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Aspiration Biopsy of Bone Marrow

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Address communications to: Staff Bulletin, 332M University of Minnesota Hospitals, Minneapolis 14, Minn.

I. ASPIRATION BIOPSY OF BONE MARROW

R. Dorothy Sundberg

Although the peripheral blood often adequately and clearly reflects the activity of its parent tissue, the red bone marrow, in many instances alterations in the blood picture lack the specificity required for diagnosis. Aspiration of marrow, a relatively new technical procedure, has become a popular as well as a useful method of procuring a sample of this major hematopoietic organ. By means of study of stained dry films and sectioned particles of marrow, it has been possible to gain much information concerning diseases of the blood-forming organs. In addition, the specific and non-specific alterations seen in the marrow as the result of a variety of conditions which are not usually associated with any major deficit in hematopoiesis have proved interesting and often valuable in diagnosis.

The importance of the red bone marrow in the production of erythrocytes, granulocytes, and platelets is seldomg disregarded, but the importance of the marrow as a blood-forming organ gainst new emphasis when quantitative values are considered. Mechanik (1926)[1] estimated that the weight of the bone marrow in man was 4.6 per cent of the body weight; this percentage value exceeds that of any other parenchymatous organ. It is, in all probability, safe to assume that the weight of the marrow in man varies from 1300 to 1500 grams.

Repeated studies of aspirated marrow have shown that the bone marrow is an extremely labile organ and that it can undergo almost complete transformation in very short periods of time (from a few hours to a few days). These rapid fluctuations of marrow pattern become almost unbelievable when one considers that changes in one portion of the marrow are reasonably representative of the alterations which have occurred in over 1000 grams of tissue.

Originally the sternum was the site chosen for most aspiration biopsies in the human. The sternum is easily accessible in most patients, and Stasney and Higgins (1939)[2] have shown that sternal marrow affords a reliable sample of the red bone marrow of the body. Within the past few years, however, aspiration biopsy of iliac and vertebral marrow has gained popularity,[3] and it has become increasingly apparent that marrow may be obtained by simple aspiration from those bones of the body which contain active hematopoietic marrow if the bones are accessible and if the cortex of the chosen bone can be penetrated without undue difficulty, discomfort, or danger.

Some of the early techniques of obtaining marrow show that attention has been turned in this direction for almost fifty years.

Almost all of the techniques in use have been employed in the University of Minnesota Hospitals. At the time of the last report (1946)[29], marrow was routinely obtained from the sternum in either adults or children. At present iliac or vertebral spinous process biopsies are done when either of these types of biopsy seems preferable. Often the latter types of biopsies prove less traumatic to the patient, and occasionally tumor cells may be more easily demonstrable.

Williams[27] in a comparative study of iliac and sternal marrow, from 20 patients in the University of Minnesota Hospitals showed, however, that in about 50% of his cases, the marrow differentials from the two sources were <u>not</u> comparable, the iliac marrow showing evidence of dilution with sinusoidal blood. In the remaining 50% of the cases, the differentials obtained on marrow from the sternum and ilium were virtually identical, but the myeloid-erythroid volume obtained on iliac marrow was never as great as that obtained on sternal marrow. The myeloid-erythroid volume of sternal marrow was generally two times as great as that of iliac marrow.

Quantitative data obtained from iliac marrow are not strictly comparable to those obtained from sternal marrow. Also

Investigators	Date	Bone	Subject	Methods
Wolff[4]	1903	Femur Tibia	Small animals Large animals	Drill 2 holes-remove bony plate
Ghedini[5]	1908	Upper tibia	Human	Trephine
Basile[6]	1910	Femur	Dog	Trephine
Caronia[7]	1922	Tibia	Children	Aspiration
Morris & Falconer[8]	1922	Long bones	Rabbit	Trephine
Seyfarth[9]	1923	Sternum	Human	Trephine
Arinkin[10]	1929	Sternum	Human	Aspiration
Alexandroff[11]	1930	Sternal manubrium	Dog	Aspiration
Nordenson[12]	1935	Sternal manubrium tibia, femur, ribs vertebra, ilium	Human	Needle puncture
Heidenreich[13]	1936	Vertebral spinous process	Human	Aspiration
Erf[14]	1937	Tibia Femur Humerus	Rabbit	Stineman pin replaced with lumbar tap needle; suction with removal of plug of marrow.
Kolouch[15]	1938	Ribs	Rabbit	Surgical biopsy
deWeerdt[16]	1939	Vertebral spinous process	Human	Aspiration
Ho, Chu & Yuan[17]	1940	Ilium	Dog,Cat,Rabbit	Aspiration
Starkoff[18]	1942	Posterior Femur	Rabbit	Removed bone with small saw. Used Site for aspiration
Suarez et al[19]	1943	Sternum	Monkey	Aspiration
Mouriquand et al[20]	1944	Femur	Guinea pig	Aspiration
Van den Berghe & Blitstein[21]	1945	Ilium	Human	Aspiration
Nelson[22]	1946	Sternum	Pig	Aspiration
Rubenstein[23]	1947	Ilium cf. sternum	Human	Aspiration
Loge[24]	1948	Vertebral spinous process cf. sternum	Human	Aspiration
Sundberg and Hodgson[25]	1949	Tibia	Rabbit, Guinea pig, Mouse Chicken	Aspiration
Carter[26]	1949	Femur	Rat	Drill & aspiration with capillary pipette
Dameshek	1948	Vertebral spinous process	Human	Aspiration
Williams[27]	1948	Sternum cf. ilium	Human	Aspiration and centrifugation
Bickel and Della Santa[28]	1949	Vertebral spinous process cf. sternum	Human	Aspiration

lymphocytosis and a larger percentage of mature granulocytes and later forms of normoblasts are common in marrow specimens which contain a large amount of sinusoidal blood. This makes interpretation of specimens of iliac marrow more difficult and less reliable in that it is very difficult to decide whether the latter changes mean real hypoplasia or dilution. Often, when there is doubt, a sternal biopsy is required. Qualitative changes in the nucleated marrow cells seem to be reasonably consistent regardless of aspiration site:[3] the megaloblasts of pernicious anemia or the various morphologic changes which occur in the leukemias have been found in marrow from either sternum, ilium, or spinous process.

Needless to say, it is advantageous to have a choice of sites for biopsy. In children it is often more convenient to aspirate marrow from the ilium or tibia than from the sternum. Occasionally in adults the site of possible tumor metastasis identified roentgenologically has been biopsied. Dr. R. J. Cullen aspirated tumor cells from the ischial tuberosity, for example, and Block and Jacobsen[30] have recommended surgical biopsy of marrow from lesions in ribs in Gaucher's disease.

Aspiration Technique--Sternal

The technique used in the University of Minnesota Hospitals is a modification of that described by Schleicher and Sharp[31] and Limarzi.[32]

The following technique has been published in various forms.[25,29] It is repeated here for convenience of reference with only a few additions.

Although marrow may easily be obtained from the manubrium or from the body of the sternum opposite the second or third interspace, the recommended site is the central or near central area of the body of the sternum below the sternal angle of Louis and opposite the second interspace. The sternum is less likely to bend at this point, congenital anomalies are seldom present, and marrow can be obtained here regardless of age. This location is, in most instances, immediately superior to the great vessels which, at this point, are covered by the fibrous pericardium. The outer lamina of the sternum is extremely variable in thickness; variations within a very limited area may be present, and variations are not infrequent between the thickness of the outer lamina of the manubrium or of the body of the sternum opposite the second or third interspaces. Wintrobe[33] stated that the thickness may vary from 0.2 to about 5 mm., and that the marrow cavity is generally 5 to 15 mm. in depth.

In a study of the area of the sterrum opposite the second interspace in 26 cadavers, I found these:

		Average
Outer lamina	0.3- 3.0 mm.	1.35 mm.
Marrow cavity	3- 11 mm.	7.5 mm.
Inner lamina	0.2- 2.8 mm.	1.42 mm.

The needle used here at present is the University of Illinois Sternal Needle obtainable from V. Mueller and Co., Chicago. The needle is a modification, (introduced by Limarzi and Bedinger[34]), of the original Klima-Rosseger needle. It is equipped with a rotating guard, which, when properly understood and employed, eliminates the possibility of penetrating the inner lamina of the sternum. A miniature edition of the Klima-Rosseger needle, designed by Dr. R. H. Reiff, is used for infants.

The patient is requested to lie on his back with his arms at his sides. The site to be punctured is located; the skin area is shaved if necessary.

The equipment used for the biopsy, with the exception of the local antiseptic (zephiran chloride or Novak's solution), 1 per cent procaine, collodion, and tape, is all contained in one sterile pack. The operator works in sterile gloves. A wide working-field over and surrounding the puncture site is painted with the local antiseptic, following which all but a small area immediately surrounding the puncture side is covered with a spinal

drape or sterile towels, the patient's face being covered. The puncture site is again carefully located. The skin and periosteum are then infiltrated with procaine. (Periosteal sensitivity is extremely variable. Some patients apparently experience no pain when the procaine needle is inserted under the periosteum. Other patients require very careful and slow infiltration of this membrane.

After the area is apparently insensitive, a very small incision is made with a Bard-Parker blade. The sternal needle is then pushed through this incision to the periosteum. (The needle is equipped with a tight-fitting stylet which is locked in position. If the stylet is not locked in position, the stylet must be kept in place while using pressure in order that the needle not become plugged.) The needle is ordinarily pushed vertically with the guard adjusted to a height approximately equal to the distance to the periosteum. When the tip of the needle touches the periosteum, the guard is screwed down until it is flush with the skin. Then the guard is screwed back $2\frac{1}{2}$ turns.

This process affords the needle an added length, beyond that necessary for the penetration of the skin and subcutaneous tissues, of 2.5 mm., just the length of the level of the needle. The adjustment generally suffices to allow penetration of the needle into the marrow cavity when pressure is applied, but with variations in the amount of subcutaneous tissue and in the thickness of the bone, added adjustments may prove necessary. Slow steady pressure is applied. When the bone has been penetrated, a definite, quick "give" is almost always felt, and the needle will, except when the bone is exceedingly thin or porous, support its own weight. The stylet is then removed, a 20 cc. syringe attached, 10 cc. of rapid suction applied, and .1 cc. of marrow aspirated. During the aspiration, the patient generally experiences a sharp pain. (Rapid suction and aspiration of only 1 cc. are generally rewarded with minimal dilution by sinusoidal blood. Dilution is unavoidable, but constant technique has produced relatively similar quantitative data.

The aspirated fluid is immediately transferred to a paraffin-lined vial containing heparin (lot #152, Hynson, Westcott, and Dunning), and the vial is gently agitated to insure mixing. The sternal needle is withdrawn and the wound sealed with a collodion-covered cotton fluff held firmly in place by tape.

Sternal marrow may be obtained from extremely young infants. For patients ranging from a few days to 3 years of age, a miniature model of the Klima-Rosseger needle made from 17 gauge spinal needle is used. Except for the sedatives which are sometimes required, the procedure of aspiration is almost identical to that already described. The sternum of the infant is largely cartilaginous. Cancellous bone is, however, found to be almost constantly present in the body of the sternum at the level of the second interspace. Frequently, it is also present near the central area of the manubrium, but this site is both less predictable and less easily biopsied. In aspirating marrow from the infant, the sternal needle is pushed carefully through the sternal cartilage until bony trabeculae are encountered. When suction is applied, a few drops of bloody fluid will rise slowly into the syringe. This fluid, which has proved to be largely marrow, is transferred to a paraffin-lined watch glass containing heparin, and direct smears are then made from the mixture. Occasionally as much as 0.2 to 0.5 cc. may be aspirated. When this much fluid is obtained, there is almost always fairly marked dilution with sinusoidal blood. However, as small an amount as 0.2 cc. may be centrifuged, and quantitative data may be obtained.

