THE SIGNIFICANCE OF RETICULOCYTES IN EMBRYONIC PIG BLOOD

by

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(Introduction)

This study was suggested by the observations of Dr. H. C. Tracy, on sections of pig embryos fixed in Bouin's solution and stained by the usual haematoxylin -eosin technique. He observed that certain large erythrocytes in embryos of the 20 to 30 mm. stage contained a small nucleus which showed a definite affinity for the acid stain (eosin); while most of these large erythrocytes with pyknotic nuclei showed a marked preference for the basic stain, (haemotoxylin). These primitive megalocytes with acidophilic small nuclei suggested to us a degenerative process in these primitive red blood cells. Villa ('29) advances with some reservation the interpretation that primary nuclear acidophilia in the hemoglobin series is the expression of the synthesis of hemoglobin in the nucleus instead of in the cytoplasm. This interpretation cannot be given for the acidophilia of the nucleus of the cells in question for their cytoplasm has long since been rich in hemoglobin and they must be regarded as mature red blood cells of the first generation ie. megalocytes. This is not a new idea, as there are several references in the literature: Michels ('31) in an extensive review of the literature to 1930 states the situation as follows:

> "Although concominent structures for a short period genetically and morphologically the primitive red blood cells differ from the definitive or ordinary type of red blood cells. Megalocytes are extremely large and have pyknotic nuclei, while the normoblasts are smaller and have a cart-wheel arrangement of the chromatin. Megalocytes never transform into normoblasts, but disappear relatively early in the embryo. Their formation is confined strictly to the hepatic and prehepatic period. Accordingly bone marrow contains neither megalocytes or megaloblasts. Structures similating them are to be interpreted as macro-normoblasts, normocytes."

> "Since in the normal individual all traces of megaloblastic tissue have disappeared before birth, the presence of megaloblasts in pernicious anemia is purely pathological, and is to be explained as due to a persistence of megaloblastic tissue in the liver of the adult. The fimilial linkage characteristic of this disease is perhaps best accounted for, according to Piney, if this viewpoint is taken."

Howell ('90) was apparently aware of this condition from his studies on a 2.5 cm cat embryo, as may be seen by the following extract from his paper:

> "In the first place there are two distinct forms of red corpuscles present in the blood: one large oval and nucleated, resembling somewhat the corpuscles of amphibia and reptiles. In shape they were biconcave, irregular, or apparently in some cases biconvex, and were so extremely plastic as to appear liquid. The size of these corpuscles in their long diameter varied from two to four times the diameter of the corpuscles of the adult mammal. The second form was circular in outline and the usual size of the cat corpuscles. - - - Some were nucleated and some had lost their nuclei. It is worthy of special emphasis that all corpuscles that were not nucleated belonged to this second class. Diligent search thru a large number of preparations failed to reveal a single large oval corpuscle that did not have a nucleus."

Hayem also observed this primitive generation of red blood cells as early as 1889, and speaks of them as giant nucleated corpuscles.

It is therefore the object of this study to ascertain if these primitive megalocytes may be identified by the supravital method of staining, how they react to the stain, and thus observe if the two generations of red blood cells may be further established or not. It is also the purpose of this study to see what relationship may be observed between the reticular substance and the nucleus of these primitive blood cells, and the nucleus of the definitive or normoblast type.

(Material and Methods)

The pig embryos were chosen for these studies for several reasons: They are used extensively in the labratory, especially in the study of embryology; hence it would be of considerable didactic value to know the details of blood formation in this particular animal. It is the most convenient type of mammal from which a complete series of blood samples might be obtained fresh. The embryos were secured at the packing plant no more than thirty minutes after the sow was killed. The embryos were still alive however, as was clearly demonstrated in one experiment when the heart could be seen beating after dilute india ink was injected into the umbilical vein of some 16 to 20 mm. specimens, which made the heart and main blood vessels stand out clearly.

The supravital technique used for demonstrating reticulocytes was that recommended by Todd & Sanford. The staining solution was made up from two stock solutions:

1% Brilliant Cresyl Blue in 0.85 % NaCl - - - - 5 drops
1% Neutral Potassium Oxilate in 0.85 % NaCl - - 25 drops

To this (3) three drops of blood was added from the umbilical cord of the embryo pig. It was found impossible to obtain blood in this way in embryos smaller than 15 mm. (crown-rump measurement unfixed and floating in saline or amniotic fluid). Various vital dyes were used but Brilliant Cresyl Blue (Grubler) was found to give the best results. The mixture was then centrifuged after allowing sufficient time for the staining to take place; and the excess fluid was drawn off until the volume of fluid and corpuscles was about equal. This allowed the material to dry rapidly when spread onto clean glass slides. Some of these preparations were then counterstained with

Wright's stain or Giemsa's technique, thus making the slides more permanent and the reticulum even more distinct. Both the not counterstained and the counterstained slides were studied and compared.

Other embryos from the same litters were fixed in Bouin's solution and Zenker's Formol (Maximow) and stained with haematoxylin and eosin, others with Wright's technique for tissues. Some were stained with Mallory's Connective Tissue stain. A few smaller specimens were stained after Kolatchev and Nassonow's Osmic acid method, since Kurashige ('30) has demonstrated a Golgi substance in erythrocytes of some Amphibia and Reptilia.

(Observations and Results)

The series of embryos studied was graded according to their crown-rump measurement as follows: 15 mm., 20 mm., 35 mm., 45 mm., 50 mm., 60 mm., 80 mm., 130 mm., 190 mm., and 270 mm. which was a practically mature foetal pig. Engel ('99) considered 25 cm., (16 wks) foetal pigs viable.

Practically 100 % of the blood cells in the 15 mm. pig embryo exhibited the reticular substance in their cytoplasm by the supra-vital technique. All the cells were conspicuously large, varying from 9 to 14 micra in diameter with an average of about 12 micra. In outline most of the cells are oval or irregular, but on the smeared preparations there is a tendency for the cells to round up, to a certain extent. Cells with various degrees of Hemoglobin formation in their cytoplasm may be seen. In the cytoplasm of the most primitive cells of the erythrocyte series there is no suggestion of hemoglobin (ie. oxophilia). These are to be considered Haemocyto-

blasts or pre-erythroblasts. The cytoplasm of these cells stains homogenously basophilic with Wright's stain on plain blood smear preparations or in sectioned embryos stained in similar ways, but with the supravital technique the basophilic substance is precipitated in the form of a dense reticulum. The nucleus of these primitive blood cells do not take the vital stain at all except for the nucleoli which stand out distinctly. The number of these nucleoli vary from 1 to 4 and they are often irregular in shape; they may be as large as 2 micra in diameter. The ground substance of the nucleus of these primitive blood cells stains a light purple with Wright's or Giemsa's stain, but can hardly be considered acidophilic. Some of the cells with a fair amount of hemoglobin in their cytoplasm have a smaller nucleus which takes the vital stain only slightly and the nucleoli are also smaller. A few cells with a comparatively large amount of hemoglobin in their cytoplasm have a small pyknotic nucleus that takes the vital dye intensely. The nucleoli are usually not visable in these cells. Blood platelets are present at this stage but are not very numerous. Some of the platelets appear quite large and they take the vital dye in the form of a reticulum, thus resembling the cytoplasm of the primitive blood cells ie. haemocytoblast or pre-erythroblast.

