The Lymphatic Tissue of the Kidney of Polyodon spathula

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BY

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With one plate No. VII and 2 figures.

Polyodon is a Ganoid fish found in the Mississippi river. Anatomically, it is of great interest on account of its many primitive characters and for that reason it was thought that an investigation of its chief haematopoetic organ would be of great interest to the science of Comparative Haematology which is yet in its infancy.

So little work has been done, by modern methods, on the lymphoid organs and the circulating blood of the lower vertebrates, that human and mammalian haematology is treated as though it had no relations to conditions found in the lower vertebrates. Many of the problems which now confront us in human haematology will probably not be solved until we have a clear idea of the relationships as they exist in the lower vertebrates. It is no more reasonable to treat human haematology as a separate science than it is to study the human nervous system without reference to the lower vertebrates.

Probably, the reason for this neglect of the study of the blood and lymphoid organs of the lower forms is due to the fact, that most of the work in Haematology is done by medical men, who are seeking for explanations of clinical facts.

Naturally, they would confine their work to man and the mammals, hence the general biological side of the work has been somewhat neglected.

In looking over the literature on the blood and lymphoid organs of the lower forms, especially of the fish, one finds that very little work with modern methods has been put out in recent times. The circulating blood has been more liberally treated, but the blood forming organs have been very much neglected.

The most extensive recent work on the lymphoid tissue of the fish is by Anna Drzewina. For a general survey of conditions as they exist in practically all groups of fish it is an excellent piece of work. What we need now is detailed work by modern haematological methods of single species of the lower vertebrates, and this is especially true of the blood forming organs; rather than a general survey of the whole field of which we now have a pretty good notion. If this detailed work is carried out with reference to relations as they exist in the mammals and in man it will undoubtedly contribute a great deal towards the solution of some of the problems which now confront us in mammalian Haematology.

Most of the investigators who have worked on the circulating blood of fish and other lower vertebrates, lament the fact that so little work has been done on the blood forming organs. It is for the purpose of supplying one stone in this gap that the present work on the lymphatic tissue of the kidney of Polyodon has been undertaken. It is intended to follow this work by investigations on the spleen and the circulating blood.

The literature which has a direct bearing on the subject in hand will be discussed in its proper place in the course of this article.

Technique.

Fixation. The most satisfactory results were obtained from paraffine sections of kidneys hardened in 10 % com. formalin for at least 24 hrs. Before dehydrating the material was washed in dist. H_2O for an hour or more and then dehydrated, and embedded very carefully on account of the extreme delicacy of the material. All of the other fixing fluids which are usually employed for mammalian tissues were used, but they gave very poor results. The only other fluid which preserved the granules in the leucocytes was 5 % trichloracetic acid. However, it was not satisfactory, because on experimenting with it on mammalian leucocytes it was found to change the chromaticity of the granules, whereas formol acts as a neutral fluid. Smears of the fresh kidney did not prove satisfactory on account of the size and delicacy of the cells. The delicacy of the material proved to be quite a stumbling block at first, and it was found that results could be obtained only by handling the material with extreme care. It is quite different from preparing a section of mammalian spleen or lymph gland, where rather rough methods will sometimes give fair results. Another difficulty encountered was, that

the connective tissue of the median septum of the posterior end of the kidney of large specimens is frequently calcified or even ossified, so it was necessary to prepare a great deal of material before a specimen was obtained which could be cut into satisfactory sections.

Staining. Mixtures of acid dyes which could be followed by toluidin blue proved to be very satisfactory for a good part of the work, for the reason that basophilic cystoplasm, basophilic granules, and basophilic cell inclusions have a very strong affinity for toluidin. This is specially true of the contents of the nucleus and its membrane. A better differentiation of the nuclear membrane was obtained by the use of toluidin than with iron-haematoxylin. The mixtures of this kind which proved most satisfactory were: the eosin-orange-toluidin combination recommended by Drzewina, and a mixture of fuchsin S and orange G followed by toluidin blue. Drzewina's combination is made up by dissolving 1 gm. of water eosin and 1 gm. of orange G in 200 cc dist. water. Sections were stained in this for from 10 min. to one half hour and the slide rinsed with water or alcohol, and then stained for about one minute in a $^1/_2\,^0/_0$ aq. sol. of toluidin blue. The fuchsin-orange-toluidin was made by mixing equal parts of sat. aq. sols. of fuchsin S. and orange G. Slides were stained for 5 to 10 min. in this, rinsed in dist. water and stained with aq. toluidin blue. This latter combination proved very satisfactory for demonstrating the reticulum and for staining the granules in the leucocytes which have a special affinity for acid fuchsin. The reticulum was especially well stained in sections which were stained for from 24 to 48 hours in Pfitzner's safranin and this followed by toluidin. With this method the granules of certain cells which were found along the endothelium of the blood vessels in some regions were stained very brilliantly in the safranin and occasionally, also, the granules in the cells described as secretory leucocyte, type III. The granules in these same leucocytes were very brillantly stained in safranin if this same method was preceded by Benda's iron-haematox. which was differentiated with acetic acid. Evidently the acid acted as a mordant for the saf. for, when the diluted liquor ferri sulphurici was used for differentiating the granules were not stained.

A good part of the work was done with the Wright-Jenner modification of Romanowsky, so modified that it could be used for staining sections. The method proved to be just as efficient as the Giemsa combination, and being a very much cheaper stain and a handier one to use it was used almost exclusively where a Romanowsky combination was desired. It proved to be very valuable for studying the changes in chromaticity of the granules during their various stages of evolution and in working out the development of the granules. It will show slight changes in chromaticity, corresponding to structural changes of the cell, when the other stains employed will show no variations whatever. It is not a good method for demonstrating nuclear structures. This stain was used as follows:

Sections are run down to water and the water drained off through filter paper, but without allowing the section to dry. The full strength stain is poured

on the section and allowed to remain for $2^1/_2$ min. Then dist. water is added, drop by drop, until a metallic scum just begins to appear (not too much water!) and the section left in this diluted stain for 3 min. The section is then washed for 1 min. in dist. water and passed directly to 95 % alcohol, then to 100 % and through xylol to damar. It must be passed through the alcohol as rapidly as possible.

Besides the above combinations the customary blood stains, such as, Ehrlich-Biondi, triacid, Ehrlich's triglycerine (indulin-aurantia-eosin), etc. were used.

For the purpose of tracing the general scheme of the circulation through serial sections injections were made through the caudal artery and the caudal vein.

General Anatomy.

The kidney of Polyodon extends throughout the length of the body cavity and even beyond the body cavity, anteriorly. Posteriorly, it forms a single thickened mass containing many urinary tubules and an abundant supply of lymphoid tissue between them. It is continued forward as two narrow strands of lymphoid and urinary tissue which unite anteriorly to form a thick mass of lymphoid tissue which contains no urinary tubules. This thick anterior mass is generally termed the head kidney, although in this case embryological investigations which would determine this point are lacking. Structurally, this anterior mass is simply an anterior continuation of the lymphoid tissue which is found between the urinary tubules of the rest of the organ. It is distinguished from the rest of the lymphoid tissue by the character of its reticulum; and by being divided into more or less complete lobules by connective tissue trabeculae which are derived from the capsule.

The renal portal vein runs through the dorsal portion of the posterior and middle, narrow part and gives off a series of venae renales advehentes which pass into the substance of the organ, ventrally and laterally. In the posterior part of the organ the dorsal portion is almost a solid mass of lymphoid tissue which surrounds the renal portal vein and extends for some little distance below it. A series of radii of lymphatic tissue extend from here to the ventral and lateral walls. Lymphatic tissue is found everywhere between the urinary tubules, but is more abundant in the radii than elsewhere. It also forms a thin layer on the surface; this layer being thickened at the points where the radii join it.

In the narrow, middle portion of the organ the lymphoid tissue is more evenly distributed, although it will accumulate here and there and will form strands which pass through the organ in various directions. The anterior "head kidney" is a solid mass of lymphoid tissue which does not differ from the rest, excepting in the features already pointed out.

Blood circulation.

It is hoped to give a more detailed account of the circulation and its direct relations to the lymphoid tissue is another paper, so that only a brief account will be given here.

As is general in the fish, the chief blood supply is venous. Most of the venous blood enters the organ through the caudal vein which becomes a renal portal vein and runs through the dorsal portion of the organ, bifurcating at the point where the kidney becomes divided into two narrow strands. The renal portal gives off a series of venae renales advehentes which pass ventrally and laterally into the lymphoid substance. The glomeruli seem to be about the only part of the organ which receives any considerable amount of arterial blood. They are supplied by small arterioles which are branches of small arteries from the dorsal aorta.

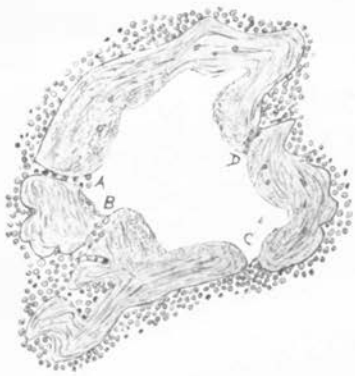


Fig. 1.
Section of a vein in the dorsal region of the kidney. The vessel is completely surrounded by lymphoid tissue. Small openings in the wall of the vessel (at A, B, C, D) furnish a direct communication between the lumen of the vessel and the surrounding lymphoid tissue.

Apparently, all of the venous blood which enters the organ through the renal portal vein must be filtered through the lymphoid pulp before it can reach the venae renales revehentes and the cardinal veins. This is probably also true of the blood in the capillaries formed by the efferent vessels from the glomeruli, although more detailed study will be required in order to determine this point with certainty.

Some of the blood in the renal portal vein can pass directly into the surrounding lymphoid tissue without first passing through the smaller branches of the vein. Occasionally an opening is seen in the wall of the portal vein which is closed only by a plug of lymphoid tissue which may extend for some little distance into the lumen of the vessel. This same relation between lymphoid tissue and vessel is found in the smaller branches of the portal vein (Text figs. 1 and 2).

It is probably this relation which accounts for the presence of so many leucocytes in the afferent venous vessels of the kidney. The venae renales advehentes lead into a series of blood spaces which are bounded only by a fibrous reticulum in the posterior end of the organ; cellular reticulum in the head kidney, which is not covered with an endothelium. It is rather coarse in the larger blood spaces (fig. 66) and very delicate in the smaller ones. Even along the larger spaces the wall is very indefinite in some places and the smaller spaces are merely more or less definite channels in the lymphoid pulp. Outside of the blood spaces the pulp contains many red corpuscles mixed in with the leucocytes, in some places fully as many

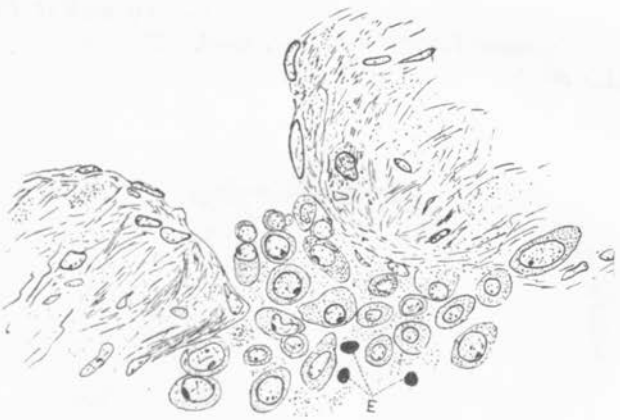


Fig. 2.
C of fig. 1 under high magnification. The passage through the wall of the vessel is lined by an endothelium. E nuclei of erythrocytes.

reds as there are leucocytes, indicating that the blood is carried into the lymphoid pulp, through which it must be filtered before it can pass into the venae renales revehentes. These latter veins are formed by the running together of many indefinite blood channels in the pulp and more or less definite spaces, all of which contain many leucocytes as well as many erythrocytes. Many of the leucocytes in the blood spaces contain haemosiderin granules and parts of erythrocytes which they have engulfed. This is specially true in the blood spaces which are connected with the efferent veins and in those which surround the kidney tubules in some parts of the section. In these latter spaces as many as 15 to 20 phagocytes containing erythrocytes and haemosiderin[1]) granules could be found in one field of the microscope (Zeiss Apoch., 2 mm, Oc. 6). These latter spaces are probably connected with efferent glomerular vessels, although this could not be determined absolutely. Phagocytosis of erythrocytes is also very active in the lymphoid pulp outside of the blood spaces.

[1]) See postscript at the end of this article.

This filtering process does not take place in all regions of the section. In some places, where the urinary tubules are close together, the lymphoid tissue is made up chiefly of non-granular leucocytes and contains no blood spaces and scarcely any red corpuscles.

It is concluded from the above, that the portal circulation of venous blood through the kidney is for the purpose of filtering the blood through the lymphoid pulp. As it passes through the pulp the worn out erythrocytes break down, or are phagocytosed; the leucocytes which are produced here are added to the blood stream; the substances produced by the secretory leucocytes are added to the blood; and the erythrocytes which are developed here are carried into the circulation.

The secretory leucocytes are very abundant in that portion of the pulp through which the blood is filtered and are not very frequent in the other regions.

The efferent blood spaces sometimes contain as many or more leucocytes as they do erythrocytes, as a glance at fig. 66 will show.

The idea of the vains functioning as lymph vessels is not a new one, as Vialleton (25) came to the same conclusion regarding certain veins leading from the kidney in Selachii. He found the veins starting from vascular lacunae which were in very close relation with the lymphoid tissue and which were bounded "only by an endothelium".

The relations as described above are of special interest to the Haematologist and the Comparative Anatomist, because they are almost identical with conditions as described by Weidenreich for the haemolymph gland and spleen. According to Weidenreich the arterial capillaries in the haemolymph glands of the sheep lead directly into spaces in the lymphoid tissue which are bounded by nothing but the surrounding lymphoid tissue and reticulum. The endothelium of the capillaries is only cellular reticulum and it communicates everywhere with the surrounding reticulum. The erythrocytes are filtered through the lymphoid tissue and pass into the blood space which is bounded, also, by a thick, fibrous reticulum which is thin and incomplete in some places. From here the blood passes back to the lymphoid tissue, through which it forms narrow channels leading into the venous lacunae. The channels are bounded only by reticulum. The blood may pass directly to the venous lacunae from the arterial capillaries, or from the blood space to the venous lacunae, but this is not its usual course. The venous lacunae contain few erythrocytes, but many leucocytes, indicating that most of the erythrocytes are destroyed in the organ and that many leucocytes are carried out by the veins. Weidenreich and Mall also believe that blood is filtered through the spleen pulp in the same way.

Weidenreich finds the reticulum in the nodules of the haemolymph glands to be purely cellular, a condition which is duplicated in the "head kidney" of Polyodon. Weidenreich further states that a fibrous reticulum developes from a protoplasmic reticulum. In a fibrous reticulum the cells which seem to be wrapped around the fibers, or to lie on them, are nothing more than the remains of the

protoplasmic cell body which was not differentiated into fibers. Accordingly, the endothelial cells which line the blood channels in the haemolymph gland, and cover the trabeculae which run across a lymph sinus of a lymph gland, are nothing more than the protoplasmic portion of reticular cells. They connect everywhere with the surrounding reticulum.

For the truth of the above described relations of endothelium and reticulum in blood and lymph sinuses the lymphoid kidney of Polyodon furnishes abundant and beautiful proof (See fig. 66 at a and c).

The above described relations of the circulation to the lymphoid tissue in the kidney of Polyodon were worked out from serial sections of injected specimens. The injection mass followed the path indicated for the blood through the lymphoid tissue. The blood spaces which immediately surround the kidney tubules in some regions of the section, did not very often get any of the injection mass, and for that reason their relation to the rest of the circulation was not very accurrately determined. Probably, these spaces are in relation with the efferent vessels of the glomeruli. The gelatine injection mass which was used did not get beyond the glomeruli and, therefore, these spaces were not injected.

A great many erythrocytes break down in the vessels of the kidney before they get into the lymphoid tissue. Fig. 60 a, b, c shows three stages in this breaking down process. Some of the vessels are almost filled with erythrocytes in the various stages of disintegration. The nucleus of a normal erythrocyte shows little structure, and is rather pale when stained with toluidin blue. The disintegrating nuclei become darker, granular, or striated, and give off lobes and small buds as shown in fig. 60 a, b and c.

