BACTERIOLOGIC AND CYTOLOGIC EXAMINATION OF STERNAL BONE MARROW.

Application in the Diagnosis of Pyrexias of Obscure Origin.

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Fever is one of the most common and apparent accompaniments of disease. As Sydenham has said: "Fever is a mighty engine which Nature brings into the world for the conquest of her enemies." The great cause of fever is infection, but it is also frequently seen in many other conditions. Of the latter, diseases of the blood and blood-forming organs comprise a notable example. In many cases of pyrexia the cause is obvious or easily discoverable by means of familiar clinical and laboratory examinations. In others however, determination of the etiology may be a most difficult and perplexing problem; such cases are 'pyrexias of obscure origin' until the cause of the fever has been explained. If no explanation is forthcoming after exhaustive investigation one is left with the unsatisfactory diagnosis 'pyrexia of unknown origin'. (P.U.O.)

A particularly difficult diagnostic problem is presented by the patient who exhibits features common to infections and to diseases of the hemopoietic system. In such instances symptomatology (malaise, weakness, sweating, vague aches and pains) and objective findings (fever, splenomegaly, altered blood picture, hemorrhagic manifestations) may be very similar. Typhoid fever, subacute bacterial endocarditis and undulant fever are common but by no means the only diseases that mimic blood dyscrasias.

Infections affect the blood picture in several ways to produce a leucocytosis, a leucopenia, or changes in the relative number and appearance of the white cells. Moreover, they may result in the sudden or gradual reduction of the red cells. Not only the infection itself, but the therapeutic agent used to com-

bat it may affect the bone marrow profoundly as is evidenced by the purpuras, anemias, agranulocytoses and leukemoid reactions produced by the sulfonamides. On the other hand, as described below, most blood dyscrasias and many anemias are accompanied by fever. Thus in many instances it is difficult to know whether one is confronted by an infection with a secondarily affected blood picture or by a blood dyscrasia associated with an elevated temperature.

Diseases of the hemopoietic system with fever as a prominent manifestation may begin acutely or insidiously. Those of abrupt onset are the acute leukemias, acute agranulocytic angina and in rare instances, Hodgkin's granuloma. Acute leukemia usually begins with sudden onset of fever and chills, inflammatory processes in the mouth and fauces, and enlargement of the cervical nodes. Its resemblance to an infective malady is noted by Piney[1] and by McCrae[2]. In agranulocytic angina the onset is also sudden with fever, chills, ulcerative angina, toxemia and marked granulocytopenia[2]. Jackson and Parker[3] state that rarely Hodgkin's granuloma begins with the symptoms of an acute upper respiratory infection, viz; chills, fever and cough. Pernicious anemia may also begin suddenly in a seemingly healthy person with a moderate rise in temperature(101) and be regarded as 'influenza' until the pallor of the pigment-stained sclerotics suggests an examination of the blood[4].

More commonly however, diseases of the hemopoietic system are of gradual onset, and in these fever may be the presenting manifestation. It may occur in any of the blood dyscrasias but the common examples are the chronic leukemias, Hodgkin's disease and other lymphomas, and pernicious anemia. In chronic mye-

logenous leukemia fever is present in two-thirds of the cases;[2] it occurs at irregular intervals for irregular periods and may be persistent, intermittent, or remittent.[5] In chronic lymphatic leukemia there is also usually some fever but no characteristic curve. In aleukemic leukemia the ordinary signs and symptoms of leukemia are present, but the characteristic peripheral blood picture is lacking. In Hodgkin's disease Jackson and Parker[3] state that "fever may be the presenting and indeed the only symptom for weeks or months. Its presence, particularly if it is relapsing in type and is not obviously due to one of the more obvious causes, should arouse the suspicion of Hodgkin's disease." They also note the difficulty in differentiating it from subacute bacterial endocarditis and undulant fever. In their series fever occurred at some time or other in 43% of cases; the curves varied widely - most frequently it was "typeless", but occasionally it was intermittent, remittent or continuous. Gall and Mallory[6] in surveying the clinical and pathological features of 618 cases of malignant lymphomas of various types, state that febrile manifestations (fever of 101 or higher) occurred at some time during the course of the disease in cases from all subgroups. In pernicious anemia fever is also present in about two-thirds of the cases.[7] It is most common in the acute stages, but bears no relation to the severity of the case or to the actual condition of the blood. It may be irregular, remittent or continuous. An irregular low grade curve may occur suggesting a mild septic condition, or it may show daily swings from 97 to 101 and remain thus for days hand-running, or it may be sustained around 99.5 to 101. The presence of such a fever has sometimes suggested typhoid or secondary syphilis.[4] In the diseases

discussed above fever occurs independently of secondary infection; it is obvious that if and when the latter occurs the fever curve will be altered and will therefore require re-interpretation.

From the foregoing it is evident that the differential diagnosis in pyrexias of obscure origin requires consideration of a wide range of diseases. In some of these, the ordinary clinical and laboratory findings may be lacking or atypical and additional information is needed. In particular, it has been repeatedly demonstrated in recent years that in some hematological disorders the peripheral blood picture does not accurately mirror the condition of the central organs of hemopoiesis. It has also been shown that bacteria and parasites may sometimes be recovered from the bone marrow when they are absent from the peripheral blood. For these reasons, combined bacteriological and cytological examination of the bone marrow can reasonably be expected to assist in the diagnosis of these problem cases.

Bone Marrow Biopsy.

Intensive study of bone marrow in vivo was long delayed, perhaps due to its inaccessibility. Very little was known about the physiology of bone marrow until Neumann[8] in 1868-9 showed that it was the site of erythropoiesis and leucopoiesis. Studies of bone marrow changes in disease which were done subsequent to his work were not satisfactory because the material was obtained at post-mortem examinations. The widespread interest in the bone marrow in disease which is prevalent today was to await new methods for its study.

Among the earliest attempts at marrow biopsy was that of Ghedini[9] who in 1908 demonstrated the parasites of malaria and

leishmaniasis by trephing into the tibial marrow of very young persons. This technique never became very widely popular although it was used by Zadek[10] and Peabody[11] in 1922 and 1927 respectively in their classical studies on the marrow changes in pernicious anemia. Seyfarth[12] in 1923 first advocated the sternum as the ideal biopsy site. His method of sternal trephination required an operating room technique but nevertheless re-awakened interest in marrow biopsy. It remained for the introduction of a simple procedure of needle puncture by Arinkin[13] in 1929 to popularize marrow study. There have followed many very complete reports of bone marrow changes in blood dyscrasias and diseases of the hemopoietic and reticulo-endothelial systems and its diagnostic usefulness in these has been well established. There have also been reports, largely in European and tropical medical literature, of its bacteriological and parasitological examination. For some reason, bacteriological culture of bone marrow which has been well established as a valuable diagnostic method in certain infections by European and South American workers has been almost entirely neglected elsewhere as evidenced by the paucity of reports in American, British and Canadian literature. In this age of specific chemotherapies early bacteriological diagnosis has assumed an increased importance and any procedure which increases the likelihood of this deserves consideration.

Bacteriological Examination of Bone Marrow.

The localization of bacilli in the bone marrow in infections has been known for many years, the first observations having been made in post-mortem studies on animals and man. It is in typhoid fever that this has been most clearly recognized.

Landau et al.[14] state that as early as 1903 Frankel and his pupils Israel and Muller had demonstrated typhoid bacilli in the marrow of patients dead of typhoid fever. Longcope[15] in 1905 noted the presence of large numbers of characteristic macrophages here as well as in Peyer's patches in the intestine, and also observed areas of necrosis in the marrow similar to those of the spleen and lymph nodes. Ludke[16] reported the persistence of typhoid bacilli in lymph nodes and bone marrow of guinea pigs following intravenous inoculation, and their proliferation in these situations after disappearance from the blood. Gay[17] further noted that the organisms were present in the marrow of infected rabbits not only after their disappearance from the blood, but after their disappearance from the lymph nodes and spleen. The clinical significance of this localization has been stressed in regard to the resultant leucopenia and neutropenia occurring during the acute phase of the disease and the osseous lesions which occasionally occur as sequelae of the infection. The first report of bacteriologic culture of bone marrow biopsy material was made by the Italian pediatrician Gerbasi[18] who in 1925 obtained positive cultures for typhoid fever from the tibial marrow in 13 children. In 1935 Debre et al.[19] cultured sternal marrow as well as blood in 7 cases of typhoid and obtained positive medullocultures in every case. This initial study showed that marrow cultures may become positive sooner than a simultaneous blood culture, presumably due to a heavier infection with bacteria. There have followed several reports[14,20,22-25] in which it has become clear that marrow culture may often be positive even when blood culture taken at the same time is negative. This is well demonstrated in the series reported by Ling and his asso-

ciates.[20] In a series of 110 marrow cultures and an equal number of blood cultures done on 38 patients with typhoid or paratyphoid the marrow culture was positive in 44 instances in which a simultaneous blood culture was negative. It was also noted that the average time required for growth to occur in marrow was less than that for blood, thus making for earlier diagnosis. It was found that marrow culture might be positive two weeks or more after the temperature had returned to normal, enabling the diagnosis to be made even during the convalescent period. Positive cultures in the 60th to 65th day of the disease have been reported.[21]

In the only American paper encountered on this subject Sacks and Hachtel[26] review the literature and report three cases of their own. They state that in all approximately 200 have been recorded. They point out that it is in the latter part of the second week when the diagnostic efficiency of current laboratory measures (agglutinations, blood and stool cultures) average about 70% that marrow culture has its greatest usefulness.

Other organisms which have been recovered from the bone marrow are those of the salmonella group[27] (which now includes paratyphoid[20,25,28]), undulant fever,[23,29] subacute bacterial endocarditis,[14,21,29,30,31] various septicemias,[21,31,32,38] tuberculosis,[19,33,34] leprosy,[35,36] and tularemia.[37]

Of particular interest are those diseases in which

A number of European papers published during the war have not become available at the time of writing. Among these is a reference to marrow culture in plague. (Modica,R.: Gazz. d osp., 62:11,1941.)

organisms have been recovered from the marrow when simultaneous blood culture was negative. In addition to typhoid this has been reported in paratyphoid, undulant fever, subacute bacterial endocarditis and tuberculosis.

Organisms of the paratyphoid group resemble typhoid in being readily obtained by marrow culture. Ott[25] records three cases in which organisms were recovered from the marrow when peripheral blood was sterile. In the only British paper encountered on the subject of marrow culture, MacDonald[28] describes two cases of paratyphoid in which sternal marrow cultures were positive. In both cases the patient had apparently recovered from the infection, bacteriological studies were negative, and they were discharged from hospital. Subsequently however, local symptoms due to an abscess in the breast in one case, and in the pelvis in the other, led to surgical drainage. Bact. paratyphosum B was recovered from the pus in both instances. A few days post operatively marrow cultures were done; in one case a positive result was obtained when the blood and feces were negative, in the other no simultaneous blood culture is recorded.

