THE BONE MARROW IN PREGNANCY AND THE PUERPERIUM

by

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Thesis

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INTRODUCTION

The changes in the blood of pregnant women have fascinated many obstetricians and hematologists. A high incidence of anemia has been observed in pregnancy throughout history. Moreover it has been acknowledged that the study of the blood may contribute to the establishment of a proper diagnosis and satisfactory treatment in many disorders of pregnancy.

An increasing awareness of the essential importance of prenatal care for the pregnant woman led to the performance of routine hematological studies along with other examinations necessary to detect the earliest deviation from normality. It was soon realized that the hematological determinations of the blood of pregnant women yield different results from those of normal men and non pregnant women.

The present work was part of a project undertaken by the Hematology Service of the Department of Medicine of the Royal Victoria Hospital and forms one aspect of a general survey of blood changes in physiological and pathological pregnancies.

The blood of a large number of pregnant women is routinely examined in the Bessborough Laboratory of the Montreal Maternity Hospital by the Hematology Service. These patients are sent by the Maternity Out-Patient Clinic and come to this laboratory at least twice antepartum, once at the time of the first visit to the Out-Patient Department and once at approximately the eighth month of pregnancy. Another routine hematological examination is performed

on the second day after delivery and a final one at approximately six weeks postpartum in the Out-Patient Department.

The routine hematological examination consists of the determination of the hematocrit, hemoglobin, sedimentation rate, red cell, reticulocyte, white cell and differential counts. It is possible to detect many early cases of anemia by these screening measures.

Bone marrow aspiration is performed in the majority of cases presenting a borderline anemia. This procedure is often necessary for the determination of the type of anemia and the institution of rational specific therapy.

The hematological study of so many of these cases has prompted the examination of the bone marrow of normal women during pregnancy and the postpartum period. The concensus of opinion in the literature is that the hemoglobin, hematocrit and red cell values of the peripheral blood fall during normal pregnancy; the reports conflict, however, concerning the changes in the bone marrow of normal pregnancy. In view of this lack of agreement an attempt was made to determine whether the bone marrow during pregnancy and the postpartum period differs significantly from the bone marrow of normal non pregnant women; if so the establishment of the physiomorphology and of standard values for the differential counts of bone marrow in pregnancy might prove useful.

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CHAPTER I.

HISTORICAL-

Bone Marrow Examination.

The importance of the bone marrow in the formation of the blood was first demonstrated in 1868 by Neumann (1), who showed that it was the site of origin of the circulating red cells. He also observed the fact that fatty marrow may be transformed into red marrow in the presence of anemia.

In 1877 Ehrlich introduced his staining method of dried blood preparations and published his concept of the development of polymorphonuclear leukocytes and lymphocytes from bone marrow cells (2). This publication started the heated discussion about the origin of blood cells which has continued until the present time.

At first most observations were made on postmortem material, but this method was proven to be of limited value due to rapid morphological changes which occur after death (3) (4) (5).

In 1903 Pianese (6) first studied the human bone marrow in vivo obtained by means of a trocar. His report was followed by those of several other workers, many of whom obtained their material by trephining the upper end of the tibia as initially proposed by Ghedini in 1908 (7) (8).

Both methods required quite a considerable convalescence period after the operation and it was also soon shown that active marrow is not always present in these bones in the adult.

Ghedini's method, however, has proven to be very useful

in infants and young children whose tibial epiphyses still contain red marrow. Caronia (9) modified this method in 1922 and used it in children.

In 1923, Seyfarth (10)introduced the method of trephining the sternum. This provides an easily accessible site for biopsy and one which normally contains active marrow. The method consists of trephining the outer table of the sternum at the level of the third or fourth rib and removing marrow with a curette. His method launched a new phase in the development of the technique of bone marrow biopsy. However it requires the services of an able surgeon for its performance and proves to be an inconvenient operation for the patient.

With Arinkin's (11) sternal marrow aspiration devised in 1929, a simple method became available for inspecting the " factory of the blood ". Under local anesthesia, he punctured the outer table of the manubrium sterni with a lumbar puncture needle, and films were made from the aspirated mixture of blood and marrow. This method is quite rapid, permits repeated examinations, causes only momentary discomfort to the patient, requires no surgical assistance and usually affords adequate information as regards the functional morphology of the bone marrow. For these reasons this method is now employed extensively in hematological investigation. It should be added that both Arinkin's aspiration method and Seyfarth's trephining technique have their limitations. These limitations will be discussed later.

In 1932 Custer (12) examined the structure and function of the bone marrow in the various parts of the skeleton in relation to advancing years of life. At birth, practically all bones were found to contain hematologically active red marrow. In early infancy, however, fat cells appear in the bones of the extremities and increase gradually in number until they replace most of the hematopoietic tissue of the long bones. After the age of twenty years active marrow is normally only found in the sternum, vertebrae, clavicles, ribs, scapulae, skull and pelvis. Small areas of active tissue may still be present in the proximal ends of the femora and humeri. Custer expressed the opinion that this involution may be due to temperature differences between extremities and torso. Transition of fatty marrow to red marrow was observed by him under experimental conditions.

The puncture of the upper end of the tibia will therefore yield active marrow in young children. Caronia performed tibial punctures in children below the age of 15 years, but it seems likely that other sites would have been preferable in many cases. Today, tibial puncture is rarely performed after the age of 4 - 5 years.

Van den Berghe and Blitstein (13) first successfully aspirated the iliac crest. Shortly thereafter Rubinestein (14), on this continent, reported excellent results with this method in a large series of cases.

Recently, satisfactory aspirations have been obtained from the spinous processes of the vertebrae by Loge (15), Bickel and Della Santa (16).

Bone Marrow Findings in Pregnancy: Review of Literature.

At the same time that Arinkin presented the new method of obtaining bone marrow, he published the results of the differential counts he had performed on marrow smears on a group of normal patients. These ranges varied widely, probably for reasons which are now better understood. The marked variation and the obvious need for normal standards stimulated other investigators to perform the same type of work. It was soon realized, due to factors which will be discussed later, that it was quite difficult to come to an agreement as to what was the actual cellular distribution of normal bone marrow.

Because of this disagreement or the wide range of variation, many workers in clinical hematology have rejected the performance of differential counts, on the basis that they are very time consuming and often give no more valuable information than careful observation of the bone marrow smears.

Many research workers consider the differential count a necessity, and under these circumstances the standard evaluation of normal bone marrow is of basic importance. The disagreement in this matter caused many investigators to make up their own series of normal, as a prerequisite to any study in the pathological field. From the work of these authors there is now available numerous data on the normal bone marrow differential count. It still seems essential to perform a number of punctures on normal persons under identically controlled conditions in any series of precise comparative bone marrow differentials. The proper control of preparation and interpretation of marrow samples will be discussed later.

The problem of anemia so often seen in pregnancy has fascinated many workers. In the last century the various morphological changes seen in blood during pregnancy aroused interest, and it was soon realized that the anemia of pregnancy was far from a nosological entity.

In 1936, a Russian obstetrician,Daniachy (17), became interested in the bone marrow of pregnant women with various disorders (especially eclampsia) which were accompanied by a profound anemia. From this work he suspected that the bone marrow of normal pregnancy may divert from the non pregnant normal. From differentials performed on bone marrow smears of fifty normal pregnant women he came to the conclusion that these marrows showed characteristic quantitative and morphologic changes in both the white and red cell series in early pregnancy. The metamyelocytes,particularly,showed a relative increase as compared with the non pregnant normal (twice the normal value in the third month, three times in the sixth month and six times in the last trimester).

Evidence of further shift to the left of granulocytes appeared in the fourth to the sixth month of pregnancy, although the percentage of myelocytes and promyelocytes did not exceed the normal physiological values as given by Arinkin. He also noted an increase in eosinophilic cells. Anisocytosis, vacuolization and asynchronism in the white cell series are striking changes according to Daniachy. Frequent occurrence of mitotic figures in both the erythropoietic and granulopoietic series were evidence of increased marrow activity. Although the red cell precursors rose to a relatively lesser extent, he felt that they could still be considered within the upper limit of normal. Daniachy found that the outstanding morphologic feature in the red cell series,however,

was the appearance of megaloblasts in a frequency of 0.2 to 1 %. They increased in number with increase in length of pregnancy. It is impossible to define Daniachy's criteria of megaloblasts from his article. The peripheral blood remained normocytic. All of these qualitative changes were more evident in primigravidae.

Unfortunately the conclusions he drew from the quantitative changes mentioned above were erroneous. Daniachy compared his results with Arinkin's values for normal men and women. He probably would have reached different results had he used the standard values of another author, or his own.

In 1939 Pitts and Packham (18) of Vancouver reported a study of the bone marrow in pregnancy. This work was started because they had been faced with the problem of interpretating the marrow picture of a 24 year old woman who developed acute lead poisoning in the fourth month of pregnancy. They felt that there was a lack of knowledge about precise data concerning the bone marrow in pregnancy and decided to provide the literature with the normal standard figures obtained from 40 healthy pregnant subjects. For control they used differentials from marrow aspirations which they performed on 24 healthy non pregnant females. They concluded that there was a slight generalized hyperplasia in normal pregnancy which, however, affects all types of cells about equally. Morphologic cell changes during pregnancy were apparently not observed by these workers.

In 1939 Markoff (19) in Switzerland reported his findings. He pointed out that the results which had been published thus far, had not yet solved the problem of whether or not there was such an

entity as a " bone marrow of pregnancy". This statement was based on his comparison between the myelograms published by Daniachy and Hansen and the myelogram given by Rohr for normal men and women. The wide range of the latter seemed to overlap completely the mean values calculated from the results of the differential counts performed by Daniachy and Hansen. Markoff concluded that the method of differentiation probably was inadequate and approached the problem from a purely morphological viewpoint.

He came to the following conclusions: in the second month of pregnancy a few large early basophilic normoblasts start to appear and the promyelocytes begin to show a striking anisocytosis. In the third month the plasma cells increase and the early basophilic normoblasts are present in clusters. By the fourth month the marrow is very cellular. Both the erythroblastic and granuloblastic series are hyperplastic and show a large number of mitotic figures. From the sixth month onwards, he noted marrow eosinophilia and found the reaction of the whole marrow to be at its peak of activity. The anisocytosis of the promyelocytes, the clusters of polychromatic normoblasts and large basophilic normoblasts and the increase of plasma cells were especially characteristic. No further changes were observed during the last month of pregnancy. The marrow in the puerperium was again normal but no comment is made concerning the time at which these samples were taken.

In 1939, Forsell (20)of Finland reported an extensive investigation concerning the morphologic changes of the blood and bone marrow in anemia caused by acute blood loss. A bone marrow

puncture was performed in eight normal pregnant women as material for comparison with a group of fifty patients with anemia following abortion. This group of eight normal pregnant cases consisted of women who were in their fourth month of pregnancy or less, except one who was eight months pregnant.

On the basis of comparison the bone marrows of 20 healthy men and non pregnant women and of these eight pregnant women, Forsell claimed that no significant changes, either quantitative or morphological, occur during pregnancy.

In 1945 Wolff and Limarzi (21) (U.S.A.) examined the bone marrows of 105 pregnant women. Of these, thirty had serial punctures throughout pregnancy, puerperium and at 6 weeks postpartum. All these women were healthy, had a normal peripheral blood smear and showed no evidence of iron deficiency.

These authors found a decrease in the values of hemoglobin, red cells and hematocrit of the peripheral blood, beginning at the 3rd and 4th month of pregnancy, with values falling considerably at the 6th and 7th month and persisting until after delivery. Since the peripheral blood had a normal morphology these low values were considered to be due to the relative increase in plasma volume during pregnancy, causing the " physiological anemia of pregnancy ". The mean corpuscular hemoglobin concentration remained at the lower limit of normal in all cases. A moderate leukocytosis was usually present, together with an increased sedimentation rate, which rapidly returned to normal after delivery. No increased reticulocytosis was found however, as was previously reported by others.

The bone marrow differential counts of these women showed no morphological changes which differed from the non pregnant normal. It was noted, however, that erythropoiesis and granulopoiesis increased in early pregnancy and became more pronounced during the last stages of pregnancy as was evidenced by the increased myeloid - erythroid ratio of the hematocrit (normal approximately 6.8 %; in pregnancy 14 % average with occasional values as high as 45 %) and an increase of the total nucleated count of the bone marrow (normally 300.000/cmm; during pregnancy 600.000/cmm. with a maximum of one million/cmm.).

Granulopoiesis remained morphologically normal throughout pregnancy, but an insignificant myeloid immaturity was observed a short time before delivery.

The megakaryocytes showed an evident hyperplasia in the last months of pregnancy and during the early puerperial days. A definite increase in bone marrow platelets was also noted.

The peripheral blood was found to be normal again at the sixth week postpartum, but the myeloid and megakaryocytic hyperplasia did not disappear for some three months postpartum.

In 1946, Callender (22) published her studies made in Britain. She performed differential counts on the bone marrows of 19 healthy pregnant and puerperal women and 10 healthy non pregnant volunteers. Because reports of several authors seem to indicate that there might be an erythroblastic reaction of the bone marrow in pregnancy, especially during the later months, she compared the percentages of all the red cell precursors found in the following groups:

1. 10 non pregnant and 15 pregnant women.
2. 10 non pregnant and 4 puerperal women.
3. 10 non pregnant and 9 pregnant women in the last eight weeks of pregnancy.
4. 4 pregnant women in the second trimester and the same during the last eight weeks of pregnancy.

Statistical analysis showed that a doubtful significant difference in erythropoietic activity could only be shown between the marrows of non pregnant women and those in the last eight weeks of pregnancy. She reported a P- value which lay between 0.05 and 0.02, but noted that because there were many uncontrollable factors in the preparation of the marrow smears and performance of the differential counts, it would be safer to accept only P-values of 0.01 or under, as significant. In this case no significance could be attributed to the found difference. She also tested the evidence of a shift to the left of the erythroblastic cell series during pregnancy as suggested by Markoff, but was not able to show a significant statistical difference.

From morphological examination of sections of bone marrow particles, she concluded that there appears to be a tendency to slight hyperplasia of the red cell precursors during the last weeks of pregnancy and in the early days of the puerperium.

Another series of 12 bone marrows from healthy pregnant women was reported by Leitner (5) in Switzerland in 1949. Three of these women were in the first trimester, six were in the second, and three were in the third trimester (bone marrow puncture was performed twice on one patient).

No definite conclusions were drawn from the results except that there seemed to be a slight hyperplasia of the erythroblastic and/or the granuloblastic cell series in some of these 12 cases. For normal non pregnant standards Leitner used his results obtained in 22 healthy men and women. In not one instance were megaloblasts seen, in contrast to the previous report by Daniachy; Leitner observed large cells similar to giant metatrophils in those marrows in which there was hyperplasia of granuloblasts.

Two more studies concerning this subject have been published by Hansen (23) and Pignoli (24).* Callender states that " Hansen compared his results with the normal ranges of Segerdahl and Nordenson and deduced that there was a shift to the left of the granulocytic series. He also thought that increased erythroblastic activity was indicated by the occurrence of clusters of macro-normoblasts, but he found no megaloblasts". It is Callender's opinion that Hansen might have come to different conclusions, if he had applied Arinkin's standard values for normal men and women. These latter values were used by Daniachi for his study as earlier mentioned. It is to be emphasized that both studies of Arinkin and Nordenson were performed on the bone marrows of men and women, hospitalized for various diseases, but in which no evidence was found of hematological abnormalities. It should

* No further remarks can be made regarding Hansen's and Pignoli's work. We have seriously attempted to obtain the original articles of both these authors, but without success.

also be noted that Arinkin's figures for the normal range differ considerably from those of Nordenson and Segerdahl.

Leitner states that Pignoli performed a study concerning the bone marrow in normal pregnancy in 1942, but does not comment regarding it.

CHAPTER II

METHODS AND TECHNIQUES.

Techniques of Bone Marrow Aspiration.

No essential changes have been made in Arinkin's original method of obtaining marrow from the sternum. A series of special bone marrow needles have been designed, most of which consist of a short, strong needle, fitted with a stylet, to prevent blockage. Arjeff (25) devised the first bone marrow needle with a guard, as a protection against accidents. Various modifications of the latter needle are now in existance. Henning and Korth (26) used a non-guarded needle, which is gauged in millimeters to give an estimation of the depth of the needle in the outer table of the sternal cortex. This needle has no stylet, but there is an opening near the tip to facilitate irrigation with sodium-citrate or heparinized plasma in case no marrow can be obtained otherwise.

Most authors direct the needle vertically into the sternum; others find it preferable to direct the needle cephalad at an angle of 45° to diminish the sense of pressure experienced by the patient.

Arinkin aspirated his marrow samples from the manubrium sterni, but as it was later found that transformation of red marrow into fatty marrow occurs earlier in the manubrium than in the gladiolus and that its spongiosa is often very thin in the middle, the mesosternum was elected as the better site. Some authors have chosen the distal part of the sternum; however, it has been found to have a central foramen in 20% of all cases, due to incomplete fusion of the two sternal plates.

Rarely the osseous union between the plates is absent over the entire length of the sternum (27).

Thus the site of choice of most authors is the proximal part of the corpus from the second to the fourth intercostal spaces. The puncture should not be performed at the level of the costochondral insertions, as cartilaginous attachments are apt to occur at these areas (27).

Young and Osgood select the sternomanubrial junction purposely because of the soft cartilaginous structure at this site. For cases in which malignant metastases are suspected, other sites of the skeleton may be more preferable (13) (14).

A few authors (29) (30) (31) feel that local anesthesia is unnecessary; Vogel and his associates (32) stated that anesthesia seems more indicated in cases from which large quantities of marrow are withdrawn. On the other hand the sensation of pain due to the suction, is not affected by local anesthesia and thus cannot be avoided. Whitby and Britton (33) also administer a sedative to their patients.

Most authors prefer to push the needle into the marrow cavity manually, using a steady, rotatory movement. A few others drive the needle through the outer table by means of gentle taps from a small hammer. This latter method has the disadvantage that the operator does not feel the sudden diminution of the resistence as the needle enters the marrow cavity; this method also causes the patient more discomfort than the manual method.

Methods of Preparing Aspirated Material for Study.

Opinions differ widely regarding technical aspects such as the amount of marrow to be withdrawn and the preparations of smears. These aspects are reported in Chapter V. The common aim of all methods is to reduce to a minimum the variable of dilution with peripheral blood.

Some authors add exalate, citrate or heparin to the marrow aspirates to prevent rapid clotting; concentration techniques require the addition of anticoagulants (18) (28) (34) (32) (35). Many others reject the use of anticoagulants on the basis that they alter cellular morphology.

The choice of staining techniques is often personal, depending upon the workers experience with a given staining method. Wright's stain or one of the variations of Pappenheim's staining technique are most commonly used.

Methods of Study of the Aspirated Material.

