THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

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

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1918

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

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

Minneapolis, Minnesota

May 25, 1920 ~~191~~

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A COMPARATIVE STUDY OF AZURE GRANULES UNDER NORMAL AND PATHOLOGICAL CONDITIONS.

A Thesis Submitted To The Faculty Of The Graduate School Of The University of Minnesota

by

Hymen Shalit Lippman

In Partial Fulfillment Of The Requirements For The Degree Of Master Of Arts.

A COMPARATIVE STUDY OF AZURE GRANULES UNDER NORMAL AND PATHOLOGICAL CONDITIONS.

Hymen Shalit Lippman.

Table of Contents.

I. Introduction.

The study of the azure granulation of the cells of the circulating blood and the bone marrow has been carried on intensively by the majority of hematologists since their discovery in 1901. Most of the work has been done on leukemic blood, for here every type of cell found in the blood and bone marrow is available, and can be demonstrated clearly and easily by means of simple technique.

The study of the cells of the bone marrow is made difficult because of the complications that arise in making satisfactory preparations, and especially those that will show clearly the azure granulations.

In my work, I have attempted to include bone marrow preparations from normal untreated animals, which study has been made possible by a technique introduced by Dr. Fineman and myself during the past year and which will be discussed later.

This subject is especially interesting when we consider the number of views expressed by the various investigators and the many conclusions reached.

My part in this problem has been to consider these opinions from an entirely impartial point of view and to try to confirm or disprove these conclusions by a comprehensive study of all the types of cells from every available source.

II. Literature.

Michaelis and Wolf in 1901 reported the presence of azure granules in the lymphocytes of the blood when stained with the Romanowsky dye. These granules were not constant, appeared only in about one third of the cells and varied between the size of neutrophilic and eosinophilic granules. They further showed by means of triacid stain that these granules were not neutrophilic in character.

Papperheim later showed that corresponding granules were to be found in the lymphoidocytes and leukoblasts in myelogenous leukemic blood. Originally Pappenheim held the view with Turk that these granules were specific just as the neutrophilic and eosinophilic granules, but soon gave up this view, and now thinks that they are figurative, functional, facultative products of cell activity coming, most likely, from the chromidial substance, i.e., the chromatin component of the nucleus.

According to Heidenreich, the azure granules differentiate from the cytoplasm of the cell.

Hynek agrees with Pappenheim but adds that the nucleus also participates. He believes that the basophilic component of the granule is derived from the nucleus whereas the acidophilic component is furnished by the cytoplasm.

Hynek and Pappenheim do not believe that the azure granules are derived from the paraplasm of the cell, that is from that portion of the cytoplasm which gives rise to the special granules. They believe that the azure granules are formed in the spongioplasm of the cell.

Pappenheim distinguishes two types of azure granules:

1- Myeloid type,

2- Lymphoid type.

The myeloid type are coarse, numerous, diffuse, bluish purple, often blended together in short strands or clusters, and not single, distinct and isolated.

The lymphoid type are fine, pointed, light red, isolated and distinct.

As a matter of fact, Pappenheim is not definite in his description of the two types of granules and hematologists differ as to the significance of his statements. By reading the literature on the subject one is convinced that Pappenheim distinguishes the two types, but adds that they are not always present in their typical cell, i.e., myeloid azure granules may be found in lymphocytes and lymphoid azure granules in myeloid cells, but in both cases this is the exception rather than the rule.

Kardos agrees with Pappenheim.

Ferrata claims that myeloid cells contain only coarse and dark staining azure granules.

Pappenheim has found that the large mononuclears and transitional cells contain the myeloid and lymphoid type of granule but that the lymphoid type is the more common and concludes that since the azure granules are not constant in either the lymphocytic or myeloid cells they cannot be used as a differential point.

Pappenheim, Kardos and Hertz believe that the very coarse azure granules found after staining with Romanowsky dye are a result of precipitation of stain. They are merely lipoid granules with the dye concentrated on their surface, and only those granules that remain after alcohol differentiation are true azure granules.

Hertz believes that azure granules are derived from nuclear material and his figures show that those cells undergoing autolysis contain the greatest number of these granules.

Naegeli at first believed that the azure granules in the transitionals and the large mononuclears were neutrophilic in character and that the transitional cell represented a stage between the granular and non-granular series.

Pappenheim proved that this view was incorrect by means of the triacid dye which does not stain azure but clearly stains the neutrophilic granules.

Naegeli abandoned his earlier view and in his later work found that in addition to the azure granules, the large mononuclear and transitional cells contain a fine, diffuse, specific granulation neither neutrophilic or azurophilic in character, which practically fills the entire cell. These granules are apparent when stained intensively by Giemsa stain.

Pappenheim has never observed these granules.

Naegeli further states that myeloblasts do not contain a true azure granulation but that the coarse granules in their cytoplasm are unripe neutrophilic granules. He comes to this conclusion from observing clinical material in which he made counts of one thousand cells and found that by using either triacid or Giemsa stain he obtained the same number of myeloid cells with neutrophilic granules.

Schridde, Schleip, Klein, Meyer and Butterfield agree with Naegeli in this view. Pappenheim, Grawitz, and others disagree with Naegeli, and believe that the neutrophile granules in later stages merely replace the coarse azure granules of the myeloblasts.

According to Pappenheim the azure granules are too coarse and arranged too disorderly as opposed to the fine, diffuse, orderly arrangement of the neutrophile granules. Even the unripe neutrophile granules in the promyelocytes are fine and diffuse. Pappenheim and Hertz prove that these granules were not unripe neutrophile granules by showing that they were present in animals as the rabbit, guinea pig, and mouse which had no neutrophilic granules in their cells; and again found by the use of triacid stain that the cytoplasm of the myeloblasts was empty. Only a few cells contained granules, but these were neutrophilic promyelocytes.

Ferrata, Hertz and Scarlatto find the same to be true. In spite of these objections Naegeli still believes his view to be correct.

Decastello and Krjukoff in 1905 found that with alcohol fixation an azure net was present in the cytoplasm of all lymphoid cells. They reported that part of the spongy network fades entirely and part stains red and gives rise to rod-shaped azure granules. Hynek, in attempting to verify this finding, says that neither he nor Pappenheim could ever demonstrate this network, but that with heat fixation they could show an azure spongy network in the "splenocytes" but not in the large mononuclears of Ehrlich. Hynek uses this as a distinguishing point in differentiating between these types of cells. He does not believe that the granules are the result of the breaking down of the network, but that they form in the spongioplasm surrounding these strands of azure.

