SOME HISTOLOGICAL AND PHYSIOLOGICAL CHANGES OF THE BLOOD OF THE DEVELOPING CHICK FROM FOUR DAYS OF INCUBATION THROUGH TWENTY-EIGHT DAYS OF INCUBATION (SEVEN DAYS POST-HATCH)

By

Kenneth Thompson Stringer

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INTRODUCTION

In the studies of the development of the chick embryo, many aspects of the physical and chemical composition of the blood have been investigated but others have been neglected. Hanon (1921) investigated the blood glucose values in the 14-16 day embryos. Fiske (1926) determined that the production of uric acid was initiated at five days of incubation. Blain (1928) developed a method for making leucocyte counts on adult avian blood. Dukes and Schwarte (1931) established the blood glucose levels for the adult hen and cock. In 1934, Dyer and Roe were studying pullorum disease of the adult fowl, and since no data was available regarding the chemical composition of chicken blood, a study of the normal adult hen's blood was made. This included non-protein nitrogen, glucose, cholesterol, and pH, and was the first complete chemical study of chicken blood.

Olson, in 1937, studied the normal blood of the adult fowl in order to establish the total erythrocyte count, total leucocyte count, and differential leucocyte count. Prior to this date, the available data on these normal values of adult chicken blood were inconsistent. In 1937, Zorn and Dalton studied the biochemistry of the developing chick blood from nine days of incubation through 27 days of development (six days post-hatch). In this study, the cholesterol, urea, uric acid, hemoglobin, and glucose were determined as well as the total erythrocyte counts. As stated at that time, methods were not available for studies on earlier stages of development because the small amount of blood obtained could not be utilized in the existing methods of analysis.

Erickson, et al, (1937-38) published their observation on chick blood, including total erythrocyte and leucocyte counts, hemoglobin, corpuscular measurements, and specific gravity. In 1939, Schoger reported the total leucocyte and erythrocyte counts, and hemoglobin values on young chicks. In 1948, Zwilling carried out a study on the effect of insulin induced micromelia and the associated hypoglycemia on the developing chick embryo. Tests were made to determine the glucose levels from the fifth day of incubation through hatching. There are no reported observations on the determination of leucocyte counts, of specific gravity of whole blood and blood plasma, of the hematocrit, of differential counts of leucocytes and of mean corpuscular measurements of developing chick blood from four days of incubation through hatching to seven days post-hatch.

It was with the purpose of furthering the histological, physiological, and biochemical studies of the early developing chick embryo blood that this study was undertaken.

MATERIALS AND METHODS

Source of Eggs

The eggs for the study, obtained from the University of Maryland Poultry Farm, were predominantly those of New Hampshire Red Chickens known on the Poultry Farm as "Replacement Stock," although occasional White Leghorn eggs were used. The eggs, uniform in size, weight and appearance, were incubated under standard conditions for incubation as practiced by the Poultry Department of the University of Maryland and others (Rugh, 1948).

A total of approximately 2400 eggs were incubated in this study. Of these, over 70% were observed to be fertile by candling at three days, or by hatching. All non-fertile eggs were removed from the incubator. The blood of the chick embryo was studied from eggs of four days of incubation to seven days post-hatch. One blood sample was utilized from each egg studied and ten eggs were studied for each day of incubation from four to twenty days. Of the observations made on post-hatch chicks, ten chicks for each observation were used in determining coagulation time, and erythrocyte and leucocyte counts. Ten chicks for each observation were used to determine differential leucocyte counts, specific gravity of whole blood, and hematocrit. Each count was made separately and averages determined.

As a means of comparing the embryonic blood with blood of an older chick, some observations were made on chickens of fifteen weeks of age. The new observations made and not previously reported were:

1. Differential leucocyte counts
2. Coagulation time
3. Specific gravity of whole blood

4. Hematocrit

5. Total leucocyte counts

In this study, erythrocyte counts were made from four days of incubation through seven days post-hatch, repeating part of the work done by Zorn and Dalton (1937). The hemoglobin values were calculated for this study as given by the formulas of Kolmer, et al, (1951). The other calculated observations were the specific gravity of blood plasma, plasma protein, and mean corpuscular measurements.

To secure blood for study, an egg of the desired length of time of incubation was candled to determine the position of a good vascular area on the blastoderm and this area was circled in red pencil. This was wiped with distilled water, allowed to dry and then a clean hacksaw blade used to saw a square on the surface of the shell. This square was lifted off the shell membrane and the shell membrane moistened with a drop of physiological salt solution prior to the next step in the process. The shell membrane was slit with a dissecting needle, the edge of the membrane grasped by thumb forceps, and lifted free of the underlying structures. With eggs of seven days of incubation and longer, it was found necessary to puncture the air chamber to allow the embryo and other structures to be freed from their close proximity to the shell membrane where it was to be opened.

Method of Obtaining and Staining Blood

Blood was obtained from the embryo of from four to nine days of development by the use of a glass micropipette. The blood vessel was nicked by the end of the micropipette and the blood drawn into the pipette by capillary attraction controlled by tubing connected to the pipette and to the plastic mouthpiece. If any fluid other than blood was obtained, this diluted sample was discarded and a new egg opened. From nine days of development through twenty-one days of development, blood was obtained from the allantoic circulation in the same manner as previously described. From twenty-one days of incubation, i.e. after hatching, through twenty-eight days (seven days post-hatch), the blood was obtained by lightly anesthesizing the chick and obtaining blood from the right external jugular vein using an eighteen gauge needle and a one cubic centimeter tuberculin syringe. From twenty-one days of development through twenty-eight days (seven days post-hatch), the coagulation time was so rapid as to prevent accurate manipulations; therefore, liquid heparin of a concentration of 1 mg. per 1 ml. was used in the tuberculin syringe and needle to prevent coagulation, except in tests on coagulation time.

After extraction, a drop of the blood was placed upon a clean slide, a thin smear made, and stained with Wright's stain (Cowdry, 1948). The differential counts were made at 660 magnification since the cells of the chick embryo were sufficiently large to be readily observed without oil immersion. When possible, the differential counts included the acidophiles and heterophiles, the basophiles, the large and small lymphocytes, and the monocytes. The number of cells counted and recorded in each specimen was well above one hundred, or if less than one hundred were found, the complete area of the smear was scanned to insure as complete observation as possible. The results of the differential counts were reported as the percent of the total observed leucocytes.

Specific Gravity of Whole Blood

Specific gravity was measured by using the copper sulfate method developed by Phillips, et al (1950). This method consists of the use of a graded series of copper sulfate solutions ranging from a specific gravity of 1.008 up to 1.064 in units of .001. Tests were made using drops of whole blood until the specific gravity of the sample was established. This was evident when the drops remained stable in the solution for approximately ten seconds.

This method is considered as accurate and as easily reproducible as other specific gravity methods and is much less cumbersome in its manipulation (Powers, 1949).

<u>Hematocrit</u>

Blood was injected immediately into Kato hemomicropipettes, and after being clamped into the Kato holders was centrifuged in an International Centrifuge, size 2, at 3000 rpm for thirty minutes. At the end of this time, the total of blood cells and plasma volume was read in units of one cubic millimeter and the blood cell volume was read individually. The amount of blood cells was compared to the total plasma and cells and the results were recorded in percentage of blood cells.

<u>Erythrocyte Counts</u>

The erythrocyte counts were made using the standard Thoma blood pipette, a Levy counting chamber and using Toisson's solution as the dilutant according to Olson (1935). A total of 400 small squares equivalent to one square millimeter were counted to assure an accurate representative sample count of the erythrocyte from the fourth day of development through the seventh day post-hatch.

<u>Leucocyte Counts</u>

Since the leucocytes do not appear in appreciable quantities in the circulating blood of the chick embryo until the sixth day of incubation (Sabin, Cunningham and Doan, 1925), (my own observations verified this), no leucocyte counts were made prior to this time. At six days of incubation through seven days post-hatch, the leucocyte count was made using a Thoma pipette, a Levy counting chamber, and using Toisson's dilutant. Five of the one millimeter squares were counted to assure the proper representative sampling.

Coagulation Time

Coagulation time was measured by the capillary tube method and by the coagulation-bleeding time method. In the capillary tube method, the blood was collected in the tube by capillary action and in the coagulation-bleeding time, the blood vessel was nicked and the blood allowed to flow until coagulation occurred.

Calculated Measurements

The value of specific gravity of blood plasma, plasma protein, hemoglobin and mean corpuscular measurement were calculated as given by Kolmer, et al, (1951). The formula for specific gravity of plasma is $GP = \frac{100\ Gb - 1.0964\ H}{100 - H}$ where Gb is specific gravity of the whole blood and H is the hematocrit; the formula for plasma protein is $P = 373\ (Gb\ 1.0070)$. P equals grams of protein per 100 ml., Gb equals the specific gravity of the sample of whole blood; the formula for hemoglobin is $Hb = 33.0 \times \frac{(Gb - Gp)}{(1.0964 - Gp)}$ where Gb equals specific gravity of whole blood and Gp equals specific gravity of plasma.

The formula for mean corpuscular volume (given in cubic microns), is: $\frac{\text{hematocrit}}{\text{erythrocytes in million per cu. mm.}} \times 10.$

The formula for mean corpuscular hemoglobin, (given in micromicrograms), is: $\frac{\text{Hemoglobin in gram/100 ml.}}{\text{erythrocytes in million per cu. mm.}} \times 10.$

The formula for mean corpuscular hemoglobin concentration is:

$$\frac{\text{hemoglobin in grams per 100 ml.}}{\text{hematocrit}} \times 100 = \text{percent mean corpuscular}$$

hemoglobin concentration.

It is recognized that the formulae for the various calculated measurements of hemoglobin, plasma protein and specific gravity of plasma, were originally formulated for application to human blood. The chemical methods for the determination of hemoglobin, plasma protein and specific gravity of plasma involve difficulties in manipulation of micro-quantities of reagents and blood samples. In the determination of avian hemoglobin, the difficulty is in the presence of a nucleus in the erythrocyte which leads to false values. Rostorfer, 1949, compared the methods for the determination of avian hemoglobin and concluded that the manometric method was the most accurate. Olson (1935) found that the most accurate method was the manometric method; between the various methods tested there was as much as 25% variation in high and low results. Olson cautioned that the results were possibly in error by any method due to the nucleated erythrocytes. Thus, the calculated measurements are included as approximate values in the interest of giving a more complete picture of the blood of the developing chick embryo.

OBSERVATIONS

Erythrocytes

The term erythrocyte is customarily used to denote the red blood corpuscle. The Committee on Nomenclature in Hematology as reported by Osgood (1948), however, has changed the name of the erythrocyte to rubricyte and the developing stages of the red blood corpuscle to rubriblasts. Since this new terminology is not in general use, the author continues to use the terms erythrocyte, erythroblast, etc.

At four days of incubation, the cellular elements of the blood were composed entirely of erythrogenic cells of the basophilic erythroblast type. The cytoplasm was basophilic and agranular, the nucleus large and vacuolated, and the chromatin stained deeply basophilic with a granular or filamentous appearance, (Figure 17). At five days of development, (Figure 18) the basophilic erythroblasts were changing to acidophilic erythroblasts and the cytoplasm exhibited a gradual transition from basophilic staining to acidophilic staining while an occasional erythroblast was observed to be a mottled blue-pink. At six day of incubation, the erythroblasts were mainly of the acidophilic type and by seven days, the erythroblasts were mostly of the acidophilic type with some few basophilic erythroblasts. The nucleus changed its appearance by becoming more compact, less vacuolated and taking a deep basophilic stain (Compare Figures 17 through 21). By nine days of incubation, all basophilic erythroblasts had disappeared from the circulating blood. Various erythroblasts and erythrocytes can be seen in Figures 21 through 34 inclusive.

Leucocytes

The acidophiles were spherical cells with a slight basophilic cytoplasm that stained pale blue but contained some small spherical granules that stained pink. The nucleus was pale blue and lobed, (Figure 31). The heterophiles were spherical with a pale blue cytoplasm, rod or crystal-like granules stained a deep red that obscured the nucleus, although in cells that were broken during the smearing process, the nucleus was lobed and stained blue, (Figures 30, 33, 34). The basophiles were large cells that contained coarse, dark blue granules in a pale blue cytoplasm. The nucleus was spherical or occasionally indented and stained blue.

The small lymphocytes were spherical with a sparse quantity of pale blue cytoplasm and a deep blue compact nucleus; the large lymphocytes were spherical and had a large amount of pale blue basophilic cytoplasm in which was a large ovoid or indented nucleus stained deep blue, (Figures 19, 21, 23, 25, 29, 30, 31, 32). The monocytes had a large quantity of pale blue basophilic cytoplasm in which were occasional vacuoles; the nucleus was large and took a deep blue basophilic stain, and the chromatin occurred as scattered granules, (Figures 24, 31, 33, 34). Various leucocytes can be seen in Figures 19 through 34 inclusive.

Differential Leucocyte Counts

1. Acidophilic Leucocytes

The acidophilic leucocytes were found in the embryonic chick blood beginning at seven days of incubation (Table I, Figure 1). They varied from 0.49% to 3.6% of the total leucocytes and were within the range of the normal adult differential count of acidophilic leucocytes made by Blain (1928) which was 1.4% to 13.0%.

2. Heterophilic Leucocytes

The original concept by Blain (1928) and others was that no heterophiles (neutrophilic leucocytes) were present in chicken blood. Olson (1937), by the use of the dye phloxine, which is a metachromatic stain, determined that there was a sufficient difference in staining properties to establish the cell containing the rod-shaped granules of the former acidophilic leucocyte as being the heterophilic leucocyte. In this study, the rod-shaped granules are considered to be the identifying feature of the heterophile.

The deep acidophilic rod-shaped granules of the heterophilic leucocytes were first seen in the embryonic chick blood at six days of incubation, (Table I, Figure 1.).

The heterophilic differential leucocyte count varied between a low of 11% and a high of 66%. This is beyond the range of the normal adult differential count as reported by Blain (1928). The developmental heterophilic leucocyte count showed a rise from 20.9% at six days of incubation to a peak of 47% at ten days while at eleven days a drop to 40% occurs. At twelve days a rise in value is noted to 55%, little variation occurs from this time through twenty-eight days.

3. Basophilic Leucocytes

The basophilic leucocytes in the embryo were similar to the normal basophilic leucocytes of the adult chicken. They had a percentage range of 0.4% to 5.0% well within the range of the findings of Blain (1928) for the adult, and of Olson (1937). The basophilic leucocytes appeared at the sixth day of incubation and were not significantly altered during the period of the study, (Table I, Figure 1).

4. Lymphocytes

The differential value of the lymphocyte as reported by

Olson (1937), was recorded merely as lymphocytes and not as small and large lymphocytes. Blain (1928) reported the majority of lymphocytes as being in the range of 3u to 5u; although some were larger in the range of 6u to 12u. In this study a similar finding was made. At four days of incubation, there were no lymphocytes present, while at five days of incubation 81% of the differential count were small lymphocytes and 19% were large lymphocytes (Table I, Figure 1). A steady drop in the percentage of small lymphocytes occurred from six to nine days from 81% to 36%; slight variations occurred from this time through twenty-eight days with a high of 43% at eleven days and a low of 26.8% at fourteen days. The majority of percentages were within the range of 28% to 33%.

Large lymphocytic differential counts changed in value in the early days of incubation as shown by Table 1, Figure 1. There was little change in the large lymphocyte counts after twelve days of incubation except one value of 15% at sixteen days of incubation.

The combined lymphocyte count showed a gradual decline in value from 100% at five days to 46.8% at ten days. It fluctuated between 55.2% and 35.7% but remained close to 41% from 17 days on as shown by Table I and Figure 1.

5. <u>Monocyte</u>

The monocytes were infrequent in occurrence and varied in percentage from 0 to 6.4. The higher counts occurred in the 7th, 8th and 9th days of development, dropping later to a percentage of 2 to 5% where it remained through twenty-eight days of incubation.

On the basis of differential counts, the only definite changes that occurred were in the counts of the small lymphocytes and heterophiles. The significant changes that occur are found from the 7th through the 12th day of incubation.

Specific Gravity of Whole Blood

The specific gravity of whole blood was a function of time in the development of the chick embryo from four days of incubation through hatching. As shown by Table II, Figure 2, specific gravity of whole blood increased from 1.0150 at five days of incubation to 1.0245 at twelve days of incubation. Between twelve and thirteen days of incubation, a great change occurred. This was an increase from 1.0245 to 1.0289 or a change of 0.0044. After this increase, the change was gradual and the increment was not higher than 0.002 for any subsequent day. This rise in specific gravity was steady up to the seventh day post-hatch when the specific gravity was 1.044 which is the same as that for chickens fifteen weeks old.

Hematocrit

The hematocrit values for the developing chick blood showed a variation at seven, twelve and fifteen days of incubation (Table III, Figure 3). Between seven and ten days of incubation a drop occurred from a value of 19.2% to 15.1%; at twelve to thirteen days, an increase in percentage of cells was noted from 16.55% to 23.7%. Again at fifteen days of incubation, a rise to 31.48% was observed. This increase continued to twenty days when the hematocrit percentage was 37.9%. It varied between 32.6% and 36% until twenty-eight days.

This was close to the adult values as given by Erickson, et al, (1938) of 35% and that of Albritton (1950). Tests carried out on chickens of fifteen weeks of age maintained the same hematocrit values.