Many authors report the total number of nucleated cells per cmm of bone marrow fluid, as an estimation of its cellularity.

All of these counts show a marked individual variation which is even larger if the data of the various authors are compared.

Segerdahl (36) found a normal range of 10.600 to 238.200 total nucleated cells per cmm and there was no significant difference in the total nucleated count of her three groups of young men and young women and of elderly men and women combined. Gormson (37) found 18.000 - 216.000 per cmm. Both workers aspirated small amounts (0.1 - 0.2 ml) of marrow.

The comparative values are lower in the data given by those authors, who preferred to aspirate larger quantities, due to a greater degree of admixture with peripheral blood, but the ranges are still considerable: eg. Napier and Gupta (38) found 32.000 - 116.000 (20 ml aspirated) whereas Pitts and Packham (18) reported 7.550 - 46.000 (10 ml aspirated.)

In addition to their data on the marrow aspirates of 24 non pregnant women, Pitts and Packham reported the results of total nucleated counts, performed on marrow samples from 40 pregnant women. They found a range of 14.400 to 125.000 per cmm with an average that was slightly higher than that found in the non pregnant group.

The information obtained by the performance of a total nucleated count, is probably more important in pathological cases with counts of over 300.000 (34) . In the present series total nucleated counts were made in approximately two-thirds of the cases, but it soon became evident that the range of variation was great enough to question the significance of these values. Consequently it was decided to omit the total nucleated cell counts in the interpretation of the results of this study.

TECHNIQUES USED IN THIS STUDY.

Bone Marrow Aspiration.

The patient's skin over the mid-sternal region is washed with iodine and alcohol and draped with sterile towels, leaving a small area around the site of aspiration uncovered. All sternal punctures were performed at the second or third intercostal region, in the midline, under sterile conditions. No sedative or other premedication was given.

A small amount of two percent novocain solution was injected intracutaneously with an intradermal needle at the chosen sitep following this a small amount was deposited under the periosteum with a 20 gauge needle.

For the aspiration a 4 - 6 cm long sternal marrow needle of simple dwsign (16 gauge) fitted with a stylet was used. The anesthesized skin was penetrated in the midline of the sternum above the level of the attachment of the lowest rib of the interspace chosen for the puncture. The needle was then directed vertically or at an angle of 45° cephalad and after reaching the anesthesized periosteum the outer table of the sternum was penetrated by means of a to and fro rotary motion of the needle using moderate manual pressure. Often, the " sudden give ", as described by almost all authors, was not felt, but was replaced by a sensation of gradual diminishing resistance during perforation of the outer table of the cortex.

Markoff noted that the sternum during pregnancy seems to be less solid; this also is our impression as less pressure is needed for penetration into the marrow cavity, particularly in the latter months of pregnancy. In these cases also the feeling of the " sudden give " was usually absent.

When the needle seemed to be in the correct place the stylet was withdrawn and a 1 ml (Old Tuberculin) syringe was attached. The plunger and inner barrel of the syringe were coated with vaseline.

The removal of the stylet is usually a good indication that the marrow cavity has been reached, because the patient experiences this as a slight pain. Also if the needle tip is in the marrow cavity usually the end of the stylet will be coated with blood upon its withdrawal.

With the application of forcible suction the subject invariably experienced a sharp short pain and 0.1 - 0.2 ml. of marrow fluid was taken. The needle was then withdrawn and a small bandage was applied to the puncture wound.

Occasionally the needle had to be advanced a few millimeters to obtain marrow and only in a very few cases was a second attempt necessary after a first failure. The irrigation technique of Henning and Keilhack was never used. When necessary,the second marrow puncture was performed one intercostal space below or above the first one, in order to avoid possible hemorrhagic or disruptive influences from the first puncture.

Quite often the patients were somewhat apprehansive before the precedure and there was considerable difficulty in attaining volunteer subjects. After the puncture was performed most of the subjects admitted that they had only suffered a moment of discomfort at the time that the periosteum was anesthesized or as the marrow sample was withdrawn.

Preparation of the Aspirated Material for Study.

Immediately after obtaining the bone marrow aspirate the needle was detached from the syringe and the content of the syringe was expelled into a paraffin block with concave surface. Anticoagulants

were not used.

Cover glass preparations were made with the aid of a pipette. Some of the aspirate was placed on slides which were tilted until the marrow particles adhered to the surface of the slide; surplus blood was drained off and blotted with gauze pledgets and the particles were smeared between two slides. Both slides and cover glasses were allowed to dry at room temperature and were then fixed and stained with Jenner - Giemsa, according to the following technique:

1. Jenner stain : 6 - 8 drops for 3 minutes.
2. Buffered water (pH 6.8): 5 - 6 drops for 6 minutes.
3. After thorough removal of the stain by washing the stain off with buffered water, add:
4. Giemsa stain (10 drops diluted with 10 drops of buffered water) for 15 minutes.
5. Wash well with running tap water and then with buffered water.

Differential Counting Technique.

Marrow particles were selected under low power magnification for the differential counts, which were performed under oil immersion at 1390 x magnification.

The cover glass smears are usually superior to the slide smears in that they are thinner and allow better observation of the details of nucleus and cytoplasm. For the differential counts only cover glass smears were used. The slide preparations were useful, however, in studying marrow architecture, cellularity and megakaryocyte distribution, but cellular details could not always be differentiated.

Those areas in the cover glass smears, containing marrow units were selected in order to reduce the errors due to dilution with peripheral blood to a minimum.

The neutrophilic and eosinophilic stab cells and polymorphonuclear granulocytes were not counted for the same reason. All basophilic granulocytes were omitted, since they represent only such a small percentage of all nucleated cells. Megakaryocytes were included, but these also are too few in number to be accurately counted by this method. No importance therefore can be attached to the percentage of these cells, listed in the Tables. A total of 500 nucleated cells was differentiated, using at least two different smears and the results were expressed as percentages of the combined red and white cell series.

Granulocytes and erythrocytic cells showing mitotic figures were included in the 500 cells but a note was made separately regarding the frequency of the mitoses observed in both series.

The differential counts of all bone marrow preparations were performed by the author. As a control of the accuracy of these counts, separate counts were performed upon approximately half of the total number of the preparations, by Dr. Louis Lowenstein, Hematologist-in-Charge. The results were only considered acceptable if the counts performed by the two separate observers were in satisfactory agreement. During the early phases of this work the dual differential counts were performed more frequently than during the later phases, as it became apparent that the differential counts of the two observers were consistantly in satisfactory agreement.

Examination of the Peripheral Blood.

The peripheral blood in all cases was examined by the same two technicians. A determination was made of the hemoglobin concentration (Hgb) hematocrit (PCV) and red blood cell count (RBC), from which values the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Also a reticulocyte count, white blood cell count (WBC) and differential was obtained in each case.

The hematologic determinations were performed with National Bureau of Standard pipettes and carefully standardized equipment; often duplicate determinations were done. The hemoglobin concentration was determined with the Evelyn photoelectric colorimeter, 15.6 gm. percent being equivalent to 100 percent. The hematocrit was performed by the method of Wintrobe and the reticulocytes were counted by Dameshek's method. Leukocyte differential smears were made by the cover glass method and were stained with Jenner - Giemsa.

The work of the two above technicians was directed and supervised and results which varied questionably or definitely from the normal were personally checked by the Author and/or Dr. Lowenstein.

CHAPTER III.

SELECTION OF SUBJECTS.

Pregnancy and Postpartum Series.

This group consisted of women attending the obstetrical outpatients' department of the Montreal Maternity Hospital. They were divided into the first, second and third trimesters of pregnancy. Each trimester group consisted of 30 women. Ten bone marrow punctures were performed on each of the first eight postpartum days, a total of 80 marrow aspirations. Only a few women had more than one puncture.

These bone marrows, taken during the puerperium, were subclassified into two groups: postpartum days I - IV and V - VIII, so that each group represented 40 bone marrow examinations.

Bone marrow aspiration was performed on another 30 women 6 weeks postpartum at the time of their routine check-up in the Out - Patient Clinic.

Normal Series:

For comparison with the pregnancy and postpartum groups a series of bone marrow punctures was done on 30 non pregnant women, all in the childbearing age. Of these, 14 were out-patients attending various clinics, 16 were women belonging to the technical and administrative staff of the Royal Victoria Hospital and 2 were women interns from l'Hospital St. Justine.

In summary the material consisted of the following:

30 bone marrows : first trimester
30 bone marrows : second trimester of pregnancy
30 bone marrows : third trimester

40 bone marrows : first to fourth postpartum day
40 bone marrows : fifth to eighth postpartum day
30 bone marrows : six weeks postpartum
30 bone marrows : non pregnant normal women of childbearing age.

Criteria of Health.

The pregnant woman, as stated, were all controlled at regular intervals by the obstetrical clinic. After having been thoroughly examined, they were referred to the Hematological Service where a careful history was taken regarding past illnesses and pregnancies, social status, dietary habits, menstrual periods and the current pregnancy. The average age of the women belonging to this group was 27 years; there were 12 primigravidae in the first trimester group, 6 in the second trimester and 5 in the third trimester.

If the patient was entirely healthy, both to medical and obstetrical examination, bone marrow aspiration biopsy and blood examination were performed.

The following criteria were followed in the hematology screening:

The patient's blood must show an entirely normal morphology. Previous work, performed by the members of the Hematological Service

of the Royal Victoria Hospital (39) and others, showed that as a result of a variable hydremia, the hemoglobin, red cells and to a lesser extent, the hematocrit values fluctuate widely during physiologic and pathologic pregnancy. Consequently the degree of hydremia must be known in order to properly interpret these values. The performance of blood volume studies provides guidance in this problem, if undertaken in each individual case. This represents a practical impossibility in the routine hematological screening process. Tysoe and Lowenstein therefore concluded from their work, that the following hematological criteria in pregnancy should be followed : the cell size, shape, corpuscular hemoglobin content and concentration should be normal. If abnormalities are discovered, then further studies should be undertaken.

The same hematological criteria were followed in the series of puerperal women, all of whom remained hospitalized for eight days after delivery. The average age of these groups was 26 years; there were 11 primiparae in the group of the 1st to 4th day and 11 in the group of the 5th to 8th day.

The above mentioned authors found that the normal non pregnant blood volume was attained by one week postpartum, or even earlier. Thus the determinations of hemoglobin, hematocrit and red cell values will vary individually, depending upon the hydremia present antepartum and the speed with which it decreases postpartum.

Another variable factor may be the amount of blood loss after delivery and during the puerperal days. Lowenstein et al performed serial hematologic and blood volume determinations and showed that the

actual blood loss,as measured by the T 1824 dye method greatly exceeded the visually estimated and the directly measured blood loss (40).

All other necessary information, as to whether these patients could be considered normal, was available in the hospital chart and was verified by a history and examination. The temperature curve of the patient was scrutinized to exclude the presence of an inflammatory process, realizing that the determination of a white cell count and differential is often of no decisive value in the discovery of a minor infection during pregnancy or the postpartum period.

The 30 women, all approximately six weeks postpartum, had a routine check-up at the postnatal clinic, and were found to be healthy. They underwent a second medical and hematological screening before a bone marrow puncture was undertaken. The average age of this group was 26 years. 11 women were primiparae.

Several authors reported that the hemoglobin, hematocrit and red cell values have reached the normal non pregnant ranges by 6 weeks postpartum. The hematological standards, applied for these women were therefore the same as those used in the non pregnant series.

An attempt was made to obtain as many bone marrows as possible from healthy non pregnant women of the same social status as the pregnant women. Thus we were able to find 14 women, attending the various out - door departments for minor complaints, who passed the screening process and were considered entirely healthy. Considerable

effort was required to find " patients " who could meet satisfactory criteria of health ; it was finally decided to recruit hospital employees in order to obtain the desired number of 30 subjects. The remaining 16 women were all technicians or clerks in the various departments of the Royal Victoria Hospital and 2 were women interns of l'Hospital St. Justine. The same medical screening process was undertaken before the selection was made. These women can be considered healthy non pregnant individuals and were of childbearing age, average 28 years. All had a normal history as regards menstrual periods; 18 of them had never been pregnant; the others had been pregnant one or more times and had a normal obstetrical record. No attempt was made to perform a bone marrow biopsy on a certain day of the menstrual cycle. Segerdahl showed that the bone marrows of women, obtained at the 4th and 18th day after the onset of a menstrual period, showed no significant differences in erythropoiesis, but that a probable significantly lower percentage of myelocytes was present during menstruation. Her differences were too small to influence the individual case. No definite difference could be found in the leukocyte values of the peripheral blood. (36)

All women studied were white. A large number of them were immigrants who had lived at least several years in Canada and the remainder were native born Canadians.

CHAPTER IV

BONE MARROW MORPHOLOGY AND TERMINOLOGY.

Anyone trying to describe the morphology of the normal constituents of a bone marrow smear is met by an important obstacle: the classification of cells.

Numerous criteria have been given by many authors; the fact, however, that a certain cell, as seen in a fixed smear, represents only a temporary picture in a series of minute changes with time, has led to a baffling discrepency and often to contradictory opinions.

The use of various methods of staining have increased this discrepency considerably and one is still left with a problem which may be decided only by subjective criteria. These criteria are often inherited from the particular school in which the individual received his morphological training.

It is not our purpose to discuss the speculative theories of the origin of the blood cells. It suffices to state that they have led to such a variety of nomenclatures that in 1948 a special committee was formed for clarification of the nomenclature of cells and diseases of the blood and of the blood forming organs (41). A nomenclature was proposed, which was based,supposedly,on the " simplest, clearest and most descriptive terminology ". The new nomenclature of the red cell series seems revolutionary, but any change in tradition will need time to gain general acceptance. In making its recommendations regarding terminology it was not the intention of this committee to imply that the origins of the various cells have been settled. It

was recommended tentatively that the blast cells should be classified according to their association with the more mature bone marrow cells.

In order to clarify the nomenclature used in this paper, a description of cells will be given for the Pappenheim method of staining. In succeeding paragraphs the cellular constituents of the normal bone marrow will be described; some abnormal bone marrows were found during the screening process and pathologic cells found in these marrows will be described briefly.

Description of Cells.

Marrow Reticulum Cells:

This term is used in conformity with Rohr (42), Markoff (43) and others to designate the primitive undifferentiated mesenchymal cell.

In 1933, Ferrata described an undifferentiated cell, which he believed to be a connective tissue cell with multipotent properties capable of forming both connective tissue and blood cells (44). This concept caused much disagreement and the actual existance of this cell was questioned.

Naegeli (45) considered this cell to be an artefact, whereas others think it has the appearance of a crushed myelocyte or a damaged fixed reticulum cell (20) (30) (34) (36) (46) (47) (48).

Rohr (42) further subdivided these cells:

1. the phagocytic reticulum cells.
2. the large and small lymphoid reticulum cells.
3. the plasma cells.

Leitner (49) denied the lymphoid character of these cells but otherwise agreed with Rohr. His primitive reticulum cell is probably identical to Rohr's large lymphoid reticulum cell. He also added two more varieties of this cell: fat storage cells and endothelial cells.

Recently Heckner (50) shed new light on this subject. He clearly demonstrated two forms of reticulum cells by using Gomori's silver impregnation technique. He had noticed that several of these primitive cells showed a faint indication of reticulum fibres in their stroma with the Pappenheim stain, which could be demonstrated properly with silver impregnation. On the basis of this work he was able to state that there are only two kinds of reticulum cells which conform to Rohr's description of his primitive reticulum cell and his small lymphoid reticulum cell. The first one seems to be a bone marrow stroma cell, whereas the latter belongs to the sinusoidal cells. Any other element such as plasma cell, mast cell, fibrocyte, osteoclast or endotherlial cell, once or still believed to belong to the reticulum cells, should be excluded from this group on the basis that no reticulum fibres were demonstrated. Heckner is not able to give an explanation for the fact that plasma cells are so often observed in the areas of these two kinds of reticulum cells. He also concludes from his study that both kinds of reticulum cells have strong phagocytic tendencies and therefore considers it not essential to subdivide them into phagocytic and non-phagocytic. No data are given about the number of these cells present in the bone marrow. They are observed with the help of this staining technique more frequently than was originally thought.

Our reticulum cell as visualized with the Pappenheim staining technique represents most likely Rohr's and Leitner's primitive reticulum cell. It is characterized by a large size (15 - 30 micra in diameter) usually has abundant cytoplasm, which is very fragile and stains pale-blue to greyish. It may contain a few azurophil granules, but is often non-granulated. The nucleus is large, oval or round, central or eccentric, with a spongy vesicular, pale-staining nucleus. The strands of chromatin may be fine or a little more coarse often causing an irregular and wide parachromatin pattern. There are usually one or two nucleoli, although the number may vary from zero to six. These cells were subdivided by us into phagocytic and non-phagocytic because it was wondered whether phagocytosis might more frequently be observed during pregnancy and the puerperium than in the non pregnant state.

Hemocytoblast:

Failure of agreement as regards terminology and pathways of differentiation of the primitive bone marrow cells has resulted in a state of confusion in the literature. Thus, Ferrata (44) regards his hemocytoblast as multipotential and feels that it corresponds to the myeloblast of Naegoli and the lymphoidocyte of Pappenheim. Downey's (51) multipotential myeleblast seems somewhat more primitive morphologically than Ferrata's hemocytoblast. Dameshek and Valentine (52) regard the erythrogone as the earliest differentiated red cell precursor but describe three stages of maturation in these cells : they relate their earliest maturation stage to the hemohistioblast;

their next maturation level of erythrogones corresponds to the so-called hemocytoblast or proerythroblast and they found the third level indistinguishable from the promegaloblast or the macroblast. Others feel the hemocytoblast is a somewhat later form of primitive reticulum cell.

From the above it is apparent that there is much overlapping of terminology and much difference of opinion as regards the level of maturation at which differentiation to the red cell series occurs. Not infrequently different authors use the same term to describe different cells.

Consequently, it would seem desirable for each author to clearly define his terminology and to accurately describe the morphology of each primitive bone marrow cell.

The term hemocytoblast as used in this study is a large cell (15 - 30 micra) with more basophilic and uniform and less fragile cytoplasm than is seen in the earliest primitive reticulum cells; the nucleus may be indistinguishable from that of the primitive reticulum cell or may show beginning condensation of chromatin resulting in a fine regular stippled appearance.

It is our impression that this cell probably belongs to the reticulum cell series. These cells were not found with sufficient frequency in the marrows reported in this study to permit statistical conclusions and consequently they were included in the non phagocytic reticulum cell series.

Granulocytic Series.

Myeloblast:

The term myeloblast applies to the earliest recognizable precursor of the granulocytic series.

It is a moderately large cell (10 - 18 micra) with a large round or oval light lavender staining primitive nucleus. The nucleus usually contains nucleoli; the chromatin pattern is extremely delicate and reticular; clumping such as is exhibited by the pronormoblast is not seen. The rim of cytoplasm is usually narrow and stains pale to deeply basophilic. No specific granules are present; occasionally a few purple azurophilic granules are seen in the cytoplasm.

