THE HISTOPATHOLOGY OF LESIONS IN THE BONE MARROW OF PATIENTS HAVING ACTIVE BRUCELLOSIS

By R. Dorothy Sundberg, Ph.D., and Wesley W. Spink, M.D.

Although it has been known for many years that brucella may invade the bone marrow, there is a paucity of precise information concerning the pathological changes that take place in this organ as a result of brucellosis. The available descriptions have resulted from a study of human material obtained at autopsy and investigations of tissues from experimentally infected animals. The present study of the lesions in the bone marrow is based upon aspirations of the sternal marrow from patients having active brucellosis which was proved bacteriologically in 8 of 9 cases. The cases described appear to be the first in which a detailed study of the bone marrow obtained from living human subjects with brucellosis has been made. These investigations constitute part of a general program aimed at clarifying the pathogenesis of human brucellosis and, also, have resulted from attempts to add further aid in the diagnosis of chronic brucellosis.

REVIEW OF THE LITERATURE

Invasion of the human bone marrow by Br. melitensis was recorded by Eyre in 1908. While the marrow appeared normal grossly, more detailed study showed numerous nucleated red cells, giant cells, mononuclear cells, and lymphoid cells with a decrease in the number of myeloid cells. Smith and Fabian and Fabian described inflammatory nodular foci in several organs of guinea pigs infected with Br. abortus, and Fabian further described and illustrated the manner in which the lesions in the marrow might precipitate the destructive and proliferative lesions in bone. Although, according to Sharp, several reports have described the osseous lesions in human brucellosis, remarkably few descriptions of the marrow pathology have appeared. Epithelioid nodules in human bone marrow were first described in 1932 by Wohlwill in material obtained at autopsy from a case of brucellosis. In 1935, Wegener reported the presence of a small number of focal accumulations of epithelioid cells with early necrosis but with no decrease in the number of megakaryocytes. Neither Wohlwill nor Wegener recorded the finding of any giant cells in the marrow other than megakaryocytes. Sprunt and McBryde, in 1936, described a fatal case of brucellosis in a 4 year old child in whom femoral, vertebral, and costal bone marrow had been replaced by atypical mononuclear cells, but granulomatous lesions were not encountered. In 1939, Rabson described changes at the sites of fractures in the humerus and radius in a fatal case of brucellosis. The lesions, which were described as "similar to those in the liver and spleen," contained large confluent areas of fibrinoid necrosis, phagocytes, giant cells with lobated solid nuclei believed to be megakaryocytes, and non-
necrotic periarteriolar granulomas formed of epithelioid giant cells and lymphocytes. In a provocative summary of the information at hand up to 1943, Meyer suggested that the continuous bacteremia might be maintained by focal lesions in the bone marrow and stressed the need for studying the various stages of the disease as reflected in the marrow and other organs.

MATERIAL AND METHODS

The sternal bone marrow of 9 patients with active brucellosis was studied. Eight of the 9 patients had a demonstrable bacteremia due to Br. abortus. The hematopoietic activity of the marrow was estimated by correlating the percentage of nucleated marrow cells in heparinized marrow with the histologic structure of fixed marrow particles. In each instance, the morphologic alterations in the marrow were compared with those of the peripheral blood. In addition, samples of aspirated marrow from 7 patients were cultured for brucella. Biopsies of the liver were performed in 5 of the 9 subjects, the results of which will be detailed elsewhere.10

Technic of Bone Marrow Aspiration and Examination of Material. The technic used was a modification of the methods described by Schleicher and Sharp11 and Limarzi.12 The details have been presented elsewhere.13 Using a Klima-Schleicher needle, approximately 1 cc. of marrow was aspirated from the marrow cavity of the body of the sternum at the level of the second intercostal space. The material was immediately heparinized, and, as soon as possible, the fluid was poured out on a clean glass plate. If particulate marrow was present, approximately one-half of the particles and fluid were transferred to a Wintrobe hematocrit tube. The remaining particles were fixed in Zenker's fluid and then prepared in the same way as is routine histological material except that the exposure to each medium was very short, and sections were cut at 5 or less microns in thickness. The fluid portion was centrifuged, and readings corresponding to the height of the various strata were taken from the Wintrobe tube. Four main layers (fat, plasma, myeloid-erythroid, and erythrocytes) were present. Smears were made from a mixture of the myeloid-erythroid layer and the plasma. The smears were stained with Wright's stain and with the May-Grunwald-Giemsa combination. The sections were stained routinely with hematoxylin and eosin. Differential counts on marrow cells were made. The number of cells counted varied from 500 to 1,500 depending upon the agreement of the differential counts with the approximations, made from a comprehensive review under low and high power, of the various cell types in six to eight slides. Megakaryocytes and their precursors were enumerated in the terminal 18 x 18 sq. mm. of the smears.14 In relatively normal marrows, cells of the megakaryocytic series have been found to vary from 50 to 75 in this area. The latter figures give only a relative estimate of the total number of megakaryocytes; the myeloid-erythroid volume must be considered in order that the degree of absolute alteration in the numbers of megakaryocytes may be approximated.