Erythrocyte Count

The total red blood corpuscular count exhibited an extremely low value at four and five days of incubation (Table VI, Figure 4). The counts on those days were recorded as red cells due to the difficulty of visually segregating erythrocytes and leucocytes, if any be present, at this period of incubation. At six days of incubation, an increase was noted to 1.274 million/cu. mm. from 0.488 million/cu. mm. of the previous day. Decreases in total erythrocyte counts were observed at eight, twelve, and eighteen days of development. At twelve days of incubation, a drop occurred to 1.662 million/cu. mm. from 1.794 million/cu. mm. of the eleventh day. A decided rise occurred on the thirteenth day to 2.092 million/cu. mm. This rose to 2.952 million/cu. mm. on the sixteenth day and dropped to 2.874 million/cu. mm. on the seventeenth day. On the eighteenth day, the total erythrocyte count plummeted to 1.878 million/cu. mm. It rose markedly on the nineteenth day to 2.276 million/cu. mm. and increased to 2.742 million/cu. mm. by the twenty-first day. It maintained this level through the twenty-eighth day of incubation. This was close to the adult total erythrocyte counts as given by Olson, 2.74 million/cu. mm. and Erickson, 2.9 million/cu. mm. The average for chickens of fifteen weeks of age was 2.70 million/cu. mm.

Leucocyte Count

The total leucocyte count exhibited a high value at six days of incubation (Table VI, Figure 5). This value of 55.5 thousand per cu. mm. increased to 67.6 thousand per cu. mm. at eight days of incubation. A general decrease occurred in total leucocyte value to 31.4 thousand per cu. mm. at eighteen days except for a variation of 58.6 at fourteen days. From the eighteenth day, the total leucocyte count varied between 29.0 and 32.6 thousand per cu. mm.

Coagulation Time

Coagulation time was tested beginning at eleven days of incubation.

Neither the capillary method nor the coagulation-bleeding method resulted in coagulation from the eleventh through the thirteenth day of incubation. At fourteen days, as shown in Table IV, Figure 6, coagulation by the capillary method occurred between ten and fifteen minutes after extraction of the blood, while at fifteen days of incubation, the time dropped to four minutes and thirty seconds. There was a graded decrease in the coagulation time of four minutes and thirty seconds at the sixteenth day to twelve seconds at seven days post-hatch which is higher than the coagulation-bleeding method. The coagulation time by the coagulation-bleeding method was four minutes and forty-eight seconds on the fourteenth day (Table V, Figure 6); at fifteen days, coagulation time had decreased to four minutes and six seconds; at sixteen days, a marked drop to one minute and fifty-four seconds had occurred. From the sixteenth day of incubation through seven days post-hatch, there was a graded decrease in coagulation time to seven and one tenth seconds.

Calculated Measurements

1. Specific Gravity of Blood Plasma

From four days of incubation to seven days of incubation, the specific gravity of blood plasma did not change markedly, remaining no greater than 1.0017 (Table VI, Figure 7). At eight days, an increase in specific gravity was noted to 1.0072. This value remained the same with little change until fifteen days when a decrease was noted to a value of 1.0041. At sixteen days a value of 1.0069 was obtained but no marked change from this was noted until twenty-one days when the value of 1.0097 was reached. With slight changes, it increased to a high of 1.0171 at twenty-seven days.

2. Plasma Proteins

The plasma proteins of the embryonic blood showed a gradual rise in value from four days of incubation through twenty-eight days (Table VI, Figure 8). There was a steady rise in small increments from a value of 2.984 grams per 100 ml. at four days to 13.801 grams per 100 ml. at twenty-eight days.

3. Hemoglobin

Hemoglobin was calculated from the recorded specific gravity of whole blood and the calculated specific gravity of blood plasma. Hemoglobin values showed a general increase during the period studied. Slight variations occurred at seven, twelve and fifteen days of incubation as shown by Table VI, Figure 9. An increase in hemoglobin was noted from 5.18 grams/100 ml. at six days to a value of 6.51 grams/100 ml. at seven days. This dropped to 5.4 grams/100 ml. at eight days and it remained steady until eleven days when it began increasing through the twenty-first day of incubation, when the value was 12.9 grams/100 ml. Slight variations occurred from this time through twenty-eight days.

4. Mean Corpuscular Volume

The mean corpuscular volume showed a large variation in the measurement of the cells. This variation was due to the fact that this value is calculated from the hematocrit and total erythrocyte count. As these values vary in periods of incubation of the embryo, then the mean corpuscular volume shall also vary (Table VI, Figure 10).

5. Mean Corpuscular Hemoglobin

The calculated mean corpuscular hemoglobin varied as the value for hemoglobin and total erythrocyte varied in the periods of incubation of the embryo (Table VI, Figure 11). The values for mean corpuscular hemoglobin were, with several exceptions, higher than the values reported for the adult by Erickson, et al, of 34.3 micrograms.

6. Mean Corpuscular Hemoglobin Concentration

Mean corpuscular hemoglobin concentration did not vary to the extremes but remained fairly stable from the fifth day of incubation through seven days post-hatch (Table VI, Figure 12).

DISCUSSION

Blood Cells

The blood cells of the developing chick have been previously studied as to their origin. Only a few studies have been made concerning the histological appearance and corpuscular measurements of the erythrocytes and leucocytes. The findings of Sabin (1920), Doan, Cunningham and Sabin (1925) and Sugiyama (1926), were that the erythrocytes developed in the blood islands of the area vasculosa of the one to two day old embryo. The circulation of blood does not occur until 33 hours of development. As development of the chick embryo progresses, the circulatory system becomes more complete. At four days of incubation, blood circulation in intra-embryonic and extra-embryonic blood vessels has been achieved. Concurring with the findings of Doan, Sabin and Cunningham (1925), the author found the blood of the four day chick did not show discernable leucocytes but exhibited only basophilic erythroblasts or hemoblasts. At the fifth day of incubation, the blood showed occasional lymphocyte cells that were of a size and histological appearance of the adult lymphocyte as reported by Blain (1928) and by Olson (1937). It was not until the sixth day of incubation that granular leucocytes were seen in the blood. This agreed with the findings of Cunningham, Sabin and Doan (1926). The erythrogenic series of cells seen in the fourth day of incubation began with the basophilic erythroblast as described by Maximow (from Cowdry, 1948). At five days of incubation, polychromatic erythroblasts were evident. This is due to the presence of both basophilic material and hemoglobin which was acidophilic, being in the same cell. At six days of incubation, the acidophilic erythrocytes were in the majority and by the ninth day, all basophilic erythroblasts and polychromatic erythroblasts had disappeared from the circulatory blood.

These findings were quite different from those of Sugiyama (1926) who maintained that the mature erythrocyte does not occur in the circulatory blood until the seventeenth day of incubation. Fennel (1947) indicated that primitive erythroblasts containing medium hemoglobin were 96% and 95% at three and four days of incubation. The value dropped at six days of incubation to 66% and at nine days to 5% when these cells contained much hemoglobin. Fennel gave no values to designate "medium" and "much" hemoglobin. Since Fennel described the entrance of a "definitive erythroblast" and "definitive erythrocyte" into the circulatory peripheral blood at six days of incubation, he recorded a total value of 27.89%. This value of the definitive erythrocyte increased as the value of the definitive erythroblasts and primitive erythroblasts declined. At seventeen days of incubation, he recorded a value of 99.16% definitive erythrocytes and a slight lowering of value on twenty days of incubation to 98.52%. On a cytological basis, the differences shown by Fennel with vital staining were not critical enough to warrant segregation of cells on a "medium" and "much" quantities of hemoglobin. Wright's stain exhibited a variation in the reaction of basophilic and acidophilic cytoplasm of the developing erythrocyte. These variations were sufficient to designate basophilic, polychromatic, and acidophilic erythroblasts. Sugiyama (1926) reported two stages of erythroblastic development based upon the use of vital dyes. The erythroblastic stage I began on the second day of incubation and lasted through the fourth day and the erythroblast stage II began about the fifth day of incubation. The correlation between Fennel's work and Sugyama's work was fairly close.

The difference in time of introduction of the definitive erythroblast was only twenty-four hours. It might be important that Sugyama mentioned, but did not emphasize, the presence of mitotic cells in the circulating blood. These mitotic figures abruptly disappeared at five days of incubation. This indicated another possible source of basophilic erythroblasts than that of the angioblasts of the blood islands. This second source of erythroblasts beginning at five days of incubation was reported by Wieman (1930) as the mesenchymal clumps near the aortic arches, the aortic roots and the anterior half of the dorsal aorta at 100 to 110 hours of incubation. These cells originating in such an area would possibly be post-mitotic cells when they entered the circulating blood and could account for the loss of mitotic cells from the circulating blood. However, this source of post-mitotic erythroblasts could not supply the requirements of the circulating blood. The other possibility is that the erythrogenic area of the yolk sac gives rise to post-mitotic cells after five days of incubation. The erythrogenic stem cells would remain as the mitotic precursors of the erythroblasts.

The leucocytes were histologically the same as described by Blain and by Olson except that Blain classified the acidophilic rod granular leucocyte as one of two types of acidophiles, the other being acidophilic spherical granular leucocytes. Olson (1937) separated the cells and called the acidophilic rod granular leucocyte, the heterophile. He based his interpretation of the heterophile on the use of the dye phloxine which differentially stained the two cells and clearly showed the difference between the heterophile and the acidophile. The lymphocytes showed a variation in size and in differential count to be divided into small (3-6u) and large (6-12u) lymphocytes. The general appearance of the lymphocytes, monocytes, and basophiles was almost identical with the same cells of the adult.

Differential Leucocyte Count

The first appearance of leucocytes occurred at five days of incubation in the form of lymphocytic leucocytes. These lymphocytic cells were of the same histological appearance as described by Cunningham, Sabin and Doan (1926) and Blain (1928). Since they comprised all of the leucocytes present at that time, they were counted as 100% lymphocytes and subdivided into 81% small lymphocytes and 19% large lymphocytes (Table I, Figure 1).

At the sixth day of incubation, the heterophiles and monocytes made their first appearance. The differential count of the heterophiles was 20.9% and that of the monocytes was 1.9%. As a result, the lymphocyte differential count was lowered to 72.4% small lymphocytes and 4.7% large lymphocytes. On the seventh day of incubation, the acidophiles and basophiles were observed.

From the seventh day of incubation, through hatching, there was no significant variation in the differential values of the acidophilic, basophilic leucocytes, the large lymphocytic leucocytes and the monocytic leucocytes. The heterophilic leucocyte differential count did show a gradual increase up to fourteen days of incubation with a value of 56% except for a value of 40% at eleven days. The differential heterophilic leucocyte count appeared to level off at about 52% to twenty-eight days. The period of high heterophilic leucocyte counts between twelve and fourteen days could be attributed to the hemopoietic activity of the bone marrow which is developed at that time (Danchakoff, 1916). It is the principal hemopoietic organ of the later stages of incubation and also of the adult chicken.

The small lymphocytic leucocytes were seen to decline in differential value from five days to ten days of development. A sudden rise in percentage value occurred at eleven days of incubation to 43% but the value dropped at twelve days to 26%. There was an increase to 30.8% at eighteen days of incubation, then remained fairly stable.

The values for the differential acidophilic and basophilic leucocytes agreed with those values of Olson (1937) and were close to the values of Blain (1928). The heterophilic, lymphocytes and monocytic leucocyte differential counts agreed with the range of values of Blain (1928) for the adult, but not with those values of Olson (1937) for the young chick. The author does not account for the discrepancy in differential values between the results of Blain (1928) and Olson (1937).

The author was in accord with Fennel (1947) and Sugiyama (1926) that the leucocytic blood picture appeared stabilized from the ninth day of incubation through hatching although neither investigator performed differential counts.

Specific Gravity of Whole Blood, Hematocrit and Calculated Measurements

The specific gravity of whole blood was a measurement of the combined specific gravities of its components. The hematocrit value was the percentage of cells compared to the percentage of plasma. Variation in these values affected the values of the calculated specific gravity of plasma, plasma protein and hemoglobin in grams per 100 milliliter of sample.

The specific gravity of whole blood and the hematocrit demonstrated a close correlation as shown by comparison of Figures 2 and 3. The greatest variation in each occurred at twelve to fourteen days of incubation. Previous to this time, the specific gravity of whole blood increased gradually to 1.024 at nine days of incubation and then dropped to 1.022 at eleven days of incubation. The hematocrit at this time showed a greater fluctuation in value but no increase until twelve days. This could be accounted for in the beginning functioning of the bone marrow as hemopoietic organ during the twelfth to the fourteenth day of incubation. From the fifteenth day through twenty-eight days of incubation, the hematocrit remained at the same approximate level of 35% (Erickson 1937) and 32% (Albritton 1950) as recorded for the adult.

The calculation of specific gravity of plasma was based on the recorded value of the specific gravity of whole blood and hematocrit. Since the values of the specific gravity of whole blood was almost a linear relationship, the variation in specific gravities of plasma resulted from the variations in the hematocrit values. This was evident in the fifteenth day of incubation when the specific gravity of plasma dropped to 1.0041 from a value of 1.0101 of the previous day (Table VI, Figure 7). After the fifteenth day of incubation, there was little fluctuation in the hematocrit value but an increasing value was maintained for the specific gravity of whole blood. This caused a stabilizing effect on the values of specific gravity of plasma until the twenty-first day when the specific gravity of plasma was 1.0097. It then increased with slight variations to a value of 1.0170.

The specific gravity of plasma as established for chickens of fifteen weeks of age was 1.017 to 1.020. The calculated values for the chickens in the latter period of the project was approaching the adult values.

Since the plasma protein was calculated from only one variable, the change in plasma as calculated could not be extreme. The calculations were based on specific gravity values of whole blood. These values were almost a linear function, thus the calculated values of plasma protein were almost a linear function. At twelve days of incubation, a slight rise in plasma protein value occurred (Table VI, Figure 8). This was in conjunction with a slight rise in specific gravity of whole blood (Table II, Figure 8).

The rise in specific gravity of whole blood and blood plasma could be explained by the work of Pickering and Gladstone (1925). The presence of prothrombin, globulin and fibrinogen in the embryonic blood at twelve or thirteen days of incubation might account for the increased values (Tables II, V and Figures 2 and 7). Moore, et al (1945) by electrophoretic studies determined the introduction of a protein fraction into the circulating blood on about the eleventh day of incubation. The electrophoretic mobility of this faction indicates it was similar to B-globulin. Two more fractions were reported at seventeen days of incubation. These were low in quantity and motility and no evidence of their presence could be seen in the calculated plasma protein values.

The calculated value of hemoglobin showed a gradual increase from five days of incubation through twenty-one days of incubation (Table VI, Figure 9). Within this period, there was a marked increase at twelve days from 6.13 gms./100 ml. to thirteen days of 8.044 gms./100 ml. At fourteen days an increase occurred to 8.4 gms./100 ml. and on the fifteenth day to 10.67 gms./100 ml. These changes reflected the change in the value of the specific gravity of plasma since the specific gravity of whole blood was almost a linear function. The variations that did occur were at times of generally known embryological development of the hemopoietic organ, the bone marrow and the functioning of the liver. The steady rise in hemoglobin values did not continue after twenty-one days of incubation but remained generally stable. These were within the values of the fifteen week bird which was 10.8 to 12.2 gms./100 ml.

The hemoglobin values as calculated were not in agreement with the results of Zorn and Dalton (1937). The earliest recorded values at nine days were 6.5 gms./100 ml. while the recorded values of this project were 5.8 gms./100 ml. The values of Zorn and Dalton (1937) increased to twenty-one days and then decreased in value to twenty-nine days. The calculated values increased as previously noted and then leveled off in the range as calculated for fifteen week chickens. The normal adult range is between 8.9 gms./100 ml. and 11.2 gms./100 ml. as given by Dyer (1934), Erickson et al (1937), Olson (1937), Dukes and Schwartz (1931) and Albritton (1950).

<u>Erythrocyte Count</u>

Total erythrocyte counts were made from four days of incubation through twenty-five days of incubation. For the fourth and fifth day of incubation, all cells were counted and recorded as total erythrocyte counts in million per cubic millimeter. The recorded value for these days was very low as indicated (Table VI, Figure 4). At six days of incubation, there was a large increase in erythrocyte count from .488 million/cu. mm. at five days to 1.274 million/cu. mm. There was a steady increase with slight decreases in total erythrocyte counts at twelve and eighteen days of incubation. The decrease at eighteen days might be explained in the beginning regression of the allantois. However the decrease was not maintained through the hatching period and an increase was observed to the former values. The count increased at nineteen days and stabilized at 2.71 million/cu. mm. Although the total erythrocyte count did not agree with the findings of Zorn and Dalton (1937), there was a similarity in decreased count at eighteen days of incubation and a subsequent increase to 2.6 million/cu. mm. at twenty-two days. At this day the similarity ceased, Zorn and Dalton's (1937) values decreased to 1.9 million/cu. mm. at twenty-nine days and these results at twenty-eight days were 2.71 million/cu. mm. The total erythrocyte count as given by Olson (1937) were 2.74 million/cu. mm. for the young chick.

Leucocyte Count

The total leucocyte count showed an initial value of 55.5 thousand/cu. mm. at six days of incubation (Table VI, Figure 5). This increased to 67.6 thousand/cu. mm. at eight days of incubation. A decrease in total leucocyte count followed to a value of 49 thousand/cu. mm. at thirteen days. An increase was observed at fourteen days of incubation and then a continued decline in total leucocyte value through eighteen days of incubation.

The values from eighteen days were close to the values for the young chick as reported by Olson (1937). The extreme limits for total leucocyte count reported by various investigators are as follows: Albritton (1950), range 16,000-40,000/cu. mm; Blain (1928), 10,000-29,000/cu. mm.; Olson (1937), 19,800/cu. mm.; Kyes (1929), 8,000-13,000/cu. mm.; and Schoger (1939) 21,400/cu. mm. These wide ranges indicate the need for the standardization of counting technique and a statistical study of the total leucocyte counts.