Aspiration Technique--Iliac

The same equipment is used. The patient is asked to lie on one side. The crest of the ilium is palpated, and the site of biopsy is chosen. Some prefer the area about 1 cm. inferior to the crest and posterior to the anterior iliac

spine. Others have more success with an area about 1 cm. below the crest and about 1 or 2 cm. posterior to the anterior gluteal line. Actually the ilium can be penetrated without too much difficulty in a variety of places; the crest, itself, however, offers too great resistance for it to be a practical site for penetration except in infants and children.

Aspiration Technique--Vertebral

A modification of the technique described and illustrated by Loge is used.[24] The patient may be sitting up and leaning forward in order to get good convexity of the lumbar region, or the patient may lie on his stomach.

The most prominent of the lower thoracic or lumbar vertebral spinous processes is chosen. (If there is x-ray evidence of an osteoclastic lesion in any vertebra, this vertebra might, of course, be the preferred site.)

The procedures are then similar to those described for aspiration of sternal marrow. The needle is advanced with a rotary motion and firm pressure until it is firmly fixed in the spinous process. Loge claimed it was never necessary for them to go beyond a depth of $2\frac{1}{2}$ cm.

Bickel and Santa claimed to have punctured the spinous processes of all vertebra between D10 and L4 with equal success.[28] They puncture either the dorsal or the lateral surfaces of the processes.

Loge,[24] Dameshek,[3] and Bickel and Santa[28] claim that results of marrow differentials, etc. are similar in marrow whether it is obtained from the sternum or from the spinous processes.

Aspiration Technique--Tibial

This technique may prove preferable for some reason in infants or children. When, for example, there appears to be hypoplasia, one may wish to check several sites. In general however, tibial biopsy is more traumatic for the patients than is iliac.

One of the most satisfactory sites of biopsy is the superior medial surface of the tibia, inferior to the medial condyle and medial to the tibial tuberosity.

We have found this site to be ideal in a large variety of laboratory animals[25] (rabbit, dog, monkey, guinea pig, mouse, chicken).

Preparation of Marrow for Study

Heparin is perhaps the least offensive of the common anti-coagulants insofar as alterations of cell morphology are concerned. A lapse of thirty minutes between heparinization of the marrow and centrifugation will not produce unintelligible preparations. Generally, however, the marrow is prepared for examination in the shortest time possible.

After it has been thoroughly agitated, the heparinized marrow is poured out on a clean glass plate. Grossly visible particles of marrow are usually present. The fluid and about half of the particles are transferred to a Wintrobe hematocrit tube by means of a chemically clean capillary pipette. The remaining particles are prepared for microscopic examination by the following method:

Fix particles in Zenker's fluid--30 minutes to 1 hour.

In the remainder of the procedure, remove the fluids from the small vial by means of a capillary pipette. Do not attempt to transfer the particles. The timing varies with the size of the particles. A suggested timing is: several changes of distilled water--30 minutes. 30% alcohol--30 minutes. 50% alcohol--1 hour or longer. 70% alcohol--1 hour or longer. 95% alcohol--10 minutes. 100% alcohol (1)--5 minutes. 100% alcohol (2)--5 minutes. (Add about an equal volume of xylol to this last alcohol almost immediately.) Xylol (1)--10 minutes. Xylol (2)-- 10 minutes.

Remove xylol, pour paraffin (M.P.54°) in vial, and allow tissue to become infiltrated in oven for 30 to 45 minutes. Remove paraffin with heated capillary pipette, add fresh paraffin, and leave in oven for 30 to 45 minutes. Do not leave tissues in oven over 1½ hours.

Remove particles from paraffin with heated capillary pipette. Place tip of pipette at bottom of paraffin-filled boat and force particles out of pipette. The particles should be made to aggregate in a relatively compact mass near the bottom of the boat. Let paraffin harden, pare blocks, cut at 5 micra, and mount. Stain as desired. Any of the special blood stains can be used following Zenker's fixation. During the staining procedure, remove the precipitated mercury. Immediately before staining, immerse slides in dilute alcoholic iodine. When the tissue is yellow, place slides in a 5 per cent aqueous solution of sodium thiosulphate. Leave slides in thiosulphate until yellow color has faded. Wash in distilled water and continue staining procedure.

The fluid portion is centrifuged at 2500 r.p.m. for eight minutes, and readings corresponding to the height of the various strata are taken from the Wintrobe tube. Four main layers (fat, plasma, myeloid-erythroid, and erythrocytes) are present. Sometimes, immediately below the fat layer, a layer which consists of a mixture of fat, perivascular cells, and nucleated marrow cells is found. (These layers give a rough idea of the cellularity of the marrow, but sections provide more accurate information in this respect. One of the main advantages of centrifugation is the concentration of nucleated marrow cells in the myeloid-erythroid layer.) The fat and mixed layer are removed and discarded, but one smear is made of the mixed layer in order that its relative composition may be estimated. With a second pipette, the myeloid-erythroid layer and a small amount of plasma are transferred to a paraffin-lined watch glass. Smears are made from this mixture. The smears are dried rapidly by whipping them through the air by means of a fan. The smears may be stained with any of the common blood stains. Wright's or the May-Grünwald Giemsa stains are excellent.

Preparation of satisfactory section material is the most difficult part of the routine technique, but the almost constant presence of some particulate marrow has made possible not only a correlation of the quantitative data obtained from the hematocrit tube with the histologic structure of the marrow but also a broader knowledge of marrow pathology. If marrow is routinely prepared in this matter, the simple technique of aspiration becomes a true biopsy of the marrow; easily examinable smears as well as sections are obtained in almost all instances. The older method of trephining need almost never be used.

The method used here is by no means universal. Other methods which involve less equipment and fewer movements are commonly employed. Scott[35] recommended aspirating only 0.1 cc. (to prevent dilution) and then making direct smears. He believed that total counts of nucleated marrow cells published by many authors showed too much variability in mormal persons to be considered precise, and that the cellularity of direct smears can serve as an adequate index of the composition of the marrow. Dameshek and Miller[36] also aspirated a small quantity of fluid (0.3 cc. or less) and made direct smears. The method described here yields information not obtainable from direct smears, but there is no wish to dispute the value of direct smears, for qualitative alterations in blood cells are often more significant than any amount of quantitative data.

Various different methods for preparations of sections have been described. Gormsen allowed a portion of the aspirated marrow to coagulate and sectioned the clot.[37] Berman and Axelrod have advocated the use of topical thrombin for concentration and coagulation of the tiny particles of marrow.[38] Schleicher aspirated up to 5 cc. of marrow in an effort to obtain a large number of "marrow units."[39]

Interpretation of Quantitative Data

The percentage values of the various constituents of the aspirated material, if interpreted with a reasonable degree of caution and an understanding of the limitations of their accuracy, are often of great significance. The normal myeloid-erythroid volume (M-E volume), the volume occupied by developing leukocytes, erythrocytes, and megakaryocytes and by lymphoid cells, varies from 5 to 8%. The fat volume varies from 1 to 3%. The volumes of the plasma and of the erythrocyte layer are extremely variable. The erythrocyte volume is generally less than the hematocrit per cent of peripheral blood, but because centrifugation time is shorter, and because a certain amount of dilution with sinusoidal blood does occur, variations in this layer are difficult to interpret.

The total leukocyte count of the peripheral blood must also be taken into consideration. If the peripheral count is 10,000, the volume occupied by the "buffy coat" layer in a hematocrit tube would be approximately 1%. Thus, in a leukemic condition in which the total leukocyte count was 500,000, white cells would occupy almost 50% of the volume of the blood, and a M-E volume of 50% might be obtained on the "marrow" even if only sinusoidal blood had been aspirated. Qualitative differences in the aspirated fluid serve almost always to eliminate this source of confusion, but the leukocyte count of the peripheral blood cannot be disregarded.

High M-E values in the presence of a normal or low fat percentage and in the absence of a pronounced peripheral leukocytosis are indicative of at least local hyperplasia. Moderately high M-E values accompanied by high fat and low erythrocyte percentage often simply indicate that aspiration was attended by minimal dilution. Low M-E values are more difficult to interpret. If they are accompanied by high fat percentages, the portion of the marrow aspirated is very probably hypoplastic. If, however, the M-E value is low (0 to 2%), and fat is absent, the "marrow" may well consist largely of sinusoidal blood. The value of section preparations as a check on this quantitative data cannot be over emphasized.

Differential counts of nucleated marrow cells can only be considered as approximations, for the distribution of the various cell types on the smears is extremely difficult to control. One of the most common sources of inaccuracy is based on the fact that mature neutrophils and platelets show more tendency to aggregate in groups than other cell types. However, with experience, general estimates of cell types obtained from comprehensive low power examinations of many slides will be found to check fairly consistently with actual differential counts. In many instances, a low power examination of an entire slide provides a far more accurate conception of the alterations in the relative percentages of developing cells than does a differential count.

It is not surprising that the literature contains almost one "normal" bone marrow differential per investigator. It is felt that these so-called "normal" differentials are only normal to the particular investigator's technique and manner of interpretation and counting. Nothing approaching the regularly accepted normal differential count of peripheral blood has yet been attained insofar as marrow smears are concerned. There is, however, sufficient agreement on the relative percentages of "myeloid" to erythroid cells and on the various expected percentages of the cell types in different phases of maturation to afford a reasonable working basis.

As has been indicated, the cells comprising the M-E layer include normoblasts and developing and mature leukocytes, megakaryocytes, and various cells which we have called lymphoid cells for convenience. Many investigators refer to the "myeloid-erythroid ratio." This is the ratio of developing and mature granular leukocytes to erythroid cells (or developing erythrocytes). With the use of constant technique, studies indicate that the normal ratio in the adult is 4:1 to

3:1, the acceptable normal range being rather wide. This is in good agreement with percentages given by Rohr,[40] Moeschlin,[41] and Markoff[42] and with the "grand mean" calculated by Scott[35] from an analysis of percentage values of both myeloid and erythroid cells given by nineteen investigators.

The differential which follows is a "normal" taken from Scott[35] and corrected to the nearest 0.5 per cent and for the terminology used here:

Myeloblasts	1.0%	1.0%
Leukoblasts	2.0	2.0
Neutrophils (total)		63.0
Promyelocytes	4.0	
Myelocytes	13.0	
Metamyelocytes	15.0	
Bandforms	16.0	
Mature forms	15.0	
Eosinophils (total)		3.0
Myelocytes	2.0	
Mature	1.0	
Basophils (total)	0.5	0.5
Normoblasts (total)		18.5
Pronormoblasts	0.5	
Basophilic normoblasts	2.0	
Polychromatic normoblasts	12.0	
Orthochromatic normoblasts	4.0	
"Lymphoid" cells (total)		12.0
Lymphocytes	10.0	
Plasma cells	1.0	
Macrocytes	1.0	

Reticular cells, histiocytes, macrophages ---- present

Gross alterations from the "normal" pattern are usually immediately apparent. Lesser alterations, minor alterations only in the percentage of cell types unaccompanied by distinctive qualitative alterations, are generally of little significance insofar as diagnosis is concerned.

For routine study of marrow in the University of Minnesota Hospitals, a differential count of 500 nucleated cells is done on each specimen of marrow. The cells are classified as:

1. Developing erythrocytes
2. Neutrophils and precursors
3. Eosinophils and precursors
4. Basophils and precursors
5. Lymphoid cells (lymphocytes, monotyes, plasma cells, reticular cells, histiocytes, and macrophages).

A five-key counter makes this type of differential the most practical for routine work. Any of the cells (viz., lymphocytes, plasma cells, myeloblasts, etc.) can be enumerated on the "basophil key" without much difficulty since basophils are ordinarily sparse. Basophils are counted routinely because of their significance to the diagnosis of myelogenous leukemia; even a slight increase in basophils must be regarded critically.

Differential counts including all cell types are often of value in the classification of leukemias, and in the thorough evaluation of the effects of any condition or therapy on the marrow. Such differentials are, however, cumbersome and time-consuming. They seem unnecessary for routine purposes.