The essential clinical data and pertinent findings in the 9 cases are presented in table 1.

### Table 1.—Summary of Data on 9 Patients with Active Brucellosis

<table>
<thead>
<tr>
<th>Case, age, and sex</th>
<th>Duration of illness prior to marrow aspiration</th>
<th>Blood culture</th>
<th>Bone marrow culture</th>
<th>Maximum titer of aggl.</th>
<th>Histology of marrow</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M. M., 66, female</td>
<td>3 months</td>
<td>Br. abortus</td>
<td>Sterile</td>
<td>1 to 2,560</td>
<td>Granuloma</td>
<td>Biopsy of liver—granuloma</td>
</tr>
<tr>
<td>2. A. S., 47, male</td>
<td>6 weeks</td>
<td>Br. abortus</td>
<td>Sterile</td>
<td>1 to 5,120</td>
<td>Granuloma</td>
<td>Biopsy of liver—granuloma, fat infiltration and low-grade hepatitis</td>
</tr>
<tr>
<td>3. J. J., 41, male</td>
<td>3 months</td>
<td>Br. abortus</td>
<td>Sterile</td>
<td>1 to 640</td>
<td>Granuloma</td>
<td>Developed subacute bacterial endocarditis with involvement of aortic valve due to Br. abortus</td>
</tr>
<tr>
<td>4. K. E., 33, male</td>
<td>21 years</td>
<td>Br. abortus</td>
<td>Br. abortus</td>
<td>1 to 5,120</td>
<td>Granuloma</td>
<td>Development of subacute bacterial endocarditis with involvement of aortic valve due to Br. abortus</td>
</tr>
<tr>
<td>5. L. N., 29, male</td>
<td>11 months</td>
<td>Br. abortus</td>
<td>Sterile</td>
<td>1 to 2,560</td>
<td>No particles for section</td>
<td>Biopsy of liver—granuloma</td>
</tr>
<tr>
<td>6. E. S., 55, female</td>
<td>2 years</td>
<td>Sterile</td>
<td>Sterile</td>
<td>1 to 640</td>
<td>No abnormality</td>
<td>Biopsy of liver—hepatitis and granuloma</td>
</tr>
<tr>
<td>7. F. H., 28, female</td>
<td>13 months</td>
<td>Br. abortus</td>
<td>Sterile</td>
<td>1 to 640</td>
<td>No abnormality</td>
<td>Biopsy of liver and sections of spleen following splenectomy—granulomas in both</td>
</tr>
<tr>
<td>8. W. M., 23, male</td>
<td>20 months</td>
<td>Br. abortus</td>
<td>Br. abortus</td>
<td>1 to 1,280</td>
<td>No particles for section</td>
<td></td>
</tr>
<tr>
<td>9. E. D., 24, male</td>
<td>1 month</td>
<td>Br. abortus</td>
<td>Br. abortus</td>
<td>1 to 1,280</td>
<td>No particles for section</td>
<td></td>
</tr>
</tbody>
</table>

**PATHOLOGICAL FINDINGS IN STECNAL MARROW**

Although aspiration of the sternal bone marrow was performed in 9 cases of brucellosis, only 4 cases showed granulomatous lesions. The histopathological changes in the marrow particles were similar in these 4 cases. Because of the large size of the particles of marrow recovered from case 2, in contrast to the smaller particles in the other 3 positive cases, and because there was a greater variability manifested in the lesions of case 2, the descriptions and illustrations deal largely with the material from this case.

Case 1 is of particular interest because the aspirated particles of marrow revealed the presence of a nonspecific granuloma, and this finding prompted further laboratory studies which led to a diagnosis of active brucellosis in a patient with a very atypical clinical history. The recognition of this granulomatous lesion was of diagnostic value, and following this observation, the marrows in other proved cases of brucellosis were studied in order to define more accurately the histopathology of the lesion.