In the 20 mm. pig blood the picture is very similar to that just described; however there is an apparent decrease in the number of the most primitive representatives (haemocytoblasts) in the circulation. Still more than 99 % of the cells show the reticular substance. There is an apparent increase in the number of red blood cells with more hemoglobin and pynotic nuclei. Most of these megalocytes are re-

ticulated at this stage. These cells are still all large and tend to take on an oval shape in fresh preparations and in serial sectioned material, but this is not so evident in the smeared preparations, though it is still noticable. These cells resemble the red blood cells of Amphibia and Reptilia in many ways: Some of them have pyknotic nuclei which take the vital dye and some slightly younger forms have small nuclei which take the vital dye only slightly, and have anobvious reticular substance in their cytoplasm. This condition is seen in both the Amphibia and Reptilia and in the more mature red blood cells of this first generation of the pig which resemble the former in also containing a particulate substance which blackens more readily with osmic acid, (considered by Kurashige '30 to be Golgi substance). These cells in the pig I prefer to call megalocytes as Michels '31 has used the term. Erythroplastids are present to the extant of about 0.2 % in this stage, while to a less extent in the preceeding stage (15mm.). The number of these plastids increases directly with the age of the embryo.

In the 35 mm. stage the red blood cells are mostly all still large cells with a definite increase in the number with pyknotic nuclei. The increased oxiphilia of the cytoplasm indicates a greater concentration of hemoglobin in the plastid. Some of the nucleated red blood cells of this stage appear smaller and considerably rounded with a richer supply of hemoglobin in their cytoplasm. These must be regarded as normoblasts in accord with the classification adopted, as they are generally recognized as such. Cells resembling the lymphocytes of the adult are seen in blood smear preparations of this stage,

but there is still no sign of the granulocytic series in the circulating blood. The blood platelets in this stage resemble those of the adult. They are comparatively numerous and usually smaller than in the earlier stages, but do not show the same vital staining activity as those in the younger stages.

One may still find a few megalocytes in the 45 mm. stage, but their maximum number appears to have been reached by the 35 mm. stage. There are many degenerated forms that cannot be recognized clearly, in this stage, but would appear to be degenerating nuclei. They show up most distinctly in the counterstained preparations. Normoblasts are still present in the circulating blood and the plastids have increased considerably. The results of reticulocyte counts may be observed most readily from the table below. From the 30 mm. stage on there is a remarkably abrupt increase in the plastids and a decrease in the number of reticulated forms. (See graph in back). These red blood cells represent the definitive corpuscles of the adult.

TABLE I.

Reticulocyte Counts Percent of a Thousand R.B.C. in Embryonic Pig Blood:

Crown-rump length in millimeters	Reticulated r.b.c.%	Non-reticulated nucleated megalocytes %	red blood cells: plastids %
15 mm.	100 %	0.2%	00.0 %
20 mm.	99.2 %	0.6%	0.2%
35 mm.	87.0 %	6.0 %	7.0 %
45 mm.	62.0 %	0.2 %	38.0 %
50 mm.	63.0 %	00.0 %	37.0 %
60 mm.	40.0 %	—	60.0 %
80 mm.	27.0 %	—	73.0 %
130 mm.	12.0 %	00.0 %	88.0 %
192 mm.	10.0 %	—	90.0 %
270 mm.	8.0 %	—	92.0 %

The cells listed as 'Non-reticulated nucleated megalocytes' are considered the examples of the mature cells of the megalocyte line or the first generation of red blood cells. These cells are designated by arrows ( ) in figures 2, 3, and 4. They are easily distinguished from the normoblasts by their greater amount of cytoplasm, acidophilia of the nucleus in the counterstained preparations and in smears with Wright's stain. In the reticulocyte preparations not counterstained the nuclei of these cells appear very pale, while in the normoblasts the nuclei are more pyknotic and dark blue with the vital dye.

By the time the embryo has reached 45 mm. the definitive type of red blood cells are in marked predominence. Although 70 % of the nucleated red blood cells present are not reticulated at this stage of development they may easily be recognized as normoblasts ie. belonging to the second generation. It was also observed that there was a greater percentage of reticulated normoblasts in the 35 mm. stage than there were non-reticulated normoblasts. A sharp line cannot be drawn between the stages of embryos containing the different generations separately, for they overlap considerably. The elements of the second generation seem to begin to appear in the circulating blood from the 15 mm. stage on through the foetal stages, while the remnants of the first generation may be found as late as in the 45 mm. stage; however, they are very few in number at this stage of development.

Some idea of the development of the second generation may be obtained from an observation of the progressive development of the plastids rich in hemoglobin, as shown in the following table:

TABLE 2.

Embryo length crown-rump:	Nucleated red blood cells:	Plastids:
15 mm.	99 %	1 %
20 mm.	86 %	14 %
35 mm.	26 %	74 %
36 mm.	14 %	86 %
45 mm.	5 %	95 %
110 mm.	1 %	99 %
130 mm.	1 %	99 %
190 mm.	00 %	100 %

(DISCUSSION)

The result of these two above tables are represented graphically at the end of this paper. Shipley '16 found the 35 mm. pig blood to contain but 50 to 60 % plastids, but this may easily be accounted for by a very slight variation in measurement as the crown-rump measurement cannot be considered a very uniform indication of the development of the embryo. Furthermore, this is the time when the plastids are increasing most rapidly as is shown plainly by the graph.

In fishes the erythrocytes have been described as undergoing a plasmolysis in which the cytoplasm gradually disappears, associated with either simultaneous or subsequent karyolysis of the nucleus by Rawitz ('99, '00) Jordan and Flippin ('13) and Jordan ('19) Jordan and Speidel ('31), also described similar 'senile types' in the circulating blood of Chelonia and the frog. The senile changes, as described by these authors, involved an increase in nuclear size or volume and a

simultaneous cytoplasmic cytolysis. This condition has been observed in the pig embryo of the 20 to 45 mm. stage. Examples of these senile primitive blood cells (termed megalocytes in this discussion for reasons sited above) are to be seen in figures 2, 3, 3a, 4, 4a, and 7, and 10, designated by the arrow.