Progranulocytes:

This is the next stage in maturation. The cell may be larger than its precursor (14 - 21 micra) but differs from it mainly in that it has fewer or no nucleoli, which, if present, are less prominent. The chromatin strands are coarser and consequently the pattern may appear a little more irregular. Azurophilic granules may be present, but at this stage dark staining granules begin to appear. These are the future specific granules. No granules are present in some cells but are represented by decreased basophilia in the region of the centriolar apparatus when stained by Pappenheim's method. The cytoplasm has increased in relation to the nucleus and has lost some of its basophilia.

Early Myelocytes:

This cell also shows a wide range in size (12 - 18 micra) and normally nucleoli are no longer present. The chromatin strands are more

condensed and coarser. Azurophilic granules may or may not be present, but the specific granules have increased in number. The cytoplasm is still abundantly visible and usually stains a pale blue. In a small number of these cells large, coarse, purplish to purplish red granules are present, which later develop into eosinophilic granules. These cells were grouped with the mature eosinophilic myelocytes in the differential counts.

Late Myelocytes:

The overall size has decreased, but varies donsiderably (10 - 16 micra) and occasionally this cell may be larger than its precursors. Specific granules are abundant and can be differentiated as neutrophilic, basophilic or eosinophilic.

The neutrophilic granules stain pink, the eosinophilic granules orange red and the basophilic granules coarse, dense and dark purple. The cytoplasm may still be abundant and its color is more greyish. The chromatin of the nucleus is now of a condensed pattern; its strands are often coarse and of a deep purple color. The shape of the nucleus is still round or oval, but may show a beginning indentation. Basophilic cells were not counted in these differentials.

Metamyelocytes:

At this stage the nucleus shows obvious indentation, which creates a reniform shape. The chromatin forms a coarse network, staining deep purple. The cytoplasm is abundant and the specific granules have increased in number. The size may be equal to that of the late myelocyte or a little smaller.

Band Cells and Segmented Granulocytes:

These mature forms occur normally in the peripheral blood and are not included in our series. Their identity is not in dispute.

There may be a difference of opinion about the definition of a metamyelocyte and that of a stab cell. The cells in which the nuclei had the form of a rod or ribbon but showed no filamentous segmentation, were considered to be band cells. The chromatin network is often coarser than in the metamyelocytes but may be of similiar appearance.

Lymphocyte Series:

Much disagreement exists concerning the relationship of the lymphocyte to the other blood cells. For our purpose it is sufficient to state that this cell is thought to be derived from the lymphoblast. Early lymphoblasts may be difficult to distinguish from early myeloblasts and early monoblasts. This is well known in the acute leukemias. It is Wintrobe's opinion that it is often impossible, even with the present staining methods, to make an accurate cytological diagnosis (53).

Most publications on normal bone marrow differentiation include only the mature lymphocytes. Initially, while doing the counts for this work, the lymphocytes were subdivided into three groups, according to size. These were further classified into young and mature lymphocytes. It has not been proven that these forms represent a maturation cycle, as has been suggested, or that they arise from a different site of origin. Since so few definite facts are known which justify this subdivision, it

was decided to present them together in one single group. It will not be necessary to describe these cells. The opinion of various investigators as to whether their presence in the normal bone marrow is due to admixture with peripheral blood has been mentioned previously.

Plasma Cells:

Leitner (49) gives a thorough description of the plasma cell and its maturation stages, but does not list these stages separately in his table representing the differentials of normal bone marrows. The matter seems to be more important in the presence of bone marrow pathology. In the present series of normal non pregnant and normal postpartum bone marrows it was observed that there was a striking variation in the size of these cells, caused mostly by variations of the cytoplasm - nuclear ratio. Occasionally the nucleus showed some differences in what could be considered the degree of maturity - i.e. coarsness of chromatin network, thickness of the nuclear membrane or size of the nucleus. Nucleoli were not observed. Usually the cytoplasm had the characteristic dark purplish blue color, but seemed somewhat lighter in the larger cells. Plasma cells were not subdivided further in our studies as it did not seem essential for our purpose and would have caused an unnecessarily complicated vocabulary. Other studies of normal bone marrow have presented plasma cells as a single group and it is to be remembered that they constitute a small percentage of the total nucleated cells. The following criteria were followed for identification:

The cell is spherical or elliptoid and its cell size varies from that of a large reticulum cell to that of any smaller bone marrow cell

normally present. The color of the cytoplasm is a dark purple blue and often there is a large, pale staining perinuclear area. The cytoplasm is abundant and frequently contains vacuoles.

Usually the nucleus is eccentric, coarse and has a thick and often irregular chromatin network. The wheel spoke arrangement is rarely seen with this staining technique. An occasional bi-nucleated cell was observed. The latest stages showed a pyknotic nucleus and were only identifiable by their type of cytoplasm.

Monocytic Series:

The monoblast, which was believed by Sabin and her associates (54) to be the stem cell of the mature monocyte, is again a subject of dispute. Its existence in non-pathological bone marrows has often been questioned. Inasmuch as it was not observed in these studies its morphology need not be described. The monoblast of so-called monoblastic leukemia is difficult to differentiate from the myeloblast. Usually it is easy to identify the mature monocyte, although it may be confused with the late myelocyte or the metamyelocyte and the large lymphocyte. The cell size is comparable to that of the myelocyte and metamyelocyte. The large nucleus is usually reniform or horse-shoe shaped, but may be round or polylobular and is often eccentric. Two characteristics are useful in distinguishing this cell from the myelocyte or metamyelocyte. The nucleus has a finer more open structure with greater amounts of parachromatin and numerous extremely fine azurophilic granules are often present (" Azurophil Dust "). The cytoplasm is grey blue often with a violaceous tinge and does not contain spedific granules.

Red Cell Series.

Erythrogone:

The erythrogone of Dameshek and Valentine (52) closely corresponds to Ferrata's hemocytoblast (44) and is believed by the former authors to be the most immature cell, belonging to the erythroblastic series. They observed this cell in normal marrow and in those of pernicious anemia in relapse. They regard this cell as the stem cell of both the normoblastic and megaloblastic series.

Downey (55) describes these erythrogones as deeply basophilic cells derived from the reticulum cells with a type of nucleus and nucleoli suggesting that it is more primitive than pronormoblasts or promegaloblasts. He rarely encountered them in normal bone marrow, but observed them more frequently in normoblastic hyperplasia showing a shift to the left. He agrees with Dameshek and Valentine that these cells are most numerous in pernicious anemia in relapse, and wonders whether this cell represents the " megaloblast " of Sabin and her school (see megaloblastic morphology). The nucleus shows a very uniform distribution of the chromatin granules, giving it an extremely delicate stippled appearance. He could not decide on the basis of the structure of this cell whether it belongs to the megaloblastic or normoblastic series.

In this study the term erythrogone is reserved for a cell which corresponds to the erythrogone of Downey and of Dameshek and Valentine. This nucleated cell may be present in both normoblastic and megaloblastic marrows. Although primitive, it may be differentiated

from the hemocytoblast and the ordinary pronormoblast and yet clearly belongs to the red cell series. Morphologically this is the earliest clearly differentiated erythrocyte precursor, and, on the basis of morphological grounds alone with presently available staining methods, it cannot be determined whether this cell will mature to megaloblast or to normoblast. We have arbitrarily called this cell the erythrogone (macropronormoblast (19) (56) proerythroblast (44) and possible promegaloblast (17) of other authors).

It is a large cell (circa 20 micra). The nuclear cytoplasm ratio varies considerably. The cytoplasm is deeply basophilic and contains no granules. The nucleus is vesicular, fairly large and round, and contains one or more nucleoli. It was decided to group the erythrogones and pronormoblasts together in this study. Erythrogones are found too infrequently to permit statistical conclusions.

Pronormoblast:

Ferrata (44) called the earliest recognizable red cell the proerythroblast, believed that it is derived from the hemocytoblast and that it develops through erythroblast stages to the erythrocyte.

This classification is confusing, since it does not clearly separate the normoblastic red cells from the pathological megaloblastic series. Ferrata's erythroblastic series corresponds to the normoblastic series of most authors.

The pronormoblast is a moderately large cell, circa 10 - 15 micra in diameter, with deep blue, non granulated cytoplasm, which is present as a relatively narrow rim around the nucleus. There is no

evidence of hemoglobin formation. The nucleus is round or oval, light lavender staining, mostly centric and shows a strandlike pattern of chromatin. Nucleoli are present, but may be only faint. The nucleus of the normoblast is coarser with more clumping of chromatin than the nucleus of the myeloblast. The parachromatin is distinct from the chromatin and is present in small amounts. The appearance of the nucleus is the most important criterion in distinguishing this cell from the myeloblast. Compared to the erythrogone it is a smaller cell and the cytoplasm is darker but less abundant. The chromatin pattern of the nucleus is more condensed and the nucleoli are less distinct.

Basophilic Normoblast:

In its earlier stages this cell is often quite similiar to its precursor, the pronormoblast. The main difference is that the nucleoli are absent. The cytoplasm is still very basophilic, but may show the earliest detectable hemoglobinization. The chromatin of the nucleus tends to be a little more clumped or granular. The parachromatin has now become more irregular and indistinct.

Polychromatic Normoblast:

This is the predominant nucleated red cell in normal bone marrow. It consists of a relatively small cell (8 - 12 micra dependent on its age). The chromatin is now very coarse, of a dotted appearance, and may be arranged in a wheel spoke fashion. Parachromatin is present in small amounts, quite irregular in pattern. The cytoplasm contains hemoglobin and varies from moderately basophilic to strongly polychromatophilic,

depending upon the amount of hemoglobin formed.

Orthochromatic Normoblast:

Leitner (5), Wintrobe (53), de Weerdt (57), Dacie and White (58) and others emphasize the point that a fully hemoglobinized normoblast (= orthochromatic) is rarely seen in the bone marrow. Dacie therefore divides the normoblasts in early and late polychromatic normoblasts and pyknotic normoblasts thus trying to avoid some confusion in this way. According to him, the pyknotic normoblast is rarely fully orthochromatic, although it is considered to be the most mature nucleated red cell .

The subdivision of these latter two stages is arbitrary and depends upon whether the nuclear or cytoplasmic characteristics are used as criteria for classification. Often the degrees of maturation of nucleus and cytoplasm are not parallel. This asynchronism is often more evident in abnormal erythropoiesis.

Dacie found the following proportion of nucleated red cells in normal marrow: 2 % pronormoblasts; 5 % basophilic normoblasts; 51 % early and late polychromatic normoblasts and 42 % pyknotic normoblasts. Wintrobe reports that the polychromatic normoblasts constitute 65 - 80 % of all nucleated red cells normally present in the marrow.

In the present study an attempt was made to subdivide these two stages according to both the maturation of nucleus and cytoplasm. In view of the asynchronism normally present it is realized that this classification is often arbitrary.

Megakaryocytes:

These cells were included in our differentials but no quantitative conclusion can be drawn from the percentages found, as they are extremely large and their distribution is uneven. For more accurate information a special counting technique should be followed such as described by Dameshek and Miller (59), Pizzolato (60) and Berman et al (61). For the sake of completeness they will be described here.

The various maturation forms show a wide range in size; the mature megakaryocytes are the largest cells normally found in the bone marrow smear.

The earliest form, the megakaryoblasts, have a diameter of approximately $\pm$ 15 micra and may be indistinguishable from the hemohistioblasts and hemocytoblasts of the Italian school.

The promegakaryocyte differs from this cell in that the shape of the nucleus becomes irregular or polylobular, usually eccentric in the now abundant cytoplasm. The chromatin pattern has become coarser and may show clumping. The cytoplasm is still basophilic, but is often paler, and granulation first becomes evident as a pinkish area of hyaloplasm in the region of the centriolar apparatus. The mature megakaryocyte shows a combination of these changes and finally the cytoplasm is purplish pink and is packed with violaceous granules. Pseudopodial processes of the cytoplasm and granules are common and probably are the source of the circulating platelets.

In view of the fact that the small percentage of these cells found in the differential counts is not significant it was decided to omit an attempt to classify the megakaryocytes according to the stages of maturation.

A short morphological description of certain cells, which are considered to be pathological when found in bone marrow, is given below. This description falls within the scope of this study for two reasons: Firstly, because several of these cells have been observed by various authors (5) (17) (19) who reported a study of bone marrow in normal pregnancy; secondly, because a very occasional abnormal cell was encountered in three patients during the performance of the bone marrow differential counts of our series. It must be emphasized however, that these three were not included in the normal pregnant and puerperal series, but will be discussed separately.

Megaloblastic Series.

Megaloblastic Erythropoiesis:

The term "megaloblast" was used by Ehrlich (2) to describe the type of nucleated red cell found in the bone marrows of patients, with pernicious anemia in relapse. Since that time the term has been widely used, but much confusion was caused by the fact that this term has been applied to cells which differ morphologically.

Ehrlich believed that megaloblastosis is a reversion to the embryonic type of erythropoiesis, based upon morphological similarities between the early fetal red cells and those seen in pernicious anemia in relapse. At present, it is thought that they differ from embryonic cells in biochemical properties and represent either a pathologic form

of the adult red cell series or a new, entirely separate generation; they appear in those cased in which there is a deficiency in the body of certain food factors. The description and bipchemical properties of these factors fall beyond the scope of this paper, which is to discuss the bone marrow in normal pregnancy, but it is sufficient to state that they are necessary for effective normal growth, cellular division and differentiation. These factors may resemble enzymes or co-enzymes in their action.

Some authors deny the special character of the megaloblast, particularly the school of Doan, Cunningham and Sabin (54). Their theory originated in Sabin's initial studies on the origin of red blood corpuscules as seen in the chick embryo (62). She considers the megaloblast to represent an early stage in the development of the normal erythroblast, and that it is derived from the endothelial cell of the bone marrow. This concept would rationalize the finding of megaloblasts in normal marrow and in the marrow of various types of anemia.

These authors hypothesize that there is only one series of red cells, megaloblasts being the least mature members of the series. The bone marrow picture of pernicious anemia e.g. would represent a process of arrest in maturation. Specific therapy causes ripening of these cells, resulting in a normal bone marrow.

Jones (63) disagrees with this theory. He stated on the basis of an extensive study, that megaloblasts differ morphologically, physiologically, chemically and biologically and considers the megaloblast to be a separate pathologic strain of cells.

Davidson et al (69) believe that there exists a direct transformation of megaloblasts into normoblasts. This concept and that of others will be discussed in the following pages.

The megaloblast is derived, according to various opinions, from the hemohistioblast (Ferrata 44), erythrogone (Dameshek 52), endothelium cell (Sabin 54)(Naegeli 45) or myeloblast (Jones 63).

MORPHOLOGY.

Megaloblasts differ from normoblasts in several ways; the cells are larger, due to the increased size of both cytoplasm and nucleus and the nucleus has a more open chromatin pattern. The cytoplasm and nucleus show more asynchronism of maturation than is normally seen and frequently hemoglobinization is more advanced than nuclear maturation. These changes are observed in all stages of development.

Promegaloblast:

These cells are larger than pronormoblasts (20 - 25 micra). The cytoplasma is deeply basophilic and relatively abundant. The chromatin is arranged in a typical finely stippled manner, giving it a more delicate appearance than its normal counterpart. One to several nucleoli are present.

Basophilic Megaloblast:

This stage differs mainly from its pathologic precursor in that the nucleoli are absent. The pattern of the nucleus remains the same, but there is a slight coarsening of the chromatin network ; the reticular structure is still present.

Polychromatic and Orthochromatic Megaloblasts:

As the cell matures the nuclei become smaller and the chromatin more condensed, but the pattern remains more open than in comparable maturation stages of the normoblastic series; sufficient parachromatin is present to cause a mottled effect of the nucleus. Complete pyknosis is therefore rarely seen. The nucleus often has an irregular outline; Howell - Jolly bodies are frequently seen; multipolar and other abnormal mitotic figures are often present.

Leukopoiesis in Megaloblastosis:

In addition to normal myeloid leukocytes, extraordinarily large leukocytes are frequently seen. The large forms may be present in any stage of the myeloid series, but are especially common among the metamyelocytes. The nucleus of these cells is larger, relatively and absolutely, and may show abnormal shapes and staining properties; as in the megaloblasts, parachromatin is more prominent than in normal metamyelocytes. The maturation of the cytoplasm may appear to have lagged behind that of the nucleus, for it may be slightly basophilic and contain very few granules.

Intermediate Megaloblastic Erythropoiesis:

The given description of the megaloblasts applies to the appearance of the red cell precursors as seen in severe pernicious anemia or other megaloblastic anemia in relapse.

The changes between the normoblastic and megaloblastic series may vary however from extreme to mild, due to the degree of deficiency of certain hemopoietic factors (58).

These intermediate cells are seen in cases of mild pernicious anemia before specific treatment, in more severe cases of pernicious anemia, treated with a suboptimal dose of therapy and other megaloblastic anemias, such as sprue (64) (65) (66) and particularly in the megaloblastic anemia of pregnancy (67) (68).

The difference between the megaloblastic and intermediate type of erythropoiesis is merely quantitative. The cells are less definitely abnormal so that all grades of changes may be recognized between extremely abnormal cells and cells almost indistinguishable from the normal.

The transition from megaloblasts via intermediate types to normoblasts can be observed by performing serial bone marrow studies in any case of megaloblastic anemia, responding to specific therapy. This fact has led Davidson et al (69) to believe in a direct transformation of megaloblasts into normoblasts, without intervening mitoses; others think that the change towards normal takes place through many generations of dividing cells.

The existence of this intermediate cell type has been recognized by many other authors (55) (70) (71) (72) (73) either by observation of individual bone marrows as seen in mild megaloblastosis or by serial marrow studies as described above.

Macrocytic Hemopoiesis:

This term was at first used by Jones (63) in 1945. Macrocytic erythropoiesis is seen in various disorders, such as hemolytic anemia, liver disease, during the recovery period after hemorrhage and during response to iron therapy in the iron deficiency anemias. The causes

may vary but deficiencies of those factors responsible for the development of megaloblastic anemias are not implicated. The cells belonging to this series are normoblastic and often larger than normal. The nucleus has the same appearance as the normoblastic counterpart. These cells are believed to be manifestations of new blood formation, which is proceeding rapidly. The presence of polychromatophilia, reticulocytosis and nucleated red corpuscles in the peripheral blood often reflects the increased erythropoietic activity.

Iron Deficiency:

The bone marrow shows increased cellularity and an increased number of erythrocytogenic cells. There is a rough parallelism between the degree of hyperplasia and the severity of the anemia.

The earlier stages of the erythroblastic series have a normal appearance but may be increased proportionally. They may show a smaller rim of cytoplasm than normal; thus they have been called micro normoblasts.

