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The Origin and Differentiation of the Thrombocytes of the Chicken (Gallus Domesticus)

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THE ORIGIN AND DIFFERENTIATION OF THE THROMBOCYTES OF THE CHICKEN (GALLUS DOMESTICUS)

by

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ABSTRACT

Blood smears and bone marrow smears obtained from sixteen White Leghorn chickens varying in age from five to seventy days after hatching were stained with Wright's stain, and histological sections of the bone marrow were stained with Maximow's eosin-azure II stain.

This study of the origin and differentiation of the definitive series of thrombocytes has yielded information that confirms the work of Lucas and Jamroz (1961) which shows that the thrombocytes are a separate series of blood cells that have an origin independent of the origin of all other blood cells.

The earliest identifiable cell of the definitive thrombocyte series is the thromboblast. The stages of differentiation from the thromboblast identified in this study were the early immature thrombocyte, late immature thrombocyte, the young or early mature thrombocyte, the mature thrombocyte and the degenerating thrombocyte.

CHAPTER I

INTRODUCTION

The cellular elements, or components, of the circulating blood of vertebrates, including birds and mammals, can be divided into two groups: the hemoglobin-containing erythrocytes and the leucocytes which do not contain hemoglobin. The leucocytes include cells that are characterized by numerous specific granules in their cytoplasm. On the basis of staining of the cytoplasmic granules, these cells are called eosinophile leucocytes, basophile leucocytes and heterophile leucocytes. Other leucocytes may contain an occasional cytoplasmic granule in their cytoplasm. On the basis of their size, character of the nucleus and staining of their cytoplasmic granules, these leucocytes are called lymphocytes and monocytes. A third non-granular, non-hemoglobin containing type of cell of the circulating blood is a small cell that is sometimes called a spindle cell but more frequently is called a thrombocyte. It is thought to have the same function of blood coagulation as the platelet of the circulating blood of mammals. The study described here has been concerned exclusively with observations on the thrombocyte of the domestic chicken.

For many years the thrombocytes of birds, reptiles, amphibians, and the fishes have interested hematologists. This

has resulted from the comparison of nucleated thrombocytes with the non-nucleated platelets of mammals and their possible function in coagulation of blood. Although the origin of platelets is known, the origin and differentiation of thrombocytes has been and still remains a puzzling one.

This introduction, with special reference to thrombocytes of birds, will be presented under three headings: (1) thrombocytes: numbers, size, and structure; (2) thrombocytes: blood coagulation and hemostasis; and (3) thrombocytes: origin and differentiation.

Thrombocytes: Number, Size, and Structure

Thrombocytes occur in the circulating blood as small nucleated cells. In some animals, for example amphibians, the thrombocytes are small, nucleated, elongate cells often called spindle cells. In other animals, including the domestic fowl, the cells are small, oval nucleated cells. Lucas and Jamroz (1961) stated that the thrombocytes of the chicken average seven microns in length and four microns in width. The thrombocytes of the chicken appear to vary in number in the circulating blood. Forkner (1930) found the thrombocytes to average 34,999 per cubic millimeter for the domestic fowl and to range in number from 5,048 to 142,048 per cubic millimeter. He noted that earlier investigators of the blood of fowl, Albertoni and Mazzoni (1891) and Klienberger and Carl (1912), had found the thrombocytes of the domestic fowl to average 34,990 per cubic millimeter and to range from 22,900 to 130,000 per cubic millimeter. Lucas and Jamroz (1961) stated that they found the thrombocytes of the chicken to range from 30,000 to

60,000 per cubic millimeter. It has been suggested that the great variation in number of thrombocytes may be the result of poor technique in withdrawing blood and in counting blood cells. Also, the condition of the chickens at the time of blood collection may influence the number of thrombocytes present.

Because of the cell's small size, information on the structure of thrombocytes of animals is usually obtained by the study of blood smears as seen under a light microscope. Such smears are made by spreading a drop of blood over the surface of a microscope slide, drying the smear in air and then staining the smear with Wright's stain or other suitable blood stain.

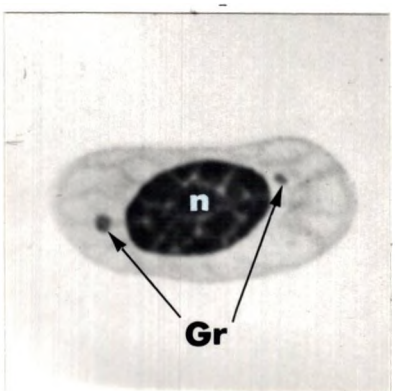


Fig. 1.--Typical mature thrombocyte of the chicken. Note centrally located oval nucleus (n), chromatin in dense masses, thin cell membrane bordered by basophilic cytoplasm, basophilic strands in cytoplasm, and two typical serotonin granules (Gr). (Photograph of Figure 73, Lucas and Jamroz, Atlas of Avian Hematology, Agriculture Monograph 25, United States Department of Agriculture, Washington, 1961.

The thrombocytes of all animals that possess them are structurally similar, except for their shape. In an extensive study of the blood cells of a number of species of reptiles and amphibians, Alder and Huber (1923) found the thrombocytes to be spindle-shaped though an occasional thrombocyte was oval in shape. They described

the thrombocytes as possessing a centrally located, dark pycnotic nucleus, a narrow rim of bluish cytoplasm and a red granule located in the cytoplasm at the poles of the cells. Yokoyama (1960) found the thrombocytes of the perch, Perca flavescens, to be spindle-shaped cells whose pycnotic nucleus was centrally located in a sparse amount of lightly stained cytoplasm. She also noted that some of the thrombocytes were rounded and fairly large. These she identified as being developing thrombocytes.

Mature thrombocytes of the chicken are oval. Rounded thrombocytes are usually present in the smears of the blood of the chicken and these are usually identified as immature developing cells.

The nucleus of mature thrombocytes and of young thrombocytes of the chicken is oval and slightly eccentrically placed in the cell. The chromatin occurs as closely packed large masses. The nuclei of the thrombocytes in blood smears, therefore, are pycnotic and dense in appearance. The cytoplasm of most mature thrombocytes appears clear and unstained and is enclosed by a narrow cell membrane. The cytoplasm of immature thrombocytes shows clear areas separated by blue-stained strands that extend from the nuclear membrane to the cell wall externally. Also, in these thrombocytes a narrow basophilic layer of cytoplasm is located near the cell membrane at the periphery of the cells. The basophilic cytoplasm is more abundant in the immature cells. Serotonin granules are present in the cytoplasm. The granules may be one to four or more in number. The serotonin granules may in some cases be clumped together in vacuoles. Vacuoles may not be present.

The thrombocytes as seen with the electron microscope present quite a different picture and Figure 2 has been prepared to show diagramatically the structures described in the various publications on that subject. The fine structure of the thrombocytes and its nuclear and cytoplasmic contents will be noted below.

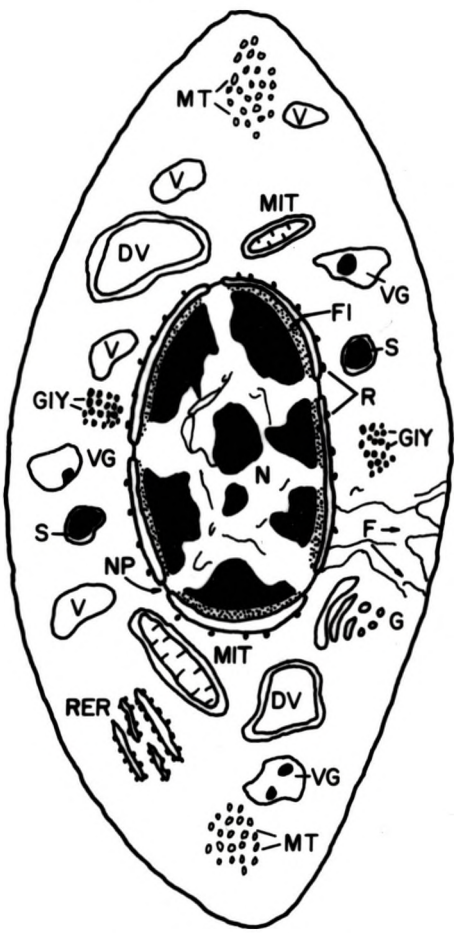


FIG. 2

Schematic representation of an Ultrathin section of a Chicken Thrombocyte. MT = Microtubules, VG = Vesicle with Granules, RER = Rough Endoplasmic Reticulum, DV = Double Membrane Bound Vesicle, MIT = Mitochondria, V = Vesicle, G = Golgi, GIY = Glycogen, S = Seratonin Granule, F = Fibrillar Filaments, N = Nucleus, R = Ribosomes on outer Face of Nuclear Envelope, NP = Nuclear Pore, FI = Fibrous Lamina.