The number of megakaryocytes and their precursors can generally be estimated by

routine examination of the area near and at the feather edge of the marrow smear. The number of megakaryocytes in this region varies considerably. We have claimed that these cells number 50-75 in the terminal 18 x 18 sqm. of the marrow smears,[43] using as a basis of our normal the figures of Limarzi and Schleicher.[44] However, Williams found that megakaryocytes, in his normal series, varied from about 30-200.[27] Numerous technical factors influence the megakaryocyte count; these factors may cause gross distortion. The commonest hazard is the low megakaryocyte count which may result from coagulation of fluid in the biopsy needle prior to successful aspiration. Coagulation is, of course, obvious when it occurs in the syringe or in the later stages of preparation of the marrow. If, however, coagulation is precipitated by numerous unsuccessful attempts at biopsy before good marrow is aspirated, the resulting low megakaryocyte count may be difficult to interpret. It is also obvious that the number of megakaryocytes in a thick smear might well be greater than the number in a thin smear. It is our impression that only gross changes in the numbers of megakaryocytes should be honored as significant. Low megakaryocyte counts in aspirated marrow specimens do not always mean that megakaryocytes are actually decreased. Various methods of counting megakaryocytes have been described by Dameshek,[36] Diggs and Hewlett,[45], etc. Berman has discussed and criticized the methods.[46]

Indications for Aspiration Biopsy of Marrow

The separation and the accurate diagnosis of the macrocytic hyperchromic or macrocytic normochromic anemias is one of the most important of the hematologic problems which has been greatly simplicied by study of the marrow. Although alterations in the peripheral blood and clinical manifestations are often unquestionably those of pernicious anemia, some of the macrocytic anemias which are accompanied by a blood picture much like that seen in megaloblastic anemias still show normoblastic regeneration of erythrocytes.

In recent years it has become increasingly apparent (Dameshek[47], Dameshek and Miller,[36] Limarzi and Schleicher,[44] Hebbel,[48], Piney,[49] Leitner,[50] Rohr,[40] Custer,[51] and many others) that bone marrow biopsy serves as a simple and effective diagnostic tool.

When there is clinical evidence (lymphadenopathy, splenomegaly, purpura, or skin lesions) of a possible blood dyscrasia, study of the blood and marrow often proves diagnostic. When routine laboratory methods reveal anemia, unexplained leukopenia, thrombocytopenia, or any combination of these findings, study of the marrow may prove of absolute diagnostic value or it may serve to exclude the possibility of a primary blood disease. The conditions which are most commonly responsible for these clinical and/or laboratory findings are agranulocytosis, aplastic and refractory anemias, megaloblastic anemias, essential thrombocytopenia purpura, and leukemia, diseases which generally produce characteristic alterations in the bone marrow pattern.

Other instances in which study of aspirated marrow may prove useful and sometimes specifically diagnostic include a wide variety of conditions. In the hypochromic anemias marrow biopsy is not usually essential. In the hemolytic anemias and in myelophthisic anemias valuable information is often obtained from study of the marrow. Conditions which may or may not be associated with anemia, leukocytosis or leukopenia, and thrombocytopenia and which may show a specifically diagnostic marrow pattern include: multiple myeloma, metastatic carcinoma, the lipoid storage diseases, Hodgkin's disease, and malignant lymphoblastoma. The changes in the marrow in polycythemia vera are often difficult to interpret, but occasionally they prove of diagnostic importance. Some of the conditions which do or may show lymphocytic reactions in the blood are also accompanied by granulomatous inflammation of the marrow; these include infectious mononucleosis, brucellosis, tuberculosis, and sarcoidosis. Infectious lymphocyto-

sis is not, insofar as present knowledge is concerned, granulomatous in nature. The presence of "L.E. cells" in the marrow in acute disseminated lupus erythematosus has proven to be of great diagnostic importance.

Interpretation

Specific information to be discussed is based on studies of marrow from approximately 1865 patients. In many cases more than one specimen of marrow was obtained from a single patient. The great majority of the marrows are sternal, but approximately 164 specimens of iliac marrow have been studied. Only a few vertebral biopsies have been done.

The cases tabulated in this report have accumulated from 1942 until the present time, and, with rare exceptions, material from the cases is on file. From Sept. 1, 1942 until Aug. 3, 1944, the special hematologic work was done by Dr. S. Stuurmans. Data from this period are, for the most part, not included here.

The tables which follow are included for the purpose of recording significant data from cases in which study of the marrow is believed to have been of diagnostic value. Although every effort has been made to make the tables as complete as possible, it is felt that a few cases may have escaped our filing system. The tables include numbers of patients, but when more than one biopsy was done on a patient, the biopsies were recorded separately and considered in the average figures. No data regarding the changes in the marrow in pernicious anemia in various phases of therapeutically induced remission have been tabulated, however. There have been 66 cases of refractory anemia; 83 biopsies were done on this series of cases. Of interest, but not apparent from the tables, is the fact that in this series some patients showed hyperplastic marrow at early examinations and hypoplastic or aplastic marrow at a later date.

Numerous abbreviations were necessary in the tables. In most cases, the explanation accompanies the table. The method of recording megakaryocytes requires clarification. Megakaryocytes were not estimated or not recorded in some of the reports, and no effort has been made to do a complete study of these cells at the present time. Thus, only the number of specimens in which estimates of megakaryocytes were available is included under "Megakaryocytes - Inc. (increased), Dec. (decreased), or N. (normal)." In addition, the number of cases with available, recorded, peripheral thrombocytopenia is indicated. For example, Table 1 : Pernicious Anemia - Megakaryocytes are increased in 18 cases, and 6 of these cases show thrombocytopenia; this is recorded 18(6).

One column, abbreviated "P.C. & M. - Inc." is also common to all the tables. In it are included the number of marrows in which an increase in plasma cells and/or monocytoid cells, histiocytes, and macrophages was recorded. This group of cells is, as indicated previously, counted as part of the lymphoid cells. When these cells are increased, as in the refractory anemias, and in those conditions in which granulomata were present in marrow, the total lymphoid cell percentage is high, but the increase in lymphocytes may not be great.

In each table, the range of variation in the percentage of each cell type or each quantity is recorded at the left. The average is at the right and underlined.

ANEMIAS

Megaloblastic Anemias

Pernicious Anemia. The marrow changes in pernicious anemia have been described by many investigators, and good illustrations of the megaloblastic generation of developing erythrocytes can be found in many of the recent atlases of bone marrow. In the older European literature and texts Ferrata and Pappenheim gave excellent illustrations of megaloblasts. The colored illustrations pro-

vided by Jones[52] have been regarded as classic by Downey and his students. More recently Berman has contributed an outstanding colored plate in which the megaloblastic generation is compared with the normoblastic generation.[53]

In brief, the changes in the marrow are as follows. There is ordinarily a moderate or a marked hyperplasia, the M-E volume is greater than normal, and the percentage of developing erythrocytes is increased. Erythropoiesis is almost completely megaloblastic in relapse although normoblasts can generally be found. Except in spontaneous or therapeutic remissions, the majority of the developing erythrocytes are basophilic and polychromatic megaloblasts. With remission, megaloblasts disappear rapidly (variable, 48 hours to about one week), and there is a period of intense normoblastic activity during which time it is often very difficult, if not impossible, to establish a diagnosis of pernicious anemia on the basis of examination of the marrow.

Neutrophils and their precursors are generally relatively decreased. Often most of these cells are abnormal forms, showing evidence of premature segmentation and hyperlobulation. Despite the great importance of the cells of the neutrophilic series to the diagnosis of pernicious anemia, they are ordinarily not well illustrated. Dameshek and Valentine offered excellent illustrations of these cells.[54] Jones has emphasized their importance in many publications, but his best illustrations are to be found in Downey's Handbook of Hematology.[52]

Evidence of hypersegmentation of nuclei is prominent in other cells as well. Eosinophils often have many lobes, and megakaryocytes are frequently unusually lobulated. Hodgson found multinucleated megakaryocytes to be more numerous in pernicious anemia than in any condition other than leukemia.[55]

Table 1 shows one very interesting feature regarding the megakaryocytes of pernicious anemia. Thrombocytopenia occurred in 6 of the cases in which megakaryocytes were increased.

Other megaloblastic anemias. As has been indicated in an earlier report with Dr. C. D. May and his associates,[56] the changes in the marrow in the various types of megaloblastic anemia are so similar to those seen in pernicious anemia that separation of the conditions is extremely difficult if not impossible. The various types of megaloblastic anemia encountered by us are included in Table 1: the quantitative similarities are obvious. The megaloblastic anemias following extensive gastrectomy may or may not be true pernicous anemia. It is felt that more data and studies are required.

Other Marocytic Anemias

Some of these anemias, particularly the macrocytic anemia which may accompany cirrhosis, may show a peripheral blood picture which cannot be distinguished from that of pernicious anemia with certainty. However, the anemia of cirrhosis is ordinarily normoblastic, and often the marrow shows a predominance of macronormoblasts. No attempt to organize our marrow specimens from cirrhosis will be made at the present time. The reader is referred to the recent studies of Berman and his associates.[53,57]

Of interest is the fact that we have recently seen a patient who had both cirrhosis and pernicious anemia. Also, several patients who presented with thrombocytopenia, leukopenia, and anemia have shown marrow patterns suggestive of cirrhosis. In these cases, subsequent liver function studies and liver biopsies have affirmed the impression that the changes in the marrow probably accompanied cirrhosis.

Other anemias which may be macrocytic and normochromic include some acquired hemolytic anemias and some refractory anemias.[58] In these conditions erythropoiesis is normoblastic, but occasional macronormoblasts with reasonably delicate nuclear structure may occur. On the basis of these cells, erroneous diagnoses of pernicious anemia may be

made. Of importance is the fact that pernicious anemia neutrophils do not occur in these conditions.

The most confusing anemia which might be placed in the general group of anemias which simulate pernicious anemia is one which seems to be the result of or to be part of a leukemic process. The peripheral blood resembles that of pernicious anemia except that the neutrophils are not typical pernicious anemia neutrophils, and several or numerous atypicalities may suggest the possibility of leukemia. The marrow in these conditions often shows a predominance of developing erythrocytes which show evidence of reticular derivation and which bear a marked resemblance to megaloblasts. Occasionally a significant increase in blast forms in the marrow, blood, or both facilitates diagnosis; often the conspicuous absence of pernicious anemia neutrophils is the major clue to diagnosis.

Hypochromic Anemias

(1) Those associated with blood loss generally show a moderately or markedly hyperplastic marrow, an increased M-E volume, an increase in the relative number of developing erythrocytes (normoblasts), and often a marked increase in megakaryocytes. Prolonged loss of iron results in progressive hypochromasia, and develping normoblasts contain less than the normal amount of hemoglobin. There is a shift to the right with a predominance of polychromatic (but not orthochromatic) normoblasts. (2) The hypochromic anemias associated with iron deficiency not explainable on the basis of blood loss (dietary anemias, faulty absorption, etc.) show changes similar to those seen with blood loss.

Noteworthy is the fact that with conditions presumably caused by a marked iron deficiency, such as the severe hypochromic anemia which occurs in premature infants, the peripheral blood may show normoblasts, basophilic stippling, and occasional immature neutrophils, features not commonly encountered in the hypochromic anemias of adults.

For an understanding of the variability in the degree of hyperplasia of the marrow and the degree of normoblastosis before and after iron therapy, the reader is referred to Hamre's excellent experimental study.[59]

Eleven of the cases of hypochromic anemia included in Table 2 are hypochromic anemias of pregnancy. These cases, the megaloblastic anemia of pregnancy, and other anemias of pregnancy have been reviewed by Dr. R. G. Holly.[60,61]

Hemolytic Anemias

Familial Hemolytic Anemia. The marrow is generally moderately or markedly hyperplastic, the M-E volume is elevated, and the percentage of normoblasts is greatly increased. There is a shift to the right in normoblasts, late polychromatic normoblasts and orthochromatic normoblasts predominating. The latter cells, in contrast to those seen with iron deficiency contain adequate amounts of hemoglobin. Although some of the normoblasts may be smaller than normal, large normoblasts also occur, and one cannot rely upon size as a particularly valuable diagnostic criterion. Often evidence of destruction of erythrocytes is prominent: macrophages containing phagocytosed erythrocytes and pigment may be numerous. In some instances, megakaryocytes and their precursors contain ingested erythrocytes.