Dacie expresses the opinion that the late red cells with a pyknotic nucleus very infrequently show a fully hemoglobinized cytoplasm in normal hemopoiesis and even less frequently in iron deficiency. Reduced synthesis of hemoglobin is probably the cause for this (58).

CHAPTER V

SOURCES OF ERROR AND PRACTICAL CONSIDERATIONS.

Before an attempt is made to gather data to establish standard values of the various cell components in any bone marrow series, normal or pathological, it should be realized that there are many variables and many sources of error inherent in the method and in its interpretation which limits its accuracy and range of usefulness.

Any article, discussing data on bone marrow of normal individuals should record the criteria by which the state of health of these individuals was evaluated. A history should have been taken and a physical examination performed in each case. Information should be given regarding the number of cases examined, the age, sex and race. The peripheral blood should have been accurately examined and results of the determinations should have been given.

It is essential to give data on the techniques used for both the examination of the blood and bone marrow. Every author should state the site of the body from which the bone marrow sample was taken and the technique involved. In those cases in which the aspiration technique was used the quantity of aspirate and the way in which it was obtained should be mentioned.

Further information should be given concerning the method used for the preparation of the marrow to be studied (particle or clot sections; particle or random sample smears; smears prepared from concentrated marrow aspirate; use of anticoagulants). If the differential counts were performed on marrow smears, it should be known whether the

smears were made between cover glasses or upon slides and which staining method was used.

As a consequence of the existent confusion in hematological terminology, a precise description of the marrow cells as well as the criteria for accurate cell identification should be included in each report. It should be stated how many cells were differentiated and in which way conclusions were drawn. The method of the statistical analysis used should be described.

It is important to know all the details concerning the selection of subjects, the techniques involved and the interpretation of results, because they represent variables, which cause considerable sources of error and because, only by doing so may the work of one author be accurately compared with that of another.

Many of these variables can be reduced to a satisfactory minimum. One variable however is less accurately or easily controlled and will be discussed in some detail.

The aspirate consists of a mixture of bone marrow and an unknown and inconstant amount of blood.

Arinkin (11) who aspirated amounts up to 10 ml. discussed this briefly when he introduced his method for aspirating bone marrow. Dameshek et al (74) stressed this point particularly and compared the relative values obtained by both the trephine and puncture methods for biopsy of the sternal marrow. They discussed the advantages of the first technique, in that the bone marrow material showed a greater cellularity and contained more early nucleated red blood cells, more

reticulocytes and relatively more erythroblastic cells as compared with the granulocytes. They believed that islands of abnormal cells, present in certain diseases, may not be seen in aspirated material, whereas they may be found in sections prepared from trephine biopsy.

The differential counts were found to be more accurate in the histological sections due to lesser dilution with blood. This work was performed on bone marrow material of pathological cases.

It is clear, however, that the trephine technique cannot be used for a project like this in which one is dealing with normal human beings. Another disadvantage of the trephine technique is that it does not allow differentiation of cytological detail and consequent identification of the more primitive cells as well as in the smears made from aspirated material. In the histologic sections, obtained by trephine, cellular relationships have been preserved, but the section is probably still too thick and the method of fixation and staining have altered cellular morphology too much to permit accurate identification and counting of all cells. Osgood and Seaman (48) and Vogel et al (32) believe that it is impossible to perform satisfactory differential counts on these sections. Thus, it seems that we are obliged to use the aspiration technique and to eliminate the factor of dilution with peripheral blood as much as possible.

After transfusing patients with red cells labeled with radioactive phosphorus (P^{32}) N.I. Berlin et al (75) attempted to measure the dilution of the sternal marrow aspirate samples taken from these

patients. They found that dilution with blood varied from 40 - 100 %, depending upon the amount of the marrow aspirated (0.25 - 0.5 ml). Fadem and I. Berlin (76) repeated these experiments and showed that the dilution (between 61 - 96 %) was not directly proportional to the amount aspirated; thus it would seem that this test should be performed in each case if it is desired to determine the exact amount of peripheral blood present in each sample. This method does seem to permit more precise determination of the ratio of the " true " to the observed percentage of a given cell type in the marrow.

The criticism of Fadem and I. Berlin of the work of N.I. Berlin and others is that the proportion of marrow cells counted on glass slide smears may differ from their proportion in the aspirate fluid, due to the difference of adhesive properties of marrow cells. This would mean that the dilution factor as determined with the radioactive technique does not necessarily express the dilution factor of a cell in a given smear.

In order to eliminate the factor of dilution with peripheral blood in the puncture technique the following methods have been tried:

1) Aspiration of only a small sample, preferably 0.2 ml or even less.

Arinkin does not state the exact amount, but indicates that it would be desirable to make the smears from the first drops obtained. Osgood (48) however, recommends that more than 1 ml should be taken for diagnostic purposes. He compared the total nucleated count of the first 0.1 ml , the first 1 ml and the last 1 ml of 10 ml samples, and concluded that a considerably lower count was found in the second

sample, but that there was no significant difference between sample 2 and sample 3.

Many authors, however, still prefer to do all the work on the first 0.1 - 0.3 ml because of the higher counts obtained in this part of the specimen.

On the basis of statistical analysis Reich and Kolb (77) conclude from their study of a series of sternal marrows that any quantitative determination on aspirated marrow samples is inaccurate. They performed two simultaneous bone marrow punctures on each of 26 patients with various diseases, one in the 2nd and one in the 3rd intercostal space of the sternum. The total nucleated cell count and the absolute number of polymorphonuclear leukocytes present in the marrow smears were determined. Their conclusion was not suprising as they must have compared samples which contained differing dilutions with peripheral blood. The total nucleated count and the absolute number of polymorphonuclear leukocytes are especially dependent on the amount of peripheral blood present in the marrow sample.

2. Separation of the marrow particles from the "marrow juice" (5) (32) (76) (78) (79).

The particles are usually easily seen, and can be picked up with the edge of a slide or a platinum loop or will adher to a glass slide when it is tilted, thus permitting separation of most of the "marrow juice." The remainder of the blood can be sucked off with filter paper or a piece of cloth (80) (81).

Fadem and I. Berlin (76) compared differential counts obtained from particle smears and random sample smears of the same (0.1 - 0.3 ml) sternal aspirate. Averages of each cell type were determined in the two series of differentials. It became evident that the particle smears consistently contained greater percentages of immature cells of both the granulocytic and erythrocytic series than did the smears prepared from the random samples. Also fewer lymphocytes were present in these particle smears in the instances where no primary disease of the lymphatic system was present.

These workers then decided to determine whether bone marrow particles contain a significant amount of peripheral blood.

They injected tagged red cells with radioactive phosphorus (P^{32}) into the antecubital vein of the patient, such as described by N.I. Berlin et al (75) and took a sternal marrow sample after ten minutes. Part of this sample was hemolysed with 0.1 N. hydrochloric acid, after which the particles were separated from the hemolysate and washed. The remainder of the aspirate served as a control. By comparing the radioactivity of particles, hemolysate and the control sample separately it became evident that the hemolysate showed a radioactivity of 100 % of the initial aspirate sample, whereas the particles only had a count which was equal to the background. This seems a convincing proof of the fact that the marrow particles are least contaminated with peripheral blood so that it would be desirable to perform differential counts on smears made from marrow fragments.

3. Preparation of smears from the buffy coat of oxalated centrifuged aspirate fluid for differentiation (35) (82) (83) (84).

It is probable however, that this procedure influences the differential count by causing damage or distortion of a relatively larger number of cells.

4. Exclusion of mature granulocytes from the differential counts (85) (86) because they are likely to be present in varying numbers, due to admixture with blood. The great variations in the means of mature granulocytes and to a lesser extent of lymphocytes as found in the differentials of many workers affords supportive evidence for this procedure. The work by Fadem and I. Berlin, as discussed earlier, strongly supports this theory.

Leitner (5) does not agree with this procedure, because he was able to demonstrate mature granulocytes in the marrow of a few patients having agranulocytosis in the peripheral blood. It could be possible that these were cases of agranulocytosis, due to an immune process with leukoagglutinins present in the serum. Thus, it would seem desirable to count the mature granulocytes in the marrow of patients who have agranulocytosis or severe granulocytopenia in the peripheral blood.

Escudero and Varela (85) and Pontoni (86) excluded the mature lymphocytes and monocytes also from their counts.

There is still considerable disagreement as to whether lymphocytes are formed in the bone marrow.

Recently Yoffrey (87) expressed his conviction in favour of this concept. He showed that very high lymphocyte counts are obtained

in the marrows of guinea pigs which were exsanguinated prior to obtaining the bone marrow. A range of 150.000 - 300.000 lymphocytes per ml was present in the bone marrow, whereas the peripheral blood contained only 5000 per ml. He concluded that lymphocytes may be formed in the bone marrow. From the data of Fadem and I. Berlin however, it seems likely that in the human most of the lymphocytes present in the bone marrow are due to admixture with peripheral blood.

5. Custer (88), Amprino and Penati and others (89) allow the aspirate to clot; the clot is fixed and sectioned. They claimed to have obtained bone marrow particles which are not distorted by the preparation of smears.

Many of these methods have their merits, but no satisfactory practical way has yet been found to avoid dilution of the marrow sample with blood.

A second possible variable which might be considered is the question of irregularities of distribution of active cellular marrow. General experience shows that the different cellular components of the marrow often occur in small islands of uneven distribution, but comparison of marrow samples aspirated from two different sites allows the conclusion that usually one marrow aspirate may safely be considered as representative of the whole mass of hemopoietic tissue. Nordenson (90) obtained marrow aspirate samples from the sternum, ribs, vertebrae, pelvic bone, and tibial epiphyses of adults. Differential counts showed a general agreement between these samples, except for the one from the tibia which contained inactive marrow. Stasney and Higgins (91) compared

the marrows from rib and vertebrae of 14 normal persons following accidental death; no significant differences in composition were found.

Bickel and Della Santa (16) compared the sternal and vertebral marrows obtained from persons with various diseases, many with hematological changes. They concluded that there were no larger variations than those found in two sternal punctures upon the same individual. The same type of experiment in healthy adults with the same results was performed by Loge (15). In patients with metastatic lesions in the marrow, Van den Berghe and Blitstein (13) and Rubinstein (14) found that the iliac crest marrow aspirate is generally more informative than the sternal marrow.

In our opinion the technique of bone marrow aspiration is such, regardless of the method used, that it would seem illogical to expect to obtain consistently reproducible total and differential counts of the marrow juice from successive punctures in the same individual. The relationship between the active circulating and fixed tissue components of the marrow is dynamic and not static. The force, its duration and the direction of its application in disrupting the marrow when suction is applied during aspiration cannot be satisfactorily reproduced in successive punctures.

CHAPTER VI

STATISTICAL METHODS USED IN THIS STUDY.

The t - test of Fisher was used with the following formulae:

$$S.D. = \sqrt{\frac{S\,x^2}{N} - \bar{X}^2}$$

$$S.E. = \frac{S.D.}{\sqrt{N}}$$

$$S.E.\ (diff.) = \sqrt{(S.E._1)^2 + (S.E._2)^2}$$

$$t = \frac{\text{the difference between two means}}{S.E.\ (diff.)}$$

S.D. indicates the standard deviation.

x represents the number of a particular cell type, expressed as a percentage of the total differential count.

$S\,x^2$ indicates the sum of the square.

$\bar{X}^2$ is the square of the mean of the percentages of cells.

N stands for the number of cases as well as the number of differential counts.

S.E. is the standard error (or standard deviation of the mean)

S.E.(diff.) is the standard error of the differende between two means.

In the analysis of the peripheral blood studies, P - values between 0.05 and 0.01 were considered to be of probable significance, those of 0.01 or less as definitely significant and those of less than 0.001 as highly significant.

It was difficult to decide what significance could be attached to an observed P - value in the analysis of the bone marrow differential counts. Callender was probably the only investigator who analysed the results of a similar study of the bone marrow. She commented that the errors due to uncontrollable factors in the preparation and differential cell counts of the marrow smears make it desirable to use more rigid criteria and she noted that P - values between 0.05 and 0.02 were probably of doubtful significance in her marrow studies. Consequently, it seemed sound in our study to regard P - values greater than 0.02 as not significant, those under 0.02 as probably significant, those under 0.01 as significant and those under 0.001 as highly significant.

The t - test was not considered valid for the megakaryocytes and the phagocytic reticulum cells because their counts were too low to allow the application of normal curve methods. No megakaryocytes were noted in 45 % and no phagocytic reticulum cells in 33 % of the differential counts of the 230 bone marrow smears studied. Treatment of these results as enumeration data showed no significance. Changes could doubtless be demonstrated had more cells been counted.

The results which varied most markedly from the normal curve were those in which only a few cells of a given classification were present in a total of 500 cells counted. This was investigated according to Mainland (93) and Snedecor (94) who recommend a transformation in such cases. The smallest count analysed was that of the mitotic figures of the granulocytic series. As these cells were expressed as absolute counts and not as a percentage of 500 cells,

the average count per case is approximately 2 mitoses. Subtraction of twice the standard deviation from the mean results in a figure well below zero. After a square root transformation this figure fell above zero and the data assumed a more normal distribution. The analysis of variance before and after transformation agreed in showing a significant variation among the studied groups at the 1 % point. Simple t - tests of pairs of groups after transformation agreed with the results of the original t - tests on the raw data.

It is therefore concluded that the original t - test is adequate for the number of mitotic figures observed in the granulocytic series. All other cells analysed have been found in greater numbers and follow a normal curve distribution more closely. Therefore the analysis is considered adequate for all cells differentiated.

The P - values reported for the shift to the left in the red and white cell series were verified by an analysis using an angular transformation.

CHAPTER VII

THE PERIPHERAL BLOOD DURING PREGNANCY AND PUERPERIUM.

PART 1.

Review of the Literature:

There is general agreement that the concentration of hemoglobin and of red cells is decreased in normal pregnancy as compared with the non pregnant state. This so-called anemia is usually regarded as " physiological ".

Before discussing the peripheral blood findings in normal pregnancy, it will be necessary to mention and discuss briefly the pathological types of anemia, caused by pregnancy:

1. Iron Deficiency Anemia:

Severe anemia in pregnancy is usually hypochromic and due to lack of iron. It is often seen in women who have borne several children (95) (96).

It has been calculated that the need of the Mother's body for iron during pregnancy increases to 3.8 mg. per day, from the non pregnant female requirements of 2.1 mg. (53). A normal diet, rich in iron-containing food will supply this increased demand and it is therefore likely that other factors such as pre-existent anemia, long standing defective diet, or decreased gastric secretion often play a role in the production of anemia (97) (95).

It will not be necessary to discuss the morphology of the blood in this type of anemia of pregnancy, since the blood picture is similar to that of other iron deficiency anemias. The red cell count may be

normal or low, but the reduction in hemoglobin is relatively greater so that the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration are below their normal ranges. The mean corpuscular volume may be normal, but usually is low.

2. Megaloblastic Anemia of Pregnancy and the Puerperium:

This disorder is much less frequently seen, at least in the mild climates. An anemia, occurring in pregnancy, with a high maternal and fetal mortality has been repeatedly described by the Indian Medical Journals (98). This anemia has been also reported in both men and pregnant women as tropical macrocytic anemia, but its greatest incidence is in pregnancy. Wills (99) (100) has demonstrated that these patients will recover from their anemia by feeding them marmite, an autolysed yeast preparation.

She was also able to show that monkeys will develop a similar anemia by feeding them a diet, composed of polished rice white bread and chappatti. This diet was chosen because it is in common use among the Mohammedan women in Bombay. The animals recovered from the anemia by including marmite in their diet.

Later reports indicated response also to injections of refined liver extract (101) but not as well as to crude liver extract (102). The tropical cases however are frequently complicated by other nutritional deficiencies, malaria and parasitic diseases.

Various etiologies have been suggested for the anemia of pregnancy and puerperium of the temperate climates. Dietary deficiency was probably responsible for the development of megaloblastic anemia

of pregnancy and the puerperium in some 25 patients studied at the Royal Victoria Hospital (68). Temporarily decreased secretion of intrinsic factor may also be of importance. (97).

It has been suggested that this type of anemia may be precipitated by the lack of one or more specific factors. The reports from centers in temperate climates vary, but it seems plausible to suppose that megaloblastic anemia of pregnancy is a result of dietary deficiency of folic acid and possibly of Vitamin B_{12} , Vitamin C and/or a still unknown factor.

The occasional classical case of megaloblastic anemia of pregnancy will show a macrocytic anemia, such as seen in Addisonian pernicious anemia. In many cases, however, the blood picture is less typical and may be quite confusing. The hematological values of the peripheral blood may suggest a hypochromic microcytic or normocytic anemia such as is seen in iron deficiency, or a normochromic normocytic anemia which in mild cases of anemia may resemble changes such as are seen in the " physiological anemia of pregnancy".

Leukopenia may or may not be present, but since the range of the number of neutrophils is wider and more variable during pregnancy, it can be difficult to evaluate slight changes of the leukocyte count. The presence of hypersegmented macropolycytes is also very suggestive of this anemia.

Because of these variable aspects, many cases are likely to be missed, unless bone marrow aspiration is undertaken.

The bone marrow picture is characterized by the presence of megaloblasts and/or abnormal granulopoiesis. Differences between the types of bone marrow as seen in Addisionian pernicious anemia and in

megaloblastic anemia of pregnancy and puerperium have been discussed in Chapter IV.

The disease is most commonly diagnosed in the third trimester of pregnancy or in the puerperium. The differential diagnosis from Addisionian pernicious anemia may be difficult. As a rule there are no neurological findings, and the response to Vitamin B_{12} is often slow or absent (68). Usually the gastric juice contains free hydrochloric acid. Recovery may take place spontaneously after delivery.

3. Hemolytic Anemia of Pregnancy:

This is a very rare disease. Only a few cases are described (103) (104) and the etiology is obscure in most cases. It is also uncertain whether this disease has any true relation to pregnancy.

The Peripheral Blood in Normal Pregnancy:

The most important physiological change in the blood during pregnancy is an increase in blood volume. Dieckmann and Wegner (105) have determined the plasma volume of pregnant women by injecting vital red intravenously and calculated the relative proportion of the plasma and red cell mass by means of the hematocrit value.

They were able to show that the plasma volume, the red cell mass and the total hemoglobin mass increase during pregnancy. The increase in the plasma volume however, is definitely greater than the increase in cell volume and hemoglobin; therefore these latter constituents show a relative decrease and an absolute increase. The hemoglobin as ordinarily measured in the peripheral blood, is

decreased maximally by 15 % from the 26th to 35th week of pregnancy. A hemoglobin value of below 10 grams/100 ml during pregnancy is therefore considered to be pathological by these authors.