In undulant fever Basserga and Barbagallo[29] obtained positive marrow cultures when simultaneous blood cultures were negative. They record three such cases in which several observations were made: (1) that blood cultures taken when the temperature does not go higher than 39 C. are seldom positive; whereas in marrow cultures positive results are obtained whenever the temperature rises above 37.5 C.; (2) that the diagnosis can be made before the agglutinations become positive, and (3) the particular type of brucella involved can be identified by culture but not by agglutin-

ation reactions. They therefore conclude that marrow culture is a most useful procedure in the diagnosis of undulant fever. Domenighini[23] recovered organisms from the marrow but not from the blood in one case of paramelitensis.

In subacute bacterial endocarditis recovery of streptococci from the marrow at a time when the peripheral blood was sterile was first reported by Basserga and Barbagallo[29] .Landau et al.[14] had a similar experience. Falconer and Leonard[30] in a report on the value of sternal marrow aspiration as a method of bone marrow biopsy incidentally mention recovery of streptococcus viridans from the marrow but not from the blood in one case. Later in the course of the disease the blood also became positive. It is evident that marrow culture done on patients suspected of subacute bacterial endocarditis may be a means of earlier diagnosis.

As previously stated, localization of bacteria in bone marrow in the course of septicemias has been long recognized. In 7 cases of fatal septicemia Lemierre et al.[38] cultured from the marrow removed at autopsy the same organisms recovered by blood culture during life. The organisms were streptococci, staphylococci, and pneumococci. Carnot et al.[31] obtained positive blood and marrow cultures during life from a case of post-abortion septicemia due to hemolytic streptococcus and from a severe pneumonia due to pneumococcus. Bock[21] in a comparative study of venous, arterial and marrow culture in a series of 41 patients with a variety of septic conditions recovered streptococci, staphylococci, enterococci, and coli from the marrow as well as the arterial or venous blood in 17 cases. He concluded that arterial

blood culture was better than either sternal or venous culture, and that sternal culture was superior to venous culture. Ling et al.[32] recovered staphylococcus aureus from both blood and sternal marrow in a case of staphylococcal septicemia. They considered this to be the first such experience to be recorded but Bock's publication preceded it by nearly a year.

Debre et al.[19] reported in their original paper the recovery of tubercle bacilli from the marrow of a child with miliary tuberculosis. In a later report[33] it is stated that a similar result had been obtained in several children, direct blood cultures taken under similar conditions giving consistently negative results. In adults, Bezancon and associates[34] performed sternal marrow cultures on 45 patients with diverse forms of tuberculosis but in no case was a positive result obtained. However, in both humans and guinea pigs post-mortem marrow cultures positive for tubercle bacilli were readily obtained. The authors suggest a terminal septicemia or even a post-mortem dissemination of bacilli as an explanation for the disparity between the results obtained on living and dead subjects.

In leprosy Gass and Rishi[35] found M. leprae in the smears of bone marrow removed surgically and at post-mortem in 17 out of 21 cases of mixed cutaneous and neural disease. In 48 cases of neural leprosy not a single marrow smear was positive. Lowe and Dharmendra[36] performed sternal puncture on 50 cases of leprosy and examined smears for acid fast bacilli. Of 32 cases of cutaneous leprosy, bacilli were found in 16; in 18 cases of neural leprosy, bacilli were found in only one. These findings are consistent with widespread involvement of the reticulo-endothelial system known

to be present in "skin" leprosy whereas in "nerve" leprosy the infection is localized to certain tissues.

No clinical studies of bone marrow in tularemia have been encountered. In experiments on rodents Lillie and Francis[37] frequently demonstrated B. tularense in marrow smears of infected animals.

Rationale of Marrow Culture.

The rationale for bacteriologic examination of bone marrow in infections exists in the fact that in this situation one may possibly secure capillary (arterial) blood as well as material rich in phagocytic reticulo-endothelial cells. These factors taken together are believed to offer greater probability of obtaining positive results than venous culture taken at a distance from the locus of infection.[26]

The superiority of arterial blood culture over venous culture has been reported by several investigators.[21,39] Cultures of blood removed from the femoral artery have shown a higher incidence of positive results than duplicate cultures of venous blood. Whether or not the procedure is justified for ordinary diagnostic purposes in view of the greater technical difficulties is problematical. In any case, in blood obtained from the marrow cavity, a certain amount of arterial blood is present, and may increase the likelihood of obtaining a positive culture.

The reticulo-endothelial system is comprised of phagocytic cells, both fixed and wandering. The fixed cells, which are specialized endothelial cells lining the blood and lymph spaces, are found in the hemopoietic organs. Thus, following a bacteremia

though the peripheral blood may be free of organisms, they may still be recovered by culture of these special phagocytic cells. Hence marrow culture is more likely to be positive than a simultaneous blood culture and therefore may render a correct bacteriological diagnosis that could not otherwise be made.

An additional advantage peculiar to marrow culture is that it may be performed on patients with thrombosed or inaccessible veins. It has accordingly a special usefulness in infants and young children.

Cytological Examination of Bone Marrow.

The development of needle biopsy of sternal marrow has already been described. Thousands of examinations in health and disease have now been done and a tremendous amount of data accumulated. It is beyond the scope of this paper to consider more than a few aspects applicable to the present study.

(1) The Myelogram.

Except in disease or embryonic life all formation of cells of the erythrocytic, granulocytic and thrombocytic series apparently occurs in the marrow, and the formation of cells of the lymphocytic, monocytic and plasmacytic series is shared with the lymph nodes, spleen and other lymphoid or reticulo-endothelial tissue.[40] The determination and tabulation of the varying proportions of adult cells and their precursors in the aspirated marrow constitutes what is known as the myelogram. Normal values for the myelogram as reported by various authors have varied widely due to (1) differences in technique, (2) variations in nomenclature and (3) study of inadequate numbers of truly normal individuals. Osgood and Seaman[40] have recently reviewed the literature pertaining to

the cellular composition of normal bone marrow as obtained by sternal puncture and propose tentative standards for use until much needed new studies are made. Other widely used normal figures are given by Scott[41] and Hynes.[42]

(2) Myelogram in Disease.

Disturbances in the myelogram in disease are the result of varied mechanisms such as hypoplasia, aplasia, hyperplasia, "maturation arrest", and myelophthisis.[43] In the course of a disease one of these may give way to another, and in some marrows more than one mechanism may be operative. For example, in pernicious anemia both hyperplasia and "maturation arrest" are present. Hypoplasia occurs as the result of chemical or physical agents, or idiopathically, and may affect one strain of cells predominantly or may depress many elements. Aplasia of course, is merely the ultimate form of such depression.

Hyperplasia occurs in both a controlled and uncontrolled manner and may affect either the erythroid or leucoid elements. The existence of an anemia for any length of time results in a controlled erythroid hyperplasia. Depending upon the etiology of the anemia, the hyperplasia will occur at different levels of the erythrocyte development. Uncontrolled erythroid hyperplasia is found in polycythemia vera. Controlled myeloid hyperplasia occurs in response to pyogenic infections; uncontrolled myeloid hyperplasia is present in myeloid leukemia.

"Maturation arrest" denotes a situation in which there is an abundance of precursor cells in the marrow but a diminution or absence of the cells of this series in the peripheral blood. Such a situation exists in agranulocytosis, pernicious anemia and

possibly other dyscrasias. Myelophthisis refers to the appearance within normal marrow of tissues foreign to this organ. The foreign tissue cells, such as those of Hodgkin's disease, multiple myeloma, and Gaucher's disease, may be observed displacing normal marrow cells in the myelogram.

Extensive studies in a wide variety of diseases have been carried out by Young and Osgood,[44] Vogel, Erf and Rosenthal,[45] Scott,[41] Hynes,[42] Sacks,[43] and many others.[30,46-51] It suffices for the present purpose to describe briefly the marrow picture in some of the diseases requiring consideration in the differential diagnosis of pyrexias of obscure origin.

Acute Leukemias.

The marrow is very cellular, 70 to 99% of the marrow cells being primitive white cells, either of the myeloid or lymphatic series.

Chronic Lymphatic Leukemia.

Here 40 to 90% of the marrow cells are lymphocytes, which may be normal small lymphocytes or show abnormalities in the size and structure of the nucleus. Israels[52] states that 50% or more lymphocytes is typical of lymphatic leukemia and is not usually present in other conditions involving lymphoid hyperplasia, (e.g. Hodgkin's and lymphosarcoma). The myeloid cells and erythroblasts show a maturation defect.

Chronic Myeloid Leukemia.

The marrow in early cases shows an increase in myeloblasts, premyeloblasts and myelocytes. Lymphocytes and monocytes are few or absent. There is a maturation defect in the nucleated red cells. In the final stages of the disease the marrow resembles that found in acute myeloid leukemia.

Aleukemic Leukemias.

Sternal puncture is of great value in the diagnosis of leucopenic leukemia. The marrow changes are similar to those in leukemia with leucocytosis.

Secondary Anemias.

Young and Osgood[44] demonstrated an erythroblastic reaction in the bone marrow in the anemia which accompanies hemorrhage, toxemias and infections. Scott[41] has found the same reaction in iron-deficiency anemias.

Pernicious Anemia.

During relapse the morphology of the bone marrow in pernicious anemia is characteristic. There is well marked hyperplasia; cells of the red series make up 30-50% of all nucleated cells, instead of 20% or less, as is found in normal marrow. Active erythropoiesis however, occurs in the marrow in many types of anemia. What is characteristic of the marrow in pernicious anemia and in the related anemias, is the preponderance of nucleated red cells of a special type, the megaloblast. There is also evidence of active and abnormal leucopoiesis.[53]

Aplastic Anemia.

The bone marrow is usually hypoplastic, showing a lack of mature cells, particularly of the myeloid type, and an increase of primitive cells resembling small lymphocytes. In other cases the marrow is hyperplastic with many primitive cells. Comprehensive studies in uncommon and atypical anemias have been made by Israels and Wilkinson[54] and by Davidson and others.[50]

Agranulocytosis.

The picture here depends upon the etiology of the a-

granulocytic syndrome and upon its severity. In severe cases there may be aplasia of granulocytic elements, in less severe cases there is a maturation arrest at some level of granulocyte development.

Infectious Mononucleosis.