Coagulation Time

The measurement of coagulation time was carried out to determine the time of coagulation and the change in coagulation time. The first day at which coagulation occurred at fourteen days of incubation in both methods. These findings agreed with the work of Schechtman (1947) which states that it is common knowledge that chick blood will not clot before the thirteenth day. No data were available on the times of coagulation in embryonic blood. Pickering and Gladstone (1925) found that fibrinogen, prothrombin and globulin could not be detected chemically before the twelfth or thirteenth day of incubation.

The coagulatory mechanisms of the blood developed very rapidly as indicated by Tables IV and V, Figure 6. The time required for coagulation by both methods decreased rapidly. From four minutes and thirty seconds, at fifteen days, for the capillary tube method, the time decreased, at twenty-eight days, to twelve and four-tenths seconds (Table IV, Figure 6). The coagulation-bleeding time (Table V, Figure 6) was even more rapid; the time at fourteen days was four minutes, forty-eight seconds. The time at twenty-eight days was seven and one-tenth seconds. The time of the reaction of coagulation mechanism must be increased at an age older than seven days post-hatch since the reported clotting time of the adult by Schonheyder (1938), was eight minutes. The rapidity of coagulation of the one day through seven days post-hatch chick suggests a regulatory mechanism to prevent excessive blood loss during and after hatching.

Mean Corpuscular Measurements

The mean corpuscular measurements were relative values for the expression of cell volume, cell hemoglobin and cell hemoglobin concentration.

Since these values were calculated from the recorded hematocrit and total erythrocyte counts and the calculated hemoglobin, the two main variants were the hematocrit and erythrocyte counts.

The mean corpuscular volume varied considerably during the developmental period of the project. The larger corpuscular volumes occurred when the total erythrocyte count was low and the hematocrit value was high. The possibility of a macrocytic or microcytic anemia existing in a developmental animal was not in this study. The mean corpuscular volume for the erythrocyte of the adult as reported by Erickson, et al (1937), was 117.6 cu. micron. The average for the period from six days of incubation to twenty-eight days of incubation was 123.62 cu. micron, the extremes were 81.30 cu. micron and 190.788 cu. micron (Table VI, Figure 10).

The mean corpuscular hemoglobin was computed from the calculated hemoglobin and the recorded total erythrocyte count. There was considerable variation during the period covered. This variation was due to the changes in hemoglobin and total erythrocyte counts. The average for the project was 44.9 micromicrograms with a range of 27.6 to 112.3 micromicrograms as compared to Erickson, et al (1938), value for the adult of 34.4 micromicrograms (Table VI, Figure 11).

The mean corpuscular hemoglobin concentration exhibited the average or "mean" hemoglobin concentration in grams per 100 ml. of packed erythrocytes or percent hemoglobin per cell. There was little variation in the mean corpuscular hemoglobin concentration from day to day during the period covered by the project (Table VI, Figure 12). At fifteen weeks of age, the mean corpuscular hemoglobin was within the range of 33.9% to 38.1%. The range of values for this study was 30.1% to 37.07%.

Correlation of Factors

The comparison of hemoglobin and erythrocyte counts showed a similarity in developmental stages except at eighteen days of incubation when erythrocyte count decreased but hemoglobin increased (Figure 13). The same comparison existed for hematocrit and erythrocyte count. The parallel development was dissimilar at twenty-two days when hematocrit decreases. Since hemoglobin was a calculated value, this may be a result of variations in values of specific gravity of whole blood, (Figure 14). Hemoglobin and hematocrit values are shown graphically in Figure 15. The changes occur at the same days and a trend toward the adult values began at twenty-two days. Although there was no direct relationship known between hemoglobin and plasma protein, the striking parallel in development is shown in Figure 16.

CONCLUSIONS

Some histological and physiological studies were made on the blood of the developing chick embryo from four days of incubation to seven days post-hatch. The following observations were made:

1. Erythrogenic stages in circulating blood were completed about the ninth day of incubation. This is earlier than formerly believed.

2. Leucocytes entered the circulating blood at five days of incubation and in greater numbers than formerly believed.

3. The differential leucocyte count showed increasing values for heterophiles from six days (20.9%) to twelve days of incubation (55.0%) when the heterophiles became stabilized. Small lymphocytes showed decreasing values from five days (81.0%) to nine days (36.7%) of incubation at which time they became stabilized. The acidophiles, basophiles, large lymphocytes and monocytes did not show significant changes during the period of the study.

4. The specific gravity of whole blood exhibited a linear relationship with time that is a result of the cumulative effect of other factors such as hematocrit, specific gravity of plasma, plasma protein, hemoglobin and total erythrocyte counts.

5. The specific gravity of plasma ranged from 1.0011 to 1.0171 with a marked increase at seven days of incubation. Hematocrit ranged from 13.6% to 37.9% and showed an increase at twelve and fourteen days. Plasma protein ranged from 2.98 grams/100 ml. to 13.8 grams/100 ml. with increases at seven and twelve days. Hemoglobin ranged from 4.94 grams/100 ml. to 13.41 grams/100 ml. with an increase at twelve days. Erythrocyte counts ranged from .440 million/cu. mm. to 2.711 million/cu. mm. and showed an increase at twelve days and a decrease at eighteen days. These

marked changes occurred at the same time as observed physiological changes such as functioning of the liver at seven days, development of the bone marrow at twelve days and the beginning regression of the allantois at eighteen days of incubation.

6. Total leucocyte counts showed a decline from a high count of 55.5 thousand/cu. mm. during the early stages of development to a lower count of about 30.0 thousand/cu. mm. at later stages.

7. The coagulation time as measured by the capillary method and the coagulation bleeding method showed a rapid and marked decrease from about four minutes and forty-eight seconds at fourteen days to seven and one tenth seconds at twenty-eight days.

8. Mean corpuscular volume varied greatly from 81.3 cu. microns to 190.7 cu. microns during the period of the study; the average for the period was close to the normal for the adult.

9. Mean corpuscular hemoglobin for the embryo was higher, averaging 44.9 micromicrograms during the developmental period investigated than the observed adult value which was 34.4 micromicrograms.

10. Mean corpuscular hemoglobin concentration was slightly higher than the observed adult values. The range of values for this study was 30.1% to 37.0%.

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TABLE I

Differential Leucocyte Counts of Chicks Indicating the Averages * for Each Day from Four Days of Incubation to Seven Days Post-Hatch

Days of Incubation	Acido-phile	Hetero-phile	Baso-phile	Lymphocytes Small	Lymphocytes Large	Lymphocytes Total	Mono-cytes
4	**0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	81.0	19.0	100.0	0.0
6	0.0	20.9	0.0	72.5	4.7	77.2	1.9
7	1.3	30.5	1.8	50.0	10.0	60.0	6.4
8	2.9	33.3	1.5	39.0	18.0	57.0	5.3
9	1.4	43.3	1.4	36.7	11.0	47.7	6.2
10	2.4	47.0	1.1	38.9	7.9	46.8	2.7
11	0.9	40.0	2.4	43.0	12.2	55.2	1.5
12	1.6	55.0	2.1	26.1	11.7	37.8	3.5
13	2.2	55.0	2.5	29.5	8.8	38.3	2.0
14	2.7	56.0	3.0	26.8	8.9	35.7	2.6
15	2.9	50.1	3.7	28.3	11.7	40.0	3.3
16	2.1	44.0	3.8	32.9	15.0	47.9	4.4
17	1.6	51.0	3.4	31.5	9.1	40.6	3.4
18	2.2	52.0	1.7	30.8	10.8	41.6	2.5
19	2.0	52.0	2.0	33.6	8.0	41.6	2.4
20	2.1	53.7	2.2	32.0	8.0	40.0	2.0
21	2.2	52.0	2.2	31.1	10.0	41.1	2.5
22	2.4	52.3	2.0	29.0	11.0	40.0	3.3
23	2.0	53.0	2.0	32.0	9.0	41.0	2.0
24	2.1	51.5	2.1	31.9	10.0	41.9	2.4
25	2.0	54.0	2.0	32.0	8.0	40.0	2.0
26	1.8	51.1	1.9	33.0	9.0	42.0	3.2
27	1.8	52.2	1.9	32.0	9.0	41.0	3.1
28	3.2	50.9	3.1	30.0	8.3	38.3	4.5

* Average of ten animals

**0 Indicates observation made, no count obtained

TABLE II

Specific Gravity of Whole Blood of the Chick from Four Days of Incubation to Seven Days Post-Hatch

Days of Incubation	*Animals A	B	C	D	E	F	G	H	I	J	K	L	Average
4	1.012	1.011											
5	1.016	1.016	1.017	1.014	1.013	1.012	1.011	1.017	1.016	1.018			1.015
6	1.014	1.019	1.016	1.014	1.014	1.016	1.017	1.019	1.015	1.018			1.016
7	1.020	1.019	1.018	1.019	1.022	1.023	1.017	1.018	1.018	1.021			1.019
8	1.020	1.019	1.021	1.018	1.021	1.021	1.023	1.024	1.023	1.026			1.021
9	1.025	1.022	1.024	1.025	1.023	1.022	1.022	1.022	1.028	1.025			1.023
10	1.025	1.024	1.023	1.022	1.021	1.022	1.023	1.022	1.023	1.024			1.023
11	1.022	1.022	1.024	1.021	1.023	1.022	1.022	1.022	1.021	1.025			1.022
12	1.024	1.029	1.025	1.023	1.023	1.025	1.023	1.025	1.023	1.025			1.024
13	1.029	1.030	1.030	1.030	1.030	1.030	1.029	1.029	1.026	1.028			1.028
14	1.031	1.032	1.033	1.030	1.033	1.033	1.030	1.025	1.031	1.031			1.031
15	1.031	1.030	1.030	1.029	1.034	1.032	1.035	1.036	1.035	1.031	1.034	1.031	1.032
16	1.037	1.037	1.034	1.035	1.037	1.035	1.035	1.033	1.032	1.036			1.035
17	1.039	1.039	1.035	1.038	1.036	1.035	1.039	1.036	1.037	1.035			1.036
18	1.037	1.036	1.039	1.040	1.040	1.041	1.040	1.038	1.039	1.038			1.038
19	1.038	1.038	1.040	1.039	1.040	1.040	1.037	1.041	1.040	1.039	1.038		1.040
20	1.039	1.039	1.040	1.042	1.041	1.042	1.040	1.039	1.040	1.039	1.039		1.040
21	1.039	1.042	1.044	1.038	1.039	1.038	1.043	1.040	1.042	1.040			1.040
22	1.043	1.039	1.035	1.038	1.035	1.038	1.040	1.038	1.039	1.038	1.040		1.041
23	1.040	1.042	1.040	1.043	1.043	1.040	1.041	1.042	1.040	1.042	1.043		1.041
24	1.040	1.040	1.042	1.041	1.042	1.040	1.042	1.040	1.041	1.040	1.042		1.041
25	1.043	1.040	1.041	1.043	1.043	1.042	1.041	1.043	1.043	1.042			1.042
26	1.041	1.043	1.043	1.042	1.041	1.042	1.044	1.044	1.042	1.042			1.042
27	1.042	1.043	1.042	1.044	1.043	1.043	1.044	1.044	1.042	1.044			1.043
28	1.045	1.043	1.043	1.045	1.045	1.046	1.044	1.043	1.045	1.045			1.044

*Separate animal used for each reading, i e. one reading per animal

TABLE III

Hematocrit Readings of Blood of the Chick from Four Days of Incubation to Seven Days Post-Hatch in Percent, Cells and Plasma

Days of Incubation	*ANIMALS A	B	C	D	E	F	G	H	I	J	Daily Average Cells	Plasma
4	14.2	16.6	10.2	11.2	16.6	22.9	13.9	10.8	12.8	9.4	13.6	86.4
5	12.5	18.0	19.6	19.8	10.4	15.3	18.7	12.5	20.0	11.0	15.6	84.4
6	12.5	21.4	11.9	13.0	10.0	19.6	18.3	21.2	12.0	11.1	15.1	84.9
7	16.7	18.3	19.0	13.4	21.6	21.0	18.0	20.8	22.9	21.0	19.2	80.8
8	9.8	13.1	10.9	22.3	13.3	16.0	13.6	18.8	22.7	20.7	16.1	83.9
9	14.5	17.3	12.5	9.6	10.9	17.7	20.9	15.7	23.4	28.6	17.1	82.9
10	14.1	13.2	20.6	14.5	14.9	12.7	12.9	18.1	12.0	18.0	15.1	84.9
11	12.2	15.0	18.2	12.2	12.5	19.0	13.1	11.5	18.8	22.0	15.4	84.6
12	17.3	13.2	16.6	23.5	14.0	16.6	15.1	17.2	14.6	17.4	16.5	83.5
13	20.9	24.3	20.5	19.3	23.9	25.0	29.0	27.5	22.2	25.0	23.7	76.3
14	28.3	25.0	20.2	29.4	21.8	20.5	29.1	23.1	29.0	21.6	25.1	74.9
15	29.4	34.0	38.0	31.5	27.1	30.4	37.0	32.6	29.3	25.5	31.4	68.6
16	27.1	23.4	33.3	32.6	30.0	31.9	28.9	35.0	36.8	33.0	31.4	68.6
17	31.6	34.6	34.5	32.9	43.2	43.0	30.0	26.0	28.0	30.0	33.3	66.7
18	40.2	34.6	39.2	35.1	38.1	32.7	32.8	32.5	36.3	36.8	35.8	64.2
19	38.6	32.7	39.4	36.2	34.6	38.7	37.8	39.4	35.5	39.4	36.8	63.2
20	38.7	37.4	36.5	39.3	37.7	39.7	35.5	38.6	37.4	38.0	37.9	62.1
21	33.3	40.0	39.0	35.0	33.6	35.7	34.0	34.8	35.5	37.2	35.4	64.5
22	35.8	31.5	34.7	35.0	37.2	36.4	32.2	33.0	34.6	36.0	34.6	65.3
23	34.6	30.5	33.4	30.0	32.0	33.0	30.0	35.2	32.4	34.0	32.5	67.5
24	35.0	37.0	37.2	35.4	34.0	35.0	36.0	36.0	37.0	36.0	35.4	64.6
25	35.2	36.0	35.1	34.2	36.5	35.0	34.9	35.2	36.0	36.0	35.4	64.6
26	34.2	32.1	32.2	34.4	35.0	35.1	35.3	33.0	32.0	34.0	33.5	66.5
27	32.0	32.7	30.2	31.2	35.4	33.0	34.0	33.6	32.1	32.0	32.6	67.4
28	35.0	35.3	34.0	34.0	36.0	35.0	37.0	34.4	35.8	34.2	35.0	65.0

* Separate animals used for each readi g, i.e. one reading per animal

TABLE IV

Coagulation Time of the Blood of the Chick from Four Days of Incubation to Seven Days Post-Hatch by Capillary Method in Minutes and Seconds

Days of Incubation	** Animals										
	A	B	C	D	E	F	G	H	I	J	Average
11	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'	*10'+
12	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'+
13	20'	18'	20'	20'	20'	20'	20'	20'	20'	20'	19'48"
14	10'	11'	12"	20'	12'	12'	12'	11'	11'	15'	11'36"
15	5'	4'	4'	6'	5'	6'	4'	3'	4'	4'	4'30"
16	2'50"	3'	3'	2'40"	2'30"	3'	3'	2'40"	2'50"	2'50"	2'48"
17	3'	1'55"	2'30"	2'40"	2'10"	2'30"	2'25"	2'50"	2'40"	2'30"	2'30"
18	2'30"	2'15"	1'45"	2'10"	1'50"	2'10"	1'40"	1'45"	1'50"	2'15"	2'
19	1'50"	2'	2'	1'30"	1'30"	1'45"	1'40"	1'35"	1'35"	1'40"	1'40"
20	50"	1'10"	60"	55"	55"	1'5"	60"	1'15"	55"	60"	1'
21	60"	40"	50"	40"	40"	50"	50"	45"	60"	40"	47"
22	60"	40"	55"	40"	40"	50"	40"	45"	55"	45"	47.5"
23	20"	20"	25"	25"	25"	20"	25"	20"	20"	25"	22.5"
24	15"	14"	16"	17"	15"	18"	19"	17"	16"	14"	16"
25	15"	12"	12"	15"	13"	12"	15"	15"	13"	13"	13.5"
26	12"	14"	13"	15"	15"	14"	15"	12"	15"	12"	13.7"
27	12"	12"	15"	15"	14"	13"	13"	14"	14"	14"	13.7"
28	12"	15"	13"	10"	11"	12"	14"	13"	13"	11"	12.4"

* Blood dried without clotting at ten minutes
** Separate animal used for each reading, i.e. one reading per animal

TABLE V

Coagulation Time of the Blood of the Chick from Four Days of Incubation to Seven Days Post-Hatch by Coagulation-Bleeding Method in Minutes and Seconds

Days of Incubation	** Animals A	B	C	D	E	F	G	H	I	J	Average
11	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'	*10'+
12	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'	10'+
13	20'	20'	20'	20'	20'	20'	20'	20'	20'	20'	20'
14	5'	3'	4'	5'	6'	4'	5'	5'	6'	5'	4'48"
15	3'	4'	4'	5'	5'	3'	5'	5'	4'	4'	4'06"
16	1'45"	2'05"	1'50"	2'	2'05"	1'50"	1'40"	1'45"	1'55"	2'05"	1'54"
17	1'30"	1'15"	1'45"	1'30"	1'30"	1'40"	1'50"	1'25"	1'30"	1'40"	1'30"
18	1'10"	1'10"	1'30"	1'20"	1'20"	1'25"	1'20"	1'25"	1'15"	1'20"	1'19"
19	1'	45"	50"	40"	45"	45"	40"	40"	45"	40"	45"
20	20"	30"	25"	30"	30"	30"	22"	22"	20"	25"	25.9"
21	10"	12"	12"	15"	14"	14"	12"	12"	10"	12"	12.3"
22	10"	10"	10"	15"	10"	10"	14"	13"	15"	10"	11.7"
23	10"	15"	10"	10"	10"	12"	12"	10"	12"	10"	11"
24	10"	10"	9"	10"	10"	11"	10"	9"	11"	9"	9.9"
25	7"	8"	10"	5"	7"	9"	7"	8"	10"	9"	8.1"
26	7"	9"	8"	9"	7"	8"	9"	6"	7"	7"	7.7"
27	9"	8"	8"	7"	7"	8"	9"	9"	8"	7"	8.0"
28	10"	5"	7"	5"	9"	9"	7"	5"	7"	7"	7.1"