Türk thinks that the azure granules of lymphocytes represent age phenomena, but Weidenreich and others do not agree with him.

Blumenthal believes that azure granules are the mother

granules of neutrophilic, eosinophilic, and basophilic granulation, but his view has few supporters.

Grawitz finds, as does Hynek, that the azure granules are often very similar in myeloid and lymphocytic cells and concludes that this is a strong argument in favor of the monophyletic point of view, i.e., the common origin of granular and non-granular cells.

Ehrlich found granules in large mononuclears and transitionals which stained with triacid dye and therefore concluded that the transitional cell represented a stage between the non-granular and granular cells. Naegeli 1906, Schleip 1907, Turk 1907, Ziegler 1910, Hertz 1911, Meyer 1911 found these same granules. Pappenheim and others could not find these granules in the normal blood cells, but found them in the myelogenous leukemic blood and called them promyelocytes.

Naegeli, in discussing this point, says that the triacid stain at present is not as good as that of ten or more years ago. This, he believes, may account for the failure of many authors to check his findings.

Pappenheim and others found that with increasing basophilia azure granules disappear. Thus, in proerythroblasts and plasma cells, one does not find the azure granulation. Hynek in 1910 and Naegeli in 1911 reported that they had seen azure granules in these cells.

There are very few who believe that azure granules can occur in oxyphilic cytoplasm. Hynek says that they can and that the coarse granules in the neutrophilic polymorphonuclear leucocytes are azure granules.

Grawitz finds azure granules only in basophilic and not in acidophilic cytoplasm. Pappenheim and Nardos have shown that he was wrong when they demonstrated azure granules in promyelocytes which contains acidophilic as well as basophilic cytoplasm. Grawtiz also believes that the fine azure granules can become neutrophilic granules. Pappenehim and Hynek disagree and say that these granules are not directly changed but merely are substituted by neutrophilic granules which may appear side by side as in the promyelocytes.

Hynek believes that the azure granule in the promyelocyte acts as a crystallization point about which the specific protoplasmic, neutrophilic substance may be deposited or adsorbed. Possibly a substance may join in with the neutrophilic substance and the two together may form the neutrophile granules, but there is no direct transition between the azure and neutrophile granules.

Ferrata disagrees with Pappenheim concerning the nature of the granules and also as to their significance.

Pappenheim's view:

Lymphoidocyte(with or without azure granules).

Leukoblast (with or without azure granules; exclusive generator of myelocytes;morphologically definable as a lymphoidocyte with a myelocytic nucleus).

Promyelocyte (with or without azure granules plus specific granules).

Ferrata's view:

Hemocytoblast(without azure granules).

Myeloblast (exclusive generator of myelocytes, morphologically definable as an hemocytoblast with azure granules).

Therefore we see that Ferrata believes that as soon as the

stem cell acquires azure granules it has started its differentiation, whereas Pappenheim depends on nuclear changes and says that the lymphoidocyte with azure granules can give rise even to erythroblasts. Ferrata believes that the myeloblasts with azure granules cannot give rise to anything but leucocytes. In other words, he cannot determine until the cell has acquired its azure granulation whether or not it will give rise to the granular cells.

Ferrata believes that there are three types of myeloblasts:

1- Pro-neutrophilic myeloblasts,

2- Pro-eosinophilic myeloblasts,

3- Mast-myeloblasts.

According to him, those myeloid cells that contain fine, numerous azure granules will differentiate into neutrophilic myelocytes, whereas those containing a few, coarse azure granules will develop into eosinophilic myelocytes, as follows:

Hemocytoblast (without azure granules).

Proneutrophilic myeloblast (Hemocytoblast with small azure granules.)	Proeosinophilic myeloblast (Hemocytoblast with large azure granules.)
Neutrophilic promyelocyte (Contains neutrophilic plus fine azure granules in a polychromatophilic cytoplasm).	Eosinophilic promyelocyte (Contains eosinophilic plus large azure granules in polychromatophilic cytoplasm).
Neutrophilic myelocytes	Eosinophilic myelocytes
Neutrophilic metamyelocyte	Eosinophilic metamyelocyte
Polymorphonuclear neutrophilic leucocyte	Polymorphonuclear Eosinophilic leucocyte

As opposed to this view Pappenheim believes as follows:

Lymphoidocyte(With or without azure granules).

Leucoblast (With or without azure granules).

Neutrophilic Promyelocyte	Eosinophilic Promyelocyte.
Neutrophilic Myelocyte	Eosinophilic Myelocyte.
Neutrophilic Metamyelocyte	Eosinophilic Metamyelocyte
Polymorphonuclear Neutrophilic Leucocyte.	Eosinophilic Polymorphonuclear Leucocyte.

We see, therefore, how the views of the various investigators differ, and that very good hematologists will often retain their views on a subject even after apparently conclusive proof has been offered to show that they are wrong.

III. Material and Methods.

Up to the present time a satisfactory method has not been in use for the study of cells of the bone marrow by using a technique similar to the one employed in studying the cells of the blood. The dry preparation made by smearing a drop of marrow across a slide has proved to be unsatisfactory for detailed study of bone marrow cells for the following reasons:

1- The cytoplasm is broken up in the majority of cells.

2- Cells are clumped together.

3- The granules are scattered over the field, and it is with difficulty that the cell to which they belong can be found.

Ordinarily, many slides must be made before a fairly satisfactory preparation is obtained. On the other hand, the moist preparations while satisfactory in that the cells are fairly well preserved, are not practicable because,

1- a comparative study of cells cannot be made since the cells are shrunken in the process of fixation,

2- the azure granules do not stain,

3- the process is long and complicated.

The method has been used of placing a drop of bone marrow on a few drops of saline and making a smear. Some use a drop of the animal's serum and many fair smears can thus be made. However, most of the cells are broken up, but occasionally many very good cells can be seen.

In the course of some studies on the attempt to produce experimental myelogenous leukemia in animals, Dr. Fineman and the writer have found a method which we feel is an improvement

over those that are in use today.