This disease,with its acute febrile onset, lymph node enlargement, frequent splenomegaly and leucocytosis, may strongly suggest a leukemia. Most authors agree that there are no characteristic marrow findings, but the absence of the typical picture of leukemia rules the latter out.[48]

Hodgkin's Disease.

Infiltration of the bone marrow occurs frequently in this condition but the lesions are commonly focal and not diffuse. Steiner[55] found involvement of the sternum in 7 of 11 cases. This suggests that at times sternal puncture might be of diagnostic assistance. However, since the marrow itself shows no picture diagnostic of Hodgkin's disease, it follows that the specific tissue must be obtained if diagnosis is to be made by this method. This must occur very uncommonly and no indisputable cases have been noted in the literature. As a rule the marrow picture is as variable and unspecific as the changes in the peripheral blood.[41]

Infections.

Morrison and Samwick[46] report the findings in 32 cases of various infections. In the majority there was a significant acceleration of leukogenesis. They point out that it is an easy matter to distinguish between the leukemoid reaction due to infection and myeloid leukemia by noting (1) increased erythrogenesis in infections, decreased in myeloid leukemia; (2) the azurophilia of the myelocytic elements in infections; (3) the distinct-

ive myeloblastosis of blood and marrow in leukemia and (4) reduction of megakaryocytes in leukemia. Scott[41] found an increase in the granulopoietic cells with a varying degree of shift to the left, the immaturity varying with the stage of the infection and the severity of the toxemia. In typhoid fever and other infections associated with neutropenia the findings are commonly a marrow poor in cells with a lack of mature granulocytes - a "maturation arrest".

Myelophthisis.

In myelomatosis an excess of plasma cells is present. Carcinoma cells occurring singly or in groups, may be found in carcinomatosis. In lipoid histiocytosis and other reticuloses cells typical of the particular condition maybe seen.

(3) Diagnostic Value of Sternal Aspiration.

The procedure of aspiration biopsy of sternal marrow, like any other new concept or technique, has been acclaimed by some and disparaged by others. Gradually its true value and its limitations have been elucidated. Widespread adoption of this method of sampling marrow is testimony to its usefulness.

One of the principal criticisms of the procedure is that patchy lesions may be missed. While this undoubtedly happens, it has been pointed out that enough material is usually obtained to permit diagnosis of such lesions as multiple myeloma and lipoid histiocytoses.[40] This is supported by the experience of Beizer and associates.[56] Ten cases of multiple myeloma each in some way atypical so that marrow examination was desirable, were encountered and successfully diagnosed. Apropos of the present study, it is stated that one of the group had chills and fever, and one had epi-

staxis and bleeding gums.

Another limitation frequently emphasized is that marrow aspiration results in a loss of cell relationships and therefore does not give a complete and accurate picture. Osgood and Seaman[40] state that in the majority of instances aspirated material is satisfactory for diagnosis if the diagnosis is possible from an examination of marrow obtained by any technique (e.g. trephining).

Failure to obtain marrow by aspiration may occur in aplastic anemia or myelosclerosis. More commonly however, such unsatisfactory results are due to inexperience of the operator. In 1,000 punctures Osgood[40] failed to obtain marrow only once, whereas in the first 62 punctures done there were 5 failures.[44]

One of the great advantages of needle biopsy lies in its ease of performance. It can be readily done, serially if necessary, on ambulatory patients in office or outdoor clinic. Its applicability to young children has already been mentioned. Another creditable feature lies in the excellence of the cytological preparation obtainable; cell morphology is better than with any other method.[40]

Attempts have been made to evaluate sternal puncture as an aid to the clinician. Kandel and Leroy[57] found it to be of relatively little value in the diagnosis of their cases and are of the opinion that "thorough clinical investigation deprives the biopsy of sternal marrow of many of its uses." On the other hand, Mendell, Meranze and Meranze[48] state that sternal marrow aspiration "is indicated in all cases suggesting disorders of the blood forming organs or reticulo-endothelial system." Propp and Schwind[51] have critically reviewed 140 cases in order to determine the usefulness of the procedure. They found that in 74 cases in which sternal

marrow punctures were indicated clinically, information of clinical value was obtained in 64.9%. Moreover in 16.2% the diagnosis was made by means of puncture when all other diagnostic means short of surgical biopsy had failed. The diseases in which the method was most helpful were the anemias and the leukemias in the aleukemic phase. Hynes[42] has likewise observed that marrow puncture is of most value clinically in aleukemic leukemia and obscure anemias.

From a study of the literature and of their own cases, Propp and Schwind[51] observe that marrow findings fall into two groups: (1) specific myelograms which are typical of a particular disease, and (2) non-specific myelograms which may occur in a variety of conditions. Specific myelograms are present in the following conditions:

1. Leukemia, especially acute and leucopenic leukemia.
2. Megaloblastic marrow, present typically in pernicious anemia in relapse, achrestic anemia and sprue.
3. Agranulocytosis in the arrested marrow stage.
4. Neoplasms: multiple myeloma and metastatic malignancies.
5. Lipoid histiocytosis.

Non-specific myelograms do not permit of diagnosis in themselves but have positive value in association with the complete clinical picture. Moreover they may provide negative information of a most valuable and comforting kind in cases where a specific myelogram was anticipated.

Parasitological Examination of Bone Marrow.

When marrow smears are being examined in patients with undiagnosed pyrexia, particularly now when military personnel who

have served in tropical and sub-tropical areas may be encountered, it is well to look for the presence of parasites. Here, as with bacteria, the marrow may reveal organisms not present in the blood; this has been observed in malaria[58-60] and relapsing fever.[61] Many reports of its value in kala-azar have been published; Scovel[62] states that sternal marrow smears were positive in 81% of 557 examinations, and constituted the most valuable means of diagnosis. Organisms have also been found in filiariasis[63] and trypanosomiasis.[64]

Investigation.

Having encountered several cases of pyrexia in which the cause was obscure from the clinical and ordinary laboratory procedures, it was decided to try sternal marrow examinations on some of these patients. In some instances punctures were done only after other methods had failed; in others examinations were done on already diagnosed cases to see what new information might be gained. The manner of proceeding and the results obtained in a series of 30 patients are as follows:

(1) Technique:

Many different techniques for needle aspiration of marrow have been described. Each has its advantages and its disadvantages. There is need for further investigation of this matter and the adoption of a standardized method as well as a standardized nomenclature for the various blood and marrow cells. In a part at least, the technique adopted depends on the purpose of the examination; in hematological research it may well differ from that used for purely diagnostic purposes. In this series, the primary concern was to make a diagnosis by obtaining a specific myelogram or a positive marrow culture. To this end the following procedure was

adopted:

(a) preparation of the patient:

In debilitated and apprehensive patients a sedative (e.g. morphine gr.1/6) is usually given 1/2 to 3/4 hr. before the puncture is done. In the majority of cases no sedation is necessary. Just before doing the sternal puncture a venous blood culture is taken in the ordinary way and 1 cc. placed in a thioglycolate broth culture bottle and 3 to 4 cc. in a bottle containing beef infusion broth. Where particular organisms such as those of undulant fever are anticipated appropriate additional culture measures are taken.

The patient lies supine on a bed or examining table with the head on a thin pillow. A small cushion placed between the shoulders is useful in obtaining the best possible presentation of the sternum. The skin over the upper and middle sternum is then cleansed with iodine and alcohol and a sterile drape laid down so that the patient is unable to see the procedure.

(b) selection of site of puncture:

It has been shown that the marrow of the sternum remains representatively hemopoietic throught normal life and that it responds early to stimuli causing hyperplasia;[65] coupled with its accessibility and the reliability of its landmarks, these features make it ideal for biopsy. Various sites may be used for puncture but ordinarily the site of election is in the body at the level of the third costosternal articulation and halfway between the midline and edge of the bone. This site is easily found even in obese patients, by using the sternal angle, which marks the second rib,as a reference point. Wherever possible at the beginning of the puncture it is advisable to grasp the lateral margins of the sternum between the first and second digits of the hand not per-

forming the puncture so that one is certain not to go too far laterally and miss the marrow cavity. The reasons for the selection of this site are anatomical:[41] the lower third of the sternum is unsatisfactory because congenital anomalies are common there. Moreover, bending may occur in the lower sternum whereas in the site of election it is less likely to bend or give. A site a little to one side of the midline is desirable because of the rare occurrence of a cartilaginous union throughout the length of the bone. The manubrium is often used but it is more likely to contain fat than the body. In infants the manubrium is recommended but after the age of one year the site of election is the same as for adults.

(c) selection of needle:

Many needles have been devised for sternal puncture but they are all essentially similar, consisting of a short strong needle fitted with a stylet and carrying an adjustable guard. A short-beveled 18 gauge needle 3 to 4 cm. in length with a lumen of 1 to 2 mm. is satisfactory. It should be short to prevent bending and the bore large enough to permit the cellular marrow to pass through. A needle of the type shown in Fig. 1 was used in this series.

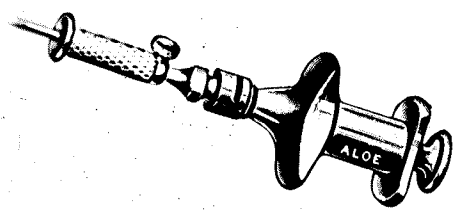

Fig. 1.
Reproduced from Wintrobe, M.M.: Clinical Hematology. Philadelphia, Lea and Febiger. 1942.

To prevent too deep penetration the adjustable stop or guard is

used. This is set at 1 cm. if the patient is an adult or at 0.2 to 0.6 cm. if the patient is a child. The outer lamina of the sternum varies considerably in thickness, ranging from 0.2 to 5 mm.

(d) actual puncture:

When the site has been identified and the skin cleansed, the puncture may be proceded with using strict surgical asepsis. The skin, subcutaneous tissue and periosteum are infiltrated with 2% novocaine. Some pain is commonly caused when the hypodermic needle penetrates the periosteum to produce local anaesthesia. The periosteum causes a firmer feeling of resistance to the needle than the subcutaneous tissue, and an appreciable sensation of thickness. Upon piercing it the needle strikes the bony anterior plate of the sternum where the final injection of novocaine is then made. Altogether about 3 cc. of solution is sufficient to accomplish adequate anaesthesia; about 5 minutes should be allowed for the anaesthetic to take effect.

A small incision is made through the skin with the scalpel and the skin edges are retracted with the thumb and forefinger of the free hand. This prevents contamination of the needle point by the skin edges and is of importance where bacteriological culture is being done although this step may be omitted where hematological examination only is performed. The puncture needle is inserted vertically and the outer table of the sternum is pierced by a rotary movement of the needle under firm pressure. A reduction of resistance or "give" is commonly felt when the marrow cavity has been entered; this may,however,be absent and boring is continued until the needle will remain upright without being supported. The needle may be passed 1 or 2 mm. into the cavity which is nor-

mally 5 to 15 mm. in depth.

