nutrition. The degenerative process seemingly does not injure the cell biologically, since in the case of the histogenous type of mast cell at least, mitotic division is not excluded. Hence it is still a question whether the mast cells are to be regarded as specific cells with specific functions, or whether like the plasma cells, they are to be regarded as temporary altered lymphocytes. Protelolytic ferments are entirely lacking in mast cells. On the other hand, the cells are bearers of oxydase and have the power of ameboid motion. Fahr even holds the histogenous mast cell to have the power of phagocytos-

ing bacteria. The nature of the mast granulation has not as yet been absolutely determined. We know nothing regarding the functions of the cell. The mast granulation is stainable in all basic dyes, even in vitro. The granules have such a strong affinity for basic dyes that in a mixture of basic and acid dyes they will at all times take the basic stain. Once stained with a basic dye, they will never give up the dye even after an attempted decolorization with acetic acid. Hence the granules are absolutely monobasic and cannot be stained with acid dyes. With mucicarmin they give the mucicarmin reaction. The granules are free from glycogen, are very sensitive to glycerin and extremely soluble in water. The latter characteristic Pappenheim holds to be a distinct proof that the mast granules cannot be regarded as specific functional biophores essential to the life of an animal. The cells evidently have nothing to do with the transportation of food material, since many of the cells may readily give off their granules to the surrounding medium. Most probably, therefore, the granules represent merely a product of cell metabolism.

The blood mast cells are not formed in the bone marrow, since its parenchyme is absolutely free from mast leucocytes. The mast cells seen in the bone marrow are simply wandering histogenous mast cells. The blood mast cells are formed in the blood stream. Hence the mast cells are the only blood cells, which to a certain extent are formed in the circulation; their ontogenetic precursors are the lymphocytes and monocytes. This being the case Pappenheim advocates a new terminology for the cell, viz., Mastlymphocyte, or mastmonocyte. Summing up his conclusions as to the nature of the mast cell he says:-

1) No haemopoietic tissue normally differentiates mast cells.

In the parenchyme of normal human myeloid tissue mast myelocytes and mast leucocytes are entirely lacking. If mast cells are present, they are of the histogenous type and are located in perivascular tissue. The mast leucocytes seen in normal myeloid tissue of rabbit and guinea pig are unripe eosinophils.

2) The mast cells seen in normal myeloid tissue (es. that of spleen and bone marrow) are histogenous mast cells which have originated from histogenous lymphocytes and monocytes located in the capsule and trabeculae of the respective organs. The tissue mast cell never enters into the blood stream. But since mast cells are entirely lacking in myeloid parenchyme, Pappenheim conjectures that perhaps after all the mast leucocytes of the normal blood have a histogenetic nature. He refers to the paremigration pictures seen by Hirschmann and dwells on the fact that histogenous mast cells are especially abundant in the neighborhood of blood vessels and that under pathological conditions they often accumulate in masses; thus around swellings, in the spleen during galactoschisis, in skin during urticaria pigmentosa. Yet in all these cases mast cells are not to be found in the blood.

3) The leukemic mast cell originates in myeloid tissue especially in the bone marrow from various lymphoid cells; lymphoidocytes, lymphocytaform micromyeloblasts, macrolymphoidocytes, lymphocytes, leucoblasts, leucocytes. All these various cells during the process of their ontogenetic development have undergone a mucoid degeneration of their spongioplasm. Hence they are true mast cells.

The leukemic mast cell having a quota of eosinophilic granules is therefore not to be regarded as an unripe forestage of the eosinophil, but as a true mast cell on the one hand and as a true eosinophil on the other. It is a true mast cell in so far as the

basophilic metachromatic granules are the result of a direct mucoid degeneration of the lymphoid spongioplasm, and it is a true eosinophil in so far as the remaining paraplasm has differentiated a few eosinophil granules. This type of cell (eosinophil with true mast granules) must, however, be clearly distinguished from the eosinophil myelocyte. The latter has also a basophilic granulation but it is not metachromatic in staining reaction as is the former, Hence the basophilic ametachromatic granules found in the eosinophil myelocyte is not a true mast granulation, but is simply a primitive "prodromale" a-granulation, which because of the youth and unripeness of the cell has a strong basophilic chromophilia. In short, in myelogenous leukemia Pappenheim distinguishes two types of mixed cells 1) eosinophils with true mast granules (=mucoid degeneration) 2) eosinophils with pseudo-mast granules (= unripe basophilic a-granules) The former cell contains two types of granules which have no genetic relationship between them, hence analogous to the condition that obtains in the pigment mast cell. The latter cell is simply a young unripe eosinophil.

4) In lymphadenoid leukemia pathological mast cells of lymphadenoid tissue are lacking.

5) The mast cells of normal blood take their origin from lymphoid cells in the blood stream thru a mucoid degeneration of their spongioplasm.

As to the tissue mast cells Pappenheim maintains that aside from the basophilic metachromatic granulation they have nothing in common with the mast cells of the blood. The tissue mast cells are purely monobasic and have a morphology similar to that of a lymphocyte. The granules are finer and more abundant than those of the blood mast cell. The granules may be readily dissolved in glycerin, but resist all treatment with water. Pappenheim distinguishes

four types of histogenous mast cells:-

1) Typical lymphocytoform mast cells. They are small structures having a morphology similar to that of the ordinary lymphocyte. They undoubtedly originate from histogenous lymphocytes and in later life develope their full amount of cytoplasm.

2) Wandering mast cells; these are larger structures with much larger granules. They originated from leucocytoid wandering cells.

Both of the above types are ameboid, since they may be seen in the intercellular spaces of epidermal and entodermal epithelium. In some cases they even migrate into the blood stream (Hirschbruch)

3) Sessile mast cells; these are the mast granulated clasmatocytes found abundantly along the blood vessels. They are fixed structures, which have undergone a mucoid degeneration of their spongioplasm. Clasmatocytes may give rise to small lymphocytic wandering mast cells.

The genetic relationship of these various cells Pappenheim portrays as follows:

clasmatocyte ——————→ fixed mast cell
↓
leucocytoid wandering cell → large wandering mast cell
↓
lymphocytar wandering cell → small wandering mast cell

4) Plasma mast cells of Krompecher. They are ordinary plasma cells which have undergone a further degeneration of their spongioplasm. As ordinary histogenous lymphocytes may metamorphose into histogenous mast cells, so a plasmolized lymphocyte may metamorphose into a typical mast cell. Pappenheim regards this as a distinct proof that a change in the cell's protoplasm does not necessarily involve a biological injury of the cell. For the mast cell in spite of the fact that it has undergone a deterioration of its spongioplasm is still capable of mitotic division. Another proof

that the mast cell is not a totally degenerated cell in the sense in which we regard a broken-down red corpuscle, is the fact that in myelogenous leukemia a lymphoidocyte which has undergone a mucoid degeneration of its spongioplasm (hence a true mast cell with basophilic metachromatic granules) may still differentiate eosinophil granules. These are typical eosinophils, but nevertheless have a true mast granulation. This again proves that the mast cell is not a " dead" cell. The two types of granules in this case have nothing in common, they have no genetic relationship, but coexist independently in the same cell, as is the case of two types of granules found in the pigment mast cell.

Pappenheim's reasons for regarding the mast cell as a partially degenerative type of cell are the following:-

1) The mast cell with its basophilic metachromatic granules is found to be morphologically identical in all animals. For in all animals they have the same habits of life as possessed by the lymphocytes. Mast cells never look like myelocytes or leucocytes, but at all times have the appearance of a lymphocyte, i.e., they have a small rim of protoplasm with a large nucleus. In this respect they are similar to the lymphocytes, monocytes and lymphoidocytes; for these too are absolutely identical in all animals. The special cells and the eosinophils on the other hand invariably show marked differences in the different animals both mammalian and vertebrate.

2) The second reason that led Pappenheim to his theory is the fact that the mast cells do not show a progressive ontogenetic development in their early life history, i.e., the mast cell does not take its origin from a non-granular basophilic stem cell or the so-called lymphoidocyte, and pass by way of the promyelocyte, myelocyte and metamyelocyte to the fully differentiated mast cell.

Furthermore, he claims that a true endogenous differentiation of mast granules from the cell's paraplasm, as is the case with the other granulocytes, has never been observed in mammals save in the guinea pig. But the mast cell of this animal Pappenheim holds to be a true granular leucocyte and not a genuine mast cell. In other words the guinea pig has no mast cells.

3) The mast cell differs both in its granulation and cytoplasm from the other granulocytes. The granules are not of a uniform size and shape, nor are they as uniformly distributed thruout the cell area as is the case with the other granulocytes. In some cells the granules are so extremely numerous as to obstruct the view of the nucleus entirely. In others the granules are very sparse, and located at intervals in the cell's cytoplasm. Morphologically therefore, they are similar to the azur granulation. The cytoplasm of the mast leucocyte (not however of the histogenous mast cell) is quantitatively restricted to a small protoplasmic rim around a characteristically large nucleus.

In contrast to this he finds the eosinophils and special cells to have a granulation that is uniform in size, shape and distribution, and a cytoplasm relatively large and uniform. From these differences he concludes that the mast granulation is not a true paraplastic differentiation product of the cell's cytoplasm, as is the case with the other granulocytes, but should be looked upon in the light in which we regard the azur granulation, which modern hematologists do not regard as a true granulation at all, but only as a temporary secretive product of the cell. Both myeloid and lymphocytic azur granules have not a specific character. They represent a mere temporary functional activity on the part of the cell. They are a prodromal granulation which appears at the time when the cell begins to differentiate its specific granules. Hence,

they have nothing to do with the specific granules. They do not pass over granule for granule into the specific leucocyte granules, but are eliminated in toto at the time when the true granules make their appearance.

To what extent does our present literature support Pappenheim's view as to the nature of the mast cell? As might be expected some uphold his contention, others deny it outrightly. Amongst the former are Pappenheim's students Prőscher, Benacchio, Kardos, St. Szécsi, Werzberg. Furthermore Weidenreich, R.Blumenthal and lately G. Graham.

Those who regard the mast cell as a true granulocyte with specific endogenous granules formed during the cell's ontogenetic development in the bone marrow are: Ehrlich, Westphal, E.Meyer, Naegeli, Maximow, Dantschakoff, Jolly, Türk, Ferrata, Ferrata-Golinelli, Levaditi, Helly, Downey, Ringoen.

A few words regarding the advocators of Pappenheim's view. Prőscher regarded the mast leucocytes of the rabbit as lymphocytes and lymphoid non-granular mononuclear leucocytes which have undergone a degeneration in the blood stream. The granules were a product of a mucoid degeneration of the lymphocytic spongioplasm. The granules are closely related to mucin. He found basophilic granulated myelocytes in the bone marrow, but does not regard them as the precursors of the mast cell 1) because they never get out into the circulation, and 2) because the true mast cells are formed from hemic lymphocytes. He claimed that the mast leucocyte of the rabbit is at all times mononuclear. Pappenheim supported this view and maintained that normally there are many mast leucocytes in the blood of rabbit. With saponin poisoning however, they become sparse and their place is taken by unripe eosinophils and special cells. Prőscher and Pappenheim are evidently wrong in

in maintaining a mononuclear mast leucocyte for rabbit. A superficial glance at rabbit's blood will convince one of the fact that the nuclei are polymorphic; mononuclear forms are entirely lacking.

