THE BLOOD OF THE GARTER SNAKE

(Eutania sirtalis and Eradix.)

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G E N E R A L   L I T E R A T U R E.

Franz Weidenreich (30)

Bei den verschiedenen sonstigen Klassen der Wirbeltiere kehren im grossen und ganzen dieselben Verhaeltnisse wie bei den Saeugern wieder, wenn sich auch im einzelnen interessante und bemerkenswerte Unterschiede ergeben. Allerdings ist auch hierbei zu beobachten, dass die Untersuchungsergebnisse bis jetzt vielfach durchaus noch ungenuegend sind und die Homologisierung der einzelnen Typen dadurch erschwert ist, dass dem faerberischen Charakter der Granula eine oft ausschliessliche Bedeutung zugesprochen wurde. Lasst man fuer die granulierten Elemente dieselben Einteilungsprinzipien gelten wie bei den Saeugern, dann kann man auch bei den Voegeln drei Formen unterscheiden und zwar Mastleukocyten, acidophile und spezialgranulierte. Die Mastleukocyten besitzen hier einen einfachen, runden und zentral gelegenen Kern; ihre Granula wechseln in Zahl und Grosse. Die acidophilen weisen einen gelappten oder einfachen Kern auf und runde Granula. Den spezialgranulierten Elementen entspricht ein zweiter acidophil granulierten Zelltypus, der bei allen untersuchten Vogelarten mehr oder weniger dominiert und dessen Besonderheit darin besteht, dass die Granula ihrem Faerbungscharakter nach zwar gleichfalls "acidophil" sind, aber in ihrer Form und Struktur kristalloiden Koerpern aehneln.

Auch bei den Reptilien kehren im allgemeinen dieselben Zellelemente wieder. Die Mastleukocyten besitzen einen einfachen, ovalen Kern. Die Acidophilen sind meist einfach kompaktkernig, seltener gelappt, wobei ihr Kern bei manchen Formen den Charakter des kleinen Lymphocytenkernes zeigt; die Granula sind rund oder elliptisch . Zellen mit Granula, die denen der spezialgranulierten der Saeuger entsprechen, fehlen hier, dagegen finden sich ungekoernte Elemente mit gelappten Kernen, die in ihrer Grosse und Struktur, ebenso wie in ihrem biologischen Verhalten und der Art ihrer Verteilung in Gewebe und Organ jenen spezialgranulierten Formen als gleichwertig zu betrachten sind.

Bei den Amphibien liegen die Verhaeltnisse aehnlich wie bei den Reptilien. Die Mastzellen zeigen keinen bemerkenswerten Unterschied. Bei den Acidophilen ueberwiegen die gelapptkernigen Formen, ihre Granula sind meist rundlich. Spezialgekoernte Leukocyten fehlen, an ihre Stelle treten gelapptkernige Formen ohne distinkte Granulationen.

Grossere Einheitlichkeit herrscht bei den Nichtsaeugern in bezug auf Vorkommen und Habitus der ungranulierten, kompaktkernigen Leukocyten. Ueberall finden sich hier Zellen vom Typus der kleinen Formen -- Lymphocyten Ehrlich's --, also Elemente mit grossem rundlichem Kern und schalem basophilem Plasmasaum, und daneben in geringerer und wechselnder Anzahl grossere Zellen mit grossem runden, ovalen oder eingebuchteten Kern und breiten basophilem Plasmaleib, also Elemente, die in ihrer Erscheinungsform durchaus den sog. "grossen mononukleaeren Leukocyten" der Saeuger gleichen. Waehrend bei den Saeugern diese beiden Leukocytentypen an Zahl hinter den granulierten zuruecktstehen, ueberwiegen sie im Allgemeinen bei den Nichtsaeugern; ja es kommt sogar vor -- bei einzelnen Voegeln und Fischarten —, dass die Zellen vom lymphocytaeren Typus da nahezu ausschliessliche farblose Blutelement bilden.

Bei den granulierten und den ihnen morphologisch nahesteh enden Formen ist noch besonders bemerkenswert, dass bei allen klassen der uebrigen Wirbeltiere auch einfache kompaktkernig Formen in einem grossen Prozentsatz in der Zirkulation vorko waehrend solche Elemente bei den Saeugern zwar nicht voellig en, aber doch verhaeltnismaessig selten sind und die Hauptma von den gelapptkernigen Leukocyten gestellt wird.

Carl Gruenberg. (14).

Grünberg's work on Amphibia, Reptilia, and Aves will be reviewed here:

Amphibia.

A. Siredon pisciformis.

1. Small non-granular leucocytes with large nucleus.

2. Mast leucocytes with two nuclei and basophil granules.

3. Large non-granular leucocytes with relatively small nucleus, usually much fragmented but seldom polymorphic.

4. Mononuclears of various sizes with eosinophil granules.

B. Rana temporaria.

1. Mononuclear non-granular leucocytes with very large nucleus.

2. Large transitional non-granular leucocytes.

3. Large leucocytes with fragmented nuclei, of two kinds:

a. Non-granular but much vacuolated cytoplasm.

b. With eosinophil granules of various sizes.

In Amphibia he finds that the granules are round and never crystalloid as is usual in reptiles and birds.

Reptilia.

A. Lacerta muralis.

1. Very small non-granular lymphocytes.

2. Transitional leucocytes with an oval to horseshoe-shaped nucleus; a few granule-like structures in the cytoplasm.

3. Mononuclear mast leucocytes with basophil granules.

(Cells smaller and granules coarser than in frog).

4. Rather large cells with round to crystalloid eosinophil granules.

B. Anguis fragilis.

1. Small and large non-granular leucocytes with a large nucleus and narrow cytoplasm.

2. Large leucocytes with or without fine neutrophil granules; nucleus varying in shape.

3. Very small mast leucocytes with relatively coarse granules.

4. Large mononuclear leucocytes with probably eosinophil granules.

C. Tropidonatus natrix.

1. Few small leucocytes with very large nucleus.

2. Large mononuclear leucocytes with or without fine neutrophil granulation; nucleus relatively large.

3. Mast leucocytes with a large nucleus and thin cytoplasm; cells small; granules finer than in Anguis. Triacid gives a bright green nucleus and bluish-green granules.

The cells of the reptiles, he finds are in general smaller than those of the Amphibia and the nuclei are less fragmented. Neutrophil granulation appears occasionally; the eosinophil granules may be round, crystalloid or rod-shaped.

Aves.

A. Hen.

1. Small leucocytes with relatively large nuclei not intensively stained as in Amphibia and Repillia.

2. Transitional leucocytes.

3. Mast leucocytes with granules of various sizes but not very coarse.

4. Large leucocytes with fragmented nuclei containing crystalloid or round eosinophil granules.

B. Sparrow.

1. Small leucocytes with relatively large nuclei and narrow cytoplasm.

2. Transitional non-granular leucocytes with nuclei large, round and usually centrally located.

3. Small mast leucocytes often mononuclear.

4. Large leucocytes; nuclei fragmented or entire; granules round, angular or crystalloid.

In the hen most of the eosinophil granules are round while those of the sparrow are often crystalloid.

Conclusions.

1. Small lymphocytes are found in all groups of animals from man to fish.

2. Leucocytes are present which represent transitional forms between lymphocytes and polymorphic leucocytes. These cells are separated from the former group by a larger amount of cytoplasm and from the latter by their

simple, round or slightly indented nucleus.

3. A sharp division of leucocytes is not possible because of these transitional forms which may have developed from small lymphocytes and be able to differentiate further becoming polynuclear cells.

4. There probably are polynuclear cells because the threads connecting the parts of the nucleus cannot always be found.

5. The author found no mitosis in the leucocytes.

6. He found spindle cells only in Scyllium catalus but suggests that they might have been destroyed in the other preparations.

7. Mast cell granules are almost invariably round.

8. Neutrophil granulation was found only in the lizard and water snake. Hirshhfeld, in ten mammals, found true neutrophils in only four and so-called 'amphophils' in two animals.

9. Asidophil granules maybe non-crystalloid or crystalloid; the latter are found in birds, some reptiles and many Selachians. These are not due to the method used in preparation because they can be demonstrated in fresh blood. Such granules usually stain in eosin. Cells with these granules are usually polymorphic or even polynuclear

10. A series showing transitional stages between the lymphocytes and polynuclears can be easily worked out in Scyllium catalus.

J. Meinertz. (21).

According to Meinertz, granules have been found in all blood of all animals so far studied. His article deals with the blood of a turtle, several fish, a lobster, insects, etc. Only his work on the reptile is of particular interest here.

Emys lutaria (Turtle).

1. Nucleus, relatively small; cytoplasm filled with numerous equal sized, almost round granules standing out sharply from the pale cytoplasm and usually about the size of human eosinophil granules. Probably corresponding to true eosinophils.

2. Nucleus and cytoplasm similar but granules rod-shaped or spindle-shaped, also visible in fresh material and having a star-shaped arrangement in the cytoplasm.

3. Relatively round, large nucleus with a small amount of cytoplasm resembling human lymphocyte.

4. Cells with basophil granules.

He says, in regard to rod-shaped granules, that they are common in birds and reptiles. Bizzozero found them in the bone marrow of the hen ; Gruenberg saw them in birds and in Lacerta muralis. Rawitz considered them to be bacteria in Scyllium which view Gruenberg strongly opposes. Sacharoff supposed them to be rods which had fallen from the nucleus of erythrocytes and had then been phagocytosed by leucocytes. If they were crystals, as some have supposed, they probably would not have such a sharp affinity for certain definite stains.