When the marrow cavity has been entered, the stylet is removed from the needle and a sterile tight-fitting 5 or 10 cc. syringe is attached and a gentle suction applied. Suction may be accompanied by a painful sensation in the chest, apparently the result of producing a negative pressure in the marrow cavity. If no marrow fluid appears the stylet is replaced, the needle advanced a little further, and aspiration again tried. This is continued until marrow fluid, which has the gross appearance of ordinary blood, appears in the syringe. About 0.1 cc. of marrow fluid is withdrawn, the syringe is removed, and the material transferred to slides. A second sterile syringe is then attached to the needle, and a further 3 to 5 cc. of marrow fluid slowly aspirated. The needle and its attached syringe are then carefully withdrawn from the sternum. It is desirable that the marrow sample in the needle be neither lost nor contaminated during the removal of the needle from the bone. To prevent this it is necessary to hold the plunger firmly in place during withdrawal so that additional material will not be aspirated when the needle suddenly comes out of the bone and so that the weight of the plunger may not force the material out of the needle before the operator is ready for it. As soon as the needle is removed from the bone the contents are quickly transferred to the culture bottles. A sterile dressing is then applied over the puncture site and held in place by adhesive for 48 hours or until healing is complete.

In this series, 1 cc. of marrow fluid was placed in thioglycolate broth and the remainder in beef infusion broth. As with the simultaneous blood cultures, where organisms requiring special media or cultural conditions were anticipated the appro-

priate bacteriological measures were taken.

Numerous criticisms of this technique are possible particularly from the hematological viewpoint. However, it is stressed that this procedure is recommended in those cases in which the diagnosis is obscure and in which both bacteriological and cytological examinations are desired. In those instances where the clinical picture strongly suggests an infection bacteriological examination alone may be done thus ensuring that more marrow is obtained for culture, and conversely where there is strong evidence for a hematological diagnosis the bacteriological culture may be eliminated and a technique ensuring more satisfactory specimens of marrow for cytological study substituted. For hematological purposes better preparations are obtained if larger quantities of marrow are aspirated; Osgood and Seaman,[40] after reviewing the results obtained with various quantities of aspirate, recommend that 1.0 cc. or more be removed. Similar amounts are given by Wintrobe[53] and Kolmer[66] though many of the reported studies have been done on the first 0.1 to 0.2 cc.[41,45,48,51]. The technique here described permits one to ascertain by examination of the smear that marrow has actually been obtained by the aspiration and therefore the bacteriological culture is more likely to be successful than if only sinusoidal blood had been obtained.

(e) safety of sternal puncture:

It is now generally agreed that when properly performed needle aspiration of marrow is safe, causes little discomfort and may be classed with such routine examinations as spinal puncture and thoracentesis. The two principal dangers are hemorrhage and infection. With regard to the former, hemophilia may be the

only real contraindication since bleeding from the patients with other hemorrhagic diseases such as the purpuras has not constituted a problem. Infection has not been recorded as a complication of this procedure done for diagnostic purposes, although it has been observed to follow the administration of infusions of blood, plasma or other substances by the sternal marrow route.

Because of the large number of reports emphasizing the safety of sternal puncture it is well to point out that accidents may occasionally occur, even in experienced hands. The first fatal case to be recorded was that of Meyer and Halpern[67] in which a patient with chronic myelogenous leukemia died a few minutes after removal of the needle. The authors believed death was due to a fatal cardiac inhibitory reflex through the vagus, initiated by fear. Post-mortem examination was not performed. Recently Scherer and Howe[68] have described a case in which death followed 30 mins. after several unsuccessful attempts at marrow aspiration. At post-mortem it was found that the wall of the right ventricle had been lacerated by the needle and death resulted from cardiac tamponade. Because of the similarity of the terminal picture to that of the case described by Meyer and Halpern the authors conclude that both cases were likely due to the same mechanism.

Posteriorly the body of the sternum is in relation to the anterior borders of the lungs and pleural membranes, to the heart, ascending aorta, and pulmonary artery, contained in the pericardial sac; and to the thymus in the child. In patients with mediastinal tumor masses or other intrathoracic pathology disturbing the normal position of these structures, special care should be taken. In the series here reported no difficulties were experienced.

(2) Results.

The main features of 30 cases on which sternal marrow examinations were done are summarized in Table 1. It is seen at once that a wide variety of diseases is included. Every case exhibited some elevation of temperature while in hospital, or had been observed to be febrile prior to admission. Simultaneous blood and marrow cultures were done on 29 patients, a marrow culture only was done in one instance. Results of cultures are shown in Table 2.

TABLE 2.

MARROW CULTURES			BLOOD CULTURES		
No. Taken	No. Positive	Organisms Recovered	No. Taken	No. Positive	Organisms Recovered
30	7	B. alkaligenes B. typhosus (2) Hem. staph. aureus Strep. viridans Diphtheroid (2)	29	3	Hem. staph. aureus Strep. viridans Diphtheroid

From Tables 1 and 2 it is apparent that marrow cultures were positive in 4 patients in whom simultaneous blood cultures were negative. The diagnostically significant cases were those in which B. alkaligenes and B. typhosus were obtained; the recovery of diphtheroids in 2 patients is of academic interest only. Also, one marrow culture was positive for B. typhosus in the case on

TABLE 1.

CASE NO.	DATE	AGE	SEX	INITIAL DIAG.	FINAL DIAG.	TEMP. WHEN MARROW TAKEN	BLOOD CULT. CULTURE	MARROW CULTURE	SPECIFIC MYELOGRAM	REMARKS
1.	20 Dec.43	21	M.	P.U.O.	Dysentery due to B. Alkaligenes	99.3	Negative	B. Alkaligenes	-----	Case report.
2.	4 Apr.44	16	M.	Septicemia Post Extraction	Typhoid fever	102.3	Negative	B. Typhosus	-----	Case report.
3.	29 Aug.42	70	M.	P.U.O. ?Typhoid fever	Typhoid fever	102	Not done	B. Typhosus	-----	Case report.
4.	27 May 43	19	F.	P.U.O. ?Typhoid fever	Staphylococcal Septicemia	105	Hemolytic Staphylococcus aureus	Hemolytic Staphylococcus aureus	-----	Case report.
5.	24 Aug.43	55	M.	Aortic regurgitation	Subacute bacterial endocarditis	100.1	Streptococcus viridans	Streptococcus viridans	-----	Case report.
6.	20 Mar.44	29	M.	P.U.O. and Unexplained anemia	Pernicious anemia	99.2	Negative	Negative	Pernicious anemia	Case report.
7.	23 Mar.44	54	M.	P.U.O. and Unexplained anemia	Leukopenic leukemia - myeloblastic	99.4	Negative	Negative	Myelogenous leukemia	Case report.
8.	10 May 43	40	F.	P.U.O.	P.U.O.	93.3	Negative	Diphtheroid	-----	Low grade fever (99) for 2 months with influenzal type onset. Apyrexial when cultures done and thereafter.
9.	27 Oct.43	43	M.	(?) Subacute bacterial endocarditis	(?) Subacute bacterial endocarditis	99.	Negative	Diphtheroid	-----	Aortic regurgitation. Insidious onset of illness with anemia, fever, petechiae and hematuria. Died - autopsy not obtained.
10.	5 Dec.43	23	F.	Typhoid fever with perforation	Typhoid fever (3rd week) with perforation	99.2	Negative	Negative	-----	Admitted to hospital with acute abdomen. Perforation of ileum found; culture from peritoneum revealed B. Typhosus. Uneventful recovery.
11.	13 June43	69	M.	? Acute endocarditis	Acute endocarditis	97.	Negative	Negative	-----	Clinically diagnosed acute endocarditis. Ran swinging temperature up to 105 for 12 days. Post-mortem done outside hospital confirmed diagnosis but organism not determined.

CASE NO.	DATE	AGE	SEX	INITIAL DIAG.	FINAL DIAG.	TEMP. WHEN MARROW CULT. TAKEN	BLOOD CULTURE	MARROW CULTURE	SPECIFIC MYELOGRAM	REMARKS
12.	13 May 43	32	M.	Rheumatic endocarditis with mitral stenosis	Rheumatic endocarditis with mitral stenosis	98	Negative	Negative	-----	Damaged heart, palpable spleen, and occasional low grade fever led to suspected subacute bacterial endocarditis. Died at a later admission - no vegetations on valves at post-mortem examination.
13.	1 May 43	39	M.	Rheumatic endocarditis with mitral stenosis	Rheumatic endocarditis with mitral stenosis	96.3	Negative	Negative	-----	Damaged heart, palpable spleen, slight icterus suggested subacute bacterial endocarditis. Died at a later admission. No bacterial endocarditis at post-mortem examination.
14.	16 May 43	25	F.	Rheumatic endocarditis with mitral stenosis	Rheumatic endocarditis with mitral stenosis. Pulmonary embolus	102	Negative	Negative	-----	Cultures done because of suspected subacute bacterial endocarditis. Died suddenly 28 May 43, apparently from pulmonary embolus. Autopsy not obtained.
15.	2 Nov.42	54	M.	Rheumatic endocarditis with mitral stenosis	Coronary thrombosis. Mitral stenosis	99.2	Negative	Negative	-----	Investigated in Nov.42 for subacute bacterial endocarditis because of damaged heart and palpable spleen. Died in March,44. Post-mortem revealed coronary thrombosis & mitral stenosis
16.	26 Aug.43	53	F.	Essential hypertension with uremia	Hypertensive nephrosclerosis due to healed pyelonephritis	99.3	Negative	Negative	-----	Suspected of subacute bacterial endocarditis because of loud systolic murmur, palpable spleen & low grade fever. No evidence of endocarditis at autopsy.
17.	29 Apr.43	20	F.	Acute pyelonephritis of pregnancy	Acute pyelonephritis of pregnancy with B. coli bacteremia	99.	Negative	Negative	-----	Developed high fever & pyuria postpartum. Blood culture positive for B.coli on 27 Apr.43. Blood & marrow cultures negative 2 days later. Uneventful recovery.
18.	27 Jan.44	72	M.	Cellulitis of arm & possible septicemia	Cellulitis of arm, streptococcal septicemia	101	Negative	Negative	-----	Blood culture taken on admission showed streptococcus hemolyticus. Blood & marrow cultures after 3 days intensive sulfanilamide therapy were negative. Died. Autopsy not performed
19.	18 Sep.43	77	M.	Bacterial broncho-pneumonia	Bacterial broncho-pneumonia	103	Negative	Negative	-----	Aged patient with emphysema & chronic bronchitis developed acute broncho-pneumonia. Rapid uneventful recovery.