Benacchio was unable to find mast myelocytes in the bone marrow of guinea pig and rabbit. Smears stained with May-Giemsa contained typical mononuclear cells with coarse metachromatic basophilic granules. These however in his opinion were not mast myelocytes, but unripe eosinophils or special cells. His reasons for assuming this were, 1) the granules are amphophilic, i.e., they stain not only with basic dyes but also with acid stains (indolin); true mast granules however are monobasophil. 2) He found eosinophil myelocytes having a quota of identical small basophilic metachromatic granules with all intergrades of these basophilic granules to the ripened eosinophil granules. In short, the basophilia of the granules was gradually lost. 3) The special cells likewise had similar basophilic metachromatic granules which in the later stages of ontogenetic development differentiated into ripened special granules.

Hence Benacchio concluded that the basophilic granulated leucocytes found in the blood stream of guinea pig and rabbit are either not mast cells (only unripe eosinophils) or if they are, they do not take their origin from the bone marrow, for in the parenchyme of that organ mast cells are entirely lacking.

Kardos made smears and sections of the bone marrow of guinea pig and rabbit. In the peripheral periostium of the bone marrow he found a few isolated small basophilic granulated histogenous mast cells, but in the bone marrow proper was unable to detect either mast cells or basophilic granulated cells. The eosinophils and special cells which in smears had a basophilic granulation, in

sectioned material did not take up the basophilic substance at all; hence the granules were not amphophilic but purely oxyphilic.

St. Szécsi maintained the same view as did Benacchio and Kárdos, altho the acitone-lucidol method given by him for the preparation of blood smears was subsequently used by Ringoen to prove the existence of mast myelocytes in the bone marrow of rabbit.

Pappenheim summing up the researches of his students came to the conclusion that there are two types of mast leucocytes.

a) Genuine mast cells found in the blood of man and rabbit; they originated in the blood stream.

b) Non-genuine mast cells found in the normal blood of guinea pig and under pathological conditions in the blood of rabbit. These are not as Weidenreich maintained, true granulocytes with specific basophil granules, but only unripe eosinophils or special cells which prematurely have reached the blood stream.

The theories established by Pappenheim and his students were disproven by Maximow, Downey and Ringoen. These men avoiding watery fixation and fluids proved conclusively that the mast leucocytes were true granulocytes fully equivalent in all respects to the other granular leucocytes. Maximow found mast myelocytes in the bone marrow of man,(also in several mammals), Downey in the bone marrow of guinea pig, and Ringoen in the bone marrow of rabbit.

Blumenthal holds that normally the basophilic granulated myelocytes develope into eosinophils and special cells. Under pathological conditions however, they differentiate into mast myelocytes and mast cells. He claims to have based this view on experimental and clinical observations.

Weidenreich has a peculiar conception as to the nature and formation of the mast cell. In mammals he distinguishes two types

of mast leucocytes viz., 1) the human type, and 2) the guinea pig type. The human mast leucocyte is not a true granulocyte but is a lymphocyte (mononuclear leucocyte) undergoing a process of degeneration in the blood stream. Its granules do not arise from a mucoid deterioration of the cell's spongioplasm as maintained by Pappenheim, but the granules originate from chromatin material extruded out into the cytoplasm. The nucleus is usually compact and nearly spherical in outline. Frequently it is stretched or indented, at times even lobulated and segmented. The segmentation however, does not follow a general plan as is the case with the other granulocytes, but extends around the whole peripheral surface of the nucleus causing many finger-like protrusions of chromatin material. Weidenreich maintains that portions of the nucleus are evidently cut off and pass out into the cytoplasm to be used in the elaboration of mast granules. Whether these chromatin particles actually become the granules or whether the cytoplasm cooperates in the formation of the granules could not be determined. At any rate, the nucleus is instrumental in the formation of mast granules. In leukemic blood this lobulation of the nucleus is still more pronounced. This accounts for the fact that normal human blood has a mast leucocyte with an irregular mononuclear nucleus, whereas leukemic blood has a polymorphic nucleated mast leucocyte. The shape of the nucleus, according to Weidenreich, depends on the degree in which the nucleus is giving off its chromatin material.

Other reasons that led Weidenreich to regard the human mast leucocyte as a degenerative type of cell are:-

1) Lack of centrosomes in the mast leucocytes, whereas they are always to be found in typical granulocytes.

2) The vacuolization of cytoplasm indicates a process of degeneration.

3) The granules have a marked variation in size, number present, and distribution in the cell body.

The second type of mast leucocyte recognized by Weidenreich he called typus guinea pig. A mere cursory view of this cell will convince one of the fact that in character of nucleus, size of granules, character and structure of cytoplasm and in presence of a central body, it corresponds to the other granulocytes. Weidenreich, therefore, logically derives it from the bone marrow where mast myelocytes may be seen in the process of division. Weidenreich however, does not want to universalize his notion of the degenerative character of the human mast leucocyte. It does not hold good for the connective tissue mast cell, nor for the mast leucocytes of all vertebrates, and not even for all mammals, but only for those animals which have the human type of mast leucocyte. Which animals have that type Weidenreich did not determine. If Maximow's descriptions are correct, then the hedge hog has the human type. As to the other animals, we need further research.

Maximow does not admit the existence of the two types of mast leucocytes established by Weidenreich. He regards Weidenreich's pictures to be the result of poor fixation and watery stains. With proper technique (100% alcoholic fixation and 70% alcoholic thionin stains) the human mast leucocyte has the morphology of a true granulocyte with mast myelocytes in the bone marrow. The nucleus is at all times polymorphic (vs. Pappenheim), has mostly three or four oval lobules which are connected with the main body of the nucleus by thin strands of chromatin. If the nucleus occasionally appears compact it is due to the fact that several lobules are overlapping one another. The granules are of a dark violet color, rather coarse in structure, tho some of them may approach the finer granulation of the histogenous type of mast cell. The granules

are decidedly not of the same size. Very rarely does one find a cell having equally sized granules. The differences however are meaningless since they are bridged over with intergrades. Maximow found no traces of vacuolization, nor of the run-together forms of granules, but at all times the granules were just as separated and sharply delineated as were the granules of the other granulocytes. He concludes therefore that the human mast leucocyte has not a varied morphology, since aside from a variation in the size of its granules, the cell has the same stereotyped and equally characteristic appearance as the other granulocytes.

Maximow admits that the mast leucocytes show decided differences in habits of life in the different animals. In man they are normally restricted to the blood stream; they do not get out into the tissues. There are, however, some exceptions to this general rule. Thus Maximow finds mast leucocytes to be very abundant in the connective tissue of rabbit. Since this animal has few tissue mast cells, the mast leucocytes evidently compensate for the sparsity of the former type of mast cell. Maximow failed to find mast leucocytes in the connective tissue of hedge hog, dog and guinea pig. In a quinea pig which had received several injections of hemoglobin Ringoen found polymorphic mast leucocytes to be very abundant both in the subcutaneous tissue and in the peritoneal cavity.* Maximow admits an emigration of mast leucocytes under pathological conditions, for in the process of regeneration which followed the introduction of a foreign body into the tissues of Axolotl mast leucocytes were seen in the tissues. Direct relationship between these leucocytes and the tissue type of mast cell was assumed because of the presence of transitional stages between the two.

* Unpublished observations.

Regeneration

The supply of mast leucocytes is maintained in two ways, viz., homoplastically and heteroplastically. As to the first method, Maximow pictures mitotic figures in bone marrow of man and other mammals. Levaditi, Helly, and Türk saw mast myelocytes in the bone marrow and, since they failed to distinguish between the two types of mast cells, derive both types of mast cells from that source. Ehrlich and Naegeli hold the bone marrow to be the only source of the mast leucocytes.

A heteroplastic development of mast leucocytes from non-granular lymphoid cells in the bone marrow of different animals is admitted by Levaditi, Maximow, Dantschakoff, Downey, Ringoen and many others. Weidenreich admits it for the guinea pig type, but not for the human type. All authors who regard the mast leucocyte as a degenerative type of cell, logically deny the presence of mast myelocytes or mast leucocytes in the bone marrow, save Blumenthal who admits the presence of eosinophil and special myelocytes undergoing a process of degeneration, thereby producing structures known as " mast myelocytes".

Dantschakoff expressly maintains that the mast cell is not a degenerative type of cell. The numerous mitotic figures seen in the young mast cells arising from a daughter generation of large lymphocytes in embryo of Trogidonotus natrix is sufficient to disprove Pappenheim's notion of the mast cell.

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THE COLD-BLOODED VERTEBRATES

In adult mammals practically all authors admit a separation of the mast cells into those of the tissues and those of the blood. A similar distinction is generally not held to be justifiable for the lower vertebrates.(Weidenreich, Dantschakoff,) According to these writers we have only one type of mast cell in the poikilothermous vertebrates which functions both as a tissue and blood cell. It migrates in and out of the blood stream. The different morphology assumed by the mast cell when it gets out into the tissues is due to the fact that in the tissues it has a tendency to hypertrophy, i.e., the cell increases the amount of its cytoplasm and very frequently throws out very irregular pseudopodia-like processes. The latter are clearly an evidence of the cell's ameboid activity.When the cell gets back into the blood stream however, it gradually pulls in these cytoplasmic processes, rounds up and becomes more compact in nature.

There seems to be some justification for the supposition that in non-mammals we have only one type of mast cell. Thus Weidenreich has shown that whether the mast cell occurs in the tissues or in the blood, it has at all times the same morphology of nucleus and granulation. Furthermore, we know for a fact that as we go down the scale of life the differences between blood and tissue cells becomes less pronounced. Thus in the lower vertebrates the special cell migrates out into the tissues very readily. They are as much tissue cells as they are blood cells. It does not require any special methods to bring them out of the blood stream as is the case in mammals, where the special cell is normally restricted to the blood stream. Hence in the lower vertebrates the mast cell seems to be an ubiquitous type of cell, i.e., it can function e-

qually well in the blood and in the tissues.

Maximow however maintains that in the lower vertebrates we also must distinguish two types of mast cells, viz., those of the tissues and those of the blood. Ontogenetically and morphologically they are totally distinct cells, hence have nothing in common save perhaps the presence of a similar basophilic metachromatic granulation. The latter property varies in the two types. Thus the granules of the tissue mast cell have a fine structure, stain rather lightly with basic dyes, and have an abundant distribution in the cell's cytoplasm. The mast leucocyte granules are, on the contrary, much larger and coarser structures; they are less numerous and stain more intensively, often a deep black. Normally the mast leucocytes are restricted to the blood stream; only a few of them are to be found out in the connective tissue.

Under pathological conditions, however, Maximow admits that a relationship may be established between the two types of mast cells. He found that when a foreign body was inserted into the body wall of Axolotl the connective tissue mast cells in the immediate neighborhood of the inserted object were the first to be phagocytosed by leucocytes and polyblasts which had come from the blood stream. During the process of regeneration mast leucocytes migrated out of the blood stream into the tissues and became transformed into typical connective tissue mast cells. Maximow maintains that this phenomenon was perhaps due to the incapacity on the part of the connective tissue mast cells of themselves to replace with sufficient rapidity the destroyed mast cells.