Schumacher (1965), Edmonds (1968), and Dhingra (1969) observed that the nucleus of the chicken thrombocyte is surrounded by a prominent nuclear envelope. The inner face of the envelope was found to be smooth and regular while the outer face appeared to be irregular and frequently studded with ribosomes on its cytoplasmic side. A fibrous lamina is located internal to and bordering on the nuclear envelope. Nuclear pores traverse the nuclear envelope. The pores are large and bridged by a single-layered diaphragm. The chromatin of the nucleus is arranged in large electron dense masses that rest on the fibrous lamina and the inner face of the nuclear envelope. Sometimes a large, dense mass of chromatin was found in the center of the nucleus. Nucleoli are usually obscured by the dense chromatin masses.

Enbergs and Kriesten (1968), Sweeny and Carlson (1968), Simpson (1968), and Maxwell and Trejo (1970) found the cytoplasm of the chicken thrombocyte to contain the organelles usually found in the cytoplasm of other types of cells. Mitochondria are small, usually rod shaped, are few in number and appear to have no definite distribution within the cell. The mitochondria are double-walled and in-folding cristae of the inner wall are present. Golgi bodies of varying size are present as are also centrioles. An endoplasmic reticulum is present and it has a general distribution in the cytoplasm. Ribosomes are present, and of general distribution although some are associated with the nuclear envelope and the endoplasmic reticulum. Microtubules are present and appear singly or in groups of ten to twenty or more. Many microtubules are distributed as a marginal band and are particularly numerous at the poles of the cell.

Glycogen granules are numerous and have a general distribution within the cytoplasm. Fibrillar filaments are present, branch and anastomose and extend from the nuclear membrane outward to the cell membrane. Harris and Brown (1971) in working with chicken erythrocytes observed fibrillar structures that extended from the outer nuclear membrane to the plasma membrane. They suggested that the fibrills of the erythrocytes served to hold the nucleus in its central position. The fibrillar filaments may serve the same function in the thrombocytes.

Sweeny and Carlson (1968), Maxwell and Trejo (1970), and Edmonds (1968) described three types of cytoplasmic vesicles. One type was a large double membrane-bound vesicle that apparently contained a large amount of lysosomal material. It was pointed out that these vesicles are found in well formed blood clots and undoubtly play an important role in clot retraction. Other vesicles were small and bounded by a single membrane. They were usually clear but some contained a small number of granules. The third type of vesicle was bounded by a single membrane and contained one or two electron dense bodies. Some of the vesicles were completely filled with a very dense granule. These bodies are called serotonin granules.

The cell membranes which frequently cannot be seen with the light microscope are distinct when observed under the electron microscope. They possess the trilaminar structure characteristic of most membranes.

From the descriptions noted above it must be concluded that the thrombocytes of the chicken are complete cells and possess a

cell membrane, a nucleus, and cytoplasm that contains all typical cell organelles.

Thrombocytes: Blood Coagulation and Hemostasis

It has generally been accepted that the nucleated thrombocytes of fish, amphibian, reptile, and bird blood correspond to the non-nucleated blood platelets of mammals and that they contribute in the same way to coagulation of the blood and hemostasis in the blood vessels. Publications that deal directly with these functions of bird thrombocytes are not numerous.

It is well established that coagulation occurs rapidly in the blood of the chicken that has escaped from blood vessels and that coagulation leads to the formation of a blood clot of fibrin and entrapped blood cells. It is generally stated that blood coagulation is completed in three to six minutes. Edmonds (1968) viewed, under a binocular microscope, the flow of blood from crushed or cut allantoic and vitelline vessels. He found that for small vessels hemorrhage ceased abruptly in thirty to sixty seconds. For larger vessels hemorrhage began to diminish in thirty to forty-five seconds and continued at a decreasing rate for two and a half to three minutes. After a series of observations on hemorrhage and blood coagulation, he concluded that the extent of hemorrhage and the clotting time of chicken blood varied with the size of the hemorrhaging vessel. Based on his observations, it can be concluded that severing the largest vessels can lead to complete loss of blood because of a failure of formation of an effective blood clot.

In 1954 Sturkie pointed out that clotting of bird blood occurs in the absence of blood platelets like those associated with coagulation of the blood of mammals. He stated that at that time some workers were of the opinion that the thrombocytes of the blood were concerned with the coagulation of the blood of the chicken. Conclusive evidence that thrombocytes performed this function was not discovered until later.

In 1968 Venkatayan and Nambiar wrote that at that time the role of the thrombocyte in blood coagulation was still unsettled. They undertook a series of studies to determine the influence of thrombocytes on blood clotting and on clot retraction. They separated the thrombocytes from other blood cells by differential centrifugation and prepared suspensions of thrombocytes for experiments on blood coagulation. They found that the addition of thrombocytes from the thrombocyte suspension decreased the clotting time of anticoagulant treated blood. They also found an increase in retraction of blood clots of blood to which thrombocytes from the thrombocyte suspension were added.

This section of the review of the literature on the thrombocytes of birds will be directed to the problem of the ways they resemble in function the platelets of mammals. The very numerous publications on the platelets of man and mammals have been reviewed and evaluated. Among these may be noted: the symposium on Blood Platelets edited by Johnson, Monto, Rebuck and Horn (1961); the colloquium on the Biochemistry of Blood Platelets by Kowalski and Niewiarowski (1967); the review on The Physiology of Blood Platelets

by Marcus and Zucker (1965); and the monograph on The Platelet edited by Brinkhous and Shermer (1971).

Associated with blood coagulation is the release of serotonin, or 5-hydroxytryptamine , a substance that is involved in constriction of injured vessels and in producing adhesiveness and aggregation of the platelets. Inouye, Kataoka, Sorimachi, and Hori (1969) obtained thrombocytes of chickens by differential centrifugation. A high amount of 5-hydroxytryptamine was found in the thrombocyte fraction of the blood. The blood of birds injected with reserpine before sacrifice contained very small amounts of 5-hydroxytryptamine. They noted that 5-hydroxytryptamine was liberated by granules isolated from platelets. Efforts to isolate granules from thrombocytes in sufficient numbers to permit study were unsuccessful.

Kuruma, Okada, Katoaka, and Sorimachi (1970) continued the studies of Inouye, et al. and confirmed their work. In addition, they included electron microscopic studies of the blood platelets of rabbits and the thrombocytes of the chicken. Electron dense granules were found in both the platelets and the thrombocytes. The granules were demonstrated to be storage sites of 5-hydorxy-tryptamine or its precursor. Study of the structure of reserpin-ized platelets and thrombocytes showed that the electron dense granules had been lost and replaced by irregular vacuoles or by irregular areas of cytoplasm. The electron dense bodies were described as stored amines and the liberation of 5-hydroxytrypt-amine to be accomplished by a process of degranulation. In an earlier study in which electron microscopic observations were also

made, Edmonds (1968) also concluded that the amines were stored in the granules of the thrombocytes and that liberation of serotonin was accomplished by a process of degranulation.

Hemostasis or the arrest of blood flow in mammals has been found to be associated with the formation of hemostatic plugs, composed of platelets, at the point where blood vessels are injured or cut. These changes are accompanied by or preceeded by constriction of the injured vessel.