No special work has been done during the course of this investigation, to determine the exact mode of origin of the erythrocytes. However, by a mere glancing over of the section one can satisfy himself that the erythrocytes are developed in the lymphoid part of the kidney. The older erythroblasts are very similar in structure to the familiar erythroblasts found in the circulating blood of frogs caught in early summer. They are large, round or slightly oval cells, having a large, perfectly round, granular nucleus, and their cytoplasm contains more or less haemoglobin, depending on the stage of development. The difference in haemoglobin percentage is shown by Wright's modification of Romanowsky. Even though the cell body is not well preserved the character of the nucleus is sufficient for determining the presence of erythroblasts among the lymphoid cells. The nucleus contains numerous, rather coarse granules which are quite evenly distributed, making the whole nucleus darker than that of the leucocytes. The older erythroblasts or young erythrocytes are smaller, and are oval or elliptical in outline; their cytoplasm is more oxyphilic; the nucleus is smaller, oval, and more homogeneous in structure. The very young erythroblasts have basophilic cytoplasm and can be distinguished from the large mononuclear leucocytes only by the character of their nucleus. The very young forms do not have as much granular chromatin

as do the older forms. It seems that there is a gradual transition from a large basophilic cell having homogeneous cytoplasm and a large, round, vesicular nucleus, to an erythroblast which gradually developes haemoglobin in its cystoplasm and in which the nucleus becomes more granular and, finally, nearly homogeneous. Further investigations will be necessary to determine the exact type of basophilic cell from which the erythroblast is developed, and to determine how this cell differs from the ordinary basophilic leucocytes of the same size and general structure.

Mitotic figures were not seen in the erythroblasts.

Older erythroblasts are found in the efferent vessels of the kidney, which shows that the final development of the erythrocytes may take place in the general circulation.

Reticulum.

It is intended to publish elsewhere a more extended and detailed account of the reticulum and its relations to the circulation, so that only a brief account of conditions as the they obtain here, without citations to the literature, will be presented at this time.

The technique which proved most satisfactory for the demonstration of the reticulum is very simple. Small pieces of the different regions of the organ were hardened in 10 % formol for three days. They were then washed in dist. water for a couple of hours and then dehydrated and embedded. Sections were stained for 48 hrs. in Pfitzner's safranin which was followed with a 1 % aq. sol. of toluidin blue for one minute. Some good results were obtained by staining first in Benda's iron-haematox. and following this with the saf. and tol. Fairly good results were also obtained by staining for 5 to 10 min. with a mixture of equal parts of sat. aq. sol's of fuchsin S and orange G, and following this with 1 % tol. for 1 min., but the most satisfactory results were obtained with the simple saf. and tol. combination.

Fig. 66 gives a good idea of the differentiation obtained by this method. Figs. 64, 65 and 67 were made from iron-haematox.-saf.-tol. preparations.

Very little connective tissue penetrates the posterior end and the middle, narrow part of the kidney, excepting where it accompanies the larger arteries and veins, forming sheaths for them. It is a very loose, white fibrous tissue, with very few elastic fibers. In the anterior, purely lymphatic end of the organ, there is a series of narrow, fibrous trabeculae which pass into the organ from the capsule. These trabeculae do not anastomose to any great extent, but pass, as narrow strands, towards the center of the organ, sometimes nearly across it.

The reticulum varies in distribution and structure in the different parts of the organ. In some parts of the posterior end, especially in the dorsal, lymphatic part of it, the reticulum seems to be entirely absent. In such regions the organ is composed chiefly of polygonal, almost colorless cells. They are generally in contact

with each other along their parallel borders and are so arranged that they appear like masses and cords of hepatic cells. These cells have been described by Drzewina for the kidney of the sturgeon.

The spaces left between the groups of these cells are filled with red corpuscles and the various types of granular and non-granular leucocytes. Their cytoplasm is colorless in most basic and acid stains and in Wright-Jenner, but may show a few very fine basophil granules and short, delicate fibrils; however, in the fuchs.-or.-tol. mixture the cytoplasm takes on a pale fuchs. color and with saf.-tol. it is a light brown. With these mixtures it is seen to be reticular, the strands of the reticulum being made up of exceedingly fine granules. The structure of the cell, excepting its general outline, is very similar to the large mononuclear illustrated in fig. 9, but the cytoplasmic reticulum and most of the fine granules have an affinity for safranin and fuchsin instead of being basophilic. The nucleus is large, round and vesicular and most of its chromatin is in small masses around the nuclear membrane. Occasionally the cytoplasmic network and the fine fibrils are slightly basophilic (with tol.) and from this, and their general structure, we may conclude that these cells are large, mononuclear leucocytes of the type shown in fig. 9 which have become slightly modified and crowded together to serve as the main supporting mass in those regions where the reticulum is particulary deficient. Without doubt they can separate from their neighbors at any time and function as leucocytes. Cells of this type are very active as phagocytes of red corpuscles, particularly in the blood spaces around the kidney tubules. These cells probably do not develop granules.

The reticulum of the posterior end and of the middle, narrow portion is chiefly fibrous, although a considerable amount of undifferentiated cytoplasm can occasionally be seen in the reticular cells. The reticulum of these regions consists of a network of branched, anastomosing fibrous strands, with the fibrils extending from one cell territory to another. Usually a small amount of granular cytoplasm can be seen around the nucleus and occasionally a very large cell will have fibrils differentiated only in its finest processes. These cells are found chiefly forming the boundary of the smaller blood spaces. Two such cells are shown in figs. 64 and 65 (from anterior end). The larger blood spaces are bounded by a very dense fibrous reticulum, which is, however, open in some places, thus allowing for communication between the blood space and the surrounding lymphoid tissue. Such a blood space (from the posterior end) with its surrounding reticulum is shown in fig. 66. By consulting the figure it will be seen at a glance that this reticulum is very fibrous. Only at three points, a, b, c, fig. 66, can undifferentiated cytoplasm be seen. The granular cytoplasm of the cell at a is continous with that of the cells on either side of it.[1]) Apparently, the fibrils have differentiated only along one side of this cytoplasmic strand.

[1]) The printed plate does not show the narrow band of undifferentiated cytoplasm along the margin of the reticulum at a which was in the original drawing.

As seen from fig. 66, the reticulum nuclei vary in size, shape and structure. All gradations are found, from small oval nuclei, like those at e, through larger triangular or irregular nuclei, like those at a and g, to large oval structures, c and f. At first glance, some of these nuclei which are located right in the wall of the blood space might be taken for endothelial nuclei; for example, those at c, e, h. However, nuclei of exactly the same type can be found out in the general reticulum, at some distance from the blood space (d, f). Absolutely no endothelial lining to these blood spaces can be demonstrated. They are lined by a fibrous reticulum, like that shown in fig. 66, which communicates with the surrounding reticulum and which does not differ from it, excepting in the thickness of its strands, or by large reticular cells (chiefly in the anterior, lymphoid end of the kidney), like those shown in figs. 64 and 65 which are fibrous only in their finer processes. Through the openings in this reticular wall the contents of the blood spaces can pass freely to and from the surrounding lymphoid tissue. This is exactly the same condition as is found by Weidenreich for the blood sinuses of the haemolymph glands of the sheep, and it certainly speaks for an "open" circulation in both cases.

Undoubtedly, all who believe that blood spaces and lymph sinuses are always lined with a distinct endothelium will deny that the conditions here described obtain. The undifferentiated part of the reticulum shown at a in fig. 66 would be interpreted as evidence for the presence of an endothelium.[1]) However, in this case, a little study of the section will show that the cytoplasm shown at a is simply a part of the general reticulum which has not differentiated fibrils. In the anterior, lymphoid end of the kidney the reticulum is chiefly cellular and contains fibrils only in the finer processes of the cells. Where such cells form the wall of the blood space they may have the general appearance of large endothelial cells (figs. 64; 65). They are drawn out into long spindle shaped structures and their lateral processes are very fine. Only the finer processes contain fibrils. Such cells do not differ from the cells of the general reticulum of this region, excepting in the fact that they are drawn out paralled to the blood space, while the cells of the general reticulum are usually more irregular in shape. Figs. 64 and 65 are two reticular cells from this region which formed the wall of a blood space. Fig. 67, a, b, c, shows three cells from the general reticulum of this same region. A comparison of the figures will give a better idea of these cells than any description could.

The technique used for demonstration of the reticulum is very positive in its action and gives a very clear differentiation between the fibrous and cellular portions of the reticulum.

Most of the granules in the reticular cells are probably due to products of phagocytosis, as many cells were found which had engulfed red and, sometimes, white corpuscles. This is true of the cells which line the blood spaces, as well

[1]) Not shown in the reproduction. See: "Correction of the plates".

as of those which are out in the general reticulum. The cell shown in fig. 64 has phagocytosed two erythrocytes and one leucocyte.

Lymphoid tissue.

As already stated, the supporting substance of the lymphoid tissue is composed of reticulum which varies in amount and structure in the different regions of the organ. In the "head kidney" it is almost entirely cellular, having fibrils only in the finer processes of the cells. It may be quite fibrous where it forms the boundary to a large blood space. In the middle, narrow portion of the organ and in the posterior end it is chiefly fibrous, the fibrils being developed in the cytoplasm of the reticular cells. In the dorsal part of the posterior end the reticulum is composed of very narrow fibrous strands, or is absent altogether. In such regions the large, almost colorless cells, which have already been described as resembling hepatic cells in their general outline and arrangement, seem to form the main supporting mass.

The meshes of the reticulum are filled in with the various cellular elements which go to make up the lymphoid tissue.

The leucocytes may be classified as granular and non-granular. The granular forms are especially abundant in that portion of the lymphoid tissue through which the blood is filtered, in some places crowding out almost all of the non-granular forms. In those regions in which there are few blood spaces (ventral and lateral regions) the lymphoid tissue is made up chiefly of non-granular forms.

The non-granular forms may be classified as 1) small lymphocytes; 2) large lymphocytes; 3) small mononuclears; 4) large mononuclears having finely granular, strongly basophilic cytoplasm; 5) large mononuclears having a fine reticular network and coarse fibers in a slightly basophilic cytoplasm; 6) plasma cells.

The small lymphocytes appear in two forms, with all possible intermediate stages between them. The two forms are shown in figs. 1 and 5. The two figures were drawn under different magnification, hence the apparent difference in size. The lymphocytes of both forms are characterized by a rather thick nuclear membrane which is generally thicker in the form shown in fig. 1 than in the other form. In the first type the nucleus is generally round, and has its chromatin distributed in thick masses around the nuclear membrane, giving the familiar "checker board" appearance. The nuclear network is generally composed of very coarse fibers and this is also true of the second type of lymphocyte (fig. 5). The cell body is very narrow and is strongly basophilic. It is generally homogeneous, but may show small vacuoles. All intermediate stages can be found between this type of lymphocyte and the large lymphocyte, and between it and the plasma cells. The large lymphocyte is developed from this by an increase in the size of the cell, especially of the cytoplasm. The nucleus remains of the general lymphocyte type; has the same distribution of chromatin, thickness of membrane, and coarseness of

network fibers, but its membrane generally becomes very much folded (figs. 1, 2, 3, 4). The cytoplasm remains strongly basophilic, but is increased, relatively, in amount.

A plasma cell is developed from a lymphocyte of the first type, by an increase of the cytoplasm on one side of the nucleus and by a change in its chemical constitution which causes it to stain metachromatically in basic anilin dyes. It has a very strong affinity for basic dyes, probably due to increase in density. The nucleus retains its general lymphocyte character and its membrane does not become folded. The cytoplasm shows the "Zellhof" and vacuoles, so familiar in the mammalian plasma cell. The plasma cells are very abundant in those regions in which the granular leucocytes are scarce, but they are also found in the other regions. The developmet of the plasma cell from the lymphocyte of the first type is shown in figs. 1, 10, 11 and 12, which are from specimens hardened in 10 % formol and stained in iron-haematox.-saf.-tol. blue. The staining reaction as shown on the figures is due entirely to the toluidin blue. If toluidin is used alone, the same metachromatic violet color is imparted to the cytoplasm of the plasma cell. The vacuoles and the "Zellhof" are of a much paler violet than is the rest of the cytoplasm and one gets the impression that some extremely dense substance is developed in the cytoplasm, but not in the vacuoles and the "Zellhof".

The second type of the lymphocyte (fig. 5) has a rather thick membrane which generally shows one or two folds. The chromatin consists of one or two irregular shaped masses which are near the center of the cell, and a few fine granules around the nuclear membrane (fig. 5). The cytoplasm forms a narrow band around the nucleus, is generally homogeneous and strongly basophilic. Consultation of fig. 5 will give one a good idea of the general appearance of these cells. The small and large mononuclears are developed from these cells, at least, all possible intermediate stages can be found between them. The nucleus of the small mononuclears is round and is often paler than that of the lymphocyte, and the chromatin shows a tendency to become concentrated into a central mass which is nearly round. The network consists of very fine fibers (figs. 6 and 7). The cytoplasm has increased in amount and contains small vacuoles and often a delicate reticular structure. The nuclear membrane is quite heavy.

The large mononuclears occur in two forms which are distinguished chiefly by the character of their cytoplasm. The first type differs from the small mononuclear by the pale, vesicular appearance of its nucleus, which has a very thin membrane and a very fine network and has its chromatin in two or three large masses in contact with the membrane. The cytoplasm is generally not vacuolated and contains innumerable, exceedingly fine granules and occasionally a fine fiber (fig. 8).

The second type of large mononuclear (fig. 9) has pale cytoplasm which shows a fine reticular network which is composed of very fine granules united to form threads which interlace to form the network. The strands of the network

are more strongly basophilic than is the general cytoplasm. Most of these cells contain rather coarse, strongly basophilic fibers, the arrangement of which is shown in fig. 9. Occasionally, there are one or more large, basophilic granules in close association with one or more of the fibers. One of these is shown in fig. 9. In the lower part of fig. 9 there is one fiber, coarser than the others, which seems to be connected with the nuclear membrane at one end. A single coarse fiber is also found occasionally in one type of granular leucocyte (fig. 44). Many cells containing these fibers were examined, but nothing was seen which can by offered as an explanation for their origin or function.

The nucleus of the large mononuclears of type 2 (fig. 9) has a very thin membrane and generally has a considerable amount of chromatin which is arranged in irregular small and large masses around the nuclear membrane. The fibers of the nuclear network are exceedingly fine.

Large mononuclears of type 1 (fig. 8) were often observed to have two nuclei which were often connected by a fine thread. Such a cell is shown in fig. 54. Whether this is an indication of amitosis, which is to be followed by cellular division, could not be determined. Such polymorphism and division of the nucleus could not possibly be interpreted as a sign of cellular degeneration in this case, because the cell showed absolutely no signs of degeneration. The nuclear network was in perfect condition. Cells like the one shown in fig. 55 are frequently met with. The smaller nucleus I interpret as belonging to a phagocytosed lymphocyte, as it has all the earmarks of such a nucleus.

Figs. 5 to 9 were drawn with the new Spencer camera lucida under a 1,5 mm Spencer objective with a Zeiss compensating ocular and were drawn as accurately as it was possible for me to draw them, so that a good idea of the relative size of the cells in this series can be gained by consulting the figures. The drawings were made from a 10 % formol preparation stained in saf.-tol. blue. The splendid preservation of the nuclear network and the finest details of cell structure show that the fixation obtained by this method is almost perfect.