The bone marrow pattern just described is that ordinarily encountered in familial hemolytic anemia. However, in recent articles[62,63] changes in the marrow and in the blood have been studied in sequence from as early as 8 days before until 34 days after the onset of illness. As early as 1941 Dameshek[64] commented on the incongruity of leukopenia, thrombocytopenia, and reticulocytopenia in the presence of an acute hemolytic process. Owren[62] described one case in which the marrow contained 4.8% and 4.2% normoblasts on days 4 and 6 after the onset of crisis. In this case, normoblasts comprised 81% of the nucleated marrow cells on day 12. Owren's impression was that the crisis

was "aplastic." Dameshek and Bloom[63] showed a marked shift to the left with a great preponderance of pronormoblasts. Their interpretation was that this could be explained adequately by a maturation arrest of the nucleated red cells of the marrow.

It is apparent, then, that low percentages of normoblasts with a preponderance of pronormoblasts can be found in cases of familial hemolytic anemia in which marrow is obtained soon after the onset of illness. The marked normoblastic hyperplasia so commonly encountered occurs during periods of increased regeneration of erythrocytes.

Acquired hemolytic anemia. The marrow pattern is extremely similar to that seen in F.H.A. Macronormoblastosis may be pronounced. Evidence of recent phagocytosis of erythrocytes may be present. Except when numerous transfusions have been given, pigment-containing macrophages are often less prominent than they are in F.H.A. where numerous previous hemolytic crises have often occurred.

In one recent patient with severe hemolytic anemia (, originally diagnosed from preliminary blood studies by Dr. Hal Downey) in whom no increased fragility of erythrocytes and no familial history was demonstrable, it is believed that the phenomenon described by Owren[62] may have been observed. In this patient, normoblasts comprised only 4% of the nucleated marrow cells; there was marked eosinophilia; and macrophages containing pigment and erythrocytes were exceedingly numerous. The depression in normoblasts and the great number of pigment containing macrophages (actually greater than that seen in known hemochromatosis) can probably be accounted for on the basis of previous transfusion; the patient had received approximately 23 liters of blood prior to her admission to the University Hospitals. However, the original degree of anemia was pronounced and was undoubtedly caused by hemolysis. When transfusions were withheld, the hemoglobin dropped abruptly, and reticulocytes remained low (ca 1%). Splenectomy was performed. Eight days later, normoblasts comprised 35% of the nucleated marrow cells. Fourteen days after splenectomy the reticulocytes had risen to 27%. The patient has been discharged, and it is believed that she has responded well. Imprints and sections of the spleen showed tremendously increased pigment-containing macrophages and macrophages containing numerous erythrocytes. None of the findings in this case has suggested the possibility that this hemolytic anemia is familial in type. Thus it is possible that we have seen acquired hemolytic anemia with reticulocytopenia and greatly decreased normoblasts.

Refractory Anemias

Aplastic Anemia. The classical changes in the marrow in aplastic anemia include a marked reduction in the M-E volume and a marked decrease in developing red cells, neutrophils, and megakaryocytes, with a relative increase in lymphoid elements (lymphocytes, plasma cells, histiocytes, and macrophages). Section preparations generally show a predominantly fatty marrow which contains scattered islands of erythropoietic cells, occasional developing granulocytes (chiefly eosinophils), tissue mast cells, and variable accumulations of lymphocytes and plasma cells. Lymphocytic follicles are sometimes encountered. These are the changes of nearly complete aplasia -- the panmyelophthisis of some authors. There seem, however, to be many stages between even hypoplasia and complete aplasia.

Refractory Anemia with normal or increased cellularity. Data on cases of this type are shown in Table 3. Of most interest is the degree of normoblastosis which may occur. The general pattern may be so similar to that seen in hemolytic anemias that no distinguishing features can be found. However, signs of pathologic regeneration of erythrocytes may be prominent, and the number of reticulocytes in the marrow is generally far fewer than that seen in hemolytic anemias. In some cases, there may be a shift to

the left in normoblasts with increased percentages of pro- and basophilic normoblasts. Macronormoblastosis may be pronounced, or peculiar large erythroblasts which have been compared with the primitive erythroblasts of the fetus may be present.

One feature which is probably of importance insofar as impeding entrance of cells into the blood-stream is concerned may be the increase in perivascular cells of various types. Dr. L. A. Long of Montreal is in the process of analyzing the cases included in Table 3. It is hoped that some of the mechanisms responsible for the various marrow patterns may be clarified.

Myelophthisic anemias show extremely variable blood and bone marrow patterns. The blood may show changes similar to those seen in pernicious anemia, aplastic anemia, or even hypochromic anemia. When both normoblasts and immature neutrophils are present in the blood, as is frequent in these cases, some invasive or malignant process is often found in the marrow. Metastatic carcinoma, multiple myeloma, primary xanthomatoses, lymphosarcoma, Hodgkin's disease and leukemia may produce this leuco-erythroblastic picture.

Leukemias

Although a diagnosis of leukemia can almost always be made from a combination of the clinical and laboratory findings and the morphologic alterations in the peripheral blood, the confirmatory evidence generally obtainable from examination of the marrow is certainly desirable in many cases. In some cases leukopenia, thrombocytopenia, and anemia may be pronounced, and the possibilities of a primary blood disease are, of course, obvious. In other cases, only the leukopenia commands attention. This has been found to be particularly true in the subacute and acute leukemias, and the problem is the most difficult in the leukemias which occur in children. Slightly immature or atypical lymphocytes can occur in the blood in a wide variety of conditions in infants and young children. For this reason, study of the marrow is extremely helpful in the diagnosis of the leukopenic leukemias of children.

Except in chronic myelogenous leukemia, the general marrow patterns encountered in the many varities of leukemia are striking. The changes which have been most regularly encountered in the cases presented in Tables 4 and 5 may be described briefly.

Acute myelogenous leukemia. The cases averaged in Table 4 are all cases in which the original picture was that of typical chronic myelogenous leukemia. In acute exacerbations the marrow is generally hyperplastic, and the M-E volume is extremely high. Myeloblasts and leukoblasts predominate; mature and immature eosinophils and basophils are generally increased. Normoblasts, megakaryocytes, and lymphoid cells are markedly decreased.

Subacute myelogenous leukemia. The variability of marrow pattern was stressed by Hebbel.[48] Although hyperplasia is generally present, low M-E volumes, suggestive of hypoplasia, have been found. The percentage of myeloblasts is almost always increased, but occasionally the numerous Rieder forms and various atypicalities of developing granulocytes are of more importance to the diagnosis of leukemia than is the increase in myeloblasts. Although it would not seem that this should be true, in many subacute myelogenous leukemias the majority of the myeloblasts and leukoblasts are cells with contorted nuclei, and the distinction between these Rieder cells and immature and mature monocytes is difficult.

Chronic myelogenous leukemia. This type of leukemia can almost always be diagnosed from study of the peripheral blood. The few cases recorded in Table 4 include those in which the results of various forms of therapy have been desired and the occasional leukopenic or subleukemic cases which have been encountered. The marrow is generally hyperplastic, and the M-E volume

is extremely high, but occasional instances in which the marrow is largely fibrotic have been encountered. The marrow is often difficult to distinguish from the peripheral blood. The presence of megakaryocytes of normal size in the marrow in contrast to the dwarf megakaryocytes or the fragments of megakaryocytes seen in the blood may be the only distinctive feature although in most instances the normoblast percentage in the marrow will be somewhat greater than that in the blood. The relative increase in myeloblasts is frequently no greater than that seen in any hyperplastic marrow. Developing granulocytes are extremely numerous, and eosinophils and basophils are usually significantly increased. Lymphocytes are markedly decreased. Megakaryocytes are often increased. Thrombocytopenia was present in only 33% of our cases. The average percentage of normoblasts encountered in the tabulated cases is 17.2. This high a percentage is actually unusual. The figure can be accounted for on the basis of the inclusion of several cases in which atypical developing erythrocytes are extremely numerous. Since other findings are comparable to those of chronic myelogenous leukemia, it seems unnecessary to separate these cases as cases of Erythro-leukemia.

A feature which I have found helpful in the separation of the hyperplastic marrows of C.M.L. from those in which there is some non-specific neutrophilic hyperplasia which may result in a greatly elevated M-E volume is the virtual absence of fat in the hematocrit tube and in the sections. Although fat is often lacking in pernicious anemia and hemolytic anemias, the decrease is most prominent and most constant in myelogenous leukemia, M.M.H.S., and polycythemia vera. It seems probable that massive uniform hyperplasia of the marrow with obliteration or destruction of the normal architecture of the marrow might easily account for the lack of fat and for the fact that the nucleated cells of the marrow are found almost only in the M-E layer rather than mixed with perivascular cells in the so-called "mixed" layer.

Myeloid megakaryocytic hepatosplenomegaly,[65] atypical myelosis[66], aleukemic myelosis,[67] or atypical chronic myelogenous leukemia is an interesting and puzzling condition. Agreement as to whether this disease is a true leukemia or a condition in which myeloid metaplasia of the spleen and liver and hypoplasia or fibrosis of the marrow coexist for some reason other than leukemia has not been reached. Downey's early papers[65,66] on the subject give excellent illustrations of the atypical platelets seen in the blood in this disease. The peculiar anemia, the presence of normoblasts in the blood, and the atypical platelets are of utmost importance in diagnosis. Young granulocytes, an increase in basophils, and abnormal dwarf forms of cells of the megakaryocyte series may be present in the blood. The latter cells often go unidentified despite the fact that similar dwarf megakarycytes are often present in chronic myelogenous leukemia.

The marrow in this condition may or may not be obtainable. In the cases included in Table 4, it can be seen that the M-E volume was never higher than 2.5%. The general changes in the cells of the marrow give no outstanding clue to the diagnosis. Dwarf megakaryocytes and their precursors have not been found in our specimens of marrow although they have been encountered in imprints of the spleen from one of the cases. Often the normoblasts, like those of polycythemia vera, appear to be somewhat abnormal, but the abnormality is not sufficiently definite to be of absolute diagnostic value.

Recent reviews on the subject of aleukemic myelosis are of interest.[67,68,69]

Acute and subacute lymphatic leukemia. Hyperplastic marrows are generally encountered, but often aspiration is difficult and only enough marrow for direct smears can be obtained. Occasionally enough fluid will be obtained, but the M-E volume may prove to be extremely low. In all cases high percentages of lymphoblasts and immature lymphocytes are found.

but when the M-E volume is low, the cells in the smears are often surrounded by a peculiar substance which tends to obscure the finer morphologic details. In these cases, diagnosis is difficult. Marrow cells other than lymphocytes are greatly decreased. From the various tables it can be seen that the percentage of eosinophils in the marrow in acute and subacute lymphatic leukemia is more greatly decreased than in any other condition studied. It seems likely that this decrease is an absolute decrease as well. In view of the recent interest in the eosinophils and their marked decrease in the blood in most severe illnesses,[70] it seems appropriate to suggest that the eosinopenia of acute and subacute lymphatic leukemia might be of importance.

Chronic lymphatic leukemia, like cyronic myelogenous leukemia, generally shows a characteristic blood picture, and the great majority of the cases of chronic lymphatic leukemia have not had marrow studies. Although the marrow is not always involved in chronic lymphatic leukemia, many cases show a hyperplastic marrow with an increased M-E volume. Smears show a relative increase in lymphocytes which are monotonously similar in size and in morphology. Immature lymphocytes are not numerous. When the maturation pattern is studied, it is generally found that cells comparable to the lymphoblasts of acute lymphatic leukemia are rare although reticular lymphocytes and hematopoietic reticular cells may be increased.[71] An accentuation of this pattern is seen with what might be considered exacerbation.