Stalsberg and Prydz (1963) studied the results of injury to vessels of the allantoic or vitelline circulation observed under a binocular microscope. The vessels were either crushed with a forceps or cut and observed until bleeding ceased when the injured segment of the vessel was removed and fixed for histological study. Histological sections showed hemostatic plugs to be composed entirely of thrombocytes. The hemostatic plugs were found to be composed of thrombocytes adhering to the cut edge of the vessel but none were found to adhere to the endothelium. By additional thrombocytes adhering to the thrombocyte mass, the hemostatic plug bridged across the injured area of the vessel, thus preventing additional loss of blood from the vessel.

Edmonds (1968) also studied the formation of hemostatic plugs in the vessels of embryo chicks. His work confirmed the observations of Stalsberg and Prydz (1963). He extended his observations to include electron microscope studies of the hemostatic plugs. The thrombocytes were not ruptured or destroyed but retained intact cell boundaries. However, he did find the granules of the thrombocytes had been replaced by vacuoles and irregular areas of

the cell that had formerly been occupied by serotonin granules. He described liberation of 5-hydroxytryptamine as having occurred by a process of degranulation.

The above observations show that the nucleated thrombocytes of birds do indeed have the same functions as the non-nucleated platelets of mammals. It is interesting to note that not only do those elements of the blood of mammals and birds resemble each other but that other factors may influence blood coagulation. Griminger and his co-workers (1953) in studying the hemorrhagic syndrome of chickens found that it could be overcome by feeding Vitamine K (methadione) to the chickens; the clotting time of the blood returned to normal, and all hemorrhagic symptoms disappeared. Vitamin K is therefore essential for normal blood coagulation for both birds and mammals.

Thrombocytes: Origin and Differentiation

The origin and differentiation of the nucleated thrombocytes of fish, birds, reptiles, and amphibians have for many years been the subject of investigations and of speculation. In 1925 Hartman reviewed and summarized the observations and opinions of forty-four publications on the origin of thrombocytes of lower vertebrates. The earliest paper on this subject reviewed by Hartman was published in 1843 while the latest paper on the subject was published in 1924. Hartman summarized the views on the origin of thrombocytes given in the early publications as follows:

1. That thrombocytes are formed from white blood cells, primarily lymphocytes.

2. That thrombocytes arise from the endothelium of blood vessels.

3. That erythrocytes by differentiation become thrombocytes of the blood.

4. That thrombocytes have an independent origin without genetic relation to other cell forms.

5. That thrombocytes are defective cells.

6. That thrombocytes correspond to and resemble the megakaryocytes of mammals.

After having reviewed and evaluated earlier studies, Hartman (1925) undertook studies of the blood cells of birds (chicken and pigeon), reptiles (turtle), and amphibians (toad and frog). He examined the fresh blood of the animals and determined the proportion of thrombocytes to leucocytes and erythrocytes of the circulating blood. The average per thousand cells was 979 erythrocytes, 7 leucocytes, and 13 thrombocytes. He also studied stained blood smears and imprint and histological preparations of bone marrow, spleen, and liver. He regarded the thrombocytes to be an independent cell series and concluded: (1) that the thrombocytes of lower vertebrates and the platelets of mammals have the same function and (2) that the thrombocytes of lower vertebrates and the megakaryocytes of mammals are genetically identical and have their origin extravascularly in the bone marrow.

Sugiyama (1926) carried out studies on blood formation in chick embryos. He studied: (1) the origin of blood vessels and blood cells in the blood islands of the blastoderm and yolk sac in hanging-drop preparations, (2) the blood cells of later embryos in mixtures of fresh blood mixed with supravital stains, and

(3) smears of the blood of the embryos stained with Wright's stain.

The blood island of early embryos, according to Sugiyama, undergoes a type of liquification as the result of which the outer cells become the endothelium while the inner group become the megaloblast precursors of blood cells. The megaloblasts increase in number by division and by the addition of megaloblasts from the endothelial cells. Erythroblasts (stage I) appear in the blood of embryos of eighteen to twenty somites. These continue to be numerous through the fifth day when a second series of erythroblasts (stage II) become numerous. Young nucleated erythrocytes appear in the blood of seven day embryos.

Sugiyama found that there was present in the blood of eighteen to twenty-one somite embryos a new type of cell formed from the megaloblasts. This type of cell was smaller than erythroblasts. These he called transitional cells because they were found to be transformed into thromboblasts. Mature thrombocytes, usually found in groups, were found in three and four day incubated embryos. Mature thrombocytes were found in small numbers in the blood of all older embryos.

The time of appearance in the circulating blood of embryos was noted for the various types of leucocytes. Eosinophiles were found in the blood of embryos incubated for three or four days. Basophiles appeared on the fourteenth day while lymphocytes and monocytes were present on the seventh day of incubation. Because of the time of appearance, Sugiyama concluded that thrombocytes are formed as a separate and independent series of blood cells and

are not formed from other blood cell types such as the erythrocytes or lymphocytes.

The most extensive and authorative study of all types and varieties of blood cells of the chicken appeared in 1961 as an Atlas of Avian Hematology published by Lucas and Jamroz. They used standard techniques and stains of the blood of chick embryos and adult chickens and also studied imprint preparations of blood forming organs. They found thrombocytes to be an independent cell series and that their origin and stages of differentiation to mature thrombocytes could easily be recognized. They identified an embryonic series of thrombocytes and a definitive series of thrombocytes of adult chickens and chicks following hatching. The thrombocytes of the embryonic series do not attain the degree of maturity found for the thrombocytes of the definitive series but do show the adhesiveness and tendency toward aggregation or clumping shown by mature thrombocytes. The stages of differentiation of the embryonic and definitive series, each with its own morphological characteristics, recognized by Lucas and Jamroz are shown below.

TABLE I

ORIGIN AND DIFFERENTIATION OF THROMBOCYTES
(Lucas and Jamroz)

Embryonic Series	Definitive Series
Thromboblast	Thromboblast
Large embryo thrombocyte	Early immature thrombocyte
Medium embryo thrombocyte	Mid-immature thrombocyte
Small embryo thrombocyte	Late immature thrombocyte
	Mature thrombocyte

The work of Lucas and Jamroz (1961) must be regarded as the most complete study of the blood cells and hematogenesis of the chicken that has appeared in the literature. However, it would be expected that their description of the thrombocytes about which so many conflicting views are found in the literature would be confirmed by later investigations. Confirmatory studies have not appeared in the literature. It has been the purpose of the present study to duplicate and, if possible, to confirm the Lucas and Jamroz studies of the thrombocytes of the chicken.

As the originally planned investigation of the thrombocyte of the chicken had been almost completed, a publication entitled "Studies on Chick Embryo Thrombocytes" came to the attention of this investigator. It was published in 1963 by Stalsberg and Prydz of the University of Oslo.

In preliminary studies Stalsberg and Prydz noted that thrombocytes contain abundant PAS-positive material in contrast to all other cells of embryonic blood. They used the PAS staining method to identify thrombocytes and their percursors in blood and bone marrow. They were able to trace the development of thrombocytes of the embryos from the precursor prothromboblasts to the mature thrombocytes. They identified five stages of thromogenesis, which they compared to the developmental series given by Lucas and Jamroz, as follows: stage I, prothromboblast; stage II, thromboblasts; stage III, large embryo thrombocytes; stage IV, medium and small embryo thrombocytes; stage V, small embryo thrombocytes and mature embryo thrombocytes.

It can be concluded that the studies of thrombocytes by Stalsberg and Prydz support and confirm the studies by Lucas and Jamroz. However, the materials and methods of the two studies are quite different. It was decided that the present studies should be completed because they duplicate the materials and methods of the Lucas and Jamroz studies and therefore would provide direct confirmation of their studies on the thrombocytes of the chicken.

CHAPTER II

MATERIALS AND METHODS

The materials examined in this study were obtained from sixteen White Leghorn chickens ranging in age from five days to seventy days after hatching.