Conclusions.

1. In the cell body of the leucocytes of man, mammals, birds, reptiles, Amphibia and many fish as well as in a number of lower forms, are found inclusions of different sizes, generally as granules, sometimes as rods, which always have an affinity for certain stains.

2. There are transitional forms between these cells and those without granules so that the one group cannot always be sharply separated from the other.

3. A cell appears nearly constant which has a round nucleus and narrow, non-granular, basophilic cytoplasm not completely homogeneous. In man this cell is known as a lymphocyte.

4. Because of the heterogeneity of there appearance it follows that the granules are not bearers of a specific function or cell activity but must be judged separately for each form.

I. J. Eberhardt. (9).

A. Eberhardt found the following cell forms in the blood of the turtle:

1. Adult nucleated erythrocytes.

2. Young erythrocytes showing mitotic figures and little haemoglobin.

3. Non-granular leucocytes of three kinds:

a. Small lymphocytes.

b. Large lymphocytes.

c. Large mononuclears.

4. Granular leucocytes.

a. Acidophils.

b. Basophils.

c. Special cells.

5. Thrombocytes.

B. Blood regeneration is energetic in the spring but almost ceases in the winter. It takes place as follows:

1. Erythrocytes develope from large lymphocytes in the bone marrow and blood vessels and also by karyokinesis of erythroblasts already in the circulation. He adds that under normal conditions the bone marrow is the only source of granular leucocytes and of cells containing haemoglobin.

2. New non-granular leucocytes are developed in the spleen, in the lymphoid tissue of the kidneys, liver and other organs and in the subcutaneous reticular

tissue. Karyokinesis in lymphocytes is seen here everywhere.

3. Acidophil leucocytes are developed by the production of granules in cells similar to the large mononuclears in the outer reticulum of the bone marrow and at times in the circulating blood. The author observed mitosis in cells in the bone marrow having a few acidophil granules (acidophil myelocytes). He says it may yet be shown, since there is as yet no proof against it, that acidophils are formed in the subcutaneous loose connective tissue--at least in young turtles.

4. Basophil leucocytes arise through the production of metachromatic granules in non-granular leucocytes found exclusively in the connective tissue of the spleen. Karyokinesis was found here also in cells already containing granules.

5. Thrombocytes are apparently formed in the spleen and stand close in genetic relation to small lymphocytes.

C. In the loose subcutaneous connective tissue he found: fibroblasts, wandering cells (lymphocytes), Maximow's resting wandering cells, Ehrlich's mast cells, acidophil cells, pigment cells and fat cells.

D. On introducing a foreign body into the connective tissue he noticed first an increased emigration of acidophil leucocytes with rod-shaped granules and of lymphocytes from the blood vessels. The acidophils became enlarged, were actively amoeboid, crawled to the foreign body and began degeneration. The lymphocytes also became enlarged and with the wandering cells previously existing in the connective tissue formed a group of polyblasts which were active phagocytes. By fusing and amitosis the latter formed giant cells which later changed into fibroblasts or wandering cells. Mast cells were taken up by the polyblasts but were found later near the introduced body after the formation of a connective tissue capsule around it. Mast leucocytes of the lymphocyte type which had migrated to the inflamed region, hypertrophied, changed considerably and assumed the characters of histogenous mast cells.

Hanna Hirschfeld-Kassman. (15).

She has described the blood of a number of animals including representatives of the Pisces, Amphibia, Reptilia, Aves and Mammalia. Her results follow:

A. Cerassius vulgaris (fish),

1. Non-granulas cells corresponding to human lymphocytes.

2. Granular cells which are undoubtedly neutrophils.

3. No pronounced eosinophils or basophils although certain honey-combed cells may perhaps be eosinophils.

B. Triton viridescens (newt).

1. Non-granular mononuclear leucocytes corresponding to human lymphocytes.

2. Non-granular transitional leucocytes of whose later development into polynuclears there is no definite proof.

3. Polynuclear leucocytes which have at times a segmented nucleus but are usually free from granules.

4. Mononuclear (only) mast cells.

5. Polynuclears with eosinophil granules (occasional).

C. Emys lutaria (tortoise).

1. Non-granular leucocytes.

2. Granular leucocytes.

a. Basophils.

b. Eosinophils with round and crystalloid granules.

Cells prevailingly mononuclear showing only an occasional slight indentation of the nucleus. No polynuclears present.

D. Tropidonatus natrix. (Lizard).

1. Large non-granular leucocytes with small nucleus.

2. Granular leucocytes.

a. Basophils (granules few and fine).

b. Neutrophils (granules very fine).

c. Acidophils (granules round and angular; when isolated, rod-shaped).

The cells are all mononuclear with a round or indented nucleus.

E. Senegalfinch.

1. Lymphocytes.

2. Transitional leucocytes with homogeneous cytoplasn and indented nucleus.

3. Mononuclear and polynuclear leucocytes with basophil granules.

4. Eosinophils with segmented or much indented nucleus and granules usually round, at times crystalloid.

A. Werzberg. (34).

Werzberg has rather recently made a haemotological study of a number of Amphibia, Reptilia and Pisces. After considering in detail the blood of sixteen toads, frogs and salamanders he draws the following conclusions for the Amphibia:

1. The blood is uniform in its cell content.
2. All Amphibia lack granular special cells.
3. Granular polynuclears (eosinophils and basophils) are present; non-granular special cells are found.
4. The size of the various cells varies with the species.
5. Spindle cells are independent elements of the blood which are not related either to lymphocytes or erythrocytes.
6. Mast cells are of two kinds (lymphoid and histoid) but apparently only the first type (a small cell with coarse granules) is derived from the small lymphocyte.
7. All non-granular leycocytes are derived from the small lymphocyte as well as the eosinophil leucocytes which are differentiated from the large lymphocyte through the eosinophil myelocyte.

He found the following leucocytes in the blood:

1. Mast cells of two types (lymphocytoid and histoid or histogenous); always mononuclear.
2. Eosinophils: mononuclear, polymorphic and polynuclear.
3. Large lymphocytes and plasma cells.
4. Small and medium sized lymphocytes (pigmented at times).

5. Leucocytoid lymphocytes, sometimes showing azurophil granulation or pigment.

6. Lympholeucocytes.

7. Polymorphic and polynuclear non-granular special cells.

From the blood of eighteen reptiles he drew the following results:

The leucocytes of reptilian blood are:

1. Only mononuclear mast leucocytes (two types).

2. Eosinophil cells; usually mononuclear but occasionally polymorphic and even polynuclear cells are found; granules, round, polygonal, or needle-like — in the latter case arranged in a definite form in the cytoplasm.

3. Large mononuclear non-granular leucocytes:

   a. Weakly basophilic lympho-leucocytes occasionally having a polymorphic or even polynuclear nucleus.

   b. Weakly ampho-oxyphil special cells, rarely showing an indented nucleus. Probably an older form of 3a.

4. Medium and small lymphocytes and leucocytoid lymphocytes.

5. Large lymphocytes often with large cell body.

6. Myeloblasts of <u>Pappenheim</u>.

In three cases only, non-granular, mononuclear, weakly oxyphilic special cells were found.

He draws the following conclusions for reptiles:

1. The blood of the reptiles is prevailingly mononuclear but polynuclear eosinophils have been found in a few species.

2. The mast leucocytes are of two types:

a. Lymphocyte form containing a few irregular, coarse granules; cell body narrow.

b. Histogenous form containing many fine granules; cell body wide.

Both or only one of these types may be present in the same animal. One type is not derived from the other and cells which are intermediate between the two are found only in Algiroides and in Anolis. According to Niegolewski and Hirschfeld-Kassmann the nucleus in Tropidonatus natrix maybe invaginated on one side; in all other cases the nuclei are mononuclear and either central or excentric in position. In a few species (Lacerta muralis, Algiroides, Anolis, Hemidaktylus, Platydaktylus) the mast granules stain very slightly in triacid but have an affinity for methyl-green pyronin or a metachromatic modification of it. According to Pappenheim, methyl-green pyronin is a specific stain for chromatin hence this reaction may show a relation between the nucleus and the granules.

3. The eosinophils are the most numerous and at the same time the most variable form of leucocytes and because of these two characteristics they appear to

accept to a certain degree the role of special cells. In quantity and specific quality they are the most noticeable reptilian leucocytes and are the characteristic cells of the blood.

4. There is an extensive amount of well-defined azurophil granulation, the presence of which is characteristic for this group of animals. It is found in the mononuclear special cells which are a new element for the Reptilia.

Comparison of Blood of Amphibia and Reptilia.

1. The polynuclear, non-granular, weakly oxyphilic special cells of Amphibia are not present in Reptilia except in Anolis where only one bisegmented nucleus was found.

2. In three reptiles mononuclear, non-granular, amphooxyphilic special cells were found.

3. In Reptilia, lympholeucocytes with indented nuclei and polymorphic or polynuclear leucocytes which are weakly basophilic are relatively few.

4. Reptilian blood is prevailingly mononuclear but polymorphic and polynuclear eosinophils are found.

5. The mononuclear special cells of the reptiles distinguish the blood of those animals from that of the Amphibia.

6. The formation of polymorphic cells is less direct in

reptiles than in Amphibia and is carried on through a middle stage -- a mononuclear, non-granular special myelocyte.