The bone marrow is placed in one to two cubic centimeters of one and a half percent Sodium Citrate in water in an ordinary test tube and very gently shaken up. Smears are then made of the mixture, the smears are dried rapidly, and stained. We find that the individual cells can be seen clearly, that the azure granules stain well, that there is little clumping of cells and that the structure of the cells very closely resemble that of the cells seen in the blood in myelogenous leukemia. The process is extremely simple, and usually, we can examine our smears, stained, ten minutes after the animal is dead. The cells are the same size as those of the blood and in order to make a comparative study even more exact, in my present work the blood of the animal studied was also placed in a one and a half per cent Sodium Citrate solution. We tried the use of other solutions, such as Ringer's fluid, Locke's solution, dextrin in Ringer's solution, various strengths of salt solutions, et cetera, but in no case obtained as good results as with the use of citrate.

In one instance serum of the animal was used and the cells obtained were fairly good, but the process was complicated and laborious, and had no advantages over the citrate method. In spite of all precautions many of the cells are broken in making the suspension of cells in the citrate solution, more so in some preparations than in others. But in practically every instance sufficient cells could be found to show all the types of marrow cells described.

In collecting my material, in most cases I proceeded as follows:

1- Direct blood smears taken from the hearts blood.

2- Smears of this blood in one and a half per cent Sodium Citrate Solution.

3- Direct bone marrow smears.

4- Smears of bone marrow in one and a half percent Sodium Citrate solution.

The bone marrow was obtained from the femur of the animals and from the ribs in the case of humans. An important fact to be mentioned is that, for the most part, the animals used were not treated by drugs that may have influenced the reaction of the bone marrow. The dogs were obtained from the physiology department and from the department of surgery following operations. The cat was obtained from the physiology department. The rabbits were obtained from the pharmacology department after blood pressure studies had been performed without the introduction of drugs. The guinea pigs were furnished by the bacteriology department after being bled to death for their serum content.

In my study the following materials were utilized:

1- Myelogenous leukemic blood.

2- Myelogenous leukemic bone marrow.

3- Lymphatic leukemic blood.(Chronic).

4- Lymphoblastic leukemic blood.(Acute).

5- Blood from several cases of mild, moderately severe, and severe secondary anemia.

6- Blood from three cases of pernicious anemia.

7- Blood of normal human adults.

8- Bone marrow of one living human adult obtained from rib at operation.

9- Bone marrow- normal and pathologic at autopsy. Nine cases, including ages from early infancy to late adult life. Marrow was obtained two to fifteen hours after death.

10- Laboratory animals:

a- Ten dogs.

b- Ten guinea pigs.

c- Six rabbits.

d- One cat.

In practically all cases bone marrow was obtained fifteen minutes to one half hour after death.

The stains used or referred to are:

1- Wright's modification of the Romanowsky dye (contains azure).

2- Giemsa's modification of the Romanowsky dye(contains azure).

3- Triacid dye.(Contains methyl green, orange G, and acid fuchsin, but no azure. The azure granules are not stained, whereas the neutrophilic granules show clearly).

4- May-Grumwald dye(acts as the triacid dye).

IV. Observations.

The results of my investigations can best be seen by a careful consideration of the cells which I have chosen to represent the diverse types of azure granulation in the various cells. I began my work on normal human adults, making blood smears and studying the lymphocytes in an attempt to find the granules which Pappenheim describes as the lymphocytic type.

Case I. Dr. C. Adult age 29 years. Normal.

In this case I chose the following cells:

a- A large lymphocyte filled with many coarse azure granules. Very deeply stained. See Fig.I.

b- Large lymphocyte with four very large coarse, dark, isolated azure granules, situated in different parts of the cytoplasm.

c- Large lymphocyte with a coarse nucleus, containing the typical block arrangement of chromatin, the cytoplasm of which contains numerous small, pointed, light staining azure granules. See Fig. π

d- Large lymphocyte with a few medium sized, dark, isolated granules.

e- Large lymphocyte with no azure granules. In no way is this cell any different than the cells containing azure granules.

In this case there seems to be a tendency towards the medium and coarse type of azure granulation.

Case II. Dr. L. Normal adult. Age 24.

a- Large lymphocytes containing the same types of granules as in case I.

b- Medium sized lymphocyte containing large pale granules which do not resemble azure granules but resemble more closely the Kurloff bodies seen in the lymphocytes of the guinea pig.

Case III. Dr. H. Normal adult age 27.years.

All the lymphocytes, both large and small, show a tendency tto light, fine azure granulation. Only occasionally is a cell seen with medium or coarse granules.

Case IV. Normal adult. Dr. F. Age 27 years.

a- Medium sized lymphocytes with many coarse, dark azure granules.

b- Same type of cell with many medium sized dark and light staining azure granules.

c- Same type of cell filled with fine light staining azure granules.

In this case the tendency is also towards a finer smaller, and lighter staining azure granulation.

Case V. Dr. S. Age 25 years. Normal adult.

Shows the same tendency towards the fine, small, azure granulation.

Case VI. Dr. S. Age 26. Normal adult.

The tendency in this case is toward the fine, light azure granulation.

Many of these cases were studied on different days to see if the granules changed in their appearance on various occasions. In most cases the above findings could not be checked, that is, the granules were extremely variable, and though there is a tendency for the lymphocytes, especially the smaller types, to contain the fine, sharp, light staining azure granulation, this

factor is not at all constant.

Having observed the granules in the lymphocytes of normal blood, I undertook a study of their appearance in the following pathological cases:

Case VII. Mrs. H. Age 26 years. Very severe secondary anemia. Hemoglobin 16 %, erythrocytes one million per cubic millimetre.

a- Large lymphocyte filled with azure granules, ~~mostly~~ mostly small, but some large, coarse and darkly stained.

b- Large lymphocyte, containing many large, coarse, dark azure granules.

c- Small lymphocytes with many fine, dark azure granules.

d- Large lymphocyte filled with fine, light staining azure granules.

e- Large lymphocyte with a few fine, light azure granules.

f- Large lymphocyte with many fine and medium sized, light and dark azure granules.

g- Plasma cell with no azure granules.

h- Large lymphocyte with a coarse nucleus containing a large, irregular, darkly staining body adjacent to the lower border of the nucleus. There is no apparent connection between this body and the nucleus. The cytoplasm of this cell contains numerous large, coarse, irregular azure granules and many fine, pointed and medium sized azure granules. Fig III

Case VIII. Mr. J.T. Age 54 years. Pernicious anemia, of the aplastic type.

a- Large lymphocyte containing a rather coarse nucleus, in the cytoplasm of which may be seen many coarse, dark staining azure granules arranged in strands and clumps, similar to the granules described by Pappenheim as the myeloid granulation. See Fig. IV.