Eberhardt, a student of Maximow's studied the phenomenon of regeneration that took place in the turtle (Emys lutaria) after the introduction of a foreign body into the animal's tissues. His

conclusions were similar to those of Maximow. During the first days of the resultant aseptic inflammation, large numbers of acidophil leucocytes and lymphocytes migrated out of the blood vessels. The lymphocytes metamorphosed themselves into typical polyblasts and immediately phagocytosed the mast cells in the vacinity of the foreign body. Contemporary with the emigration of the lymphocytes we have an emigration of mast leucocytes which, the moment they get out into the tissues, have a tendency to hypertrophy and thus gradually assume the form of typical tissue mast cells. New tissue mast cells appear in the immediate neighborhood of the foreign body however only after the formation of a connective tissue capsule, which occurs two or three months after the insertion of the foreign body.

Altho Maximow insists on a separation of the mast cell into those of the blood and those of the tissues even in the lower vertebrates, his experimental researches on Axolotl, and those of Eberhardt on turtle, give evidence to the fact that a more intimate relation exists between the two types in the lower forms than is the case in mammals. The descriptions which Maximow gives of how a mast leucocyte is converted into a typical connective tissue mast cell under pathological conditions corresponds fully to the method of procedure which Weidenreich holds to be a normal process for all the lower vertebrates. Maximow gives a complete series of pictures to show the development of a mast leucocyte into a typical clasmatocyte like mast cell. In the blood stream the mast leucocyte has a relatively large nucleus and a small rim of protoplasm. When the cell leaves the blood stream the nucleus grows considerably in size, lengthens out in its longtitudinal axis; the cytoplasm undergoes a corresdponding process of hyperthrophy. As soon as the

nucleus begins to enlarge, the cytoplasm grows considerably in size and sends out numerous pseudopodia-like processes which ramify in all directions similar to the terminal arborization found in the dendritic processes of nerve cells.

In birds Eberhardt and Soluch found that under pathological conditions the mast leucocytes likewise emigrate out of the blood stream and become transformed into typical mast cells. Ranvier found the peritoneal fluid of Triton and Axolotl to contain a small percentage of mast cells similar to those found in the peritoneal cavity of rat. They differed from the ordinary connective tissue mast cell of the Amphibia only by the fact that they did not have protoplasmic processes. The difference, therefore, was similar to that which obtains between the mast cells found in the tissues and those found in the blood stream.

Dekhuyzen maintains that in frog and Triton the young blood mast cells are small structures having a spherical nucleus surrounded by a small band of cytoplasm The older mast cells, however, are much larger, have a broader cytoplasm which at times has the tendency to cut off portions of its cell body. He regards the latter as identical with the clasmatocyte of Ranvier.

Dantschakoff speaks of a close relationship that exists between the mast cells of the blood and those found in the tissues in bird and reptile embryoes. In the embryo of Trogidonotus natrix the mesenchym gives rise to large lymphocytes; these then produce a daughter generation of small lymphocytes. Some of these immediately after their first appearance develope into mast cells. There is no sharp distinction between the mast cells found in the tissues and those found in the blood stream, save perhaps the fact that in the tissues the mast cell developes a richer protoplasm and gives off many

pseudopodia-like processes, whilst the mast cells of the blood retain a uniform and spherical outline. But even this difference is pronounced only in the adult animal.

Maximow holds a common mother cell for the two types for the embryo but denies it for the adult organism. Thus in the larval stage and in young frogs with tail vestiges, he found the connective tissue to have many wandering cells. These were partly lymphocytes, and partly typical and atypical granulocytes. Connective tissue mast cells were also present which undoubtedly had developed from lymphocytes and wandering cells. Since the tissue mast cells developed from lymphocytes, they probably are morphologically and biologically equivalent to those found in the blood stream, since they too took their origin from the selfsame lymphocytes.

Werzberg in a recent paper distinguishes two types of mast cells, viz., histogenous and lymphocytoform. He studied the blood of various cold-blooded animals. Of Amphibia he investigated 16 species, 8 of Urodela and 8 of Anura. He studied 18 species of reptiles and 16 species of fish, viz., 8 teleosts and 2 cyclostomes. Mast cells were found to exist in all species of Amphibia and reptiles. They were lacking in all fish save in Carassius auratus. In all the species studied he found the mast cells to have a rather uniform and stereotyped spherical nucleus. The bi-nucleated mast cell seen by Grünberg in Siredon pisciformis, Werzberg holds to be an artefact, due to the fact that the mast granules in that animal are very abundant and at times arrange themselves in a bridge-like formation across the nuclear membrane.

In most species of Amphibia and reptiles Werzberg distinguishes two types of mast cells, which judging from their biology and morphology of cytoplasm and granules he holds to be absolutely distinct

entities. The two types with their respective characteristics are best seen in Tropidonatus tesselatus (a reptile) and in Lacerta muralis. The histogenous type of mast cell is a rather large irregular structure having an abundant quota of cytoplasm in which is scattered a profuse fine granulation. The lymphocytoform mast cells are smaller structures. They look very much like small lymphocytes, i.e., they have a relatively large nucleus and a small band of cytoplasm. The granules are coarse in nature and sparse in distribution. The two types of mast cells are absolutely distinct; they have no genetic relationship. The lymphocytoform type therefore, cannot be regarded as the ontogenetic forestage of the histogenous type. Nor is the histogenous type to be regarded as the aged stage of the young lymphocytoform type of mast cell. The marked differences in form, size and nature of the granules speak against such a hypothesis. If the theory were true, then among the histogenous types of mast cell we would have to expect the occurence of small-bodied elements with a fine granulation (= the young forestage of the histogenous) and, on the other hand, among the lymphocytoform type of mast cell we would have to expect the occurence of very broad-bodied elements with a coarse granulation (=acting as the ripened stage of the lymphocytoform). But such cells are not to be found.

Werzberg maintains that the two types are not found in all species, for some of the animals had only one type of mast cell. This was usually of the histogenous type. In some animals Werzberg claims to have found an intermediate type of mast cell. Thus in Algiroides nigropunctatus (reptile) and to some extent in Anolis principalis he found a mast cell which he holds to be an intermediate form between the histogenous and lymphocytoform types. The cell had a relatively large amount of cytoplasm but instead of

having the characteristically fine granules usually found in the histogenous type of mast cell, the cell had a coarse granulation, found only in the lymphocytoform type. The cells therefore partake of the characteristics of both types of mast cells. Of the histogenous characteristics it has the abundance of mast granules, of the lymphocytoform characteristics it has the coarse granulation. Werzberg conjectures that perhaps here we have a case of a progressive ontogenetic development of the lymphocytoform type of mast cell, perhaps the cell is an older stage of the leucocytoid lymphocytoform.

In going over his material the writer found no evidence for the division of mast cells into two distinct types as maintained by Werzberg. It is true that the morphology of the mast cells of the different animals varies a great deal, especially was this evident in the blood of horned toad. Picking out only the smallest and largest mast cells of this animal one could possibly see Werzberg's view; but if the intergrading types are given proper consideration Werzberg's contention is seen to be erroneous.

The mast cell of the lower vertebrates has not received as much attention on the part of hematologists as has the mast cell of mammals. The reasons are obvious. Still if the nature of the mast cell is to be based on conclusions derived from a comparative study of the blood of different animals, it is evident that we must begin with the lower forms. A resume of what has been accomplished in the lower vertebrates would not be amiss.

Mast cells were found in the connective tissue of the lower vertebrates by Ehrlich and Westphal. A detailed description of these structures came only when Ranvier finally described the clasmatocyte. Ranvier studying the blood of Urodela found among

the connective tissue elements a peculiar structure which he thought to be decidedly different from the ordinary type of mast cell. He regarded the cell as a cell type sui generis and called it a clasmatocyte 1) because the cell gave off portions of its cytoplasm with granules to the surrounding medium (clasmatosis), and 2) because the cell did not have as distinct a metachromatic granulation as obtains in the mast cells discovered by Ehrlich. Ranvier describes the cell as being a large irregular structure with oval nucleus. The abundant cytoplasm was spread out into numerous, pseudopodia-like, non-anastomosing processes.

Jolly, Maximow, Romitti and Pardi showed that the Ranvierian clasmatocytes in Amphibia were simply connective tissue mast cells. Maximow and Pardi gave a very good description of the connective tissue mast cells in Amphibia (= Ranvierian clasmatocytes).

Maximow studied the connective tissue mast cells in Axolotl. He found them to be extremely irregular and large cells, measuring sometimes $\frac{1}{2}$ a mm. in length. The cell has many pseudopodia which extend in all directions. The latter are usually to be found in one plane, unless the cell is a perivascular clasmatocyte. The pseudopodia are absolutely free and not connected by anastomosing processes with neighboring cells. The cytoplasm is extremely labile, at times it can hardly be seen. The granules are fine, of uniform size and distribution in the cell's body. At times however they form dense accumulations. The irregular arrangement of the pseudopodia shows clearly that the cell is capable of ameboid activity. The isolated mast granules occasionally found trailing the cell are not an indication of clasmatosis or a giving off of granule substance to the surrounding medium as Ranvier maintained, but their presence is simply due to artefact, or they are enclosed in

a portion of the cell's cytoplasm which is still connected with the main mass of the cell by non-visible and non-granular strands of cytoplasm. The nucleus of the clasmatocyte is oval or kidney shaped. Occasionally it is round. Its inner structure is hard to determine since it is usually very dark. Still in its interior one sees several longtitudinal coarse dark chromatin blocks running parallel to one another. Longtitudinal folds of the nuclear membrane are met with. A central body with a light area around it, is occasionally found to exists in several of the cells. The cells have a varied distribution thruout the connective tissue. Great masses of them are to be found in the subcutaneous and intramuscular tissue, and to a less extent in the adventitial coats of the blood vessels.

Arnold described the same type of cell in frog. He occaionally found cells having two nuclei. The nucleus had a few dark chromatin threads which had a wheel shaped arrangement. With vital stains the granules would swell and show a tendency to run together.

Pardi investigated Urodela and Anura. The clasmatocytes of these animals are nothing else but branching mast cells. He maintains that the clasmatocytes found by Ranvier in the large omentum of mammals are not morphologically analogous to the mast cells of the Amphibia (= structures which Ranvier erroneously called clasmatocytes). Pardi gives the following reasons for his contention:-

1) The granules of the mammalian clasmatocyte have not the characteristic metachromatic staining reaction possessed by typical mast granules. 2) The granules of the mast cell are usually very abundant. The granules of the clasmatocyte vary in number, form, size and distribution in the different cells. They can easily be seen with vital stains but not so easily in fixed preparations. Pardi maintains that the mammalian clasmatocyte stands in close

relationship to the small wandering cells of the connective tissue. They resemble the lymphocytes and perhaps take their origin from them. He found that in the large omentum of mouse the distinction between a clasmatocyte and a mast cell is very clear, for here both types are found side by side.