TABLE 1. (cont.)

CASE NO.	DATE	AGE	SEX	INITIAL DIAG.	FINAL DIAG.	TEMP. WHEN MARROW CULT. TAKEN	BLOOD CULTURE	MARROW CULTURE	SPECIFIC MYELOGRAM	REMARKS
20.	24 Sep.43	41	F.	Atypical pneumonia	Bilateral pneumonitis due to unidentified virus	98.1	Negative	Negative	-----	Ran swinging temperature (99-103) for 9 days with clinical picture of virus pneumonitis. Blood & marrow cultures on 10th day negative. Afebrile from 10th day on. Uneventful recovery.
21.	4 Dec.43	15	M.	Lobar pneumonia	Lobar pneumonia due to type 4 pneumococcus	104	Negative	Negative	-----	Admitted 4 Dec.43 with clinical picture of acute lobar pneumonia - W.B.C. 47,150. Uneventful recovery.
22.	5 May 43	19	M.	P.U.O.	Reaction to sulfanilamide	104.3	Negative	Negative	-----	Admitted 5 May 43 with history of taking sulfanilamide for 10 days for infection of upper lip. Had fever, bluish cyanosis, palpable spleen, W.B.C. 7,100. Temp. normal two days after sulfanilamide discontinued. Uneventful recovery.
23.	18 May 43	26	M.	(?) Undulant fever	P.U.O.	98.2	Negative	Negative	-----	Admitted 6 May 43 for investigation of alleged chills & fever occurring in bouts for two years. Temp. of 99 on 3 or 4 occasions during first week in hospital. Clinical & laboratory tests all negative. Afebrile for 2 weeks prior to discharge.
24.	15 June 43	56	M.	(?) Undulant fever	Undulant fever	100.2	Negative	Negative	-----	History of malaise & fever (99-103) for 2 months. Agglutinations (1: 250) and skin test positive for undulant fever.
25.	22 June 43	16	F.	P.U.O.	P.U.O.	101	Diphtheroid	Negative	-----	Admitted 1 June 43 with fever & malaise for a month. Blood culture 4 June 43 and stool cultures negative. In hospital 39 days with fever chart similar to typhoid. Gradual resolution of fever and spontaneous clinical improvement. No diagnosis was established.
26.	22 Sep.43	82	F.	Pemphigus vulgaris	Pemphigus vulgaris	100.3	Negative	Negative	-----	Admitted 1 Sept.43 with crusted skin lesion of face. Developed diffuse bullous dermatitis. Died 28 Sept.43. Autopsy not obtained.

TABLE 1. (cont.)

CASE NO.	DATE	AGE	SEX	INITIAL DIAG.	FINAL DIAG.	TEMP. WHEN MARROW CULT. TAKEN	BLOOD CULTURE	MARROW CULTURE	SPECIFIC MYELOGRAM	REMARKS
27.	9 Oct.43	43	M.	Pericarditis & pleuritis	Polyserositis, probably rheumatic.	100.	Negative	Negative	-----	Admitted 21 Sept.43 with chest pains and progressive anemia for several months; obscure clinical picture with fever, anemia, pericarditis, pleuritis and lymphocytosis. Died 14 Nov.43. Pathological findings as obscure as were clinical findings. Bone marrow essentially normal.
28.	11 Oct.43	28	F.	? Septic abortion	Pelvic cellulitis. Syphilis.	102.2	Negative	Negative	-----	Admitted with fever, abdominal cramps and abdominal and pelvic mass. Long history of crime, drug addiction and psychopathic behaviour. Recovered from pelvic cellulitis and anti-syphilitic treatment given.
29.	14 Dec.43	16	F.	Portal pyemia	Multiple liver abscesses with generalized sepsis following appendectomy.	104.3	Negative	Negative	-----	Admitted 11 Dec.43 with history of fever since appendectomy done elsewhere 29 Oct.43. Clinically portal pyemia, confirmed at autopsy 12 Jan.44.
30.	19 Jan.44	29	F.	P.U.O.	P.U.O.	98.1	Negative	Negative	-----	Investigated for low grade fever of two months duration. Chronic osteomyelitis in childhood. No cause for fever found after very extensive investigation.

TABLE 1. (cont.)

which a simultaneous blood culture was not done. One blood culture was positive for diphtheroids when in a simultaneous marrow culture no growth was obtained; such an occurrence is of no importance as will considered below.

In addition, specific myelograms permitting of definite diagnoses were obtained in two patients, one with pernicious anemia and one with leukopenic myelogenous leukemia. Because of their interest and importance, cases 1 to 5 with marrow cultures positive for pathogenic organisms and cases 6 and 7 with specific myelograms, will be completely considered in individual case reports. The remaining cases may be grouped and discussed in a general way.

Cases 8 and 9.

Diphtheroids were recovered from the marrow in these patients. Such organisms are very numerous and ubiquitous and have been isolated from a great many different sources, both in connection with the human body and in nature. They can be isolated from the skin, nose and throat, urine, ascitic fluid, from normal and diseased lymph nodes, and from supposedly sterile tissues. They are not infrequently found in blood cultures as exemplified in case 25. It is not surprising that they should be found in marrow cultures but it is of interest to note that they may be obtained here as well as from other supposedly sterile tissues. It is possible of course, that they were contaminants from the skin. The lack of other contaminants in this series, and the skin incision technique used, are against this view. Specific mention of bone marrow as one of the tissues from which diphtheroids may be isolated was not noted in the references[69-71] consulted, nor has it

been reported in any of the papers dealing with bacteriological culture of marrow obtained by sternal puncture.

Cases 10 and 11.

In proven typhoid fever (3rd week) and acute bacterial endocarditis both blood and marrow cultures were negative. This demonstrates that marrow cultures may be sterile in cases where a positive result is reasonably to be expected. In patients showing a disparity between the clinical findings and the results of a single simultaneous blood and marrow culture, repetition of the procedure may be necessary.

Cases 12, 13, 14, 15, 16.

All of these patients were suspected of subacute bacterial endocarditis. The possibility of making earlier diagnosis in this disease by use of marrow culture has already been mentioned. (page 9) All cultures in this group were negative; the reliability of these results was established by post-mortem examination in 4 cases. It is of interest to note that the splenomegaly observed in 4 of these patients was apparently due to chronic passive congestion.

Cases 17 and 18.

Both of these patients had proven bacteremias 2 and 3 days prior to performance of simultaneous blood and marrow cultures. In case 17, B. coli were found in the blood on 27 April '43, treatment was instituted and marked clinical improvement occurred. Blood and marrow cultures on 29 April '43 were both negative. In case 18 streptococcus hemolyticus was isolated on initial blood culture, sulfanilimide was given and blood and marrow cultures done three days later were negative. In these cases, if any organ-

isms were taken up by marrow reticulo-endothelial cells during the bacteremia, they must either have been destroyed in the interval, or marrow aspiration failed to remove any of them.

Cases 19, 20 and 21.

Two bacterial pneumonias and one virus pneumonitis yielded negative results.

Cases 22-30.

This miscellaneous group likewise yielded negative cultures.

It is of passing interest to note that of the 30 patients, 18 were males and 12 were females. Age varied from 15 to 82 years, with an average of 39.

(3) Case Reports.

Case No. 1. Dysentery due to B. alkaligenes.

A student airman, age 21, who had never been out of Canada, was admitted to Deer Lodge Hospital on 22 Sept. '43 with a history of mild diarrhoea and daily temperature 99-100 for 4 days. He was in the 7th day of a mild respiratory infection for which he had received sulfathiazole prior to admission. Family history was negative, and past history revealed nothing but an appendectomy in 1925. General physical examination was negative. Complete blood count and sedimentation rate on day of admission were normal; blood Wasserman test was negative, and urine was normal.

It was observed that he ran an irregular low grade fever (98-101) and that he had a persistent mild diarrhoea (3-5 bowel movements daily). Further laboratory investigation was done: hemoglobin, 107%; red blood cells, 5,200,000; white blood cells, 8,200; differential blood count: polymorphonuclears, 66%; lymph-

ocytes, 29%; monocytes, 3%; eosinophiles, 2%; blood sedimentation rate normal. Agglutinations for undulant fever were negative and stool culture was negative for pathogens including tubercle bacilli. Mantoux test was slightly positive.Radiographic studies of the chest, sinuses, stomach and duodenum,and large bowel were negative. Prostatic fluid was normal. Blood cultures on 25 and 28 Oct. '43 were sterile.

On 4 Nov. '43 sigmoidoscopy revealed edematous mucosa of sigmoid and rectum with shreds of mucus and pus, but no ulceration. On 20 Nov.'43 simultaneous blood and marrow cultures were taken and B. alkaligenes was obtained from the marrow only. This was the only positive laboratory result obtained in a prolonged and intensive investigation. Repetition of agglutinations for undulant fever continued to be negative in January and February 1944. Repeated blood counts, sedimentation rates and stool cultures were unchanged.

Over the period extending from September 1943 to March 1944 this patient exhibited an irregular low grade fever and a mild intermittent diarrhoea. The only positive findings were an edematous mucosa of sigmoid and rectum revealed by sigmoidoscopy, and a positive sternal marrow culture for B. alkaligenes. He was uninfluenced by a variety of therapies but gradually recovered spontaneously.

Discussion:

Bacillus (faecalis) alkaligenes is ordinarily considered to be a non-pathogenic organism which may be cultured from the stools of healthy persons. However, it has been recovered from the blood and stools of persons with mild disease of the enteric

type. Shearman and Moorhead,[72] working among British soldiers in Egypt during the First Great War recovered B. alkaligenes from the blood in eleven cases. They considered it as "having a pathogenic role and being the definite cause of a mild pyrexia in which the true condition is a bacillemia." They found agglutinations with patients' sera were positive 1:200 whereas in controls the values did not exceed 1:50. Hirst[73] recovered the organism from the blood in an additional 12 cases and concluded that it is "an organism of low virulence, capable of multiplying in the bowel in certain favorable conditions and occasionally gaining access to the blood stream through a more or less damaged mucous membrane, thereby producing a mild enteric-like infection." Some of his cases exhibited an irregular pyrexia for 30-40 days. From these and a number of similar reports[74,75] it would appear that B. alkaligenes is capable of producing a mild enteric-like disease accompanied by an irregular, low grade fever.