* Blood dried without clotting at ten minutes

** Separate animal used for each reading, i.e. one reading per animal.

TABLE VI

Summary of Results Indicating the Averages* for Each Day of Incubation of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch

	Averages							
Days of Incubation	Erythrocytes in millions per cu. mm.	Leucocytes in Thousands per cu. mm.	Specific Gravity of Plasma	Plasma Protein in Gms.per 100 ml	Hemoglobin in Gms.per 100 ml.	Mean Corpuscular Volume in Cu. Microns	Mean Corpuscular Hemoglobin in Micro-micrograms	Mean Corpuscular Hemoglobin Concentration in percent
4	.440	**—	—	—	—	—	—	—
5	.488	—	1.0011	2.98	4.94	—	112.3	31.69
6	1.274	55.5	1.0017	3.35	5.18	118.5	106.2	34.35
7	1.419	59.6	1.0011	4.64	6.51	135.3	45.9	33.95
8	1.354	67.6	1.0072	5.44	5.47	119.0	40.4	33.94
9	1.544	62.2	1.0088	6.26	5.80	110.8	37.5	33.92
10	1.588	59.3	1.0099	5.96	5.13	95.5	32.4	33.99
11	1.894	53.1	1.0089	5.74	5.23	81.3	27.6	33.96
12	1.662	50.8	1.0078	6.32	6.13	99.5	36.9	37.07
13	2.092	49.0	1.0079	8.16	8.04	113.2	38.4	33.94
14	2.506	58.5	1.0101	9.25	8.40	110.1	33.5	33.48
15	2.796	47.0	1.0041	9.39	10.67	124.4	38.1	33.90
16	2.952	39.4	1.0069	10.48	10.68	106.3	36.1	33.93
17	2.874	35.5	1.0063	10.96	11.32	116.1	39.4	33.92
18	1.878	31.4	1.0054	11.56	12.14	190.7	64.6	33.89
19	2.276	32.6	1.0055	11.93	12.84	161.6	56.4	34.89
20	2.560	30.0	1.0057	12.30	12.81	148.0	50.0	33.80
21	2.742	31.5	1.0097	12.45	12.95	129.1	47.2	36.60
22	2.686	31.9	1.0116	12.68	10.44	128.8	38.8	30.17
23	2.710	30.0	1.0144	12.68	10.81	119.9	39.9	33.27
24	2.720	29.5	1.0125	12.68	12.26	132.3	45.0	34.06
25	2.675	31.0	1.0121	13.05	12.02	131.5	44.6	33.95
26	2.722	29.9	1.0115	13.05	12.17	123.0	44.7	36.34
27	2.685	29.3	1.0171	13.42	11.06	121.6	41.3	33.94
28	2.711	30.5	1.0156	13.80	12.43	129.6	49.5	38.25

* Average of ten animals

**— Indicates no observation made

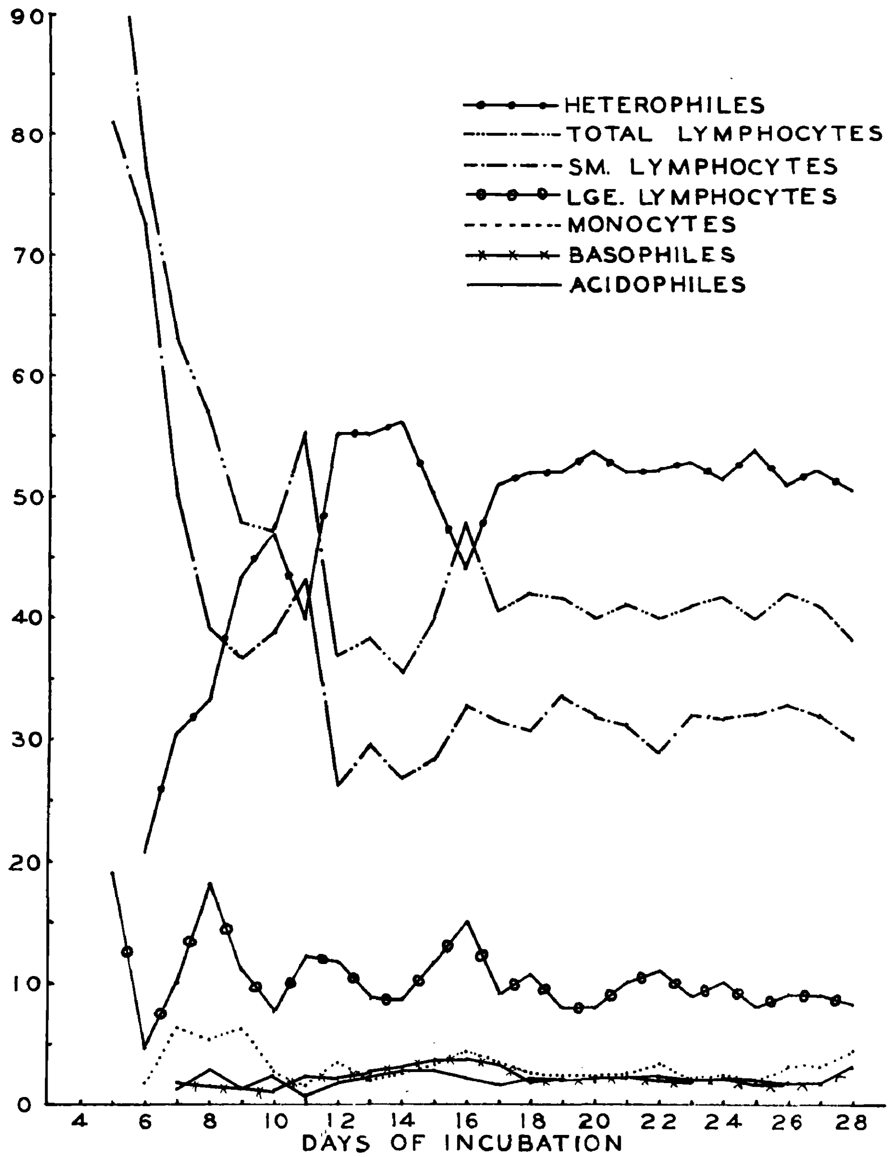


FIG. I DIFFERENTIAL LEUCOCYTE COUNT

FIGURE 1

Differential Leucocyte Count of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. Each Measurement is Average Value from Ten Chicks.

FIGURE 2

Specific Gravity of Whole Blood
of Young Chick from Four Days of Incubation to Seven Days Post-Hatch.

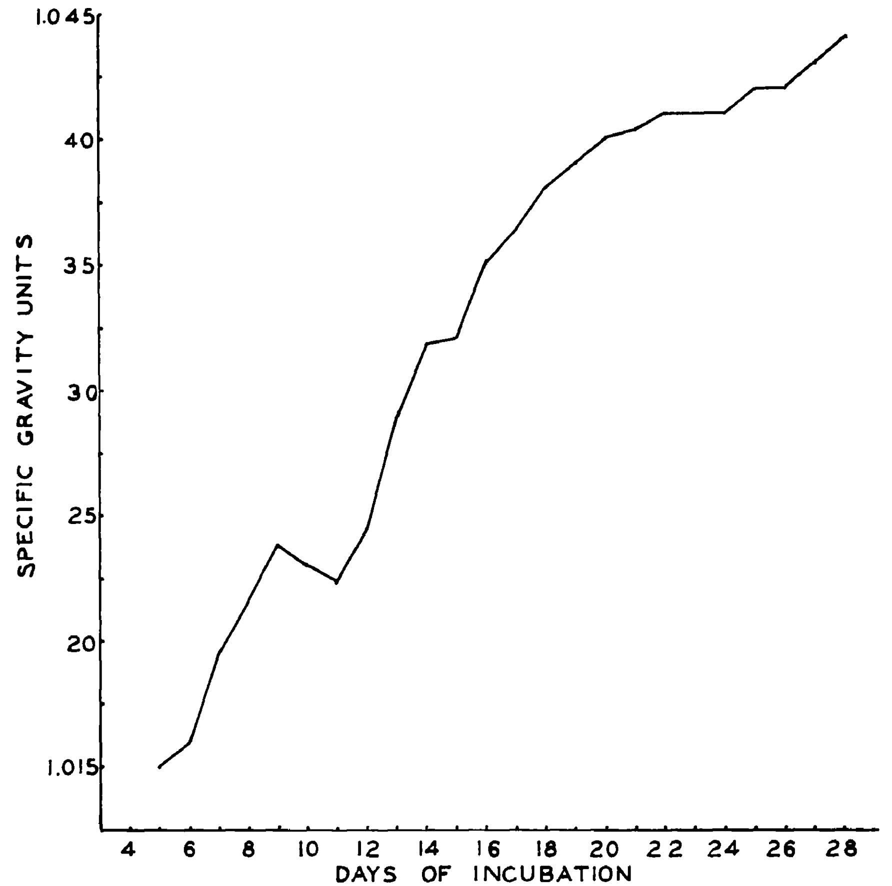


FIG.2 SPECIFIC GRAVITY OF WHOLE BLOOD

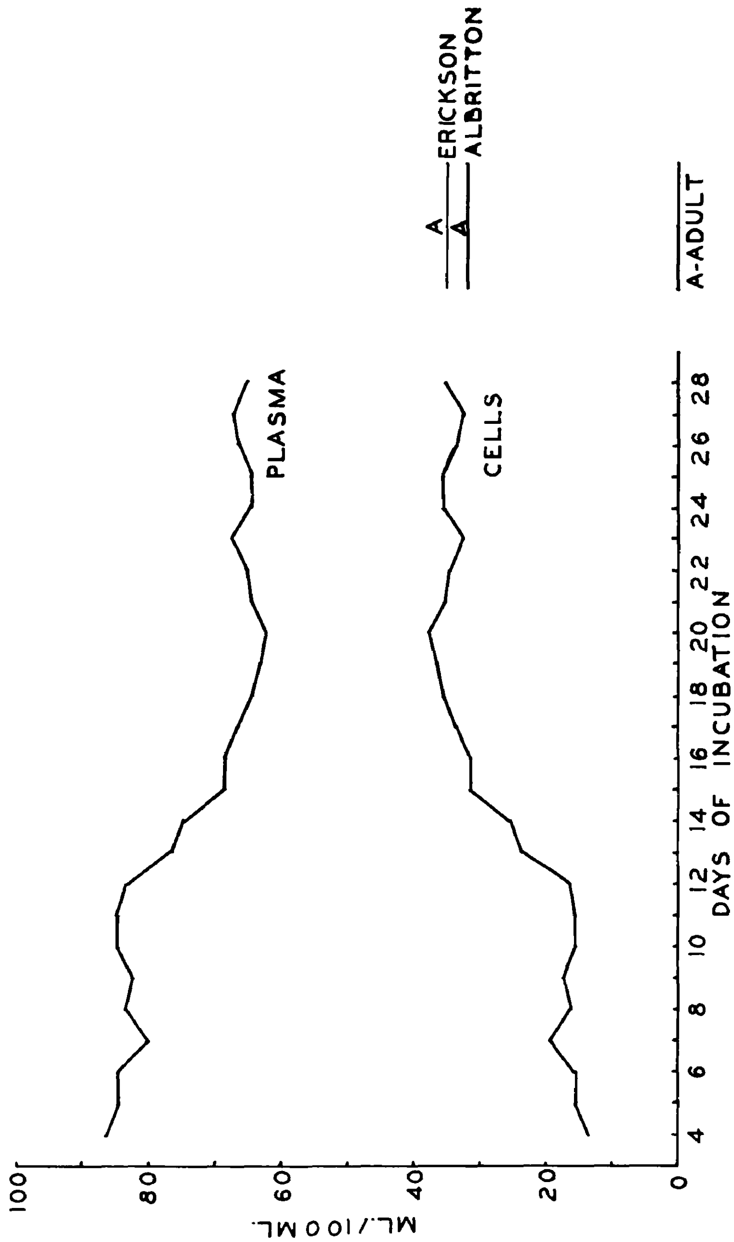


FIG. 3 HEMATOCRIT READINGS

FIGURE 3

Hematocrit

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. At right Indicated are Adult Values Obtained by Other Investigators.

FIGURE 4

Erythrocyte Count

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. At right are Values Obtained by Other Investigators.