Origin of Mast Cells

As to the origin of mast cells in the lower vertebrates we know that they regenerate by homoplastic and heteroplastic means. A homoplastic regeneration is assured for Maximow saw many mitotic figures in the connective tissue mast cells of Axolotl which were undergoing a process of regenerative proliferation three or four weeks after the introduction of a foreign body.

Dantschakoff saw abundant mitotic figures in mast cells in the embryo of Trogidonotus natrix and concludes from this that the mast cell cannot be regarded as a degenerative type of cell. Arnold saw mitotic figures in the mast cells of frog.

Evidences for a heteroplastic development of mast cells from lymphoid cells has likewise been reported. Thus Werzberg in Gongylus ocellatus pictures a broad bodied large lymphocyte which has a diffuse "myeloid" nucleus (myeloblast) and a cytoplasm containing weak violet (not azur) granulation. These granules Werzberg holds to be immature mast granules.

Eberhardt maintains that the mast cells of the turtle are exclusively formed in the spleen from non-granular leucocytes. A homoplastic regeneration, however, also takes place in that organ, since occasionally mast granulated cells were seen in division.

The writer observed in the blood of hellbender a special cell with a few basophilic metachromatic mast granules. This evidently shows that in the lower vertebrates the special cell is not as

highly differentiated as it is in mammals. In the lower forms the special cell retains some of its primitive plasticity, and altho it has started to differentiate special granules, yet it can diverge into the mast cell line of blood cells. A condition, therefore, similar to that which obtains in the mammalian plasma cell.

Freidsohn maintains that in Anura and Urodela the polymorphic leucocytes, mast leucocytes and perhaps also the pigment leucocytes take their origin from morphologically similar cells, viz., the lymphocytes. Ontogenetically and phylogenetically the lymphocyte is the oldest type of cell. Hence the lymphocyte of the Amphibia is not, as Ehrlich maintained, a fully matured cell incapable of further differentiation, but must be regarded as the mother cell of both the leucocyte and erythrocyte series of blood cells.

In Amphibia the metamorphosis of a lymphocyte into a typical mast leucocyte takes place out in the circulation. During the process the parent lymphocyte grows considerably in size. The nucleus undergoes a corresponding growth, retains its rotundity and central position. Occasionally a few nuclear protrusions are developed, which however do not have the appearance of true lobules found in typical neutrophils. The first metachromatic granules to appear are small, sparse, and rather irregular in size. Gradually with the loss of the cell's basophilia, they become more numerous and assume a character similar to the granulation found in the normal human mast leucocyte. Freidsohn found no evidence for nuclear participation in the formation of mast granules as observed by Weidenreich in the development of the human mast leucocyte. According to Freidsohn the formation of the mast granules did not seem to be related in any way to the size of the cell, or to the size of the nucleus.

Aside from Freidsohn many other authors maintain that lymphocytes can differentiate into mast cells. Thus Werzberg in Hemidactylus saw evident intermediate stages between lymphocytes and mast cells. Neumann saw a development of mast cells from lymphocytes in the blood of Amphibia.

Dantschakoff in embryo of Trogidonotus natrix admits a heteropastic development of mast cells from small lymphocytes. During the first stage of blood formation in the body of the embryo the mesenchym gives rise to large wandering cells. These accumulate around the brain vesicles, aorta, thymus and thyroid glands and around the vertebrae. Thereafter these large lymphocytes give rise to small lymphocytes, some of which then immediately differentiate into mast cells, i.e., they do not pass thru the myelocyte stages.

Maximow found the same thing to be true of embryos of Salachii and Amphibia. In the larval stage and in young frogs with tail vestiges, wandering cells or lymphocytes were seen to differentiate into typical mast cells. In Batrachia Jolly says there is a genetic relationship between lymphocytes and clasmatocytes. In these animals the clasmatocytes correspond to the lymphocytes or resting wandering cells. Drzewina claims that in the lymphoid organs of Ichthyopsidae we have a differentiation of basophilic granulated cells from non-granular cells. In the mucosa of the horned toad's stomach the writer found abundant evidence for a heteroplastic development of tissue mast cells from fixed connective tissue cells.

As to the nature and origin of mast granules we have very little information. Werzberg maintains that the mast granulation of the cold-blooded vertebrates is decidedly different from that of the mammals, for in some of the lower forms the granules stain in triacid and methyl-green-pyronin. In the latter stain the methyl

green is specific for chromatin, and the pyronin stains all other basic substance of both nucleus and cytoplasm. Using that stain Werzberg noted that the mast granules of several reptiles stained with methyl green, some of them being metachromatic. From this he concluded that the granules had a nuclear origin and that they contained chromatin material, a conclusion therefore similar to the one held by Weidenreich regarding the origin of the human mast leucocyte granules.

Von Staffel and Rheindorf see a relationship between mast granules and pigment granules and maintain that mast cells can differentiate into pigment cells. They base their theory on the fact that mast granules and pigment granules were found in one cell. Pappenheim denies their contention and regards the cell as having two distinct types of granules. Meirowsky holds the two types of granules to be different developmental phases of the same nucleolar substance. Freidsohn and Weidenreich maintain that at least in Amphibia mast leucocytes and pigment leucocytes have no genetic relationship.

An opinion often voiced is that in the lower vertebrates eosinophils gradually differentiate into mast cells. Werzberg opposes that view decidedly, since in none of the bloods he studied, could he find any evidence that such a phenomenon actually took place. In Siredon pisciformis the theory could be shown to be entirely erroneous. The eosinophils and mast cells of this animal were totally distinct as to habits of life and morphology of granulation. The theory could likewise be refuted in Hemidactylus. Tho this animal's blood is literally teeming with eosinophils and small mast cells, yet there is not the slightest indication of a genetic relationship existing between the two types of cells. Even in Lacerta viridis which has only one type of mast cell, viz., lympho-

cytoform with large irregular granules, no genetic relationship between the two types was found to exist.

Arnold studying the blood of cold-blooded animals found in the blood of frog a cell which had acidophilic, basophilic and non-stainable granules. The presence of these basophilic granules in an acidophil granulated leucocyte he regarded as a regressive mutation change of the granules. Werzberg however failed to find this particular type of cell. Meirowsky came across a cell with basophilic granules which were not metachromatic in staining reaction. He regarded them as unripe mast cells, corresponding therefore to the unripe eosinophils of Pappenheim.

In birds Dantschakoff distinguishes two types of eosinophils, viz., 1) spherical and 2) rod shaped or crystalloid granulated elements. The spherical granules are amphophilic in staining reaction, i.e., they stain not only in acid but also in basic dyes. Since the spherical granules gradually metamorphose into the crystalloid type, she assumes that the acidophil leucocytes in their ontogenetic development from primitive lymphocytes, pass thru a basophilic metachromatic stage. As far as the metachromatic staining reaction and water solubility of granules is concerned, the spherical granules are closely related to the ordinary type of mast granules.

Hirschfeld-Kassman studied the blood of Emys lutaria. Blood smears stained with May-Grünwald contained typical mast cells having a fine but dense granulation. In addition to these she found non-granular structures having a few isolated basophilic granules. She conjectures that these cells might perhaps function as transition cells between the non-granular cell and the mast cell.

Material and Methods

The present investigation was restricted to a few representatives of the cold-blooded animals. Of Amphibia the writer investigated frog, Amblystoma, hellbender (Cryptobranchus alleghaniensis) and mudpuppy (Necturus maculosus). Of Reptiles, turtle (Chrysemys picta) garter snake, and horned toad (Phrynosoma coronatum).

In studying the blood of the lower vertebrates the writer took special care to follow out the technique prescribed by Maximow for the preservation of mast granules. A summary of the technique given by Maximow in his paper of 1913 is as follows:

From beginning to end one should avoid everything below 70% alcohol, for even after a 100% alcoholic fixation a short stay in water will distort the mast granules and lead to run-together forms. Slides containing the blood smears should be dropped into the absolute alcohol with blood films to the top. They may be left in the alcohol for several days until ready to stain. This should be accomplished with a 70% solution of alcoholic thionin. But since alcoholic thionin gives poor results as to nuclear structures, the solution should be rectified by adding two drops of a 2% solution of sodium carbonate to every 10 c.c. of the alcoholic thionin solution. The stain should stand for 24 hours during which time a precipitate is formed. The solution is good for two or three weeks, but should be filtered each time before use. Stain from 10 to 20 minutes and differentiate in 100% alcohol.

The study of the frog was restricted to the blood, bone marrow subcutaneous tissue and spleen. The writer himself made only a few preparations of the blood and marrow of frog. Considerable time and energy was saved thru the courtesy of Dr. Ringoen who loaned the writer some 30 slides of frog blood and marrow. Ringoen made

his preparations by a great variety of methods. Ordinary dry smears were stained in Wright's stain, in Johnson's modification of Wright's, in Harris' modification of Romanowsky. Others were fixed in heat and stained with Ehrlich's triacid. Wet and dry smears of both blood and marrow were prepared by the acetone-lucidol method and subsequently stained in May-Giema.

Four frogs were used in the study of the spleen. Contrary to the usual method employed, the spleen was not sectioned. The reason for this procedure was the fact that in sectioned material the mast cells do not flatten out as they do in smears but remain relatively small and compact. In sections one seldom gets the complete morphology of an individual blood cell. With the " abklatsch" method however the whole cell comes to view and the details of cytoplasmic, nuclear and granular structure can readily be ascertained. After the spleen of frog had been removed, the blood which had accumulated on the surface of the organ was washed off with a 70% solution of alcohol. Thereupon the spleen was cut into four pieces, each of which was gently drawn over a cover glass. The preparations were left to dry, then without previous fixation, stained in Wright's blood stain. With this method the mast cells were well preserved, in spite of the fact that in blood smears of the same animal stained in Wright's stain, the mast cells were extremely distorted, if not totally dissolved.

The preparations of the frog's subcutaneous tissue were made by the department's technician. They were fixed in absolute alcohol, and stained in an 80% solution of alcoholic thionin to which had been added a 2% solution of sodium carbonate according to the method of Maximow.

Blood smears of hellbender and mudpuppy were likewise made by the department's technician. They were dry smears stained in Wright's stain and in Harris' modification of Romanowsky.

Smears of the blood of Amblystoma were made in the usual manner and stained in Wright's stain, in Johnson's, in May-Grünwald etc. These preparations however did not give as good results as those prepared according to the technique prescribed by Maximow.

Portions of the mesentery of Amblystoma and hellbender were prepared as follows. In situ the digestive tract was cut into three different parts. These were then removed, stretched and fastened by means of porcupine quills on a cork block. To prevent any danger of drying the preparations were immediately submerged into a solution of absolute alcohol. Fixation lasted from one to two hours. The mesentery proper was then removed from the digestive tract, stained from 15 to 20 minutes in an 80% solution of alcoholic thionin to which had been added a 2% solution of sodium carbonate, (2 drops to 10 c.c. of alcoholic thionin). The pieces were then differentiated in 95% alcohol and by way of 100% alcohol and xylol enclosed in damar. The mesentery was not sectioned, since by stretching the tissue fairly thin preparations were obtained. Several pieces of the mesentery were subjected to watery stains such as Dominici's and Mayer's hemalum but gave no results, since the mast granules were dissolved by the action of the water.