7. In Amphibia the eosinophils are prevailingly polymorphic while in reptiles they are prevailing mononuclear.

8. The eosinophils of Amphibia correspond in their relations to those of mammals while those of reptiles, because of their prevailing mononuclearity and great variability are characteristic for their group.

9. Without any question the reptiles, through the appearance of special, mononuclear, special myelocytes, are in sense more highly differentiated than the Amphibia; yet considering their very peculiar eosinophil type, they do appear to lie in the direct line between Amphibia and Mammalia but rather lead off to one side toward the Aves.

10. The lymphocytes, large lymphocytes(gross), leucocytoid lymphocytes, lympholeucocytes and mononuclear mast cells of both types in the Reptilia are homologous with similar cells found in the Amphibia.

Vera Dantschakoff. (6).

Dantschakoff and Maximow have done excellent work on the embryonic development of the various blood cells; both hold the monophylletic view of the origin of blood cells. The latter studied fish and Amphibia while Dantschakoff has carefully followed out the development in birds (hen) and in reptiles (adder).

Blood Formation outside of the Embryo.

She discovered that the first embryonic blood organ in the adder is the Area vasculosa which early becomes a complicated organ corresponding in its formation to the yolk sac of birds, which is there the primary blood organ. In both cases, the primitive cell is a large, round, amoeboid cell with basophilic cytoplasm and a vesicular nucleus — a large lymphocyte or "lymphoid haemocytoblast", so-called because morphologically it is identical with the large lymphocyte in the blood forming organ of the adult animal but physiologically it is the parent of all the blood cells. This primitive cell appears in the blood vessels developing there from blood island cells and also in the extra-vascular tissue where it is formed by the rounding off of mesenchyme cells of the splanchnopleure. The intra-vascular haemocytoblasts develope into erythroblasts and finally erythrocytes while those outside of the blood vessels become granulocytoblasts and granular leucocytes. The first blood development then, is identical with that of birds.

Blood Formation within the Embryo.

When the heart begins to beat, the blood vessels of the embryo are already rich in blood cells which have not arisen in loco but have been derived from the blood island cells of the yolk sac. A little later, at a stage corresponding to the three or four day chick, a rich formation of wandering cells takes place in the embryonic mesenchyme from mesenchyme cells which have become rounded off. The embryonic connective tissue of the adder is rich in these free wandering cells; accumulations of them are found between the cerebral vesicles, around the aorta, in the thymus gland and thyroids and between the vertebrae. The first free cells which develope from the mesenchyme in the body of the embryo are morphologically like large lymphocytes. These increase their numbers by mitosis and very soon begin to develope acidophil granules in the cytoplasm making them, according to the nomenclature, granulocytoblasts or myelocytes; by further differentiation they finally assume the characterictics of the adult granular leucocyte. This differentiation takes place in the reptiles principally in the interstitial tissue already mentioned as being rich in free wandering cells.

A day or two later, the differentiation of large lymphocytes takes a new direction — that of the small lymphocyte which appears in the adder at a relatively early stage (6-7 dy. chick). This differentiation takes place simultaneously in the connective tissue between the cerebral vesicles, between the intercostal muscles, around the oesophagus and in the thymus gland as well as in the connective tissue surrounding it.

At this time the connective tissue of the adder embryo contains the usual fixed tissue cells, many pigment cells, large lymphocytes, granulocytoblasts (myelocytes) and small lymphocytes.

Directly after their appearance the small lymphocytes develope into mast cells which are not degenerative stages, as has been supposed, as shown by their active mitosis. The mast cells of the adder and of birds have a round nucleus. There is no sharp differentiation between the mast cells of the connective tissue and the mast leycocytes in the blood; in the connective tissue the mast cells become rich in cytoplasm and develope many pseudopodia while the mast leucocytes in the circulation retain their round form, of course, but even this distinction is found clearly only in the adult animal.

There is no development of erythroblasts and erythrocytes in the mesenchyme of the adder such as is found in birds; instead, during the greatest part of embrynic life the development of these cells takes place in the broad vessels of the yolk sac. Further blood formation is localized in the thymus gland, the spleen and the bone marrow of the vertebrae. Lymphoid tissue developes in the thymus in a simple manner. Coetaneous with a development of large lymphocytes and granulocytoblasts in the interstitial tissue of the adder embryo, is the appearance of large lymphocytes in the mesenchyme adjacent to the thymus, and these soon make there way between the epithelial cells into the organ itself. Here they multiply and great numbers of them develope into granulocytoblasts. After a short time, when the small lymphocytes begin to appear in the mesenchyme, there is noticeable also a similar development of small lymphocytes in and around the thymus; this development is, of course, not limited to this region, but leads here to the formation of accumulations of lymphoid tissue. Small lymphocytes, similar in morphological characters, are found everywhere in the tissue bordering on the thymus and around the oesophagus. The spleen is similarly the seat of extensive formation of granular cells. At the end of embryonic life, the bone marrow in the vertebrae appears as the functional blood forming organ.

PERSONAL OBSERVATIONS.

TECHNIQUE.

A. Preparation of Smears.

The blood was procured with little difficulty by cutting off very small pieces from the end of the tails of the snakes kept alive in the laboratory throughout the winter. There are some advantages in taking the blood in this manner:

1. The preparations are usually quite thin because the blood flows very slowly and
2. For the same reason many preparations can be made without causing much loss of blood to the animal.

Although the snakes have been operated on a number of times during the course of this investigation infective conditions have arisen just once and lasted then for only a very short time.

For the study of the circulating blood the preparations were all the so-called "smears" made by drawing out a drop of blood into a thin film over the coverslip by means of another coverslip along the edge of which the drop was first allowed to spread. The coverslips and slides were all carefully cleaned by washing consecutively in acetic acid, distilled water and 90% alcohol and drying on a clean linen towel after which the coverslips were passed through the flame of a Bunsen burner.

Various fixations and stains were experimented with; only those giving good results will be described here.

Fixations.

1. Osmic vapor method of Weidenreich.

In this case, as well as in all vapor fixations, a small dish was used over the opening of which the cover-slip fitted very nicely. The cover was first exposed for about one minute to the fumes; the smear was then made upon the exposed surface and this again exposed to the osmic fumes for twenty seconds. This fixation proved very good for the majority of cells.

2. Osmic-Glacial Acetic acid of Werzberg.

Two drops of glacial acetic acid to 1 c.c. of 1% osmic acid proved especially good for the eosinophils. The preparation was exposed to the fumes in the manner described above for twenty seconds.

3. Osmic-Formaline-Iodine. (Werzberg).

Here a mixture of equal parts of 1% osmic acid, 40% formaline and iodine was used as a fixative in the manner described above.

4. Formaline. (Commercial). Used as above.

5. Heat.

Cover-slips bearing a thin film of fresh blood on one side were passed back and forth, four or five times, over a Bunsen flame, with the film side away from the flame.

Stains.

1. Giemsa.

Stain fifteen minutes in the dilute stain (1 c.c. distilled water to 1 drop of Giemsa). Rinse thoroughly in distilled water. Dry between pieces of filter paper.

2. May-Giemsa.(Pappenheim).

Fix in May-Gruenwald for three minutes.

Add distilled water and stain one minute.

Float the cover-slip on a Giemsa solution (one drop to 1 c.c. distilled water) for fifteen minutes.

Rinse in distilled water. Dry quickly.

3. Wright's stains.

Concentrated stain for 1 minute (to secure fixation in dry smears).

Add about ten drops of distilled water causing a metallic scum to form on the stain. Stain 2-1/2 minutes.

Rinse and leave in distilled water 2-1/2 minutes.

Rinse again and dry quickly.

4. Harris-Romanowsky.

Concentrated stain for 1 minute.

Add distilled water. Stain five minutes.

Rinse in distilled water and dry quickly.

5. Vital staining.

A small amount of Brilliant Cresylblau was dissolved in about 2 c.c. of 100% alcohol. This was then spread in a very thin film over one surface of a clean glass slide and the alcohol was allowed to evaporate. A small drop of fresh blood was then placed in the center of the slide and a cover-slip applied. These preparations were made permanent in several ways as follows:

1. When the granules had become stained in the erythrocytes the cover-slip was removed and both the blood on it and that on the slide was fixed in osmic fumes as described above. This preserved the granules and the dells as they were in the fresh preparation.

2. The vital stain was allowed to work for a longer time until the substantia granulo-filamentosa appeared. These preparations were fixed;

   a. Over osmic and stained with May-Giemsa.

   b. By heat and stained with Wright's stain.

Some were dried quickly and stained with Wright's stain.

These preparations show particularly well the granules of the erythrocytes and their origin, the substantia granulo-filamentosa in the erythrocytes and the detail of the mononuclear leucocytes (special cells or bacterio-phags) with the azurophil dust since bacteria were present in the blood at this time. The occurrence of these bacteria was very peculiar. Upon examination of some blood being stained with Brilliant Cresylblau great numbers of bacteria (bacilli and diplococci) were noticed, many of which were being ingested by the large cells with azurophil dust in the cytoplasm. Feeling certain that the death of the specimen was imminent, it seemed wise to take samples of the blood daily. To my surprise practically no bacteria were found in preparations made the following day. The presence of the bacteria in the first place, may have been due to the use of an unsterilized instrument in a previous amputation of a small portion of the tail. As the blood flowed very slowly from the caudal blood vessels, it was necessary, at times, to press the blood backward through the whole tail region; this may account for the large number of bacteria that were present. Some of these parasites may have been squeezed upon the cover from the injured surface. Their total absence on the second day may have been due to the pressure exerted the preceeding day which had perhaps cleared the tissue and caudal blood of the bacteria just at the right time. The animal did not die but, with its fellow companion, has recently been liberated in its natural enviroment—— the grassy field.