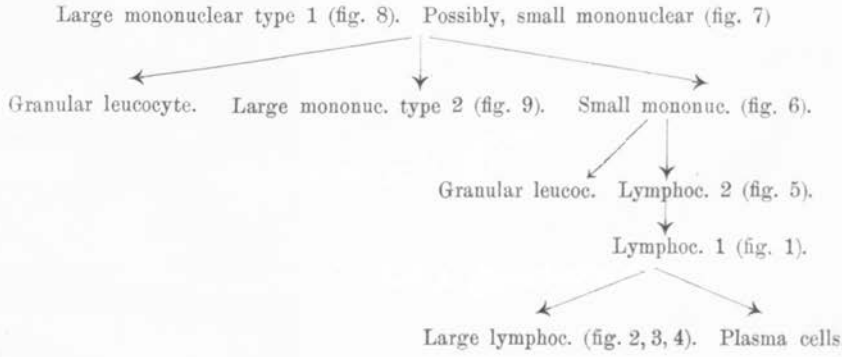
Figs. 1, 2, 3, 4, 10, 11 and 12 are drawn under a different magnification and are not camera drawings. However, they are drawn to scale after several measurements of each cell by means of the eye piece micrometer, so that the relative size of the cells in this series is fairly accurate. The lymphocytes of the type shown in fig. 1 are of about the same size as those of the second type (fig. 5), and the size of the other cells in that same series should be increased in proportion when comparing with figs. 5 to 9.

It must be clearly understood that the non-granular cells described and illustrated are of extreme types at the ends of their respective series and that all possible intermediate forms are found between the members of the same series, and between the lymphocytes of the two types. The lymphocytes of type one (fig. 1) are undoubtedly related to the large lymphocytes (figs. 2, 3, 4) and to the plasma cells (figs. 10, 11, 12). The large mononuclears (figs. 8 and 9) and

the small mononuclears (figs. 6 and 7) are related to the lymphocytes of type two (fig. 5).

The description of the relationships of the non-granular cells has been given as though the lymphocyte were the common progenitor of the other types. It must be stated, however, that mitotic figures, which are rare, were found only in the mononuclears of the types shown in fig. 7 or 8 and that they were never found in the lymphocytes. If this observation is sufficient for drawing a conclusion we may infer that the mononuclears, or possibly the small mononuclears, are the common progenitors of the other forms. The large mononuclear of type 1 (fig. 8), possibly the small mononuclear (fig. 7), by differential mitoses form the large mononuclears of type 2 (fig. 9) on the one hand, and on the other, the small mononuclears (fig. 6) and the small lymphocyte of type 2 (fig. 5). The lymphocyte of type 1 is developed from the lymphocyte of type 2 and lymphocyte 1 developes into the large lymphocytes of its series (figs. 2, 3, 4) and into the plasma cells. The granular leucocytes (at least most of them) are probably formed by the differentiation of granules in the cytoplasm of the small mononuclears and of the large mononuclears of type 1 (fig. 8), the proof of which will be given further on.

These relationships could be presented in a diagram as follows:



If we make allowances for the specific differences between the non-granular cells of Polyodon and those of mammals, and if we could call the large mononuclear a large lymphocyte, we would have praticcally the same scheme for the relationships of the non-granular leucocytes in Polyodon as Pappenheim and others have for the non-granular cells in man and mammals.

Drzewina is correct when she states that there cannot be two independent and distinct series of leucocytes in the Ichthyopsidae, for these forms do not possess lymphoid myeloid (bone marrow) tissue and, furthermore, it is seen that all the different forms of leucocytes and the erythrocytes are developed in the same region — that is, in the lymphoid tissue of the kidney and probably also in

the lymphoid tissue of other regions of the body, although the present investigation covers only the lymphoid tissue of the kidney.

In the light of the above, we must hold to the monophyletic theory of blood development, at least in so far as the Ichthyopsidae are concerned. It appears from the work of Kollmann (12) and others, that the same is true for the invertebrates, and there is certainly plenty of evidence accumulating to show that this is the only theory which will hold good for the mammals.

When one first examines the granular leucocytes it seems almost impossible to get them together into definite groups. When this study was first undertaken it seemed as though about 25 to 30 different groups would have to be made. However, after many hundreds of these leucocytes had been examined and many of them drawn, it was recognized that most of the different forms found were simply different stages of evolution of a few definite types of granular leucocytes. When the relationships of the innumerable forms of leucocytes were worked out it was found that they could all be reduced to seven distinct types, whose morphological characteristics distinguish them from each other.

The lymphoid tissue of the kidney of Polyodon, when treated by the methods used in this investigation, is almost ideal material for the study of the leucocyte granules and their various stages of evolution. The preservation of the granules and nuclei is almost perfect, so that one is not dependent on staining reactions alone as a basis of classification. Ehrlich's classification of leucocytes according to the staining reactions of their granules is well and good so far as it goes, but no one would now maintain that it tells the whole story of the morphological characteristics of the different leucocytes. The recent work of Weidenreich on mast cells and on eosinophils of man and mammals may be taken as an example of how little can be learned from staining reactions concerning the morphology of a cell. Weidenreich shows that the granules of an eosinophil and of a haematogenous mast cell of man have a totally different origin and functional significance. The morphological characters of the two cells are totally different, even though we were to entirely to neglect the staining reactions. Grouping the leucocytes according to Ehrlich's classification is a convenient method for diagnosis and it tells us that the granules in the various forms of cells are chemically different, but that is about all that it does tell us.

One is especially convinced of the truth of this when he comes to study the leucocytes of the Ichthyopsidae and finds that, although they have a tendency to follow Ehrlich's classification, they do not follow it completely. For example, granules of the leucocyte shown in fig. 32 when stained in a mixture of eosin and orange G, followed by toluidin blue, have a slight affinity for eosin (they are stained a pale red) and occassionally for the orange in the mixture. In fuchsin S — orange G — toluidin they have a strong affinity for the acid fuchsin, yet when stained in the Wright-Jenner modification of Romanowsky they are violet

(fig. 32). Granules which are only slightly acidophilic will stain reddish in the Wright-Jenner, so it is difficult to explain the staining reaction of these granules by saying that they are amphophil. The developing granules in the leucocytes of the type shown in figs. 37 and 38 are at first only slightly acidophilic, yet they are colored red with the Wright-Jenner.

I must agree fully with Drzewina's statement that the granules of the leucocytes of the Ichthiopsidae have a special affinity for certain stains, but would modify it by stating that they show a general tendency to follow Ehrlich's classification. The cell shown in fig. 32 has already been cited as being an exception to Ehrlich's classification. Another striking example of cells showing a special affinity for a stain is found in the type of leucocyte shown in fig. 16. The granules of these cells show a strong affinity for basic safranin after fixation in trichloracetic acid and occasionally also after formol fixation, but they will not stain in any other basic dye.

Kollmann (12), who has recently made a very complete study of the blood of invertebrates, takes exception to Drzewina's statements regarding the variability in staining reactions in the Ichthyopsidae. He thinks that her results were due to the fact that various fixing fluids were used, whereas Ehrlich's classification is based on heat fixation. He, himself, used Zenker's fluid containing only 0,5 % acetic acid and found that this fluid did not change Ehrlich's reactions in the mammals. The leucocytes of the invertebrates fixed by this method follow Ehrlich's classification.

To meet just this kind of an argument against the use of formol, portions of mammalian spleen, lymph glands and bone marrow were fixed in formol, then sectioned and stained by Ehrlich's methods. No change from the usual chromaticity of the leucocyte granule could be discovered. We may reasonably conclude, then, that the formol produces no essential change in chromaticity of the Polyodon leucocyte.

Drzewina finds two kinds of granules in certain leucocytes of the sturgeon kidney and makes this a strong point against Ehrlich's classification. In Polyodon only a few cases of this kind were found, and then only in cells which had all the earmarks of degenerating cells. Such a cell is shown in fig. 53, stained in Wright-Jenner. As far as Polyodon is concerned, then, the presence of two kinds of granules in the same cell indicates abnormal cellular and granular degeneration. This is in accord with the views of Kollmann and Ehrlich, who hold that the presence of two kinds of granules indicates different stages in their evolution, but not that they belong to different species of granules.

The granules in probably all of the leucocytes in the lymphoid kidney of Polyodon are of a secretory nature, homologous in all respects to the secretory granules of gland cells. There is only one type of granular leucocyte in which different stages in the evolution of the granules could not be found. This may be due to the fact that only four or five cells of this type could be found and hence

I can say nothing about the origin or function of their granules. They are cells which have a very large oval nucleus and their cytoplasm is filled with large, round granules which have a strong affinity for orange G in the eos.-or.-tol. mixture, indicating that they are strongly acidophilic. In this mixture the cytoplasm has a pale greenish blue tinge. The nucleus is very pale but has two or three large Karyosomes and several finer granules (fig. 51).

There are three other types of granular leucocytes the complete life histories of which could not be worked out. However, enough was seen of the evolution of their granules to convince me that they, too, are secretory leucocytes. They are not found very often and are not mixed in with the other lymphoid cells.

The first type (figs. 45, 46, Wright-Jenner) are found chiefly in the smaller blood vessels of the narrow part of the kidney and were found only in a few instances to be out in the lymphoid tissue.

The second type (figs. 47, 48, saf.-tol.) were found only along the inner walls of the blood vessels, and then chiefly in the smaller specimens of Polyodon. They are often found in vessels which are empty, excepting for these cells, which are often so closely applied to the inner wall of the vessel that they may be mistaken for endothelial cells. They may be endothelial cells which have developed granules and have separated from their surrounding endothelial connections.

The third type of cell to be described here (fig. 49, Wright-Jenner, fig. 50, eos.-or.-tol.) was found in a few instances in the lymphoid tissue, but they are generally found only in or between the epithelial cells of the kidney tubules.

The first type of cells described here (figs. 45, 46) is similar in many respects to the cell figured in number 32 and described as belonging to the secretory leucocytes of series I. However, its staining reactions are so different that it must be put into a separate group. The chromatic qualities of the granules and of the cytoplasm vary in the different stages in the evolution of the cell. In Wright's Romanowsky the granules are crimson and the cytoplasm is a light tan color or a light pink or, in a different stage in the development of the cell, the cytoplasm is a brilliant red and the granules appear as light spaces in the cytoplasm. With other stains the following reactions are obtained:

Indulin-aur.-eos.-granules faint reddish or purplish color in a blue-black cytoplasm.

Methyl green-fuchs. S-granules and cytoplasm stained brilliantly in the fuchs.

Eos.-or.-tol.-granules not stained, cytopl. slightly blue.

The second type of cell (figs. 47, 48, saf.-tol.), the one which is found along the endothelium of the blood vessels, contains granules which have a strong affinity for safranin when stained in Pfitzner's saf., followed by tol. blue. In indulin-aur.-eos. the granules are aurantia color, in Wright's Romanowsky they are colorless. Many of these cells were found in which the granules were very small and were contained in distinct vacuoles (fig. 48), indicating that the granules dissolve and form a substance which accumulates in cytoplasmic vacuoles, the un-

dissolved portion of the granule remaining in the center of the vacuole. The vacuoles enlarge until the cytoplasm is reduced to a narrow reticulum (fig. 48).

The third type of granular cell, which is found in the kidney tubule, has large coarse granules which are sometimes contained in vacuoles (fig. 49, W.—J., fig. 50, eos.-or.-tol.) Usually the cell body is very narrow, excepting on one side of the nucleus where it is filled with granules which form a little cap over the nucleus (fig. 50). Sometimes the nucleus is central and the granules may be all around it. Some of these cells have no granules, others only a few. In Wright's Rom. the color of the granules varies. They are brick red, red, reddish brown or brown. In Ehrlich's triglycerine they are violet to crimson; in eos.-or.-tol. they are orange or eosin color; in fuchs.-or.-tol. they stain in the orange of the mixture; in triacid they stain with the orange, the fuchsin, or with both which will give them a peculiar reddish orange color. The variations in color in the different stains are probably due to the fact that the granules are in different stages of evolution. The cytoplasm is always almost colorless, so that in the cells which contain no granules it is difficult to see much more than the nucleus which is always of the same general plan of structure, whether the cell contains granules or not.

The figures of the three types of cells just described were drawn with the camera ludica, so that a closer description of their morphological characters will not be necessary.

The changes in chromaticity of the granules in the first type of leucocytes (figs. 45, 46) and the frequent occurrence of the granules of the second (figs. 47, 48) and the third type (figs. 49, 50) in cytoplasmic vacuoles would not be sufficient evidence for concluding that these cells are secretory leucocytes, but when these changes are compared with similar changes which take place during the evolution of the next three types of cells to be described (under secretory leucocytes), we can be reasonably certain that the three types just described are also secretory leucocytes.

The complete life history of three types of leucocytes to be described under secretory leucocytes has been worked out and their various stages of evolution described in detail. The results obtained clearly indicate that the granules of these cells are not living, functional parts of the cell ("organs" of the cell), but, rather, that they represent an accumulation of material, elaborated by the cytoplasm, which, after being changed chemically will form a substance which is forced out of the cell into the plasma as an internal secretion. Many Haematologists already hold to this idea of the function of the leucocyte granule, although it is not based on a great deal of direct cytological evidence in favor of it.

The most recent advocate of this view is Max Kollmann (12), who, from his study of the blood of invertebrates, particulary the crabs, concludes that the leucocyte granules are secretory granules. K., however, concludes that the sub-

stance formed by the granules is in the nature of reserve food material and that it is not in the nature of an internal secretion in the ordinarily accepted sense of that term.

It may be, that in the invertebrates, where K. finds only a few varieties of granular leucocytes in any one species, and where there seem to be intermediate stages from one type to the other, that the granules are merely accumulations of reserve food matter. However, I could not accept that explanation for the function of the secretion formed by the leucocyte granules of Polyodon or higher vertebrates; for, if they represent reserve food matter, then we must conclude that in Polyodon six different kinds of food matter are stored up in the leucocytes, inasmuch as we have six different types of secretory leucocytes. The three types which have been worked out in detail have absolutely independent life histories and there is no changing over of one type into another. We can only conclude that the substance secreted by each type of cell is different from that secreted by the others.

Further discussion of the literature of this subject will be found with the general discussion of the secretory function of leucocytes.

Secretory leucocytes.

The three types of secretory leucocytes whose complete life histories were traced are, on account of their striking morphological characters, easily found in any section of the kidney. For want of better terms they will be described as cells belonging to series I, series II, series III. The cell belonging to series I is shown in fig. 32. Series II is best illustrated by figs. 40 and 41 and the most conspicuous types in series III are shown in figs. 15, 16, 25 and 27.

Cells like those cited above are easily found on a casual glance through the section, but the various intermediate stages, corresponding to different stages in the physiological activity of the cells can be worked out only by laborious examination of many hundreds of these cells.

These cells are quite abundant in the anterior, lymphatic part of the kidney and also in the posterior portion. In the middle, narrow part of the kidney they are quite scarce, but this may be due to a general scarcity of lymphatic tissue in this region and not to a diminution in the relative number of these cells. No attempt was made to determine the proportions of secretory leucocytes to those of other classes.

The cells belonging to all three series show a tendency to become grouped together in certain regions of the section. This is particularly true of the cells belonging to series I, which may become so numerous that they are in contact with each other and form a solid mass in the particular part of the kidney occupied by them, crowding out all other types of cells. The cells of series II and III do not congregate in any such numbers. However, they are quite numerous in some parts of the section, while other parts are quite free from them. Cells of series II

and III are always associated together in regions which are generally quite free from those of series I. This is not true of the more dorsal regions of the kidney, for here all three types may be equally well represented, excepting, of course, those parts in which cells of series I become so closely packed together.

In general, the secretory leucocytes of all three types are confined to the dorsal half of the kidney where the lymph tissue is more abundant. In the ventral half of the organ the urinary tubules are so close together that the lymphatic tissue between them is very much reduced in quantity. It is made up chiefly of lymphocytes, plasma cells and basophilic mononuclears. Secretory leucocytes are found here only occasionally and then chiefly in the thicker strands of lymph tissue which radiate out from the dorsal, lymphoid part of the organ.

A detailed account of the technique employed is given in the beginning of this paper and therefore will not be reviewed here.

At first it was not suspected that the granules of these cells were of a secretory nature and therefore the modern blood stains were employed to determine whether the granules could be classified, as are those of the mammalian leucocyte, into acidophils, neutrophils, basophils, amphophils. It was soon found that no such classification could be made. However, it was by the very use of these stains that the true nature of these granules was determined.