The inflated and ligatured lungs of Amblystoma and hellbender were fixed in absolute alcohol. To maintain an even fixation a small watch glass was used which had been filled to the top with the absolute alcohol. Fixation lasted from one to two hours. Portions of the lung were then stained in a 70% solution of alcoholic thionin (+the sodium carbonate ingredient); others were stained in

Dominici's eosin-orange G- toluidin blue.

To ascertain differences of cell morphology one of the Hellbender's inflated lungs was rolled into a ball, imbedded and sectioned. The work however proved futile since no mast cells were found to exist in the lung of that animal.

Portions of the liver, stomach, intestine and body wall of Amblystoma were fixed in two ways. One batch was fixed in Helly's fixing fluid for six hours, then washed in running water for 12 hours and imbedded in the usual manner. Other pieces were fixed in absolute alcohol for four hours, then transferred into 95% alcohol over night and imbedded on the following day. The latter method gave the best results. The various blocks were sectioned from 6 to 10 μ in thickness and the sections stained in an 80% solution of alcoholic thionin.

Pieces of the Amblystoma's skin and a portion of the underlying subcutaneous tissue were fixed in absolute alcohol, stained in alcoholic thionin, but gave no results. Mast cells were entirely lacking.

Portions of the digestive tract of Hellbender were fixed in absolute alcohol, imbedded and sectioned. The sections were stained in 80% alcoholic thionin, but gave no results. In 15 slides not a single mast cell was to be seen.

In studying the blood of reptiles the same general plan was followed. Dry blood smears of garter snake and turtle were loaned thru the courtesy of the biology department. Those of the garter snake were stained in Wright's, those of the turtle were stained in Harris' modification of Romanowsky and in Wright's stain. Since the watery stains had distorted most of the mast cells, several blood smears of the turtle were prepared according to Maximow's

technique with excellent results.

The writer found it exceedingly difficult to get good preparations of the blood and marrow of horned toad. Various stains such as Wright's, May-Grünwald, May-Giemsa etc. failed to produce clear slides. To offset this trouble the time element in the staining process was shortened and finally fairly well preserved preparations were obtained with Johnson's modification of Wright's. A still greater difficulty was met with in making the bone marrow smears. After many unsucessful attempts with various stains a shortening in the staining time of Johnson's gave a good preparation. The shortening of the time element however caused the hemoglobin of the red corpuscles and the nuclear structures to remain indistinct.

The mesentery and lung of horned toad were fixed by the same method used for the mesentery and lung of Amblystoma. Portions were stained in 80% alcoholic thionin to which had been added a 2% solution of sodium carbonate. Other pieces were stained in Dominici's but gave no results, as the watery stain dissolved the mast granules.

Portions of the tongue, liver, spleen, stomach and intestine and body wall of horned toad were fixed in Helly's, others were fixed in absolute alcohol. The sections were cut from 7 to 10 μ in thickness and stained in an 80% alcoholic solution of thionin (+ the sodium carbonate). The best preparations were obtained from material fixed in absolute alcohol.

The mesentery of the turtle was fixed by the method employed for the mesentery of Amblystoma. Several pieces were then stained in a 50%, others in an 80% solution of alcoholic thionin (+ the sodium carbonate). Preparations which were left in an 80% solution of alcoholic thionin (without the sodium carbonate ingredient) over

night, showed no apparent difference from those prepared by the quick method.

Portions of the liver, spleen and digestive tract were fixed in absolute alcohol, imbedded and sectioned from 4 to 10μ in thickness. Preparations were stained in 80% alcoholic thionin (+ the sodium carbonate), differentiated in 95% alcohol and by way of 100% alcohol and xylol enclosed in damar.

The spleen showed an enormous number of mast cells. Since most of them were distorted, attempts were made to stain several sections in a 95% solution of alcoholic thionin. The attempt however proved futile, since with that solution not a single mast cell was to be seen. Evidently the thionin does not dissolve in a 95% solution of alcohol. Maximow's warning as to the solubility of mast granules was strikingly illustrated in sections of the spleen stained with Dominici's. In sections treated with that stain the mast granules were dissolved to such an extent that not a trace of the metachromatic substance was to be seen.

Preparations of the turtle's subcutaneous tissue were prepared as follows. Several c.c. of a normal salt solution were injected under the skin. After a lapse of three minutes several pieces of the now adematous tissue were removed, spread on # cover glasses, fixed in absolute alcohol for ½ hour and stained in an 80% solution of alcoholic thionin (+ the sodium carbonate). The material contained thousands of mast cells (tissue) most of which had well preserved granules.

Observations

Observations made on the present material showed a great variation in the solubility of the mast gramule substance. In some animals the mast granules were well preserved, in others they were extremely distorted, if not totally dissolved even after a 100% alcoholic fixation and an 80% alcoholic stain. This evidently proves Maximow's former contention that the solubility of the mast granules varies in the different animals. The writer found that it varies in the same animal. For in the horned toad, snake and especially in the turtle some of the mast cells were well preserved, others were totally distorted by the small amount of water still present in the alcohol.

Why some mast granules should be more soluble than others it is hard to say. Perhaps we have here a condition similar to that which obtains in the reaction of the erythrocyte lipoid membrane to hypotonic salt solutions. When red corpuscles are treated with such a sulution some of them break up more readily than others. From this we conclude that the power of resistance varies in the different red cells. Something similar may be the case with the mast granules.

In spite of variations in chemical ar physical structure all mast granules may, nevertheless, have the same physiological function. The condition would then be analogous to that which exists in the differences of muscular tissue of different animals. We know for a fact that lamb chops taste differently than beef, i.e., the chemistry of the former is different from that of the latter, still in both animals the muscle fibers have the same function, viz contraction. Hence a difference of chemistry need not necessarily imply a difference of physiological function.

Frog

Blood: Mast cells were fairly abundant in the blood of frog. Since the various stains used produced different morphological pictures, considerable difficulty was encountered in ascertaining the true morphology of the cell. Smears stained in Wright's gave the best results as to cytoplasmic and granular structures. Nuclear structures were for the most part indistinct. The watery stain, while leaving some of the mast granules intact, caused others to become lumped together in irregular masses. The cell shown in fig. 29 represents the most common type of mast leucocyte. In most cells the granules were so numerous as to obliterate the view of the nucleus. Broken cells (fig. 28) and those obtained from the smears of spleen (fig. 10) allowed a closer study of both granules and nucleus. In slides prepared by the acetone-lucidol method, the mast leucocytes usually appeared as one solid mass of dark metachromatic substance (fig.21) At the periphery of the cell the structure of the granules could be seen rather clearly. Even with this method some of the mast granules were dissolved (fig.22). The heteroplastic development of mast leucocytes from lymphocytes reported by Freidsohn was best corroborated in slides stained in Wright's. The first granules to appear in the lymphocytes were basic in staining reaction and with further differentiation assumed a typical metachromatic tone.

Bone-marrow:- Marquis and Weidenreich maintain that the parenchym of frog marrow contains very few mast cells. This observation is evidently correct, since the microscopic field had to be changed several hundred times before a mast leucocyte was seen. In slides stained with Wright's the mast granules were extremely distorted, if not totally dissolved. In smears prepared with the ace-

tone-lucidol-May-Giemsa method the mast cell appeared as a dense black mass of metachromatic substance (fig.24). Characteristic was the presence of enormous numbers of special cells, which with the latter method showed a fine dust-like azur granulation (Jordan). Mast myelocytes were not seen. Their absence indicates an exclusive origin of the cells in the blood stream. The histogenous or lymphocitoform type of mast cell reported as present in the blood of some of the lower forms by Werzberg was found to be lacking both in the blood and marrow of frog.

Spleen:- Ordinarily the spleen of frog contains very few mast cells. In one case however such large masses of them were present (150 in a single low power field) as to approach the condition of a local mast leucocytâsis. The mast cells were clearly of the haematogenous type (fig.10). Their morphology was similar to that of the blood mast cells. Histogenous mast cells, found abundantly in the spleen of mammals, were entirely lacking.

Subcutaneous Tissue:- The tissue contained enormous numbers of histogenous mast cells. Most of them were clasmatocyte-like structures, others had the morphology of the lymphocytic type of mast cell. In the fully differentiated cells, (fig.9) the mast granules were extremely abundant, fine in structure but irregular in outline. Emigrated mast leucocytes were few in number and seemingly did not develope into tissue mast cells. This process was not necessary, since a heteroplastic regeneration of mast cells from fixed tissue cells was quite common. The subcutaneous tissue seemingly contained two types of fibroblasts. In one type the nucleus was very large, spherical in outline, and had only a few chromatin blocks. Its cell body was round. The second type had the morphology of a typical fibroblast, except that its nucleus was built more on the plan of

the one found in smooth muscle fibers. Figures 30-36 show various stages in the differentiation of the mast granules in both types of fibroblasts. As seen from the drawings the method of mast granule elaboration was practically the same in both series. Evidences for nuclear participation in the manufacture of mast granules as reported by Downey in guinea pig and cat, were found to be lacking.

Mesentery:- Tissue mast cells of both clasmatocyte and lymphocyte-like types with the usual fine granulation were abundantly present. A few of the fibroblasts were differentiating mast granules in a manner similar to that which takes place in the subcutaneous tissue. The lymphocytic type of mast cell, was found along the peripheral regions of the tâches laiteuses. Since many of them had only a few granules, they evidently were being formed from small lymphocytes. From the fact that many of the mast cells with the Marschalko type of nucleus were abundantly present in the small capillaries, one might conclude that these cells made their way into the blood vessels after being formed in the mesentery.

A large number of the clasmatocyte-type of mast cells showed considerable variations in size, shape and structure of nucleus (figs.37-39). Some of the nuclei were indented, others bilobed, while still others were more or less pyknàtic in nature. But since none of the cells had a typical lobulated nucleus, these variations in nuclear structures were considered to be of no special importance. A few of the mast cells, whilst having a large cell body, had a very small and densely pyknotic nucleus (fig.40). The perinuclear zone of such cells was at all times free from granules. The pictures are perhaps due to the fact that the absolute alcohol fixation caused a considerable shrinkage in the volume of the nucleus.

Amblystoma

Blood:- In the blood of this animal the eosinophils were about as numerous as are the mast cells in the blood of turtle. The percentage of mast leucocytes was about equal to that of frog. Morphological details as to cytoplasm and granules were best seen in smears prepared according to the technique of Maximow. Figure 25 shows such a cell. The mast granules, which were larger than those of the frog, were of a uniform round size and had an even distribution in the cell body. The nucleus was at all times spherical in outline, very large and central in position. Details of nuclear structures were, however, obliterated by the great abundance of mast granules. In dry smears prepared with Wright's stain some of the mast granules were totally dissolved, leaving only bands of metachromatic substance around the nucleus. In others the granules were reduced considerably in size and quantity (fig.26). The defect of this technique lay evidently not in the staining process but in the fixation. For if blood smears were first fixed with the fumes of osmic acid and then subsequently stained with Wright's, the mast granules were well preserved. With the latter method the spindle cells looked very much like mast cells. Their azur granules had a slight metachromatic tinge. But since the spindle cells were very much smaller in size, and since their granules were characteristically accumulated at the poles of the cell, a confusion of the two types of cells was excluded. There was no evidence for the heteroplastic development of mast cells from lymphocytes in the blood stream.