The chickens were anesthetized with anhydrous ether for one minute, and blood smears were obtained in the following manner: a cardiac puncture was made by inserting a needle through the chest wall of each chicken, and one milliliter of blood was withdrawn into a heparinized syringe. One drop of the heparinized blood was then placed on each of twenty-five clean glass slides and spread out to make a thin film. The smears were then stained with Wright's stain.

Bone marrow was removed from the tibia of each chicken while it was still under anesthesia. The marrow was processed in two ways. First, bone marrow from the tibia was placed on glass slides, spread out into a thin film, and stained with Wright's stain. Second, a block of bone marrow was removed from the tibia of each chicken and immediately immersed in Zenker's fixative. The pieces remained in the solution for six to eight hours and then were removed and washed for twenty-four hours in running tap water. After that, the pieces of marrow were dehydrated in

increasing grades of alcohol, cleared in chloroform, and embedded in paraffin or nitrocellulose. They were then sectioned at five microns, mounted on slides and stained with Maximow's hematoxylin eosin-azure II stain in the manner described by Buchsbaum and Loosli (1936). A small number of blood smears, bone marrow smears, and histological sections of bone marrow were stained with PAS-methyl green by the technique described by Stalsberg and Prydz (1963).

CHAPTER III

OBSERVATIONS

The definitive series of thrombocytes was determined largely from smears of the bone marrow since they were found to be the most suitable type of preparation for the identification of the various stages of development. The blood smears of the chickens were inadequate, possessing only mature thrombocytes and old, degenerating thrombocytes.

In this study six stages of the origin and differentiation of thrombocytes were identified and are listed below in order from their origin to their degeneration.

Stage 1. Thromboblast
Stage 2. Early immature thrombocyte
Stage 3. Late immature thrombocyte
Stage 4. Young mature thrombocyte
Stage 5. Mature thrombocyte
Stage 6. Degenerating mature thrombocyte

Each stage of thrombocyte origin and differentiation is described below. Photographs of cells in the various stages are presented on Plates I and II.

Stage I--Thromboblast (Figures 3, 4, 5, Plate I)

The thromboblast is the earliest identifiable cell of the thrombocyte series. Because the later stages of formation of

thrombocytes develop from the thromboblast, the thromboblast is the youngest cell of the series. The thromboblast arises from a cell of the bone marrow called the hemocytoblast which is the progenitor of all series of blood cells. Though an occasional hemocytoblast was found in the smears of the bone marrow, satisfactory photographs of the hemocytoblasts could not be obtained.

The appearance of cells in smears of blood and bone marrow is determined by the character of the smears. If the smears are thin and the cells of the smear are separated from each other, the cells become expanded, flattened and thin and show clearly their internal structure. In smears that are closely packed, cells remain rounded, do not become flattened and thin and will show little of their internal structure.

Figures 3, 4, and 5 of Plate I are all thromboblasts. All show that thromboblasts have rounded, sphere-shaped nuclei. The chromatin of the nuclei is not coarse but is in the form of fine punctate granules. A nucleolus is present in the nucleus of Figure 5 but, though undoubtedly present, none are visible in the nucleus of the cells of Figures 3 and 4. The cytoplasm of the thromboblasts forms a narrow darkly basophilic band around the nucleus. An occasional vacuole is present in the cytoplasm. The cells of Figures 3 and 4 appear rounded or spherical in shape. The cell of Figure 5 is not rounded but possesses cytoplasmic blebs or extensions. The presence of bleb-like processes of cytoplasm has been recognized as one of the outstanding characteristics of thromboblasts and are present in the thromboblast of Figure 5.

Stage II--Early Immature Thrombocyte (Figures 6 and 7, Plate I)

Early immature thrombocytes are the earliest cells of the series that show characteristics of thrombocytes. They are formed by transformation of thromboblasts and, therefore, show many of the structural characteristics of the thromboblasts. The nucleus is circular in shape and occupies the greater part of the cell. The chromatin is granular in character and stains more lightly than the thromboblasts. Nucleoli are present. The cytoplasm is still sparse and forms a narrow rim about the nucleus. Vacuoles in the cytoplasm have increased in number. The cytoplasm has lost some of its basophilia, as shown in Figure 7. The loss of basophilia by the cytoplasm distinguishes this stage of differentiation of thrombocytes.

Stage III--Late Immature Thrombocyte (Figure 8, Plate II)

This is a transitional stage between thromboblast-like cells and the mature thrombocyte. The shape of the nucleus has changed from a round shape to an oval shape and usually occupies an eccentric position in the cell. The chromatin of the nucleus is granular but more densely packed than for earlier stages. Nucleoli may occasionally be found in the nucleus. The cytoplasm of the cell has lost most of its basophilia and has a light clear appearance. Vacuole-like areas are present in the cytoplasm. The cell membrane is thin and distinct.

Stage IV--Young Mature Thrombocyte (Figure 9, Plate II)

The nucleus of the cell shown in Figure 9 is round in shape but other cells of this stage of development may be oval in shape. The chromatin is condensed into dense darkly stained masses. The shape of the cell varies from round to oval. The cytoplasm has lost its basophilia and is stained a light blue color. Light almost clear areas appear in the cytoplasm and strands of more darkly stained material radiate from the nucleus to the cell membrane. Serotonin granules appear in the cytoplasm. The cell membrane is thin and lined on its internal surface by a thin band of lightly stained cytoplasm.

Stage V--Mature Thrombocytes (Figures 10 and 11, Plate II)

The cell shown in Figure 10 is developmentally younger than the cell shown in Figure 11. The thrombocyte of Figure 11 has the shape and appearance usually described as the typical thrombocyte of the circulating blood of the chicken.

The nuclei of mature thrombocytes are oval in shape and located in the center of the cell. The chromatin of the thrombocyte in Figure 10 is composed of dense masses while the chromatin of the thrombocyte of Figure 11 is densely compressed to the degree that it shows no internal structure.

The cytoplasm of the two cells is acidophilic rather than basophilic as in earlier stages. The cytoplasm of the younger thrombocyte possess densely stained strands that radiate from the nucleus to the periphery. The strands are not present in the most

mature cell. The cell membrane for both cells is thin and lined by a thin layer of cytoplasm on its inner surface. Serotonin granules are present in the cytoplasm of both cells.

Stage VI--Degenerating Thrombocyte (Figure 12, Plate II)

Figure 12 shows three thrombocytes arranged in a row. This illustrates the tendency mature thrombocytes have of clumping and forming aggregations of cells. This tendency is due to the property of adhesiveness that thrombocytes have for each other.

Of the three thrombocytes shown in Figure 12, the lower thrombocyte is an early mature thrombocyte in the same stage of differentiation as the thrombocyte shown in Figure 10. The nucleus is oval in shape and its chromatin is in block-like masses. The cytoplasm is fairly abundant and clear in appearance and contains a prominent serotonin granule. A thin cell membrane is present.

The second, or middle thrombocyte, is older than the thrombocyte described above. The nucleus is irregular in shape and contains large dark masses of chromatin. The cytoplasm of the cell is clear and contains a serotonin granule. The cell membrane is very thin and appears to be lacking on some areas.

The third cell of the figure is an early degenerating thrombocyte. The nucleus is small and round in shape and the chromatin forms a large darkly stained mass. It shows the early pycnosis of the nucleus, characteristic of a degenerating thrombocyte. The cytoplasm is very clear and contains a serotonin granule. The cell membrane is thin, irregular and lacking on some areas of the cell. The cytoplasm and cell membrane are degenerating. The

process of cytoplasm and cell membrane degeneration continue for thrombocytes and in advanced stages only a naked pycnotic nucleus of the thrombocyte remains. It is not certain how the pycnotic nuclei are removed from the circulating blood.

It must be noted that the thrombocytes of Figures 10, 11, and 12 are from smears of the circulating blood. The thrombocytes of all other figures are from smears of the bone marrow and illustrate stages of origin and differentiation. The thrombocyte of Figure 8 represents the stage at which thrombocytes are discharged from the bone marrow into the circulating blood.