B. Preparation of Sections.

Small pieces of the tail, both of normal and of wounded tissue, were fixed in Helly's Zenker-Formol (2 hrs.) for comparision. The material was then washed in tap water, dehydrated, cleared in xylol, mounted in paraffine, sectioned and stained in eosin-orange G followed by toluidin and in May-Giemsa. The sections were again washed, dehydrated, cleared and mounted in damar.

General Notes.

1. Azurophil mononuclears: heat fixation$ followed by Wright's stain brings these cells out beautifully; in this case the azurphil dust is reddish but with May-Giemsa it has a more purplish hue.

2. Eosinophils: an osmic-glacial acetic fixation preserves the granules as well as the other general features of the cell. Either Wright's stain or May-Giemsa give good results.

3. Mast cells: the general characteristics and development of the cells is best shown with a fixation of osmic-formol-iodine followed by Harris-Romanowsky.

4. Erythrocytes: for general characteristics, any fixation and stain mentioned are sufficient but for the granules a vapor fixation should be applied followed by May-Giemsa or Harris-Romanowsky.

MORPHOLOGY.

The following cells are found in the blood of the garter-snake (Eutania sirtalis and Eradix):

A. Erythrocytes.

B. Leucocytes.

a. Lymphoid cells.

1. Lymphocytes.
2. Mononuclears (Ehrlich's "transitional forms").
3. Azurophil mononuclears.

b. Granular cells.

1. Mast leucocytes.
2. Eosinophils.

C. Thrombocytes or spindle cells.

A. Erythrocytes.

According to Weidenreich, the red corpuscles of the blood are present in all vertebrates except Amphioxus. They are limited to the confines of the blood vessels but may be found occasionally in blood forming organs where regeneration or degeneration is taking place. These cells have lost the power of amoeboid motion and have been specialized as oxygen and carbon dioxide carriers. "Aber auch ausser der Faerbung zeigen alle roten Blutkoerperchen der verschiedensten Tierklassen in wesentlichen Punkten uebereinstimmende Charaktere. Ueberall besitzen sie glatte Konturen und einen ausserordentlich hohen Grad von Plastizitaet, nirgends lassen sie in ihrer ausgepraegten Form eine amoeboide Beweglichkeit im gewoehnlichen Sinne des Wortes erkennen und ihrer Plasmabeschaffenheit nach machen alle einen durchaus homogenen Eindruck. Auch hinsichtlich der Form stimmen sie, wenn man von den Saeugetieren absieht, in ueberraschender Weise ueberein; bei den Ichthyopsiden und Sauropsiden--nur die Petromyzonten machen hierin anscheinend eine Ausnahme--sind es abgeplattete ovale scheiben, die nur an der Stelle des Kernes jederseits buckelfoermig aufgetrieben sind, und eine im wesentlichen gleiche Form weisen die roten Blutkoerperchen der Wirbellosen auf." "Die Faehigkeit der Erythrocyten, durch Formveraenderung auf Schwankungen des osmotischen Druckes zu reagieren, ist aber keineswegs eine Eigenttuemlichkeit der Saeugerblutkoerperchen. Auch die ovalen bikonvexen Elemente der Ichthyo- und Sauropsiden zeigen ganz aehnliche Erscheinungen. Bei starker Verduennung des Plasmas durch Wasserzusatz quellen sie unter Verlust ihrer natuerlichen Form und starker Zunahme des Dickendurchmessers zu Kugeln auf und andererseits zeigen sich bei Verdunstung oder Bei Zusatz konzentrierter Salz loesung deutliche Schrumpfungserscheinungen." (30).

The adult erythrocytes of the garter snake correspond in size and shape to those usually found in Reptilia, Amphibia and Aves. Some cells, apparently fully mature in haemoglobin content, are small and round with a central or excentric nucleus while most of the erythrocytes are at least twice as large, oval in shape and have the nucleus centrally located. The nuclei of the smaller cells usually stain more intensively and take on a different shade, a purer blue perhaps, than those of the large cells. In contrast to the pycknotic oval nuclei of the latter is the round, nucleus of regular contour found in the small round cells. As will be noted at greater length later in this article, all stages in the development of erythrocytes are found in the circulating blood; this is apparently most noticable in the spring. In the light of this observation, it seems very probable that the small round cells with round nuclei represent daughter cells of what was, perhaps, the last mitotic position since the cells have now developed such a large amount of haemoglobin. Other cells may be found with similar nuclei but less haemoglobin and hence a much paler cytoplasm; such cells will probably pass through mitosis at least once more. The nuclei of what are probably the older cells may be round, oval or oblong in shape with one or more large projections extending out into the cytoplasm and a number of bud-like processes; the chromatin is arranged in densely staining patches or knots suggesting a net work, while the karyoplasm is rather pale; near the end of the projections, a small accumulation of chromatin can usually be seen, similar in size and shape to one of the larger bodies, mentioned below, found free in the cytoplasm; the nucleus may be centrally located or somewhat excentric.

In the majority of cases the cells are strictly mononuclear but an occasional bisegmented nucleus is present (2 cases).

In a preparation stained in Brilliant Cresylblau, fixed in osmic vapor and stained again in May-Giemsa, a number of cells will show the substantia granulo-filamentosa which can be easily seen surrounding the nucleus as a sort of basophilic net-work standing out quite clearly against the acidophilic haemoglobin of the cytoplasm. (Fig.). Altho this is supposed to stain only in vital stains a possible indication of the net work is seen in some cells in ordinary preparations fixed in osmic fumes and stained with Harris-Romanowsky.

With all vapor fixations and in preparations stained first in a vital stain, one or more bluish granules are shown in the cytoplasm. These granules vary considerably in size from a mass as large as a large mast granule to a tiny dust-like particle. They may lie very near the nucleus, on either side or either end of it, or they may have a position close to the periphery of the cell. These granules do not correspond either to the "punktierten Erythrocyten" described by Luzzatto and Ravenna or to the (19) comparatively fine basophilic granulation described and pictured by Werzberg for a frog (Rana temporaria) which, (33) eight days before, had had one leg amputated. According to Werzberg, this granulation has never before been found in animals below the mammals. (Fig.).

B. Leucocytes.

The white corpuscles of the blood are still of doubtful function but they probably act as transporters of nourishment, according to Weidenreich. He says that they are not limited to the blood vessels but are found also as a large constituent of the serous glands, the lymph system and the spaces of the connective tissue. It is the general impression that these cells use the blood vessels as a quick and comfortable means of transportation which makes possible and rapid their distribution over the whole body. The fact that they have a tendency to leave the vessel of their own accord when they reach the connective tissue indicates that they are not primarily blood cells but free cells of the organism, the connective tissue of which is their natural habitat for functioning. (30)

Early workers studied the white corpuscles and their related forms as found in the lymph stream and because of this, some cells in the blood are known as "lymph" cells or "lymphocytes". It was not until Ehrlich worked out his blood stains that the cells present in the blood came to be studied. By using the relations of the granules in the cells to certain stains as a basis of classification and differentiation, he divided the granular leucocytes into three classes: basophils (mast cells), acidophils and neutrophils.

It has now been conclusively proven that the reaction of granules toward stains proves nothing about their function. For instance, all granules which stain with eosin are not true eosinophil granules; this fact is demonstrated by the two kinds of eosinphils found in the rabbit and birds. Then again, the special cells so far discovered for different animals vary greatly in the staining reaction of the cytoplasm although their function is everywhere identical. As Weidenreich says, the biological worth and function of leucocytes are not dependent upon the accident of morphological form and still less upon the manner in which the morphological form responds to certain stains. (30)

a. Lymphoid cells.

The non-granular cells are constant forms for all classes of animals, says Weidenreich. In many cases they seem to be almost the only form in which the white corpuscles appear (Gastropods of invertebrates, Perca fluviatilus of the fishes and "Sturmseglern" of birds). This observation shows that there is no essential biological difference in meaning between granular and non-granular cells since the animals mentioned are apparently not at all different from their nearest relatives. (30)

1. Lymphocytes. (Ehrlich's nomenclature).

The lymphocytes vary from about one-third the size of an adult erythrocyte to three or four times that size. The small lymphocytes have round to oval nuclei and a very limited amount of cytoplasm almost imperceptible at times, often visible only at one side of the nucleus which may be centrally located or somewhat excentric. The medium and large lymphocytes have the same relative characteristics but both the cytoplasm and nucleus have increased in amount. The nucleus shows a diffuse distribution of chromatin with no particular net-work or distinct patches present. Occasionally a nucleolus is visible. Grosslymphocytes (Pappenheim's "Lymphoidocyte") are present in few numbers; the nuclei show a characteristically, fine, arrangement of the chromatin; cytoplasm, relatively very small in amount.

2. Mononuclears.

Although no polymorphic cells are found in the circulating blood of the garter snake, leucocytes with "transitional" nuclei are present. Such cells have a relatively large amount of slightly basophilic, homogeneous cytoplasm and a large, slightly excentric, kidney-shaped nucleus staining more intensely. These leucocytes correspond to the "transitional forms" of Ehrlich; they form only a small per-centage of the non-granular cells in the blood of the garter snake.