Mesentery:- A few clasmatocyte like mast cells with fine granules were found along the blood capillaries of the mesentery. Most of the cells were broken, the free granules being distributed at some distance from the cell body. Whether this fact was due to the

technique used, or to the phagocytic activity on the part of special cells found in the immediate vacinity of the mast cells (Maximow), could not be ascertained. Emigrated mast leucocytes and the lymphocytic type of mast cells found in the mesentery of frog were entirely lacking. Heteroplastic regeneration was not observed.

Lung:- Very few mast cells were found, but eosinophils were very numerous (Downey).

Liver:- Sections of this organ contained very many pigment cells, a large percentage of which were evidently being differentiated from the parenchymal cells of that organ. Tissue mast cells were very sparse, only four to five being found in a single section.

Digestive- Tract:- Sectioned material taken from various portions of the digestive tract showed enormous masses of clasmatocyte like mast cells. Whilst many of the cells extended into the villi, the great majority of them were located in the mucosa below the villi. Their morphology was similar to that pictured by Maximow for Axolytl. In the latter animal according to Maximow the cytoplasmic processes of the mast cells were usually located in one plane. In Amblystoma however the processes ramified in all directions. Every field was practically covered with accumulations of mast granules, but due to the fact that the section went thru the cytoplasmic processes of various cells, considerable difficulty was encountered in finding a complete cell. Finally after a prolonged search two fairly complete cells were found.

Some of the tissue mast cells (fig.45) were peculiar "spider" shaped structures. Their cytoplasmic processes were long, narrow and extremely irregular. The cells had a characteristically large oval nucleus, the chromatin of which was usually arranged in longitudinal folds. In other cells (fig.46) the cytoplasm extended into

two longtitudinal processes, which quite frequently assumed the characteristic siz-zag appearance. Their nuclei were much smaller in size and did not show as plainly the arrangement of their chromatin. In spite of the great variation in the structure of the tissue mast cells, all of them possessed a common fine and abundant metachromatic granulation.

Scattered amongst these various tissue mast cells one often noted clasmatocyte like pigment cells, the cytoplasmic processes of which would occasionally intermingle with those of neighboring mast cells. From a superficial view of such cells one might be inclined to believe that here there was a case of the pigment-mast cells reported by Meirowsky. A change of focus however would soon show that the cytoplasmic processes of the two cells were simply overlapping one another. Evidence for a local origin of tissue mast cells was not obtained.

Hellbender

Blood:- Mast leucocytes were quite frequent in the blood of this animal. The cells (fig.43) were spherical structures having large central nuclei, the chromatin arrangement of which was indistinct. The fine dust like granules had a deep dark metachromatic staining reaction and were abundantly distributed thruout the cell cytoplasm. Smears prepared with the iodine-formalin (20 seconds) - Giemsa (10minutes) method showed quite a few lymphocytes differentiating mast granules. The granules usually appeared in medium sized lymphocytes and increased in number with the progressive growth & development of the cell (figs.41-43).

Mesentery:- In all the preparations studied not a single mast cell was to be seen. Special cells however were remarkably numerc

Lung:- The organ, which was studied both in sections and in

whole mounts, contained no mast cells. In their stead I found extremely large accumulations of special cells. They were about as numerous as are the eosinophils in the lung of Amblystoma (Downey).

Digestive Tract:- Sections of the digestive tract taken at various intervals contained no mast cells. Thruout the mucosa layer the clasmatocyte-like pigment cells were about as numerous as are the tissue mast cells in the mucosa of Amblystoma.

Liver:- The organ, while having a remarkably large number of pigment cells, had no mast cells.

Since mast cells were entirely lacking in the mesentery, digestive tract, liver and lungs, the hellbender either has no tissue mast cells or else they are so sparse as to excape detection.

Mudpuppy.

Blood:- The blood cells of this animal are extremely large. Mast leucocytes were so few in number that it required several hours search before one was found. As seen from fig. 13 the cells are very large structures, having an extremely abundant quota of fine dark dust like granules. The nucleus is likewise very large, spherical in outline and shows no visible chromatin structures.

Garter Snake

Blood:- Mast leucocytes, which were rather numerously present, showed very great variations in their morphological features. The majority of them were oblong structures with excentric nuclei (fig. 63) (6
Others were more spherical in outline and had a centrally located nucleus (fig.65). In both types the nuclear structures were difficult to discern because of the great abundance of mast granules. These were usually uniform in shape and size, not very soluble in

water and varied in their staining reaction from a light red to a deep purple. Occasionally the granules showed such an avidity for the basic component of the dye that they stained in a nearly black tone (fig.64).

Heteroplastic development of mast leucocytes from small sized lymphocytes occurs quite frequently in the circulation. The cells shown in figs. 67-69 give a few stages involved. The granules when first formed in the lymphocytes were metachromatic in staining reaction and generally had the same morphology as those found in the fully matured mast cell. A second type of mast cell formation showed myelocytes in which the granules when first differentiated were evidently not metachromatic but basophilic in staining reaction. A pencil sketch of such a myelocyte is given in figure 66. Most of the granules have reached the metachromatic stage, a few however still take the basic dye. In spite of the fact that the cell has differentiated most of its granules, its nucleus is still typically lymphoidocytic in character.

Horned Toad.

Blood:- In contrast to the large numbers of mast cells found in the tissues of this animal, its blood contained a relatively small number of mast leucocytes. The cells showed a varied morphology with the different histological methods used. The mast granule substance was evidently very soluble, since with watery stains most of the metachromatic substance had diffused back into the nucleus. In smears stained with May-Grünwald, the cells appeared as very small structures and were so compact in nature (fig.44) that the details of nuclear and granular structures could not be ascertained. With alcoholic fixation and alcoholic thionin the cells were much larger, the granules uniformly round and they had an even distribution

thruout the cell body. A heteroplastic regeneration from sall lymphocytes was not observed.

Bone Marrow:- The observations made on the eosinophils of this tissue are very interesting, since they corroborate for the lower forms the findings of Maximow, Downey and Ringoen in mammals. The eosinophil granules in horned toad are true endogenous products of the cell's protoplasm and are in no way related to hemoglobin or its dissociation products as claimed by Weidenreich and others. Blood mast cells of horned toad were at all times distinguishable from the "unripe" eosinophils. The latter had a granulation which was basic but never metachromatic in staining reaction. Marrow mast leucocytes were absolutely analogous to those of the blood stream. Their granules were soluble and metachromatic in staining reaction, their nuclei spherical and for the most part indistinct. Since the marrow contained no special or mast myelocytes, a confusion of the "unripe" eosinophils with the mast cells was eo ipso excluded. The difficulties encountered by Maximow, Downey and Ringoen in distinguishing between the early eosinophil and special myelocytes, were therefore not to be contended with.

The presence of basophilic granules in eosinophil myelocytes has been reported at various times by Arnold, Hirschfeld, Hesse, Bennachio, Kardos, Maximow, Pappenheim, Downey, Ringoen and others. Downey in marrow of guinea pig showed that the first granules to appear in the eosinophil myelocytes were totally different structures (morphologically and chemically) from those found in the fully differentiated cells. During their life history the granules passed thru gradual complex chemical changes, which involved corresponding changes in size, shape and structure of the granules. The first

basophilic granules would not dissapear, but remained to differentiate into typical eosinophil granules. Ringoen in bone marrow of rabbit came to a similar conclusion, the eosinophil granules were endogenous produbts. When first formed they were indulinophilic (or basic). Soon after their appearance however the granules passed thru a progressive evolutionary process and finally when they became fully differentiated stained only with the eosin component of the dye.

In corroboration of the observations of Downey and Ringoen my findings show that the eosinophil granules in hormed toad are likewise true endogenous products of the cell's protoplasm. They have nothing to do with hemoglobin or its dissociation products. In marrow smears stained with Johnson's many of the early eosinophil myelocytes showed a preponderance of basophilic granules. The granules when first differentiated were monobasophilic. Since comparatively few cells were found having all basic granules, the granules most probably begin their process of differentiation immediately after their first appearance.

Figures 1-4 show the various stages involved in the ripening process. In the very earliest eosinophil myelocytes (fig.1) the granules were all basic in staining reaction. In some of the granules however this basophilia was more pronounced than in others. The gramules tho numerous were not uniform in size and shape, nor were they evenly distributed thruout the cell's basic cytoplasm. The cells usually had a typical myeloid nucleus, the membrane of which was often thrown into irregular folds.

Figure 2 shows a cell with a mixed type of granulation. Some of the basic granules have become oxyphilic in staining reaction, i.e. have ripened into typical eosinophil granules. The ripening process evidently does not take place simultaneously in all the granules.

Many of the small basic granules ripen immediately after their appearance; others grow considerably in size and then gradually take on the oxyphilic tint. The cytoplasm has undergone a corresponding differentiation. Its basophilia is less pronounced than in fig. 1. The nucleus is structureless and poor in chromatin.

In figure 3 practically all the granules are oxyphilic, i.e., their chemical constitution has changed to such an extent that they take only the acid component of the dye. The cell therefore is evidently in its last stages of differentiation. A few granules are still basophilic, the smaller ones representing perhaps recent differentiations (Downey). Scattered at various intervals one notices granules having a mixed tone. They are intermediate in staining reaction because they take both the acid and basic component of the heterogeneous mixture. Their presence proves conclusively that the original basic granules do not disappear in toto, but become transformed granule for granule into the fully differentiated oxyphilic granules. The cell's cytoplasm is for the most part oxyphilic in staining reaction, though faint traces of the original basophilic cytoplasm are still visible.

In the fully differentiated cell (fig.4) all the basophilin granules have acquired their full amount of oxyphilin and accordingly stain only in eosin. A comparison of the oxyphilic granules in cell 3 with those found in cell 4 will show that the process of granule differentiation is not completed at the time when the granules have acquired an affinity for the eosin of the staining mixture. The granules in the fully matured cell (fig.4) are large and mostly oblong structures, whilst those in the younger cells (fig.3) are smaller and more uniform in shape and size. This gradual and progressive change in the staining reaction and in the morphological

features of the original basophilic granules proves conclusively that they in reality are the younger granules and therefore true differentiations of the cell's protoplasm.

Figure 5 shows a typical mast leucocyte. Since relatively few of them were present in the bone marrow the vast majority of them evidently are formed in some other locality. The cells have a decided different morphology from that of the precursors of the eosinophils. Usually they are smaller in size and have a more compact granulation. The latter is at all times metachromatic in staining reaction and as seen from figure 5 very soluble in water. A still greater contrast between the unripe eosinophils and the mast leucocytes is to be found in the structure of their nuclei. In the former the nuclei are of the lymphoidocytic type, in the latter the nuclei are devoid of all visible structures. A central, less granulated zone usually indicates their position.

Spleen:- Sections of this organ contained only one or two mast cells. A remarkable contrast therefore existed between the spleen of this animal and that of the turtle which contained thousands and thousands of mast cells.