Emmel ('14, '20, '24) has studied extensively the process of denucleation in erythrocytes. This work would confirm the formation of plastids by a budding and cytoplasmic constriction process as described by him. It would seem that the karyolytic process takes place in the megalocytes, ie. primitive red cells of the 'senile type", while the process of budding and cytoplasmic constriction takes place most likely in both the primitive and the definitive families of red blood cells. The primitive plastids are undoubtedly formed by cytoplasmic constriction.

Batracoseps attenuatus, a particular type of amphibian with no lungs was also studied and reported by Emmel ('24). In most amphibia the red cells are all nucleated but in this form the plastids may make up as much as 90 % of the total number of red blood corpuscles. Emmel has demonstrated quite clearly that the plastids of this Amphibian are formed by cytoplasmic constriction. He had found no evidence of extrusion of the bare nucleus; but practically all show a narrow rim of cytoplasm. These findings have been confirmed in this work and such a cell with little cytoplasm is shown in figure 3. The granulation and reticulum are present to a striking degree in the erythroplastids of Batrachoceps attenuatus, According to Emmel again, the greatest amount of reticulation is in the largest erythroplastids,

although not infrequently it may be equally pronounced even in the smallest elements. A small percent of the non-nucleated corpuscles show apparently none or at the most only a few scattered granules, and all intermediate stages are found between the latter and the corpuscles with the abundant reticulum. These studies of Emmel correlate well with the findings reported herewith that most of the plastids of the younger embryos resembles the cytoplasm of the most primitive blood cells. Possibly some of the smaller plastids have even been considered platelets, when they have come from the primitive erythroblasts before there is evidence of the oxihemoglobin.

Shipley ('16) also counted the percentage of plastids containing mitochondria as seen in embryonic pig blood stained supravitally with 1/10,000 Janus Green. His results are as follows: 73 % in 24 mm. embryo and 12 % in 100 mm. embryo. Though in this article he has been primarily interested in the plastids he makes the sweeping statement in his conclusion, "They (meaning mitochondria) are present in all nucleated red cells in vertebrates, in the blood and bone marrow of embryos and of forms normally having nucleated red cells in the blood stream throughout life, and in nucleated red cells of the bone-marrow of adult mammals." A glance at table (1) is sufficient to indicate that the above statement is not true in entirety if at all. Janus Green will also precipitate the reticular substance of red blood cells and no doubt this is with what Shipley was dealing. Key ('21) has also found Janus Green not so satisfactory for demonstrating the reticular substance as Brilliant Cresyl Blue, and concludes with the statement that "The presence of mitochondria in young erythrocytes

has not been satisfactorily proven." However, to quote him further," "In young blood cells which contain little or no hemoglobin and a large pale nucleus mitochondria may be demonstrated constantly." Furthermore, "In normoblasts containing much hemoglobin and a small pyknotic nucleus only a few minute mitochondrial granules or none at all could be seen." Key is also convinced that the reticular substance is not of mitochondrial nature, and makes the following statement in that regard: "The persistence of the reticular substance in the cells is an unchanged form for days after the blood has been shed, its resistance to heat, to solution by water, acetic, nitric, hydrochloric, oxalic and other acids, alcohol, ether, and chloroform, its morphology, and its staining characteristics when treated with various basic dyes prove conclusively that it is not of mitochondrial nature, though it can be demonstrated in fragmented form by certain mitochondrial technique."

That the reticular substance is not a result of nuclear degeneration as suggested in some text books (Todd & Sanford loc cit.) is demonstrated beyond doubt by the presence of this substance in greater amounts in the most primitive cells of the erythrocyte series ie. haemocytoblasts and pre-erythroblasts. A further difference between the chromatin of the nucleus and the reticular substance is found in the Prussian blue test and by McCallum's ammonium hydrogen sulphide reaction, Key ('21, pp. 532). These tests indicated the presence of no iron in the reticular substance while the nuclei, and chromatin in general, according to McCallum '95, always gave a strong iron reaction.

Cooke ('29) produced punctate basophilia and various reticular forms in erythrocytes with an alcoholic solution of benzidine and hydrogen peroxide. He concluded that punctate basophilia and reticulation were expressions of the same thing, since he could duplicate any form of reticulation by varying his technique. It does not seem likely that he was dealing with the reticular substance in the true sense as it is impossible to demonstrate the substance in mature erythroplastid which may be made to show punctate basophilia etc. by the benzidine and hydrogen peroxide.

Gawrilow ('29) studied the basophilic erythrocytes of Guinea Pigs blood produced by bleeding. With media of different hydrogen ion concentration he found the following interesting results:

Of 100 basophile erythrocytes

pH of the mixture	Vitalstr. Eryth. %	Difuse stained polychromatophilia %
9,18	100	—
8.67	100	—
8.34	97	3
8.04	91	9
7.73	90	10
7.38	89	11
7.17	85	15
6.98	71	29
6.81	68	32
6.64	41	59
6.47	11	89
6.24	5	95
5.91	1	99
—	—	—

"The results (of the above mentioned experiments) gave no grounds for identifying the diffuse polychromasia and vital staining with punctate

basophilia," says Gawrilow.

It is reported by Sato ('29) that potassium increases the permiability of the cell and calcium decreases it. He therefore, goes on to say that (vital staining is caused by particles of dye which have been taken up into the cell body by a physical process rather than a chemical one. Therefore the density of the tissue elements and the diffusibility of the stain used play the most important role in the staining.)

It should be interesting to know more about the actual nature of the reticular substance and what relation it actually bears to the formation of hemoglobin. This will no doubt await a quantitative precipitation and extracellular analysis, since microchemical tests have practically been exhausted in this endeavor. The condition of the blood platelets in the younger stages (15 to 35 mm.) is worthy of special consideration. The majority of platelets of this stage are of a primitive type. (See figures 1 to 3). They resemble the cytoplasm of the haemocytoblast and pre-erythroblast; they may be as large as 7 or 8 micra and by the reticulocyte technique exhibit a dense reticulum like that of the primitive blood cells (haemocytoblasts). This type of platelet disappears with the disappearance of the megalocyte series. The definitive type of platelets are more abundant at the 35 mm. stage of development and appear in every respect like those of the adult blood. Jordon '19, pp.401 reports that the granulocytes, young hemoblasts, and lymphocytes produce corpuscles in the frog resembling platelets. It may be that the budding and pinching off of cytoplasm is a biological property of blood cells in

general under the proper conditions, or at least of the more primitive type.