3. Azurophil mononuclears.

The azurophil mononuclears vary in size and shape. Some are comparatively small and round while others are longish-oval or almost rectangular in shape and equal to, or even larger than, mature erythrocytes. The simple nucleus may be round or oval and is quite regular in outline; it usually occupies about one-half of the cell and may be centrally located or excentric. The chromatin is arranged in deeply staining patches which do not seem to be connected by strands forming a net-work while the karyoplasm has a weak affinity for the stain.

The cytoplasm is basophilic and contains a fine dust-like granulation which, after heat fixation, stains a reddish-violet in Wright's stain and more deeply (reddish purple) in May-Giemsa. This fine granulation may be spread diffusely throughout the cytoplasm which arrangment is particularly true for the smaller cells. In the large rectangular cells, when the nucleus is centrally located, the granulation which has been termed "Azurophil dust", is massed at each pole; if however, the nucleus is decidedly excentric and lies at one pole close against the outer edge of the cell, the azurphil dust is found only at the opposite pole. This granulation is most dense at the periphery of the cell so that apparently pure basophilic cytoplasm may often be observed only in the vicinity of the nucleus. There may be some relation between the granulation and the chromatin in the nucleus; at any rate, the following observation has been made: when a great amount of granulation is present the patches of chromatin in the nucleus are relatively few in number and vice versa, much chromatin is present when the granulation is sparse. In the latter case the granulation in diffusely distributed through the cytoplasm.

(34) According to Werzberg, with the exception of one Amphibian, the azurophil granulation of the mononuclears is found first in the Reptilia. In the garter snake most of the mononuclears

contain azurophil dust in the cytoplasm and have become specialized as the result of their increased or new functional activity and the polymorphonuclear, non-granular, oxyphilic cells of the Amphibia have not been differentiated (Downey). However, Werzberg describes "einkernige ungekoernte, amphochromophile Spezial-Myelozytoidzellen" in Anguis and Tropidonatus which shows that even in the reptiles, in exceptional cases, a special line of cells may be differentiated alongside of the ordinary mononuclears. This specialization of some of the mononuclears as "special cells" is characteristic for the Amphibia; in such cells the cytoplasm is slightly acidophilic and non-granular while the nucleus is decidely polymorphic. These specialized mononuclears of Amphibia and Reptilia correspond somewhat in function to the neutrophils of man, the pseudoeosinophils of the birds and rabbits, the amphophils of the guinea pig and the non-granular polynuclears of the cat, rat and mouse. (34)

(1.) According to Adami, the polymorphonuclears are "actively phagocytic, particularly for bacteria; secrete bodies of the nature of enzymes and, either while active or in the process of dissolution, liberate antitoxic and antibacterial substances". This cell in man is the "special cell " and if the azurophil mononuclear of the reptile corresponds to this cell it should show some of its functions. It apparently is not particularly attracted by inflammation since such cells are not noticebly present in the wounded tail tissue. That they are phagocytic however, is demonstrated in my material from the garter snake. At the time of the bacterial infection, the blood was over run with bacilli and diplococci. The cells, noticeably increased in number, are the eosinophils and the azurophil mononuclears. The former show no phagocytosis but the mononuclears are, as Adami states, actively phagocytic for bacteria. Many of these leucocytes show ingested bacteria surrounded by clear vacuoles; others show bacteria being taken into the cytoplasm while all of these cells present show some signs of activity.

The nucleus becomes irregular, often dumb-bell shaped (Fig.) and show some changes in the arrangement and staining reaction of the chromatin. The cytoplasm loses, by degrees, its azurophil granulation (Fig.) and becomes very delicate and vacuolated showing traces of both "acidophilie" and "basophilie". The decrease in the amount of the azurophil granulation suggests that this particular structure of the cytoplasm is related to the formation of enzynes or other antibacterial substances. These cells do then have a function corresponding to that of the neutrophils of man and hence are analagous to them. One of the functions of the neutrophils that of active emigration to inflamed regions, has apparently been taken over by the eosinophils in this animal which according to Adami, in man, are always attracted early toward the inflammatory focus, but not in such great numbers as the neutrophils. This function of the eosinophils will be described later. There are then no true "special cells" in the garter snake but the functions of these cells have been taken over by the large mononuclears and the eosinophils each of which has become somewhat specialized in morphological characteristics for the added duties.

b. Granular cells.

1. Mast leucocytes.

The mast leucocytes vary in size and in number of granules. Some cells are as small as a very small lymphocyte while others are equal in size to the large mononuclears. Many of them have the lymphocyte characterictics, namely, a large nucleus with a relatively small amount of cytoplasm surrounding it. In such cells the nucleus is more basophilic than the cytoplasm and the granules are not very numerous. (Fig.). Occasionally cells are found in which no clearly defined cytoplasm is present and the granules appear like marbles adhering to the surface of the nucleus. (Fig.). The latter shows no definite structure; chromatin is suggested by indistinct areas in the nucleus staining a little more intensively than the karyoplasm which is usually basophilic. The cytoplasm has a pale purplish tinge and may contain just a few or great numbers of granules. These stain metachromatically in basic dyes and vary in size, often in the same cell, from a tiny dust-like particle to a granule, twice or even three times the size of an eosinophil granule. Some of the mature cells contain almost entirely rather fine granules while others have a majority of coarse granules, but neither the size nor the number of the granules stands in any definite relation to the other morphological characterictics of the cell.

The mast leucocytes of the garter snake do not agree then with the general observations for Reptilian mast cells which Werzberg makes based upon his study of the blood of eighteen reptiles. He decides that the mast leucocytes of these animals are of two types, namely, a large lymphocyte form with a few coarse granules and an histogeneus form containing many fine granules in a wide cell body. Only one or both of these may be present in the same animal. It is stated by all who have studied Reptilian mast leucocytes (Werzberg[34], Niegolewski, Hirschfeld-Kassmann[15], Gruenberg[14], Eberhardt[9], Meinertz[21], Kollmann[17]) that these cells are mononuclear with a central or excentric nucleus. In Tropidonatus, Niegolewski and Hirschfeld-Kassmann found the nucleus slightly invaginated on one side.

2. Eosinophil leucocytes.

With an osmic-glacial acetic fixation followed by May-Giemsa, the eosinophils are fairly well preserved. The granules are very hard to save and it was only after a great many unsuccessful attempts that finally a fixation and stain were found which would preserve and color the granules in any definite way; even with this method the cells are not evenly fixed, some always showing the substance of the granules as a mass of undifferentiated material. Occasionally a cell is shown which demonstrates definitely the characters of the eosinophil leucocyte. In this respect, the eosinophils of the garter snake are similar to the mast cells of higher animals which are also very hard to preserve.

The eosinophil leucocytes of the garter snake are round cells about equal in size to the majority of erythrocytes. When not well fixed the cell swells greatly being then from two to four times the size of a red corpuscle. The cell is always mononuclear and the nucleus excentric and very small, in proportion to the cytoplasm. The nucleus is always decidedly excentric being closely pressed against the outer surface of the cell at one pole so that it is, in general, free from granules, although occassionally a few may be seen on top of, or at the sides of, it. The nucleus, at times, lies depressed in the cytoplasm and granules, reminding one of a chick embryo after it has become sunken in the yolk mass; often it is possible to see the lower contour of the oblately spheroidal nucleus through the cytoplasm. In other cases, the nucleus bulges out from the cell so that the cytoplasm forms only a very thin covering over it. With some preparations, a distinct halo can be demonstrated around the nucleus; some cells have the appearance of being hallow spheres with the granules arranged entirely at the periphery. The chromatin is arranged in purplish-blue masses while the karyoplasm is very pale.

The cytoplasm is practically colorless and contains a number of coarse granules which are large, round, acidophilic, and stain a rich rose red in May-Giemsa. Although with Ehrlich's triacid, the granules are more compact and quite irregular in shape, no genuinely crystalloid or spindle-shaped granules were observed in the circulating blood. However, a section of spleen, fixed in Helly's Zenker-formol, (kindly loaned by Dr. Downey) does show spindle-shaped granules in the eosinophil cells which are very numerous there; to the contrary, eosinophils found in the lung have distinctly round granules similar to those of the circulating blood.

In sections of wounded tissue from the end of the tail the eosinophil cells with round granules varying in size, are very numerous, while in sections of normal tissue from the same region such cells are scarcely present. The abnormal tissue is a mass of eosinophil granules. Here are found small compact cells with reddish-orange granules and large loosely constructed eosinophils with yellowish granules. In some cells the granules are very much swollen and are apparently disintegrating. The migration of these leucocytes from the subcutaneous connective tissue into the outer epitheleim is very noticeable and the cells are found in every conceivable nook of the wounded region. While the cells are crawling between the muscle fibers and the cells of the other tissues, the nuclei adapt themselves to the amoeboid motion of the leucocyte by becoming elongated. They return again to their compact form as soon as the cell regains its ordinary outline. Even though the granules are disintegrating there is no apparent sign of nuclear degeneration.

The characteristic cells for the blood of the garter snake are these peculiar eosinophils and the large mononuclears with the azurophil dust. They are about equal in numbers in the circulation. Neither of them is present in the Amphibian, bird or fish blood that I have examined; in fact these cells seem to be leucocytes which have been specialized for this particular species and group of animals.