In addition all the other types of azure granules as seen in the lymphocytes of the normal blood can be demonstrated in this case. Blood smears of this case were studied almost daily for a period of one month. No changes were seen in the types and arrangement of the azure granulation of the lymphocytes.

Case IX. Mr. F. Age 33 years. Pernicious anemia.

a- Small lymphocyte with coarse, dark azure granules.

b- Large lymphocytes with many distinct, dark staining, isolated, coarse azure granules.

The other types of azure granulation are seen in this slide also as well as in other slides of the same case.

Many more cases of varying degrees of anemia, ranging from mild to moderately severe and severe have been studied, and in all cases, the same cells are seen as have been described in the cells of the normal human adult. It would seem that in those cases where there is an increased production of lymphocytes some change in the character of the azure granulation should be in evidence. The next two cases will demonstrate this point.

Case X. Mr. J.H. Age 45 years . Chronic lymphatic leukemia.

The lymphocytes, which at times reached 150,000 per cubic millimeter, showed, although a scarcity of azure granules was present, the character of their size, number and distribution did not differ markedly from those of the normal lymphocytes.

Case VI. Miss F.J. Age 20 years. Acute lymphoblastic leukemia. This case which is extremely interesting because of its rarity, was the subject of a very careful study last year at the University Hospital. During her stay her white blood count varied from 646,000 to 6,000 per cubic millimeter. Her illness began during January 1919 and she died 72 days later.

a- Smear taken on April 4, 1919 when her white blood count was 578,000 per cubic millimeter, showed for the most part lymphoidocytes and lymphocytes in which the nucleus almost completely filled the cell leaving only a narrow rim of cytoplasm. Occasionally a cell that resembled a large lymphocyte was seen although its nucleus was of a younger type than the one ordinarily met with in the adult lymphocyte. Azure granules could be demonstrated in very few of these cells. One or two transitional cells were located which contained a few azure granules.

b- Smear taken on March 27, 1919 when her white blood count was 6,000 per cubic millimeter showed that the early type of cell with the fine sieve-like, open nucleus which contained many nucleoli, was present, although much less frequent than in the first slide described. There were many more cells of the lymphocytic type which contained a greater amount of cytoplasm and these cells in many instances contained numerous azure granules of all types. The cytoplasm of these cells was much lighter stained than that of the earlier cells. In a very few

minutes spent in examining the slide many cells could be found containing azure granulation.

c- Smears taken February 19, 1919, when the blood count 200,000 showed practically the same findings as those described in(a). The azure granulation is extremely rare, in fact, after examining the slides for twenty minutes not a single cell which contained the granules was seen.

d- Smears taken February 20, 1919, when the white blood count was 6,800 per cubic millimeter, showed, as in the other smear described when the white blood count was low, a greater frequency of those cells which contained the azure granulation.

It is interesting to note, that though the author came in contact with but two cases of lymphatic leukemia, one acute and the other chronic, in both instances azure granules were found in the cells.

Case XI. Mr. C.H. Adult age 45 yrs. Chronic myelogenous leukemia. The slides in this case show every type of bone marrow cells described in the literature. The azure granulation in many of the myeloid cells is very rich and often occurs in the characteristic grouping as described by Paprenheim. On the other hand cells are seen in which the granules are of almost every possible variety described, i. e., large and small, dark and light staining, isolated and in clumps. In the plasma cells and prosrythroblasts seen no azure granules were ever demonstrated by the author. About two hundred slides were examined during the course of the patient's stay at the hospital.

There is considerable to be said concerning the types of cells and granulation in this case of myelogenous leukemia. However, in the study of the bone marrow, both in the human and the experimental animals, these same cells are to be seen. So much has been said in the literature of the findings in leukemia, in fact, the greater portion of the myeloid cells described in the literature, are taken from cases of leukemia. I have therefore decided not to discuss to any extent my leukemia case. I wish to add, however, that in the bone marrow of this patient, obtained at postmortem, no appreciable changes could be detected in the cells that were not found in the cells of the blood.

In the study of the lymphocytes of the blood of the dog very little can be seen that differs in any way from the azure granulation in the human. In my series I have found that there is probably more of a tendency towards the finer type of granules and also possibly to a fewer number. However, occasionally cells are to be seen which contain moderately coarse azure granulation.

In the rabbit's blood, on the other hand, in those animals that I have studied, the lymphocytes contain many coarse azure granules. This is the case even in the medium and smaller types of lymphocytes which often contain many large, dark staining granules which resemble the myeloid type described by Pappenheim.

In the guinea pig's blood the same is true. In addition the Kurloff bodies are to be seen in the lymphocytes. On staying with May-Grumwald which does not stain azure substance, the azure granulation disappears in these cells but the Kurloff bodies remain.

In the cat's blood, as in the other animals, lymphocytes are found containing all types of azure granules.

In studying the bone marrow, I have observed absolutely nothing that would lead me to believe there is a myeloid granulation in any way different from the granulation of the lymphocytes, except perhaps in the number of the granules. For I have noted that there is a tendency towards a greater number of these granules in the myeloid cells. In the dog, cat, guinea pig, rabbit and human the myeloid cells contain, in most cases, diffuse, often isolated and distinct, either coarse or fine, dark or light staining azure granules. As others have found, many of the myeloblasts contain no azure granulation. In the promyelocytes the azure granules are seen alongside of the special granules. In the case of one of the dogs, the myeloid cells contained only a fine azure granulation. Only here and there could cells be seen with coarse azure granules.

In most cases, the azure granules are not very prominent as a constituent of the cells. I recall that in many slides much time was spent before any of the granules were seen. On the other hand, some of the slides are very rich in cells containing azure granules. In the case of a child that died of miliary tuberculosis, the myeloblasts were very numerous and were practically filled with a coarse azure granulation. In this case the dark, coarse granule predominated in the myeloblasts, although the other types were also to be found.

Many more points that I have observed during the course of my study of this problem will be further taken up in the discussion.

I next attempted to substantiate the view of the origin of azure granules from nuclear material by allowing the cells of the blood of my myelogenous leukemia case to stand in citrate solution for several days. After about fifteen hours definite destruction of cells could be observed. Smears of the blood were made twice

daily after the collection of the specimen until ten days later when an extensive destruction of the cells had occurred. It is of interest to note that the blood settled into three distinct layers. The top layer, about forty per cent of the total was almost pure serum and citrate containing a few isolated cells; the middle layer about twenty per cent, was grayish white in appearance and on study proved to be made up, almost entirely, of leucocytes, with only here and there a few red blood cells; the bottom layer was composed of a mixture of red and white blood cells, the red cells predominating.