CHAPTER IV

DISCUSSION

It was noted in the introduction that the thrombocytes of the chicken function in the same manner as the platelets of mammals in the coagulation of blood and in the maintainence of the blood vascular system. Although there has been an agreement in the literature on the function of thrombocytes, there has been no agreement, as pointed out by Hartman (1925) and Sugiyama (1926), on their manner of origin and differentiation. In an extensive study of all types of cells of the blood of the chicken, Lucas and Jamroz (1961) concluded that thrombocytes of the chicken are an independent blood cell series that have no relation to other cells of the blood, and that the thrombocytes arise in the bone marrow and pass through a series of changes to be later discharged into the circulating blood as mature thrombocytes.

The observations of Lucas and Jamroz (1961) show that the thrombocytes of the chicken develop as an independent blood cell series and thereby resemble the development of other blood cells of the chicken and the blood cells of mammals. It appears that their conclusions on the cytogenesis of thrombocytes are very logical ones. Since research confirming their studies had not appeared in the literature, the purpose of the present study was

to duplicate and to confirm the observations of Lucas and Jamroz (1961). Our studies of the thrombocytes of the chicken as seen in blood smears and smears of the bone marrow confirm the work of Lucas and Jamroz. However, the present study did not include observations on thrombocytogenesis in the chick embryo and was limited to observations on thrombocytogenesis of the definitive series of thrombocytes.

A small number of studies of thrombocytogenesis have contributed to the conclusion that thrombocytes arise and develop as a separate blood cell series that is independent of and has no relation to the origin and development of other series of blood cells of the chicken. Four studies supplement each other in establishing the thrombocytes as a separate blood cell series. These studies are those of Sugiyama (1926), Lucas and Jamroz (1961), Stalsberg and Prydz (1963) and the present study. The studies of Sugiyama, Lucas and Jamroz and Stalsberg and Prydz made observations on the origin and development of the embryo series of thrombocytes. Lucas and Jamroz also made observations on the definitive thrombocyte series, and the present study was limited to the definitive series of thrombocytes. The manner in which the four studies duplicate and supplement each other is shown in Table II on page 28. The table also shows the stages of differentiation of thrombocytes recognized in the different studies.

It must be noted that the blood islands of the chick embryo and the bone marrow include cells that have the potentiality of giving rise to all types of blood cells. These are usually called hemocytoblasts or megaloblasts. These may undergo change in the

TABLE II

SUMMARY OF CONTRIBUTIONS TO ORIGIN AND DIFFERENTIATION OF THROMBOCYTES OF THE CHICKEN

Sugiyama (1926)	Lucas and Jamroz (1961)	Stalsberg and Prydz (1963)	Present Study
Embryo Series of Thrombocytogenesis			
Megaloblast Thromboblast Embryo Thrombocyte	Prothromboblast Thromboblast Large Embryo Thrombocyte Medium Embryo Thrombocyte Small Embryo Thrombocyte	Hematocytoblast Thromboblast Large Embryo Thrombocyte Medium Embryo Thrombocyte Mature Embryo Thrombocyte	
Definitive Series of Thrombocytogenesis			
	Thromboblast Early Immature Thrombocyte Mid-immature Thrombocyte Late Immature Thrombocyte Mature Thrombocyte		Thromboblast Early Immature Thrombocyte Late Immature Thrombocyte Young Mature Thrombocyte Mature Thrombocyte Degenerating Thrombocyte

direction of a specific blood cell type. The earliest recognizable cell type related to thrombocytes is named thromboblast. These, by a series of morphological changes, lead to the formation of mature thrombocytes. The various stages of differentiation of thrombocytes are listed in Table II on page 28 and are described in Chapter III, Observations, of this thesis.

CHAPTER V

SUMMARY

The present study of blood smears, bone marrow smears and sections of the bone marrow of sixteen White Leghorn chickens varying in age from five days to seventy days after hatching has yielded information on the definitive series of thrombocytes. The thrombocytes were found to be a separate series of blood cells that have an origin independent of the origin of all other blood cells of the chicken. The earliest identifiable cell of the definitive thrombocyte series was the thromboblast. The stages of differentiation from the thromboblast identified in this study were the early immature thrombocyte, late immature thrombocyte, the young or early mature thrombocyte, the mature thrombocyte and the degenerating thrombocyte. The present study fully confirms the studies of Lucas and Jamroz (1961) made on the definitive series of thrombocytes.

APPENDIX

LEGEND TO FIGURES

n	Nucleus
v	Vacuole
Bl	Bleb-like Cytoplasm
Nu	Nucleolus
cm	Cell Membrane
Gr	Granule

PLATE I

Fig. 3.--Thromboblast. This cell shows a spherical-shaped nucleus (n) with fine punctate chromatin granules. The cytoplasm forms a dark basophilic band around the nucleus. A vacuole (v) is present in the cytoplasm. 600X.

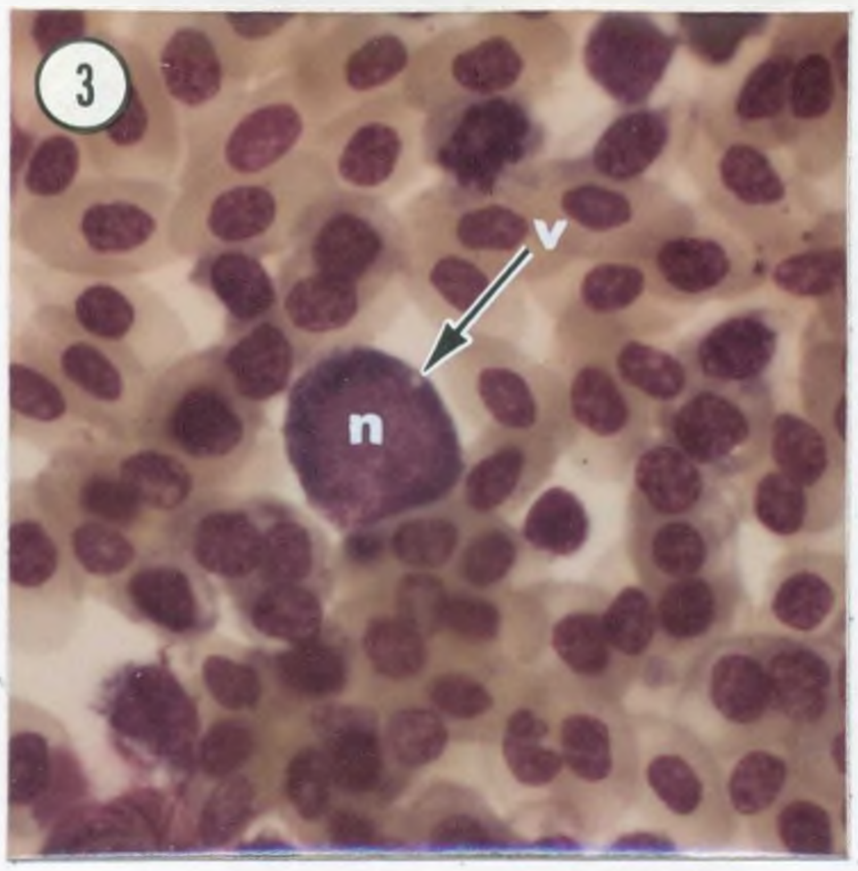
Fig. 4.--Thromboblast. The nucleus (n) shows a rounded shape and fine punctate chromatin granules. The cytoplasm forms a narrow basophilic rim around the nucleus. Note that erythrocytes crowd the cell so that it is not expanded fully and the internal features are somewhat obscured. 600X.