Werzberg came to a similar conclusion in 1911. He states that the eosinophils are the most numerous and at the same time the most variable form of leucocyte in the reptiles. They are the cells which give character to the blood and seem to replace the special cells in so far as their variability in different species is concerned. This variability distinguishes the Reptilia from the Amphibia where the eosinophils correspond more to those of Mammals in number and general morphology. Reptilian eosinophils are usually mononuclear but occasionally polymorphic and even polynuclear cells are found. The granules may be round, polygonal or needle-like; in the latter case, they are arranged in a definite form in the cell. He adds this interesting bit of theory that without any question the reptiles, through the appearance of special myelocytes, are in a sense more highly differentiated than the Amphibia yet considering their very peculiar eosinophil type they do not appear to lie in a direct line between Amphibia and mammals but rather, lead off to one side toward the birds. (34).

¶ 24. Pappenheim (1909) states that the eosinophils may form the bulk of the leucocytes of reptiles. The granules are very delicate; they swell easily and get lumped together, but he concludes in spite of these characteristics that they are true eosinophil granules in contrast to the pseudo-eosinophils of the guinea pig or the rod-shaped granules of birds, both of which are generally considered special granules. My material does not support this view. In the first place, true eosinophils are repelled by bacteria but in the preparations of the blood where, perhaps, millions of bacteria are present, these cells are much more numerous than in normal blood. Werzberg found great numbers of (34) eosinophils in the blood of Emys lutaria which contained many parasites; the author draws no conclusions from this fact but it seems to me that this is but more evidence for my theory. The eosinophils do not phagocytose bacteria but as may be easily seen in some of the sections of wounded tail where bacteria are present, the granules swell and break down liberating, possibly, some antibacterial substance. In their morphological characteristics and attraction toward bacteria then, they are similar to special cells and not at all like true eosinophils.

Adami claims that the eosinophil leucocyte is "actively attracted and that at an early period, toward the inflammatory focus, migrating from the surrounding tissues and also from the vessels, but it is never the preponderating cell present". Later he says, "Very rarely is it seen to ingest bacteria, so that, to all intents and purposes, it is non-phagocytic. Kanthack and Hardy, and Hardy and Wesbrook have ascribed a secretory activity to these cells, associated with a reduction in number and apparent discharge of the coarse granules, but later observers have failed to confirm these observations." (1.) This agrees in part with the results obtained in my work upon the garter snake. Sections of the tissue of the wounded tail region show great numbers of eosinophils which have made their way into the region because of the inflammation caused by the injury. Normal tissue from the same region shows almost no eosinophils while the inflamed tissue is packed with them. Eberhardt found, after introducing a foreign body into the connective tissue of the turtle, that the first result was an increased immigration of acidophil leucocytes with rod-shaped granules and of lymphocytes from the blood vessels. (9.) If these cells have partially assumed the functions of the polymorphonuclear cells of man as suggested above, then that accounts for their active emigration in both cases and for the lack of the azurophil mononuclear special cells at the seat of inflammation. Citing again from Adami, (1.) "In acute inflammation, when the irritant is not too intense, this (polymorphonuclear leucocyte or neutrophil) is the form most often attracted to the focus of irritation, migrating most rapidly and in the greatest numbers." It seems very probable then that in the garter snake, the eosinophils are not true eosinophils but function, to some degree, like the special cells of man; this theory helps to explain also their peculiar morphological characters.

Hollman believes that the crystalloid granulation found in reptiles is derived from the round granulation through intermediate stages in which both granules are present. (16,17) Sacharoff and Venzlaff for birds, believe in the production of eosinophil granules from the paranuclein of the erythrocytes. (27) and (29). Weidenreich (31) found in one mammal that eosinophil granules were formed from phagocytosed pieces of the cytoplasm and haemoglobin of degenerating erythrocytes.

C. Thrombocytes or Spindle Cells.

It is very hard to draw a line/of separation between the lymphocytes and spindle cells since all stages and transitions between the two are found. The adult spindle cells will be described here. Such cells are spindle-shaped at times but, are, in general, more cylindrical in shape. The nucleus is oval and entire, oval and slightly indented, oval and apparently split partially at one end, or even bisegmented (one instance only). The chromatin is arranged in rather deeply staining patches suggesting a net-work while the karyoplasm is less basophilic. The nucleus is centrally located in well preserved, mature cells but may be excentric in younger stages. The cytoplasm is very pale, vacuolated (one to several vacuoles) at the poles of the cell and azurophil granules are sometimes visible in the vacuoles as well as in the cytoplasm itself. (Fig.).

Thrombocytes, or spindle cells, have been considered by many to be analogous to blood platelets but this seems improbable. In this connection J. H. Wright has a very good support for his (35) opinion that they are homologous to the megakaryocytes of the bone marrow and not analogous to the blood platelets. He bases this theory upon the following facts:

1. The forerunners of megakaryocytes are circulating cells in the embryonic blood hence the megakaryocyte was probably a circulating cell at one time in the history of the race.

2. The cytoplasm of the spindle cells of certain Amphibia has a similar staining reaction to that of the megakaryocytes

(Wright's stain), that is, a granular reddish-violet endoplasm and a hyaline blue ectoplasm. Then in Batrachoseps attenuatus, spindle cells regularly lose parts of their cytoplasm by a pinching-off process and fragments appear which look much like blood platelets in form and have the same characteristics, namely, a central reddish-violet portion with vacuoles and a hyaline blue marginal portion with an irregular or jagged outline. This similarity was first noticed by Eisen.(10)

Spindle cells have been considered as intermediate stages of erythrocytes by Neuman(23) and others while Werzberg is of the opinion that they are elements of the blood which are not related either to lymphocytes or erythrocytes.

DEVELOPMENT IN THE CIRCULATING BLOOD.

1. The development of erythrocytes in the circulating blood is particularly noticeable at certain times in the spring. A long series of many stages can be easily worked out from blood taken when the regeneration period is at its height. Young forms of the red corpuscles are round except just before mitosis when they assume an oval shape; at this time they are very large; daughter cells formed by division, are small and round with a small amount of cytoplasm. The very young cells are decidedly basophilic and have dense nuclei; as the development progresses the cytoplasm becomes less basophilic and less homogeneous while the chromatin structure of the nucleus takes on a looser arrangment with distinct chromatin knots and net work. In a Harris-Romanowsky preparation, the presence of the haemoglobin is first apparent by the gray color of the cytoplasm; this gray later becomes tinged with yellow and finally the latter predominates and soon after this mitosis ceases, probably because of the large amount of haemoglobin present. The round cell then begins to assume the characters of the adult erythrocytes, namely, oval form, homogeneous cytoplasm, and rather dense chromatin structure showing distinct chromatin knots. (Figs.)

This development in the circulation is very similar to that described by Freidsohn (12) for the frog where he finds erythrocytes developing from lymphocyte-like cells in the blood. This view is supported by many: Weidenreich, Dantschakoff, Pappenheim, etc. Bizzozero believes that the red corpuscles are formed from cells containing haemoglobin.

2. <u>Spindle cells</u> develope from small lymphocytes in the circulation. Almost parallel changes occur in the nucleus and cytoplasm during the development which takes place as follows:

Stage 1. A typical small lymphocyte with a relatively large, dense, excentric nucleus and a very limited amount of cytoplasm, scarecely visible around the nucleus. (Fig.)

State 2, Cell retains its round outline; excentric nucleus slightly irregular showing at least an indication of further changes; cytoplasm more extensive. (Fig.)

Stage 3. Cell oval; nucleus roundish oval but still showing a dense structure; cytoplasm retaining its basophilic properties and somewhat more extensive. (Fig.)

Stage 4. Cell oval; nucleus oval and still somewhat excentric; cytoplasm not appreciably increased. (Fig.)

Stage 5, Cell longish oval; nucleus showing little definite structure; cytoplasm increased, slightly less basophilic and showing one or two vacuoles. (Fig.)

Stage 6. Cell oval but larger; nucleus less excentric, less dense, showing several chromatin knots; cytoplasm more weakly basophilic, vacuoles and a few fine azurophil granules present. (Fig.).

Stage 7. Adult or mature spindle cell; longish oval, almost rectangular in shape: nucleus more centrally located, less dense and showing distinct chromatin knots; cytoplasm very pale, vacuolated at the poles and showing at times fine azurophil granules in the vacuoles and in the surrounding cytoplasm. Nucleus often having a split appearance. (Fig.)

This development of spindle cells from small lymphocytes has been recognized by a number of authors. Eisen (1896) (10.) worked on Batrachoseps attenuatus where he claims that cells corresponding to the ordinary spindle cells were formed from arithrocytes by the loss of the cell membrane and the haemoglobin content of the cytoplasm. MacCallum (cited from Eisen) formed a similar condition. The majority of haematologists however, do not agree with these authors.

Riess believe that spindle cells represent a definite form of leucocyte since transitional forms are found between leucocytes and spindle cells. (26). Loeweitt and many others hold this view.

Neumann (1903) (23.) says that the lymphocytes (frog) are always easily confused with spindle cells but particularly so after a discharge of blood because of the changes which occur. He suggests that some of the cells having lymphocyte characteristics may have passed from spindle cells. Again he says, small lymphocytes show all transitional stages to spindle cells which he adds are nothing other than development stages of erythrocytes (haematoblasts of Hayem).

Eberhardt says that the thrombocytes of the turtle are (9.) apparently formed in the spleen and stand in close genetic relation to small lymphocytes. According to Freidsohn and Dekhuyzen spindle cells can scarcely be differentiated from lymphocytes in the frog's blood (12). Both Weidenreich and Freidsohn apparently believe in the formation of spindle cells from lymphocytes in the circulating blood.