The reticulocyte count has already received considerable practical application. Krumbhaar ('22) is credited with attaching the name "reticulocyte", to the red blood cells containing this basophilic substance, and has considered the significaance in reticulocyte counts in disease. With the introduction of liver therapy in Pernicous Anemia, the enumeration of reticulocytes is a very valuable procedure. Spohr ('30) recommends that it be done daily to determine whether a given case will respond to liver therapy and to avoid missing the crisis. Eaton and Damren ('30) have used the reticulocyte count to determine the age of erythrocytes. After a severe bleeding they found a flood of reticulocytes reoccured in the circulation every eight days in the case of the rabbit.

(Summary and Conclusions:)

1. It has been shown that the most primitive cells of the erythrocyte series possess a reticular substance in considerable amount.

2. That the reticular substance is not of nuclear origin ie. a result of nuclear degeneration is demonstrated beyond doubt, by the presence of this substance in the most primitive red cells (haemocytoblasts).

3. Further evidence has been presented for the existence of a degenerative process in the primitive red blood cells (megalocytes), suggesting further that Ontogeny recapitulates Phylogony,

specifically with regard to blood formation in a mammal (Pig).

4. Morphologically the reticular substance is associated with the formation of hemoglobin in the cell; whether directly or indirectly one cannot say at present. At least, it is safe to say that it is associated with the differentiation of the erythrocyte series.

5. Reticulation is certainly a sign of youth on the part of the cells containing it.

6. It is generally agreed that diffuse polychromasia and reticulation are expressions of the same substance; whether this is identical with the punctate basophilia found in lead poisoning is still uncertain. The majority of evidence would seem to indicate that it is not.

7. The percentage increase in the non-reticulated plastids as the pig matures is inversely proportional to the decrease in reticalated corpuscles. This has been represented graphically and the most rapid change in these elements is seen to occur between the 30 mm. and the 45 mm. stage.

It is a pleasure to acknowledge the inspiring council of Dr. H. C. Tracy, throughout this course of study. I am also especially grateful to Edson C. Carrier, of the Kansas City Biological Supply Company for assistance in securing the materials, and to George A. Walker for assistance with the microphotographs.

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PLATE I

Fig. 1 and 1a. are cells of 15 mm. Pig embryo stained by the reticulocyte technique. The cells in Fig. 1a, have been counterstained with Wright's stain. Magnification, x1450.
1.- Haemocytoblast (or pre-erythroblast)
2.- Megaloblast.
3.- Megalocyte.
4.- Plastid
5.- Platelet? (primitive type)
x.- Embryonic connective tissue cell?

Fig. 2. and 2a are cells of 20 mm. Pig embryo stained by the reticulocyte technique. The cells in Fig. 2a have been counterstained with Wright's stain. Magnification x1450.
1.- Haemocytoblast.
2.- Megaloblast.
3.- Megalocyte.
4.- Plastid.
5.- Platelet.
6.- Normoblast.
x.- Degenerated forms (nuclei).

Fig. 3. and 3a. are cells of 35 mm. Pig embryo stained by the reticulocyte technique. The cells in Fig. 3a have been counterstained with Wright's stain. Magnification x1450.
1.- Megalocyte.
2.-Normoblast.
3.- Plastid.
4.- Platelet?
4a.- Definitive platelet.
5.- Degenerated forms (nuclei).