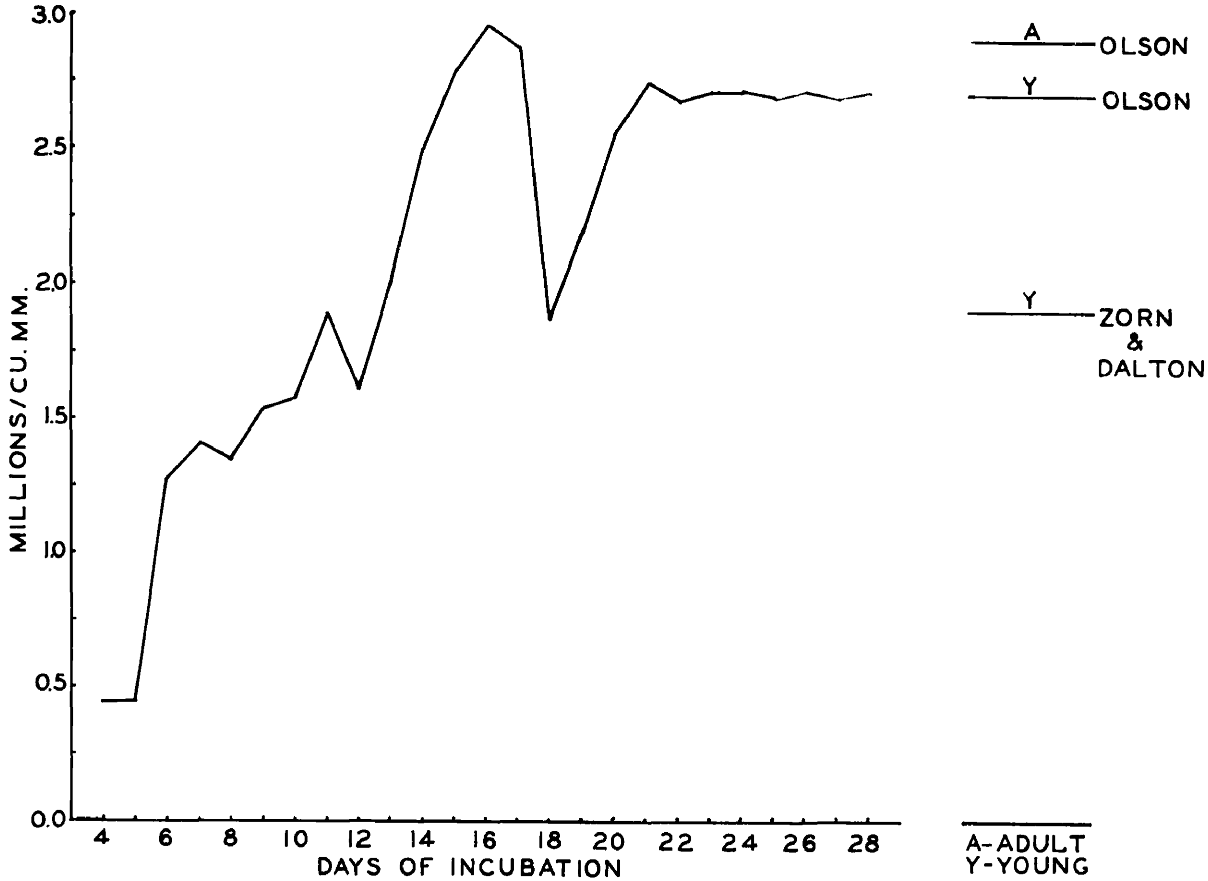


FIG. 4 TOTAL ERYTHROCYTE COUNT

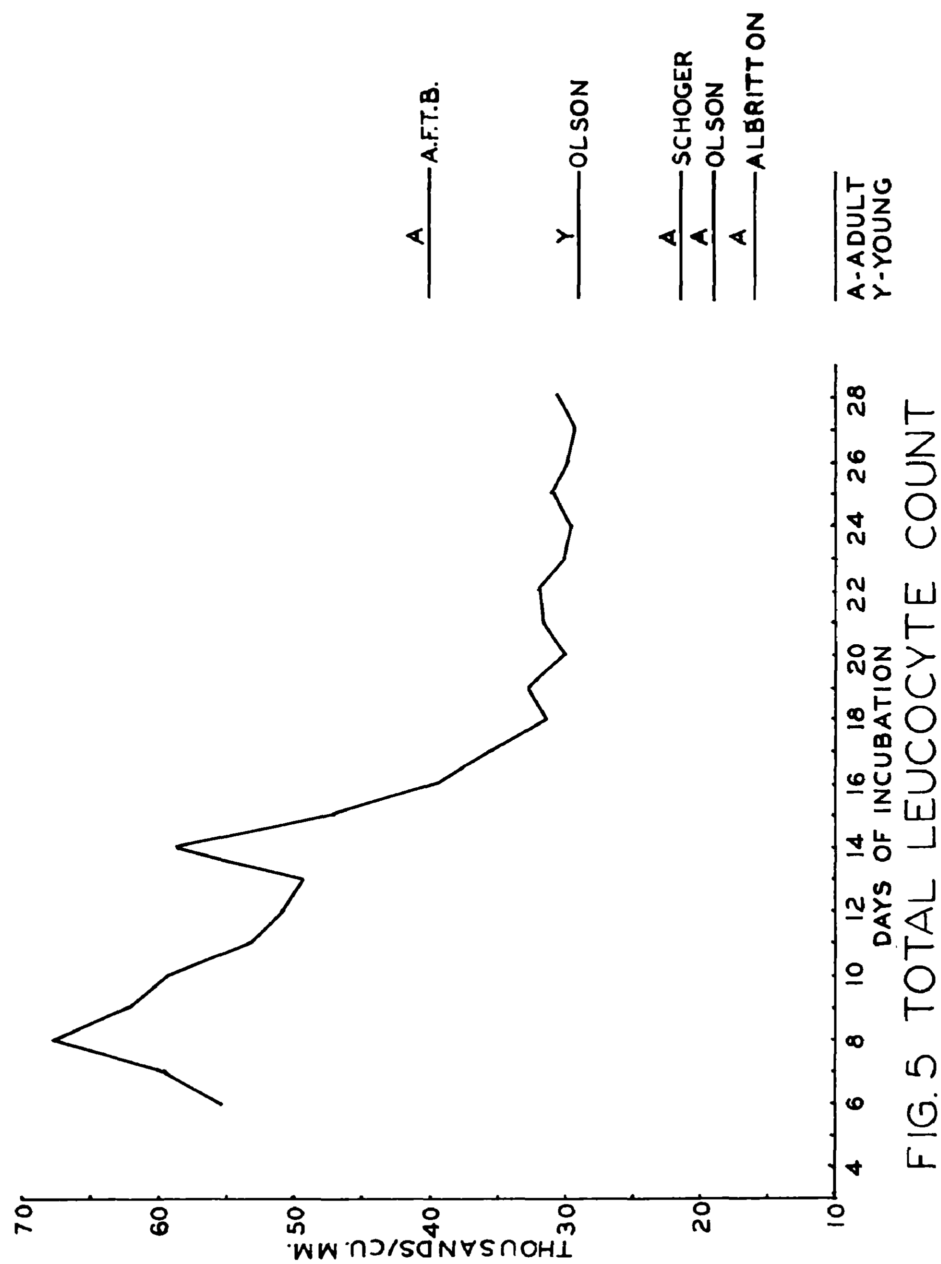
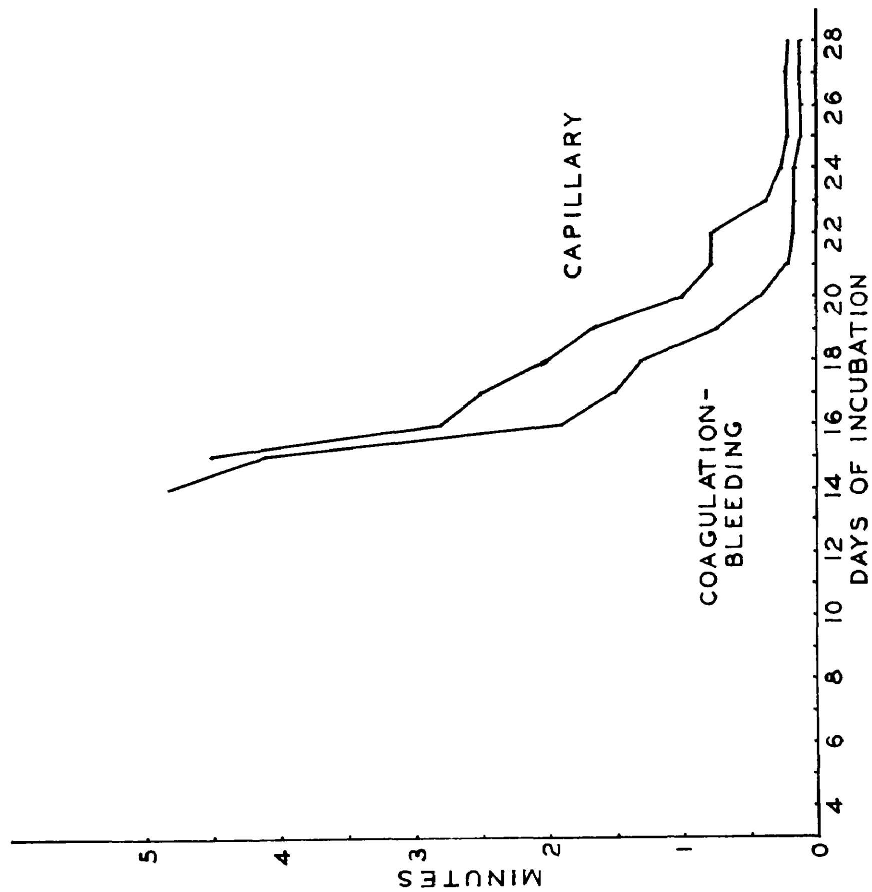


FIGURE 5

Leucocyte Count
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.
At Right are Values Obtained by Other Investigators.



F IG. 6 COAGULATION TIME

FIGURE 6

Coagulation Time
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

FIGURE 7

Specific Gravity of Blood Plasma
of Chicks from Four Days of Incubation to Seven Days Post-Hatch.

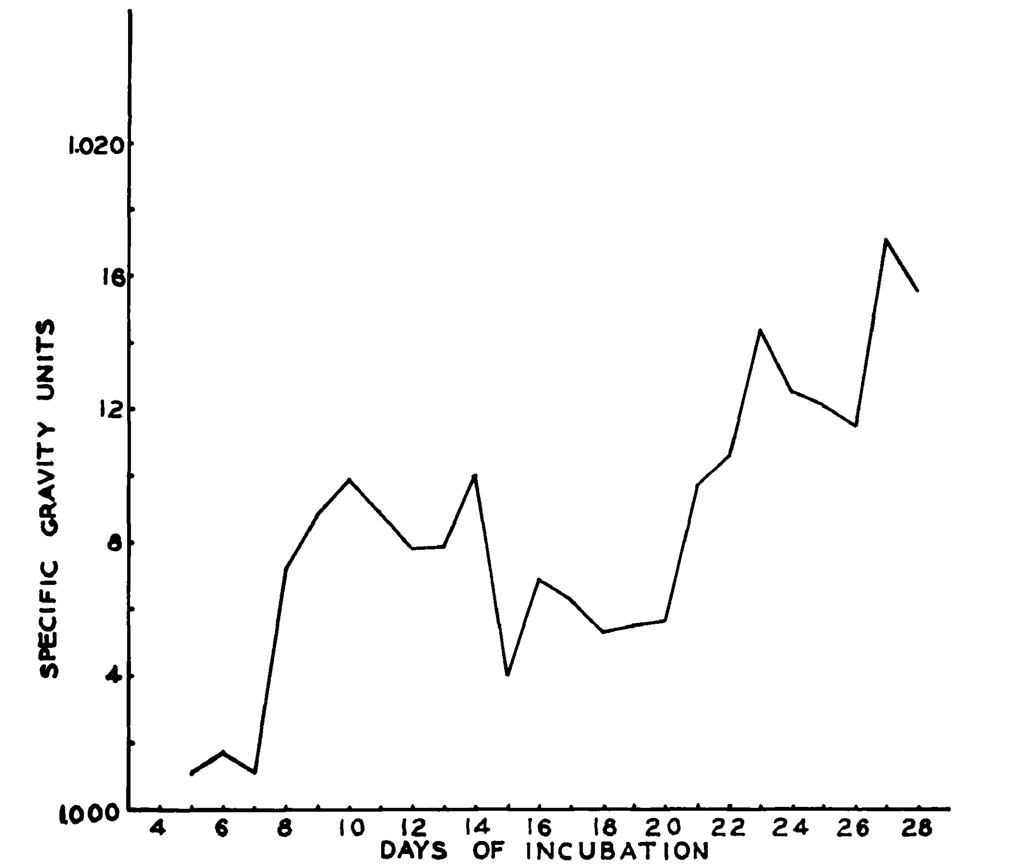


FIG. 7 SPECIFIC GRAVITY OF BLOOD PLASMA

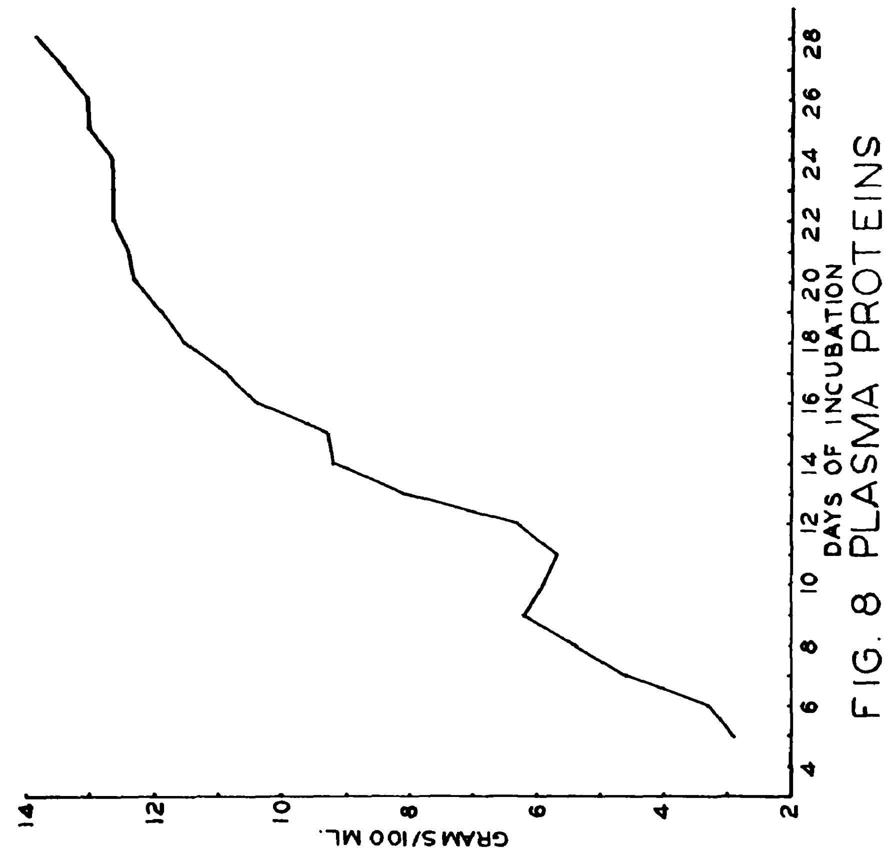


FIGURE 8

Plasma Protein

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

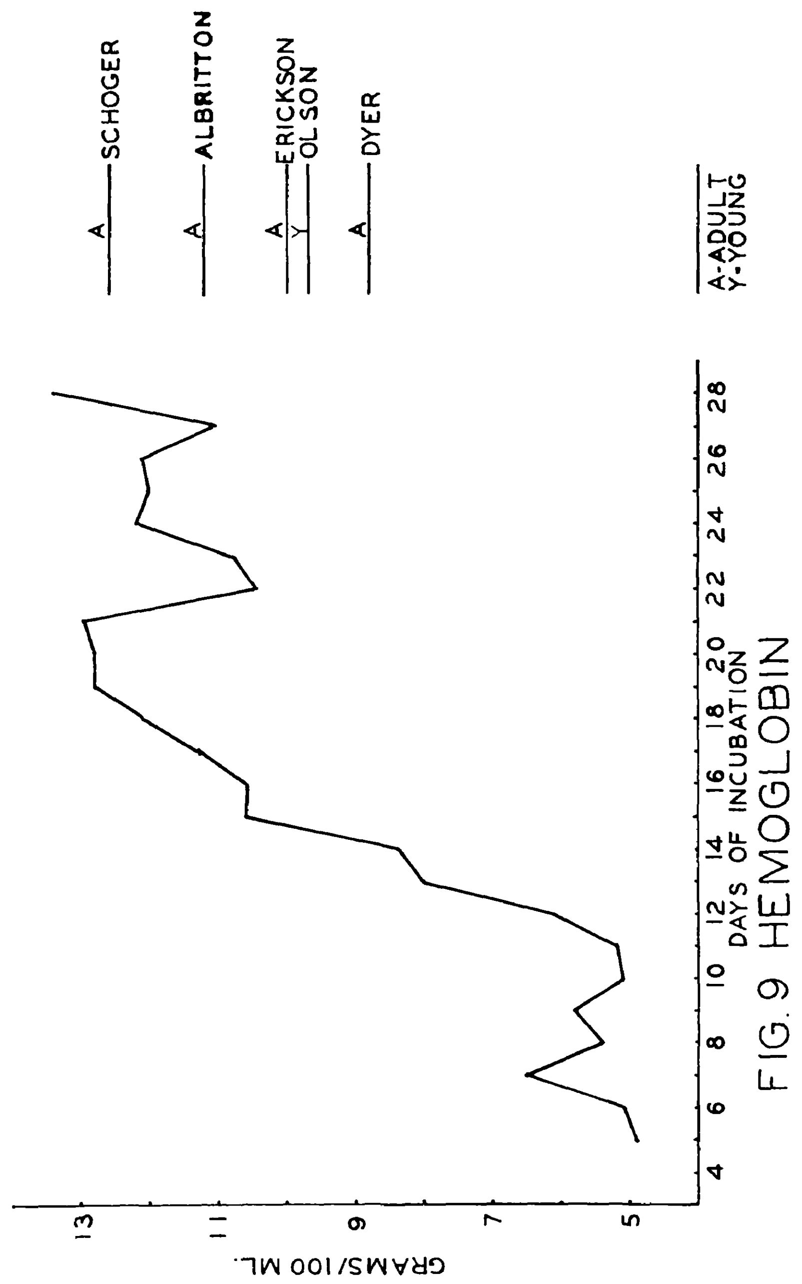


FIGURE 9

Hemoglobin
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. At Right are Values Obtained by Other Investigators.

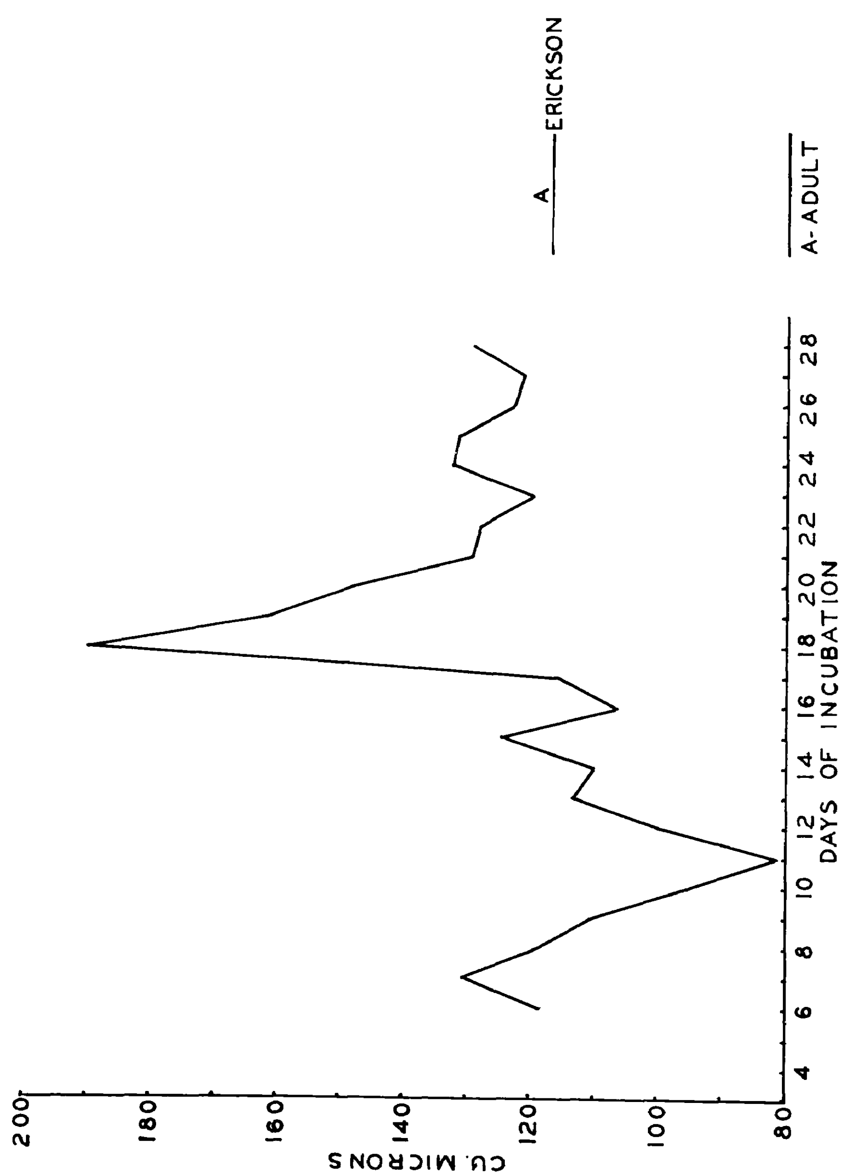


FIG.10 MEAN CORPUSCULAR VOLUME

FIGURE 10

Mean Corpuscular Volume
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.
At Right is Adult Value as Obtained by Another Investigator.