Lung:- Tissue mast cells were abundantly present, 75 to 100 being counted in every low power field. Here, as in the mesentery, the cells had a tendency to accumulate in masses along the blood vessels (fig.52). Most of the cells were of the histogenous type, a drawing of which is given in fig.48. The cells have a small dense spherical nucleus and a cytoplasm densely filled with fine and coarse granules. Evidence for emigration of mast leucocytes with subsequent hypertrophy of the cells in the tissues was quite frequent. Figure 47 shows a few stages involved in the process. Cell a is clearly

within the lumen of the blood vessel. Cell b is partly within the capillary and partly outside of it. Cell c has reached the tissue and has begun its period of growth. The intermediate stages between the emigrated mast leucocytes and the fully hypertophied tissue mast cells are seen in figs. 49–51. During this process of transformation there is a decided change in the structure of the granules (Maximow). Whilst the granules of the blood mast cell were uniform in size and shape (fig.47,a), those of the fully differentiated tissue mast cell vary in these two respects (fig.48).

Mesentery:– Enormous numbers of tissue mast cells were present in the mesentery of horned toad. Since large masses of them were found accumulated along the blood vessels many of them evidently migrated out of the blood stream. The process was essentially similar to that which takes place in the lungs.

Mopphologically the cells were oblong in outline and usually devoid of cytoplasmic processes. A few lymphocyte like mast cells were found (figs. 17–18), but since all of them had a fairly abundant quota of granules, an inference of heteroplastic development of tissue mast cells was not deemed justifiable. The mast cytoplasm was generally so densely filled with a mixture of fine and coarse granules as to obstruct all details of nuclear structures.

Great variation was found to exist in the solubility of the mast granules. In most of the cells (fig.6) the granules were well preserved; in others they were lumped together in irregular masses. The solubility of the mast granulation brought about peculiar structures. In figure 7 we have a cell which morphologically has two cytoplasmic zones, an outer zone in which the granule substance is totally diffused in the cell's cytoplasm and an inner zone still showing fairly well preserved mast granules. Figure 8 at first

sight looked like a binucleated mast cell. The picture is due to a confluence of two mast cells. The outer zone of figure 7 has fused with the corresponding zone of another cell and formed one common metachromatic zone seen in fig.8. The two nuclei and their perinuclear granules are still intact. Cells with dissolved mast granules as seen in fig.7 were few in number and were usually found in isolated patches. Their chemical composition was evidently different from that of the greater majority of mast cells, the granules of which did not dissolve. What exactly the significance of this variation in the chemistry of the granule substance is, could not be determined.

Digestive Tract:- The mucosa and the muscularis layers of the digestive tract contained very many mast cells. They were especially numerous in the vacinity of the larger blood vessels (fig.59). The material offered an excellent opportunity to study the great variations which could exist in the solubility of the mast granule substance. In most of the cells the mast granules were completely dissolved (figs.55–57) and the metachromatic substance had either diffused back into the nucleus or had accumulated in large irregular lumps. A large percentage of the cells, however, showed such a dense and well preserved granulation that all nuclear structures were totally hidden from view (fig.53). Again other cells seemed to be developing heteroplastically from small lymphocytes. A complete series of differentiating cells from those having only a few granules and a nucleus similar to that of the small lympocyte to the fully differentiated tissue mast cell is reproduced in figures 60–62. Since the majority of the mast cells however showed dissolved granules, the apparent differentiation might perhaps be due to the fact that some of the mast granules were more resistant than others to the action of the water and consequently remained intact. Small

bands of metachromatic substance found occasionally in the nuclear membranes of some of the lymphocyte-like mast cells favoured the latter interpretation. A few of the mast cells had a small and extremely pyknotic nucleus. They were evidently undergoing a process of degeneration.

Liver:- The liver contained very many pigment cells, but few mast cells. Those found were small in size, had a relatively small amount of granules and a nucleus similar to that of the surrounding hepatic cells.

Tongue:- The intermuscular tissue of the tongue of horned toad contained enormous numbers of tissue mast cells (fig.54). Morphological details of these cells however cannot be given since nearly all of them consisted simply of a diffused mass of metachromatic substance. They were so totally different from those found in the other tissues that at first sight one was inclined to believe that the horned toad had two types of tissue mast cells. But since this evidently cannot be the case, the morphological differences must be explained either by the fact that the mast granule substance is more soluble in the tissue mast cells of the tongue, or by the fact that the tongue was cut into too large pieces, thereby allowing the tissue fluid to mingle with the absolute alcohol and thus produce a total dissolution of the mast granules.

Turtle

Blood:- The various blood smears contained enormous numbers of mast cells. Since special cells were relatively few in number, this preponderance of mast leucocytes inclines one to believe that in the turtle the mast cell functions as the special cell. The morphological features of the mast leucocytes varied not only with the

different histological methods used, but also when using a single staining combination (figs.11-12). Nuclear, cytoplasmic and granular details were best seen in preparations stained according to the technique of Maximow. Smears prepared by his method showed a great variation in the size of the mast cells. The majority of them were similar to the one pictures in fig.14. Others were considerably larger in cell area (fig.16); whilst still others were smaller than a small sized lymphocyte (fig.15). In all these cells however the granules were well preserved. Tho small in size, they were abundantly distributed thruought the cell's cytoplasm.

Nuclear structures were invisible in cells having a full quota of mast granules, but could be seen plainly in the less differentiated cells. Since many of the mast cells had a typical Marschalkó type of nucleus and only a few mast granules, a heteroplastic development of mast leucocytes from small lymphocytes may have taken place. The Marschalkó type of nucleus is shown in fig. 19; the few granules in fig. 20. The remarkably small percentage of lymphocytes found in the blood of turtle favours the above interpretation..

Spleen:- Eberhardt held the spleen of turtle to be the exclusive sourge of the animal's mast cells. Here, according to him, regeneration takes place thruout life by both homoplastic and heteroplastic means. Since his paper was not available, further inquiry as to his conception of the process of regeneration was excluded. My observations on the blood of turtle however, showed that at least some of the mast cells are developed heteroplastically in the circulating blood from small sized lymphocytes.

Since the spleen contained thousands and thousands of mast cells most of which were of the lymphocytic type, Eberhardt's contention might, however, be substantially correct. The cells were usually re-

stricted to the pulp regions (fig.72) and could therefore be readily washed out into the blood stream. The mere fact, however, that mast cells were extremely abundant in the pulp regions does not justify us in concluding that that process actually took place here. Because in mammals we get many mast cells in the sinuses of the lymph nodes and in the pulp regions of the spleen and yet they do not get out into the blood stream but remain in the tissues.

Great difficulties were encountered in trying to procure well preserved mast cells. Since the thionin dye failed to dissolve in a 95% solution of alcohol, the concentration of the latter was reduced to 80%. But even with this staining combination the mast granules were either entirely distorted or had diffused back into the nucleus. Usually the cells presented a mass of diffused metachromatic substance in the center of which was located a fairly well preserved nucleus (fig.72,a) My descriptions of the mast cells therefore must of necessity be largely restricted to the morphological features of their nuclei.

In some cells the nuclei were much darker than in the neighboring lymphocytes. Such cells (fig.72,b) usually had a dark blue band different in color from that of the surrounding metachromatic substance. ,If these structures were merely diffused mast granules, they should likewise have stained in purple. The pictures therefore suggested the granule elaboration method found in guinea pig mast cells (Downey). But since the mast granules were practically totally dissolved in all the cells, that conclusion could not be drawn here. A mixture of the granule substance with the chromatin material might result in a difference of chemistry and hence bring about a difference in staining reaction.

Another item which favoured a heteroplastic development of mast

cells from lymphocytes was the great abundance of mast cells having a typical Marschalkó type of nucleus (fig.72,c) As far as nuclear structures were concerned these pulp mast cells corresponded fully to the lymphocytes found in the follicles which also had the Marschalkó type of nucleus (fig.72,d). But until such time as a proper technique has been devised to preserve the mast granules intact, the method of heteroplastic development of mast cells from lymphocytes in the spleen of turtle must remain unsolved.

Digestive Tract:- Mast cells were even more numerously present in the mucosa and muscularis layers of the digestive tract. They accumulated in large masses at the bases of the villi and extended thruout the propria (fig.73). The cells, morphologically, were similar to those found in the splenic pulp. Their granules were extremely distorted, most of them being totally dissolved. Hence all that has been said in regard to the origin of the mast cells found in the spleen might equally well be applied here.

Mesentery:- Tremendous numbers of mast cells were met with (fig.80). The great majority of them evidently came from the blood stream and hypertrophied in the tissues. Figures 74–79 show various stages involved in the process of transformation. While still in the capillaries (fig.80,a) the mast cells were very small and compact in nature. Their granules were uniform in size and distribution; their nuclei spherical in outline. In the hypertrophied cells the character of the granules has undergone a decided change. Most of them are fine, tho many of them are coarser structures. The nuclei of many of these cells have changed from a spherical to an oval outline. Evidence for heteroplastic regeneration from fixed tissue cells was entirely lacking.

Liver:— The liver contained very many pigment cells, but only a few mast cells. The latter were usually of the lymphocytic type, had comparatively few granules and nuclei simular to those found in the surrounding hepatic cells.

Subcutaneous Tissue:- Preparations of this tissue showed tremendous accumulations of tissue mast cells with remarkably well preserved granules (fig.70). Except for those found along the blood vessels, nearly all of them were comparatively large structures. The pseudopodia-like cytoplasmic processes found in many of the cells gave evidence of their ameboid motion.

Since a heteroplastic regeneration from fixed tissue cells was not observed, most of the cells undoubtedly came from the blood stream. Figure 70 shows two mast leucocytes leaving the blood stream. Cell a is evidently in its first stages of hypertrophy. In the fully matured cells (fig.71) the granules were remarkably uniform in size and distribution. Their nuclei were usually smaller and had a chromatin arrangement similar to that of the small lymphocyte.

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General Discussion.

With the exception of Türk, Helly and Levaditi all modern haematologists agree that in mammals the mast cells of the blood are different from those of the tissues. Aside from the dommon basic metachromatic staining reaction of the granules, the two kinds of cells have nothing in common either morphologically or genetically. A similar conclusion, however, is not generally made in regard to the mast cells of the lower forms. Weidenreich, Dantschakoff and others maintain that in the poikilothermous animals the mast cell is an ubiquitous type of cell, i.e., it is as much a tissue cell as it is a blood cell. The cell's varied morphology is due, according to Weidenreich, solely to the surrounding medium in which the cell happens to be located. When in the blood stream, the cell pulls in its cytoplasmic processes, rounds up and becomes compact in character. When in the tissues the cell tends to hypertrophy, sending out cytoplasmic processes in various directions. Possession of the latter, according to Weidenreich, is the only distinguishing characteristic between the two types of mast cells in the lower forms.

Opposed to this view, Maximow maintains that even in the lower vertebrates under normal conditions tissue and blood mast cells are morphologically and genetically distinct types of cells. Under pathological conditions however Maximow admits that one type may pass over into the other. A mast leucocyte may leave the blood stream, migrate out into the tissues and there by a gradual change in the structure of its granules and in the character of its nucleus become transformed into a typical histogenous mast cell.

Observations made on the present material show that the process which Maximow regards as occuring only under pathological conditions often takes place in untreated animals. I found abundant evidence

for the metamorphosis of mast leucocytes into typical tissue mast cells in the mesentery and lungs of horned toad and in the mesentery and subcutaneous tissue of turtle. The process was essentially similar to that described by Maximow for Axolotl.