Recently Favour et al.[76] reported a case of subacute bacterial endocarditis due to streptococcus viridans in which penicillin was given. Prior to administration of penicillin blood cultures were positive for Strep. viridans; after penicillin therapy was commenced the blood culture became negative for the original organism but positive for B. alkaligenes. At autopsy, both Strep. viridans and B. alkaligenes were cultured from valvular vegetations. This case is cited here as additional evidence that this organism can and does enter the blood stream.

With regard to recovery of B. alkaligenes from the bone marrow a consideration of its place in the classification of micro-organisms is of interest. Its morphology, active motility

and normal habitat relate it to the bacteria of the coli-typhoid group. But its fermentative and other physiological properties relate it to the Brucellae.[71] It therefore occupies a position intermediate between these two groups. Since positive sternal marrow culture has been shown to be most readily obtained firstly in typhoid-paratyphoid infections, and secondly in undulant fever, it is not surprising that an intermediate organism should also be obtained in this way.

Thus, in a mildly febrile enteric illness which could not be diagnosed in any other way, sternal marrow culture provided an organism which is capable of producing such a clinical picture. Unfortunately, agglutinations of the organism with the patient's serum were not done, so that the etiologic evidence is not absolute. However, since the likelihood of this organism occurring as a contaminant in a sternal marrow culture is slight, and since the clinical picture is that which one would anticipate from infection with such an organism, it seems reasonable to presume that B. alkaligenes is the causative agent. Recovery of B. alkaligenes by marrow culture has not previously been reported.

Case No. 2. Typhoid Fever.

B. W., age 16, an Indian youth from a remote community was admitted to the Winnipeg General Hospital on 31 March '44. He gave a history of having had 3 teeth extracted two weeks previously (15 March '44). Following this he began to feel feverish and on 17 March developed anorexia and nausea. He reported to a physician who noted a temperature of 104 and gave him sulfapyridine. From then until he was admitted he felt fairly well except for feverishness and occasional chills.

On admission, temperature was 103 and pulse was 80. Examination revealed no other positive physical findings. X-ray of the chest and urinalysis were normal. The white blood cells numbered 12,300 with a normal differential count. The tentative diagnosis was septicemia following tooth extraction. Two venous blood cultures were taken the day of admission.

In hospital he ran a sustained fever around 103 for three days. On 4 April '44 simultaneous blood and sternal marrow cultures were taken. Agglutinin titre with H antigen, taken the same day was positive 1:500. The following day, 5 April, the blood cultures taken on admission were reported positive for B. typhosus.

The temperature chart is shown in Fig. 2.

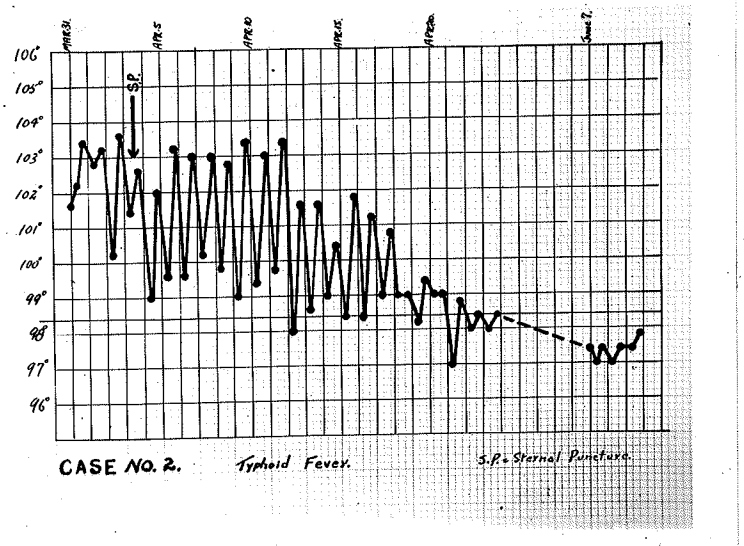

Fig. 2.

Discussion:

Results of various cultures are shown in Table 3. It is seen that blood cultures taken on the 15th, 17th, and 18th, day

of the disease were positive. On the 19th day, however, the blood was sterile, but a sternal marrow culture was positive. Obviously marrow culture was not instrumental in establishing the diagnosis in this instance, but it is apparent that had the patient been received a few days later, its importance might have been very great indeed.

TABLE 3.

Date	Day of Disease.	Blood Culture.	Marrow Culture.	Stool Culture.	Agglutinations.
31 Mar.	15	x			
2 April	17	x			
3 April	18	x			
4 April	19	-	x		x
9 April	24	-			
12 April	27	-		-	
17 April	32	-		x	
10 May	55	-		x	
13 May	58			-	

Case No. 3 Typhoid Fever.

E.S., a man age 70, was admitted to the Winnipeg General Hospital on 27 August '42 from an itinerary through rural areas, complaining of fever, anorexia, malaise and insomnia of a weeks' duration. On admission he was seen to be a big, drowsy, red-faced man in obvious respiratory distress, with a temperature of 102, pulse 60 and respirations 44. There were many fine crepitations in both lung bases and the heart sounds were weak and irregular. Abdomen was moderately distended and tympanitic; spleen not

palpable. Laboratory examinations were as follows: Blood: hemoglobin, 74% (11.20 gm. per 100 cc.); white blood cells, 7,000; differential, mature polymorphonuclears, 31%; young polymorphonuclears, 38%; lymphocytes, 24%; monocytes, 7%. Urine: albumin, .01%, otherwise normal. Agglutination titres were 1:20 for H antigen and 1:80 for O antigen. X-ray of the chest revealed elevation of the right diaphragm and slight displacement of the heart to the right, and some infiltration in the right base (interpreted as atelectasis). Electrocardiogram showed left ventricular preponderance and auricular fibrillation.

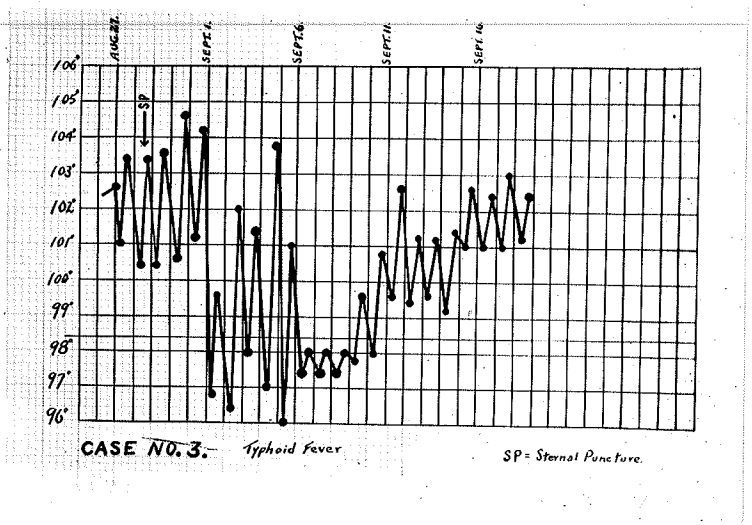

Fig. 3.

It was considered that he might have typhoid fever and a sternal culture was done on 29 August '42. A positive result was obtained within 30 hours.

The clinical course was progressively downward and he died on 19 September '42. The temperature chart is shown in

Fig. 3. Necropsy confirmed the diagnosis of typhoid fever. The ileum showed small mucosal ulcers most numerous near the ileocecal valve. The sternal bone marrow was only moderately cellular with an active erythroblastic and monocytic reaction. Sections of the bone marrow were stained and examined for typhoid bacilli but none were found.

Discussion:

This case was the first in the series investigated by sternal marrow culture and unfortunately a simultaneous blood culture was not performed. It is of interest however, that typhoid organisms were readily cultured from the marrow on the 10th day of the disease, and at a time when agglutinations were still not diagnostic. It served to diagnose this case of pyrexia and to stimulate further interest in investigation of the method.

Case No. 4. Staphylococcal Septicemia.

A young married woman, age 19, was admitted to the Winnipeg General Hospital on 25 May '43 with a history of fever and drowsiness of three days duration. On 22 May '43 while working in a store she had struck her forehead on some object but the injury was of trivial nature and she continued her work as usual. About four hours later she began to feel ill and nauseated and went to bed. She became feverish and confused and began to have frequent epistaxis and emesis of blood-streaked vomitus. She was in this severely ill condition on arrival at the hospital.

Examination revealed a flushed, lethargic, obviously ill patient with a temperature of 103.3, pulse 116 and respirations 20. Careful physical examination showed no other abnormalities.

Laboratory examinations on admission were as follows: Blood: hemoglobin, 70%, (10.4 gm. per 100 cc.); red blood cells, 3,680,000; color index, 0.97; white blood cells, 6,450; differential, mature polymorphonuclears, 52%; young polymorphonuclears, 33%; monocytes, 2%. Urine: albumin 0.03%, 10 pus cells and 4 red blood cells and a few granular casts per high power field. Agglutinations for typhoid, paratyphoid, and undulant fever were negative. X-ray of the chest was normal. Lumbar puncture revealed a pressure of 250 mm. of water and a normal fluid. A blood culture was taken and sulfadiazine therapy instituted.

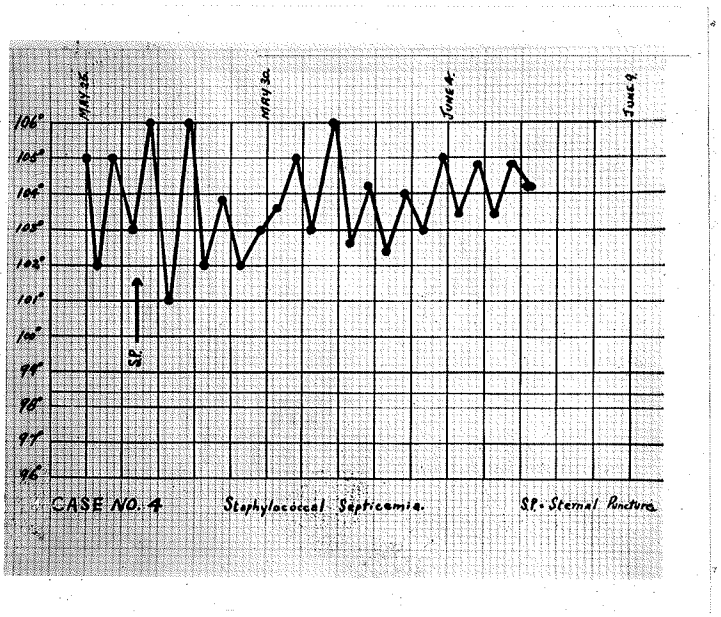

Fig. 4.