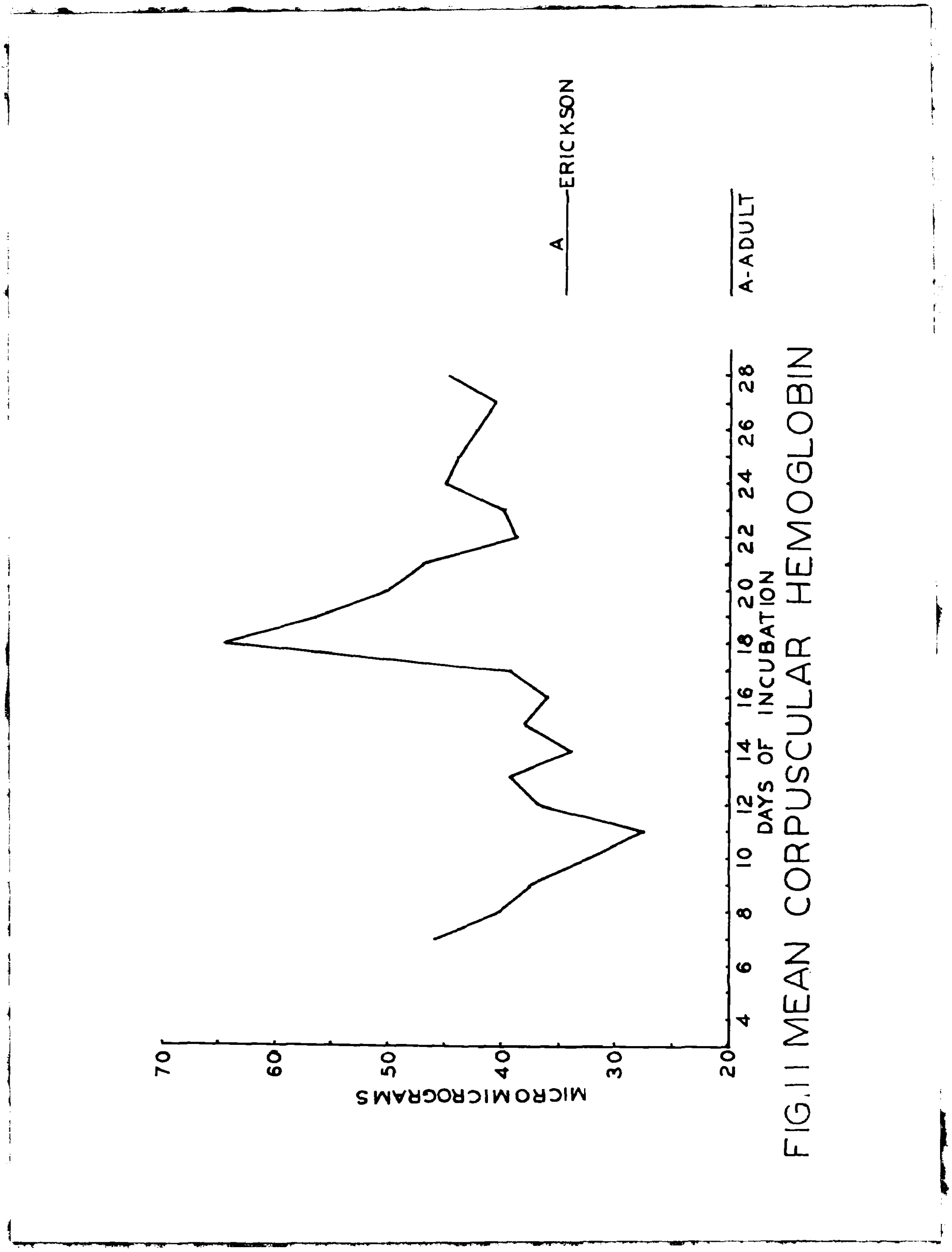


FIGURE 11

Mean Corpuscular Hemoglobin
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. At Right is Adult Value as Obtained by Another Investigator.

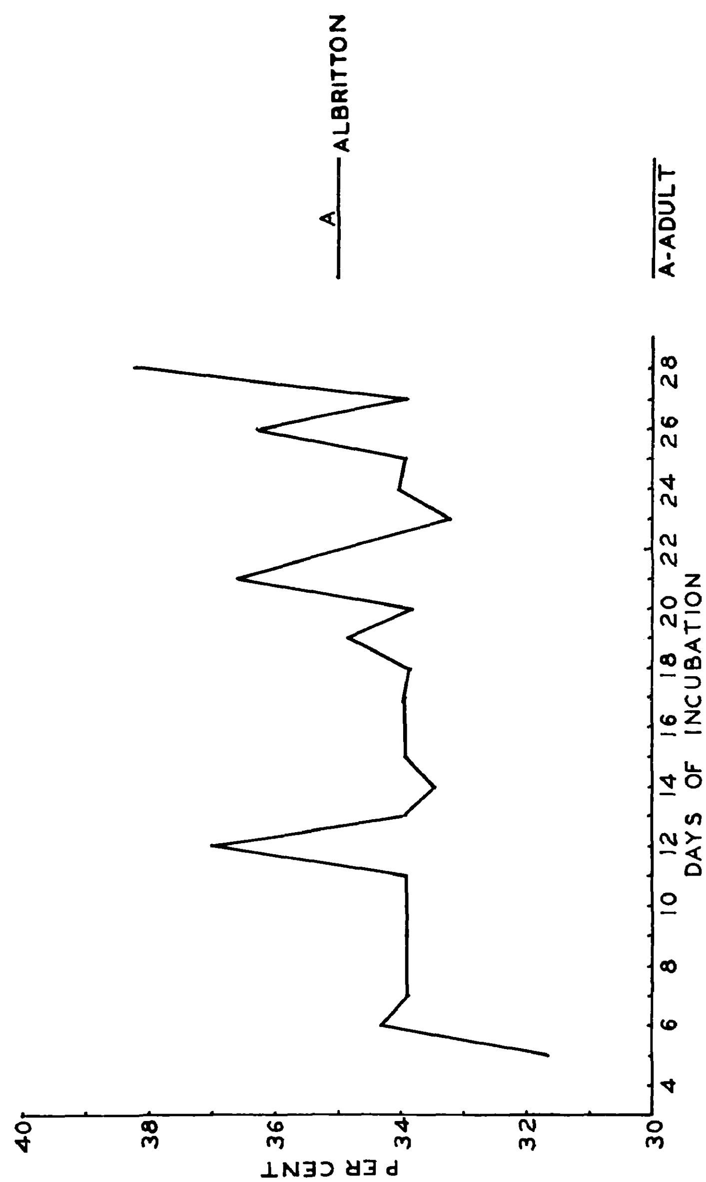


FIG.12 MEAN CORPUSCULAR HEMOGLOBIN CONC.

FIGURE 12

Mean Corpuscular Hemoglobin Concentration
of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch. At Right is Adult Value as Obtained by Another Investigator.

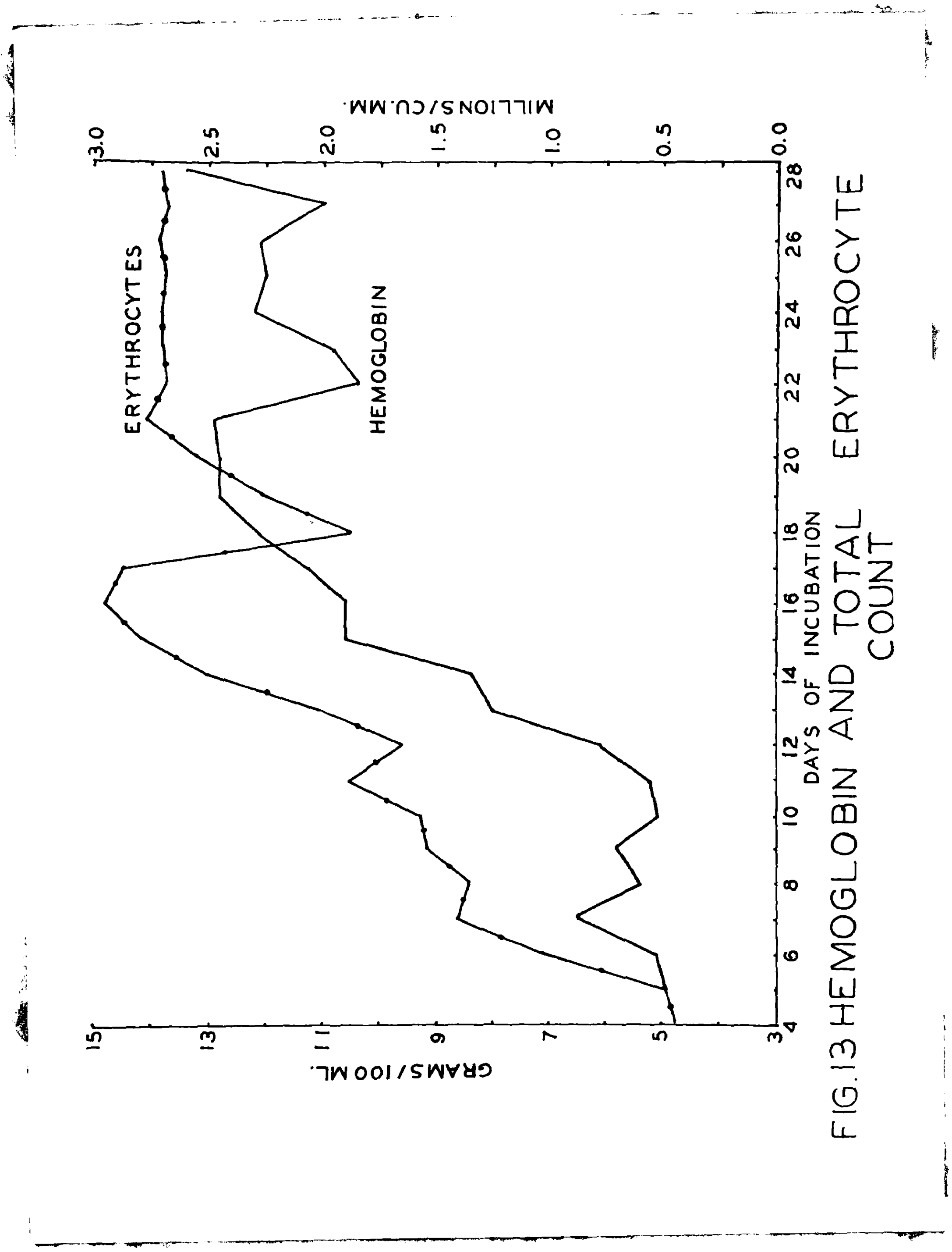


FIGURE 13

Hemoglobin and Erythrocyte Count

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

FIG.14 HEMATOCRIT AND TOTAL ERYTHROCYTE COUNT

FIGURE 14

Hematocrit and Erythrocyte Count

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

FIGURE 15

Hemoglobin and Hematocrit

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

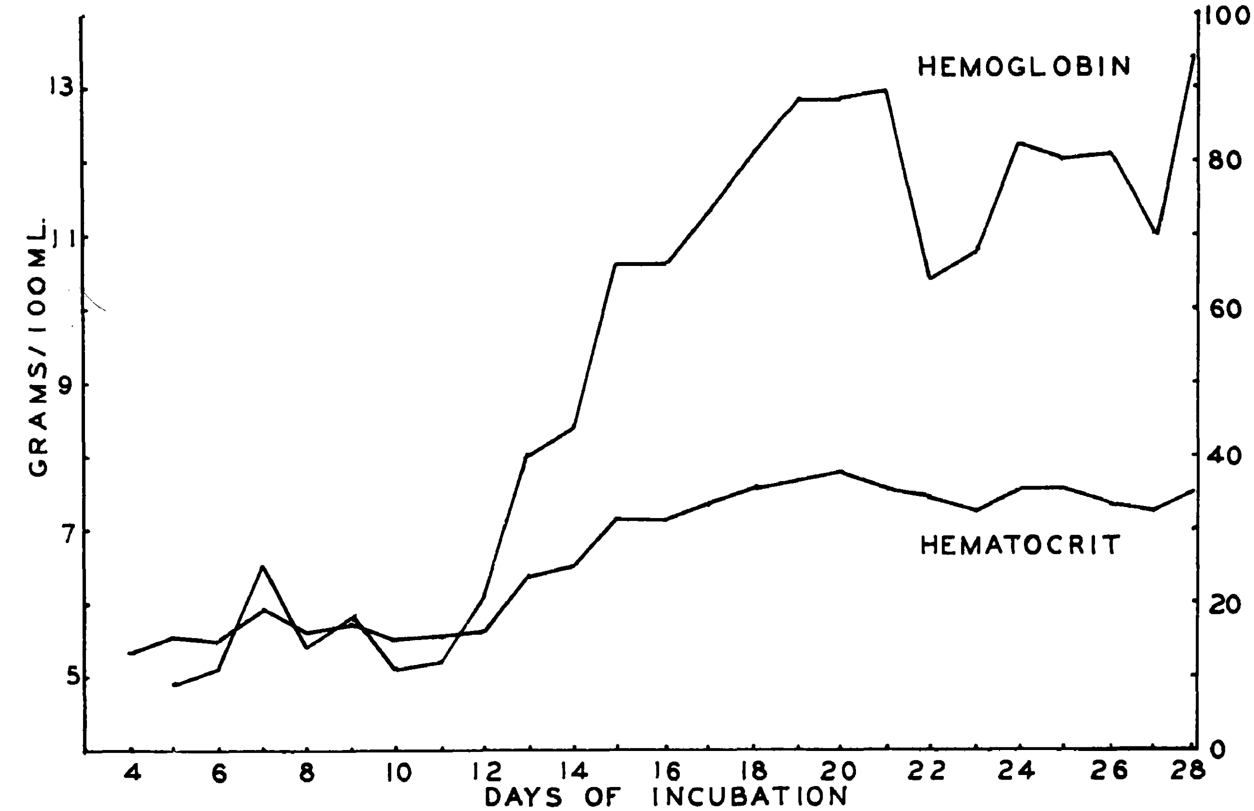


FIG.15 HEMOGLOBIN AND HEMATOCRIT

FIGURE 16

Hemoglobin and Plasma Protein

of Chick Blood from Four Days of Incubation to Seven Days Post-Hatch.

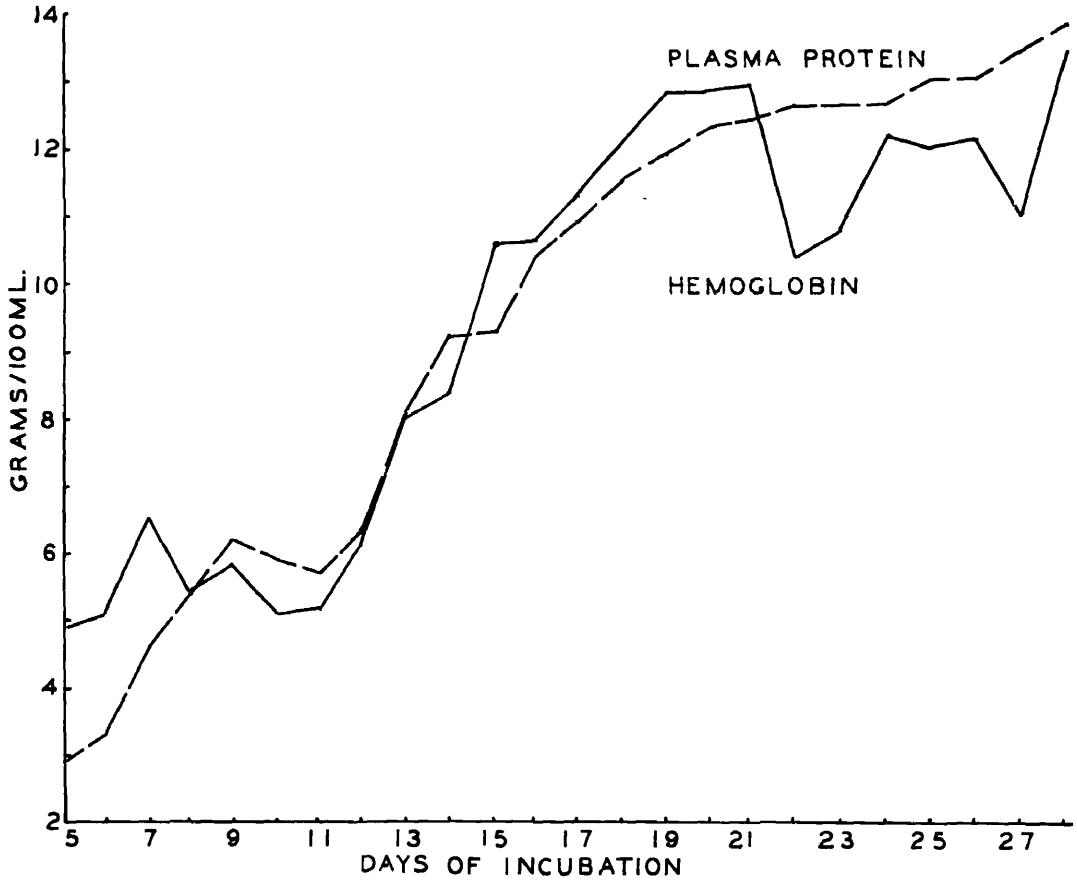


FIG.16 HEMOGLOBIN AND PLASMA PROTEIN

BLOOD OF THE DEVELOPING CHICK

FIGURE 17

Erythroblasts at four days of incubation

BLOOD OF THE DEVELOPING CHICK

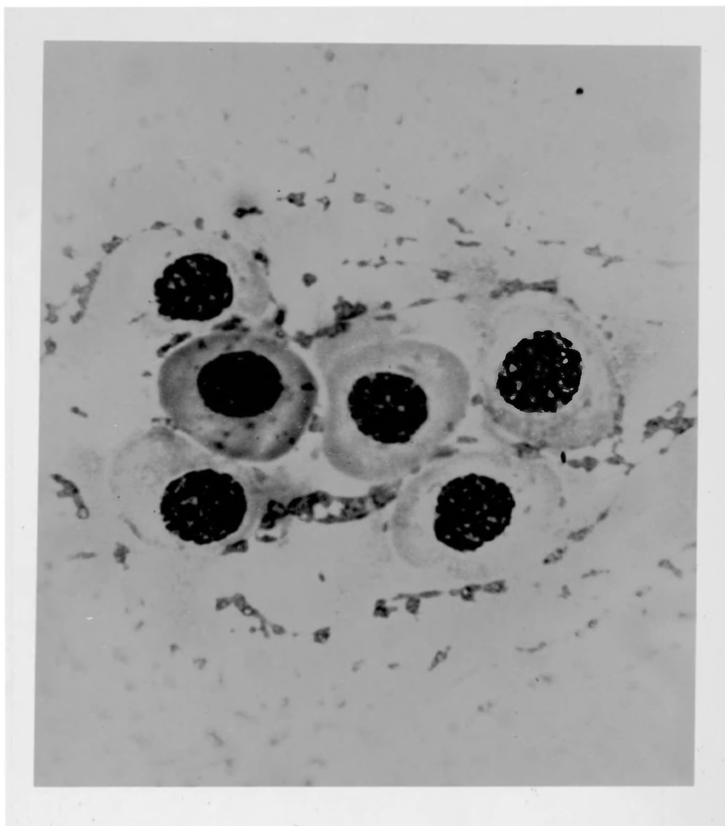


FIGURE 18

Erythroblasts at five days of incubation. (1200X)