Opposed to the view of Weidenreich, the writer maintains that in the fully differentiated condition tissue and blood mast cells in the lower forms are decidedly different structures and this for the following reasons:- 1) In all the material studied I never saw a tissue mast cell migrating into the blood stream. If, as Weidenreich maintains, the mast cell in the lower forms is an ubiquitous type of cell, then an occasional immigration of tissue mast cells should have been observed. 2) Mast granules are differentiated in cells which morphologically and genetically are totally distinct from blood cells, viz., in fibroblasts and clasmatocytes. The former remain for the most part fixed tissue cells. Hence at least some of the tissue mast cells in the lower forms are genetically fully analogous to the mammalian type of connective tissue mast cells.

It is commonly conceeded that in the connective tissue of mammals clasmatocytes and different types of lymphoid cells can develope different types of mast cells. The same is undoubtedly true in the lower forms. But while the tissue mast cells of the mammals are comparatively uniform in their morphological appearances, those of the cold blooded vertebrates are decidedly different in size, shape and structure. In the frog and horned toad the tissue mast cells are comparatively small and have only a few cytoplasmic processes. In Amblystoma however the 'spider' shaped histogenous mast cells are so large and their cytoplasmic processes ramify in such diverse directions, that it requires considerable search before a complete

bell can be found.

The mast leucocytes in the lower forms likewise show marked variations in size and structure, differing in this respect from the rather uniform type of mast leucocyte found in the blood of mammals,(excepting of course the guinea pig mast leucocyte). The cells vary morphologically not only in the different animals, but also in the same animal (turtle). This phenomenon is perhaps due to the fact that many of the mast cells are formed in the circulating stream from hemic lymphocytes which then subsequently grow into larger structures. In spite of the great variations in the size and structure of the turtle's blood mast cells, the writer found no evidence for Werzberg's contention that in some of the lower forms histogenous mast cells are to be found in the blood stream.

Maximow has shown that in some of the mammals the frequency of the tissue and blood mast cells vary in inverse ratio. Thus the rabbit has very few histogenous mast cells, but its blood carries a relatively high percentage of mast leucocytes. On the other hand the cat, mouse and rat have very many tissue mast cells, but relatively few blood mast cells. In mammals, therefore, a functional correlation seems to exist between the two types of mast cells.

In some of the lower vertebrates the frequency of the respective types likewise varies in an inverse ratio. Thus the Amblystoma, frog and horned toad whilst having many tissue mast cells, have a comparatively small number of blood mast cells. The inverse ratio condition, however, is not universally true. It does not apply to the mast cells of the hellbender and turtle. The turtle has enormous numbers of tissue mast cells and in spite of this, its blood carries a very high percentage of mast leucocytes. The hellbender on the other hand, has few mast leucocytes and its tissues either

have no histogenous mast cells or else they are so few in number as to escape detection.

Michaelis, Pappenheim, Maximow, Downey and others have shown that in some of the mammals the mast granules are very soluble in water. The granules of the mast leucocytes (es. those of the guinea pig) are much more resistant in this respect than those of the tissue mast cells. In the latter type of cells, however, the granules likewise show considerable variation as to their solubility. Thus in the rabbit the tissue mast cells are extremely soluble, while in man they are not.

The present study shows that in some of the lower forms the solubility of the mast granules varies not only in the different animals but also in the same animal. While all of the poikilothermous animals investigated have extremely soluble mast leucocytes, not all of them have equally soluble tissue mast cells. Thus for an example, the tissue mast cells of horned toad and turtle are for the most part extremely distorted, whilst those of the Amblystoma and frog are well preserved. Interesting is the observation that in the subcutaneous tissue of turtle the histogenous mast cells are not soluble, whilst those found in the intestinal mucosa and in the splenic pulp are extremely distorted, if not totally dissolved by the small amount of water still present in the alcohol.

As in mammals, so in the lower vertebrates the supply of the mast cells is maintained by heteroplastic means. Mast granules may be differentiated in the lymphocytes, clasmatocytes and fibroblasts of the tissues, as well as in the lymphocytes of the circulating stream. The clasmatocytic type of mast cell and the intermediate stages in their differentiation are especially abundant in the subcutaneous tissue of frog. Nuclear participation in the manufacture

of the mast granule substance does not take place in the lower forms.

A homoplastic form of regeneration by mitosis of pre-existing mast cells, generally admitted in mammals, apparently takes place very rarely in the poikilothermous animals. Maximow reports mitotic figures in the tissue mast cells of Axolotl, but in all my material I failed to come across a single mast cell in division.

Maximow, Downey and Ringoen have shown that in mammals the mast leucocytes form an independent line of granulocytes which is developed in the bone marrow from non-granular cells. Absolutely nothing was seen in the bone marrow of some of the lower forms which would indicate that a similar process is carried on there. Frog and horned toad marrow contains only a few mast cells. Typical mast myelocytes are not to be seen. The vast majority of the blood mast cells are therefore evidently formed in some other locality. In the turtle, frog and hellbender many of the mast leucocytes are formed from hemic lymphocytes. A few mast myelocytes, morphologically similar to those found in the bone marrow of mammals, are to be found in the blood of turtle. This fact seems to corroborate the contention of some authors that during the months of May, June and July the blood of the lower forms is " myeloid" in character, i.e. that all three types of granulocytes are developed in the circulating stream from non-granular cells by the gradual differentiation of granules in their cytoplasm. But since my blood smears were made during the fall and winter months, a further corroboration of that theory was eo ipso excluded.

Maximow, Downey and Ringoen have shown that in mammals the mast cells of the blood are in no way related to the eosinophils. My observations show the# same thing to be true in the lower forms. In

marrow of horned toad the fully differentiated mast cells and eosinophils are very different types of cells. This observation led to the further question whether in the lower forms the " unripe" young eosinophils are in any way related to the mast cells as claimed by Pappenheim, Benacchio, Kardos and Szecsi. Here again my observations corroborate the findings of Downey and Ringoen. The granules of the eosinophil leucocytes in marrow of horned toad are true endogenous formations. The granules when first differentiated are basophilic in staining reaction. Soon after their appearance the granules undergo gradual and complex chemical changes which involve corresponding changes in size, structure and staining reactions. The original basic granules do not disappear, but remain to become transformed granule for granule into the mature oxyphilic granules. Weidenreich's theory that the eosinophil granules are derived from hemoglobin or its dissociation products is therefore proven to be erroneous even in the lower forms.

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Summary.

The mast cells in the cold blooded vertebrates are of two distinct types, viz., those of the blood and those of the tissue. A close relationship however exists between the two, since mast leucocytes can hypertrophy into tissue mast cells.

In some of the lower forms the frequency of the two types of mast cells stands in inverse ratio, in others it does not.

Both blood and tissue mast cells show greater variations in their morphological features than they do in mammals.

A heteroplastic regeneration of mast cells from non-granular cells takes place both in the blood stream and in the tissues. In the blood the granules are differentiated in lymphocytes, in the tissues they are differentiated in fibroblasts, clasmatocytes and

lymphocytes.

A homoplastic form of regeneration by mitosis of pre-existing mast cells takes place very rarely in the lower forms.

The marrow of horned toad and frog do no contain any mast myelocytes.

The eosinophil granules in horned toad are true endogenous products of the cell's protoplasm; hence they are not related to hemoglobin or its dissociation products. The " unripe" eosinophils are chemically and morphologically different from the mast leucocytes.

Very great variations exist in the solubility of the mast cells of poikilothermous animals.

Reptiles have a larger percentage of tissue and blood mast cells than Amphibia.

Haematogenous and histogenous mast cells are so extremely numerous in turtle that the mast cell probably functions as the animal's special cell.

The mesenteries and lungs of horned toad and turtle show extraordinary accumulations of mast cells. Most of these are emigrated mast leucocytes which have become transformed into typical tissue mast cells.

Histogenous mast cells are entirely lacking in hellbender. The animal, however, has very many special cells both in its tissues and in the blood stream.

Tissue mast cells, while abundantly present in the spleen of turtle, are entirely lacking in the spleen of horned toad. Yet both animals belong to the reptile group.

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Explanation of Plates I-VII

Figures 1-8 were outlined with the camera lucida and drawn with the same magnification (save fig.8 for which drawing a Huygenian eyepiece 4 was used). Leitz achrom. objective 1/12 and Huygenian eyepiece 2. All other figures (9-80) are free hand drawings, made with an approximate same magnification.

Explanation of Plate I

Figs.1-5 from the bone marrow of horned toad. Johnson's modification of Wright's.

Figs.1-3. Eosinophil myelocytes (unripe eosinophils) in various stages of their differentiation. Notice size, shape and staining reaction of their granules and compare with those found in the mast leucocyte of fig.5. The granules of the mast cell are metachromatic, those of the unripe eosinophils are not.

Fig. 1 Early eosinophil myelocyte with basophilic cytoplasm, and basophilic granules. The granules are not uniform in size, shape, staining reaction or in distribution.

Fig.2 Shows the transformation from basophilic to eosinophilic granules. The transformation does not take place simultaneously. Some of the eosinophil granules are very small, others are nearly the size of those found in the fully matured cell of fig. 4. The nucleus is still very large, but the chromatin arrangement is indistinct.

Fig. 3. A late eosinophil myelocyte in which most of the granules have changed their staining reaction and become eosinophilic. A few of the basophilic granules are just being cut out of the cell's protoplasm. Others have grown decidedly in size and are located in colorless vacuoles. Scattered

at various intervals are granules having a mixed tone. Since their transformation is as yet not complete, they take both the acid and basic dye.

Fig. 4. Typical eosinophil leucocyte in which all the granules have become eosinophilic. Notice the large size of the granules, compare with those found in fig.3 and note that the granules may be oxyphilic before their differentiation is completed.

Fig.5. Typical mast leucocyte, comparatively few of which were found in the marrow. The water of the stain has caused most of the granules to become lumped together. Note the metachromatic color of the granules and compare with the corresponding structures in figs.1-3.

Figs.6-8 Histogenous mast cells from the mesentery of horned toad. The preparation was fixed in absolute alcohol and stained in 80% alcoholic thionin.

Fig.6 Shows the most common type of tissue mast cell. The granules are very numerous, well preserved and uniform in distribution. A few of the granules have broken away from the cell body. The nucleus is oblong in outline, its chromatin arrangement is indistinct because of the abundance of mast granules.

Fig.7 Tissue mast cell apparently showing two cytoplasmic zones, viz., an inner zone in which the granules are fairly well preserved and an outer zone in which the granules are dissolved, leaving a dense mass of metachromatic substance. The nucleus is very small and pyknotic.

Fig.8 Shows an apparent bi-nucleated mast cell produced by a confluence of two mast cells of type shown in fig. 7. The

inner zone of the two cells involved remain intact; their outer zones have fused to form a common metachromatic zone. The granules of the inner zone are fairly well preserved; those of the outer zones have become diffused with the cell's cytoplasm. The nuclei are very small, only one however is pyknotic.

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Note: Plates I-VII are filed in the archives of the Animal Biology Department.