**CASE 1**

*Essential Clinical Data.* M. M., U. H. no. 766805, a 66 year old white widowed female had an episode of pain in the left lower quadrant about six months prior to admission to the hospital. There was no
Bone Marrow Pathology. Several large clumps of cells were visible in the smears of the marrow (fig. 1). These cells had none of the morphological characteristics of tumor cells, but their presence in aggregates could only be clarified by a study of the material in sections. Such preparations showed multiple nodular accumulations and fused masses of large-bodied cells with indistinct cytoplasmic boundaries, vaguely granular and eosinophilic cytoplasm, and pale nuclei. The hyperchromatism and pleomorphism of tumor cells were lacking. Mitoses, though present, were normal in appearance and not numerous. In some areas, these masses of cells were surrounded by plasma cells and eosinophils.

The lesions in case 1 showed much less variability than those in case 2. They consisted primarily of almost sheet-like aggregates of epithelioid cells and variable
numbers of lymphocytes (fig. 2) with only very occasional giant cells. In one section, this epithelioid type of lesion replaced the normal marrow between and around 14 fat cells. The epithelioid cells which spread rather diffusely through what Wohlwill referred to as the partitions between the fat cells are comparable to those illustrated by him in his figure 4 and to those in the present case as shown in figure 2.
I2. BONE MARROW OF PATIENTS HAVING ACTIVE BRUCELLOSIS

CASE 2.

Essential Clinical Data. A. S., U. H. no. 768094, a 47 year old white married male confectioner entered the hospital on June 22, 1946, with the complaints of fever, night sweats, weakness, nervousness, headaches, and anorexia of one month's duration. When seen shortly after the onset of his illness by a physician, a diagnosis of "summer flu" was made, and the patient was treated unsuccessfully with penicillin. The probable source of his infection was the ingestion of raw milk obtained from a herd of cattle having Bang's disease. On entry to the hospital, he appeared well developed and slightly obese. Temperature, 101.6°F; pulse, 118. The only abnormal physical finding was a slightly tender liver that was palpated 4 cm. below the right costal margin in the midclavicular line. The spleen was not palpated. The initial laboratory findings were as follows: normal urine analysis; hemoglobin, 13.9 grams; leukocytes, 4,100, with 49 per cent polymorphonuclear neutrophils, 39 per cent lymphocytes, 6 per cent monocytes, 5 per cent polymorphonuclear eosinophils, and 1 per cent basophils. The erythrocyte sedimentation rate (Westergren) was 68 mm. in one hour. Blood serum revealed agglutinins for Br. abortus present in a titer of 1 to 5,120. A culture of venous blood showed Br. abortus. Intradermal tests with brucella antigen were positive. After the diagnosis of active brucellosis was established, he was treated with streptomycin beginning on the tenth hospital day. He was given 400 mg. of streptomycin base intramuscularly every three hours for a total of 2.8 Gm. Coincident with this therapy, he became more febrile, a diffuse macular rash developed, and purpura was apparent over the lower part of the legs. Treatment was discontinued on the nineteenth hospital day. The skin eruption subsided and he became afebrile. Sternal marrow was aspirated on the eighteenth day, and a biopsy of the liver performed on the twenty-sixth day. Because therapy with streptomycin was interrupted by the appearance of toxic manifestations, he was given sulfadiazine for a total of 87 Gm. in 15 days. His recovery was uneventful. He remained afebrile for four months, but following a mild exacerbation of fever, a specimen of blood revealed the presence of Br. abortus. His only complaint is weakness.

Bone Marrow Pathology. A large solid mass measuring 6 mm. in diameter was recovered. This is in contrast to the minute particles of marrow which were obtained from the remaining cases. Therefore, extensive studies were made with this material.

The lesions varied in size from that of a large nodule measuring approximately 0.5 mm. in diameter, as shown in figure 3, to minute lesions consisting of accumulations of only 3 to 4 epithelioid cells. It is of interest that the lesions described by Wohlwill in the spleen, lymph node, liver, and bone marrow from a fatal case of brucellosis were seen in the marrow sections of case 1. The peculiar hyaline "fibrinoid material" described and illustrated by Löffler and v. Albertini was also present. The increase in cells, interpreted by Rabson as megakaryocytes, was likewise recognized.