BLOOD OF THE DEVELOPING CHICK

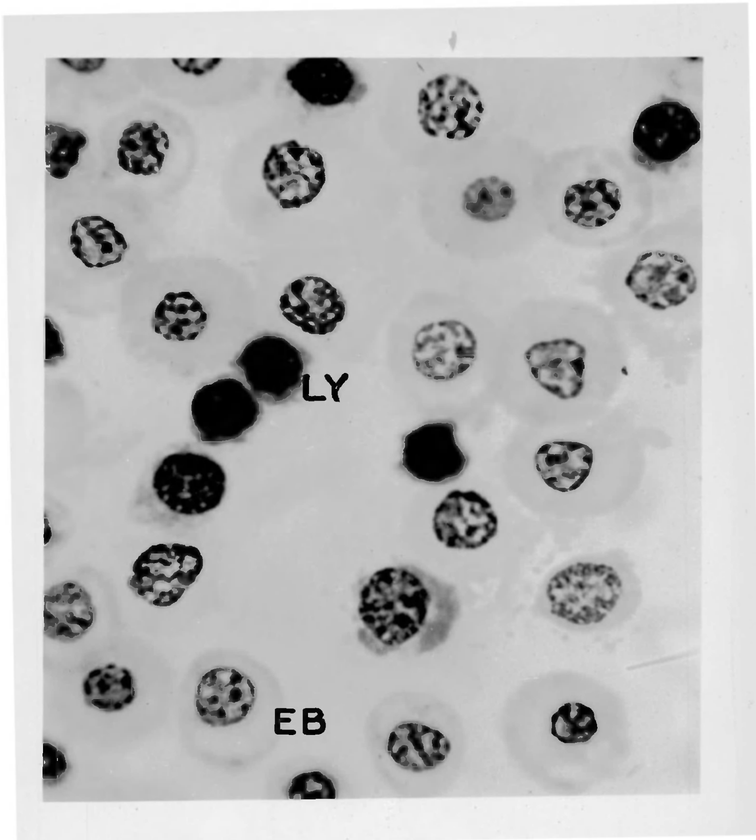


FIGURE 19

Erythroblasts (EB) and lymphocytes (LY) at six days of incubation. (1200X)

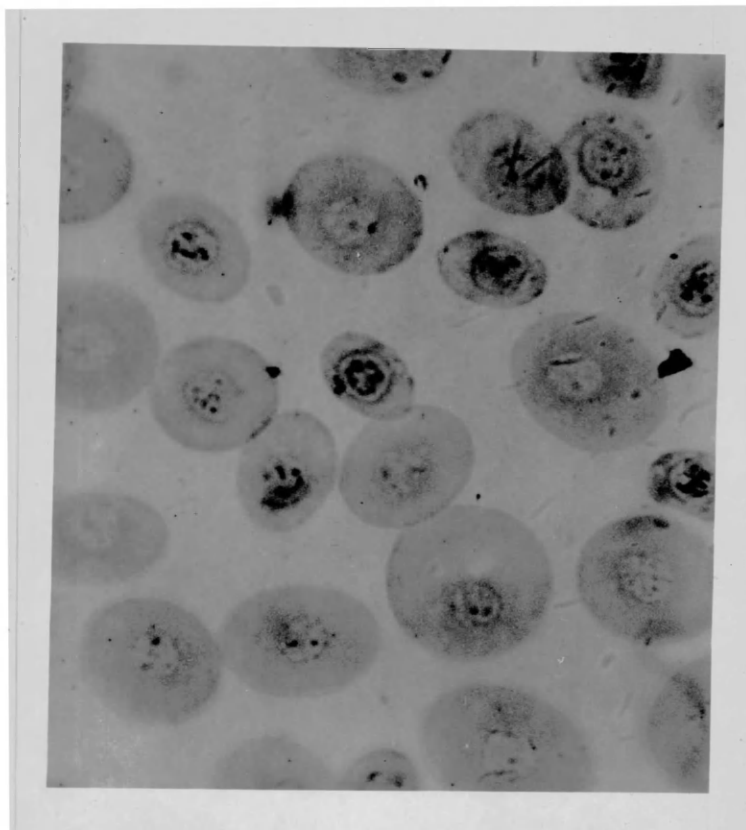


FIGURE 20

Erythroblasts at seven days of incubation. (1200X)

BLOOD OF THE DEVELOPING CHICK

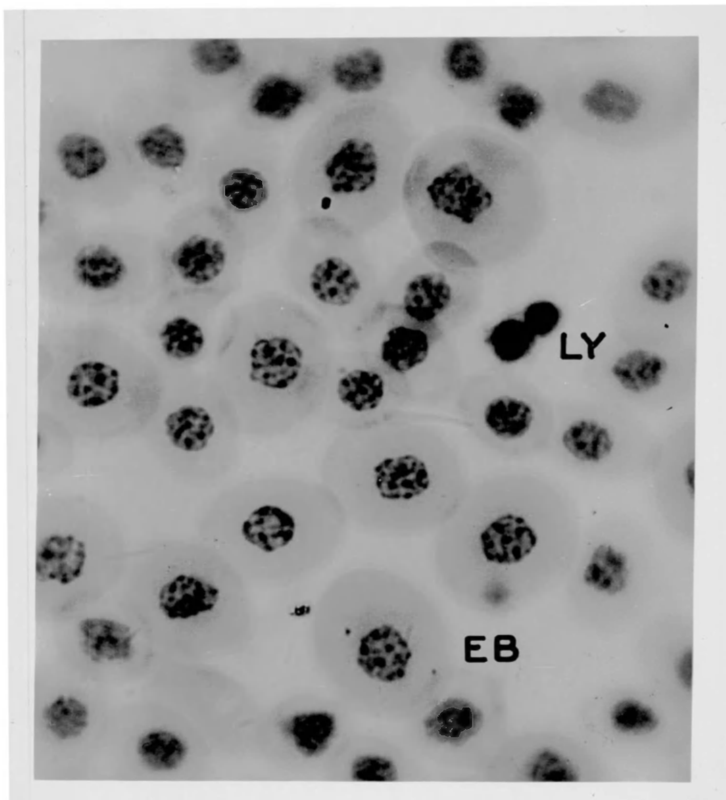


FIGURE 21

Erythroblasts (EB) and lymphocytes (LY) at eight days of incubation. (1200X)

BLOOD OF THE DEVELOPING CHICK

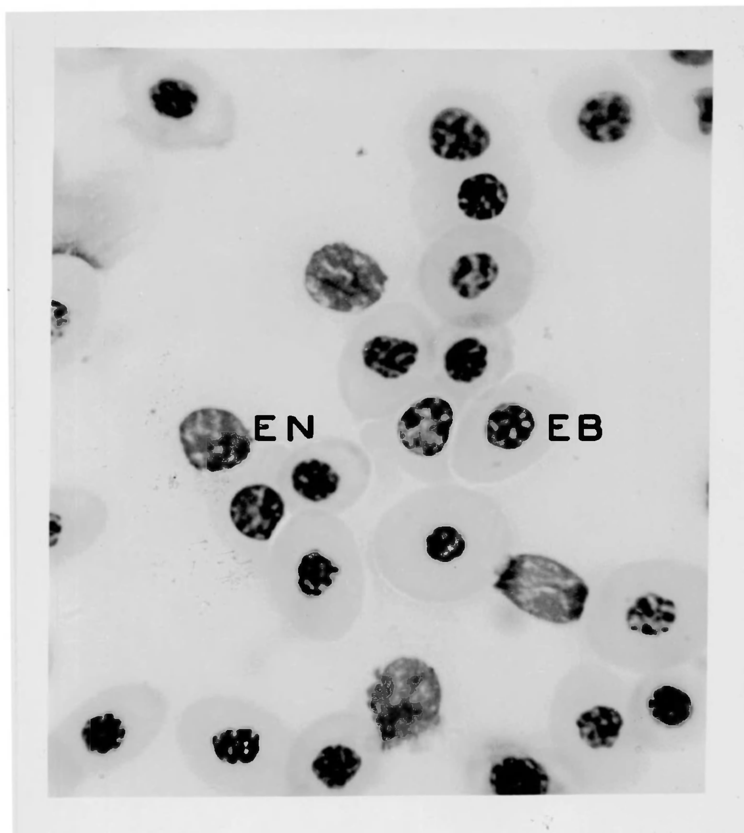


FIGURE 22

Erythroblasts (EB) and endothelial cells (EN) at nine days of incubation. (1200X)

BLOOD OF THE DEVELOPING CHICK

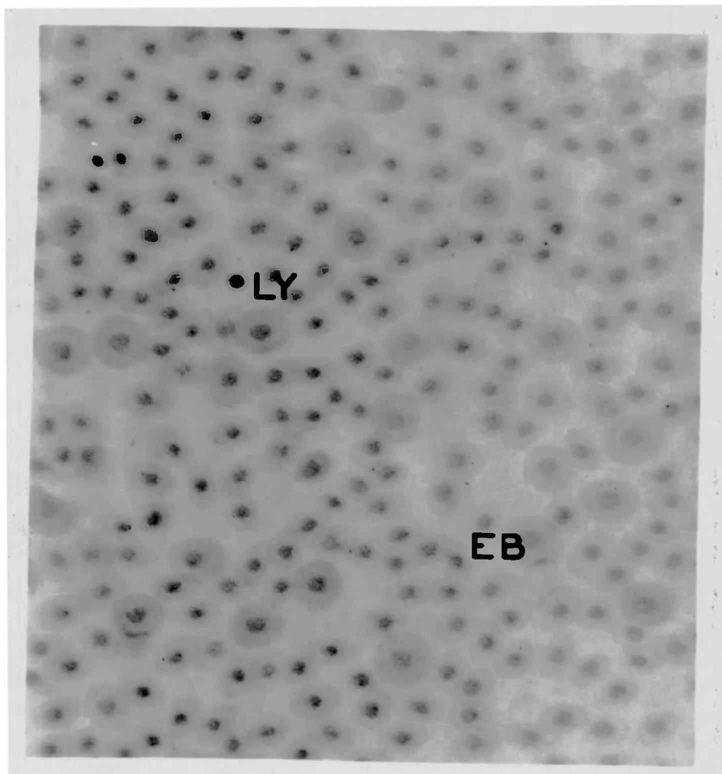


FIGURE 23

Erythroblasts (EB) and lymphocytes (LY) at ten days of incubation. (500X)

BLOOD OF THE DEVELOPING CHICK

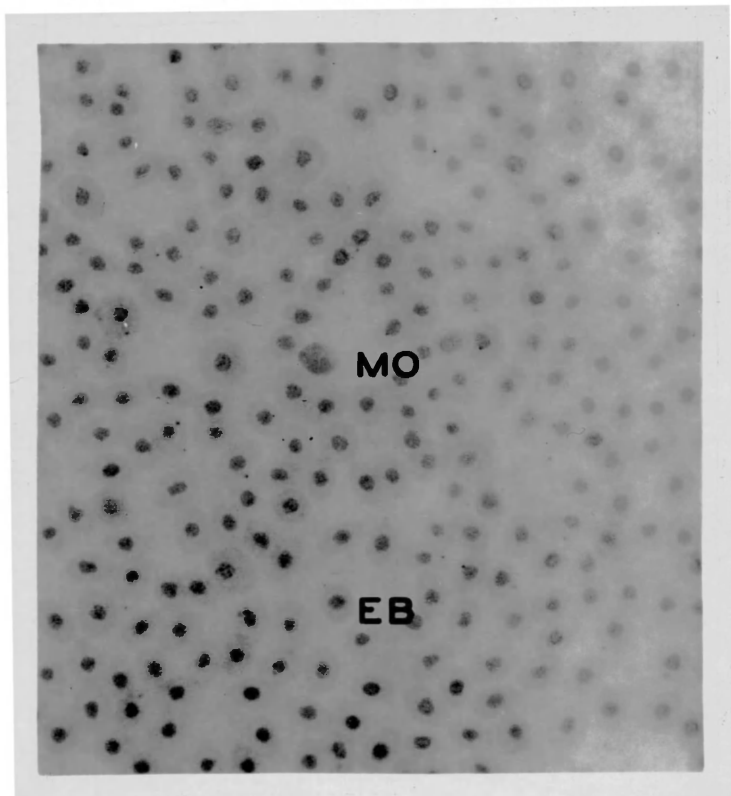


FIGURE 24

Erythroblasts (EB) and a Monocyte (MO) at twelve days of incubation. (500X)

BLOOD OF THE DEVELOPING CHICK

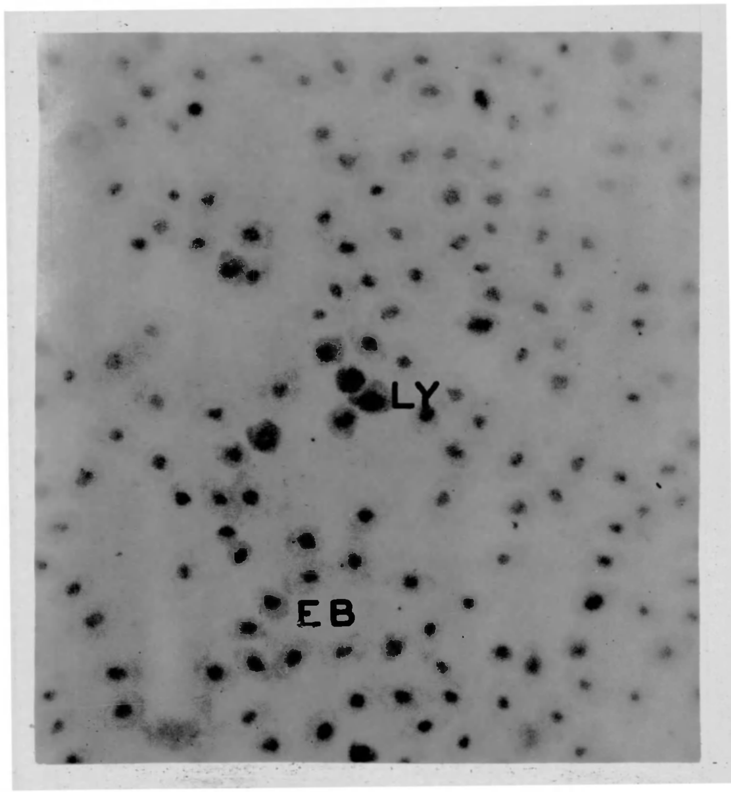


FIGURE 25

Erythroblasts (EB) and lymphocytes (LY) at fourteen days of incubation. (500X)

BLOOD OF THE DEVELOPING CHICK

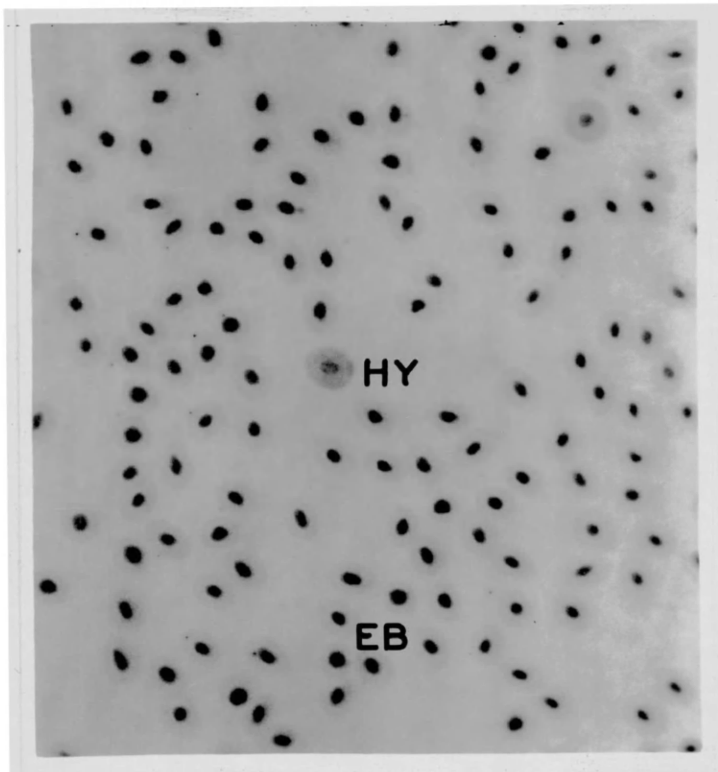


FIGURE 26

Erythroblasts (EB) and hyperchromic (HY) erythroblasts at sixteen days of incubation. (500X)