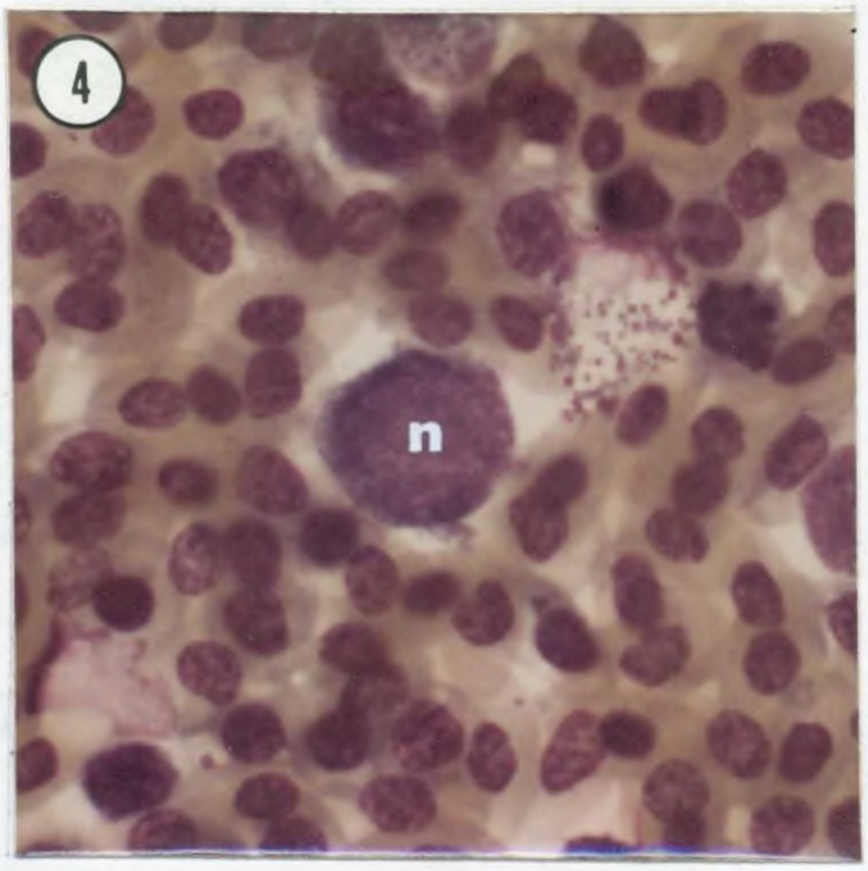
Fig. 5.--Thromboblast. This cell was chosen to illustrate the dark, basophilic, bleb-like processes of cytoplasm (Bl) that is one of the outstanding characteristics of thromboblasts. Also note the nucleolus (Nu) in the center of the nucleus. 600X.

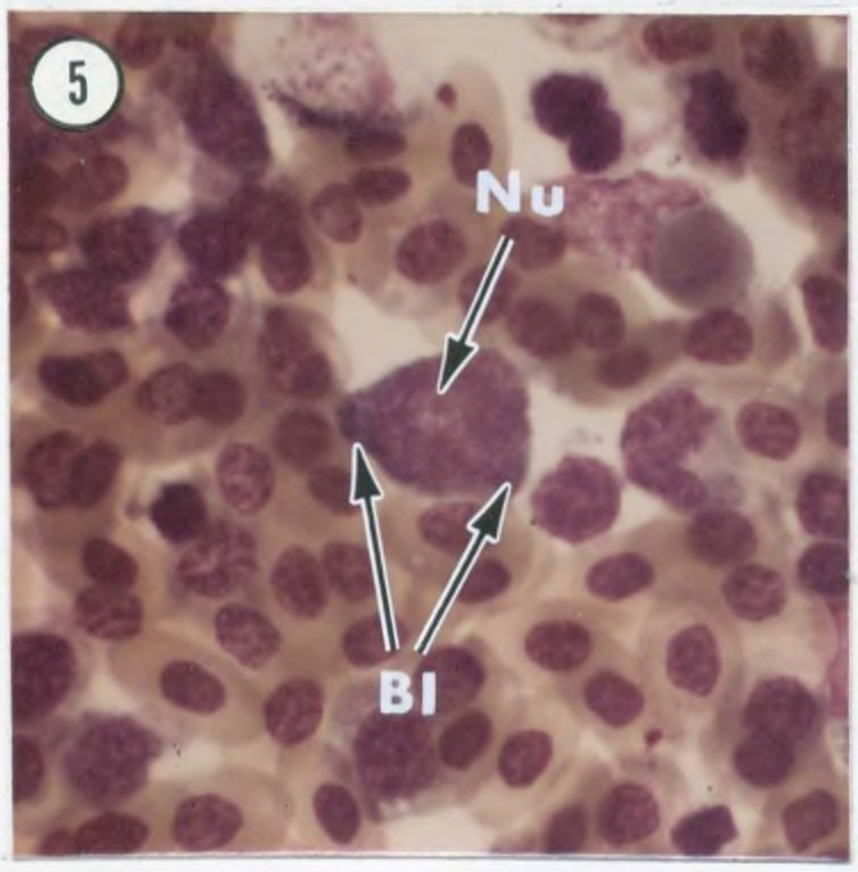
Fig. 6.--Early Immature Thrombocyte. The nucleus is circular in shape and occupies the greater part of the cell. The chromatin is granular and more lightly stained than that of the thromboblasts. Two nucleoli (Nu) are present. The cytoplasm is sparse and less basophilic. More than one vacuole (v) exists in the cytoplasm. 600X.

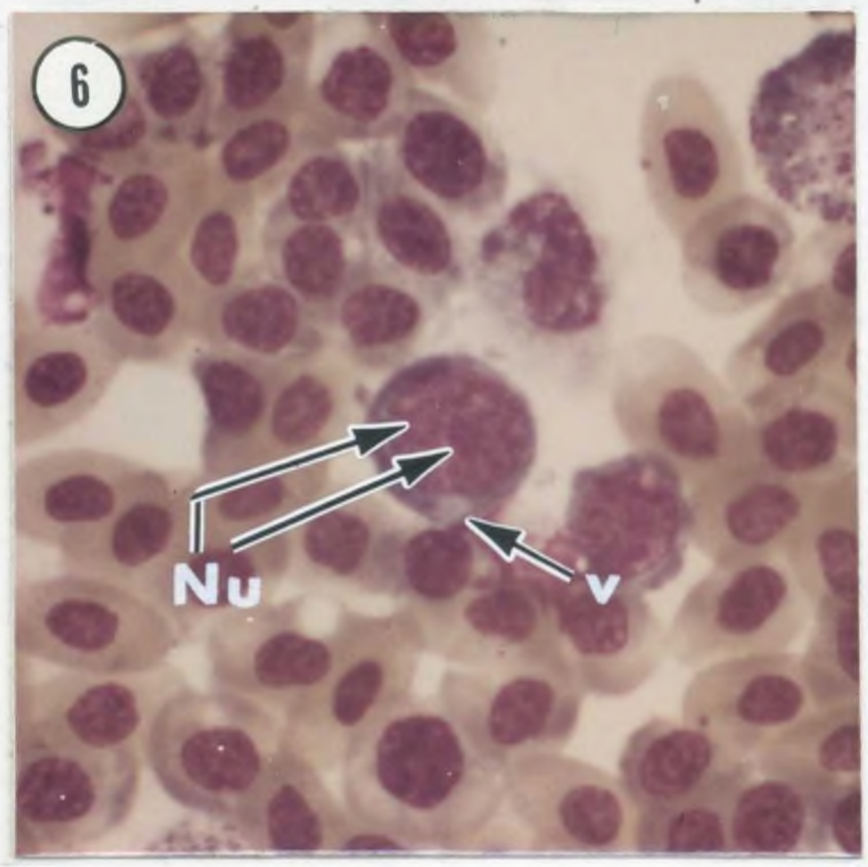
Fig. 7.--Early Immature Thrombocyte. This cell shows many of the characteristics of the cell in Figure 6, circular nucleus two nucleoli (Nu) and sparse cytoplasm which is even less basophili The vacuoles (v) have increased in number and are easily distinguishable. 600X.