The largest granulomatous lesion encountered is presented in figure 3. The area shown was apparently the central and largest part of the lesion, but it was possible to trace this lesion through many serial sections in which the cellular aggregates, although fundamentally similar, showed marked variations in the relative percentages of cell types. The nodule consisted primarily of epithelioid and giant cells, but these elements were abundantly surrounded by lymphocytes and other mononuclear cells. Reticulendothelial cells with irregular cell outlines were also prominent. Some of the reticuloendothelial cells appeared to be free cells, while others were syncytially arranged.

The nucleus of the epithelioid cells was characterized primarily by a distinct nuclear membrane which outlined a nucleus containing such sparse chromatin that, except for one or two small and distinct nucleoli, the nucleus appeared almost
colorless. However, with higher magnifications fine chromatin particles could be seen near the nuclear membrane. The shape of the nucleus was more often irregular than round. Wohlwill described these nuclei as 'egg-shaped,' "hook-shaped," "curved or dumbbell," and "sausage-shaped." The cytoplasm was generally pale, but assumed a faint pink color with eosin. There was little cytoplasmic homogeneity; the cytoplasm usually appeared to be granular, fibrillar, or, occasionally, vacuolated. Lymphocytes and, more rarely, neutrophils were seen within the cytoplasm of these cells.

Present in the section of the granulomatous nodule in figure 3 was one very large foreign body type of giant cell, as shown in figure 4. As this cell was traced through consecutive serial sections, the centrally located nuclei disappeared, and numerous nuclei were seen along approximately one third of the peripheral portion of the cell. Later sections revealed a group of epithelioid cells, similar to those seen to the right of the foreign body giant cell in figure 3, in this location.

At 'a' in figure 3 can be seen a cell with three visible peripheral nuclei. This cell, in later sections, assumed the characteristic appearance of the Langhans' type of giant cell and was almost identical with that shown in figure 12. There was no remarkable and constant morphological difference between the nuclei of the giant cells and the nuclei of the epithelioid cells. Even the more rounded shape seen in the giant cell in figure 4 was not a constant finding.

Rare degenerating neutrophils and occasional eosinophils and plasma cells could be found in the central part of the lesion shown in figure 3. However, there was a definite, narrow circumferential zone of degenerating cells, neutrophils, eosinophils, lymphocytes, and plasma cells which contained fewer reticuloendothelial cells than did the central portion of the nodule. Peripheral to this zone, there was relatively unchanged hyperplastic marrow.

In tracing the granulomatous nodule in figure 3 through serial sections, the following changes in structure became apparent.

1. The number of whorls of epithelioid cells increased and then decreased. Occasionally small giant cells with three to five peripheral nuclei could be found.

2. Gradually the nodule containing prominent epithelioid cells became one which was primarily lymphocytic despite the prominent and characteristic reticuloendothelial stromal cells. At this point in the nodule, neutrophils and plasma cells were more numerous, and there was some evidence of cell destruction in the form of nuclear fragments. Changes which might be interpreted as proliferative were, however, also marked. Numerous mitoses in large cells, probably reticuloendothelial cells, were present. The peripheral zone was similar to that shown surrounding the nodule in figure 3. In addition, the capillary bed was either more developed or more functional in this region of the marrow; intra- and extravascular erythrocytes were numerous. The central portion of this variation of the lesion is shown in figure 1. Here can be noted the pale fibrillar cytoplasm containing the nuclei of epithelioid cells and the similarity of the pale staining nuclei of epithelioid cells. One prominent mitotic cell can be seen (a). Although lymphocytes predominated, neutrophils were fairly numerous. Note neutrophil
Bone Marrow of Patients Having Active Brucellosis

(b). Other neutrophils are present, and several degenerating cells are visible (c). Plasma cells can be readily identified (d).