For a detailed study of chromatic changes in granules and cytoplasm during the evolution of these cells, by far the best results were obtained with the Wright modification of Romanowsky. The Giemsa modification was also used, but as it did not give better results it was soon abandoned in favor of the Wright-Jenner, the latter being more convenient to use. In working out the stages of series II very excellent results were obtained with the eos.-or.-tol. combination as used by Drzewina, also with fuchsin S-orange G-toluidin. These combinations are especially valuable for this series, because in the early stages of the evolution of these cells the cytoplasm has a strong affinity for toluidin blue; also the acidophilic nature of the granules is more evident than with the Wright-Jenner. These two combinations were also used for detailed study of nuclear structures in all three series, as the Wright-Jenner does not bring out nuclear structures very satisfactorily.

For the study of the granules in the early stages of cells belonging to series III (figs. 13, 15, 16, 17) iron-haematoxylin, followed by safranin and toluidin was found very valuable, because the granules when stained in this combination showed a very strong affinity for safranin. Apparently the acetic acid used in the differentiation of the Benda's iron-haematoxylin acts as a mordant for the safranin, for, when stained in safranin alone, or safranin and toluidin, or when the liquor ferri was used for differentiating the iron-haematox. the granules would not hold the safranin.

Further staining reactions will be given under the detailed descriptions of these cells.

Series I.

(Figs. 31, 32, 33, 34, 35, 36.)

The cell shown in fig. 32 is one of the type which accumulates in great numbers in a portion of the dorsal lymphatic tissue and is also frequently met with in the entire peripheral portion of the kidney. They are the cells which may accumulate in such great numbers that they will crowd out all other types of cells.

In a section through the post. end of the kidney of a large animal, large numbers of them are found in the dorsal parts of the section to the right of and above the renal-portal vein, which here is almost completely surrounded by the lymphatic tissue.

These cells are not very abundant in the interior of the organ, in the lymphatic tissue which fills up the space between the urinary tubules. In the anterior, lymphatic part of the organ they are found in great numbers and chiefly in the surface layers. In the middle, narrow part of the kidney they do not seem to have any definite distribution and are not present in any great numbers. In sections through the post. end of the kid. of a very small animal they have no definite distribution and are not abundant.

These cells are nearly round. Their granules are large, round and of about equal size and fill up the cell to such an extent that the nucleus is crowded to one side and the whole cell appears as though tense with the intracellular pressure caused by the accumulation of the large granules.

The nucleus is always at one edge of the cell and is usually kidney shaped, convex externally, concave internally. Chromatin not very abundant; a distinct chromatin granule at either end of the nucleus and two or three very small granules may be scattered through the nucleus. Other cells of this same type may have an oval nucleus, seldom round. The distribution of the chromatin may be somewhat different, although it is never abundant.

In W.-J. (fig. 32) the nucleus of these cells is stained a light bluish green; cytoplasm-pale blue, almost invisible; granules — dark violet. This reaction of the granules may be regarded as amphophil.

In Ehrlich's triacid the granules stain a dark violet or purple (neutrophile). In Ehrlich-Biondi the granules of the majority of these cells are of a chocolate color (neutrophile) with a tinge of violet.

Variations from this can be found — violet — greenish gray to greenish, the older cells having the pale green granules. Occasionally all shades are found in the same cell. It seems that the older the cells the more the granules resemble the cytoplasm in their color.

In thionin the granules are very pale green, hardly stained.

In Ehrlich's ind.-aur.-eos. the granules are stained a pale gray with a slight tinge into the indulin color.

With fuchs. S-or. G-tol. the granules are stained intensely with the fuchsin (oxyphil).

In eos.-or. G-tol. the granules of these cells are stained pale blue, violet, reddish, orange, depending on fixation and length of staining.

In iron-haematox.-saf.-tol.-deep violet.

From the above staining reactions it is seen that the granules of these cells are slightly basophilic, neutrophilic or acidophilic according to the stains used, and that they have a special affinity for acid fuchsin.

Drzewina in her work on the lymphatic tissue of the Ichthyopsidae finds the same thing to be true for most groups of fish.

Grünberg (7) found in the circulating blood of Scyllium catulus, that the granules in the different groups of leucocytes have an elective affinity for different acid stains. For ex., in triacid they may have a special affinity for the fuchsin or the orange G, and in eos.-aur.-nig. for the eosin or the aurantia or, for a mixture of the two. Evidently, it is impossible to classify these leucocytes according to the classification adopted for the mammalian granular leucocytes. This becomes still more evident when one studies the development of the granules and the changes which they undergo during the further evolution of the cell.

Fig. 31 is an early stage of the cell shown in fig. 32. Various intermediate stages between 31 and 32 can be found, and between 31 and a mononuclear with strongly basophilic cytoplasm and a round, somewhat vesicular nucleus. Fig. 31 is drawn under a slightly higher magnification than is fig. 32, hence the apparent difference in size.

In 31 the elaboration of the granules is evidently not yet completed. The cytoplasm is still strongly basophilic (fuchs. S-or. G-tol.) and is filled with vacuoles of a variable size which contain a homogeneous substance stained a light gray color. The nucleus appears to be almost crowded out of the cell, the cytoplasm around its outer edge being so thin that it can hardly be seen. The nucleus is kidney shaped and very regular in outline. Between the stages shown in figs. 31 and 32 there is a reduction in the total size of the nucleus and in the amount of its chromatin. This may be due to a participation of the nucleus in the elaboration of the granules. This reduction in the size of the nucleus and in the amount of the chromatin is the only evidence which might be in favor of the view that the nucleus participates in the elaboration of the granules. There is absolutely no evidence which would favor the view held by P. Stephan (22) in regard to the formation of the eosinophile granules in the lymphoid tissue of Protopterus. According to Stephan the large granules seem to develope in the interior of the nucleus by the direct transformation of a karyosome; sometimes a portion of the nucleus becomes detached and breaks up into small particles which give origin to a mass of small granules.

In the type of cell under discussion in Polyodon there is no appearance which could be interpreted in the light of the above statement by Stephan. However, the nuclear changes in these cells do agree with Stephan's further statements in regard to nuclear changes in the eosinophils of Protopterus. According to these statements, the nucleus during the elaboration of the granules is large and shows distinct chromatin in the form of thick cords, karyosomes and more or less fine granules. After the disappearance of the granules the nucleus is very much condensed, more homogeneous and contracted like an exhausted element.

In Polyodon, for cells of series I, the nuclear changes agree very well with this description, excepting that in the early stages "cords" of chromatin are not very evident. As stated above, nothing was seen in the way of the detachment of particles from the nucleus for the formation of granules. Occasionally, budding of the nucleus as in fig. 28 (another type of cell) was seen in stage 32 or later, but this can be interpreted in the light of nuclear degeneration, as will be discussed later.

According to Weidenreich (28) irregular budding of the nucleus, such as is found in certain leucocytes of the Polyodon kidney (secretory leucocytes, series III) is found in the basophil leucocytes (mast cells) of man and certain mammals and is associated with the formation of the granules. However, these granules are, according to W., to be interpreted as degenerative products and not as functional elements of the cell. Speaking of these granules (29, page 285) he says: „Wie die Körnelung der Mastleukozyten zu beurteilen ist, habe ich an dieser und an anderer Stelle nun schon genügend auseinander gesetzt: mir scheint die Granulation der Ausdruck einer besonderen degenerativen[1]) Umsetzung des Plasmas mit sehr starker Beteiligung des Kernes zu sein; eine besondere normal physiologische Bedeutung und Wirksamkeit dürfte demnach dieser Körnelung kaum zuzusprechen sein."

According to the same author the finely granulated leucocytes (neutrophils) in mammals develop their granules as a special differentiation of a basophilic cytoplasm without any particular participation of the nucleus. The first appearance of granules is in the concavity of the kidney shaped nucleus (young forms) in the region occupied by the centrosphere. They are grouped around the centrosphere, which is interpreted as indicating a special influence of this body in their elaboration.

According to Stephan the eosinophil granules of Protopterus are developed from the nucleus, or they first appear in a niveau of cytoplasm more dense and more stainable than that of the rest of the cell. The granules, which are at first very small, grow and become distributed throughout the cell. After the cell becomes filled with the granules the latter lose their affinity for stains, become smaller and are finally completely dissolved and one sees nothing in the cell but the cytoplasmic network which was interposed between them.

[1]) S. Pappenheim, Atlas I, S. 47, 48; Fol. Haem. 1908, Bd. V, S. 158.

The granules of the cells in series I (fig. 32 and following) in Polyodon do not originate from detached portions of the nucleus or in a special niveau of the cytoplasm as described by Stephan; nor are the developing granules grouped around a centrosphere as described by Weidenreich[1]) for the neutrophils in mammals, for a centrosphere could not be found in any of the granular leucocytes of Polyodon. If a central body were present iron-haematox.-saf.-tol. and the W.-J. would surely reveal it. In spite of careful search for such a body nothing of the kind could be found, nor was there anything in the arrangement of the cytoplasm, such as the radial arrangement described by W., which would indicate that a central body might be present. In Polyodon the granules of series I originate simultaneously throughout the cytoplasm. In the earliest stages which can be definitely placed in this series the substance of the granules is homogeneous and slightly basophilic, while the surrounding cytoplasm is strongly basophilic. In the next stage (fig. 31) the granules are almost colorless and the surrounding cytoplasm is still strongly basophilic. In the intermediate stages between fig. 31 and fig. 32 the granules gradually gain their affinity for acid fuchsin and for the violet color when stained in W.-J. In the meantime, the cytoplasm loses its affinity for basic stain, becoming almost colorless in stage 32. The nucleus becomes smaller and loses chromatin by solution or otherwise. In stage 32 the nucleus is always as close to the edge of the cell as it is possible for it to be. It is usually kidney shaped, but may be oval. In W.-J. it is stained a greenish blue, indigo blue in any of the combinations containing toluidin blue.

In the further evolution of these cells only the W.-J. combination shows adequately the variations in chromaticity of the granules. In staining combinations containing acid fuchsin the granules show no diminution in their affinity for this dye, but with the delicate W.-J. the stages shown in figs. 33 and 34 show clearly a diminution in staining capacity of the granules, which here appear quite pale in comparison to those of fig. 32. At this stage the granules are occasionally found to vary in size (fig. 33). The cytoplasm again increases in chromaticity, varying from a pale violet to a pale blue when stained with W.-J., whereas in stage 32 it is almost colorless. The nucleus increases in size and becomes irregular in outline and may become slightly removed from the margin of the cell (fig. 33). Quite a few cells in this stage of their evolution have their nucleus stained indigo blue instead of greenish blue (compare figs. 34 and 33).

In the stage shown in fig. 35 the granules have clearly begun to dissolve, the solution forming a clear vacuole in the cytoplasm, with the undissolved portion of the granule in its center. The cytoplasm and granules are strongly basic, both staining deep violet in W.-J. The nucleus is of the same size as in the previous stage described (figs. 33 and 34), is irregular in outline and is generally somewhat removed from the edge of the cell. As stated above, it may be colored indigo blue or greenish blue.

[1]) S. Pappenheim, Virch. Arch. 1899, Bd. CLVII, S. 65—66 (Pl. II, III).

The cell shown in fig. 36 is undoubtedly in its final stage of physiological activity and is ready to extrude into the plasma or lymph the secretion which has been formed by the chemical and physical changes of its granules, which in turn were elaborated by the cytoplasm. The vacuoles are very large and almost colorless. They are separated from each other by narrow partitions of cytoplasm which are still strongly basophilic. Three or four of the vacuoles still contain undissolved remains of the granules. Many cells of this same type can be found which are quite irregular in outline and in which the cytoplasmic network has more of a stringy appearance. They are probably cells which have extruded a portion of their secretion. In this stage the nucleus is in nearly all cases stained indigo blue in W.-J., violet in eos.-or.-tol. Structurally it may be in the same condition as in the previous stage, but usually is more granular.

In the stages following, in which most of the secretion has been expelled and the cell becomes very irregular in outline, the nucleus is still more irregular in shape. The chromatin is either more granular than in the previous stages, or the granules have run together to form two or three large masses. In the latter case, the membrane and chromatin masses have generally begun to lose their affinity for stains and are often quite pale. Still later stages are found in which the cell body is very much shrunken and the nucleus forms a nearly homogeneous mass.

A good many cells in these late stages of degeneration were found to be contained in phagocytes. No attempt was made to determine accurately whether all of these cells were finally phagocytosed or not, but from what has been seen in looking over the slides for other purposes I would conclude that many of these cells undergo fragmentation and final dissolution without being taken up by phagocytes.

From the above observations on the chromatic and structural changes of the cells belonging to series I, the conclusion is reached that the function of these cells is: the formation of an internal secretion which is to be poured out into the lymph and plasma.

From the staining reactions alone, one would conclude that the granules of these cells are not specific, highly organized, permanent differentiations of the cell itself, but, rather, that they are products of metabolism, temporary accumulations of a substance elaborated by the cytoplasm, which substance is to be changed chemically into a fluid which will be secreted by the cell into the blood and lymph when needed by the animal organism.

The diminution in the size of the nucleus during the elaboration of the secretory granules may be taken as favoring the view held by Stephan, who maintains that the granules in certain types of leucocytes in Protopterus are formed from detached particles of the nucleus. In Polyodon there seems to be nothing more than a condensation of the nucleus during the formation of the granules.

During the time in which the secretion is being formed by solution of the granules the nucleus increases in size, becomes irregular in shape, and moves in, a short distance, towards the center of the cell, all of which indicates that the nucleus is playing an important role in the chemical reactions going on in the cell. There is certainly a striking similarity between the cytoplasmic and nuclear changes enumerated above and the changes which the same structures in a gland cell of the higher animals pass through during the process of secretion. This is particularly true when they are compared with the secretory processes of a gland cell of the serous variety.

I think that abundant proof has been given to show that the process of secretion in these leucocytes, or better, unicellular glands is followed by complete destruction of the cell. Degenerating cells are very frequently taken up by phagocytes.

Further discussion of the secretory process in leucocytes with citations to the literature will be taken up after the remaining two types of secretory leucocyte in Polyodon have been described.

The evolution of these cells is not necessarily completed while they are located in the lymphatic tissue. The blood vessels (veins) and blood spaces contain many of them in all stages of evolution.

Series II.

(Figs. 37—43.)

In series II we have a type of cell which, even in the comparatively early stages of its evolution, is clearly differentiated from the cells of series I. Both originate from basophilic mononuclears having a large, round, vesicular nucleus. In the very early stages, before the granules are developed, it is difficult to differentiate the two types. Those belonging to series I probably have a more eccentric nucleus, but otherwise there seem to be no distinguishing characters. However, as soon as the granules begin to develop the two types are easily distinguished. In series I the developing granules are very large, quite irregular in size and closely crowded together, filling up the entire cell. They soon crowd the nucleus to the edge of the cell, indenting its inner border and thus making the nucleus kidney shaped.

In the cells belonging to series II the developing granules are not so large as those of series I and are not so closely packed together. In most cases they develop all around the nucleus which, therefore, remains round and nearly central.

The staining reactions will very soon enable one to distinguish the two types of cells. With W.-J., for example, the granules of series I will very soon take on a violet tinge, whereas those of series II will stain reddish.

In series I there is a condensation of the nucleus during the elaboration of the granules, but in the cells of series II no such change takes place. In the

latter the nucleus remains large and round, and generally shows no changes in its chromatin and network until the granules are fully developed.

In series II there is considerable variation in the behavior of the nucleus during the elaboration of the secretion from the granules. The changes which it passes through seem to be due entirely to degeneration and not to active participation in the formation of the secretion. There is no increase in size, such as we have in the cells of series I, nor does it become irregular in outline until an advanced stage of degeneration is reached.

There is considerable variation as to the time in which the degenerative changes begin. Cell 37 is a young cell in which the granules are not yet fully developed, yet the nucleus shows degenerative changes. It has lost its network and the chromatin has begun to break up into smaller masses. In cell 38 the granules have already begun to dissolve, but the nucleus is still in the condition in which it occurs in the young cells, of which the fine preservation of its network is the best evidence.