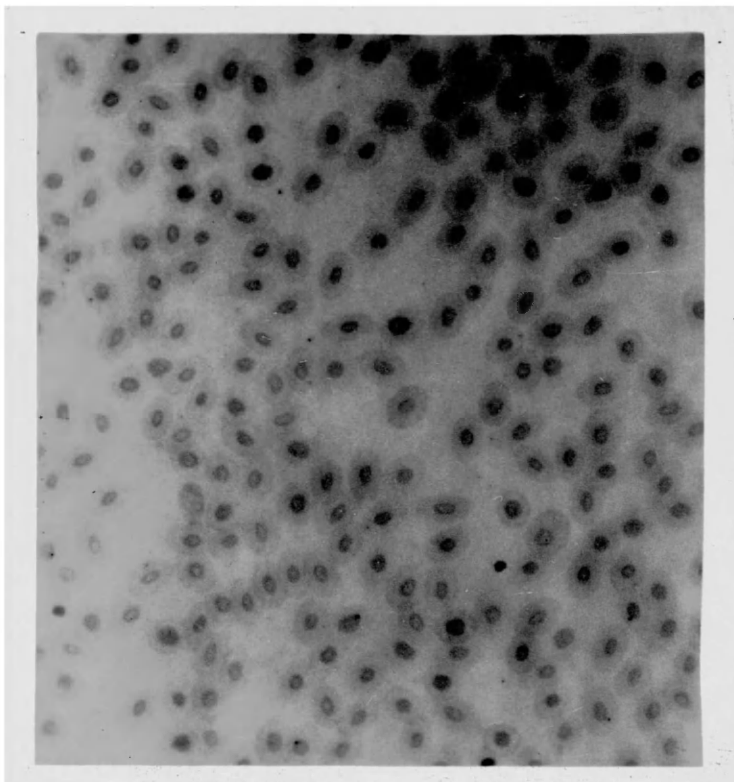


FIGURE 27

Erythroblasts at eighteen days of incubation. (500X)

BLOOD OF THE DEVELOPING CHICK

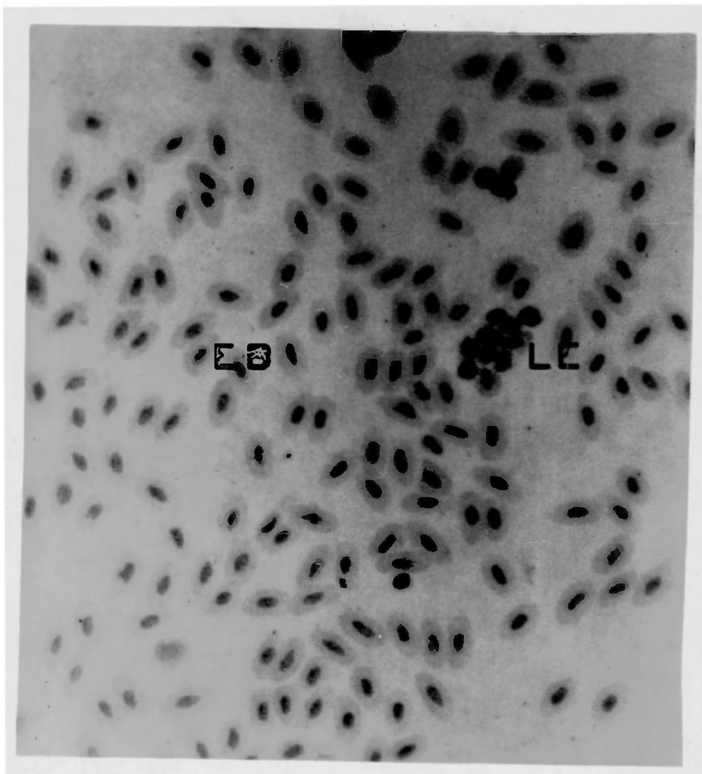


FIGURE 28

Erythroblasts (EB) and leucocytes (LE) at twenty days of incubation. (500X)

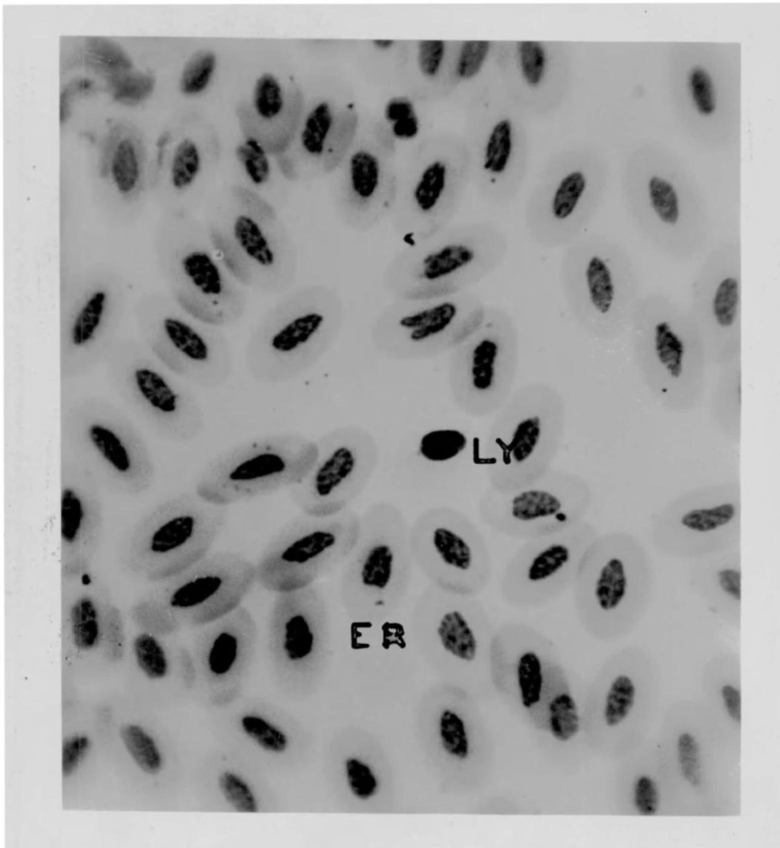


FIGURE 29

Erythrocytes (ER) and lymphocyte (LY) at one day post-hatch. (1200X)

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BLOOD OF THE DEVELOPING CHICK

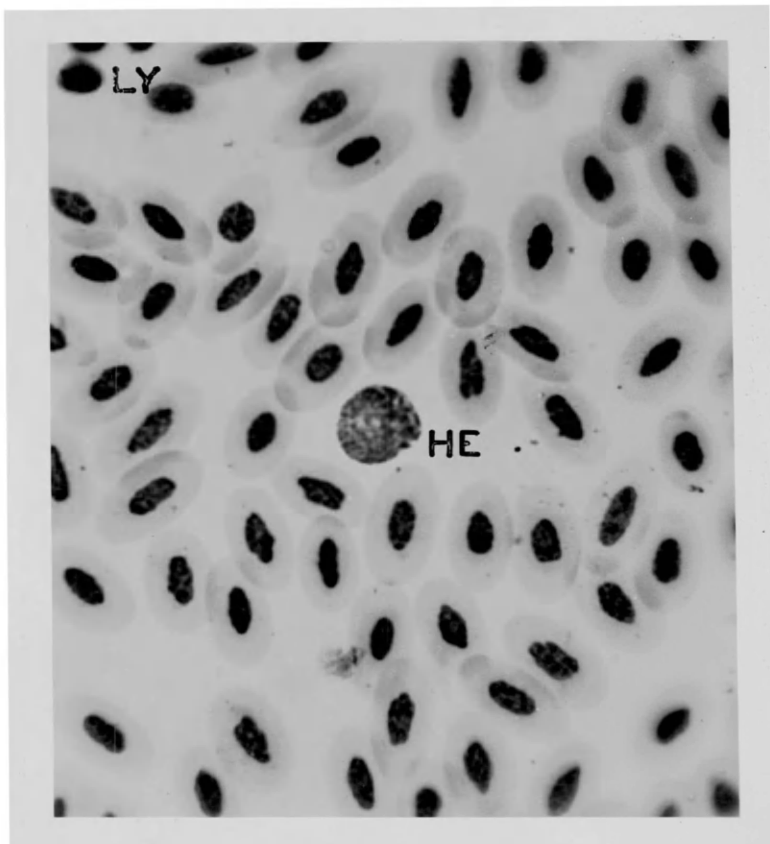

FIGURE 30

Erythrocytes heterophile (HE) and lymphocyte (LY) at three days post-hatch (1200X)

BLOOD OF THE DEVELOPING CHICK

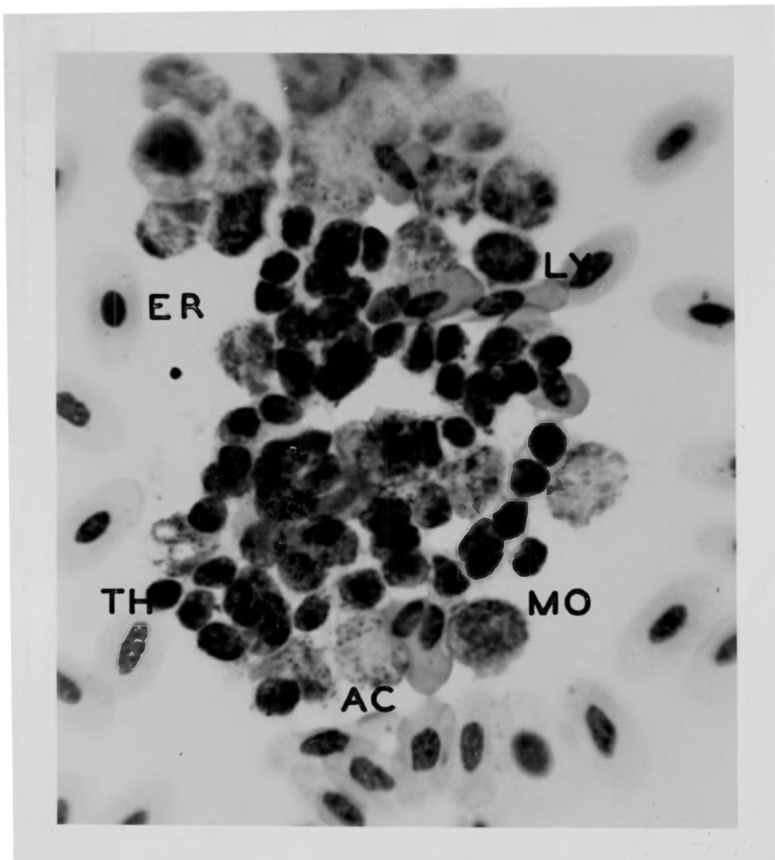

FIGURE 31

Erythrocytes (ER), acidophiles (AC), lymphocytes (LY), monocytes (MO) and thrombocytes (TH) at five days post hatch (1200X)

BLOOD OF THE DEVELOPING CHICK

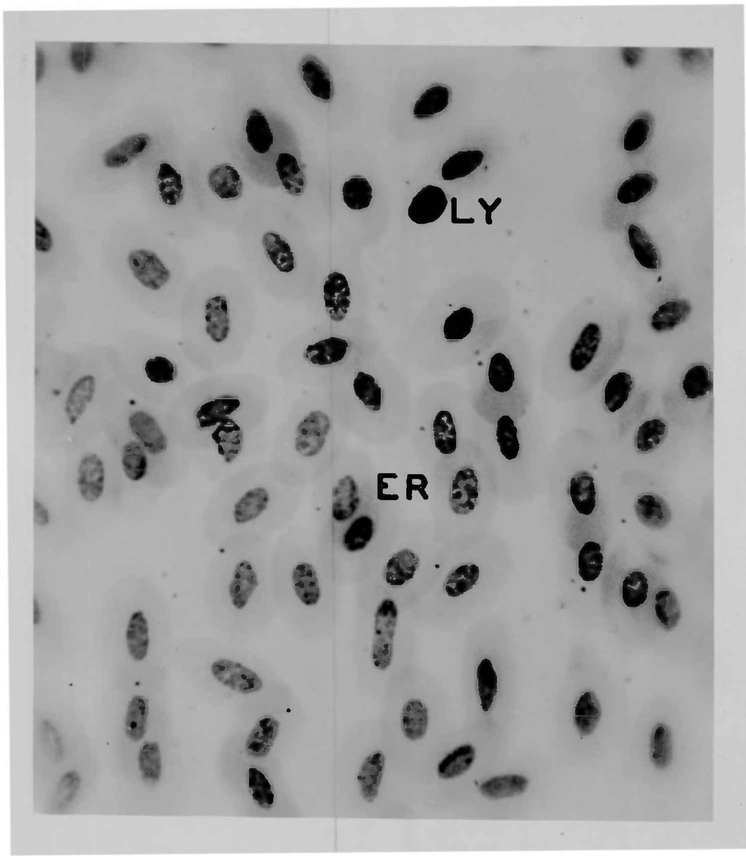

FIGURE 32

Erythrocytes (ER) and lymphocyte (LY) at seven days post-hatch (1200X)

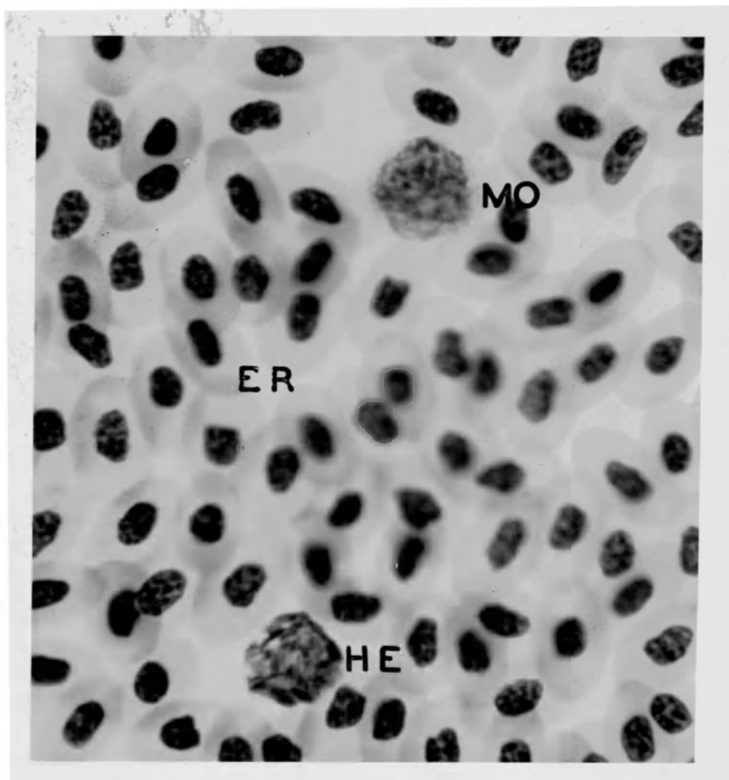

FIGURE 33

Erythrocytes (ER), heterophile (HE) and monocyte (MO) of the adult chicken (1200X)

BLOOD OF THE DEVELOPING CHICK

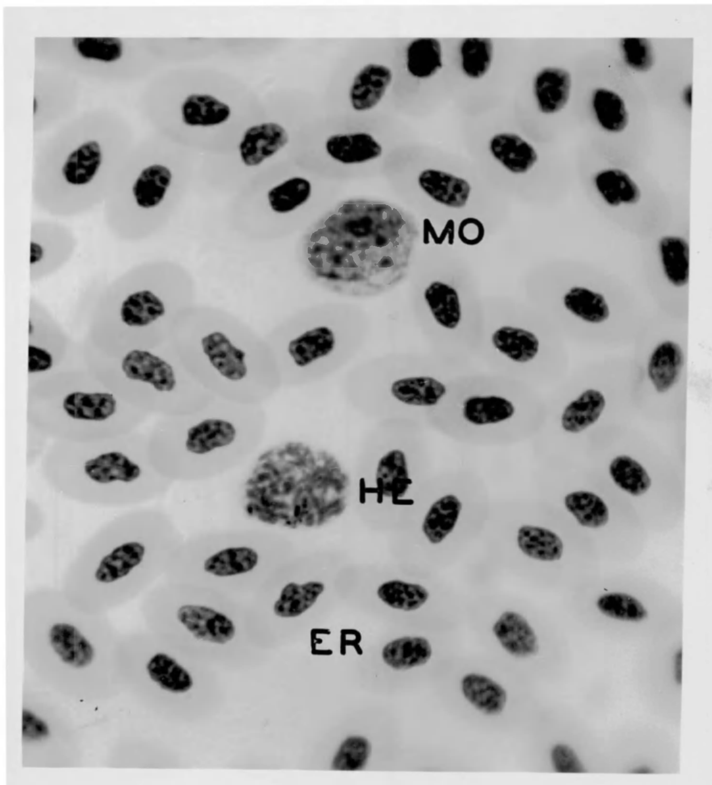

FIGURE 34

Erythrocytes (ER), heterophile (HE) and monocyte (MO) of the adult chicken (1200X)

VITA SHEET

Name	Kenneth Thompson Stringer
Degree and Date	Doctor of Philosophy, August 1953
Date of Birth	September 22, 1922
Place of Birth	Baltimore, Maryland
Secondary Education	San Pedro High School San Pedro, California
	Portsmouth High School Portsmouth, New Hampshire

Collegiate Institutions Attended	Date	Degree	Date of Degree
University of Maryland	1940-1943 1945-1946	B.S.	June 1946
University of Maryland	1946-1948	M.S.	June 1948
University of Maryland	1948-1953	Ph.D.	August 1953

Publications	None
Positions	Graduate Assistant in Zoology 1946-1948
	Instructor in Zoology 1948-1953