At two weeks postpartum the hemoglobin is 17 % below normal and at eight weeks it is 14 % below normal according to these authors. The changes in the hematocrit value and erythrocyte count are similar to those of the hemoglobin. The recovery, however, postpartum of these values was found to be more rapid. The hematocrit is still slightly below normal at eight weeks postpartum, whereas the erythrocyte count has reached the normal level at three weeks after delivery.

All these hematological data reach their minimum in the third trimester, and rise slightly in the last month towards term. The calculations of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration show that there are marked individual variations, but that the average remains close to normal in normal pregnancy. These authors stress the point that in physiological and in pathological anemias the morphology of the red cell should be of the greatest diagnostic importance.

Adair and W.J. Dieckmann (106) concluded from a large series of hematological studies in pregnancy that statistically the minimum figures in normal pregnancy should be 10.16 grams per 100 ml of blood for hemoglobin, 33.11 % for the hematocrit and 3.36 million for the erythrocyte count. Their mean values were respectively: 11.56 grams per 100 ml, 37,31 % and 3.77 million.

The normal changes could not be altered by various forms of therapy (iron, liver, Vitamin B. or ammonium-citrate as suggested by Strauss and Castle (97).

A small discrepancy between clinic and private patients was found in this study. Of the clinic patients 12 % had a hemoglobin below 10 grams, whereas tin the private cases there were only 7 %. This discrepancy was assumed to be largely due to differences in hygiene and nutrition. According to the observations of these workers, diet and hygiene were of far more importance in preventing anemia than any medication.

Wolff and Limarzi (21) studied 105 pregnant women and followed 30 of these throughout their pregnancy. According to them the hematological values started to fall by the 3rd and 4th month, which fall greatly increased by the 6th and 7th month of pregnancy and persisted until after delivery. They found normal values by the 6th week postpartum. The mean corpuscular volume was hormal throughout pregnancy and the mean corpuscular hemoglobin concentration was at the lower limits of normal.

The leukocytes during pregnancy were usually found to be within normal limits, but at times there was a slight leukocytosis.

In most cases there was an increased percentage of polymorphonuclear leukocytes towards the end of pregnancy, with an additional slight increase of the percentage of the stab cells. A mild leukocytosis was observed during puerperium, which had disappeared at six weeks postpartum. The sedimentation rate was increased during the last trimester. They found no reticulocytes. The erythrocytes

were of normal size, shape and staining qualities throughout pregnancy and the puerperium. The platelets were normal or slightly increased in number.

Serial blood volume studies during pregnancy and postpartum were performed by Tysoe and Lowenstein (39) with Evans Blue (T-1824). These authors also reported an increase of blood, plasma and red cell volumes during pregnancy. The maximal values occurred in the last trimester and decreased slightly during the last 60 days before term. The plasma changes were greater than the red cell volume changes.

By one week postpartum the normal non pregnant blood volume was attained again according to this study.

The red cell count, hematocrit and hemoglobin concentration fell during pregnancy, and this drop was caused by and varied directly with increase of plasma volume. After the 8th day postpartum no significant changes of the red cell count and hematocrit were observed, but the hemoglobin concentration continued to rise slightly to the 60th day postpartum. The sedimentation rate increased during pregnancy; it did not begin before the 6th month and was back again to normal by 30 days postpartum. The leukocytes rose progressively during pregnancy due to a neutrophilia. The maximal leukocytosis was observed on the second postpartum day.

The mean corpuscular volume, mean corpuscular hemoglobin,mean corpuscular hemoglobin concentration and the appearance of the red cells did not vary from the non pregnant normal in any stage of pregnancy or puerperium. Since a variable hydremia may cause wide fluctuations of

of either the hemoglobin, hematocrit or red cell values, it was suggested by these authors that the criterion of normal morphology of the red cell is a useful screening measure to detect many of the pathologic anemias of pregnancy and the puerperium.

Lowenstein et al (40) measured the blood volume of 37 patients shortly before and at varying intervals after delivery. They were able to show that the blood loss from the active circulation, calculated from antepartum and postpartum blood volume changes, averaged 990 ml, which amount greatly exceeded both the visually estimated blood loss and the directly measured blood loss. It was suggested by these workers that permanent loss of blood from the active circulation accounted for this discrepancy, possibly due partly to trapping of circulating blood in the body of the uterus.

N.I. Berlin et al (107) performed a study on blood volume during pregnancy and puerperium, using P^{32} labelled red cells. In contrast to the reports of other authors, they found that there is a distinct decrease in total red cell volume in the first trimester, but following this there is a rise above normal to a maximum value in the 9th month. Shortly before term total red cell volume decreases slightly. During delivery there is a rapid decrease of red cell volume which continues more slowly into the puerperium. The total red cell volume was still slightly below normal at 6 weeks postpartum.

The plasma volume followed a similar pattern and had not yet completely reached the normal non pregnant value at 6 weeks postpartum but was still slightly above normal.

These workers were able to calculate from their data that the total blood volume decreases by approximately 1000 ml at delivery, a finding which is in agreement with the results of the work of Lowenstein et al. The basis for the discrepancy of blood loss as measured by blood volume and observation still remains to be investigated.

Summarizing it may be concluded that the hemoglobin concentration, red cell and hematocrit values of the peripheral blood definitely decrease during pregnancy due to a relative increase of the plasma volume. This increase of plasma volume exceeds the increases of total red cell mass and hemoglobin mass. Thus there is a variable hydremia which makes it difficult to predict normal hematological values for a given patient at any stage of pregnancy and to a lesser extent during the puerperium. It seems practical therefore to perform a careful morphological study of the erythrocytes. The mean corpuscular volume, mean corpuscular hemoglobin content and mean corpuscular hemoglobin concentration, computed from a routine hematological study will be of great value in detecting hematological abnormalities during pregnancy and puerperium.

PART II

Results and Discussion of the Hematological Determinations of the Peripheral Blood:

The results of the examination of the peripheral blood in all 230 cases were statistically analyzed and are shown in Table I. Table II presents those probability values which were smaller than, or are approximately equal to 0.05, resulting from a comparison between the mean values of the various groups and the non pregnant normal group. In Graph I curves for each type of determination are shown. For reasons mentioned in Chapter VI P values between 0.05 and 0.01 are considered of probable significance in the analysis of the peripheral blood findings. P values equal to or smaller than 0.01 may safely be taken as significant. The following conclusions can be drawn:

1. There is a significant fall of the hemoglobin during pregnancy. The degrees of this fall, compared with the normal non pregnant standard value, is greatest in the second and third trimester. The hemoglobin values in the last two trimesters are found to be the same.

As discussed earlier in this chapter a slight increase of the hemoglobin, hematocrit and red cell count has been reported during the last antepartum month due to a decrease of the plasma volume. It seems likely that this latter fact has been responsible for the comparable hemoglobin values found in the second and in the third

trimesters in this study.

Immediately after delivery, there is an abrupt return towards normal, probably due to the rapid disappearance of the hydremia. Separate hemoglobin values for the eight consecutive days postpartum are not shown in the tables, but it was found that the highest mean value of 14.42 g/100 ml was obtained in the blood of those 10 women which were examined on the first day after delivery. The minimal mean value of 12.61 g/100 ml occurs on the 4th day postpartum. Following this there is a gradual increase towards normal.

The mean values for the hemoglobin in the I - IV days and V - VIII days postpartum compared to the normal non pregnant values are not significantly different. At 6 weeks postpartum however, a slightly lower and probably significant value is obtained as compared with the non pregnant ($P = 0.05$).

2. The same fall occurs during pregnancy with respect to the erythrocyte count. The mean value obtained in the third trimester however is slightly higher than in the second trimester. This difference is statistically not significant ($P > 0.05$). Postpartum the red cell count rises considerably compared to the value obtained during the last trimester antepartum ($P < 0.001$), but normal non pregnant values are not yet attained. The maximal red count occurs on the first day of the puerperium (4.40 million) and the minimal on the fourth day (4.09 million). After this there is a slow rise and at six weeks after delivery the count is the same as the normal non pregnant values.

3. Analysis of the mean values of the hematocrit, determined at the various stages of pregnancy and the puerperium, shows the same changes as the red cell counts. A significantly lower value than the non pregnant normal found during all three trimesters; the minimal value occurred in the second trimester, with a tendency towards a slight, but probably insignificant rise during the latter part of pregnancy. Postpartum there is an abrupt return towards non pregnant values. The maximal and minimal values also occur on the 1st and 4th day of the puerperium (43 % and 39.1 %) . The mean values for the I - IV day, V - VIII day and 6 weeks postpartum do not differ significantly from the normal non pregnant value.

The results of the calculation of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration are not shown in the tables, but it is sufficient to state that the values of these indices remained within normal limits.

4. There is a moderate leukocytosis in all stages of pregnancy. The mean values, obtained for the first, second and third trimester are all approximately equal, ranging from 11,000 - 12,000 but differ significantly from the non pregnant normal. The highest values are obtained postpartum yielding 14,110 for the first to the fourth and 11,870 for the fifth to eighth days. The analysis for each separate day showed that the highest leukocyte count is obtained on the first day after delivery (17,160); a gradual recession occurs towards the eighth day (11,200).

The leukocyte count is within normal limits six weeks postpartum.

It has been observed that the leukocytosis of pregnancy is more pronounced in primigravidae (17). The number of primigravidae in this work was too small to allow any definite conclusions in this regard.

In the first trimester there were 12 primigravidae; their mean WBC was 11,440; the respective values were 13,000 and 13,100 for those in the second and third trimester. These data are indeed slightly higher than the mean WBC, calculated for the multiparae of the various trimesters, but statistical analysis showed that the increases were insignificant. (The mean values for the multiparae from the first to the third trimester were respectively: 11,200; 11,200 and 11,500).

For the purpose of this work it was decided to omit a statistical analysis of the white blood cell survey; consequently only the results of the total leukocyte counts are presented in the tables and graphs.

There is general agreement among those authors, who found a leukocytosis during pregnancy, that this leukocytosis is due to a neutrophilia with a shift to the left of neutrophils. Tysoe and Lowenstein were able to draw the same conclusions in their study and further observed that as the neutrophils rose the lymphocytes reciprocally decreased during the antepartum and early postpartum periods.

The findings of these authors were used as criteria of normality. In each case examined, a white blood cell survey was

performed in order to discover any existent abnormalities. The individual results of the differentials in this study were found to be in agreement with the conclusions of the above mentioned authors.

5. A probable significant increase of reticulocytes during pregnancy can only be discovered in the first trimester of this study. The values found in the second and third trimester are slightly above normal, but do not differ significantly from the non pregnant values. During the puerperium the highest reticulocytosis (2.39 %) was attained on the 4th day, following which a gradual decrease occurred. The values found in the I - IV days and V - VIII days postpartum are definitely higher than normal. No reticulocytosis is present at 6 weeks after delivery.

To Summarize:

1. The red cell count, hematocrit and hemoglobin concentration fall during pregnancy. The lowest values are reached during the second trimester; following this there is a tendency to a slight rise.

2. Immediately after delivery a significant rapid increase occurs and normal non pregnant values are almost attained. The highest values are found on the first day postpartum, probably due to hemoconcentration, following which there is a slight fall until the fourth day postpartum.

No significant difference can be detected comparing the hemoglobin and hematocrit values during the I - IV days postpartum and the V - VIII days postpartum with normal non pregnant values. The red blood cell

count however is still slightly below normal during the 8 days postpartum period.

3. At 6 weeks postpartum the red cell count reaches normal non pregnant values. The hemoglobin concentration,however, seems to be somewhat lower compared with the values of the 8 days of the puerperium and is slightly and probably significantly lower than normal.

The hematocrit which reaches the normal non pregnant range during the first eight days postpartum, is slightly higher at six weeks postpartum but this difference is not statistically significant.

4. There is a moderate, but significant leukocytosis during pregnancy and the puerperium, the peak of which is attained during the first few days after delivery. Following this there is a gradual recession towards normal.

5. A slight reticulocytosis occurs during the three trimesters of pregnancy, but a significant rise can only be demonstrated in the first trimester. Postpartum the reticulocytes reach the maximum value on the 4th day. A normal value is found 6 weeks after delivery.

These conclusions are in satisfactory agreement with previous reports and a study by Tysoe and Lowenstein, also performed in the Bessborough Laboratory , and discussed in Part I of this chapter.

Graph II reproduced from the paper of these two authors shows the lowest hematologic values are actually reached in the first part of the third trimester, whereas the maximal recession in the present series appears to occur during the second trimester. This can easily

be explained by the fact that the women studied, were grouped according to the three trimesters of pregnancy. Small serial changes within a given trimester could therefore not be demonstrated.

It appears that at 6 weeks postpartum the hemoglobin has not yet reached normal non pregnant values, which is in conformity with the work of Dieckmann and Wegner and of Tysoe and Lowenstein.

The conclusion seems justified that the normal hematological equilibrium is not attained by six weeks after delivery.

CHAPTER VIII

Results and Discussion of the Bone Marrow Findings in This Study.

The results of the 230 differential counts, performed on the bone marrows of non pregnant, pregnant and puerperal women are presented in Table III, which shows the mean values, their standard errors and standard deviations for each cell type. In Table IV are listed the probability values, approximately equal or smaller than 0.05. These values were obtained by comparing the means of each cell type present in the pregnant and puerperal bone marrow series with the means of the non pregnant series.

For reasons discussed in Chapter VI it was decided to report all P - values of 0.05 or smaller with the suggestion that values between 0.05 and 0.02 are not significant and that values between 0.02 and 0.01 have doubtful significance. P - values between 0.01 and 0.001 are considered significant, whereas values smaller than 0.001 are highly significant.

Red Cell Series:

The proportions of the red cell precursors and their mitotic figures, as found in the bone marrows of the groups studied are shown in Tables III and IV and Graphs III, IV and V.

Pronormoblasts + Erythrogones:

The values found for the three trimesters of pregnancy and compared with the non pregnant normal show only slight differences. Their range fluctuates between 0.92 and 0.85 %. The means for the

two postpartum periods are slightly lower than normal; the maximal recession seems to occur during the fifth to eighth day (mean: 0.70). At 6 weeks following the delivery a percentage is found approximately equal to the normal non pregnant value. Statistical analysis shows however that no significance can be attributed to any change in percentages of these early erythroblastic precursors obtained in the differential counts of pregnant and puerperal series.

Basophilic Normoblasts:

This type of cell seems to increase gradually during pregnancy. The mean value of 1.08 % for the non pregnant series rises to 1.85 % during the first trimester, to 2.31 % in the second and to 2.34 % in the third trimester. These percentages are statistically highly significant compared with the non pregnant normal. They appear to decrease slightly postpartum particularly from the fifth to eighth day (mean: 1.86 %) but do not return to the non pregnant normal. The mean percentage of 2.12 % found at the sixth postpartum week is higher than immediately after delivery.

Polychromatic Normoblasts:

These cells, which represant the predominating type of nucleated red cell, are probably the best indicators of erythropoietic changes occurring during pregnancy.

From the curve, showing the mean values for this type of cell, it appears that they exhibit exactly the same changes as the basophilic normoblasts, during the three trimesters of pregnancy, the

first eight days of the puerperium and at the sixth week postpartum.

There is a slow increase during pregnancy, compared with the normal non pregnant mean value which is 13.47 %. A highly significant peak is attained in the third trimester, the mean value being 19.93 %. Immediately after delivery a gradual decrease occurs but normal values are not yet attained. At the fifth to eighth day postpartum a percentage of 16.43 is found, a value which may fall within the normal non pregnant range. Following this there is a similiar rise to that which is observed for the basophilic normoblast. The mean percentage is 20.69, which is a higher value than was obtained during the third trimester and which is significantly higher than the non pregnant normal.

Orthochromatic Normoblasts:

The most mature red cell precursors show the same proportional changes as discussed in the case of the basophilic normoblasts and polychromatic normoblasts during pregnancy and the puerperium.

These cells exhibit a gradual increase and a peak is reached during the third trimester. The non pregnant value is 8.10 %; values for the first and second trimester are higher but differences found are too small to draw definite conclusions. In the third trimester a mean percentage of 9.72 is obtained, which is higher than normal, but of doubtful significance. After delivery there occurs a slight and relatively insignificant increase to 10.12 % during the first to fourth day, in contradistinction to the decrease of basophilic normoblasts and polychromatic normoblasts. Following

this the orthochromatic normoblasts drop to 7.92 % on the fifth to eighth day. This value and the relatively higher value of 8.59 % for the sixth week postpartum are not significantly different from the non pregnant normal.

Mitoses:

The mitotic figures, observed in the erythroblastic cells are expressed in absolute numbers - i.e. the value of 3.1 obtained in the normal non pregnant series stands for a mean of 3.1 mitotic figures counted in 500 cells.

From the data given in the table it appears that mitoses occur more frequently during pregnancy. A peak of 4.8 per 500 cells is reached in the third trimester, which is statistically significant. Immediately after delivery their value is found to be within normal limits but slightly higher values are calculated for the fifth to eighth day and the sixth week postpartum.

Graph IV shows curves for the early and late erythroblastic cell series, obtained by combining the erythrogones, the pronormoblasts and basophilic normoblasts in one group of cells and the polychromatic and orthochromatic normoblasts in the other. It will be seen that both curves follow a similar pattern, rising during pregnancy, falling off during the puerperal days and rising again at six weeks postpartum. During pregnancy, however, it appears that the basophilic precursors of the erythrocytes increase relatively more rapidly during the first and particularly the second trimester, which is suggestive of a shift to the left within the red cell series. Postpartum there is no evidence

of this divergence.

An attempt was made to demonstrate a significant shift to the left statistically. The distribution of the various red cell precursors in the bone marrow during pregnancy and the puerperium was examined further. The early red cell precursors (erythrogones + pronormoblasts + basophilic normoblasts) were expressed as the percentage of the total nucleated red cells present. Comparison between the means of the early red cell precursors, found in the bone marrows of the normal non pregnant group and those of the second trimester, showed that there is a significant shift to the left of these nucleated cells during the first two trimesters of pregnancy ($P = 0.01$). This could not be demonstrated in the six weeks postpartum group.

Graph V shows the relation of the erythrogenic-leukogenic ratio of the bone marrow and the hemoglobin values of the peripheral blood, as found in all groups studied. The erythrogenic-leukogenic ratio represents the total percentage of all nucleated red cells of the bone marrow divided by the total percentage of all white cells present, from which the lymphocytes and monocytes have been subtracted. This ratio indicates the proportion of the erythrocytogenic and granulocytogenic bone marrow cells.