In the stages shown in figs. 37, 38 the cytoplasm is strongly basic, staining an intense blue in fuchs. S-or. G-tol. (fig. 37) and in eos.-or.-tol. (fig. 38). When the granules are nearly dissolved the cytoplasm becomes metachromatic (figs. 39, 40), staining violet in the above combinations. With the W.-J. the cytoplasm in the early stages varies from a light robin's egg blue to a greenish blue, but in stage 39 it is violet. In the later stages it is generally violet, but may be of various shades of pink or brick red.

Figs. 37 and 38 show the staining reactions of the granules in the early stages in fuchs.-or.-tol. and in eos.-or.-tol.

While these granules will stain reddish or pink in a fuchsin combination they do not begin to have the brilliant fuchsin color that the granules of cell 32 do. With the eosin combination they show greater affinity for this dye than do the granules of cell 32. However, they are not stained brilliantly with the eosin, their color is rather a reddish purple which would indicate that the toluidin has acted upon them to a certain extent. In W.-J. they stain reddish or purplish. They will stain with safranin, but not very brilliantly.

From the above it is seen that the granules are slightly acid in W.-J., fuchs.-or.-tol., and eos.-or.-tol. and that they are slightly basic when safranin is used. Further, it is seen that they have no special affinity for fuchsin as do those of series I, nor for safranin as do those of series III.

In the stages shown in figs. 39—41 the granules have lost their slight affinity for acid dyes and for safranin and have become basic and metachromatic, just as is the cytoplasm. In W.-J. (fig. 39), eos.-or.-tol. (fig. 41) and in fuchs.-or.-tol. they stain violet just like the cytoplasm. In the later stages (fig. 43) where the cytoplasm may be found to vary considerably in its staining properties, the undissolved remainder of the granules will always be found to be of the same color as the cytoplasm.

In the behavior of the granules during the regressive evolution of the cell, we again see important differences between cells of this series and those of series I. In series II the fully developed granules show only slight affinity for stains, while the cytoplasm is strongly basic. In series I the granules have a strong affinity for stains, especially fuchsin S, while the cytoplasm is almost colorless (fig. 32).

During the formation of the secretion in the cells of series I the nucleus goes through a series of changes which are very similar to the nuclear changes in a serous gland cell of the mammals. In series II the nuclear changes are of a degenerative nature. In cell 38 the granules have begun to dissolve, but the nucleus shows no changes. In 39 the nucleus is metachromatic, staining violet in toluidin blue mixtures and in W.-J. The chromatin has begun to break up, but the nuclear network is still pretty well preserved. In 40 the chromatin has become more granular and the nuclear network has almost disappeared. Chromatin and membranes stain a deep violet in toluidin mixtures and the whole ground tone of the nucleus is of a deep violet color. In 40 the nucleus shows indications of polymorphism, which process is carried on further in 41 and 43.

The nuclear changes described above prove that during the elaboration of the secretion the nucleus becomes pycnotic and finally degenerates completely. Later stages of degeneration than the ones figured were found, but no attempt was made to figure or describe them, because my object here is to simply determine the fact that the elaboration of the secretion is accompanied by nuclear and cellular degeneration.

The changes which granules and cytoplasm pass through during the elaboration of the secretion are very similar, excepting for staining reactions, to those described for the cells of series I. The granules are at first large and are not surrounded by clear spaces (fig. 37). As the peripheral portions of the granules dissolve the solution forms clear vacuoles which gradually increase in size as the granules diminish (figs. 38, 39). Finally the granules disappear almost entirely and the vacuoles enlarge until they are separated from each other only by narrow partitions of metachromatic cytoplasm (fig. 42). The conclusion regarding the function of these cells is, that they are secretory in nature and that the elaboration of the secretion is accompanied by a cellular degeneration. As in the case of the cells of series I many of them are found in the veins and blood spaces of the kidney in all stages of their regressive evolution.

Series III.

The most conspicuous stages in the secretory leucocytes of series III are shown in figs. 14—17. The complete series of changes which these cells undergo during their development and during their functional activity is shown in figs. 18—21 a, 14, 16, 22—30. The granules of these cells are the ones which stain brilliantly in safranin when the preparation is fixed in formol and stained in Benda's iron-

haematox., followed by safranin and toluidin blue. However, this reaction is obtained only when acetic acid is used for differentiating the iron-haematox. If the liq.-ferri sulph. is used as a differentiating fluid the granules will be stained blue as is the cytoplasm. With the exception of their peculiar affinity for basic safranin, after treatment with acetic acid, these granules show a distinct oxyphil reaction.

The affinity for safranin can hardly be regarded as a basophil reaction in this case, since the granules must be acidified before they will take up the safranin. This is further shown by the fact that when trichloracetic is used for fixation the granules of all three series stain in safranin. In W.-J. the granules stain a brilliant red or crimson and are about the same color in eos.-or.-tol. and fuchs. or.-tol. In ind.-aur.-eos., however, the granules do not give the strict eosinophil reaction, since they show all gradations from the blue black indulin color to a reddish eosinophil color. This varies in different parts of the section and may be due to variations in fixation. In triacid they are fuchsin color.

The stages referred to above as being the most conspicuous stages of this series show considerable variation in the shape, size and distribution of the granules. The granules may be large, round and of about equal size and they may occupy most of the cell or, the granules may consist of large spindles, about half the diameter of the cell in length, each spindle having a nodular swelling in its central portion (fig. 16). These spindles may be quite evenly distributed throughout the cell as in fig. 16 or, they may leave a portion of the cell, near the nucleus, free, as in fig. 14. Again, the spindles may be much smaller and more numerous than in the previous case and will not have the nodular swellings in their central portion (fig. 14). Other cells of this same stage contain both spindle shaped and round granules, and others contain granules of different sizes, or the two conditions may be combined in the same cell, as is shown in fig. 17. This cell contains large and small granules some of which are spindle shaped, others round. They are quite unevenly distributed through the cytoplasm.

Morphologically, the cells of this stage must be regarded as having reached their fullest development.

Variations in the cytoplasm are due to the age of the cell. The fully developed cell which has not yet begun its regressive changes has basophilic cytoplasm and a round or oval nucleus which shows a distinct network and two or three karyosomes with several smaller granules. Not a great many cells will be found in this ideal condition owing to the fact that there is no close relation between the regressive changes of the cytoplasm and those of the nucleus. In many cases the nucleus will be in perfect condition, showing a distinct network and karyosomes, but the cytoplasm is no longer basophilic and the granules are contained in large vacuoles or are losing their chromaticity, showing that they are undergoing the chemical changes necessary for the formation of the secretion. Such a cell is shown in fig. 17. In other cells the cytoplasm is strongly baso-

philic and the granules are in perfect condition, but the nucleus shows signs of degeneration: its karyosomes are breaking up into smaller granules and the nuclear network has nearly disappeared and frequently the nucleus is polymorphous or has one or two buds attached to it (fig. 14). A great many of the cells in this stage have polymorphous nuclei, but polymorphism may be delayed until later, or it may not take place at all, many of the nuclei degenerating completely without becoming polymorphous or without giving off buds.

The granular, secretory leucocytes in series III originate from round or polygonal cells, having basophilic, nearly homogeneous cytoplasm and a large, round, vesicular nucleus. These cells seem to be identical with the non-granular mononuclears of the type shown in figs. 7 and 8. Their nucleus is large and nearly round and contains two or three irregular shaped karyosomes and a few small chromatin granules at the nodes of the linin network and at the point of junction of the linin threads with the nuclear membrane, or there may be only one large karyosome in the center of the nucleus with the nuclear threads radiating out from it like the spokes of a wheel as in fig. 19 (Radkern). The cytoplasm is homogeneous and basophilic, staining dark blue in toluidin mixtures and greenish blue or pale blue in W.-J.

The granules first appear as exceedingly fine bodies, each one located in a little vacuole or mass of colorless cytoplasm. These vacuoles are concerned with the elaboration of the granules and are not related to the secretory vacuoles which appear later in association with the dissolution of the granules. These primary vacuoles generally disappear completely when the granules have reached their full development.

The granules are irregularly distributed throughout the cytoplasm and apparently have no relation to the nucleus or to a centrosphere. In the very early tsages the granules are not numerous, but new ones are added as those which were first formed continue to enlarge. The older cells, in which the granules are more numerous, contain both, small and large granules, but, generally, they all come to be of about the same size before they show any signs of regressive changes. In the very early stages of their development they stain a brilliant red or crimson in W.-J., eos.-or.-tol., fuchs.-or.-tol. (figs. 18, 19, 20, W.-J.) and they maintain this staining reaction until their regressive changes begin.

When these cells are stained in mixtures containing toluidin the basophilic part of the cytoplasm is seen to consist largely of exceedingly fine basophilic granules. Occassionally, exceedingly fine oxyphil granules can be found right in among the basophilic granules. This condition is shown in fig. 52 (fuchs.-or.-tol.). This cell is somewhat abnormal in that the oxyphil granules have developed so close together in one region of the cell that the vacuoles which contain them have run together, forming one large vacuole containing many small granules. Outside of this vacuole are a few red granules scattered among the basophils of the same size. This would certainly lead one to believe that the granules are first de-

veloped as basophilic differentiations of a basophilic cytoplasm and that they gradually become oxyphilic. This corresponds exactly with the development of the neutrophils in the mammalian leucocyte as described by Weidenreich, excepting that in Polyodon the granules are not in any relation to a centrosphere. In spite of careful search, a centrosphere could not be found in the cells of series III any more than it could be found in those of I and II.

Only a few cells like the one in fig. 52 could be found. These are exceptionally favorable cells for the determination of the origin of the granules. In the majority of cells the granules are so far apart that it is impossible to determine their first appearance, but where the granules are so close together, as in these cells, it is clearly seen that they are differentiated out of the basophilic cytoplasm.

As development proceeds the granules enlarge and the cytoplasm remains basophilic, probably until the granules have reached their full size. The granules may remain spherical or they may become spindle shaped. Probably in the majority of cases they become spindle shaped, but, still there are a great many fully developed cells in which the granules are spherical.

As stated above, all of the intermediate stages between the cells with round granules and those with spindle shaped granules can be found and occassionally a cell containing both forms of granules, which shows that these granules are not bacteria, as Rawitz states them to be in certain leucocytes of Scyllium catelus (18, page 506).

The staining reactions of these structures are the reverse of what they would be if they were bacteria, as has been pointed out by Grünberg (7), Knoll (11) and Meinertz (14). They have been fully discussed by the above named authors. They are of such common occurrence in fish, reptiles and birds that they cannot possibly be classed as bacteria.

In the further changes of the cell there are a good many irregularities. The nucleus may become polymorphous and show other degenerative changes before the cytoplasm and granules show any regressive changes at all, or the cytoplasm may become colorless and the granules show a slight loss in chromaticity, but the nucleus still be in perfect condition. However, whichever part of the cell starts its regressive changes first, it will soon be followed by regressive changes in the other parts of the cell, so that there is no doubt but what the regressive changes in the nucleus are associated with the changes in the granules and cytoplasm for the purpose of forming the secretion. Probably in the majority of cases nuclear and cytoplasmic changes take place at the same time and the following detailed description is based on these cells.

As the granules begin to change they lose in chromaticity and at the same time they seem to swell slightly, so that they take on the appearance of reddish drops in a pale, greenish blue cytoplasm (figs. 21, 22, 23, W.-J.). The cytoplasm gradually loses its affinity for basic stains until it becomes almost

colorless and then it gradually becomes oxyphil until it is finally almost as strongly acidophil as are the granules (fig. 24, eos.-or.-tol.). As the granules dissolve, the substance which they form accumulates around them in the form of a globule which pushes the cytoplasm away from the granule and which increases in size as the granule diminishes. In sections this will give the cytoplasm a reticular appearance. The meshes of the reticulum are filled with a homogeneous pale substance, while the strands of the reticulum are stained much darker (figs. 26, eos.-or.-tol., fig. 25, W.-J.). The accumulation of so much secretion causes the cell to swell, so that it is generally much larger than it was in the previous stages and it pushes the nucleus over to one side of the cell. Compare fig. 25 and 26 with figs. 24 and 23. Fig. 25 should be larger than the drawing would indicate, because it was drawn under a lower magnification than were figs. 26, 24 and 23. Some of the vacuoles may contain small undissolved portions of the granules in their center (fig. 25).

At this stage the cytoplasm with its secretion shows great variation in staining properties, although it is always acidophilic. With W.-J. (fig. 25) it will stain various shades of pink, red or brick red, and about the same shades are obtained with the eos.-or.-tol. and fuchs.-or.-tol.

In the stages which follow (figs. 27 and 30) the cytoplasm again becomes more uniform in its staining reactions, generally staining reddish violet or purplish in W.-J. (figs. 27, 30).

The stages shown in figs. 27 and 30 represent the exhausted cells after the secretion has been thrown out. They are smaller than during the previous stage and are very irregular in outline and their whole appearance indicates an exhausted element. Their cytoplasm is generally homogeneous, but it may contain a few small vacuoles and small bodies—undissolved remains of granules. Finally, the appearance of the nucleus indicates that we have an exhausted and degenerating element.

The next two figures (28, 29) are of later stages of degeneration. Here the cytoplasm again becomes vacuolated, but these vacuoles are not in any way associated with the formation of secretion. They contain no granules and are very irregular in outline and are due to the degenerative changes going on in the cytoplasm.

At this stage the cytoplasm again shows great variation in staining reactions. In the W.-J. mixture they will stain various shades of brick red, reddish violet, or orange. The structure of the nucleus alone, would indicate advanced stages of degeneration.

Nuclear degeneration may not start until the cytoplasmic changes are pretty well under way or, it may start very early. If it starts early it is generally initiated by polymorphism or budding; if later, budding and polymorphism will generally be delayed until the later stages of degeneration.

The first stages of degeneration are recognized by a disappearance of the network. This is followed by a breaking up of the karyosomes into smaller granules and a thinning of the nuclear membrane (figs. 21, 22, 23). The granules and membrane stain darker, becoming indigo blue or dark greenish blue in W.-J. and indigo blue in toluidin mixtures (figs. 21, 22, 23, 25, 26, etc.). Later the membrane becomes thickened and the granules swell (fig. 26).

The type of polymorphism which takes place in the later stages of degeneration is shown in fig. 29. Budding of the nucleus is shown in figs. 28, 30 and 61. Polymorphism, as it takes place in the earlier stages of nuclear degeneration, is very well shown in fig. 14. In these stages the nuclear lobes are regular in outline and nearly round. There are generally only two of them. In the other type of polymorphism, which comes in the later stages, there may be several lobes which are quite irregular in outline. When polymorphism comes early in the regression of the nucleus the disappearance of the network, breaking up of the karyosomes, etc., come in the same order that they do in the nucleus that remains round. In the other case these processes are well under way before polymorphism takes place.

Without doubt, many of these cells can degenerate completely without their nuclei becoming polymorphous or giving off buds, as many cells in advanced stages of degeneration are seen in which the nucleus is large and quite regular in outline, but in the majority of cases polymorphism is the rule. Many cells, such as those shown in figs. 25 and 27, which have small nuclei, are probably polymorphonuclear cells in which only one lobe of the nucleus or one bud came in the plane of the section.

The later stages of nuclear and cellular degeneration can be followed out very nicely in this material, but they have not been figured or described, because the object of this study is not a detailed determination of the exact changes which the nucleus undergoes during its degeneration, but, rather, the determination of the method by which a secretion is formed.

The method of budding in series III is very well shown in fig. 61 which is the same type of cell as 26, excepting that the nuclear network is still distinct and the nucleus has not yet become metachromatic. Here two buds are present in different stages of growth. The smaller bud is merely a bulging out of the nuclear membrane. The bud may remain broadly attached to the nucleus and a polymorphous nucleus like those shown in figs. 29, 43 and 62 may result, or the bud may become almost separated from the nucleus, remaining attached to it only be means of an exceedingly fine thread (figs. 28, 30, 61). In this condition it will continue to grow until it reaches the size of the original nucleus which has not been reduced to an appreciable extent (fig. 43). In the small bud there are only one or two exceedingly fine chromatin granules present, but as the bud enlarges its chromatin increases in quantity. In many cases the connecting thread between the nucleus and its bud is so fine that it is difficult to distinguish it even

with the highest powers (figs. 28, 30), therefore, we must conclude that the bud elaborates its own chromatin, or that the chromatin passes in solution along or through the connecting thread. In the other type of budding (figs. 29, 43, 62) the original nucleus may retain its original size, the bud becoming of almost equal size (fig. 43) or, the original nucleus may be reduced in size and the buds be of the same size as the reduced nucleus, so that it is difficult to determine which is the original nucleus and which is bud (figs. 62 and 29). As the nuclear degeneration progresses it may be accompanied by fragmentation of the cell body and nucleus, but in no case was cell division seen in this type of cell which could be interpreted as being the formation of new cells which would function, physiologically, as young elements.