PLATE 1









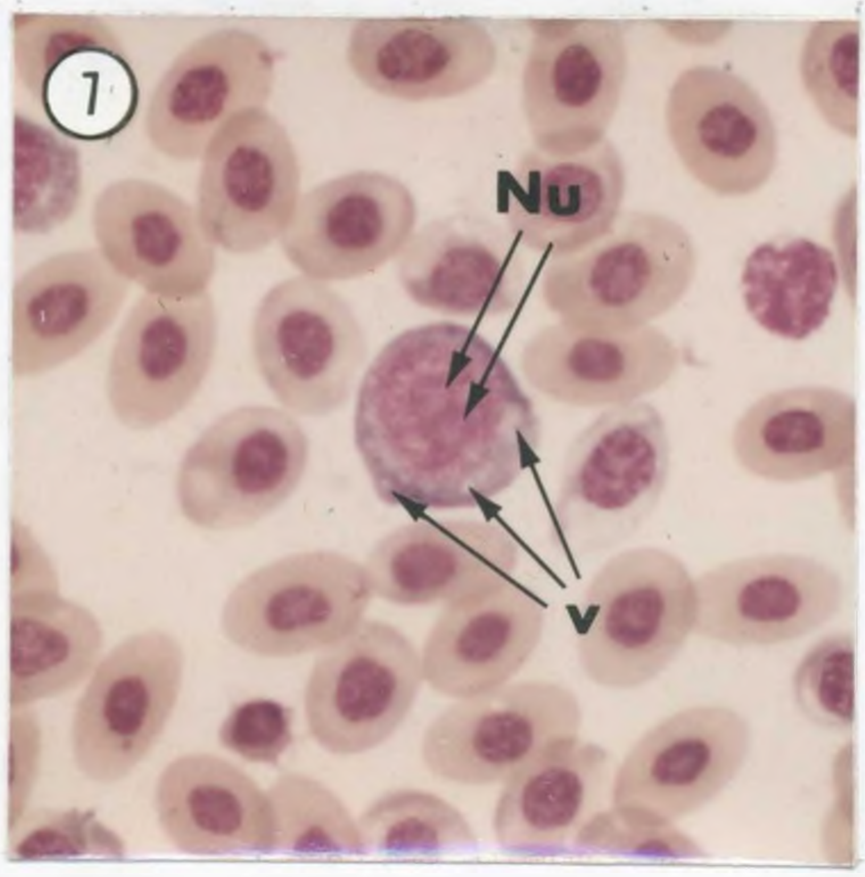


Fig. 8.--Late Immature Thrombocyte. The shape of the nucleus (n) has changed from round to oval and is in an eccentric position. The chromatin is granular and more densely packed than earlier stages. The cytoplasm has lost most of its basophilia and clear areas are present. 600X.

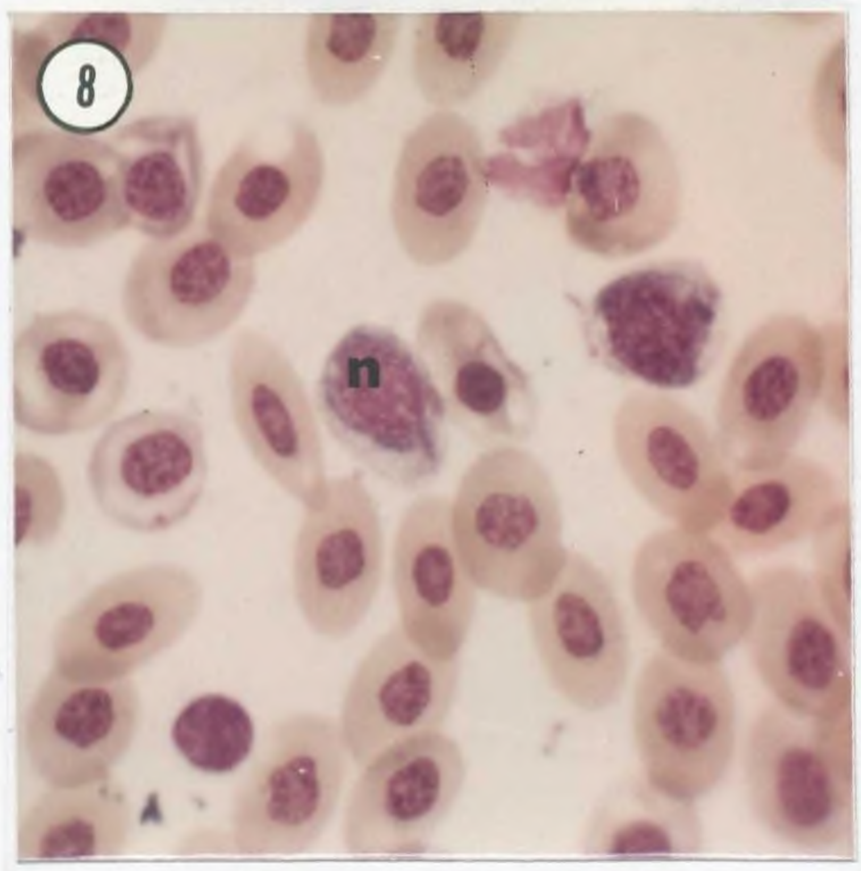
Fig. 9.--Young Matura Thrombocyte. The nucleus is round but other cells of this stage may show an oval nucleus. The chromatin is condensed into darkly stained dense masses. The cytoplasm contains vacuoles separated by darkly stained material that radiates from the nucleus to the plasma membrane. Note the fine serotonin granule (Gr) in the cytoplasm. 600X.

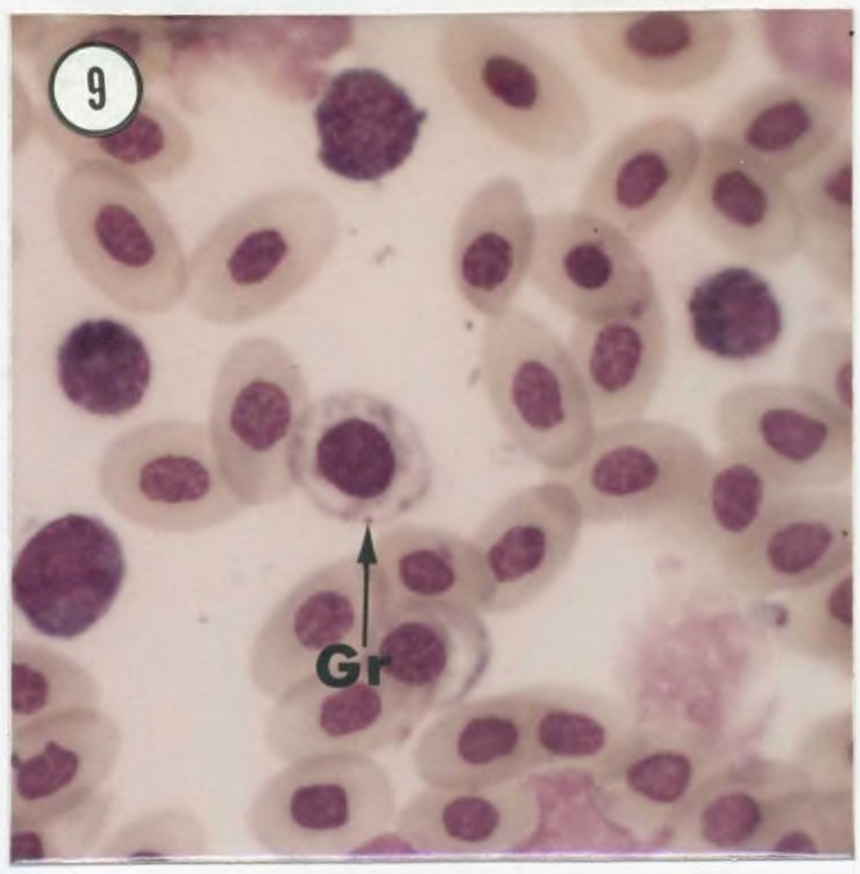
Fig. 10.--Mature Thrombocyte. A very young mature thrombocyte with an oval centrally located nucleus (n). The chromatin is composed of the characteristic darkly stained block-like masses. The cytoplasm is now rather acidophilic and contains fine serotonin granules (Gr). The cell membrane (cm) is thin. 600X.