3. Mast Leucocytes also develope in the circulating blood from cells of the lymphocyte type . Granules may develope in the cytoplasm of small, medium or large lymphocytes and without any apparent increase in size such cells may become pure mast leucocytes. (Fig.) Granules of various sizes may be found in one of these differentiating cells, from fine dust-like particles to very coarse granules. The number of granules present varies, of course, with the age of the cell hence mast leucocytes in circulation may contain a very few (Fig.) or a great many granules (Fig.).

No definite indication of the method of formation of the granules was observed. The nucleus retains its spherical shape and smooth contour but looses its dense chromatin structure while the cytoplasm becomes less basophilic. There is apparently then, some relation between the formation of the granules and the structure of the nucleus in the cytoplasm, but the question still remains: "what is that relation?" Werzberg (34) found that in a few species (Lacerta muralis, Algiroides, Anolis, Hemidaktylus, Platydaktylus) the mast granules have an affinity for methyl-green pyronin or a metachromatic modification of it. According to Pappenheim, methyl-green pyronin is a specific stain for chromatin hence the above reaction may also be an indication of a relation between the nucleus and the granules.

This development of mast granules in lymphocytes is similar to that proved for the mast leucocytes of man by Weidenreich and for those of the frog by Freidsohn. (12). There is no fragmentation or lobing of the nucleus in the lower forms (frog and snake) such as Weidenreich found in pathological donditions in man.

Freidsohn started the development of mast leucocytes in the circulation of the frog from the lymphocyte by the appearance of a few irregular granules. The cell then enlarges and later the nucleus increases in size showing a few processes but no real lobes. The cytoplasm looses its basophilia as it becomes broader but the formation of granules, he says, does not appear to stand in any relation to the nucleus or cell body. (12.) This differs in one respect particularly from the development found in my material from the garter snake where any sized lymphocyte may become an adult mast leucocyte with practically no change in its morphological characteristics.

Eberhardt (9.) found that basophil leucocytes in the turtle arrise through the production of metachromatic granules in non-granular leucocytes found exclusively in the connective tissue of the spleen. Karyokinesis was found here also in cells already containing granules.

Weizberg, (34) after studying the blood of eighteen reptiles, concludes that there are two types of mast leucocytes:

1. Lymphocyte form containing a few coarse, irregular granules in a narrow cell body.

2. Histogeneous form with a wide cell body and many fine granules.

Both or only one of these types may be present in the same animal. One type is not however, derived from the other and cells which morphologically, are intermediate between them are found only in Algiroides and Anolis. In my preparations of the garter snake however, both types are found and the transtional stages are also present that Werzberg describes for Algiroides negropunktatus which have a wide cell body and numerous coarse granules. These cells are not necessarily intermediate stages between the other two however, because in the snake lymphocytes of any size may develope mast granules and become mature mast leucocytes without adding any appreaciable amount to the cytoplasm. Werzberg suggests that the lymphocyte type is developed from the small lymphocyte.

According to Downey, histogeneous and haematogeneous mast cells are apparently identical in Amphibia, reptiles and birds and although different in mammals they take their origin from the same lymphoid cell. (8.). Maximow and Dantschakoff find that mast cells in the embryos of birds, reptiles and mammals develope from lymphocytes.

4. There is some evidence for the development of azurophil mononuclears in the circulating from lymphocytes. A complete series could undoubtedly be made out for these cells with sufficiant time, similar to that described by Freidsohn (12) for the development of the special cells in the frog. He finds them developing from cells corresponding to lymphocytes by changes in the nucleus resulting in polymorphy and by an increase in the cytoplasm which looses its affinity for basophilic stains and becomes slightly acidophilic. In the case of the azurophil mononuclears of the garter snake, changes in the structure of the nucleus are noticeable but no polymorphy occurs. The changes in the cytoplasm differ also since it does not entirely lose its basophilia but at the same time part of it becomes strongly acidophilic.

Basophil Granules in the Erythrocytes.

Granules have been reported in erythrocytes by several authors, particularly by those experimenting with vital stains.

O. Schultze (1887) (28.) after using a very thin watery solution of methylen blue, found solitary blue granules in the colored blood cells which were apparent in the fresh blood of the same larvae. He suggested that they might be considered remains of yolk granules if they did not appear also in the blood of the adult Trytons. He was able to demonstrate the same granules in the erythrocytes of the adult form which had lived in a solution consisting of one part methylen blue to 100,000 parts water for ten days.

Ludwig Bremer (1895) (3.) described "paranuclear-koerperchen" in the erythrocytes of the turtle which Meves suggests may correspond to the "chromatoid granules" of the triton and salamander. According to the author, "Das Paranuclearkoerperchen, oder Kuegelchen, wieich es nennen moechte, ist ein kleiner Koerper von Kugelform, welcher in der Naehe des Kernes in Diskoplasma der voellig ausgebildeten Erythrocyten, --- aber auch in den nicht ganz entwickelten, und sogar in den juengsten Formen, den Haematoblasten, gefunden wird." In the erythrocytes of Testudo Carolina and Chelydra serpentina, examined when the blood is in a fresh condition, small spherical bodies may be seen lying usually, near one of the poles of the nucleus. Bremer says, "wenn man schnell manipulirt, nimmt man nur ein einziges derartiges Koerperchen fuer je einen Erythrocyten wahr. Nach einigen Minuten jedoch, und noch mehr nach einigen Stunden, sieht man Erythrocyten, welche mehrere Kuegelchen von ansch einend derselben Art und Groesse enthalten. Diese neuentstandenen Gebilde sind Kunstproducte. Sie sind entweder zertruemmerte Fragmente des Paranuclearkoerperchens, welches sich beim Absterben des Erythrocyten in zwei, drei und mehi Kuegelchen theilt, oder es sind wirkliche Vacuolen, imndem Sinne, den man gewoehnlich mit diesem Worte verknuepft, oder aber es sind auf-oder eingelagerte Fibrin-Kugeln. Der Beweis kann leicht vermittelst der Faerbung geliefert Werden." "

"Seiner Natur nach ist das Paranuclearkoerperchen ein vom Innern des Kernes in das Biskoplasma ausgewanderter Nucleolus oder vielleicht ein Nucleolusfragment, umgeben von einer dem Kerne entnommenen Huellsubstanz. In den Kernen mancher Blut-Koerperchen kann in der That der Nachweis zweier Nucleoli geliefert werden. In diesem Falle existirt kein paranucleares Koerperchen."

Later in the same year, influenced particularly by Dehler's work on Centrosomes, Bremer withdraws the name of "Paranuclearkoerperchen" and substitutes for it "Centrosom der gekernten rothen Blutzelle." (4.)

Julius Arnold (1897) in examining frog's blood in fixed preparations found by careful observation one or several granules at the poles in many erythrocytes. Since he was studying coagulated blood and these same cells show evidences of degeneration, it is impossible to decide whether or not he found the conditions similar to the one described later in this article. (2.)

F. Fischel (1901) (11.) with Bismarck-Brown, found small groups of intensively stained brown granules at one end of the nucleus in the red corpuscles of Siredon which he regarded as analogous to those described under various names by Bremer, Dehler, Horsley, etc. Fischel seems to doubt the plausibility of Bremer's interpretation of the granules as centrosomes, speaking of it as "eine Deutung, die wohl nicht zu halten ist."

F. F. Weidenreich (1903) (32.) claims that the granules found in the erythrocytes of the salamander are degeneration products due to the effect of the stain. He says, "der Kern stirbt ab und faerbt sich, aus dem Inhalt werden Substanzen in Form kleiner Troepfchen ausgefaellt, dieddie Farbe ebenfalls annehmen."

Prowazek,(1907) (25) while searching the Javanese reptiles for haemogregarine parasites, noticed that most of the older erythrocytes of the snakes as well as the geckos, had nuclei which were not oval but "vielfach zerklueftet" and had many deep furrows in them similar to the nuclei of the epithelium of the Axolotl larva. He found that the peripheral projections of the nucleus became more and more cut off from the surface until they entirely separated from it in drop-like forms Then,according to the author,these severed parts wander out toward the Periphery, become fragmented and soon lose their affinity for the red component of the Giemsa stain; they stain reddish violet, then bluish, becoming constantly smaller and less distinct until they finally are invisible. The nuclei of the erythrocytes of Reptilia, he says, are then continually sending out parts of their substance into the cell body toward the periphery; there is a possibility then that the chromatin thus liberated, after further metabolic processes furnishes the lipoid componant for the cell membrane.

F. Meves, (1911) (22) in the fresh blood of the fire salamander, found the yellow glittering body, usually at one of the poles of the erythrocyte; sometimes two, three or several smaller granules were grouped together. When a watery solution of an aniline dye was added to the fresh blood the granules stained intensively; with methylen blue and neutral red, the granules were tinged before the nucleus showed a trace of color. With certain stains (Gentian and methyl violet, thyonin, toluidin blue) the granules were metachromatic while the nucleus was bright blue at first. Near the granule after a short time, a clear space appeared and soon after moleculer motion of the granules could be seen. These are considered by Meves to be "chromatoid granules".

Concerning their interpretation he says, "In den letzten Jahren ist in nunmehr schon zahlreichen Fallen beobachtet worden, dass Nukleolen in Beginn der Teilung aus dem Kerm ins Cytoplasma ueber-treten koennen; hier koennen sie liegen bleiben und der allmaehlichen Aufloesung anheimfallen.