This patient died January 28, 1920. Blood was removed from the median basilic vein at 10 P.M. immediately after death and was collected in a solution of one and a halfh per cent Sodium Citrate, and was allowed to stand in the ice box until January 29, 1920 at 1 P.M. when the first smears were made. These smears showed the following:

a- The nucleus was fairly intact in a leucoblast containing very many coarse azure granules

b- Myeloid cell beginning to degenerate the cytoplasm of which is filled with coarse and medium azure granules. See Fig. VI

c- Leucoblast in which the nucleus is beginning to undergo degeneration. The upper border of the nucleus is irregular and is surrounded by large numbers of coarse azure granules blending with the nucleus and giving the impression that they are coming from nuclear material.

d- Degenerating myeloid cell containing few fine, coarse light staining azure granules. See fig VII

On January 30, 1920, the smears showed the same changes as observed on the day previous. The degeneration is slightly more advanced. The nuclei stain fairly well; the nucleoli appear as they usually do, but the cytoplasm is rapidly disintegrating.

On February 1, 1920, the smears made were accidentally overstained but showed the details in which I am interested very clearly. In fact, I have observed that though overstaining often conceals the finer structure of the nucleus, the granules seem to be more distinct and more clearly visualized. The cells show about the same changes as in the other slides of the series, and in the next slide the cells of which will be discussed more fully.

On February 2, 1920 the degeneration had progressed to the extent that few entirely normal leucocytes could be observed. The following were chosen to represent the relationship between nuclear degeneration and the number of azure granules:

a- Myeloblast the nuclus of which is fairly intact, the cytoplasm of which is filled with dark, large, azure granules.

b- Myeloblasts the nucleus of which is beginning to degeneration and in which the cytoplasm is filled with coarse azure granules.

c- Same type of cell with a markedly degenerated nucleus, with the cytoplasm filled with coarse azure granules.

d- Early type of cell in which the degeneration is advanced, the cytoplasm of which contains only a few fine azure granules.

During the next few days the degeneration was so marked that the cell structure could not be clearly seen. It is interesting

to note that the eosinophiles and nucleated red blood cells seem much more resistant than the other nucleated forms. More intact eosinophiles can be seen in this slide than any other type of cell. The non-nucleated red blood cells show no changes whatever which is of clinical interest in that blood which is collected in citrate to be used for transfusion can stand for several days without a destruction of the most important element of the mixture.

To determine whether or not the coarse azure granules are a result of overstaining, as Kardos states, I studied the effect of staining the cells for various leggths of time, using for my material the blood of my myelogenous leukemia patient. The slides were stained for one, two, three, five, ten, twenty and fifty-three minutes respectively. The bone marrow in citrate from the case of miliary tuberculosis was stained for two minutes, washed, dried and then restained for another two minutes. This was repeated five times. In those cases where the staining was aparently only superficial, many of the cells showed a coarse azure granulation, although most of the field was definitely understained. Where the slide was overstained the same coarse azure granulation was present, though very dark. In many cells the granulation was medium and fine, even in the overstained slides. After it was noted that overstaining did not produce coarse azure granules, slides were stained of the same material and then treated with ninety-five per dent alcohol and then allowed to stand in the alcohol for two, ten, and twenty five minutes. It was observed that when the slide was treated with alcohol for two minutes, the nucleus was pale in most of the cells though the azure granules were often coarse as well as medium and fine. When the alcohol had

been acting for ten and twenty five minutes, some of the cell was faded, but the azure granulations, though less distinct, still showed the definitely coarse type. During the course of this work, the idea suggested itself of using a strong chemical agent, such as concentrated ammonium hydroxide to act on the cells after they were stained, to note what effect would be produced on the azure granulations. This was done and it was noted that while other parts of the cell became faded and understained the azure granules were distinctly seen.

When the same material was treated with glacial acetic acid, after staining, the entire field became decolorized. Every cell appeared, apparently as before being stained. When this slide was restained, the nuclear structure appeared muddled, though the granules stained fairly well. The eosinophilic granules seemed to be washed out but the azure granules persisted. One of these slides was overstained and the granulation became more distinct.

Another of the slides was placed in the hot flame of a bunsen burner for fifteen seconds. On staining, it was observed that most of the granules had disappeared. In two or three cells, several azure granules were present.

Finally, I thought it would be of interest to check the findings of Hynek who believed that the coarse granules, often seen in polymorphonuclear neutrophilic leucocytes were azure granules. During the course of my work I often found cases in which the neutrophilic granules were fairly coarse, but one case in particular presented itself in the surgical ward whose blood contained large, coarse neutrophilic granules which very closely resembled azure granulation. However, after staining with May-Grunwald these granules persisted in the neutrophiles while the

lymphocytes contained no granules in their cytoplasm.

Using the May-Grunwald stain in both the myelogenous leukemia smears and in the bone marrow of the case of miliary tuberculosis, I found that the myeloblasts, which with the Wright's stain were often filled with azure granules, now contained none. The neutrophilic granules stained well. This was done to attempt to corroborate Naegeli's statement that the coarse azure granules of the myeloblasts were unripe neutrophiles.

In my observations I have left out the description of many cells that showed individual changes when these same changes, when these same changes could be detected in cells showing other findings. The method of making bone marrow smears in citrate, I believe, made this problem possible. More work is being done at present, to try to modify the technique in such a way that a fewer number of cells will be destroyed in the process.

V. Discussion.

I had originally intended to devote my entire time in an attempt to determine whether there was such a thing as a myeloid and lymphocytic type of azure granulation. As time went on, however, my problem broadened so that before I finished almost every phase of the question had been studied. There is no doubt in my mind but that I have clearly shown that such a classification of the azure granules is not justifiable. There is nothing about the granulation that is constant either in size, color or distribution, and often the cell which one would expect to contain the coarsest granules contains the finest ones. In the bone marrow, as has been discussed, the granules are described as being coarse and dark violet, usually occurring in strands and groups in the myeloid cells. Pappenheim admits that a modification of his original statement must be made because of the variation that exists in the granulation but still maintains that the tendency towards his first finding is very marked. One point in particular he emphasizes, namely, that the cell can not be recognized by its granulation, because many of the cells contain no granules and so would remain unclassified. In my opinion the granules can not be used to classify the cells because firstly they are not constant and secondly the reverse of what Pappenheim says occurs in about fifty per cent of the cases.