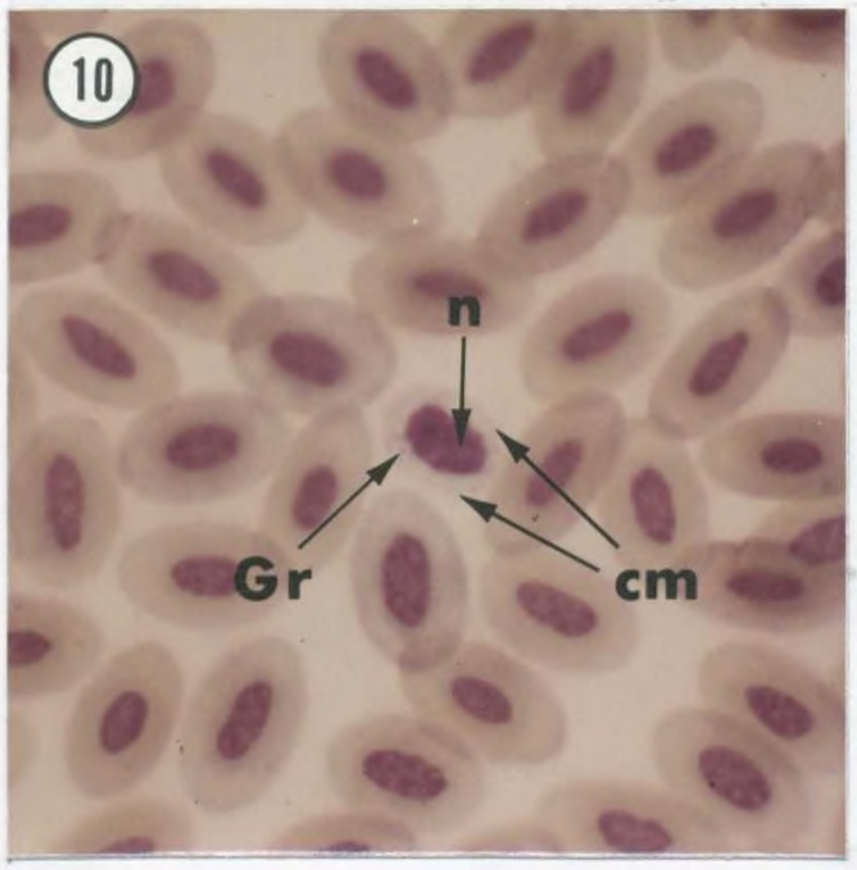
Fig. 11.--Mature Thrombocyte. This thrombocyte possesses most of the characteristics of the cell of Figure 10 but is more mature. The nucleus (n) is oval and centrally located. The cytoplasm is also oval giving it the characteristic spindle shape. Three serotonin granules (Gr) can be seen. The cell membrane (cm) is very thin. 600X.

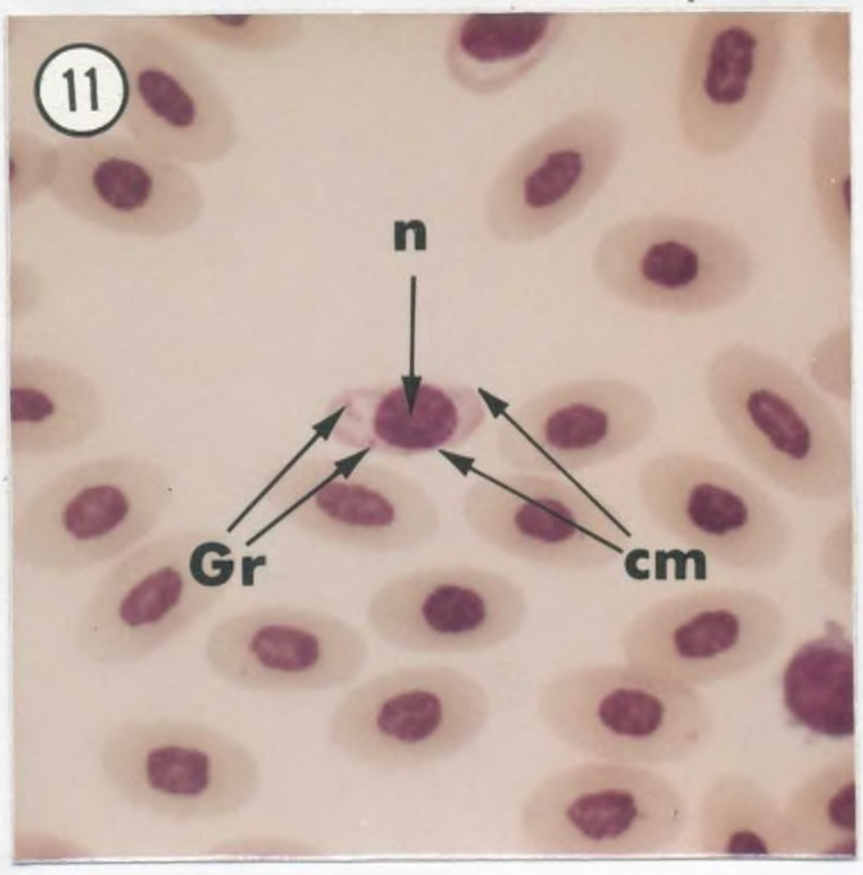
Fig. 12.--Degenerating Thrombocyte. As the process of degeneration takes place, nuclear detail is lost and the chromatin becomes one solid mass. The cell membrane is irregular and not present on all surfaces. Cytoplasmic detail is lost and all that seems to be left is serotonin granules (Gr). 600X.

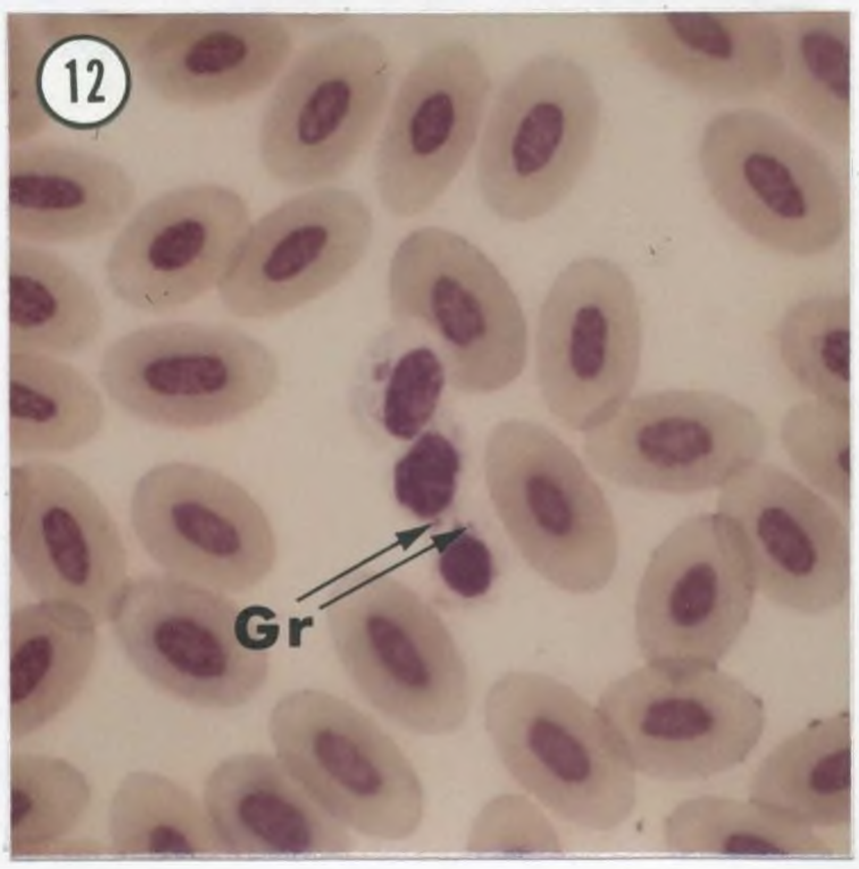
PLATE II











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