Following admission she ran a high fever with frequent chills as shown in Fig. 4. Epistaxis occurred frequently and rectal bleeding was noted. She was obviously very ill. On 27 May '43 a simultaneous blood and sternal marrow culture was done; these

and the blood culture taken on 25 May '43 were all reported positive for hemolytic staphylococcus aureus on the following day, 28 May '43.

In spite of intensive sulfadiazine therapy, blood transfusions, staphylococcal antitoxin and various supportive measures (penicillin was not yet obtainable) she gradually became worse. Bilateral bronchopneumonia developed and pustules (from which hemolytic staphylococcus aureus was cultured) occurred on the skin. Death occurred on 7 June '43, the thirteenth day of hospitalization.

Discussion:

Positive blood and sternal marrow cultures taken 48 hours after initiation of sulfonamide therapy, in a fatal case of staphylococcal septicemia were obtained. Since blood culture taken on admission also became positive it cannot be said that marrow culture in this instance was of diagnostic importance. It is of academic interest in that simultaneous recovery of staphylococci from blood and marrow during life has been reported in but a few instances. Bock[21] records 3 cases in which staphylococci alone were recovered, and 1 case of osteomyelitis in which both staphylococci and streptococci were obtained. Ling et al.[32] report a single case which they considered to have been the first to be reported. (page 10

Case No. 5. Subacute Bacterial Endocarditis.

B. G., a male aged 55, was admitted to Winnipeg General Hospital on 9 August '43 complaining of generalized weakness of a months' duration. Direct enquiry elicited only that he had noticed excessive sweating at night for two weeks. Past history revealed that he had served in tropical Africa and in France during

World War 1 but had never had any illness then nor since. He had had rheumatic fever in 1914. In May 1943 he had a herniorraphy with fascial graft performed and this had been uneventful. Family history was irrelevant.

Examination showed a heavily built, slightly pale man in no apparent distress; temperature was 99.3, pulse, 65 and respirations 20. The head, neck and lungs were normal on examination. The heart was regular, forceful and of normal size but there was a harsh systolic apical murmur and a soft diastolic murmur in the 4th interspace to the left of the sternum. B.P. was 110/60. Peripheral vessels were moderately thickened, the character of the pulse was within normal limits. Spleen was not palpable; there were no embolic phenomena. Remainder of examination was negative. He thus appeared to have a mild aortic regurgitation, presumably rheumatic in origin.

Laboratory studies on admission were as follows: Blood: hemoglobin, 73% (10.9 gm. per 100 cc.); red blood cells, 4,380,000; color index, 0.85; white blood cells, 10,250; differential: mature polymorphonuclears, 40%, young polymorphonuclears, 28%; lymphocytes, 23%; eosinophiles,5%; monocytes 4%. Urinalysis was negative. Electrocardiograph showed left ventricular preponderance. Radiographical studies revealed a normal upper gastrointestinal tract and a few diverticulae in the lower sigmoid. The gall bladder did not visualize; no calculi were seen. Films of the teeth did not show periapical infection. Chest plate showed extensive dense calcification in the right upper lung field which was interpreted as healed tuberculosis.

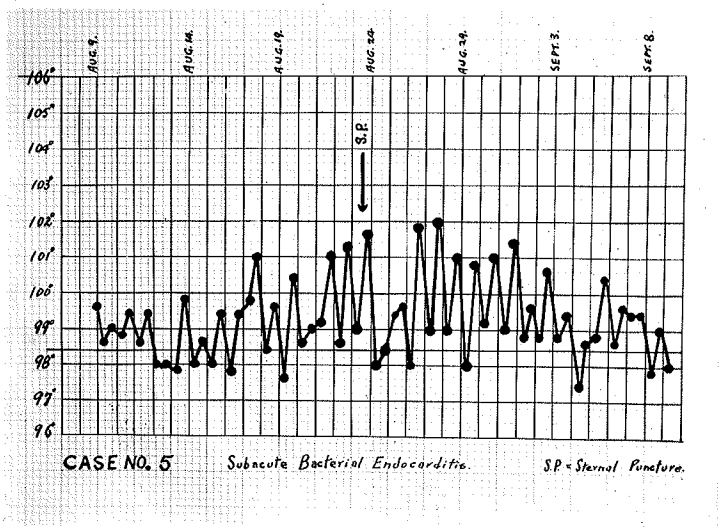

Fig.5.

For the first two weeks in hospital he did not appear very ill but he ran a low grade irregular fever which reached 101 on two occasions. As seen in Fig. 5 his temperature was gradually rising and he felt correspondingly worse. Sedimentation rate on 21 August was found to be 78 mm. in 1 hr. in 200 mm. tube, agglutinations were negative for undulant fever. Agglutinin titres were positive 1:50 for typhoid O antigen, 1:250 for H antigen and 1:125 for paratyphoid B antigen. To clarify the picture simultaneous blood and sternal marrow cultures were done on 24 August; both of these were reported positive for streptococcus viridans on 30 August. That he was suffering from subacute bacterial endocarditis was now apparent and he was started on sulfonamide therapy. He left the hospital on 11 September '43 and treatment was continued at home. He subsequently developed all the classical evidences of the disease and died at home several months later. Autopsy was not performed.

Discussion:

This man with a mild hypochromic anemia, low grade fever, and mild aortic regurgitation was not at first thought likely to have subacute bacterial endocarditis, partly because of his age and partly because his symptoms were vague and he did not appear particularly ill. In retrospect however, the diagnosis is obvious enough. The frequency of subacute bacterial endocarditis in older age groups has been recently stressed.[77] Since both blood and marrow cultures were positive no superior diagnostic value can be attributed to marrow culture in this instance. However, interest in the investigation of cases of this type by bacteriological marrow culture perhaps led to earlier performance of cultures than might otherwise have occurred. Recovery of streptococcus viridans from the marrow is of academic interest in that such an occurrence has been but rarely reported. Bock[21] records recovery of streptococci from the marrow in six cases of subacute bacterial endocarditis. In five of these it was also present in a simultaneous venous blood culture; in one it was absent in the venous but present in the arterial blood. Carnot et al.[31] recovered streptococcus viridans from both marrow and blood in one case.

Case No. 6. Pernicious Anemia.

S. M., a farmer, age 29, was first admitted to Winnipeg General Hospital on 4 February '42 complaining of numbness of the left hand for six months, (since August '41) and blurring of vision for one week. Family history was negative except that his mother had hypertension. Past history was negative. Physical examination revealed some dilatation of the right pupil and left

facial weakness of supranuclear type. Visual fields, x-rays of skull and cerebrospinal fluid examination were normal. During the ten days of hospitalization he had a temperature of 99.2 on five occasions. He was discharged with a tentative diagnosis of intracranial neoplasm.

He was readmitted on 14 March '44 with a history of occipital headaches and blurring of vision of right eye since January '42. He still complained of numbness in the left arm. He had been vomiting after meals for a week prior to admission. Examination revealed a pale feverish man of 31 with a temperature of 102, pulse of 90 and normal respirations. The right optic disc showed atrophy and the left facial weakness was again noted. Remainder of physical examination was negative.

Laboratory examinations were as follows: Blood: hemoglobin, 34%,(5.1 gm. per 100 cc.); red blood cells, 1,880,000; color index,0.97; white blood cells, 3,650; differential: mature polymorphonuclears, 15%; young polymorphonuclears, 4%; lymphocytes, 74%; monocytes, 3%; immature cells, 2%; degenerated cells,2%. The blood smear showed many microcytes,(average cell diameter 7.2u), anisocytosis and poikilocytosis. Price-Jones curve showed a microcytic distribution. This blood is shown in Fig. 7. In Fig. 8 is shown blood from a typical macrocytic pernicious anemia (average cell diameter, 8.2 u) for comparison.

X-rays of the skull, chest and lumbar spine were negative. Intravenous pyelogram and barium enema showed no abnormality. Spinal fluid examination was normal. Stools were negative for occult blood.

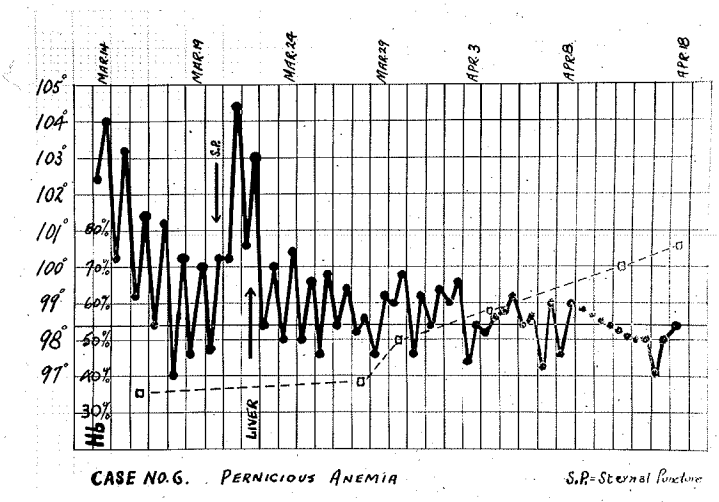

Fig. 6.

The clinical picture was thus (1) fever (see Fig. 6), unexplained microcytic anemia with leucopenia and relative lymphocytosis and (2) right optic atrophy and left facial weakness. In an attempt to clarify the diagnosis a simultaneous blood and marrow culture was done and a marrow smear was examined. The latter revealed a typical megaloblastic picture and a diagnosis of pernicious anemia was made. Bone marrow from a case of pernicious anemia is shown in Fig. 9. Both cultures were negative. To confirm the diagnosis the icterus index was determined and found to be 11, and gastric analysis showed achlorhydria after histamine. Parenteral liver therapy was instituted and a reticulocyte response of 45% was achieved on the third day. The resulting improvement in the anemia and subsidence of the fever are shown in Fig. 6.

Discussion:

The clinical features and the hematological findings in this case are very unusual, yet they have been described in a

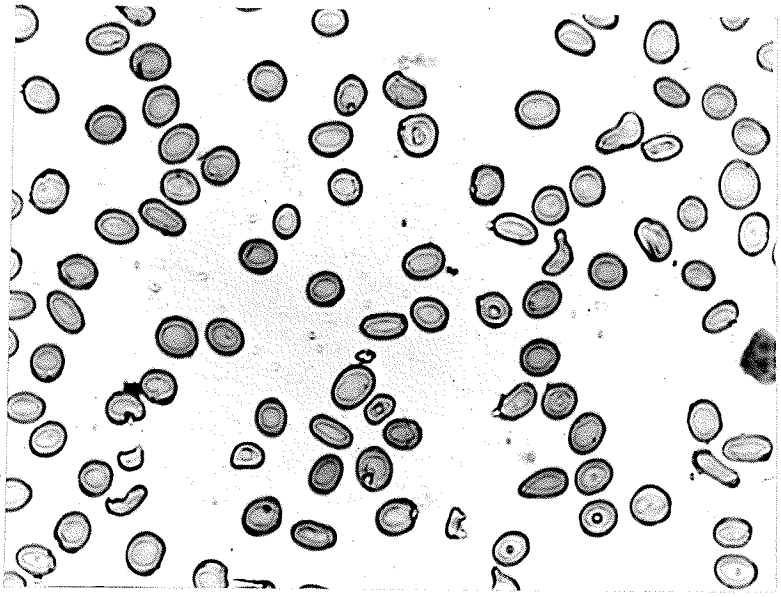

Fig. 7. Smear of peripheral blood from case 6. (x 600)
Average cell diameter 7.2 u.