In series III the nuclear degeneration is not as rapid as in the case of the cells belonging to series II. In this latter series of cells the nucleus become pycknotic and metachromatic while the granules are still of considerable size, but, on the other hand, the nucleus of this series of cells does not become polymorphous until a rather advanced stage of degeneration has been reached.

From the above observations it is concluded that the leucocytes belonging to series III, which have oxyphil (safranophil) granules during the state of highest morphological differentiation, are secretory cells and that the physiological activity of the cell is associated, morphologically, with cellular degeneration. The metachromatic properties of the cytoplasm in stages shown in figs. 25—29 would, alone, indicate degeneration, and one is still more convinced of this after a study of the nuclear changes.

There is nothing to indicate that the polymorphism or budding of the nucleus is for the purpose of cellular reproduction; on the contrary, the evidence favors the view that polymorphism of the nucleus in all three series of secretory leucocytes is associated with cellular senescence and is to be followed by complete degeneration. The degenerating cell may be taken up by phagocytes, or it may first undergo fragmentation and the fragments be taken up by phagocytes.

I do not care to enter into a discussion of the significance of amitosis and polymorphism of the nucleus of the leucocyte to any great extent, as the present paper has other problems in view, but will refer to a very recent work of Weidenreich (29), dealing chiefly with the nucleus of the mammalian granular leucocyte.

It is certain that polymorphism of the nucleus in cells of series I, II and III in Polyodon is accompanied by or followed by nuclear degeneration. According to Weidenreich, in the article referred to above, the same is true in the granular leucocyte of man and mammals.

According to Weidenreich the nucleus of the granular leucocytes in mammals (excepting mast cells) undergoes a regressive development of which five different types can be distinguished.[1]) The young forms have compact, almost round nuclei which pass through a definite series of types as the cells grow older. The types

[1]) Pappenheim, Virch. Arch. 1900, Bd. CLIX, S. 68; Atlas I, S. 15, 16, 27, 28, 31.

that Weidenreich distinguishes are 1) kidney shaped; 2) horseshoe shaped; 3) S shaped; 4) loop shaped; 5) spiral shaped.

Nuclei of these different types may be "compact" or "lobed". Following type 5 the lobes seperate and at the same time there are degenerative changes in the membrane and chromatin leading to pycknotic nuclei. The compact nuclei, following type 5, break up into several pieces which become pyknotic. The cells then undergo complete degeneration, with or without fragmentation. Only the young forms, with round or kidney shaped nuclei, can divide by mitosis.

In Polyodon there is no such regularity in the series of changes which the nucleus passes through and yet it does undergo a pretty definite series of changes which are associated with the age of the cell.

The granular leucocytes of mammals, excepting, perhaps, the mast leucocytes of man, which, according to Pappenheim and Weidenreich are degenerative forms and not true granular leucocytes, may divide by mitosis. According to Weidenreich only the young forms having "compact" nuclei have been seen in mitosis. In Polyodon, I could not find a single example of mitosis of a granular leucocyte in the lymphatic part of the kidney. The only mitotic figures which were seen were in the large non-granular, basophilic mononuclears. From this it would seem, that in Polyodon the power of mitosis is lost in the leucocytes as soon as they develop granules.

Regarding this condition in the mammalian leucocytes Weidenreich says: „Aus der bisherigen Betrachtung ergibt sich, dass die granulierten Leukozyten nur in ihrem Jugendstadium, das durch die kompakte Kernbeschaffenheit charakterisiert ist, mitotischer Teilung fähig sind, dass dagegen die sogenannte direkte Kernteilung, gleichviel ob ihr eine Zellteilung folgt oder nicht, als ein degenerativer Vorgang gedeutet werden muss, d. h. als der Ausdruck einer zur schliesslichen Auflösung der Zelle führenden und durch die Lappenbildung eingeleiteten besonderen Karyorrhexis" (29, page 278). According to Flemming (8), leucocytes which have once divided by fragmentation of their nucleus are no longer capable of further multiplication, but are destined to complete destruction, although they may live in the tissues and circulating media for some time before their final destruction is initiated.

Important for the present purposes, is the fact, that in mammals polymorphism of the nucleus is followed by fragmentation which is accompanied by degenerative changes. This is exactly what takes place in two types of granular leucocytes of Polyodon. However, here the process is not so regular as in the mammals. In Polyodon degeneration may take place without polymorphism and fragmentation; and the converse is also true, that polymorphism is not necessarily followed by cellular and nuclear degeneration. In the large non-granular leucocytes a series of stages can be seen, which indicate that a small bud may be given off from the nucleus and that this bud will enlarge and finally separate completely from the nucleus. Before separation, the round bud is generally attached to the nucleus by a very fine structure which appears to be nuclear membrane drawn out to a

very fine thread. There is no indication of degeneration in the nucleus or its bud. Many cells can be found having two nuclei of the same size, with no signs of degeneration in cell or nuclei.

We have here a plain case of amitosis which is not followed by cellular degeneration. Whether cellular division follows or not, could not be determined but probably it does, because mitotic figures are rare, while stages of amitosis are quite frequent.

From the case in hand, it must be concluded that nuclear polymorphism or budding, alone, are not sufficient evidence for nuclear degeneration, for, these processes may represent cellular reproduction, that is, a youthful condition of the cell. Further, the facts here indicate, that nuclear degeneration is generally, but not always, accompanied by budding and polymorphism of the nucleus.

That polymorphism of the leucocyte nucleus does not necessarily indicate degeneration, nor even amitosis was determined by O. van der Stricht (24). He found in the border of the liver of larval salamanders and in the larva of axolotl, in the same location, leucocytes with polymorphous nuclei which were in the spireme stage of mitosis.

Polymorphonuclear leucocytes in the spireme stage have also been figured by Heidenhain and by Flemming. Maximow, in an article published recently (13), figures various stages of amitosis and budding of the nucleus which were found in certain regions of the mesenchyme of rabbit embryos of 12 to $12^1/_2$ days. Maximow describes and figures conditions which are very similar to those found in Polyodon. The nucleus may give off one or more buds, which, while they enlarge, remain attached to the nucleus by means of a very fine thread. When they have reached the size of the nucleus they may separate from it and cellular division will follow. Simple constriction of the nucleus may also take place. This process is very active during the time indicated, but is then followed by mitosis. Mitosis may even take place in polymorphonuclear and polynuclear cells. No indication of nuclear degeneration was seen by Maximow, except in the case of very small buds which became detached from the nucleus and degenerated in the cytoplasm. Maximow's results show that in this case, budding, polymorphism and amitosis of the nucleus represent active growth and not degeneration.

Patterson, in his work on the pigeon's egg (15), came to the same conclusion. He found amitosis very active in certain regions of all three germ layers which were growing very rapidly. There was no indication of degeneration and amitosis was followed by mitosis.

Reuter (20) found many examples of amitosis in all the tissues of Alytes during metamorphosis of the intestine. Edward Reichenow (19) worked over some early stages of the same form and also Rana esculenta and Bufo vulgaris, but could find no indication of amitosis which was followed by cell division. He found many examples of polymorphism and budding and constriction of the nucleus and even complete nuclear division, but this was always associated with cellular de-

generation. He concludes, therefore, that amitosis does not take place in the frog and that where it is found it is exceptional and is in tissues which are not very highly differentiated.

From the present observations on the leucocytes of the haematopoetic organ of Polyodon and from the works cited above, the conclusion is reached that polymorphism and budding of the nucleus may be an indication of growth and cellular reproduction or, it may represent cellular senescence and degeneration, depending on the material under observation. Even in the same animal, as in Polyodon, it may indicate growth in one type of leucocyte and degeneration in another.

Evidently, Weidenreich's conclusions regarding the significance of polymorphism in the mammalian leucocytes do not apply to all the vertebrates

Accompanying the nuclear changes in the Polyodon secretory leucocyte are the cytoplasmic changes which lead to the formation of the secretion.

The stage of highest physiological activity corresponds to one which, morphologically, must be regarded as degenerative. The evidence for this is very convincing in these three types of leucocytes in Polyodon. This is also true for the granular leucocytes of mammals, according to Weidenreich and Metschnikoff. To quote Weidenreich, page 268: ". . . physiologisch wirksam kann die Zelle in jeder Lebensphase sein und wenn Metschnikoff Recht hat, entfalten gerade die zugrunde gehenden granulierten Leukozyten durch Freiwerden der sogen. Mikrozytase noch eine ausserordentliche Wirkung. Dass mit einer Zellumwandlung, die morphologisch als Degeneration bezeichnet werden muss, überhaupt erst die volle physiologische Leistungsfähigkeit erreicht werden kann, lehren uns in besonders deutlicher Weise die Erythrozyten der Säugetiere. Zweifellos fällt wohl bei dem granulierten Leukozyten der Höhepunkt der Lebensäusserung mit der morphologischen Phase der ausgesprochenen Kernlappung zusammen, und von diesem Gesichtspunkte aus mag die spezifische Kernumformung als Reifung gedeutet werden; berücksichtigt man aber, dass die weitere Entwicklung zu einem Zerfall der Kernmasse führt, so wird auch gegen die Auffassung der Lappenbildung als eines degenerativen Vorganges kaum etwas eingewendet werden können; ..."

In Polyodon, the secretion (Mikrozytase of Metschnikoff?) is liberated before the cell is destroyed, that is, in the earlier stages of its regressive evolution, but in the mammals the ferment is probably not liberated until the cell undergoes final dissolution, either in a phagocyte or, free in the connective tissue or in a serous cavity.

In the lymphatic portion of the kidney of Polyodon phagocytosis of red corpuscles and of leucocytes is extremely active, especially so in those portions of the lymph tissue which are pretty well filled with the secretory leucocytes. It is, therefore, not unreasonable to conclude that the secretion formed by these leucocytes is identical with the "Mikrozytase" of Metschnikoff which is formed by dissolution of the granular leucocytes in the mammals. Its function then, is to put the worn out red corpuscles and leucocytes into such shape that they can be

phagocytosed by the large non-granular basophilic leucocytes. Whether that is the only function is, of course, impossible to say. There are, in Polyodon, at least three distinct types of secretory leucocytes, each having a life history peculiar to itself, and so we may conclude that the secretion formed in each type of cell has its own specific function, different from that formed by the other cells.

The question as to whether leucocytes do form a secretion or not, is still being debated and this is particulary true for the mammalian leucocyte. In the mammals the evidence for the formation of the secretion is more circumstantial than otherwise. No such series of cellular changes during the formation of the secretion, as observed in Polyodon, can be found in the mammalian leucocyte. Nevertheless, certain bodies found in the mononuclears and lymphocytes of all mammals have been interpreted as products of secretion. Whether these bodies are to form a secretion which is to be extruded from the cell as an internal secretion having a definite function or, whether they are simply products resulting from the protoplasmic activity of the cell, is not yet clear. In the large mononuclears of the guinea pig and in the transitional leucocytes are found large bodies, the socalled Kurloff-bodies, which on account of their behavoir towards Giemsa and towards methyl green-pyronin, have been interpreted by Pappenheim to be secretory vacuoles filled with a kolloid or mucoid secretory contents. The contents retract from the wall of the vacuole after fixation in alcohol and appear perfectly homogeneous and structureless, taking on the azurophil color with Giemsa.

Cesaris-Demel holds that the same bodies have at least the value of a highly differentiated and complicated giant granule (from Pappenheim's Referat, Fol. Haem., Bd. V, Nr. 1). This conclusion is reached from their appearance with vital stains.

Ferrata holds these bodies to be specially enlarged "Plasmosomes" which, in turn, are identical with the azurophil bodies found in the lymphocytes, lympholeucocytes, large mononuclears, myeloblasts and promyelocytes. Ferrata, with Pappenheim, holds them to be products of secretion or, at least, expressions „eines allgemein vitalen Funktionszustandes" (Pappenheim). However, Pappenheim is not certain that the azurophil bodies in lymphocytes are identical with the plasmosomes of Ferrata which are colored in vital stains.

Patella states that the plasmosome bodies are not products of secretion „sondern degenerierte Bällchen der sterbenden leichenähnlichen Zellen sind" (from Pappenheim's Referat).

The whole question of the relation of the azurophil bodies and plasmosomes to the secretory activity of the mononuclears and lymphocytes in mammals is pretty well covered in the "Referata" of Folia Haematologica, Bd. V, Nr. 1, so it will not be necessary for me to go into further details here.

Metchnikoff concludes from experiments with mammalian leucocytes which have been introduced into normal salt solution containing bacteria, that the leuco-

cytes secrete the opsonin of the blood whenever there is a bacterial invasion. He also concludes that the granules of the granular leucocytes in undergoing dissolution liberate a substance, the "Mikrozytase" which acts on cells which have served their physiological usefulness in such a way that they can be taken up by phagocytes. However, detailed cytological observations which would support these views are lacking.

From the above it is seen that the question of the secretory activity of the leucocytes, so far as the mammals are concerned, is still very much "up in the air". In the lower vertebrates, more particularly in the fish, the evidence is more positive, but, still, it has not been very convincing.

Rawitz (18) in working on the circulating blood of fish described and figured leucocytes which are undoubtedly of a secretory nature, but he did not recognize them as such and did not work out their complete life cycle. Such cells are figured in V, 2c and d, Tafel VI of his article. On page 160 he says of them: „Die Zellensubstanz, die sich in Eosin-Hämatein meist dunkel-, selten hellpurpurn gefärbt hat, ist entweder homogen, und das ist bei dem letzteren Farbenton der Fall, oder sie zeigt eine ganz eigentümliche Strukturandeutung. In dunkel gefärbter Grundsubstanz sieht man zahlreiche, unmessbar feine Fädchen, die sich an vielen Stellen durch einander schlingen und dadurch eine Netzstruktur anzeigen. Die Kerne, in Eosin-Hämatin dunkelblau gefärbt, sind zuweilen gross und dann fast kreisrund, zuweilen klein und dann unregelmässig konturiert. Manchmal zeigen sie Andeutungen amitotischer Teilung oder sie sind leicht halbmondförmig eingebogen; nur selten geht diese Einbiegung bis zur zwerchsackförmigen Gestalt. Immer aber haben sie einen netzförmigen Bau, der bald sehr deutlich, bald nur angedeutet ist." These he saw in Scorpena porcus.

In Sargus vulgaris, fig. VIII, the same type of cell was found with the nucleus showing great variation in its structure.

In Crenilabrus pavo, fig. X, leucocytes were found which showed a distinct network in the cytoplasm. The appearance of this is associated with nuclear and cellular degeneration. As degeneration proceeds the network breaks up into granules (some of them spindle shaped according to the figures) which stain brilliantly in eosin.

From Rawitz' figures it would seem that the cytoplasmic network which he describes as being made up of interlacing threads is nothing more than the undifferentiated cytoplasm which remains between the secretory vacuoles. The degenerative changes in the nucleus, which accompany the appearance of the network in the cytoplasm, would further lead me to believe that exactly the same processes were going on as in the secretory leucocytes of Polyodon.

The presence of threads in the cytoplasm which would form a network cannot be absolutely disputed, for, in Polyodon a few cells were found, belonging to the secretory leucocytes of series II and III, which contained in their cytoplasm a long heavy thread which was coiled into one or two loops, but in no

case did the loops interlace to form a network (fig. 44). This thread was independent of the cytoplasmic reticulum between the vacuoles and in no case did its one or two loops give any such appearance as is shown in Rawitz' figures V, 2 c and d and X, 3 c and d. However, the cytoplasmic reticulum between the vacuoles often appeared very much the same as is figured by Rawitz.