![Image](image_url)

Fig. 3. Section of Marrow from Case 2 Showing Granulomatous Lesion 0.5 mm. in Diameter. (a) Epithelioid Giant Cell (X 150)

Fig. 4. Foreign Body Giant Cell from Lesion Shown in Figure 3 (X 800)

3. The primarily lymphocytic "nodule" just described appeared to continue into a more diffuse lesion which resembled the peripheral portion of the large giant cell nodule shown in figure 3. This portion of the lesion appeared, for several
sections, to spread peripherally among the fat cells. Then the cell aggregates coalesced to form a solid nodule (fig. 6) about one half the size of the original giant cell nodule. This nodular aggregate was traced until no definite nodule was...
visible, the fat cells being more prominent than the radiating portions of the lesion. This section of the lesion contained numerous neutrophils, many of which were degenerate. Remains of relatively normal marrow, such as normoblasts, were also apparent. Mononuclear cells with contorted nuclei, lymphocytes, occasional plasma cells, and very occasional eosinophils were also present. As can be observed in figure 6, reticulo-endothelial cells were present but less prominent. Neutrophils were more numerous here than in any other lesion in this case or in any other part of the marrow, but they were more diffusely distributed than those seen in miliary abscesses encountered in other conditions. Evidence of disintegration of many types of cells was present, but proliferative changes appeared more pronounced than in most chronic suppurative processes. Nothing which resembled the caseous necrosis of tuberculosis could be found. Although the degenerative processes are not pronounced, those present suggest that this portion of the lesion might be comparable to Wegener's nodules which showed early necrosis. Löffler and von Albertini have described a similar "inflammatory cellular infiltrate" thickest in the peripheral zone of the nodules occurring in organs other than the bone marrow. However, they did not emphasize the degenerative processes which were demonstrated peripherally in the present case.

Two other lesions from the marrow of case 2 are illustrated. One relatively large granulomatous nodule (figs. 7 and 8) shows approximately the same structure as that seen in figure 3. However, in the lesion shown in figures 7 and 8, a typical, large Langhans' type of giant cell containing phagocytosed neutrophils was present, and in the aggregate of epithelioid cells, a small giant cell with five peripheral nuclei could also be seen (fig. 8 [a]). This nodule had no conspicuous peripheral collar of neutrophils and cell fragments. In contrast, immediately adjacent to the lymphocytes was a zone containing large numbers of eosinophils and only a few neutrophils. Eosinophils could also be identified within the epithelioid portion of the nodule. An idea of the number of eosinophils surrounding the lesion can be gained by examining figure 8 (b). Both mature and immature eosinophils were numerous in this region, and several plasma cells could be found. The remaining cells in this vicinity are, for the most part, lymphocytes and pyknotic normoblasts. This lesion could only be traced through twelve consecutive serial sections, and no remarkable change in its cellular composition was apparent. Nevertheless, the large Langhans' giant cell, in tangential section, exhibited centrally located nuclei, and thus resembled a small, foreign-body giant cell. Particular attention was given to giant cells of all types because Löffler and von Albertini believed that masses of hyaline substance surrounded by peripheral nuclei represented not true giant cells but rather solid vascular capillaries. However, no evidence in support of their interpretation could be found in the present study.

The small lesion shown in figures 9 and 10 is of interest primarily because of its size. Its composition is similar to that of those shown in figures 3, 7, and 8. Eosinophils and plasma cells were prominent in the peripheral zone.

No lesions were encountered which consisted only of lymphocytes or of epi-
thelioid cells. However, often epithelioid cells could only be found among the lymphocytes if the lesions were traced through three or four serial sections. One lymphatic nodule as large as the nodule shown in figure 7 was present. Only a few epithelioid cells and no giant cells were present in this nodule, but peripheral eosinophilia was pronounced. This lymphatic nodule was of particular interest because of its great vascularity. Patent capillaries and intra- and extravascular erythrocytes were numerous. No clear-cut relationship between the epithelioid cells and the capillaries could be demonstrated.

Small lesions consisting primarily of fairly compact masses of centrally located epithelioid cells, the nuclei of which were concentrically located, were also seen.

**Fig. 7. Section of Marrow from Case 2 Showing Granulomatous Lesion Containing Langhans' Giant Cell (X 250)**

These lesions were very similar to the "tuberculoid nodules" in the spleen as illustrated by Löffler and von Albertini in their figure 4. Peripherally, the epithelioid masses were surrounded by lymphocytes and eosinophils.