PLATE I

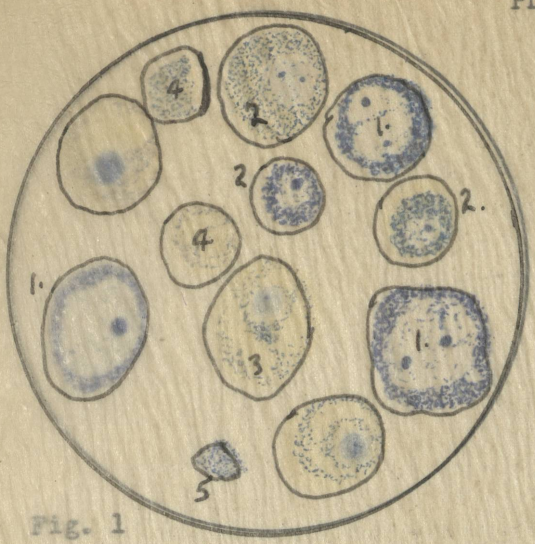

Fig. 1

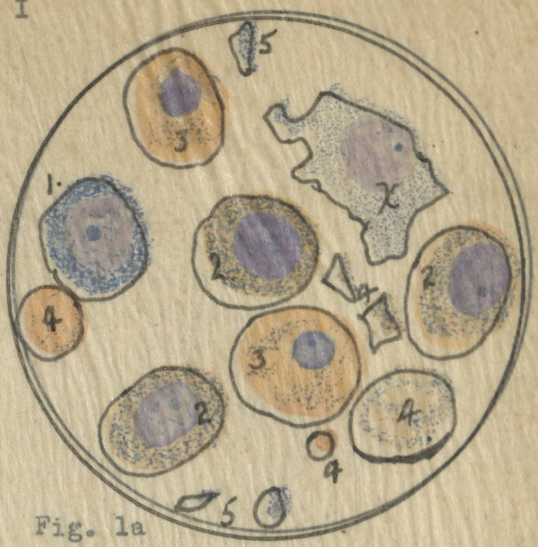

Fig. 1a

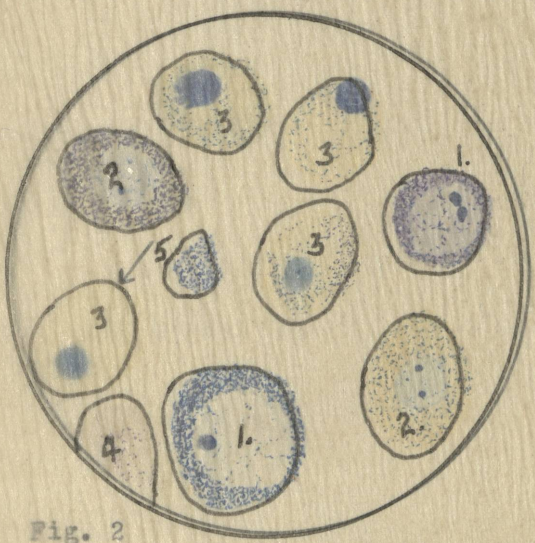

Fig. 2

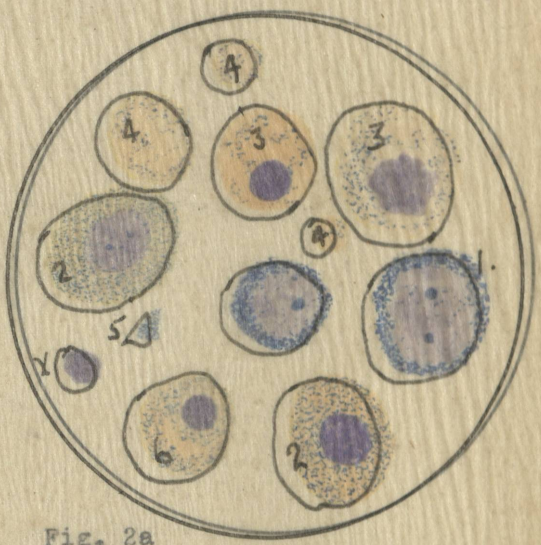

Fig. 2a

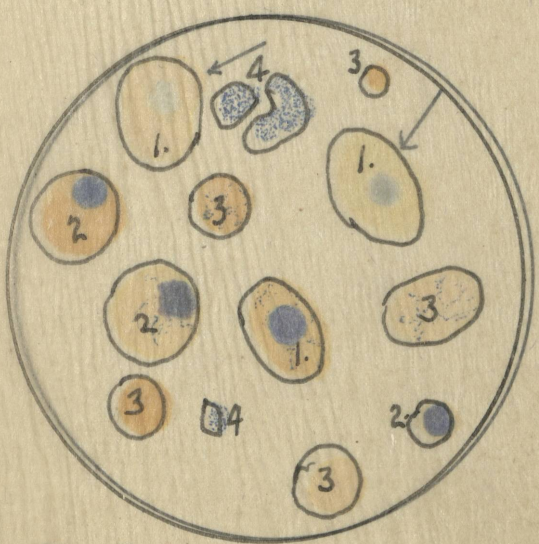

Fig. 3

Fig. 3a

PLATE I

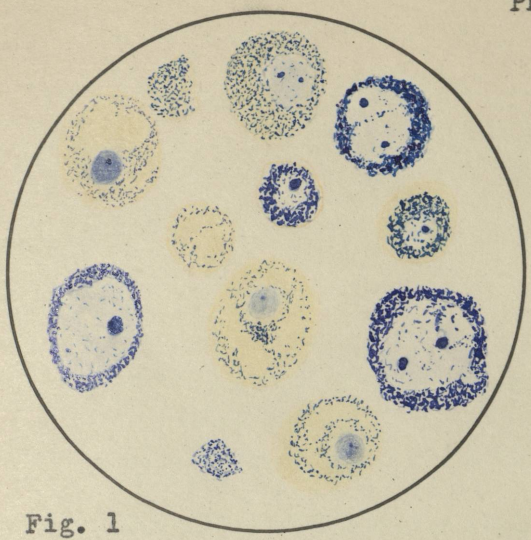

Fig. 1

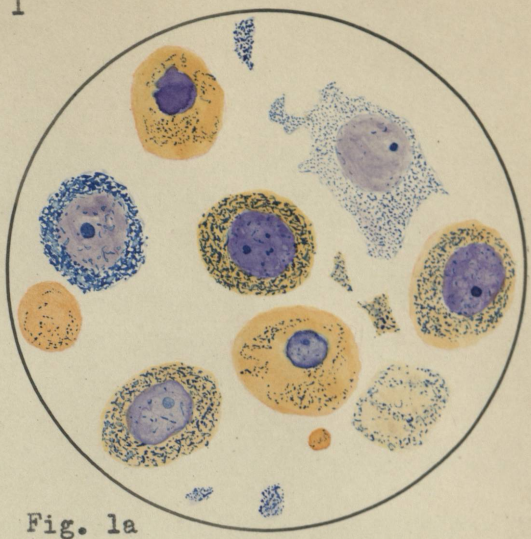

Fig. 1a

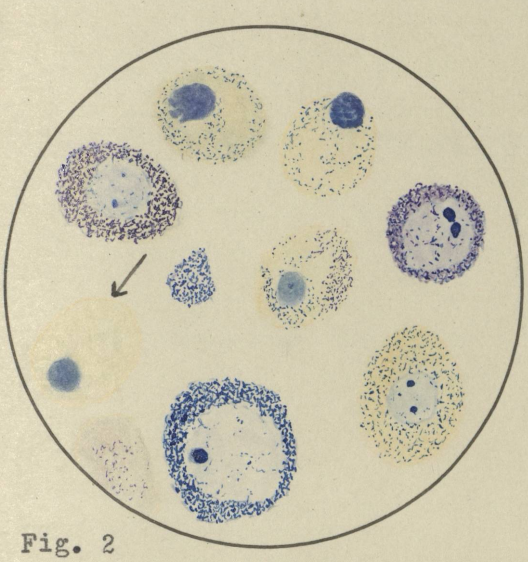

Fig. 2

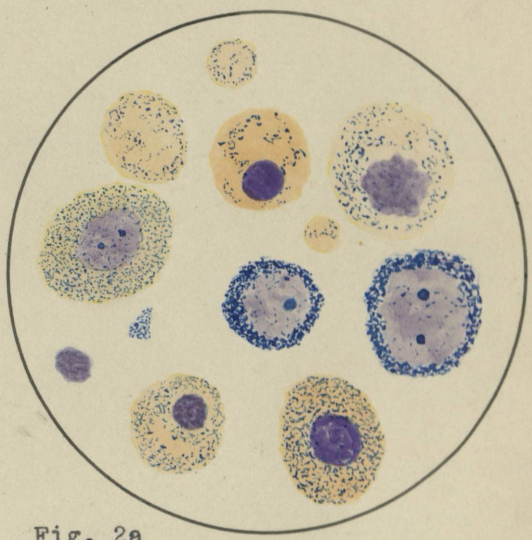

Fig. 2a

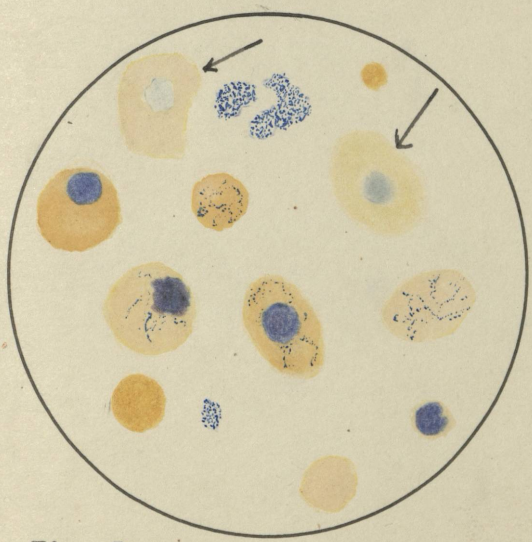

Fig. 3

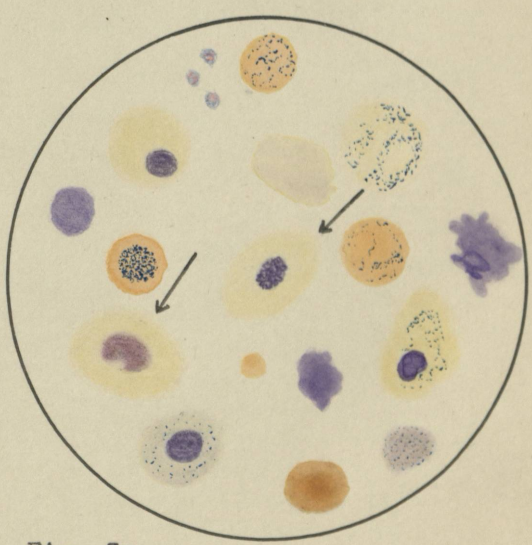

Fig. 3a

PLATE II

Fig. 4. and 4a are cells of 45 mm. Pig embryo stained by the reticulocyte technique. The cells in Fig. 4a, have been counterstained with Wright's stain. Magnification x1450.
1.-Megalocyte.
2.-Normoblast.
3.-Plastid.
4.-Platelet?
4a.-Definitive type platelet.
5.-Degenerated form (nucleus).

Fig. 5. Cells from 130 mm. Pig embryo stained by the reticulocyte technique and counterstained with Wright's stain. Magnification x1450.
All cells are plastids a few of which are reticulated.

Fig. 6. Cells from 270 mm. Pig embryo stained by the reticulocyte technique, and counterstained with Wright's stain. Magnification, x1450.
All the cells are plastids. Reticulated forms are less numerous.

Fig. 7. A field from a section of a 20 mm. Pig embryo liver, fixed in Bouin's solution and stained with haematoxylin and eosin. Magnification, x1000.
1.-Haemocytoblast.
2.-Megaloblast.
3.-Megalocyte.
4.-Plastid.
5.-Normoblast.
6.-Endothelium of sinusoid.
7.-Megakaryocyte.
8.-Liver cells.

Fig. 8. Cells from a Frog (Rana pipiens) stained by the reticulocyte technique, (no counterstain).
Magnification, x1000.
1.-A granulocyte.
2.-Reticulated red blo d cells with pyknotic nucleus.
3.-Spindle cell (reticulated and nucleus not stained).
4.-Non-reticulated red cells with pyknotic nucleus.
2a.-Red cells containing vacuoles, in one cell near the nucleus and in the other in the peripheral cytoplasm.