For the normal non pregnant series an erythrogenic-leukogenic ratio of 0.37 is found. This value is higher during all trimesters of pregnancy; a maximum of 0.58 is attained during the third trimester, due to the increased erythropoiesis already discussed. A gradual

decrease occurs during the 8 days after delivery, but a higher value is again found at six weeks postpartum (0.96). Comparing the values of this ratio with the hemoglobin of the peripheral blood for all groups studied, it becomes apparent that the erythrogenic-leukogenic ratio rises as the hemoglobin falls, and vice versa. This would suggest that the fall of the hemoglobin during pregnancy (which has been shown to be due to hydremia) may be related to an increase of the erythropoietic activity. It is well known that increased erythropoietic activity may be present in bone marrows of individuals suffering from various anemias, such as anemia associated with systemic infection, nephritis etc., iron deficiency anemia and pernicious anemia. In these anemias the increased erythropoiesis is usually abnormal and inefficient in contrast to the increased erythropoiesis of normal pregnancy. Since the lowered hematologic values during pregnancy are not due to an absolute but only to a relative decrease of the erythrocytes and an absolute anemia is not present, it seems likely that another mechanism is involved.

It has been observed that the total hemoglobin and red cell masses of the peripheral blood increase during pregnancy, although to a lesser extent than the plasma volume.

Eddington and Lowenstein (108) have shown that red cells transfused into normal pregnant women have the same survival time as when transfused into the non pregnant normal subject. Thus, the increased erythropoietic activity of the marrow in pregnancy is not

due to decreased life span of the red cells of the pregnant woman; nor do the morphological characteristics of the red cell series of blood or bone marrow during pregnancy suggest that erythropoiesis in normal pregnancy is pathologic or inefficient.

In view of the above the increased erythropoietic activity of the bone marrow must be related to the increase of total circulating hemoglobin and red cell volume in normal pregnancy. It is interesting to speculate upon the possible role of the placental circulation, the arterial oxygen saturation of the blood of mother and fetus and certain endocrine changes in the mother in providing the stimulus for this increased erythropoiesis.

The increased erythropoiesis which develops between the 8th day and 6 weeks postpartum is more difficult to explain due to the absence of available data. The loss of blood at delivery and the decrease of total circulating hemoglobin and red cell mass may well be of prime importance in stimulating this increase of erythropoietif activity.

White Cell Series:

The results of the differential counts of these cells are presented in Tables III and IV and Graph VI, VII and VIII. Graph VI shows the proportions of the granulocytic cells and their mitoses, as calculated for each group. The following results were obtained after statistical analysis:

Myeloblasts:

The mean percentage for the non pregnant normal is 0.48. During pregnancy and puerperium only minimal changes occur, which

are statistically insignificant.

It is nevertheless interesting to note that these cells show the same proportional changes as the progranulocytes and the early myelocytes during the antepartum and postpartum periods.

Progranulocytes:

These cells show an increase during pregnancy as compared with the non pregnant normal of 1.24 %. The maximal value of 1.9 % is found during the first trimester and the increase is statistically significant. (P less than 0.01). During the second and third trimesters a slight decrease occurs from the first trimester value to 1.63 % and 1.51 % respectively. Following delivery, significantly higher percentages are found (1.82 % for the I - IV postpartum days, 1.89 % for the V - VIII postpartum days and 2.05 % at six weeks postpartum.)

Early Myelocytes:

A similar and highly significant increase of these cells occurs from the non pregnant normal (3.37 %) to the first trimester of pregnancy (4.54 %). During the following two trimesters there appears to be a smooth decline of the curve. A value is found for the third trimester which falls within the normal non pregnant range. As observed for the earlier stages of maturation, another significant increase is seen following delivery, with a maximal value in the I - IV days postpartum period of 4.47 %.

Late Myelocytes:

These cells (as the more mature metamyelocytes) follow a curve which is different from the curves of the earlier granulocytic cells. Compared with the non pregnant value of 21.07 % the late myelocytes seem to decrease during pregnancy, starting in the first trimester (19.39 %), rise slightly, however, during the second (20.76 %) and show a highly significant regression (17.49 %) during the third trimester. A gradual increase towards normal occurs during the early days of the puerperium, and from the V - VIII day a percentage is found, which may fall within the normal non pregnant range (19.11 %). At 6 weeks postpartum a lower than normal value is found (16.46 %) which is highly significant.

Metamyelocytes:

These cells show the same quantitative changes as the late myelocytes. They decrease slightly during pregnancy, compared with the normal non pregnant value (31. 13 %); throughout pregnancy, they represent approximately 28 % of all nucleated marrow cells counted. A gradual increase towards normal occurs shortly after delivery, however. At six weeks post partum these cells, like the late myelocytes have decreased significantly as compared with the non pregnant normal values.

Eosinophilic Cells:

The eosinophilic myelocytes seem to fall slightly during pregnancy; the maximum decrease is found in the third trimester

(0.93 % compared to the non pregnant normal of 1.29 %) and is statistically significant. After delivery, the percentage found during the second four days of the puerperium falls well within the normal non pregnant range. At 6 weeks postpartum a value lower than normal is found (0.87 %). The eosinophilic metamyelocytes show a relative but not significant increase during the first trimester. The values found for the second and third trimester and the early postpartum period are well within normal non pregnant ranges (1.47 %). A slightly higher but insignificant percentage is obtained 6 weeks after delivery. Combining all eosinophilic cells counted, there may be a slight decrease during pregnancy and a gradual increase towards normal during the postpartum period.

Other White Cells Present:

The lymphocytes show a considerable and highly significant fall during pregnancy. The lowest value occurs in the third trimester (9.86 %) and persists through the first 8 days postpartum. At 6 weeks postpartum no significant difference from the non pregnant normal can be demonstrated.

The monocytes show no important quantitative changes during pregnancy or the puerperium, except in the first trimester in which an isolated significant increase is found which is difficult to explain.

The plasma cells increase slightly during the latter part of pregnancy, but highly significant elevated values are only obtained.

in the postpartum period and are maximal V-VIII days after delivery (1. 67 %). The percentage found at 6 weeks after delivery (1.43 %) still differs significantly from normal.

The reticulum cells increase gradually during the latter part of pregnancy and the early postpartum period. Maximal counts are obtained from the first to fourth day after delivery; this peak in the curve is statistically significant. Comparing the mean percentages of the phagocytic reticulum cells, it appears that there is a significant increase in phagocytosis during the last part of pregnancy and to a lesser extent during the early puerperium. The non phagocytic reticulum cells only show minimal changes.

The quantitative changes as just described became apparent after statistical analysis. It must be emphasized that some of these results were not expected. While performing the differential counts the following impressions were obtained:

1. The marrow is definitely more cellular during the latter part of pregnancy and the first 8 puerperal days. This impression is further supported by the fact that more and larger particles are present in the bone marrow aspirate. Total nucleated counts were performed in approximately 75 % of all cases. A slightly increased mean value was obtained for all pregnant and puerperal groups studied (ranging from 154,000 to 177,000) compared to the normal non pregnant group (the average count was 130,000). Although this slight difference cannot be discarded it became apparent that the individual results showed such a large degree of

variation that it was decided not to utilize the total counts in presenting the data.

2. There is a definite increase of erythropoiesis with a shift to the left of nucleated red cells during pregnancy and the puerperium. This was verified by the analysis, as discussed before.

3. There is probably a slight increase of plasma cells and phagocytic reticulum cells. Both observations were supported by the analysis.

4. There is no increased eosinophilia during pregnancy. From the statistical analysis it appears that these cells show a moderate decrease.

5. There is some hyperplasia of the granulocytic series during pregnancy. From the results of the differential counts and the statistical analysis, it becomes apparent that the late myelocytes and metamyelocytes seem to drop significantly during pregnancy and at the sixth postpartum week. This requires further explanation.

Earlier in this chapter it was shown that erythropoiesis is increased during pregnancy and at 6 weeks postpartum. If one compares Graphs IV and VII it is seen, that as erythropoiesis increases during pregnancy and again at 6 weeks postpartum, there is a relative and possibly reciprocal decrease of late myelocytes and metamyelocytes. This decrease is accompanied, however, by an increase of the immature granulocytic precursors (Graph VII). These findings suggest that there is a shift to the left of the granulocytic series during pregnancy and the puerperium. This suggestion is supported by

statistically comparing the immature granulocytic precursors (myeloblasts, progranulocytes and early myelocytes), as percent of total granulocytes, found in the first trimester with those of the non pregnant normal. A P-value well below 0.01 is obtained which indicates that the shift to the left of granulocytes is definitely significant. This significant increase of the more immature granulocytes, the increase of mitotic figures, the observed increase of marrow cellularity during pregnancy, and the early puerperium support the conclusion that granulopoiesis is increased during pregnancy and the early puerperium, although the increase of erythropoiesis is greater than that of granulopoiesis. The leukocytosis of the peripheral blood is consistent with this concept. The apparent decrease of late myelocytes and metamyelocytes then would seem relative rather than absolute. Had stab and segmented neutrophilic forms not been excluded in the differential counts the above changes from the non pregnant normal would not have been so readily demonstrated.

Abnormal Cases.

In the 200 bone marrows of pregnant and puerperal women studied, 3 cases were found which showed some abnormalities. These were women who were examined on the fourth or fifth day after delivery. They did not differ from the other women studied medically, obstetrically or hematologically, except in one case in which there was a M.C.V. of 97 cu. micra. All other hematologic determinations were well in the normal ranges.

From the study of these bone marrows it became evident that the earliest changes towards megaloblastosis was found in one case on the 5th postpartum day. Some of the red cell nuclei showed beginning intermediate transitional changes. Also a few intermediate macrometamyelocytes and macrostabs were present. By comparing the differential count with the results obtained from those of the 40 women studied from the fifth to eighth day after delivery, it became evident that the only quantitative abnormality present was an increase of basophilic red cell precursors to 4.0 %. The M.C.V. was 91 cu. micra.

The other two bone marrows contained a very occasional intermediate macrometamyelocyte and macrostab but erythropoiesis was entirely normoblastic. The only hematological abnormality of the blood of one patient who was 4 days postpartum was a M.C.V. of 97 cu. micra; 4.4 % basophilic normoblasts were present in the marrow, which is higher than normal for this stage of the puerperium. Two bone marrow aspirations were previously performed on this patient: the first in the third trimester and the second one on the first postpartum day. Both bone marrows showed no abnormalities, quantitatively or qualitatively. The results of the differential counts did not differ from the other bone marrows studied during similar stages of normal pregnancy and puerperium. Aside from the occasional macrometamyelocyte and macrostab, the bone marrow from the third patient (5 days postpartum) showed no quantitative or qualitative differences from the other cases studied at this postpartum period.

It is beieved that the marrow findings in these three patients may have been the first reflection of hematological abnormality, which could have developed had the pregnancies not been terminated.

CHAPTER II

COMPARISON WITH OTHER AUTHORS

I. Studies on Normal Bone Marrows:

Numerous reports concerning bone marrow differential counts in normal individuals have appeared in the literature. Comparison of these reports with the data of this work seems useless in many instances, due to the differences in technique, terminology or in the selection of material. Several authors failed to mention how many subjects were examined (30, 33, 43, 53, 79, 81, 88) and others reported a study compiled from one to ten cases (42, 85, 109, 110, 111, 112, 113, 114). No further information was given by a great number of authors regarding the sex (11, 31, 34, 42, 53, 57, 88, 109, 114, 115) or the age (11, 29, 53, 88, 109, 110) of the individuals studied. Several articles stated that their supposedly normal subjects were patients who were hospitalized for minor illnesses (11, 15, 90). In many instances, no information was given regarding the amount of bone marrow material aspirated (11, 15, 29, 31, 34, 88, 110, 116, 117). Most authors prefer to withdraw less than 0.5 ml ; a few are of the opinion that larger amounts are desirable (18, 28, 35, 38, 118, 119). In some instances, the data given in the report were compiled from bone marrow obtained with different techniques (32, 34, 88). A great number of authors failed to mention how many cells were differentiated (29, 30, 33, 34, 38, 53, 79, 81, 88, 110, 120); others counted fewer than 500 cells (43, 109, 114).

Apart from the different terminology used in the differential counts by various authors, comparison of their data is difficult because of the different methods of presentation. Several articles expressed their results in ranges and did not report the means (11, 29, 47, 79, 121). Others express the mean percentages of the red and of the white blood cell series separately or express the number of nucleated red cells as the percentage of 100 white cells present.

The results of the bone marrow differential counts on 30 healthy non pregnant women, presented in this work, were compared with the reports of those studies which permitted satisfactory comparison on the basis of similar techniques and interpretations. It was necessary to exclude most of the numerous studies of normal non pregnant bone marrows for the reasons already discussed.

Studies which were accepted were required to conform with the following criteria:

1. The amount of marrow material aspirated must have been 0.3 ml or less.
2. The number of cases reported must have been 10 or more.
3. The number of cells counted in each differential must have been 500 or more.
4. The description of cells and of their terminology must be comparable.
5. The statement must have been made, or adequate evidence must have been reported that all examined individuals were healthy.

6. The differential count must have been performed on marrow smears (cover glasses or slides), prepared directly from the aspirated sample.

7. The marrow should have been obtained by the aspiration technique at the corpus sterni (second or third interspace).

In Table V are shown the results of bone marrow differentials from normal individuals performed by Segerdahl (36), Forsell (20), Leitner (49), Fontein (122), Fadem and Berlin (76) and by the author. It was necessary to recalculate the data given by these authors, since they included the polymorphonuclear and stab neutrophils in their differential counts. The cells have been grouped according to the description given by these authors, but it is realized that unavoidable errors have been made, since the criteria for cell identification were too incomplete in some instances to allow accurate grouping. Segerdahl's series concists of young healthy women; the other authors drawn from the literature included both men and women in their series. There is one report on the bone marrow of healthy women, by Pitts and Packham, but these authors aspirated 10 ml of marrow fluid (18). Fadem and Berlin compared differential counts of smears made from 0.1 to 0.3 ml of " marrow juice " with those of smears made from marrow particles of 10 normal subjects.

A few conclusions can be drawn by comparison of the data of bone marrow differential counts performed by these authors.

The individual results vary considerably; this is not suprising since the comparison is based on mean values for each type of cell and not on ranges. Some of the authors also reported their ranges, calculated by adding and subtracting one or two times the standard deviation found for each type of cell. Segerdahl has suggested that it is safer to determine the upper limit of variability as the mean plus four times the standard deviation but this would mean that many differential counts of pathologic bone marrow would be covered by the wide normal range calculated in this manner.

Inspection of the individual results shows that the greatest degree of variability seems to occur within the granulocytic and erythrocytic series of cells. The degree of maturity can often be decided by subjective criteria only since changes within one series of cells occur gradually. By adding all granulocytid cells and all erythrocytic cells as shown in Table V, it becomes apparent that the differential counts performed in this study are in general agreement with those reported by Segerdahl and Forsell. Segerdahl found 51.53 % granulocytic and 16.81 % erythrocytic cells, resulting in a ratio (erythrocytic/ granulocytic cells) of 0.33. Forsell found 55.80 % granulocytic and 19.97 % erythrocytic cells, yielding a ratio of 0.36. In this study 57.29 % granulocytic and 23.57 % erythrocytic cells were found with a ratio of 0.41. Fadem and Berlin, and Fontein obtained a relatively higher percentage of nucleated red cells in their counts and fewer granulocytic cells, but the ratio still remains well below 1.

Leitner's results are such that a ratio higher than 1 was obtained (1.08). There is fair agreement between all authors regarding eosinophilic cells. Fontein and Leitner found more plasma cells than others. Segerdahl found 26.45 % lymphocytes in her counts of normal marrows. Fadem and Berlin found 25.5 % lymphocytes in marrow juice and 3.63 % lymphocytes in marrow particles, which illustrates the different values which may be obtained with different techniques and with different degrees of dilution by the circulating blood.

II. Studies on Bone Marrows in Pregnancy and Puerperium:

A review of the literature regarding bone marrow in pregnancy has been given in Chapter I. The conclusions of all reports and some of the technical details, both important for a comparison with the results of this study will be discussed.

Daniachij performed differential counts on 40 bone marrows obtained from healthy pregnant women from the first to third trimester and on 10 marrows taken 7 to 8 days after delivery. He obtained the marrow material by aspirating 0.5 - 1.0 ml from the manubrium sterni and differentiated 500 to 1500 cells, including all nucleated cells present, on glass slides, stained according to Pappenheim. He compared the results obtained with the ranges given by Arinkin for apparently healthy mena and women. From these counts and his morphological study he concluded that a striking increase of erythropoiesis , together with an obvious change towards megaloblastosis, starts to occur early in pregnancy. He further claimed that the greatest number of megaloblasts

were found in the 7th to 8th month of pregnancy (1 % of all cells differentiated). Daniachij states that these cells, which resembled the red cell precursors of the embryo, are normally absent in the bone marrows. Unfortunately he does not describe the characteristics of these cells and only presents a diagram showing the derivation of the erythrocytes. No deductions can be made regarding morphologic characteristics, but it appears from the scheme that his megaloblast is derived from the macroblast which he believes to be the precursor of both normoblastic and megaloblastic cell series. According to him the macroblast is a lympho-erythroblastic cell. His megaloblast might well have represented a cell which has been called erythrogone, macropronormoblast etc. by others. This subject has been discussed in Chapter V.

The normoblastic nucleated red cells reached the upper limit of Arinkin's ranges (16.7 %), but it is obvious that no definite conclusions can be drawn on this basis. Daniachij stated clearly that the erythrocytes of the peripheral blood showed no abnormalities. The hemoglobin (given in percent) and RBC were within the range of normal. The color index varied from 0.8 - 0.9. Further evidence for increased erythropoiesis was an increase of observed mitoses during the latter months of pregnancy.

Evidence for increased leukopoiesis was a higher percentage of neutrophilic metamyelocytes, as compared with Arinkin's normal values. During the 8th month of pregnancy a maximal percentage was obtained, following which a slight decrease occurred. Further

evidence of a shift to the left was a higher percentage of myelocytes and progranulocytes during the second and third trimester compared with the first trimester. Increased mitotic figures of the granulocytic cell series were also observed.

Daniachij obtained a relatively high percentage of eosinophilic cells during the first six months of pregnancy (maximal 4.7 %) but this conclusion was again drawn on the basis of Arinkin's figures.

The monocytes were fewer in comparison with Arinkin's data for normal bone marrow and also in comparison with the percentage of the monocytes of the peripheral blood of the subjects examined. Daniachij concluded therefore that it is evident that monocytes are not found in the bone marrow.

The lymphocytes appeared to be decreased in 50 % of all cases, when compared with Arinkin's figures; the lymphocyte count of the blood, however was always within normal ranges in these cases.

The peripheral blood showed neutrophilia and lymphocytopenia, initiated in the 7th month of pregnancy. At the same time a moderate fall of the hemoglobin and RBC was observed. These changes in the peripheral blood, according to Danachij were explicable by the quantitative and qualitative changes of the bone marrow. He concluded that it is justified to speak of a " bone marrow of pregnancy ". At 6 days after delivery the bone marrow had not yet returned to normal. The same changes in the leukopoiesis were found as during pregnancy; yet megaloblasts had disappeared and erythropoiesis had regressed to normal.