Marrow cells other than lymphocytes are generally relatively decreased. Absolute decreases in normoblasts, granulocytes, and megakaryocytes are often apparently not great, for anemia, thrombocytopenia, and absolute granulocytopenia are in many cases absent insofar as the blood is concerned.

It can be seen from Table 5 that normoblasts may be greatly increased. The case with 53.4% normoblasts and two additional cases with increased normoblasts were accompanied by hemolytic anemia. These cases account for the relatively high average percentage of normoblasts obtained in the recorded cases.

Leukemic reticulo-endotheliosis (Downey[72]). The M-E volumes obtained are variable, but section preparations generally show evidence of hyperplasia. Smears show a marked increase in reticular cells with evidence of the transformation of these cells to leukocytes and often also to erythrocytes, megakaryocytes, and plasma cells. In a few cases, it has been possible to trace the development of the leukemic cells to undifferentiated reticular cells. More often, blast forms of reticular origin (hematopoietic reticular cells) predominate. Although occasional cases of leukemic reticulo-endotheliosis are chronic in type, most show a subacute clinical course. Despite the immaturity and atypicalities of development seen in the cells of the blood and marrow, only a few cases prove to be acute.

One of the interesting and distinguishing features of leukemic reticulo-endotheliosis is the tendency for the multipotent stem cell to develop into both lymphocytic and myeloid elements. In those cases classified as "myeloid," the developmental pattern is largely towards granulocytes, megakaryocytes, and, possibly, erythrocytes, but it can be seen from Table 4 that lymphocytes are not decreased as they are in other myelogenous leukemias, and often the peripheral blood shows an absolute lymphocytosis. In the lymphatic type of leukemic reticulo-endotheliosis, there may be some abnormal development of granulocytes and erythrocytes, but ordinarily the mixed developmental pattern is less prominent here.

Acute basophilic leukemia. In a previous report,[29] one case (.) of acute basophilic leukemia was described. Two additional cases (and) have been seen. In both of the recent cases hemorrhagic manifestations also dominated the clinical picture and the clinical course proved even

more acute than in the original case. Doan and Reinhart[73] have described two similar cases.

Polycythemia Vera

The distinction between polycythemia vera and erythrocytosis is reputedly difficult. The distinction between polycythemia vera and chronic myelogenous leukemia on the basis of alterations in the bone marrow is also difficult. The marrow in P.V. is generally markedly hyperplastic, but this is variable, and often adequate material can not be aspirated. In one case in which aspiration of fluid seemed impossible, the third attempt at aspiration was rewarded with a solid core of hyperplastic marrow which had plugged the needle. Whether viscosity, hyperplasia, or occasional areas of decreased cellularity or fibrosis are responsible for some of the difficulties in aspiration biopsy is naturally difficult to determine for the case in question. When good marrow is obtained, there is evidence of an absolute increase in all nucleated marrow cells with, however, a fairly regular relative decrease in lymphocytes. Erythropoiesis is relatively increased, but this increase has been found to be less striking than that encountered in erythrocytosis or secondary polycythemia. Granulocytopoiesis is often relatively increased, and myeloblasts, eosinophils, and basophils may be increased. Kienle[75] believes that the regular increase in megakaryocytes and platelets is of value in diagnosis. Abnormal and atypical normoblasts, developing granulocytes, and megakaryocytes are not uncommon. The changes are often so similar to those of chronic myelogenous leukemia that it is difficult to conceive of polycythemia vera as anything but a primary process. Often, when anemia occurs late in the course of the disease, the marrow and blood patterns become strikingly similar to those seen in myeloid megakaryocytic hepato-splenomegaly. Also, in some of the cases which show all of the hematologic features of M.M.H.S. as well as massive splenomegaly, there may be slight erythrocytosis rather than anemia.

From the standpoint of changes in the blood and bone marrow, there seems to be a very definite relationship between chronic myelogenous leukemia, myeloid megakaryocytic hepato-splenomegaly, and polycythemia vera.

Malignant Lymphoblastoma

Diagnosis of malignant lymphoblastoma on the basis of study of the marrow is possible, but it usually proves more difficult than diagnosis by examination of biopsies lymph nodes. Cooper and Watkins[76] have reviewed this subject in detail and have given illustrations of abnormal lymphocytic cell types in bone marrow smears and sections. Our series of 20 probable cases of lymphoblastoma includes only 6 in which abnormal lymphocytes were considered sufficiently definite to be of absolute diagnostic significance. In one of these cases, section material was also positive. One case, believed to be lymphosarcoma on the basis of marked hyperchromatism and abnormality of lymphoblast-like cells has proved of interest in that following x-ray therapy, the marrow showed 96% lymphoid cells (most of which were abnormal) in contrast to the 12.2% abnormal cells seen prior to therapy. At the time of the second examination, approximately 32% of the circulating leukocytes were hyperchromatic lymphocytes similar to those in the marrow. The subject of lymphosarcoma terminating in leukemia has been discussed in a recent paper.[77]

A recent case of reticulum cell sarcoma should be mentioned in that a single osteoclastic lesion of the spine was the indication for aspiration biopsy. Blood findings were entirely negative.

Hodgkin's Disease

Recent reviews of the literature[76,78] have emphasized the difficulty of the diagnosis of Hodgkin's disease on the basis of aspiration biopsy. Reports of the presence of Reed-Sternberg cells in marrow smears would indicate that these cells have been identified in smears in

only 10 cases[79,82] other than those which have come to our attention.[29,83] In only two of our three cases, diagnosed as Hodgkin's disease on the basis of finding typical Hodgkin's tissue in sections of aspirated marrow, could Reed-Sternberg cells be found in the smears.[83]

Granulomata of the Marrow

Literature concerning this subject has been reviewed elsewhere.[43,84] The granulomatous lesions found in brucellosis[43] caused interest in other known granulomatous conditions, and as Table 6 indicates, two cases of known sarcoidosis and two cases of tuberculosis have shown granulomata in sections of aspirated marrow. In one of the cases of tuberculosis, tubercle bacilli were demonstrated in the lesions. The number of cases which showed epithelcid cells in smears as well as granulomata in sections is indicated in the Table. Seven cases which have shown granulomata in sections of marrow are not definitely classified.

It is to be noted in Table 6 that except in infectious mononucleosis and Hodgkin's disease, only the cases in which granulomata were demonstrable in sections are included.

The increased regeneration of lymphocytes and the presence of numerous leukocytoid lymphocytes in the marrow as well as in the lymph nodes in infectious mononucleosis has been discussed previously.[29,71] Corroboration of the original impression that the marrow was involved in infectious mononucleosis has been presented in a recent article.[85] Foci of developing lymphocytes were found in the sections in 14 of 23 cases, and granulomatous foci were found in 9. Epithelioid cells similar to those seen in lymph node imprints were found in the latter 9 cases and in two additional cases in which sections of marrow were not available. Findings which appear to be similar were reported by Campbell in 1948[86] and by Schleicher in 1949.[87]

The findings are of particular interest in that despite the lack of giant cells in the granulomata of infectious mononucleosis, the lesions strongly resemble the more diffuse and the smaller lesions seen in brucellosis. Brucellosis can show a blood picture which strongly resembles that seen in infectious mononucleosis.

Lipoid-Storage Diseases

The few cases encountered here have not been tabulated. The characteristic storage cells of Gaucher's and Niemann-Pick's disease[33] are shown in many of the atlases of bone marrow. Excellent colored illustrations of Gaucher cells were provided by Groen and Garrer[88] and by Block and Jacobsen.[30] Knowledge concerning the changes in the marrow in Hand-Schüller-Christian's disease is based, for the most part, on study of section material.[89] However, imprint preparations of lymph nodes show a characteristic reticular hyperplasia, variable numbers of lipoid-filled macrophages, and peculiar giant cells[55] which show some resemblance to osteoclasts. Abnormalities of this type have been seen in marrow smears from five cases. Only two of these cases are patients in the University Hospitals. In one, only the epithelioid cells associated with the granulomatous lesions have been found. In th other, abnormal reticular cells, epithelioid cells, macrophages which may or may not have contained lipoid, and abnormal giant cells were encountered.

Multiple Myeloma. It is well-known that this condition can be diagnosed on the basis of study of aspirated marrow even when x-ray findings are negative. The range and average of the findings in 38 biopsies of 25 cases are given in Table 3. As is generally stated,[33,90] the plasma cells of multiple myeloma can generally be recognized by various morphologic features and by their excessive numbers. However, non-myelomatous plasmocytoses can occur, and often immature plasma cells seen in cases in which plasma cells are markedly increased strongly resemble the plasma cells of multiple myeloma. The most reliable aid to differential diagnosis has been found

to be the perivascular location of the plasma cells seen in secondary plasmocytoses. The perivascular location of plasma cells in section material appears to be reflected in smears by the tendency of plasma cells to cluster in a petal-like arrangement around reticuloendothelial cells.

One of our most interesting cases of multiple myeloma showed only 1% and 2% plasma cells in sternal and iliac marrow respectively. The cells were sufficiently abnormal to permit diagnosis. Sections of the marrow showed multiple foci of developing plasma cells.

Metastatic Tumor. One of the earliest descriptions of metastatic tumor cells in the marrow is that of Rohr and Hegglin.[80] Now the possibilities of diagnosis of metastatic carcinoma on the basis of marrow aspiration are well-known and illustrations of the various types of tumor cells can be found in many of the atlases of the bone marrow. Rohr's illustrations are particularly good.[40] The leukemoid reaction generally encountered with metastatic carcinoma is an excellent index of the likelihood of osseous metastases. We have almost never seen metastatic carcinoma in the marrow when blood morphology was negative. It is of interest that the average figures in the 18 positive cases recorded in Table 3 show a slight tendency towards hyperplasia with a relative increase in normoblasts.

Purpura

Essential Thrombocytopenic Purpura

Agreement regarding the role of the spleen in the production of essential thrombocytopenic purpura has not been reached. Dameshek and Miller,[36] believe that the primary role of the spleen is inhibitory. Wiseman, Doan, and Wilson[91] and Doan and Wright[92] believe that the phagocytic and destructive function of the spleen is the most important. Evidence for both theories can be obtained.

Within recent years, megakaryocytosis accompanied by a lack of maturation of the cells of the magakaryocyte series[36,44] has been stressed as an important feature in the diagnosis of E.T.P. More recently Diggs and Hewlitt[45] have been able to demonstrate the left shift in megakaryocytes but not the megakaryocytosis in their series of 36 patients.

Our findings have, in general, agreed with those of Dameshek and Miller,[36] but it should be emphasized that the megakaryocyte count is widely variable. Megakaryocytosis can occur in a large variety of conditions in which there may or may not be thrombocytopenia and in which it is difficult to consider the thrombocytopenia "essential." It remains possible, of course, that when there is megakaryocytosis and thrombocytopenia, there may also be hypersplenism. This could easily be true in cirrhosis where splenectomy may alleviate thrombocytopenia and the bleeding tendency.

Most of the information available from our series can be extracted from Table 7.

Included in the series of 31 cases are 2 cases of cirrhosis, 3 of Grave's disease, and 1 case in which sarcoidosis was diagnosed from the removed spleen. Splenectomy was performed in 27 of the 31 cases, including 2 of the cases of hyperthyroidism and both of the cases of cirrhosis. Platelets increased and purpura disappeared in 23 of the splenectomized patients. In the remaining 4 cases our data are not complete. One patient in whom the marrow showed only 144 cells of the megakaryocytic series which, for the most part, were megakaryoblasts and promegakaryocytes expired one week after splenectomy. One patient has had a submaximal response. She retains a marrow pattern comparable to that seen in untreated E.T.P. and variable thrombocytopenia remains. She was reexplored for accessory splenic tissue, but none was found. Approximately 10 years after splenectomy, the patient experienced an uneventful pregnancy. Her child was born with purpura and marked thrombocytopenia.

Both purpura and thrombocytopenia were transiroty; the infant is well at the present time. The classification of this case is difficult. In two recent cases, the results of therapy can not be evaluated at the present time.