As I have stated elsewhere, it is very difficult to know exactly just what Pappenheim really intends to say, for in his own article he makes contradictory statements. When one reads the interpretations of what he writes, the situation becomes even more complex. It seems clear that though Pappenheim does not

believe that the arrangement always occurs as he originally described them, their color usually is as he first stated, that is, that the myeloid granules are dark, while the lymphocytic type are finer and lighter. Even this does not hold true in all cases, for cells containing large, light staining granules and darkly staining fine granules are commonly met with. They are found in the blood of the myelogenous leukemia case studied and in the bone marrow of the humansand laboratory animals.

According to Ferrata, all the coarse granules in the myeloblasts are bluish violet in color, a fact with which the author disagrees.

As concerns the question of the clumping of the coarse azure granules and their tendency towards strand formation, I can only say that such arrangements occur almost as commonly in the lymphocytes of the blood as they do in the myeloblasts of the bone marrow. There is one distinguishing point, and only one, that may be used as characteristic of the coarse granulation, namely, the number as found in the myeloid cells of the bone marrow. I have observed that the large lymphocytes of the blood of all the animals studied and also the human, showed a coarse, dark azure granulation in many instances. However, in these cells, the granules tend to be scarce and isolated. The color, shape, and size are of no significance. Their position as regards their relationship to the nucleus means nothing.

In the myeloblasts there is a marked tendency towards large numbers of granules, in many instances crowding the cell. For example see Figure VIII . In no single instance have I observed the lymphocytic type of cell filled with this coarse azure granulation.

The large number of azure granules described by Pappenheim in the transitionals and large lymphocytes does not hold true in a large per cent of these cells in which I have found discrete, isolated, either dark or light staining granules. I believe that these cells are characterized, if by anything at all, by the irregularity in the size and staining of the azure granules that they contain.

The attempts of authors to discuss both large mononuclears and large lymphocytes in the same sentence, in my opinion, should be discouraged, because there is no characteristic difference between the two cells and the nomenclature is misleading.

Hynek has attempted to distinguish the mononuclear from the splenocyte by means of heat fixation, but his work has not been confirmed by others. The charts that he uses to illustrate his point show that the cells are definitely overstained. The author has attempted the use of heat fixation and only obtained a difference after heating the slide in the direct flame. In this case most of the granules disappeared but the network described by Hynek is not realized. Any attempt to differentiate the two types of cells by the azure granulation is without foundation.

According to Pappenheim, as mentioned above, the function of the azure granule is not definitely known, but very likely, it is a secretion product. This view seems reasonable to the writer. Pappenheim states, however, that the leucoblasts, filled with coarse azure granules, illustrates his point in that this cell is at a stage of increased activity and is functionally hyperactive. When we realize how many of the very early cells contain few or none of the azure granules, we see that the example is not a very apt one. At any rate the early cell is in a state of irritation

not because of any secretion that is to be used by the body, but because it is going to divide and give rise to new cells in which act the granules play a v ry little part.

I believe that the case of lymphoblastic leukemia cited tends more to emphasize the functional side of the granules as secretion products of the cell. In the course of the disease when the white blood count was extremely high, when the cells were rapidly thrown out into the circulation, there was no time for functional activity, whereas during the lower counts the activity of the individual cell was apparently increased due to the fact possibly, that it remained in one condition for a greater length of time and did not divide so rapidly.

It has been noted that in those cells in which the cytoplasm is extremely basophilic, as in the erythroblasts and plasma cells, azure granules are not seen, although Naegeli and Hynek have reported them. The author has not observed azure granules in these cells during the course of his work. The idea has suggested itself that in view of the fact that the granules rarely occur in those cells with a very small amount of cytoplasm are not often present in cells with deeply basophilic cytoplasm, and are usually most numerous in cells with a light basophilic cytoplasm that this part of the cell probably plays a part in the production of the granules. There is no definite proof for this view and the author merely states it as a suggestion, for there are cases in his collection showing azure granules in the small lymphocytes with with deeply staining cytoplasm.

According to Pappenheim, Mardes and Hertz, the coarse azure granules are due to a precipitate of the dye which is adsorbed on the lipoid granules where the stain is concentrated on

the surface. They find that when these slides are treated with alcohol after staining, the coarse granules disappear and only those that remain are the true azure granules. In my experiments I have been unable to demonstrate these coarse azure granules and have used the various methods for overstaining. In fact, I found that after just flashing the slide with the stain, the large granules were present, though pale. In the later stages, as for example, after staining the slide for fifty three minutes, small irregular precipitates of stain were seen, but these usually almost covered the cell and certainly would never have been mistaken for granules. The application of alcohol failed to remove the coarse granules and the same picture remained as before though every element was faded.

As regards the view that the azure granulation comes from a degenerating nucleus, I feel that the experiments cited, shows conclusively that these granules are entirely independent of nuclear degeneration. There is nothing about the autolysis of the cell that shows the relationship to granule formation. In comparing the slided taken during the life of the myelogenous leukemia patient and those taken after the cells were allowed to stand and degenerate I have found that the former contained more azure granules than the latter. Were my method for allowing the cells to autolyze to be questioned, I would still contend that there is no foundation for this view, since the very early forms of cells, with intact nucleus, are often filled with coarse azure granules.

Another point which is emphasized in my work is that azure granulation may occur in the lymphocytes of the blood in chronic lymphatic leukemia. This is in contradiction to what Mangeli has

reported. As stated they are scarce, but are more frequent when the cell count is low. In examining several slides in the case of lymphatic leukemia, the author had no difficulty in finding cells with azure granules.

Another point of interest is that in anemia, either accondary or primary, the azure granulation does not differ from the normal.

In the blood of the animals studied no changes were found as regards the lymphocytes and their granulation that could not be observed in the human. The Kurloff bodies in the guinea pig are of interest in that they are very common and resemble the coarse azure granules. Pappenheim says that they are azure, but in my work I have shown that these Kurloff bodies persist in those slides treated with May-Grunwald stain whereas the azure granulation disappears.