It is difficult to assess Daniachij's evaluation of the bone marrow differential counts, since they were based on a study of another author. From his morphological description of the bone marrow during pregnancy and the puerperium it can be deducted that incfeased leukopoiesis and, to a lesser extent, increased erythropoiesis were observed.

Forsell compared the differential counts performed on the marrows of 8 pregnant women, all of whom except one, were four months pregnant,with the counts obtained from the bone marrows of 10 healthy men and 10 healthy women.

He estimated that the cellularity of these bone marrows was normal. His bone marrow samples were obtained by aspiration from the corpus sterni (second intercostal space) and 0.1 to 0.2 ml were aspirated. In each case 1200 white cells were counted; nucleated red cells, plasma cells, reticulum cells, and all mitotic figures observed were expressed in numbers per 400 white cells.

He was not able to show any changes in the bone marrows of these pregnant women, either morphologically or quantitatively.

Pitts and Padkham examined 40 healthy, pregnant women. The marrows were obtained by the aspiration technique from the sterno manubrial junction in a quantity of 10 ml and oxalate was used as an anticoagulant. In each case a total of 500 cells were counted.

On the basis of their own non pregnant normal values obtained from the bone marrows of 24 healthy young women, they concluded that there is a general hyperplasia of the marrow during

pregnancy, which involves all cells equally. Total nucleated counts of the bone marrow of the non pregnant group range from 7,750 to 46.000 and those of the pregnant group varied from 14,400 to 125,000. This difference was found to be significant. It is to be noted that these authors aspirated an extremely large amount of marrow, which consequently must have had a high degree of dilution. The neutrophilic stabs and polymorphonuclear cells amounted to an average of 49 % and the lymphocytes to 12.7 % . After adding 12.93 %, the percentage of the disintegrated cells (which might well have been due to the oxalate used), it becomes evident that these cells constituted 74.63 % of all cells counted. It seems likely that small quantitative changes of all other marrow cells could not have been detected.

Markoff reported morphologic studies on the bone marrow in pregnancy. No information was given as to how many cells were observed nor are other technical details discussed. He emphasized that there were no megaloblasts present in the bone marrow of normal pregnancy as stated by Daniachij. He observed an increase of normoblastic erythropoiesis, often with cluster formation of polychromatid normoblasts. As pregnancy advanced more of these clusters were found, some of which consisted of large erythroblasts, his so called macroblasts. A second characteristic during early pregnancy were changes in the granulocytic cell series, which showed a striking anisocytosis, often with giant forms, such as are seen

in pernicious anemia. This was particularly observed in the promyelocytes. The anisocytosis of the myelocytes and metamyelocytes was much less conspicuous. The leukocytes of the peripheral blood showed no abnormalities. Markoff was not able to give an explanation for these observations and suggested pregnancy as a possible cause.

Further deviation from normality was an increase of immature eosinophilic cells, after the 6th month of pregnancy, which, according to Markoff indicated a hypersensitivity to proteins. He believed that this latter hypothesis was supported by an increase of plasma cells, starting in the 3rd month of pregnancy. Some of these cells showed mitoses and contained 2 or more nucleoli; others were larger than normal. According to Markoff plasma cells belong to reticulum cells, but no changes were observed in phagocytic and non phagocytic reticulum cells. No abnormalities were found in the postpartum period; it is not stated at what time after delivery these observations were made.

Wolff's and Limarzi's conclusions regarding the bone marrow in normal pregnancy are that both erythropoiesis and leukopoiesis increase during pregnancy. This was deducted from the increased total nucleated cell count and the increased bone marrow cell volume in the hematocrit. Erythropoiesis and leukopoiesis were morphologically normal, but showed a slight but insignificant immaturity towards the end of pregnancy. A megakaryocytic hyperplasia was also found. The bone marrow did not return to normal for at least six weeks postpartum

Callender's conclusions were based on the statistical analysis and comparison of differential counts of 29 bone marrows, obtained from 19 healthy and puerperal women and 10 healthy non pregnant women. These marrows were aspirated from the sternum; the quantity of aspirate is not stated. Presumably the amount was a small one since she criticizes Pitt and Packman who aspirated 10 ml. 500 cells were differentiated and Fisher's t- test was applied for the analysis. She was unable to verify the findings of other authors that there is an erythroblastic hyperplasia in the marrow in pregnancy. Also she was unable to demonstrate a shift to the left of erythropoiesis. From morphological observations, of marrow sections, she stated that there appeared to be a slight tendency towards hyperplasia in the late weeks of pregnancy and in the early puerperium. She observed occasional large erythroblasts showing premature hemoglobin formation and the proerythroblasts seemed to have increased somewhat in number. No megaloblasts, however, were observed. Occasional clump formation of erythroblasts, large progranulocytes and groups of plasma cell type reticulum cells were observed. These changes were sufficiently striking to justify descriptions of a characteristic marrow of pregnancy.

Leitner's study was compiled from 12 dases, 3 of which were in the third trimester of pregnancy. The marrow was aspirated in a quantity of 0.1 - 0.3 ml from the sternum (second or third interspace). Counts of 500 cells were performed from smears made on glass slides. He presents the differential counts individually and draws no definite conclusions from the data. Comparing the results with a series of 22

bone marrows of men and non pregnant women it appeared to him that erythropoiesis and/or granulopoiesis may increase during pregnancy, but not to such an extent that it is characteristic. The nucleated red cells were normoblastic and early basophilic seemed to have increased somewhat. He observed some large granulocytes, similar to giant neutrophils.

To Summarize:

Variable general cellular hyperplasia has been observed in the bone marrow during pregnancy by all authors but one. Forsell studied a small group of patients; only one of these had advanced to the third trimester of pregnancy. Most authors agree that quantitative changes, if present, reach their maximum during the last stage of pregnancy. Markoff stated that erythropoiesis increases to a greater extent than leukopoiesis: this statement was based on his visual estimate, not on the result of differential counts. No author found megaloblasts other than Danachij. Callender and Leitner and Markoff observed an increase in numbers and size of the basophilic erythroblasts, which however retained their normoblastic characteristics. Wolff and Limarzi state that there is a minimal and insignificant shift to the left of all bone marrow cells.

Danachij found that leukopoiesis increased to a greater extent than erythropoiesis during pregnancy. This was evidenced by an increase of mitotic figures and a shift to the left of the granulocytic cell series. According to Markoff striking anisocytosis of the progranulocytes occurs ; Callender observed only an occasional

large progranulocyte, but Leitner found giant neutrophilic cells. Increase of plasma cells was observed by Markoff and increase of plasma cell type reticulum cells was found by Callender. Daniachij noted marrow eosinophilia and Markoff observed that the immature eosinophilic myelocytes seemed to have increased somewhat in number.

Only three of these authors commented on the appearance of the bone marrow after delivery. Markoff stated that it did not differ from normal, but did not indicate what stage postpartum these observations were made. Daniachij found that the marrow had not returned to normal and that leukopoiesis was still increased at 6 days postpartum.

Wolff and Limarzi's findings indicated that the normal non pregnant state of the marrow was not attained until at least six weeks after delivery.

In this study an increased normoblastic erythropoiesis was found during pregnancy. A shift to the left in both the erythrocytogenic and granulocytogenic cell series was demonstrated, indicating general marrow hyperplasia. From morphologic observations it can be concluded that there may exist a slight anisocytosis of the immature white cell precursors, but giant metamyelocytes or stab cells were never observed, other than in the three pathologic cases discussed.

It was also shown that plasma cells and reticulum cells are increased during pregnancy; eosinophilia, however, could not be demonstrated and our findings suggest that the eosinophilic cells

decreased. Our observations that the bone marrow does not return to normal for a considerable time after delivery (> 6 weeks) are in agreement with those of Wolff and Limarzi.

SUMMARY AND CONCLUSIONS

1. The bone marrow findings of normal subjects and of women during normal pregnancy and puerperium have been critically reviewed from the available literature.

2. The morphological findings of bone marrow aspirates, obtained from the second or third interspace of the corpus sterni, from healthy pregnant and puerperal women were compared with those of marrow aspirates from healthy non pregnant women.

The material studied consisted of bone marrow aspirates, obtained from thirty subjects during each of the three trimesters of pregnancy, from ten subjects on each of the first eight days postpartum, from thirty subjects at six weeks postpartum and from thirty non pregnant normal women of childbearing age.

3. Differential counts were performed on 500 bone marrow cells in each case and the results were statistically analysed.

4. The results showed that there are significant quantitative changes in the cellular components of the bone marrow during pregnancy and the puerperium, which are maximal in the third trimester.

5. There is a significant increase of erythropoiesis during pregnancy; this was deducted from both observations and statistical analysis of the differential counts. A significant shift to the left of the nucleated red cells was demonstrated.

6. A significant shift to the left of the precursors of the neutrophilic cells was also demonstrated. This result and the increased cellularity to observation are suggestive of an increase of granulopoiesis.

7. During the early days of the puerperium there is a tendency towards restoration to the normal non pregnant findings.

8. The bone marrow, examined at six weeks postpartum, has not returned to normal; in fact both erythropoiesis and to a lesser extent granulopoiesis are more active than early in the puerperium.

HEMATOLOGICAL DETERMINATIONS OF THE PERIPHERAL BLOOD

Mean Values, Standard Deviation and Standard Errors

in

Pregnancy, Puerperium and 6 Weeks Post Partum

Compared With

Normal Non Pregnant Women

	NORMAL			1st TRIMESTER			2nd TRIMESTER			3rd TRIMESTER			I - IV DAY P.P.			V- VIII DAY P.P.			6 WEEKS P.P.		
DETERMINATIONS	M.	S.D.	S.E.	M.	S.D.	S.E.	M.	S.D.	S.E.	M.	S.D.	S.E.	M.	S.D.	S.E.	M.	S.D.	S.E.	M.	S.D.	S.E.
HGB.(gm/100ml)	13.6	0.77	0.14	12.8	1.10	0.20	12.24	1.03	0.19	12.23	0.98	0.18	13.43	1.65	0.26	13.64	1.47	0.23	13.20	0.78	0.14
R.B.C.($x10^6$)	4.53	0.27	0.05	4.1	0.31	0.06	3.87	0.35	0.06	3.95	0.31	0.06	4.27	0.46	0.07	4.28	0.45	0.07	4.45	0.26	0.05
HEMATOCRIT (%)	41.8	2.26	0.41	39.1	2.92	0.53	36.6	2.78	0.51	37.7	2.67	0.49	40.7	4.26	0.67	41.3	3.82	0.60	41.3	2.12	0.39
W.B.C.($x10^3$)	9.11	2.10	0.38	11.11	2.62	0.48	11.36	2.90	0.53	11.22	3.06	0.57	14.11	4.32	0.68	11.87	3.25	0.51	9.87	2.24	0.41
RETICULOCYTES(%)	1.22	0.81	0.15	1.80	0.96	0.18	1.62	1.08	0.20	1.69	1.07	0.20	2.10	1.26	0.20	1.82	1.11	0.17	1.66	0.98	0.18

TABLE I

P-VALUES OF THE DETERMINATIONS OF THE PERIPHERAL BLOOD

Comparing
the

THREE TRIMESTERS OF PREGNANCY, PUERPERUIM AND 6 WEEKS POST PARTUM

with

NORMAL NON PREGNANT WOMEN

DETERMINATIONS	1st Tri	2nd Tri.	3rd. Tri.	1-1V DAYS P.P.	V-V111 DAYS P.P.	6 WEEKS P.P.
Hgb.	0.01	0.001	0.001	-	-	0.05
R.B.C.	0.001	0.001	0.001	0.01	0.01	-
P.C.V.	0.001	0.001	0.001	-	-	-
W.B.C.	0.01	0.01	0.01	0.001	0.001	-
Retics.	0.05	-	-	0.01	0.02	-

P. values found are less or approximately equal to the values stated.
P. values greater than 0.15 are not shown in this table.

TABLE 11

STERNAL MARROW DIFFERENTIAL COUNTS

MEAN VALUES, STANDARD DEVIATIONS & STANDARD ERRORS

IN

THREE TRIMESTERS OF PREGNANCY, PUERPERIUM AND 6-WEEKS POST PARTUM

COMPARED WITH

NORMAL NON PREGNANT WOMEN.

Cell Types (%)	NORMAL			1st TRIMESTER			2nd TRIMESTER			3rd TRIMESTER			1st - 4th DAY p.p.			5th - 8th DAY p.p.			6 WEEKS p.p.		
	M	S.D.	S.E.	M	S.D	S.E.	M	S.D	S.E.	M	S.D.	S.E.	M	S.D.	S.E	M	S.D.	S.E.	M	S.D.	S.E.
Myeloblasts	0.48	0.31	0.06	0.52	0.38	0.07	0.50	0.34	0.06	0.49	0.42	0.08	0.50	0.38	0.06	0.54	0.34	0.05	0.64	0.43	0.08
Progranulocytes	1.24	0.50	0.09	1.90	1.06	0.19	1.63	0.77	0.14	1.51	1.07	0.20	1.82	0.81	0.13	1.89	0.91	0.14	2.05	0.89	0.16
Early Myelocytes	3.37	1.17	0.21	4.54	1.45	0.27	4.25	1.22	0.22	3.57	1.22	0.22	4.20	1.52	0.24	4.47	1.98	0.31	4.30	1.80	0.33
Late Myelocytes	21.07	4.09	0.75	19.39	4.53	0.83	20.76	3.58	0.65	17.49	3.95	0.72	18.20	3.45	0.55	19.11	5.06	0.80	16.46	3.82	0.70
Metamyelocytes	31.13	4.56	0.83	28.25	5.48	0.00	28.47	4.38	0.80	27.95	5.41	0.99	27.95	5.09	0.80	30.11	6.01	0.95	25.63	5.09	0.93
Eosinophilic Myelocytes	1.29	0.62	0.11	0.99	0.45	0.08	0.97	0.47	0.09	0.93	0.38	0.07	0.95	0.44	0.07	1.10	0.98	0.15	0.87	0.53	0.10
Eosinophilic Metamyelocytes	1.47	0.57	0.10	1.71	0.86	0.16	1.55	0.77	0.14	1.39	0.75	0.14	1.30	0.76	0.12	1.55	1.06	0.17	1.89	0.86	0.16
Lymphocytes	13.45	2.66	0.49	11.57	3.78	0.69	10.03	3.49	0.64	9.86	3.00	0.55	9.97	3.51	0.56	9.76	2.99	0.47	11.86	3.86	0.70
Monocytes	1.01	0.52	0.09	1.87	1.14	0.21	1.37	0.99	0.18	1.25	0.87	0.16	1.19	0.80	0.13	1.33	0.77	0.12	3.45	0.86	0.16
Megakaryocytes	0.12	-	-	0.15	-	-	0.11	-	-	0.19	-	-	0.25	-	-	0.32	-	-	0.19	-	-
Plasma Cells	0.86	0.57	0.10	1.12	0.63	0.11	0.91	0.57	0.10	1.24	0.74	0.14	1.59	0.87	0.14	1.67	0.89	0.14	1.43	0.83	0.15
Reticulum Cells (phagocytic)	0.19	-	-	0.17	-	-	0.22	-	-	0.39	-	-	0.56	-	-	0.29	-	-	0.18	-	-
Reticulum Cells (non-phagocytic)	0.76	0.33	0.06	0.89	0.54	0.12	0.85	0.54	0.10	0.80	0.60	0.11	1.02	0.54	0.09	0.91	0.47	0.07	0.70	0.44	0.08
Mitoses	1.87	1.52	0.28	2.67	1.85	0.34	1.47	1.18	0.21	2.40	1.91	0.35	2.20	1.75	0.28	2.72	1.34	0.21	2.90	1.51	0.28
Pronormoblasts	0.92	0.41	0.08	0.85	0.65	0.12	0.86	0.41	0.07	0.87	0.41	0.08	0.88	0.53	0.08	0.70	0.59	0.09	0.96	0.57	0.10
Basophilic Normoblasts	1.08	0.41	0.08	1.85	0.86	0.12	2.31	1.52	0.28	2.34	1.43	0.26	2.17	1.05	0.17	1.86	1.01	0.16	2.12	0.89	0.16
Polychromatic Normoblasts	13.47	3.82	0.70	15.97	5.18	0.95	15.54	4.89	0.89	19.93	5.16	0.94	17.55	5.91	0.95	16.43	5.05	0.94	20.69	6.10	1.11
Orthochromatic Normoblasts	8.10	2.47	0.45	8.25	3.19	0.58	9.58	4.31	0.79	9.72	3.54	0.65	10.12	4.24	0.67	7.92	4.63	0.75	8.50	4.26	0.78
Mitosis	3.10	1.82	0.30	4.00	2.72	0.50	3.57	2.29	0.42	4.80	3.50	0.64	3.62	2.18	0.34	4.42	2.25	0.36	4.77	2.45	0.45

TABLE III

P - VALUES OF STERNAL MARROW DIFFERENTIAL COUNTS

Comparing
the

THREE TRIMESTERS OF PREGNANCY, PUERPERIUM AND 6 WEEKS POST PARTUM

with

NORMAL NON PREGNANT WOMEN

CELL TYPES	1st Tri.	2nd Tri.	3rd. Tri.	I-IV DAYS p.p.	V-VIII DAYS p.p.	6 WEEKS p.p.
Myeloblasts	-	-	-	-	-	-
Progranulocytes	0.01	0.05	-	0.001	0.001	0.001
Early Myelocytes	0.001	0.01	-	0.01	0.01	0.02
Late Myelocytes	-	-	0.001	0.01	-	0.001
Metamyelocytes	0.05	0.05	0.02	0.01	-	0.001
Eosinophilic Myelocytes	0.05	0.05	0.01	0.02	-	0.01
Eosinophilic Metamyelocytes	-	-	-	-	-	0.05
Lymphocytes	0.05	0.001	0.001	0.001	0.001	-
Monocytes	0.001	-	-	-	-	0.02
Plasma Cells	-	-	0.05	0.001	0.001	0.01
Reticulum Cells (phagocytic)	-	-	0.01	0.02	-	-
Reticulum Cells (non-phagocytic)	-	-	-	0.02	-	-
Mitoses	-	-	-	-	0.02	0.01
Pronormoblasts	-	-	-	-	-	-
Basophilic Normoblasts	0.001	0.001	0.001	0.001	0.001	0.001
Polychromatic Normoblasts	0.05	-	0.001	0.001	0.02	0.001
Orthochromatic Normoblasts	-	-	0.05	0.02	-	-
Mitoses	--	-	0.001	-	0.01	0.01

* P. values found are less or approximately equal to the values stated.
P. values greater than 0.05 are not shown in this table.

TABLE IV

DIFFERENTIAL CELL COUNTS
REPORTED ON
STERNAL MARROWS OF HEALTHY INDIVIDUALS.