In one case Rawitz found the cytoplasmic threads breaking up into granules which stained intensely in eosin. He associates the appearance of the network in the cytoplasm entirely with cellular degeneration and makes no suggestion whatever that it is associated with the secretory activity of the cells.

Drzewina (6), who has recently worked over the lymphatic tissue of practically all groups of fish, says nothing about a secretory function of the leucocytes, nor do her figures show anything which would indicate that she had seen stages in the life cycle of the leucocyte which would suggest such a function. However, her figures 14, 15, 17, 29 are very suggestive when interpreted in the light of conditions as they are found in Polyodon.

Grünberg (7) found in the circulating blood of Scyllium catulus leucocytes having spindle shaped granules and vacuolated cytoplasm. He found the cytoplasmic reticulum to be always basophil. He thinks that the vacuoles are due to the presence of uncolored granules. He found no indications of "grains de ségrégation" or of changes in the granules or cytoplasm. In Triton and in Rana he found close relations between the granular and non-granular cells. In the non-granular cells the structure of the nucleus and cytoplasm was the same as the nucleus and cytoplasm of the granular cells, and their homogeneous cytoplasm took the same color in triacid and acid stains as did the intergranular cytoplasm of the granular leucocytes. He concludes, that in Rana and in Triton there are close relations between the non-granular and the granular, acidophilic, polymorphous and polynuclear cells. Whether the latter originate from the first or, whether the granules disappear from the cell and its cytoplasm becomes homogenous, could not be determined from the blood preparations.

P. Stephan (22) (Referat in Fol. Haem. Bd. V, No. 1, page 20) who has published a paper on the function of the large eosinophils in Protopterus, states that these cells behave like elements having an internal secretion. During the time of full activity of the animal the lymphoid tissue contains many cells having eosinophil granules. At the end of the period of sleep the number of these is very much reduced. A short time after the awakening one can see the development of the granules in many of the large cells. At the same time the granules in many of the elements undergo a progressive destruction, this destruction continuing during the time of sojourn of the animal in its cocoon. From this it is concluded that the granules are composed of a substance, the elaboration of which is limited to the period of activity and nutrition of the animal, but which is consumed at all times by the organism.

After the cell becomes filled with granules the latter lose their affinity for stains, become smaller and are finally completely dissolved and one sees nothing in the cell but the cytoplasmic network which was interposed between them. Mitotic figures during all stages of activity are found in those cells which are filled with granules and those which are empty. Finally, a certain number of the large cells show signs of degeneration, especially so at the end of the period of sleep and present various stages of pyknosis. However, these nuclear degenerations are exceptional and the exhaustion of the granules seems to be followed by a new period of elaboration. The nucleus participates in the formation of the secretion and shows signs of trophic activity, similar to the changes of the nucleus in a gland cell. During the elaboration of the granules the nucleus is large and shows distinct chromatin in the form of thick cords, karyosomes and more or less fine granules. After the disappearance of the granules the nucleus is very much condensed, more homogeneous and contracted like an exhausted element. Its relation to the formation of the granules, as Stephan sees it, has been described in another place in this paper.

The above description of the formation of the secretion could be applied to the same process as found in certain types of leucocytes in Polyodon. However, in Polyodon, mitotic figures are not found in any of the granular leucocytes, nor in the leucocytes containing secretion; also, in Polyodon, degeneration of the cells which are forming the secretion is the rule and not the exception.

In Polyodon there is no such periodicity in the formation of granules and secretion as is found in Protopterus. Polyodon caught in summer and winter show exactly the same condition of the lymphatic tissue. Stephan states that the aspect of the secretory cells (eosinophils) in Protopterus is so special that one cannot homologise them in structure or function with the granular cells in the tissues of other vertebrates.

Unfortunately, Stephan's description is not sufficiently detailed and is not accompanied by figures.

At the nineth reunion of the "Association des Anatomistes" at Lille, 1907, A. Policard and J. Mawas presented a paper on "Le Tissue Lymphoide du Rein des Téléostéens" (16) in which they conclude that certain mononuclears having an alveolar cytoplasm, apparently pierced with holes like a skimmer, are secretory leucocytes. This conclusion is based on the appearance of these cells when stained in a fresh condition with the vital stains, neutral red and toluidin blue. They dissociated the fresh kidneys in serum to which the vital stains were added. After this treatment certain of the mononuclears showed a spongy cytoplasm, the vacuoles of which were stained with neutral red. Some of the vacuoles contained granules and others not. According to the authors this may represent different stages of secretion. The neutral red stained only the liquid contents of the vacuoles and not the granules.

As to the significance of these elements, the authors conclude that the manner in which these cells behave towards neutral red and other vital stains is proof of the secretory function of these elements. They possess a "rhagiocrine" function, described in a series of works by M. Renaut (Communications à la Société de Biologie, 1905—1906).

The published article does not contain a detailed description of the intermediate stages to be found during the formation of the secretion and is not accompanied by figures.

The fact that the contents of the vacuoles stains in neutral red is no absolute proof of the secretory nature of the vacuoles. In the discussion which followed the reading of the paper M. van der Stricht thought that the spongy aspect of the cytoplasm might be due to products of phagocytosis. M. Policard denied this, pointing out that the vacuoles are very small and do not contain anything in the way of cellular débris. There are no intermediate stages between these and the cells which are undoubtedly phagocytic. M. Dubreuil in a measure confirmed the results of M. Policard in regard to the secretory nature of the vacuoles in the larger elements of the kidney of Teleosts. The vacuoles are filled with a liquid, holding in suspension a granule corresponding to the "grain de ségrégation" described by Renaut and Dubreuil in the Rhagiocrine connective cells of the connective tissue (C. R. de la Soc. de Biolog. 1905—1906 and C. R. de l'Assoc. des Anat. 1906). The function of phagocytosis is always represented in these cells and there exist two kinds of vacuoles: 1. vacuoles of phagocytosis — large and more irregular than the others; 2. vacuoles of "ségrégation", characterised by liquid and a granule. The regularity of these latter granules and their property to stain electively in neutral red make these granules secretory and not phagocytic.

Without doubt, Policard and Mawas have seen the secretory leucocytes in the Teleost kidney, but their conclusions regarding the secretory nature of these leucocytes are based on the rather slim evidence that the cells contain granules and vacuoles which stain in fresh the condition with vital stains. At any rate, they have not worked out the life history of the cells concerned and without that, it seems to me, it would be difficult to tell in all cases whether the granules and vacuoles are due to a secretory activity of the cells, or are simply due to products of phagocytosis.

The best account of the secretory activity of leucocytes has been given in the above mentioned article by Stephan on the secretory function of the large eosinophiles in Protopterus.

The history of the granules and vacuoles in the cytoplasm with the correlated changes in the nucleus, prove conclusively that these leucocytes have a secretory function, but, as he himself says, the habits of Protopterus are so exceptional that we have here a special case from which we would hardly dare generalize for the rest of the fish, much less for all of the vertebrates.

J. Meinertz (14) finds in Tinca vulgaris a series of leucocytes which are very similar to those of series I of Polyodon. They are illustrated in figs. 47, 48, 49, Taf. XI of his paper. Fig. 47 corresponds to fig. 32 of Polyodon and the staining reactions are the same in triacid. M. describes this leucocyte as follows: „Wir sehen eine Zelle etwa von der Grösse der menschlichen multinukleären Leukozyten mit einem Zellleibe, der im Vergleich zum Kerne gross und dicht mit violett gefärbten, annähernd regelmässig runden, gleich grossen Granula erfüllt ist, die, grösser als die menschlichen neutrophilen, sich von dem kaum gefärbten Untergrunde deutlich abheben. Eine Struktur des Kernes ist, wie gewöhnlich, bei Triazid nicht wahrzunehmen."

The cell shown in fig. 48 corresponds pretty well, excepting for the shape and position of the nucleus with fig. 35 of Polyodon. The figure would indicate that the granules are located in vacuoles and that the cytoplasm has stained a dark violet, about the same color as the granules. This is the same condition as is found in Polyodon, fig. 35.

Regarding this leucocyte M. states: „Bei anderen Zellen (Taf. XI, Fig. 48) ist die Granulation nicht so regelmässig, die Granula sind weniger gleichmässig, weniger vom Grunde abgehoben, der, mitgefärbt, stellenweise in die Granula übergeht."

„Bei anderen wieder, ist kaum mehr etwas von Granulis zu sehen, der Grund des Zellleibes ist violett gefärbt, aber nicht gleichmässig, sondern mehr in Gestalt eines Netzwerkes, das stellenweise sich mehr verdichtet, stellenweise auch granulaähnliche dunkle Flecken zeigt (Taf. XI, Fig. 49). Es ist kein Zweifel, dass diese Formen ineinander übergehen, dass keine scharfe Abgrenzung zwischen den beiden vorgenommen werden kann."

Excepting for the structure and position of the nucleus, the cell shown in fig. 49 corresponds to a stage in secretory leucocytes of series I in Polyodon which would be intermediate between figs. 35 and 36 of Polyodon.

M. recognizes the connection between the leucocytes shown in figs. 47, 48 and 49, but does not suggest in any way that the granules are taking part in the formation of a secretion.

In some of the leucocytes of this same fish, Tinca vulgaris, M. finds peculiar structures which are quite variable in their size, shape, number and staining reactions. They are illustrated in figs. 65 to 73, Taf. XI and seem to correspond to irregular structures found occasionally in the basophil leucocytes of Polyodon. A Polyodon leucocyte containing them is shown in fig. 58 (iron-haematox.-saf.-tol.). Here these structures have a very strong affinity for safranin and are very clearly differentiated from the dark violet cytoplasm. M. finds the structures in Tinca vulgaris in cells which otherwise resemble lymphocytes. M. thinks that they are not products of phagocytosis but that they have been produced by the cytoplasm, but no longer form a living part of it nor are they an integral

part of the cell. He finds all the intermediate stages between these bodies and the ordinary granules.

In Polyodon these structures have the same staining reactions as the granules of secretory leucocytes of series III and for that reason I would not count them as phagocytosed remains of degenerating cells. To me it seems that the irregular structures in the Polyodon leucocyte, which are found only occasionally (fig. 58), are composed of the same substance as are the round and spindle shaped granules of the secretory leucocytes of series III. This substance, instead of being deposited in a regular shape of round or spindle shaped granules, more or less evenly distributed throughout the cell, is, in this case, deposited in a few irregular masses which vary in size and distribution. On account of the fact that so few cells of this type are found in Polyodon, the process may be regarded as abnormal for Polyodon. In Tinca vulgaris it is a normal method for depositing the granular substance.

Irregular structures in Polyodon leucocytes which are undoubtedly of phagocytic origin are shown in figs. 59a (W.-J.) and 63 (fuchs.-or.-tol.). These structures are the remains of red corpuscles. In fig. 59a the cytoplasm shows as a faint pink substance surrounding the degenerating nucleus. Fig. 59b is the same cell drawn at a higher focus to show its nucleus. In fig. 63 the cytoplasm of the phagocytosed corpuscles has disappeared, some of the nuclear substance has broken up into finer granules and some of the remaining larger masses have become vacuolated. Further evidence that these structures are degenerating, is the fact, that they stain metachromatically which is especially evident in fig. 63, where the phagocytosed nuclear substance is stained green with toluidin blue.

The case with the structure shown in the leucocyte shown in fig. 58 is quite different.

These structures have none of the earmarks of degenerating phagocytosed substances. Their reactions to stains are exactly like those of the granules in secretory leucocytes of series III and they occur in the same type of cell, which has a strongly basophilic cytoplasm. Leucocytes which have phagocytosed whole cells or large fragments of them, generally lose their basophilic qualities to a certain extent.

Figs. 83 and 84, Carassius vulgaris look very much like secretory leucocytes in Polyodon. Fig. 83 corresponds to fig. 25 of Polyodon; figs. 86 and 87 are very much like fig. 36 of Polyodon. M. describes the cytoplasm as containing many, almost colorless granules which have dark borders. The granules cannot be stained with any of the Ehrlich mixtures, but the borders always appear dark. From the fact that similar "granules" and "dark borders" in Polyodon leucocytes were, on closer study, found to be vacuoles separated by a small amount of dark cytoplasm, I should judge that the same conditions obtain here. For Cyprinus carpio M. figures three large leucocytes in figs. 109, 110, 111 which evidently represent three different stages of a secretory process. The cells are

large and have eccentric nuclei. The granules are fine and stain violet in triacid. In fig. 109 the granules are very numerous and the cytoplasm is almost colorless. In fig. 110 the intergranular substance is of about the same color as the granules and shows indications of small vacuoles. At least some of the granules are apparently located in the vacuoles, although the figure is not very definite on this point. Fig. 111 is more definite in this regard and shows a reduction in the number of granules. (Some of them have completely dissolved, their substance forming the contents of the vacuoles which contain no granules.) The remaining granules are, according to the figure, located in large vacuoles, the intergranular substance being reduced to a small amount, so that it appears like a reticular network.

The point here is, that in Cyprinus carpio, leucocytes are found which pass through practically the same changes as do the Polyodon leucocytes of series I and III. At the stage of highest development of the granules the intergranular substance is almost colorless, but as the granules dissolve it will gradually assume the same color as the granules and will remain in that condition until the granules are completely dissolved.

Regarding the leucocytes in Cyprinus carpio M. states: „Es ist hervorzuheben, dass hier, ebenso wie bei Carassius, sich die einzelnen Gruppen nicht scharf scheiden lassen, sondern Übergänge vorkommen von mehr zu minder granulierten Zellen. Ebenso wenig wie dort, möchte ich hier entscheiden, ob wir dabei Stadien verschiedener Reife der einzelnen Zellen anzunehmen haben, oder ob die anderen, oben berührten Verhältnisse vorwalten."

Hesse (9) takes the position, based on Arnold's investigations on cell granulations and on his own investigations on the marrow of rabbits and on a lymphosarcom, that the different granula represent structural constituents of the protoplasm — elemental organs of the cell. He thinks that Ehrlich's conception of the granula as specific cell products has been disproven by the demonstration of several kinds of granula in the same cell. He thinks that Arnold's researches prove that the granula are structural constituents of the cell.

M. does not think that Hesse has proven his point very well; for example, he thinks that the presence of two kinds of granules in the same cell does not show that these granules are not products of secretion by the cell.

It is true, as M. states, that we do not yet possess sufficient evidence to judge the whole question from the standpoint of a definite theory and it is equally true that the most important part of the question is not, whether the granules are products of secretion of the protoplasm or, whether they are structural elements of the protoplasm; yet we are on the road to a solution of the question if we can determine definitely that, at least, certain classes of leucocytes have granules which are identical with secretory granules of gland cells; that these granules are simply intermediate stages in the chemical formation of a specific secretion which is to be poured out of the cell and which has nothing whatever to do with

the ordinary metabolism which is concerned with the life of the cell itself. Perhaps the immediate purpose of the granules is a convenient form of storage of the secretion in a concentrated condition from which the final product can be rapidly manufactured as the organism needs it.

Whether these granules are derived from living structural elements of the cell, the plasmosomes, as Arnold (2) is inclined to believe they are, or whether they are products which have been secreted by or precipitated from the protoplasm as Ehrlich believes, is impossible to determine from the Polyodon material. The determination of this point is not of very great importance to the problem of the function of the granules after they are once formed.

If we take the special case of Protopterus as representing the Dipnoids, Polyodon for the Ganoids, Policard and Mawas findings in Abramis brama and Cyprinus carpio, and Meinertz' figures and descriptions on Tinca vulgaris, Carassius vulgaris and Cyprinus carpio for the Teleosts, Drzewina's figures for lymphoid tissue of Selachii and Teleosts, Rawitz' figures for circulating blood of Teleosts and Scyllium catulus, and Grünberg's figures and description for the same form, we have considerable evidence which would lead us to conclude that all fish have certain leucocytes whose function is the formation of an internal secretion. In some cases the authors have recognized this function, but in most cases they have not recognized it, which may be due chiefly to the fact that so little work has been done on the lymphoid organs. In most cases where the authors have not recognized this function, their figures and descriptions show, nevertheless, that secretory processes were going on.

The next step is to determine definitely the exact nature of the secretion and its exact function. Here is where a large field is opened up to experimental physiology.