The views of Naegeli are unique in that they differ markedly from the findings of Pappenheim and many other noted hematologists. His original view, namely that the lymphocytes contained neutrophilic granules did not last long even with him. But the question of the nature of the coarse granules in the myeloblasts is still disputed by him, in spite of the fact that so many have shown that these granules are not unripe neutrophilic in character. The very fact that they occur in the cells of those animals which have no neutropoile granules would seem to be sufficient proof that Naegeli's view is unsound. His argument that in counting one thousand cells in a case of leukemia he obtained the same number of cells containing granules with the use of both the triacid and the Giemsa stains, is not of very much moment when we realize that in some cases there is a predominence

of neutrophilic myelocytes or promyelocytes. If this does not account for Naegeli's conclusions, the author does not see how the result was obtained unless there was an error in technique. Many attempts were made during my work to check this finding, but in every case those cells containing coarse azure granules showed an empty cytoplasm when the May-Grunwald stain was used. As has been mentioned, many cells showed granules but these were not nearly so coarse as the granules of the myeloblasts. Perhaps an important step in Naegeli's experiment has been left out in his description, for otherwise, it would seem that this argument, to which he attaches such significance is valueless.

The author has also failed to reproduce the fine granules which entirely filled the transitionals and large lymphocytes when stained intensely with Giemsa stain, according to Naegeli. Others have failed to reproduce what Naegeli has described. In the case of miliary tuberculosis, the bone marrow contained many cells in mitosis, many of these containing azure granules throughout the entire cell, with no area about the chromosones free from granules. See fig. I It would hardly be likely from such findings, that Turk was correct in calling the azure granules age phenomena.

The work of the author has shown that Hynek is incorrect in concluding that the coarse granules often seen in neutrophiles are azure in character. The May-Grunwald preparations of the blood smears showing these coarse granules when stained with Wright's stain still showed these granules.

Probably the most extreme view cited in the literature on this subject is that of Ferrata in his discussion of the significance of these granules. His theory that the coarse azure granules are

found

found in those cells destined to become eosinophiles and the fine azure granules in those cells which will later develop into neutrophiles, is entirely unfounded. In an earnest search for such a relationship, the author has never found a fact that would tend to support such a view. On the other hand, many points stand out clearly which are in direct opposition to the stand taken by Ferrata. There are those cells in the marrow that contain both the coarse and the fine azure granules, cells which Ferrata does not describe. No steps in his process can be traced either in the myelogenous leukemia blood or in the bone marrow. Surely the process is not nearly so apparent as the one showing the evolution of the eosinophilic granules from the immature basophilic granules. On the other hand, neutrophilic promyelocytes, in many cases, contain coarse azure granules, though according to Ferrata, this cell would differentiate into an eosinophile.

In the bone marrow obtained from a patient's rib at operation, occasionally cells are seen containing pale eosinophilic granules and fine azure granules. Such a combination would seem impossible according to Ferrata's view. Finally, the case in which the coarse azure granules were so numerous in the bone marrow showed no increase in the eosinophilic myelocytes or polymorphonuclear eosinophiles. The blood of the patient showed no eosinophilia. It is difficult to believe that the differentiation stopped at this point and had only been at this stage of activity for a very short while for this would be mere speculation.

Ferrata admits that the coarse azure granules are characteristic of myeloid cells, whereas the fine granules are present in the lymphocytes. Such being the case, either the eosinophiles are not formed in the bone marrow, for here the coarse type of azure

granulation predominates even thought the eosinophiles are rare. On the other hand, many of the lymphocytes of the blood contain coarse, isolated azure granules. According to Ferrata, these cells probably would develop into eosinophiles.

We see, therefore, that the question of a definite relationship between the azure and the specific granules is a very uncertain one indeed, and if such a relationship does exist, the various steps in its development can not be satisfactorily traced. Investigation of recent date tends to show that mitochondria are the result of the phagocytosis of colloidal substance from the tissue fluids. In view of the fact that the azure granulations has never been definitely traced, it would seem that such an origin might be applied to them.

According to the author, Pappenheim's theory of the significance of the azure granules, i.e., as a functional, temporary product of secretion, seems the most logical, whether the granulation is the result of nuclear or cytoplasmic activity can not be definitely stated.

Before concluding, I wish to briefly mention a few points of interest noted in examining bone marrow smears:

1- The early types of eosinophiles, namely those containing unequal, irregular granules, staining with different intensities, are very rarely seen.

2- The nucleated red blood cells are not nearly as common as expected.

3- Mitotic figures are very infrequent.

4- Mitotic figures have been found in bone marrow twenty-one hours after death.

VI. Summary.

1- Azure granules are inconstant and can not be used to characterize a cell.

2- There is no such thing as a myeloid and lymphocytic azure granulation.

3- Either the myeloid or lymphocytic can be of any size, shape, or color.

4- The number of azure granules, usually tending towards a coarser type, is the only point that may characterize the myeloid cells in the bone marrow.

5- The cytoplasm probably as well as the nucleus, plays a role in the production of the azure granules.

6- In chronic lymphatic leukemia, the lymphocytes may contain azure granules.

7- The small number of lymphocytes containing azure granules when the white blood count was high, and the much greater frequency of these cells when the count was low (in the case of acute lymphoblastic leukemia) would tend to point towards the theory that the azure granulation is a functional product of secretion.

8- The occurrence of azure granules in the same in anemia as in the normal blood of the human.

9- Mitotic figures may occur in cells containing azure granulation. There is no definite area about the chromosones which do not contain the azure granules.

10- Kurloff bodies are not azure granules.

11- The coarse granules in the myeloblasts are not unripely neutrophilic in character.

12- The coarse granules in the neutrophiles are not azure.

13- Ferrata's theory, that the coarse azure granulation is found in those cells which will become eosinophiles, whereas the fine granulation appears in those cells which will develop into neutrophiles, can not be substantiated.

14-The azure granulation is not a result of the autolysis of the cell.

15- The coarse azure granules are not due to a precipitation of stain.

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numerous medium sized, dark staining and very many fine, light staining azure granules.

Fig. VI. Myeloid cell in the blood of the myelogenous leukemia patient. Case XI. The nucleus has begun to undergo degeneration. The cytoplasm is filled with every type of azure granulation.

Fig. VII. Myeloid cell from Case XI in the same smear as the one from which Fig. VI was taken. This shows the nucleus in a more advanced stage of degeneration. The cytoplasm contains one or two small clumps of coarse azure granules. Here and there are a few isolated azure granules of the medium sized and light staining type.