Secondary Thrombocytopenic Purpura

In secondary thrombocytopenic purpura, the megakaryocyte count has been decreased or normal in most of the cases. In one of the cases no information regarding megakaryocytes was obtained because only poor direct smears were made but the patient had an uneventful recovery. In the remaining 13 cases, the following information is available. Splenectomies were performed in 5 of the cases. In one case with a normal number of megakaryocytes[142] adenocarcinoma of the liver with metastases to the spleen was found at the time of splenectomy. The platelet count rose following splenectomy. In one case with increased megakaryocytes (473), no rise in platelet count followed splenectomy; the patient died of lymphoblastoma. In one case with a normal number of megakaryocytes (137), platelets have remained decreased for 2 months following splenectomy. In two cases with decreased megakaryocytes (none in one, and only a few in the sections in the other), platelets remained decreased and the patients expired.

Of the remaining 8 cases, 3 have expired. One, ., a case with decreased megakaryocytes which has been documented by Watson, Schultz, and Wikoff[93] was believed to have been caused by administration of estrogenic substances. One case, an infant with a normal number of megakaryocytes showed evidence of internal hemorrhage at autopsy. Although lymphedema of the extremities was prominent in this infant, it is possible that splenectomy might have proven of some benefit. One case with increased megakaryocytes (594) and with astrocytoma showed evidence of internal hemorrhage at autopsy. Splenectomy may have been of some benefit, but the patient was moribund upon admission.

Of the remaining 5 cases sufficient data are available in only 3. One case with increased megakaryocytes (350) has recovered. One, the infant born of the mother who has thrombocytopenic purpura which may or may not be essential in type, appeared on the basis of our material to have had decreased megakaryocytes, but the biopsy was not entirely satisfactory. The infant, as stated previously, recovered. One case with a normal number of megakaryocytes (194) was pregnant. Recent reports indicate recovery. One case with decreased megakaryocytes had carcinoma of the breast. A follow-up history was not available. In one remaining case with decreased megakaryocytes no information as to the clinical course was found.

Anaphylactoid Purpura

The cases in Table 7 are included in order that they may be compared with the cases of essential and secondary thrombocytopenic purpura. Since there is no thrombocytopenia in the anaphylactoid purpuras, the conditions are actually not comparable. Eosinophilia of the marrow and blood has been a constant finding. It may be noted that megakaryocytes appear slightly increased. This could be a compensatory phenomenon.

Neutropenia and Agranulocytosis

Only 8 cases of neutropenia are recorded. There have been many more, but most have been transitory, and blood studies have sufficed. It is also possible that neutropenias have been filed under categories not considered in the present report. When the 8 cases of neutropenia are compared with the 6 cases of agranulocytosis (Table 7), it appears that the main difference is one of degree. Of interest is the striking eosinopenia in the cases of agranulocytosis.

No discussion of neutropenia will be attempted. Some of our cases were secondary mild varities of agranulocytosis definitely relatable to some drug or toxin. Others may or may not have been primary neutropenia.

Three types of alterations in the maturation pattern of neutrophils may occur in agranulocytosis. There may be: (1) complete absence of granulocytes with a relative increase in lymphoid cells; (2) absence of mature neutrophils and metamyelocytes with an increase in myelocytes and promyelocytes (a picture which often superficially suggests leukemia); or (3) moderate decrease in metamyelocytes, band, and segmented forms. Normoblasts may be markedly decreased, but more often they show no alarming changes. The reasonably constant increase in lymphod cells often proves helpful in the exclusion of leukemia. An understanding of the changes in the peripheral blood is of utmost value in diagnosis. Relative monocytosis in the peripheral blood has long been considered a favorable prognostic sign. Monocytosis is, in agranulocytosis, generally associated with an hyperplastic marrow. Of interest is the fact that macrophages containing ingested neutrophils may be found in the peripheral blood.

Acute Disseminated Lupus Erythematosus.

The presence of L.E. cells in the marrow in A.D.L.E. was originally documented in a thesis by Morton and later published by Hargraves, Richmond, and Morton.[94] I had seen the L.E. cells in June of 1946 in a patient (Mrs. .) with a profound leukopenia and lymphocytopenia. I believed the inclusions in the neutrophils to be unique and thought they might be remains of phagocytosed lymphocytes, but I was unable to find transitional stages between lymphocytes and the peculiar amorphous substance contained in the neutrophils. I was delighted to learn from Morton's thesis that these cells had been observed by others, for at that time we had again seen these peculiar cells, this time in a case of known A.D.L.E. Haserick initiated a study concerning the value of the L.E. cells in the diagnosis of A.D. L.E.; L.E. cells were found in 4 out of 5 cases of A.D.L.E. but not in other conditions studied at that time or at any previous time.[95] Because I could not believe that such a large percentage of mature neutrophils could contain inclusions and remain confined to the marrow I decided to try by means of concentrating the leukocytes in the buffy coat to find them in the circulating blood. Mrs. Norma Lick found the first L.E. cell in smears of the buffy coat of venous blood. In our report of this finding we suggested that study of the buffy coat for L.E. cells might supplant marrow biopsy as a diagnostic measure in patients who were extremely ill.[96] At this time it was believed that L.E. cells circulated as such. Communications from investigators who were unable to find L.E. cells in their marrow specimens from cases of typical A.D.L.E. convinced me that something about our method was responsible for their presence. It was recalled that only direct smears were obtained in the case of A.D.L.E. reported as negative in a previous study,[95] and the idea that our concentration technique was important developed. The anticoagulant seemed unimportant since both heparin and oxalate had been employed.[95,96] Hargraves reported similar experiences concerning technique.[97] Recently Lorraine Gonyea has sent a communication to the effect that L.E. cells can be found in smears made from reagitated and centrifuged L.E. marrow which has been allowed to clot.[98] Hargraves found no L.E. cells in direct smears of marrow, and our experience has been similar.[97] It would seem then that cells must be exposed to plasma for a short time in order that L.E. bodies (free amorphous material) and L.E. cells be formed.

Haserick and Bortz[99,100] and Hargraves[97] have shown that when plasma from a patient with A.D.L.E. is added to normal marrow, L.E. cells are produced. Haserick and Bortz[100] emphasized the value of the L.E. plasma-normal bone marrow mixture in the diagnosis of A.D.L.E. and felt that the fraction of the plasma which induces the phagocytic phenomenon or the production of L.E. cells might be related to, or actually be, the etiologic agent in A.D. L.E. Plasma from 14 patients with A.D. L.E. caused the appearance of L.E. cells in at least 2 different bone marrow preparations. Refrigeration for as long as

three weeks did not destroy the potency of the plasma.

Before the latter reports appeared,[99] [100] Haserick wrote me of his findings. We[101] have confirmed Haserick's findings, and have found that plasma or serum, fresh or frozen, from the four cases which have been available for study will produce the L.E. cells in normal marrow. Frozen serum from one of our patients has remained potent for approximately one year. Plasma or serum from normal persons or from the one case of multiple myeloma tested did not cause the formation of L.E. cells. Gamma globulin prepared by precipitation according to the method introduced here by Dr. E. Frame from the plasma or serum of patients with A.D.L.E. has produced positive results even when the gamma globulin was diluted ten times. Gamma globulin from multiple myeloma and from normal controls did not produce L.E. cells.

Of most interest was the fact that we have been able to produce L.E. cells in marrow aspirated from rabbits[102] and monkeys[103]. This was done by mixing plasma, serum, or gamma globulin from patients with A.D.L.E. with the aspirated marrow allowing the mixture to stand for approximately 10 minutes, centrifuging, and then removing the buffy coat.

The most interesting experiment[102] was the addition of a large amount of lupus serum to a rabbit. Marrow obtained from the left tibia was separated into two portions. One portion was prepared routinely as a control and the other portion was mixed with 0.5 cc of L.E. serum. Then 25 cc. of L.E. serum were administered intravenously to the rabbit during a period of about 5 minutes. This was not tolerated well, and because the rabbit appeared moribund, aspiration biopsy of the right tibia was done 20 minutes after the serum was given. L.E. cells comparable in every way were found in the smears made from the marrow obtained from the left tibia (and mixed with L.E. serum) and in marrow obtained from the right tibia (prepared as if it were control marrow). No L.E. cells were demonstrable in the control marrow from the left tibia. The rabbit was quiet and apathetic 17 hours later. Blood was drawn and tested for the presence of the L.E. factor. No L.E. cells were produced in human marrow specimens by the rabbit's serum. The rabbit died during the following night. Autopsy was attempted, but the results were not considered significant. One interpretation of the results of our experiment might be that the vascular system and the marrow sinuses of the rabbit served as a simple receptacle for the interaction of the L.E. serum and whatever factor is responsible for the morphologic appearance of the L.E. cell. The changes in vivo were similar to those in vitro. The potency of the injected lupus serum had disappeared in 17 hours.

On the basis of our experiments we can only say that gamma globulin from patients with A.D.L.E. will produce L.E. cells in normal marrow. Haserick and Lewis[104] have been in the process of analyzing this more carefully; they have found with Tiselius fractionation it is only the gamma globulin fraction which will induce the L.E. phenomenon. Haserick[105] has also indicated that he has been able to induce the formation of specific antibodies against the L.E. factor. He used gamma globulin. We[106] have independently attempted a similar induction experiment using whole serum, but the patient from whom we obtained our original serum had only a weak L.E. factor, and our results might better be interpreted as negative.

Moffatt, Barnes, and Weiss[107] have reported the induction of the L.E. phenomenon in peripheral blood. We[101] pooled buffy coats in several types of experiments in an effort to devise a test which might prove satisfactory from all respects, the attempt being to eliminate the necessity of using bone marrow as one ingredient of the mixture and to avoid the strain of searching for L.E. cells in blood from patients with acute disseminated lupus erythematosus. We found L.E. cells in our preparations, but we felt that the method might be subject to error.

Our positive cases of A.D.L.E. are recorded in Table 7. Two additional cases showed strongly positive findings in the buffy coat of venous blood, and marrow biopsy was considered unnecessary. The lack of pronounced eosinopenia of the marrow seem odd in view of Williams' findings.[70] An average eosinophil count of 0.7% was found in the blood, however.

A communication from Dr. Harry Agress told of his finding a method of making section preparations of L.E. cells. Since his communication, a similar method has been developed here.[108]

Osteoblasts and Osteoclasts in Smears of Marrow

Although these cells might be expected in smears of bone marrow, they only rarely occur under normal conditions. Their identity has been obscured for many years, during which time they have been classified as cells of the megakaryocyte series (prepolykaryocytes and polykaryocytes of Di Guglielmo[109]). The importance of these cells was stressed by Esser,[110] and now they are included in the latest edition of Rohr's atlas.[40] Wo[111] have been interested in these cells partly because of the existing confusion concerning their identity and partly because they can prove of value in diagnosis. In one instance the identification of numerous osteoblasts in the marrow led to a diagnosis of Paget's disease in a patient who was thought to have had a primary splenic neutropenia. In our studies of the marrow in vitamin C deficient monkeys,[56] it is believed that a peculiar alteration in osteoblasts occurs prior to the development of frank scurvy. In one child, abnormal osteoblasts were encountered in marrow smears; x-rays revealed osteonephropathy. Osteoblasts and osteoclasts are common in the marrow when metastatic tumor cells are present. Proper understanding of the alterations of these cells may prove a valuable diagnostic tool.

Conclusion

Historical and technical aspects of biopsy of the marrow have been described. Some of the more typical changes seen in the marrow in primary blood diseases have been discussed. Various unusual lesions and changes of the marrow which have proven of diagnostic value have been given much attention primarily because little information concerning these changes is readily available. The most readily classifiable marrow patterns obtained from a survey of 1865 cases have been recorded in Tables 1-7.

Note: I am indebted to Dr. C. J. Watson and Dr. I. McQuarrie of the Departments of Medicine and Pediatrics, respectively, for permission to use the clinical data in the cases reported here.