PLATE II

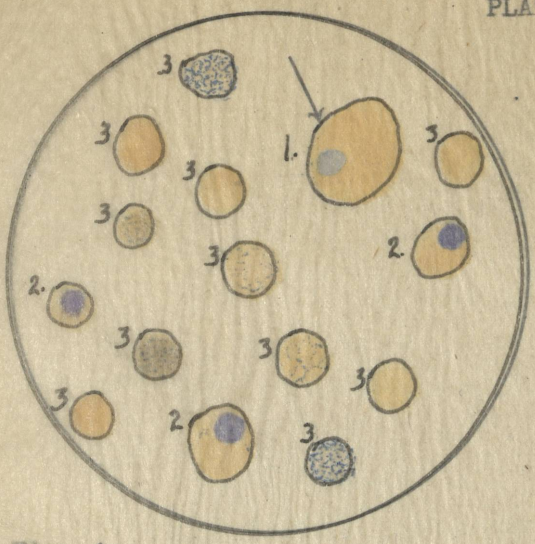

Fig. 4

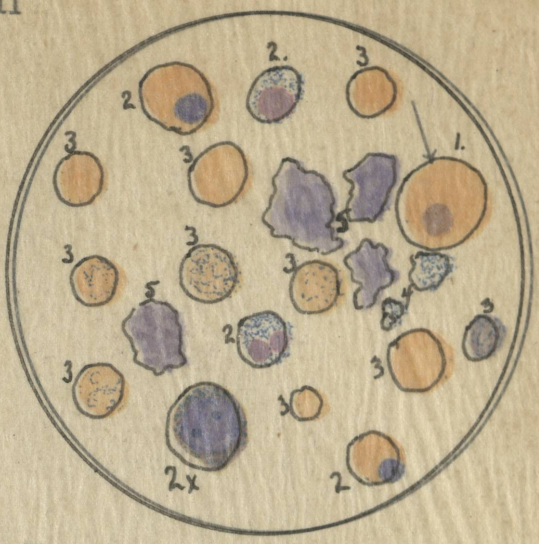

Fig. 4a

Fig. 5

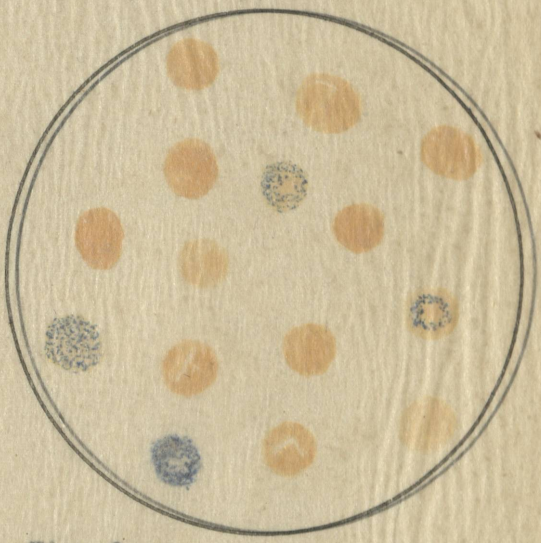

Fig. 6

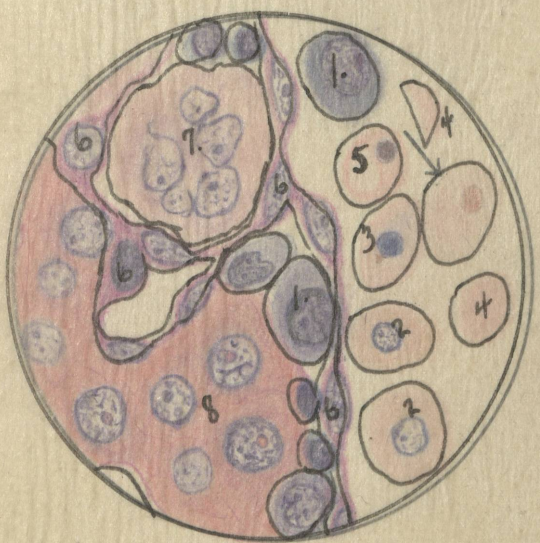

Fig. 7

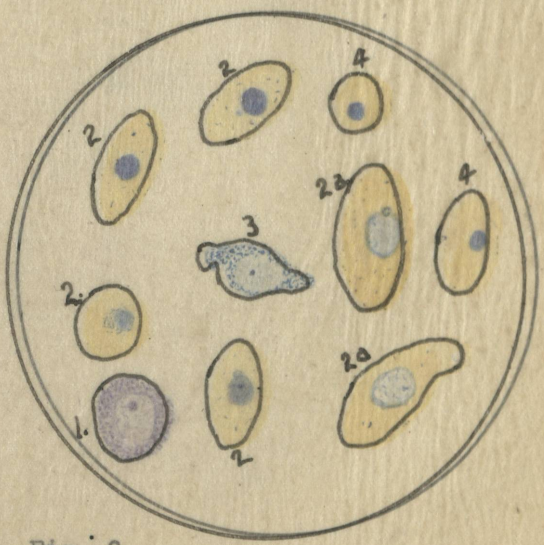

Fig. 8

PLATE II

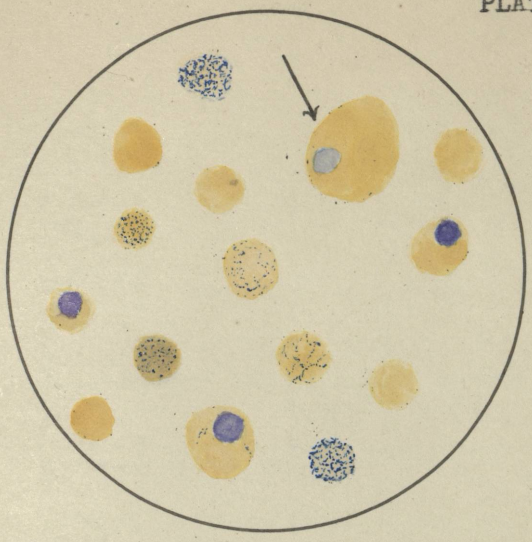

Fig. 4

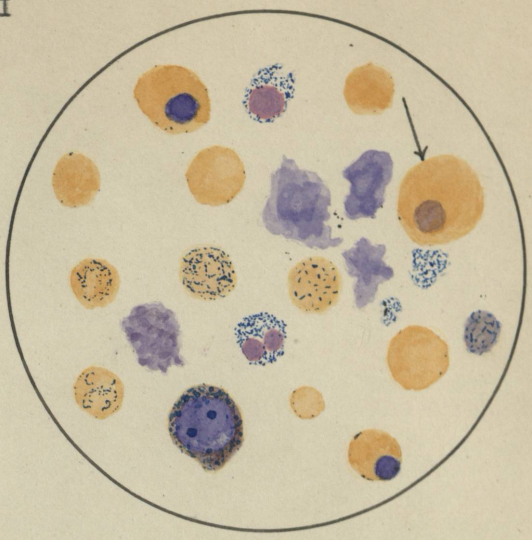

Fig. 4a

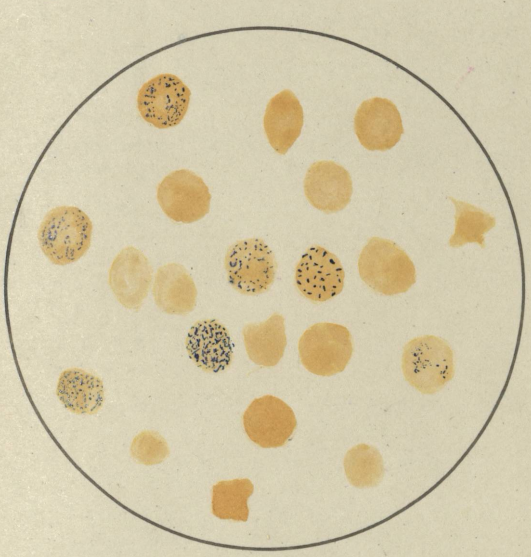

Fig. 5

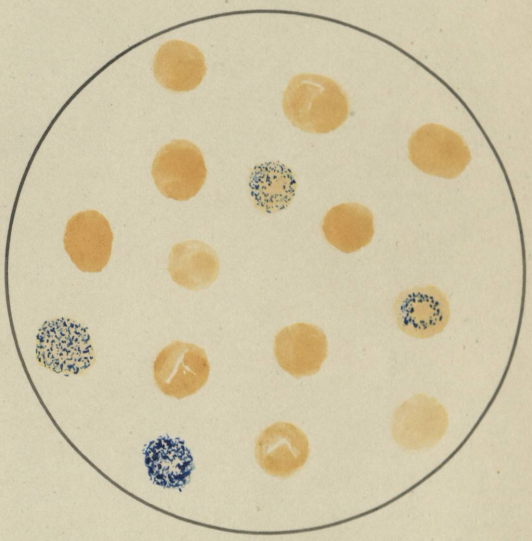

Fig. 6

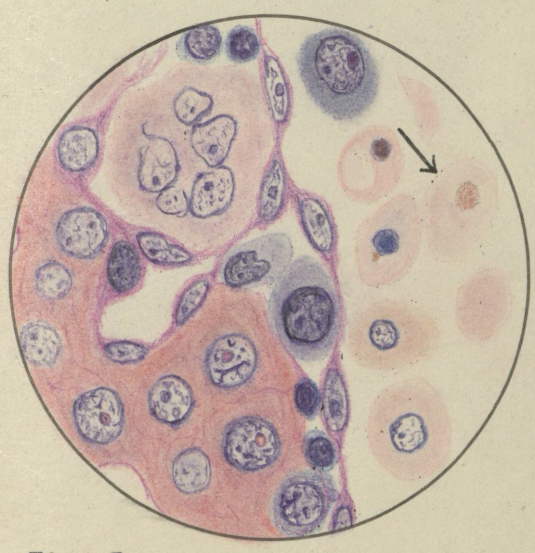

Fig. 7

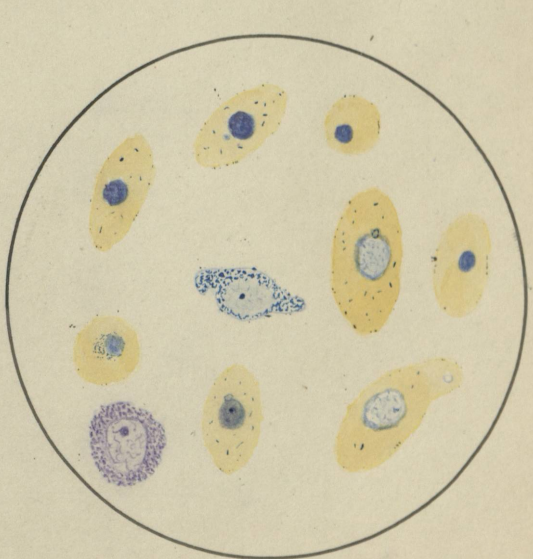

Fig. 8

PLATE III

Figures 9, 10, 11, and 12 are all microphotographs. The magnification is the same in each, (x1450).