Solche ausggstossenen und "verrottenden' Nukleolen koennten auch die chromatoiden Kuegelchen der Triton-und Salamander-blutkoerperchen sein. Jedenfalls kann schon aus der starken intravitalen Faerbbarkeit derselben Geschlossen werden, dass es sich um abgestorbene Elemente handelt."

Personal Obsesvations.

In the erythrocytes, especially in those showing nuclei of irregular outline, granules of various sizes are present in the cytoplasm. The larger of these take on a blue color when fixed osmic vapor and stained in May-Giemsa, while the smaller ones stain very intensively. In many cases, the nuclei of such cells show one or more projections which usually contain a greater or less amount of deeply staining chromatin. In other cells these projections have become differentiated into two parts, a rather narrow, weakly staining steak and a rounded distal portion containing chromatin and hence staining more intensively. Since large granule-like bodies similar in size and staining reaction to the rounded distal part of the projection are found in the cytoplasm, often close to the nucleus. It is reasonable to suppose that the rounded distal part is finally entirely constricted off as a mass of chromatin and nuclear sap surrounded by a small part of the nuclear membrane. This larger mass may become fragmented into several parts which become smaller and smaller, staining quite intensively until they finally disappear; this process is probably due to the action of the cytoplasm upon the granule causing the latter to dissolve. I do not find these granules at any time, having an affinity for the red component of the Giemsa stain as was noted by Prowazek. At all times theybstain a blue color, naturally varying in shade with the edge of the granule, but always similar in staining reaction to the chromatin in the nucleus. The author mentioned the fact that he had found this fragmentation of the nucleus in a number of Javanese reptiles and, as I have also noted it in the snake and alligator, I am inclined to agree with him in his general statement, "Der Kern der Rotzellen der Reptilien stoesst demnach bestaendig Teile seiner Substanz in den Zellleib gegen die Peripherie ab." He suggästsight be well to mention here that in two respects, at least, the granules prescribed for the snake correspond to the so-called "Jolly Koerperchen" of mammals as described by Dora Friedstein.(13) According to her, these bodies are intraglobular remains of the erythrocyte nucleus and are found in fixed preparations in which they stain red with May-Giemsa. The erythrocyte granules are of neuclear material and are found in fixed preparations, in which, according to Prowakek, they have an affinity for the red componant of the Giemsa stain.

Bremer has apparently found a similar condition in the erythrocytes of Testudo Carolina and Chelydra Serpentina to that which I have demonstrated in the garter snake. His earlier interpretation of the granules as "Paranuclearkoerperchen", that is, as the nucleolus or a nuclear fragment which has found its way into the cytoplasm from the nucleus, seems much more plausible than his later designation of it as "Zentrosona der gekerntenroten Blutzellen." But as yet I have been unable to demonstrate a true nucleolus and if none is present the granules could not of course be of nucleolar material. His change of opinion as to the interpretation of these granules seems to have been due almost entirely to his complete acceptance of Dehler's work on the red corpuscles of the chick embryo. Undoubtedly, with an iron-haematoxylin stain and embryonic blood which much have shown many mitotic figures, Dehler was able to demonstrate genuine centrosomes in the (1) erythroblasts and even in young erythrocytes but Bremer, using only aniline dyes and experimenting with apparently mature blood blood cells, would hardly be able to prove conclusively the presence of centrosones. His ascertion that the centrosone may come from the nucleus by nuclear budding, shown in his figure 10, is,cI believe, untenable.

Schultze undoubtedly saw similar granules in the Triton erythrocytes as these granules can be easily demonstrated in frog blood. He does not report any observations on their production. The granules which Fischel found in Siredon may correspond to those of the garter snake but they are not like them in morphological characteristics.

POLYMORPHISM.

There has been much discussion concerning the cause for the change in the contour of the nucleus of most of the granular leucocytes from the large round form characteristic of the myelocyte to the lobed and much fragmented nucleus ofthe mature cell. Neuman (1903) asserts that a change in nuclear form does not necessarily mean a new stage of development because such a change in form may occur without a definite law in any cell. Lavdowsky, Ranvier, Arnold, Deckhuyzen, Heidenhain and Jolly believe that the change of nuclear shape was due to the amoeboid motion of the cell. Metschnikoff and Jolly consider the lobed nucleus an arrangment to facilitate the migration of cells through the walls of the blood vessels. (23)

The following extracts from Weidenreich's "Die Leucocyten und verwandte Zellformen" give a comprehensive view of the situation. "Indem ich einen entscheidenden Wert fuer die Klassifikation und die Beurteilung der Leucocytentypen, besonders auch der granulierten Formen, auf das Verhalten des Kerns und speziell auf die Art seiner Lappung lege, entferne ich mich vonder frueher besonders auch unter den Anatomen weit verbreiteten Ansicht, dass der Kern der Leucocyten ein ausserordentlich wandelfaehiges Gebilde sei, dessen Lappung und Art der Lappung in erster Linie ein Ausdruck und eine Folge der amoeboiden Zellbewegung waere. Diese Vorstellung stuetzt sich in der Hauptsache auf die Beobachtung des lebenden bezw. ueber-lebenden Objektes und zwar ausschliesslich der Amphibienleucocyten. So beschrieben Ranvier und Lavdowsky Kernveraenderungen an den kriechenden Leucocyten des Axolotls, wobei sie gesehen haben wollten, dass die kugeligen Kerne in polymorphe uebergehen koennen und umgekehrt. Auch Arnold, Flemming, und Neumann aeussern sich in aehnlichen Sinne. In neuester Zeit hat sich M. Haidenhain auf Grund von Beobachtungen einesLeucocyten im Schwanz der Tritonlarvae auf den gleichen Standpunkt gestellt; nach ihm entstehen bei der Bewegung die absonderlichsten Kernformen mit den mannigfachsten Ausziehungen und Aufteilungen der Kernmasse, wobei zwischendurch immer wieder einmal der kugelige Zustand mehr oder weniger erreicht werden koenne. Von den Klinikern ist besonders Grawitz fuer dieselbe Auffassung der Polymerie des Kernes eingetreten. Andrerseits liegen aber auch Aeusserungen von Autoren vor, die in der Kernlappung nicht eine beliebige und wieder ohne weiteres ruechgaengig zu machende passive Bewegungserscheinung sehen, sondern sie fuer den Ausdruck einer Charakteristischen Progressiven Kernunbildung Halten, als deren Ausgangsform der kugelige oder besser einheitlich kompakte Kern zu gelten hat. Diese Auffassung wurde besonders von Ehrlich und seiner Schule vertreten; sie sahen den polymorphen Kern als eine Folge des direkten Wachstums der sog. Mononucleaeren Elemente an; in diesem Sinne Hat sich besonders auch Pappenheim ausgesprochen." (31).

From my own observations on the blood of the garter snake the view that changes in nuclear form are due to the Amoeboid motion of the cell is decidedly untenable. In the first place, cells with polymorphic nuclei are found neither in the blood nor in the tissues studied up to this time. The leucocytes, both granular and non-granular, are strictly mononuclear when normal, showing only in pathological conditions any sign of polymorphy and then exhibiting only an indication of it.

Secondly, in studying the wounded tail region, numerous eosinophils were found which normally are not present there. These had wandered through between the muscle fibers, through the subcutaneous connective tissue and from there had worked their way among the cells of the outer epithelium where in some regions they were very plentiful. In spite of this extended emigration no polymorphic nuclei were observed. The latter of course adapted themselves to the amoeboid motion of the cell by a change in form, becoming elongated while the cell was crawling but when the latter assumed again its compact characteristics the nucleus also took on its normal form; and, even though the granules had begun to disintegrate in several cases, the nuclei were still intact showing their characteristic structure.

The following view expressed by Weidenreich seems most plausible to me: "Ich komme also zu dem Ergebnis, dass die Hufeisenbildung, Ringbildung und Kernlappung ein innerer Vorgang ist und dass die amoeboide Bewegung nur eine Verlagerung der kompakten oder gelappten Masse herbeifuehren kann, keineswegs aber die Lappung selbst verursacht. Aus diesen Gruenden ist auch eine Rueckentwickelung zum kom- (31)

pakten, kugeligen Kern im Berlauf der Bewegung unmoeglich." (For further information see: "Beitraege zur Kenntnis der granulierten Leucocyten," by F. Weidenreich. Arch. f. mikr. Anat. Bd. 72. S. 209.) The findings of the author upon which he bases this ascertion, have been corroborated by other workers such as, Brugsch, Schilling, W. Knoll, etc.

CONCLUSIONS.

1. The blood of the garter snake (Eutaenia sirtalis and radix) is mononuclear. Normally no polymorphic cells appear.

2. The erythrocytes develope in the circulating blood especially in the spring.

3. Basophilic granules are found in the erythrocytes due to a degeneration of the nucleus.

4. Mast cells are developed from all sizes of lymphocytes and any of these cells may become filled with granules without broadening its cell body.

5. The eosinophils and azurophil mononuclears have each assumed part of the function of the special cells of other animals. Both have become specialized for this duty and are characteristic cells for the blood.

6. The lymphoid cells present are lymphocytes of all sizes, "grosslymphocytes", mononuclears with indented nucleu and mononuclears containing azurophil granulation.

7. Spindle cells are developed in the circulation from small lymphocytes.

8. There is evidence of a similar development for the azurophil mononuclears.