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TABLE 1

MEGALOBLASTIC ANEMIAS	No. Cases	Fat		M-E		RBC M&N		PMN		E		B		LYM		PC&M Inc.	Megakaryocytes Inc.	Dec.	N.
PERNICIOUS ANEMIA	146	0 ' 8	1.4	0.3 ' 86.0	16.2	7.3 ' 63.6	36.2	19.6 ' 71.3	45.1	0.4 ' 6.9	3.8	0 ' 1.0	0.2	2.4 ' 45.3	17	(24) (24)	(18(6)	29(24)	38(15)
INFANCY	9	0 ' 1.5	.9	0 ' 12.5	8.6	31.0 ' 50.0	30.7	25.0 ' 36.0	30.5	2.6 ' 4.0	3.3	0	0	19.6 ' 21.0	20.3	?	?	?	?
GASTRECTOMY	3	0 ' 2.0	0.6	6.0 ' 31.0	15.3	35.2 ' 48.0	39.7	35.2 ' 48.0	43.5	1.6 ' 5.4	2.9	0 ' 0.6	0.2	11.4 ' 15.4	13.7	(1)	1	1(1)	1(1)
SPRUE	3	0 ' 1.5	0.8	7.0 ' 25.0	12.8	23.7 ' 47.6	38.6	35.2 ' 66.5	47.1	1.4 ' 3.2	2.1	0 ' 0.1	0.03	6.5 ' 18.8	12.1	(?)	0	0	3(1)
PREGNANCY	1		1.0		18.0		29.6		57.4		2.8		0.2		10	(1)	1	0	0

Rbc-includes megaloblasts and normoblasts. In almost all of the cases there had been no previous therapy, and the majority of the developing erythrocytes were megaloblasts.

TABLE 2

ANEMIA	No. cases	Fat		M-E		RBC NBL		PMN		E		B		Lym		PC&M Inc.	Megakaryocytes Inc.	Dec.	N.
F.H.A.	5	0 ' 1.5	0.4	5.0 ' 47.0	17.8	31.0 ' 85.0	60.1	12.0 ' 58.5	34.5	1.0 ' 4.0	2.0	0 ' 0.6	0.2	5.8 ' 9.8	8.0	(4)	6(1)	0	9(1)
OTHER H.A.	25	0 ' 6.3	2.0	3.0 ' 42.0	13.3	4.0 ' 78.0	50.6	12.8 ' 63.8	33.1	0.4 ' 24.9	3.9	0 ' 1.3	0.3	8.0 ' 28.4	13.5	(11)	13(2)	3(1)	6(1)
HYPO-CHROMIC	52	0 ' 14.0	2.2	0.2 ' 42.0	13.3	7.6 ' 52.6	33.8	21.6 ' 80.2	50.0	0 ' 13.1	3.5	0 ' 1.2	0.3	3.0 ' 41.5	13.9	(32)	34(2)	2	13
SPRUE (normo-blastic)	5	3.5 ' 9.5	4.3	7.0 ' 31.5	18.8	8.5 ' 34.5	19.4	47.5 ' 73.3	62.7	1.0 ' 1.6	1.0	0 ' 0.4	0.2	9.2 ' 18.8	16.2	(3)	3	0	2

F.H.A.- Familial hemolytic anemia
H.A. - Hemolytic anemia

TABLE 3

REFRACTORY ANEMIA	No. Cases	Fat	M-E	RBC NBL	PMN	E	B	Lym	PC&M	Megakaryocytes Inc.	Dec.	N.
R.A. Hyperplastic	16	0.0 ' 2.5 ' 7.0	8.0 ' 14.5 ' 24.0	5.9 ' 42.8 ' 70.4	16.8 ' 37.2 ' 61.8	0.7 ' 3.4 ' 11.5	0.0 ' 0.4 ' 2.5	5.0 ' 15.9 ' 40.0	(17)	12(2)	3	11
R.A. Normal Cellularity	14	0.0 ' 3.2 ' 8.0	4.0 ' 5.8 ' 7.5	4.7 ' 22.7 ' 43.0	37.0 ' 53.3 ' 80.0	1.0 ' 3.6 ' 13.2	0.0 ' 0.8 ' 2.0	10.4 ' 19.8 ' 41.5	(12)	3(1)	5(3)	7
R.A. Aplastic	36	0.0 ' 2.0 ' 17.0	0.0 ' 1.6 ' 4.5	0.0 ' 15.8 ' 62.6	0.5 ' 41.9 ' 82.2	0.0 ' 2.3 ' 14.5	0.0 ' 0.6 ' 2.5	0.0 ' 37.6 ' 80.+	(22) 0-39	3	36(20)	6(1)
Metastatic Tumor	18 (+)	0.0 ' 1.1 ' 5.0	0.0 ' 9.2 ' 20.0	2.4 ' 31.1 ' 56.6	21.8 ' 39.2 ' 65.0	1.0 ' 2.5 ' 5.8	0.0 ' 0.4 ' 1.0	5.6 ' 26.6 ' 64.6	Tumor (6)(18)	1	6(3)	3(1)
Multiple Myeloma	25	0.0 ' 1.3 ' 10.0	0.0 ' 7.4 ' 25.0	1.3 ' 15.5 ' 36.2	1.4 ' 41.6 ' 77.6	0.0 ' 2.8 ' 12.4	0.0 ' 0.3 ' 1.3	.3 ' 12.5 ' 27.0	*P.C.-M.M. 0.2 ' 28.5 ' 97.0	4(1)	7(5)	6(1)

45 Negative biopsies for tumor in cases where clinical history was suggestive of metatatic tumor. In most of these 45 cases, the blood was negative. In an occasional one of these cases, the blood showed a leukemoid reaction, but no marrow could be obtained by aspiration.

* P.C.-M.M. - Plasma cells of multiple myeloma.

TABLE 4

DISEASE	No. Cases	Fat	M-E	RBC NBL	PMN	Blasts	E	B	LYM	PC&M	Megakaryocytes Inc.	Dec.	N.
A.M.L.	6	0.0 ' 0.2 ' 1.0	7.1 ' 41.1 ' 83.0	.4 ' 1.5 ' 3.0	8.0 ' 10.8 ' 13.0	68.0 ' 75.4 ' 80.2	4.0 ' 7.5 ' 11.0	0.4 ' ' 7.0	0.0 ' 1.0 ' 3.0	(0)		5(5)	
S.M.L.	9	0.0 ' 0.5 ' 3.0	4.0 ' 24.2 ' 50.0	0.0 ' 8.2 ' 20.4	5.0 ' 28.1 ' 52.0	30.6 ' 48.3 ' 74.0	0.0 ' 2.4 ' 7.2	0.0 ' ' 22.4	3.4 ' 7.4 ' 17.2	(6)	3(1)	5(4)	2(1)
C.M.L.	21	0.0 ' 0.2 ' 1.5	2.0 ' 21.1 ' 53.0	1.0 ' 17.2 ' 55.4	26.6 ' 65.1 ' 88.0	0.0 ' 2.8 ' 10.6	0.4 ' 4.1 ' 7.8	0.2 ' ' 18.4	0.0 ' 4.5 ' 14.9	(0)	14(4)	3(3)	4
M.M.H.S.	10	0.0 ' 0.2 ' 1.0	0.0 ' 1.4 ' 2.5	9.0 ' 22.8 ' 35.0	44.2 ' 59.7 ' 78.0	0.8 ' 1.6 ' 2.5	0.0 ' 0.7 ' 1.4	0.0 ' ' 1.2	6.6 ' 14.7 ' 29.0	(2)		10(3)	
P.V.	20	0.0 ' 0.3 ' 5.0	1.0 ' 13.9 ' 49.0	5.6 ' 28.5 ' 67.0	17.0 ' 60.9 ' 88.8		0.0 ' 2.3 ' 6.8	0.0 ' ' 2.0	0.0 ' 7.8 ' 18.3	(0)	13	2(1)	3
Leuk. R-E (myeloid)	23	0.0 ' 0.5 ' 2.5	0.0 ' 15.0 ' 43.0	0.0 ' 13.4 ' 65.4	0.0 ' 28.8 ' 66.4	2.6 ' 35.4 ' 90.8	0.0 ' 2.3 ' 12.2	0.0 ' ' 7.0	3.0 ' 19.1 ' 59.0	(4)	2(1)	9(6)	1

A.M.L., S.M.L., C.M.L. -- Acute, subacute, and chronic myelogenous leukemia
M.M.H.S. -- Myeloid megakaryocytic hepato-splenomegaly
P.V. -- Polycythemia vera
Leuk. R-E -- Leukemic reticulo-endotheliosis

TABLE 5

DISEASE	No. Cases	Fat	M-E	RBC NBL	PMN	E	B	LYM.	Blasts	PC&M Inc.	Megakaryocytes Inc.	Dec.	N.
A.L.L.	9	0 ' 0.3 ' 2.0	0 ' 25.4 ' 52.0	0 ' 1.2 ' 5.4	0.2 ' 2.4 ' 11.3	0 ' 0.1 ' 0.8	0 0	88.7 ' 96.4 ' 99.8	86.2	(0)	0	3(2)	?
S.L.L.	25	0 ' 1.1 ' 5.0	0 ' 9.3 ' 26.0	0 ' 5.0 ' 25.0	0 ' 3.0 ' 7.0	0 ' 0.05 ' 0.8	0 0	69.2 ' 92.7 ' 100.0	40.0	(0)	0	12(9)	?
C.L.L.	41	0 ' 1.7 ' 25.0	0 ' 8.7 ' 34.0	0 ' 16.0 ' 66.0	2.6 ' 21.4 ' 53.4	0 ' 2.0 ' 7.9	0 ' 0.3 ' 1.0	19.2 ' 58.5 ' 90	Rare	(3)	3	12(6)	1
Leuk. R-E (lymph.)	14	0 ' 1.0 ' 5	0 ' 17.8 ' 49.5	0 ' 11.5 ' 29.0	0 ' 19.1 ' 53.4	0 ' .1 ' 2.6	0 ' .1 ' 0.5	27.4 ' 68.5 ' 99:3		(3)	2(1)	9(6)	1
Lympho-blastoma	20 6+	0 ' 3.8 ' 25.5	3.0 ' 6.7 ' 18.0	0 ' 21.2 ' 42.4	16.0 ' 48.3 ' 60.9	0 ' 3.0 ' 5.2	0 ' 0.4 ' 1.2	9.0 ' 27.0 ' 84.0		(11)	5	3	9
Reticulum cell sarcoma	1	0	4.0	0	6.0	0	0	43.0	51.0 (ret)	?	absent, but platelets Normal in Bl.		

A.L.L., S.L.L., C.L.L. - Acute, subacute, and chronic lymphatic leukemia. Leuk. R-E (lymph) - Leukemic reticulo-endotheliosis of the lymphatic type. Blasts, when included, are also included under total lymphoid cells. Mature and immature, normal and abnormal cells of the lymphocytic series in leukemic reticulo-endotheliosis and in lymphoblastoma are included under total lymphoid cells.