Fig. 9. 15 mm. Pig blood stained by the reticulocyte technique, (no counterstain).

Fig. 10. Cells of 15 mm. Pig embryo, stained by the reticulocyte technique, and counterstained with Wright's stain.

Fig. 11. Cells of the 35 mm. stage (Pig embryo) stained by the reticulocyte technique, and counterstained with Wright's stain.

Fig. 12. Cells from 270 mm. Pig embryo, stained by the reticulocyte technique, and counterstained with Wright's stain.

* The arrow in figure (11) indicates a non-reticulated megalocyte.

PLATE III

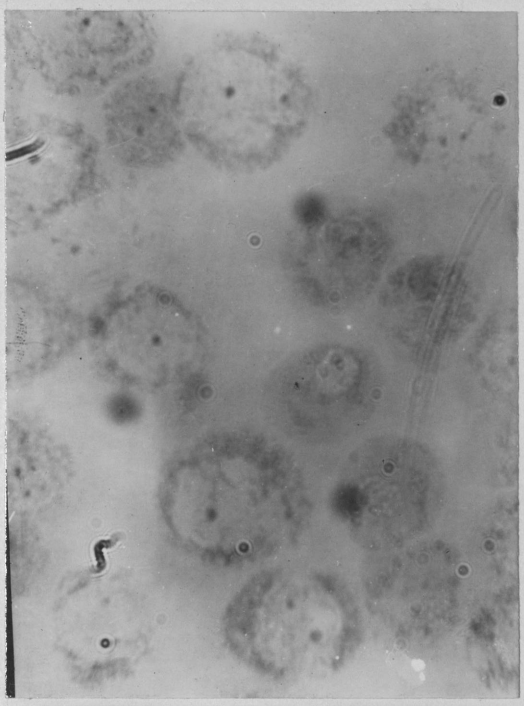

Fig. 9

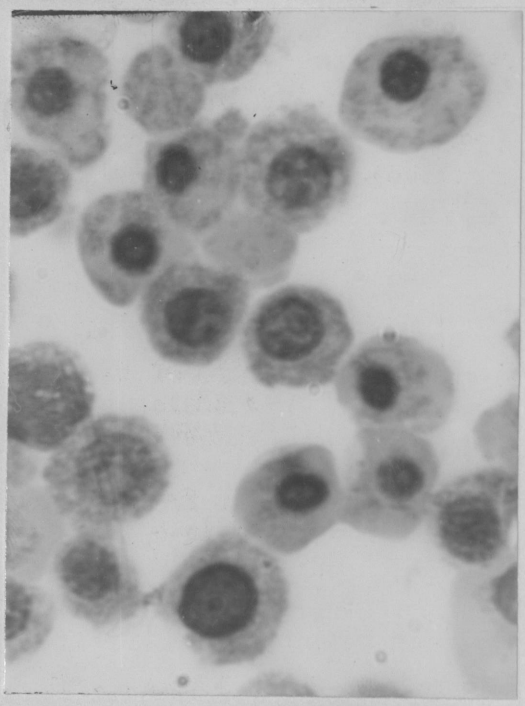

Fig. 10

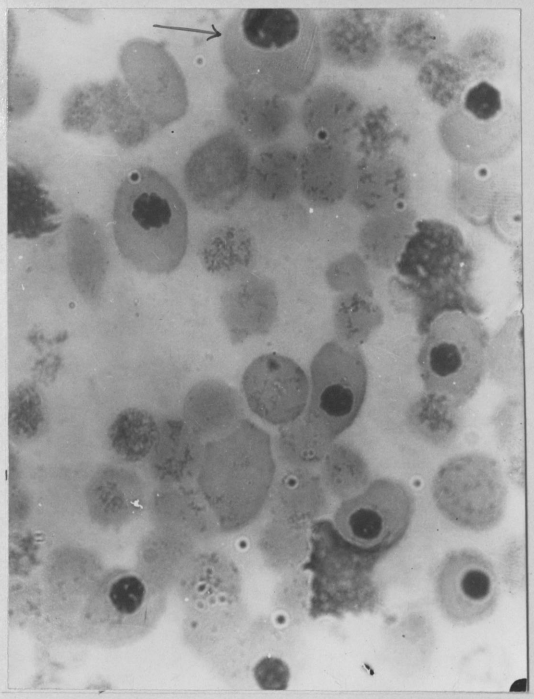

Fig. 11

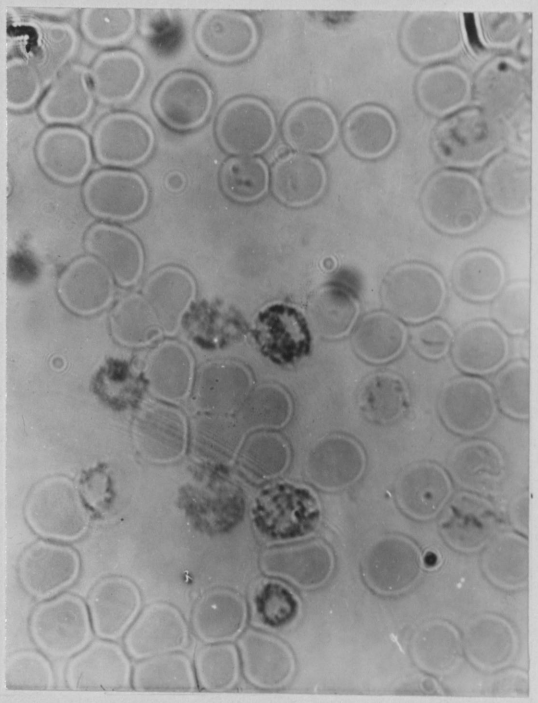

Fig. 12