AUTHORS	MYELOBLAST	PROMYELOCYTE	EARLY MYELOCYTE	LATE MYELOCYTE	METAMYELOCYTE	EOSINOPHILIC MYELOCYTE	BASOPHILIC METAMYELOCYTE	LYMPHOCYTE	MONOCYTE	MEGAKARYOCYTE	PLASMA CELL	RETICULUM CELL (PHAGOCYTIC)	RETICULUM CELL (NON PHAGOCYTIC)	PROMORRHOBLAST	BASOPHILIC NORMOBLAST	POLYCHROMATIC NORMOBLAST	ORTHOCHROMATIC NORMOBLAST	UNDIFFERENTIATED	MISCELLANEOUS	NO. OF CASES AND SEX	AMOUNT ASPIRATED (ml)	AGE IN YEARS	NO. OF CELLS COUNTED
SEGERDAHL (1935)	1.75	2.41	24.24		23.13	2.12		26.45	2.35	0.036	0.61	0.07		16.81				-	-	40 ♀	0.2	20-30	1000
FOSSELL (1939)	1.10	2.07	15.38		37.25	2.23		18.15	2.43	0.08	0.85	0.48		19.97				-	-	20 ♀♂	0.1-0.2	?	1200 (W.B.C.)
LEITNER (1941)	1.67	3.06	7.50	10.01	14.18	1.94	1.11	10.56	1.94	1.11	1.42	0.28	1.94	1.11	4.45		33.91	2.43	1.38*	22 ♀♂	0.1-0.3	?	500
FONTEIN (1945)	1.77		6.73	20.04	15.25	2.42	0.51	10.03	1.00	-	2.87	2.11		12.61			22.10	2.16	0.37**	25 ♀♂	0.1-0.3	20-45	500 (W.B.C.)
FABEN et al (1951)	1.36	2.71	19.00		22.62	-	-	23.50	-	-	-	2.26		1.58	4.98		21.04	-	-	10 ♀♂	0.1-0.3	?	500
FABEN et al (1951)	1.38	3.63	27.11		19.34	-	-	3.63	-	-	-	3.45		2.24	8.29		30.91	-	-	id.	particles	id.	id.
OWN VALUES (1955)	0.48	1.24	3.37	21.07	31.13	1.29	1.47	13.45	1.01	0.12	0.86	0.19	0.76	0.92***	1.08	13.47	8.10	-	-	30 ♀	0.2	19-41	500

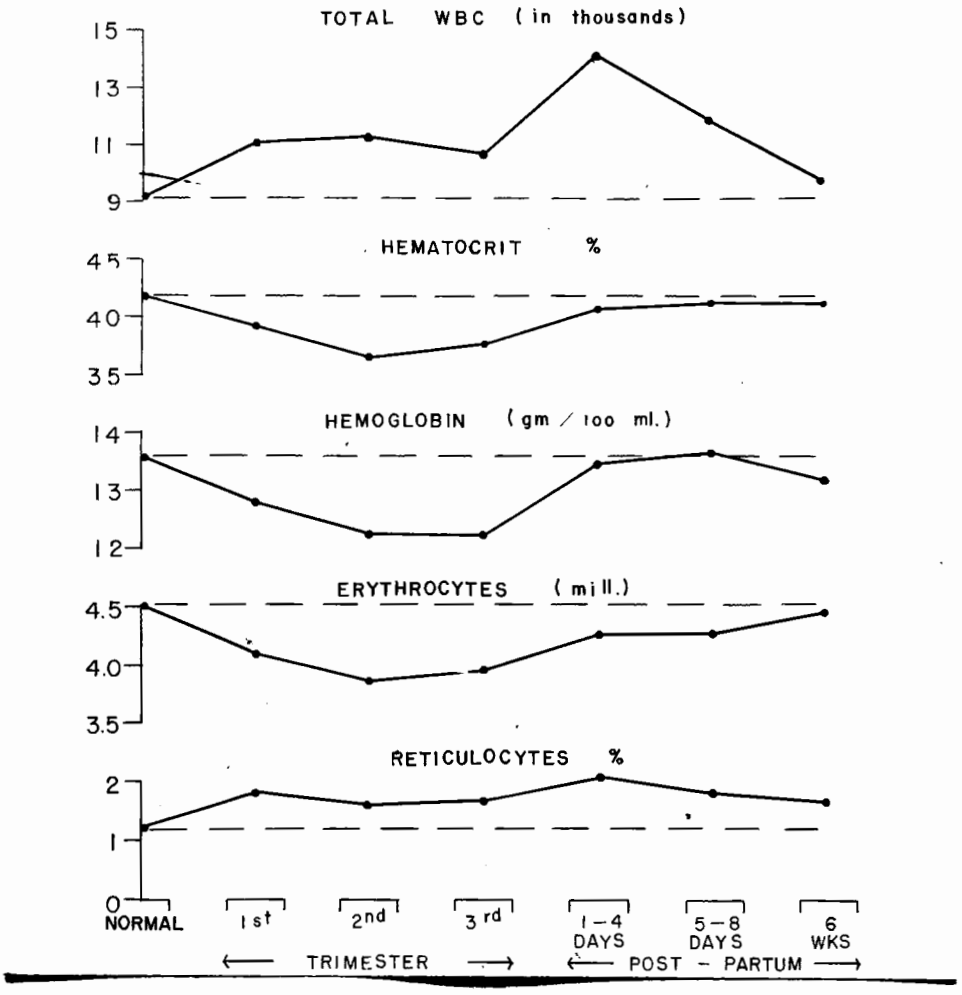
* 0.83 Ferrata Cells 0.55 Endothelial Cells

** Ferrata Cells

*** Erythrogones + Promormoblasts

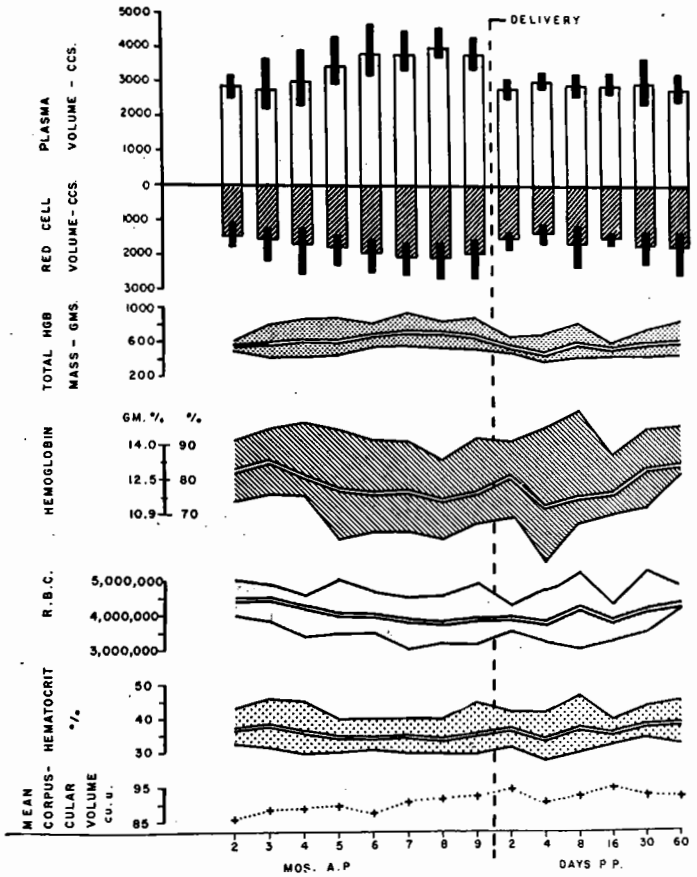
TABLE V

THE PERIPHERAL BLOOD OF PREGNANT AND NON - PREGNANT WOMEN



GRAPH I

BLOOD STUDIES IN PREGNANCY AND THE PUERPERIUM.

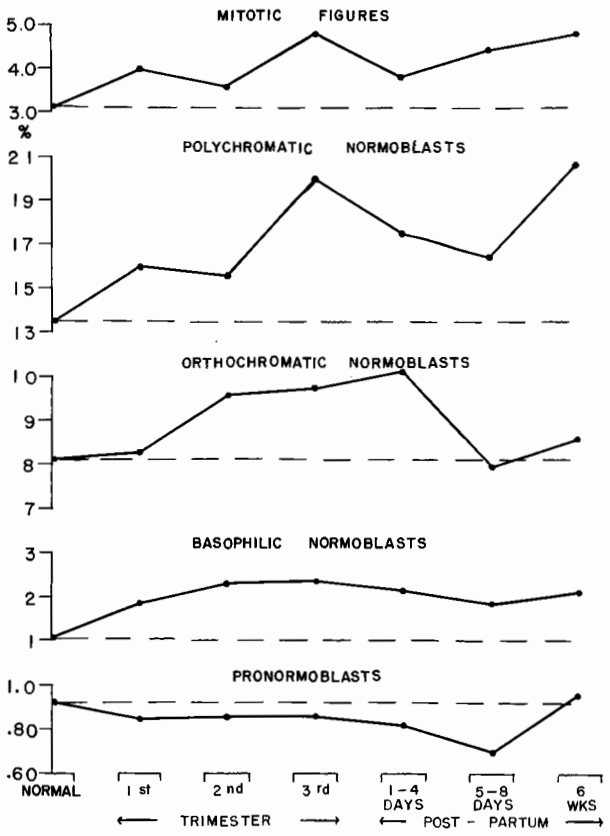


-Serial blood studies of 14 normal women in pregnancy and the puerperium. Plasma and red cell volume: Large columns represent average values. Small black columns represent minimum and maximum extremes.

Red cell volume, total Hgb. mass, Hgb., R.B.C., and hematocrit: Shaded areas represent minimum and maximum extremes. White lines represent the average values.

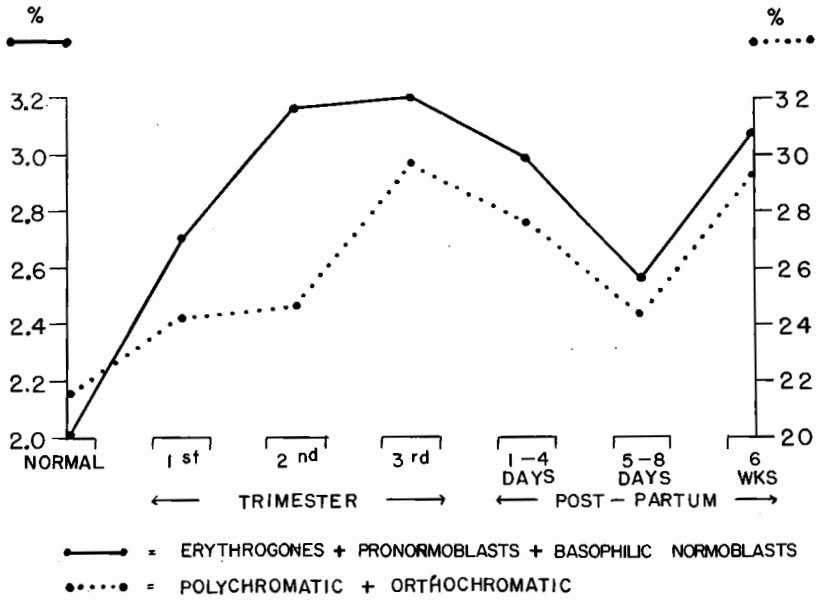
GRAPH II

THE PROPORTIONS OF THE RED CELL PRE - CURSORS AND THEIR MITOSES IN THE BONE MARROW OF PREGNANT AND NON - PREGNANT WOMEN



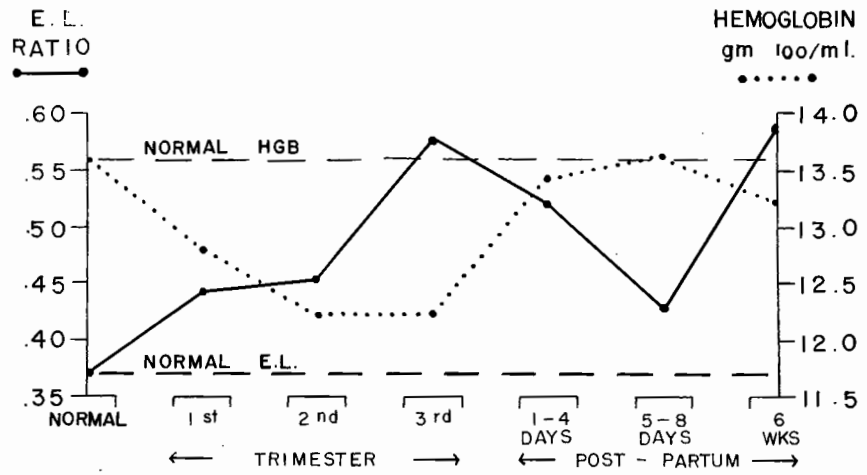
GRAPH III

THE PROPORTIONAL RELATION BETWEEN THE IMMATURE AND MATURE NORMOBLASTS IN THE BONE MARROW OF PREGNANT AND NON - PREGNANT WOMEN



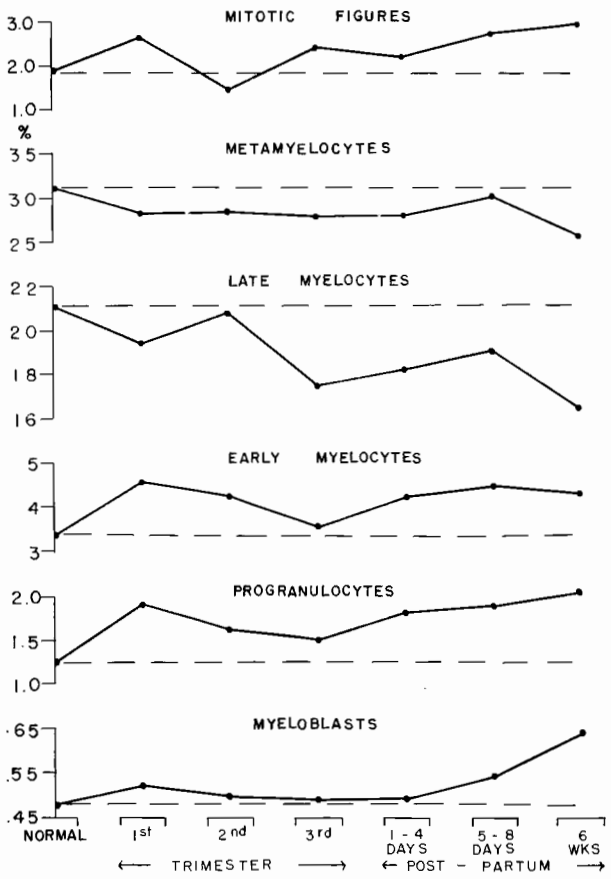
GRAPH IV

THE RELATION OF THE ERYTHROGENIC - LEUKOGENIC RATIO OF THE BONE MARROW AND THE HEMOGLOBIN VALUE OF THE PERIPHERAL BLOOD. (PREGNANT, NON- PREGNANT, AND PUERPERAL WOMEN)



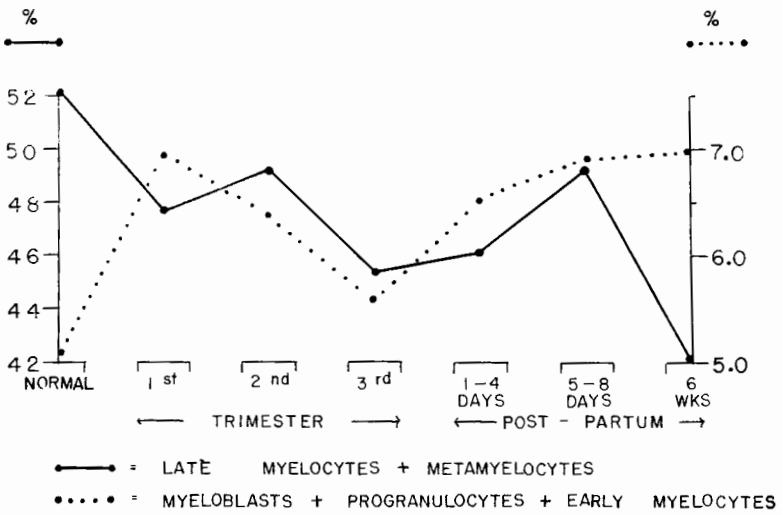
GRAPH V

THE PROPORTIONS OF THE NEUTROPHILIC PRECURSORS AND THEIR MITOSES IN THE BONE MARROW OF PREGNANT AND NON-PREGNANT WOMEN



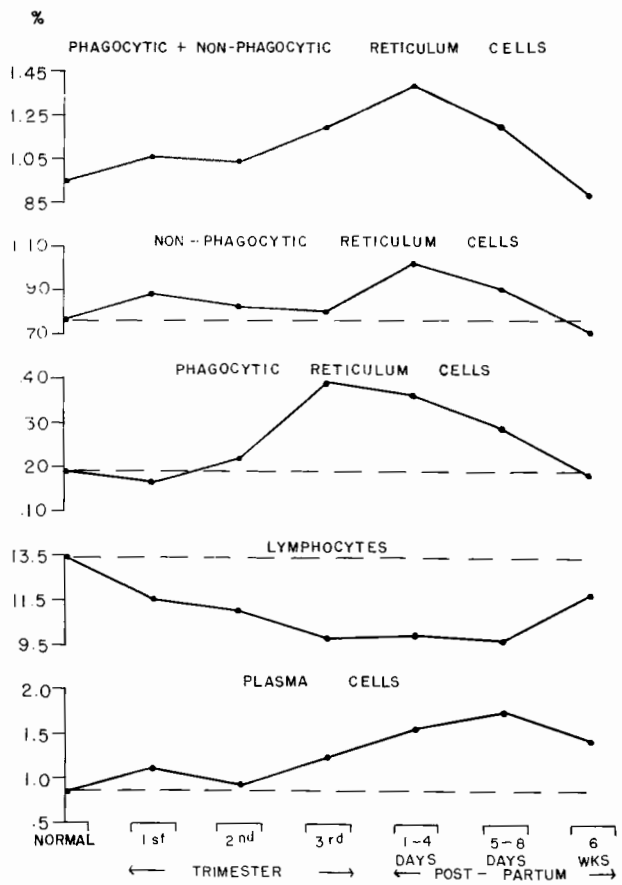
GRAPH VI

THE PROPORTIONAL RELATION BETWEEN THE IMMATURE AND MATURE PRECURSORS OF THE NEUTROPHILIC CELL SERIES IN THE BONE MARROW OF PREGNANT AND NON-PREGNANT WOMEN



GRAPH VII

THE PROPORTIONS OF RETICULUM CELLS, LYMPHOCYTES AND PLASMA CELLS IN THE BONE MARROW OF PREGNANT AND NON - PREGNANT WOMEN



GRAPH VIII

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