TABLE 6

GRANULOMA	No. Cases	FAT	M-E	RBC	PMN	E	B	LYM	PC&M Inc.	EPITH.	MEGAKARYOCYTES Inc.	Dec.	N.
TB	2	1.0 ' 3.6 5.5	5 ' 9.0 1.4	8.0 ' 11.9 17.2	58.6 ' 61.7 64.7	2.6 ' 4.4 5.8	0.6 ' 1.5 2.2	11.7 ' 20.4 29.0	(2)	(2)	2	0	0
SARCOID	2	15.0 ' 25.0 35.0	9.5 ' 14.3 19.0	21.4 ' 28.5 35.6	42.6 ' 50.2 57.8	3.2 ' 3.5 3.8	0.4 ' 0.6 0.8	14.0 ' 15.4 16.8	(2)	(1)	2	0	0
BRUCELLOSIS	8	1.0 ' 3.9 8.0	1.0 ' 7.9 13.0	7.2 ' 27.8 49.0	33.6 ' 51.2 69.0	2.0 ' 3.3 6.0	0 ' 0.1 0.6	6.5 ' 17.6 54.4	(8)	(2)	4	0	4
I.M. + sections in 9	23	0 ' 0.9 4.8	3.3 ' 10.5 21.0	10.2 ' 27.6 36.4	24.0 ' 39.8 51.4	1.8 ' 4.1 9.8	0 ' 0.4 1.0	12.2 ' 24.6 42.6	3.9	(11)	10	2	11
Unknown etiology	7	0 ' 8.6 32.0	4.0 ' 16.7 27.0	19.0 ' 41.4 65.0	13.4 ' 34.9 55.2	0.4 ' 4.0 9.8	0 ' 0.3 1.0	12.6 ' 19.4 26.0	(6)	(0)	5(2)	1(1)	1
Hodgkins + sections in 3	16		0.5 ' 14.0 37.0	9.8 ' 21.0 42.4	48.2 ' 64.4 76.4	0 ' 2.7 5.8	0 ' 0.3 0.8	5.4 ' 11.4 23.6	(8)	R-S (2)	7(1)	?	2

I.M.-Infectious mononucleosis.
Epith. - Number of cases in which epithelioid cells were found in marrow smears.
R-S - Number of cases in which Reed-Sternberg cells were found in marrow smears.

TABLE 7

DISEASE	No. Cases	FAT	ME	RBC NBL	PMN	(Blood)	E	B	LYM.	PC&M	Megakaryocytes Inc.	Dec.	N.
Neutropenia	8	0.0 ' 2.2 7.5	2.0 ' 6.2 13.5	6.0 ' 25.2 44.0	20.2 ' 46.1 80.0	(30.3)	0.6 ' 5.5 13.2	0.0 ' 0.7 2.0	10.8 ' 22.4 34.8	(4)	2	1	4
Agranulocytosis	6	0.8 ' 2.8 5.5	2.0 ' 4.4 11.3	3.2 ' 16.1 25.0	5.0 ' 29.9 86.6	(8.6)	0.0 ' .45 2.0	0.0 ' 1.6 5.0	8.2 ' 52.1 83.0	(?)	3	3	2
A.D.L.E.	7+	0.0 ' 1.5 4.0	1.5 ' 4.6 10.5	8.8 ' 21.3 35.3	29.4 ' 56.0 79.4		0.0 ' 2.0 4.8	0.0 ' 0.3 1.2	9.0 ' 20.4 37.8	(2)	1	1	5
E.T.P.	31	0.0 ' 3.0 10.5	4.0 ' 14.7 35.0	8.0 ' 30.8 55.6	15.8 ' 49.6 67.5		2.0 ' 3.8 6.8	0.0 ' 0.3 0.6	7.2 ' 15.0 36.8	(8)	(3690---144) 30(30) (1016)	0(0)	1(1)
S.T.P.	14	0.0 ' 2.5 10.0	1.2 ' 13.1 23.0	1.0 ' 26.8 54.3	17.4 ' 45.9 76.8		0.0 ' 3.8 10.5	0.0 ' 0.2 1.0	5.0 ' 23.5 53.4	(8)	(594---0) 3(3) (258)	6(6)	4(4)
A.P.	4	1.0 ' 1.3 1.8	5.0 ' 7.1 9.0	16.6 ' 21.8 26.8	49.0 ' 58.1 66.6		4.4 ' 5.1 6.0	0.0 ' 0.3 5.0	12.0 ' 14.8 17.8	(4)	(592---152) 3(0) (359)	0(0)	1(0)

A.D.L.E. -- Acute disseminated lupus erythematosus.
E.T.P., S.T.P., -- "Essential" and secondary thrombocytopenic purpura.
A.P. -- Anaphylactoid purpura.
Under megakaryocytes in purpura: Range of megakaryocyte count is above numbers of cases and average is below.

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Anat.Rec.100(4):85, '48.

II. MEDICAL SCHOOL NEWS

Coming Events

June 12 - Minnesota Medical Alumni Association and Minnesota Medical Foundation dinner - Spaulding Hotel, Duluth, Minnesota (during annual meeting of Minnesota State Medical Association) - 6:00 p.m.

June 26-30 - Continuation Course in Ear, Nose, and Throat for Specialists.

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Minnesota Medical Foundation Scholarships

The Board of Trustees of the Minnesota Medical Foundation has voted upon and authorized, at a recent meeting, the awarding of scholarships to undergraduate medical students. The scholarship awards, which were begun last year, will provide $500 to each of five undergraduate medical students selected on the basis of merit and need.

Members of the present freshman, sophomore, and junior classes are eligible to apply for the scholarships and may obtain application forms in the Medical School Office or the office of George N. Aagaard, M.D., Secretary-Treasurer of the Foundation, 3411 Powell Hall. Applications should be returned to the secretary of the Foundation on or before July 1, 1950.

Presentation of awards will be made as a part of the Minnesota Medical Foundation day activities during the opening week of the fall quarter.

Scholarship award winners for the present academic year were Alan R. Hopeman (Senior), Norman Albert Nelson (Senior), Mildred L. Schaffer (Junior), John W. Anderson (Junior), and Edward G. Huppler (Sophomore).

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Faculty News

Members of the staff of the Department of Medicine will journey to Duluth on Saturday, May 27, to attend the meeting of the Minnesota Society of Internal Medicine.

Scientific papers will occupy the morning and afternoon sessions. The special feature of the evening session will be the address, "Exploring the Universe", by Clarence B. Lindquist, Ph.D., Associate Professor of Mathematics and Astronomy, Director of the Darling Observatory, University of Minnesota, Duluth Branch.

III. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

May 28 - June 3, 1950

No. 290

Sunday, May 28

9:00 - 10:00 Surgery Grand Rounds; Station 22, U. H.

10:30 - Surgical Conference; The Diagnosis and Treatment of Parotid Gland Tumors; David State; Todd Amphitheater, U. H.

Monday, May 29

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; M-109, U. H

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:00 - Pediatric Rounds; Erling Platou; Sta. I, Minneapolis General Hospital.

11:00 - 11:50 Physical Medicine Seminar; E-101, U. H.

11:00 - 11:50 Roentgenology-Medicine Conference; Veterans Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Eustis Amphitheater, U. H.

12:00 - 1:00 Physiology Seminar; Observations on Factors Associated with the Genesis of Mammary Cancer in Mice; John J. Bittner; 214 M. H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

12:30 - 1:20 Pathology Seminar; Subject to be announced; 104 I. A.

12:30 - 1:30 Surgery Problem Case Conference; A. A. Zierold, C. Dennis and Staff; Small Classroom, Minneapolis General Hospital.

1:30 - 2:30 Surgery Grand Rounds; A. A. Zierold, C. Dennis and Staff; Minneapolis General Hospital.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - Medical-Surgical Conference; Bldg. I, Main Conference Room, Veterans Hosp.

4:00 - Pediatric Seminar; Inclusion Bodies; Marie Moorhead; 6th Floor West, Child Psychiatry, U. H.

4:30 - 5:30 Dermatological Seminar; M-436, U. H.

5:00 - 5:50 Clinical Medical Pathologic Conference; Todd Amphitheater, U. H.

5:00 - 6:00 Urology-Roentgenology Conference; C. D. Creevy, O. J. Baggenstoss, and Staffs; M-109, U. H.

Tuesday, May 30 -- HOLIDAY

Wednesday, May 31

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-109, U. H.

8:00 - 9:00 Roentgenology-Surgical-Pathological Conference; L. B. Thomas and L. G. Rigler; Todd Amphitheater, U. H.

8:30 - 9:30 Clinico-Pathological Conference; Auditorium Ancker Hospital.

8:30 - 10:00 Orthopedic-Roentgenologic Conference; Edward T. Evans and Bernard O'Loughlin; Room 1AW, Veterans Hospital.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker, Veterans Hospital.

11:00 - Pediatric Rounds; Erling Platou; Sta. I, General Hospital.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Surgery Case; O. H. Wangensteen, C. J. Watson and Staffs; Todd Amphitheater, U. H.

12:00 - 1:00 Radio-Isotope Seminar; Studies on the Nucleic Acid Synthesis in Megaloblastic Monkeys as Studied with P32, C. Lowe; 113 Medical Sciences.

12:15 - Staff Meeting; Main Classroom, General Hospital.

3:00 - Pediatric Rounds; C. J. Huenekens; Sta. I, General Hospital.

3:30 - 4:30 Journal Club; Surgery Office, Ancker Hospital.

4:00 - 5:00 Infectious Disease Rounds; Basement Amphitheater, General Hospital.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; E-101, U. H.

5:00 - 7:00 Dermatology Clinical Seminar; Dining Room, U. H.

8:00 - Dermatological Pathology Conference; Todd Amphitheater, U. H.

Thursday, June 1

8:30 - 10:20 Surgery Grand Rounds; Lyle Hay and Staff; Veterans Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-109, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Surgery-Radiology Conference; Daniel Fink and Lyle Hay; Veterans Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

11:30 - Pathology Conference Clinic; Main Classroom; General Hospital.

11:30 - 12:30 Clinical Pathology Conference; Steven Barron, C. Dennis, George Fahr, A. V. Stoesser and Staffs; Large Classroom, Minneapolis General Hospital.

Thursday, June 1 (Cont.)

12:00 - 1:00 Physiological Chemistry Seminar; Enzymatic Breakdown of ATP; Elda Jean Martin; 214 M. H.

1:00 - 1:50 Fracture Conference; A. A. Zierold and Staff; Minneapolis General Hospital.

4:15 - 5:00 Bacteriology Seminar; The Dissolution of Thrombae in vivo Through Use of Streptokinase-Activated Profibrinolysin; D. Morledge; 214 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 6:00 X-ray Seminar; Thoracic Surgery Cases; Surgery Department, University Hospitals; Todd Amphitheater, U. H.

7:30 - 9:30 Pediatrics Cardiology Conference and Journal Club; Review of Current Literature 1st hour and Review of Patients 2nd hour; 206 Temporary West Hospital.

Friday, June 2

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:20 Medicine Grand Rounds; Veterans Hospital.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:00 - Pediatric Rounds; Erling Platou; Sta. I, General Hospital.

11:00 - 12:00 Surgery-Pediatric Conference; C. Dennis, O. S. Wyatt, A. V. Stoesser, and Staffs; Minneapolis General Hospital.

11:45 - 12:50 University of Minnesota Hospitals General Staff Meeting; Subject to be announced; Osmond J. Baggenstoss; Powell Hall Amphitheater.

12:00 - 1:00 Surgery Clinical Pathological Conference; A. A. Zierold, Clarence Dennis and Staff; Large Classroom, Minneapolis General Hospital.

2:00 - 3:00 Dermatology and Syphilology Conference; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

1:00 - 3:00 Pathology-Surgery Conference; Auditorium, Ancker Hospital.

3:00 - 4:00 Neuropathology Conference; F. Tichy; Todd Amphitheater, U. H.

4:00 - 5:00 Clinical Pathological Conference; A. B. Baker; Todd Amphitheater, U. H.

4:15 - 5:15 Electrocardiographic Conference; 106 Temp. Bldg., Hospital Court, U. H.

Friday, June 2 (Cont.)

4:30 - 5:30 Journal Club; M-436, U. H.

5:00 - 6:00 Otolaryngology Seminar; Review of Current Literature; L. Younger; Todd Memorial Room, U. H.

Saturday, June 3

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; M-109, U. H.

8:30 - 9:30 Surgery Conference; Auditorium, Ancker Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; E-221, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:15 - 10:00 Surgery-Roentgenology Conference; F. Ruzicka, O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

10:00 - 11:30 Surgery Conference; O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - Contagion Rounds; Forrest Adams; Sta. L, General Hospital.

11:00 - 12:00 Anatomy Seminar; The Megaloblast-Normoblast Problem, Hal Downey; Application of Electron Microscopy to Hematology, John W. Rebuck; 226 I. A.