These cells are unicellular glands with, according to Dubreuil, the function of phagocytosis added. However, in Polyodon, granular leucocytes and those containing secretory vacuoles were never seen to be phagocytic, so the question of whether these same leucocytes can also function as phagocytes must still be regarded as open to debate.

If, in the mammals, the socalled non-granular cells (lymphocytes, large mononuclears, etc.) are secretory cells, then these same cells combine with their secretory activity the function of phagocytosis, and it would be reasonable to suppose that the same thing is true in the lower vertebrates.

In Polyodon there are at least two types of granular leucocytes which occur so rarely, that stages indicating the development of the granules and their future history could not be found, so we cannot conclude that all of the granular leucocytes in this animal are of a secretory function. However, the three main types of granular cells, those which occur most frequently, are of a secretory nature and so we may conclude that the main function of the granular leucocytes in the fish, is the formation of a substance which is in the nature of an internal secre-

tion, or an extracellular ferment, although there may be some special leucocytes set aside for some other purpose.

According to Metchnikoff and others, this is also the function of the granular leucocytes in man and mammals. However, the evidence for this view of the function of the granular leucocytes in mammals is still very incomplete. Probably more can be gained from a thoroughly worked up comparative histology of the blood and lymph organs of the vertebrates between the fish and mammals than from any amount of work upon the mammals alone.

Leucocytes having a secretory function have also been found in the invertebrates. L. Bruntz (3) states that the elements of the blood of the Arthrostraca and Decapoda are glandular and phagocytic elements.

Kollmann, in his recent article on the blood of invertebrates, expresses the same view regarding the function of the leucocytes, with the addition that the secretion formed is in the nature of albuminous reserve food matter.

Kollmann's article (12) contains a good bibliography of the invertebrate literature and also a thorough discussion of that literature, so nothing further need be said here regarding conditions in the invertebrates.

The most important results of this investigation may be summarized very briefly.

1. The portal circulation through the lymphoid part of the kidney is an „open" one. The wall of the portal vein and of its larger branches has openings which lead directly into the lymphoid tissue surrounding the vessels. The venous blood which does not pass through these openings is carried into blood spaces which are not lined with endothelium and which empty into the general reticulum of the lymphoid tissues.

After filtering through the lymphoid tissue the blood passes into another series of spaces and thence into the efferent vein. Many of the worn out erythrocytes break down in the afferent vessels, others are phagocytosed in the lymphoid tissue and in the blood spaces.

2. The lymphoid part of the kidney is the chief haematopoetic organ of Polyodon. In it, all the different blood elements are formed, leucocytes as well as erythrocytes, and the blood cells which have served their physiological usefulness are gotten rid of by phagocytosis or otherwise. The efferent vessels function, both, as lymph and blood vessels and serve to carry the young blood cells into the circulation. That the secretion formed by the granules of the leucocytes is of use in the general circulation may be concluded from the fact that secretory leucocytes, in all stages of their evolution, are found in the efferent vessels.

3. The blood elements cannot be divided into a myelogenous and into a lymphatic series, because they are all developed in the same region and there is no lymphoid bone marrow in these forms.

4. A basophilic large mononuclear cell seems to be the mother cell for all the different forms of blood cells, leucocytes as well as erythrocytes.

5. There are at least three types of secretory leucocytes in Polyodon and possibly six. The three types which were worked out in detail live out their own independent life history after they are once differentiated from the basophilic mononuclear. There is no passage from one type to another. The secretory leucocytes are found chiefly in that part of the lymphoid tissue through which the blood is filtered and which often contains as many erythrocytes as it does leucocytes. Whether the secretion formed is carried into the general circulation and is of use to the general metabolism of the animal or, whether it is in the nature of a „microcytase", could not be determined. The fact that many of the secretory leucocytes are found in the efferent vessels in all stages of their evolution, may be regarded as favoring the former view and the fact that they are especially numerous in those regions in which phagocytosis is very active may favor the latter view.

6. Polymorphism of the nucleus may indicate cellular degeneration in one type of cell, but it may be an indication of cell growth and multiplication in another type of cell.

Postscript.

While this article was in press a paper appeared in this journal by Carmelo Ciaccio in which he states that there is no phagocytosis of erythrocytes in the myeloid tissue (lympho-renal tissue of fish) of any vertebrates below mammals. As phagocytosis of erythrocytes is a very important function of the lympho-renal tissue of Polyodon, it was decided to make this the subject of a special paper, the manuscript for which has already been sent to Folia haematologica.

During the course of this study it was found that the black granules contained in the phagocytes of erythrocytes, which in the present paper are described as haemosiderin granules, are not haemosiderin granules, but pigment granules formed from the degenerating nucleus of the phagocytosed erythrocyte. They have the ordinary appearance of haemosiderin granules found in any tissue in which there is an extensive destruction of erythrocytes. A closer study of them shows that they are derived from nuclar material, and that the cytoplasm of the erythrocyte is dissolved without leaving any granular remains.

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Explanation of Figures.

Fig. 5—9, 14, 15, 17—53, 59, 60 and 63 are camera lucida drawings with Spencer 1,5 mm objective and ocular 6X. Of these, fig. 5—9, 22—24, 26, 30, 31, 37, 38, 40, 41, 44—51, 53 and 63 are drawn with the same objective, but with Zeiss Comp. Oc. 6 which gives a somewhat greater magnification. The figures which are not drawn with the camera lucida are drawn to scale after several measurements of each cell with the eye piece micrometer. All of the figures are made from material fixed in 10 perc. com. formalin.

Fig. 1 small lymphocyte, type 1. Iron-Haematox.-saf.-tol.

Fig. 2—4 large lymphocytes developed from small lymphocyte, type 1 (fig. 1).

Fig. 5 small lymphocyte, type 2.

Fig. 6—9 small and large non-granular mononuclears. Fig. 5—9 are stained in saf.-tol.

Fig. 10—12 plasma cells. Tech. same as for fig. 1.

Fig. 13—30 secretory leucocytes of series III. Tech. for fig. 13—17 same as for fig. 1; 18—23, 25, 27, 30 in Wright's modification of Romanowsky; 24 and 26 in eos.-or.-tol.; 28, 29 in fuchs.-or.-tol.

Fig. 31—36 secretory leucocytes of series I. 31 in fuchs.-or.-tol.; 32—36 in Wright's Rom.

Fig. 37—44 secretory leucocytes of series II. 37 in fuchs.-or.-tol.; 38, 40, 41 in eos.-or.-tol.; 39, 42, 43, 44 in Wright's Romanowsky.

Fig. 45 and 46, 47 and 48, 49 and 50 three types of leucocytes whose granules are probably secretory granules. The complete life history of these cells was not worked out. Granules show changes in chromaticity and are sometimes found in vacuoles. 45—46 in Wright's Rom.; 47, 48 in saf.-tol.; 49 in Wright's stain; 50 in eos.-or.-tol.

Fig. 51 eos.-or.-tol. Granular leucocyte of which only four or five were found. Nothing known about its origin or function. The granules have a special affinity for orange G and they were never found in vacuoles.

Fig. 52 secretory leucocyte of series III. Shows origin of acidophilic granules from a basophilic, slightly granular cytoplasm. Stained in fuchs.-or.-tol.

Fig. 53 degenerating gran. leucocyte containing acidophilic and basophilic grans. Wright's Romanowsky.

Fig. 54 non-granular leucocyte with 2 nuclei. Tech. same as fig. 1.

Fig. 55 non-granular mononuclear leucocyte which has phagocytosed a lymphocyte.

Fig. 56, 57 leucocytes which contain one large mass or several smaller masses of a colloid substance which is elaborated by the cell. Cells like these are not frequent. 56 in Wright's stain; 57 in fuchs.-or.-tol.

Fig. 58 leucocyte in which the granular substance has accumulated in irregular masses. Staining reactions of this substance are the same as for granules of secretory leucocytes of series III.

Fig. 59a leucocyte containing the phagocytosed remains of erythrocytes. 59b same cell drawn at a higher focus to show its nucleus. Wright's stain.

Fig. 60a, b, c three stages in the degeneration of erythrocytes in the afferent vessels. Eos.-or.-tol.

Fig. 61, 62 vacuolated secretory leucocyte to show budding and polymorphism of the nucleus.

Fig. 63 leucocyte containing products of phagocytosis. Fuchs.-or.-tol.

Fig. 64 reticular cell in the boundary of a blood space. The cell contains a phagocytosed leucocyte and 2 erythrocytes. Iron-haematox.-saf.-tol.

Fig. 65 reticular cell from the wall of a blood space. The cell contains granules which are probably products of phagocytosis. Tech. same as for fig. 64.

Fig. 66 relations of the reticulum to a small blood space (post. end of organ). The reticulum is fibrous and contains many different types of nuclei, some of which resemble endothelial nuclei. a, b, c undifferentiated cytoplasm of the reticulum; d, e, f, g, h nuclei of the reticulum; k older erythroblast; l kidney tubule; m nuclei of erythrocytes. The blood space contains various forms of leucocytes, plasma cells, erythrocytes and one erythroblast at k. Tech. saf.-tol. Zeiss Apoch. 2 mm, Comp. Oc. 6, camera lucida.

Fig. 67a, b, c reticular cells from the ant. lymphoid portion of the kidney. They all show granules in their cytoplasm and fibrils in their finer processes. Iron-haematox.-saf.-tol. Zeiss Apoch. 2 mm, Comp. Oc. 6. Camera lucida drawings.

Correction of the plates.

The fact that the proof for the lithographed plates was lost in transit accounts for the following errors in the plate.

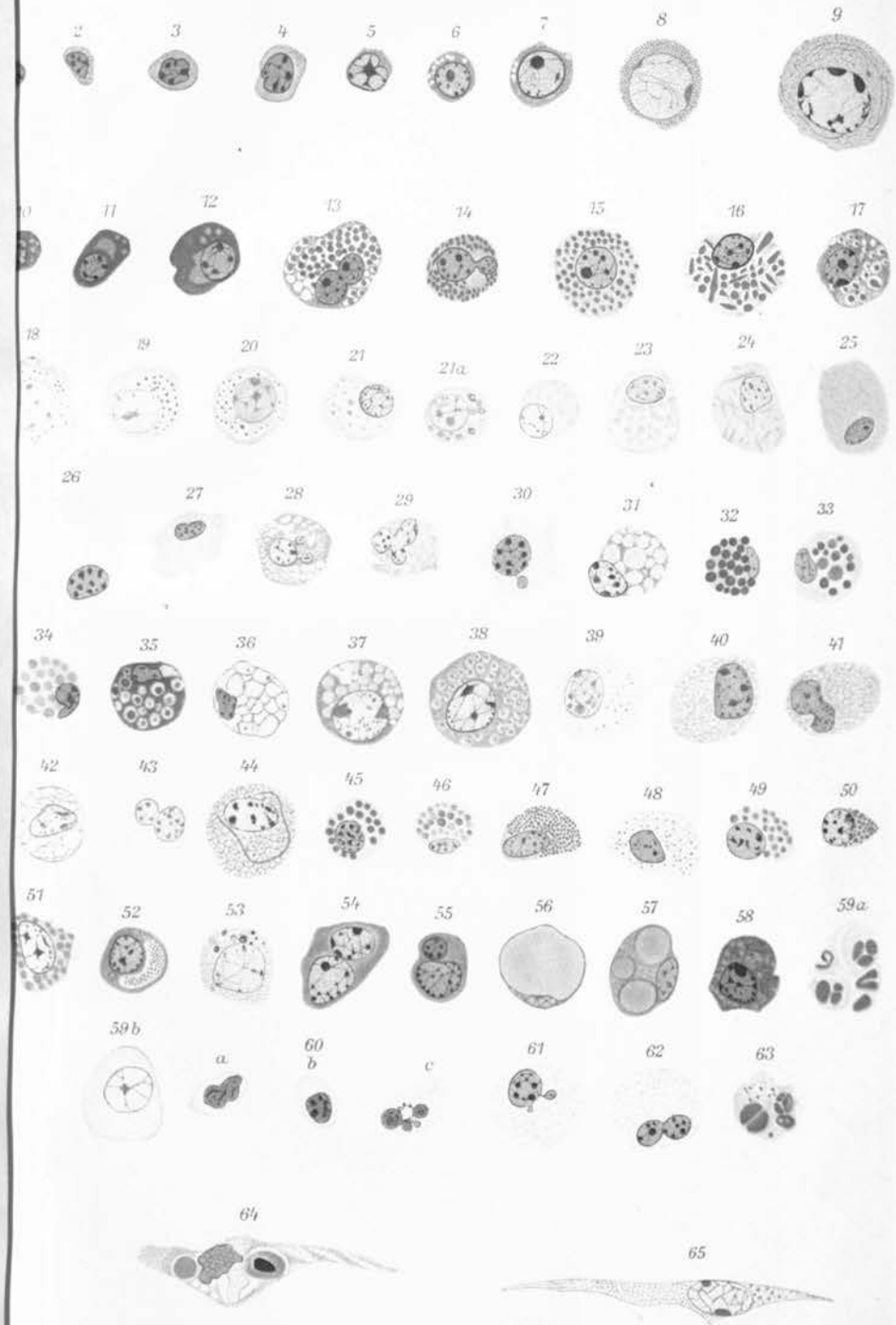
Fig. 9. The cytoplasm should be a very pale blue and the reticular network should show more distinctly.

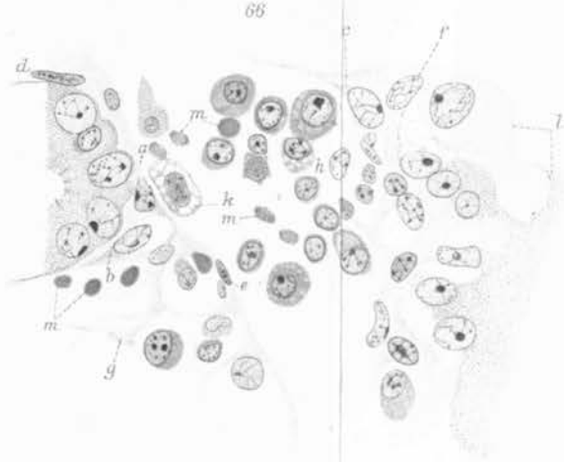
Fig. 26. The original drawing shows a vacuolated cytoplasm which is just barely indicated in the plate.

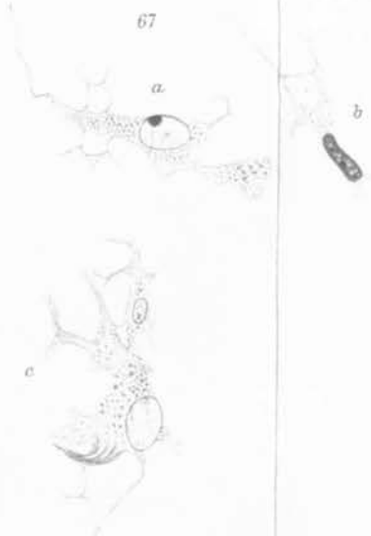
Fig. 33. The granules should be pale violet in color.

Fig. 66. The original drawing shows a narrow band of undifferentiated cytoplasm along the border of the reticulum at a. g is a nucleus of the reticulum and should contain chromatin granules and have a membrane around it.

H. Downey.







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Life

I, Hal Downey, was born at State College, Pa.,Oct. 4th, 1877. My elementary education was gained in the public schools of Minneapolis and in the Royal Realgymnasium of Hannover, Germany, which institution I attended for four years. After returning from Germany I spent a year in the Central High School of Minneapolis, from which I was graduated in 1896. In the fall of the same year I matriculated at The University of Minnesota where I remained until the outbreak of the Spanish-American war in 1898,when I went to the Philippine Islands with the 13th Minnesota Volunteer Infantry. After an absence of three years I again took up my work at The University of Minnesota, receivingthe Bachelor of Arts degree in 1903.During the same year I was elected a member of Sigma Xi. The Master of Arts degree was granted the following year, 1904.

After graduation I was made an assistant in the Department of Animal Biology of The University of Minnesota. In 1907 I was promoted to an Assistant Professorship in that department, which position I hold at present.