Fig. VIII. Myeloid type of cell in Case VIII. This cell shows a cytoplasm containing very many azure granules of all types. This perhaps may be described as the characteristic of myeloid azure granulation.

Fig. IX. Myeloid cell of case XI. The cytoplasm contains coarse, dark and coarse light, medium dark and medium light, fine dark and fine light azure granules.

VIII. Figures and explanations.

Fig. I. Large lymphocyte of normal human adult blood. Case I. Nucleus shows the typical coarse, block arrangement of the chromatin. The cytoplasm is a pale, sky blue and contains numerous, coarse, dark, isolated, azure granules with an occasional fine, dark azure granule.

Fig. II. Large lymphocyte of same blood as Fig. I. Nucleus of the same type. Cytoplasm somewhat darker blue, with a very fine, loose network. In the cytoplasm may be seen numerous very fine light staining azure granules to illustrate Pappenheim's lymphocytic granulation.

Fig. III. Large lymphocyte from blood of Case VII. The nucleus is rather coarse. In the cytoplasm may be seen numerous large, coarse, irregular, dark staining, as well as large numbers of very fine light staining azure granules. On the lower border of the nucleus but not attached to it, is a large, dark staining, irregular body, which stains slightly darker than the nucleus.

Fig. IV. Large lymphocyte from Case VIII. The nucleus is a typical lymphocytic one. The cytoplasm is pale blue and contains numerous coarse, dark staining azure granules, some of which are arranged in strands and clumps. Others are discrete. This arrangement of granules has been described by Pappenheim as being typical of myeloid granulation.

Fig. V. Cell in mitosis in the bone marrow of the case of miliary tuberculosis. The chromosones are rather distinct. The cytoplasm stains a moderately light blue, and contains

Fig. I.

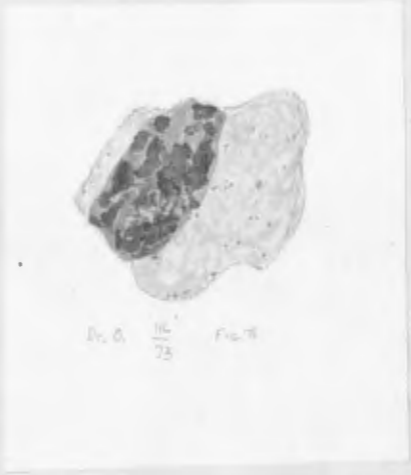

Fig. II.

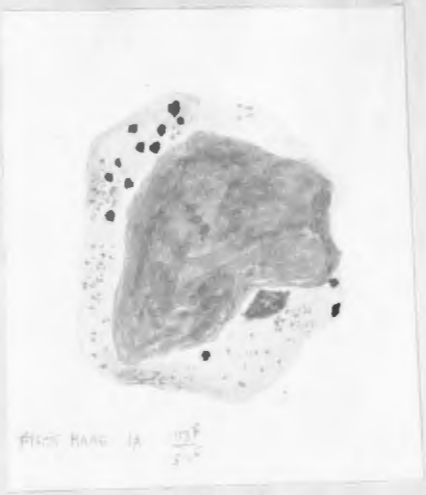

Fig. III.

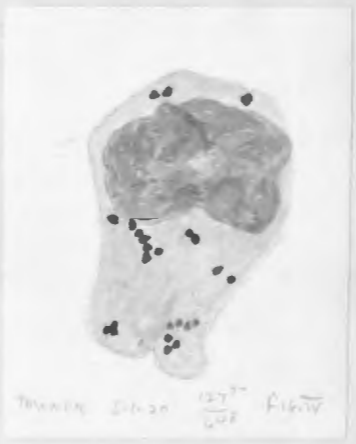

Fig. IV.

Fig. V.

Fig. VI.

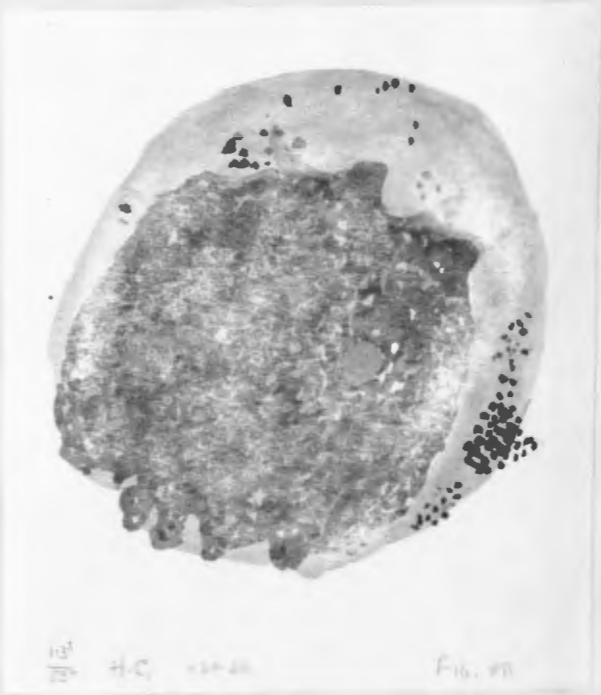

Fig. VII.

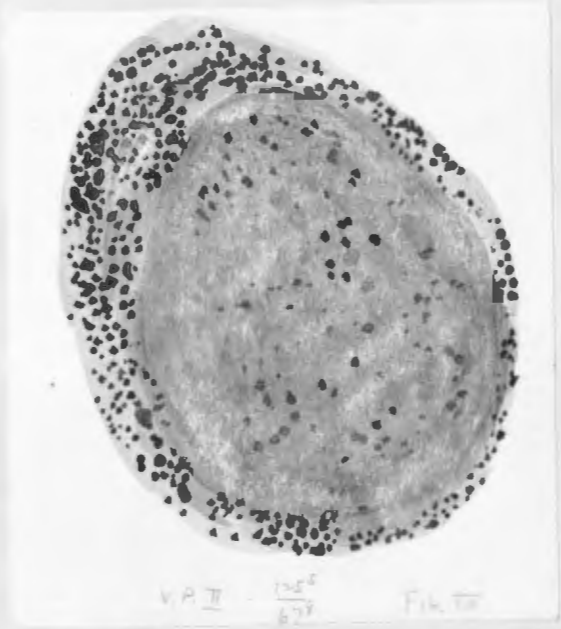

Fig. VIII.

Fig. IX.