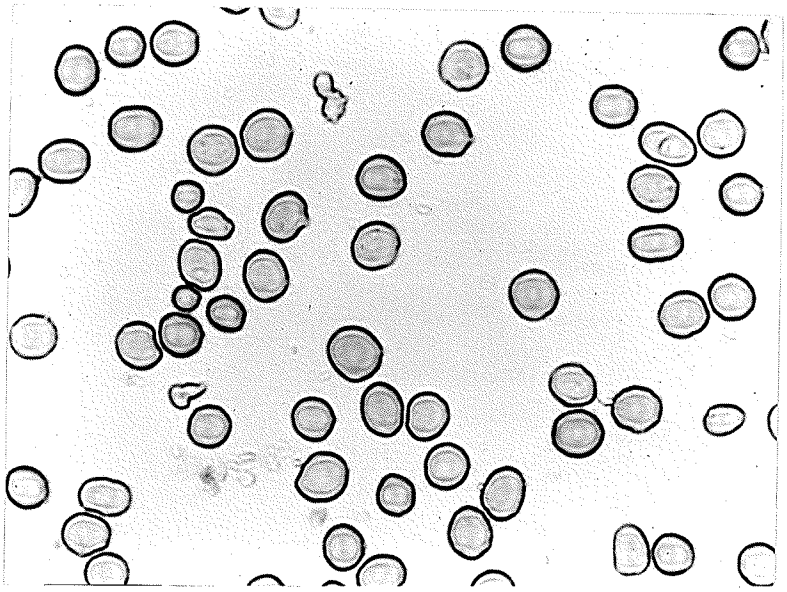

Fig. 8. Smear from typical pernicious anemia showing macrocytosis.

Average cell diameter 8.2 u. (x 600)

few cases.

Clinically, one is struck by the youthfulness of the patient, for pernicious anemia is rare under the age of 30, and by the unusual presenting symptoms. However, it has been pointed out by Kampmeier and Jones[78] that although optic atrophy in pernicious anemia is rare, it still may be the presenting symptom. It is compared to combined degeneration of the cord which may make itself manifest before clinical symptoms referable to the anemia itself. Cranial nerve lesions in pernicious anemia are very rare but do occur.[53] Fever in pernicious anemia, well illustrated in this case, has already been referred to. (page 3)

The peripheral blood picture is as atypical as the clinical picture, although some features such as neutropenia, relative lymphocytosis, marked anisocytosis and poikilocytosis are characteristic of pernicious anemia. The microcytosis and hypochromia are of course, very unusual. However, Wintrobe[58] describes five cases of pernicious anemia in which the mean corpuscular volume was normal or low, and in some of which hypochromia was present. Propp and Schwind record one case in which the color index was 0.80 and the mean corpuscular volume only 75 micra. That this constitutes a hypochromic microcytic anemia is readily seen by reference to Haden's [79] models showing the relationship between cell diameter and mean corpuscular volume.

This case thus presents an excellent example of the value of sternal puncture in the diagnosis of an obscure anemia associated with fever. It is possible that liver therapy might have been tried empirically but it is apparent that at the time he was seen that an accurate scientific diagnosis could not have been

made by any means other than marrow examination.

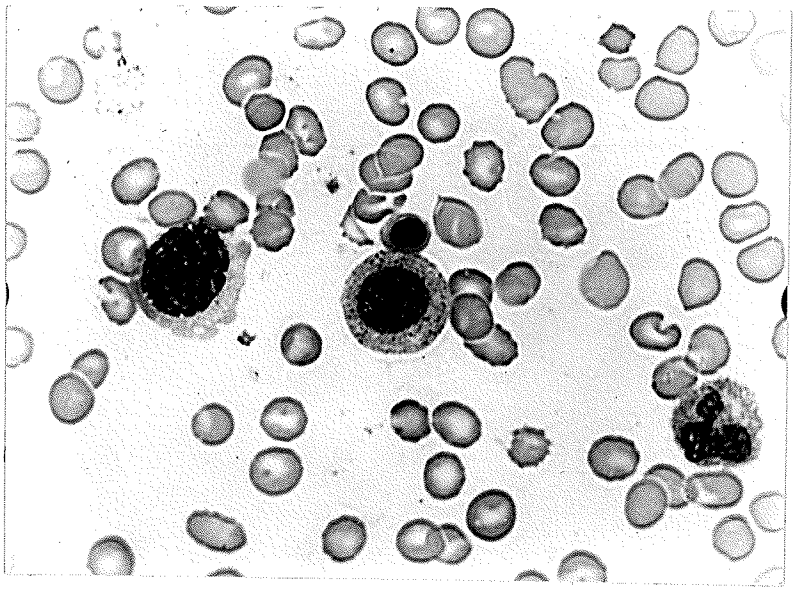

Fig. 9. Bone Marrow in Pernicious Anemia (x 700)
Two megaloblasts are seen.

Case No. 7. Aleukemic Myelogenous Leukemia.

S.K., a male, age 54, was admitted to Winnipeg General Hospital on 17 January '44 complaining of feverishness, excessive sweating and pains in the legs and arms for the past two months (since November '43). Physical examination was entirely negative. While in hospital he was observed to have a fever from 99 to 100 as shown in the first portion of the fever curve in Fig. 10.

Laboratory studies were as follows: Blood: hemoglobin, 93% (14.1 gm. per 100 cc.); red blood cells, 4,640,000; color index, 1.0; white blood cells, 6,900; differential: mature polymorphonuclears,42%; young polymorphonuclears, 31%; lymphocytes, 26%; and monocytes, 1%. Sedimentation rate was 30 mm. in 200mm. tube in one hour. Agglutinations for undulant fever and enteric group

were negative. X-rays of teeth, spine, pelvis and chest were non-contributory. The urine and prostatic fluid were normal. He was considered to have an obscure infection and was discharged on 9 February '44.

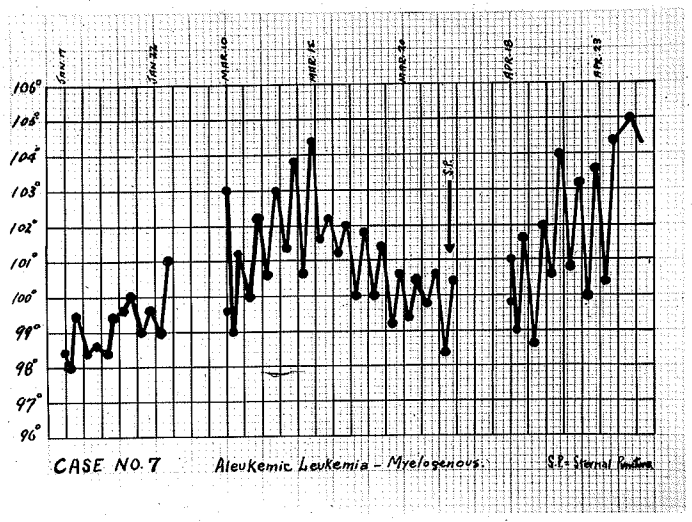

Fig. 10.

He remained in bed at home for a month but as his condition was gradually becoming worse he re-entered hospital on 10 March '44. He was now obviously quite ill and he was extremely pale. Apart from pallor and fever, physical findings were still negative except for multiple retinal hemorrhages. Examination of the blood now revealed: hemoglobin, 20% (3.01 gm. per 100cc.); red blood cells, 1,100,000; white blood cells, 4,100; differential: mature polymorphonuclears, 2%; young polymorphonuclears, 5%; lymphocytes, 60%; monocytes, 3%; degenerated cells, 16% and immature cells (? lymphoblasts) 12%. The diagnosis appeared to be either an aplastic anemia or a leukemia. A simultaneous blood and marrow culture was taken and a marrow smear examined. The latter showed

a picture of myelogenous leukemia; the cultures were both negative.

The subsequent course was progressively downhill and he died 3 May '44 without ever showing a leucocytosis. At post-mortem leukemic infiltration of the liver, kidney, spleen and bone marrow was found.

Discussion:

This case illustrates the manner in which the onset of a disease of the hemopoietic system may simulate an infection. It also demonstrates the usefulness of sternal puncture in the diagnosis of aleukemic leukemia.

Summary.

1. Fever is a common manifestation of diseases of the hemopoietic system as well as of infections. Clinical differentiation in many cases of obscure pyrexia is difficult or impossible because of features common to both. The ordinary laboratory procedures frequently fail in the elucidation of this diagnostic problem.

2. Bacteriological culture of bone marrow has been shown to yield more frequent positive results than venous blood culture. It is particularly useful in typhoid, subacute bacterial endocarditis and undulant fever which are the diseases which most often simulate hemopoietic disorders.

3. Cytological examination of bone marrow has proved to be of diagnostic value in many disorders of the hemopoietic system in which changes in the peripheral blood do not permit of definite conclusions.

4. A technique permitting of combined bacteriological, cytological and parasitological examination of bone marrow is

described.

5. Thirty cases in which marrow examinations were done are presented. The superiority of marrow culture over venous culture has been shown in two cases. (Dysentery due to B. alkaligenes and typhoid fever). Superiority of marrow examination over peripheral blood examination has been shown in one unusual case of pernicious anemia and in one case of aleukemic leukemia. A number of organisms, which have seldom been recovered in this manner, have been isolated by marrow culture.

Conclusion.

In the differential diagnosis of pyrexias of obscure origin, combined bacteriological and cytological examination is of value. Further investigation of its usefulness is warranted.

Acknowledgments.

Dr. D. Nicholson initially suggested investigation of the value of sternal marrow culture in infections. Without his interest and advice this study would not have been initiated nor pursued.

Mrs. Eira Charles Friesen did the bacteriology and Dr. J.M. Lederman identified the specific myelograms in cases 6 and 7.

Many physicians kindly permitted the use of their patients for these examinations.

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