One feature seen in the sections of the marrow from case 2 was of great interest and may be of importance insofar as the interpretation of progressive changes is concerned. The mass of tissue available for preparing fixed sections was relatively large. A comparison of the number of megakaryocytes in smears and in the sections indicated that partial coagulation of the particles of marrow must have occurred. In almost any section of the marrow a relatively smooth eosinophilic substance could be seen (fig. 11). This material did not resemble the fibrin needles and strands which had been observed in other clotted marrows, nor was it identical with true collagen. Cells of all types could be seen trapped in its interstices. One mass of this substance was almost as large as the nodule shown in figure 3.
Otherwise the substance was distributed rather diffusely. Occasionally it could be found at the periphery of the granulomatous nodules, often it was perivascular. Unfortunately, this substance was not prominent in the sections available for staining with azocarmine. In structure, however, this hyaline substance appeared completely similar to the fibrinoid material described and illustrated by Lößler and von Albertini. Similar material has been seen in the lymph nodes from cases of sarcoidosis. It appears entirely possible that this substance might be a manifestation of involvement of the marrow, perhaps comparable to the "hyaline fibrosis" in sarcoidosis described by Watson and his group, rather than a result of the coagulation of marrow particles which occurred prior to fixation. Figure 11

![Image](image_url)

**Fig. 8. Higher Magnification (× 450) of Figure 7. (a) Epithelioid Giant Cell. (b) Peripheral Zone Containing Numerous Eosinophils**

Illustrating this substance and the various cell types found in its meshes shows prominent megakaryocytes, one of which contains phagocytosed neutrophils.

**Attempts to Demonstrate Br. Abortus in Fixed Preparations of Marrow.** Sections were stained with Gram's and Giemsa's stains and also with Pappenheim's methyl green pyronin in an effort to demonstrate Br. abortus. Organisms could not be identified in either epithelioid or giant cells despite thorough examination of sections 3 to 5 microns in thickness. With methyl green pyronin, minute coccoid-like masses and rods could be seen in the peculiar fibrinoid material but no differentiation could be made between bacteria and the disintegrated cells.

**Special Stains for Fibers in the Nodules.** Silver and azocarmine stains were employed on serially sectioned material. The result of the staining with silver can be seen in figure 12. The fibers shown throughout the small nodule also stained blue with azocarmine, but their diameters were not so great as those seen in figure...
Some of the fibers were thicker than the great majority of reticular fibers. That these fibers may represent young collagenous fibers is not denied.\textsuperscript{17}

For comparison, and in order to avoid any possible technical variant, sections of the marrow from case 2 and sections of a lymph node containing the granulomatous nodules of sarcoidosis were stained simultaneously both with silver and...
FIG. 11  Section of marrow from case 2. Showing hyaline fibrinoid substance with numerous megakaryocytes (X 850)

FIG. 12. Section of marrow from case 2. Stained by Wilder's silver impregnation method showing reticular fibers in granulomatous nodule (X 850)

with azocarmine. No important differences in the character of the fibers within the nodules from the two conditions could be demonstrated. The argyrophil fibers present in the granulomatous nodule from the present case of brucellosis
were also compared with those in a nodule from brucellosis described by Wegener and with those shown for a sarcoid 'tubercle' by Watson et al. No apparent differences could be elicited.

The marrow obtained from case 2 has been described in detail because it is believed that the variability in the structure of the lesions in different portions of the marrow affords a partial explanation of some of the varying types of pathological changes which have been described in the literature. Here, in one lesion, could be seen giant cells of the Langhans' and foreign body types, epithelioid cells, reticulo-endothelial hyperplasia, accumulations of lymphocytes, evidence of cell destruction which might be termed partial necrosis, abscess-like accumulations of neutrophils, and variable numbers of predominantly peripheral plasma cells and eosinophils. The fact that these changes were present in one relatively large lesion does not, by any means, indicate that this is always the case, but it might explain the variable histopathology of single sections of either marrow particles or of other organs or tissues.

OTHER CASES

The remaining cases did not contribute any further basic information concerning the nature or genesis of the lesions of the marrow in patients with active brucellosis, and for that reason only the essential features will be summarized.

CASE 3

Essential Clinical Data. J. J., a 41 year old male truck driver was admitted to a local hospital on September 7, 1946. He was in good health until two months before admission, when he felt unusually fatigued. One month later, there was a sudden onset of shaking chills, fever, and sweats which recurred each evening thereafter. In addition, he had spells of dizziness, occipital headaches, and marked anorexia. On August 1, 1946, his blood serum had agglutinins for brucella present in a titer of 1 to 1,280. From October 1945 to March 1946, he had worked in a packing house handling freshly cut pork and rolling beef hides. On entry into the hospital, his temperature was 98°F, pulse 65. There were no abnormal findings on physical examination. His blood revealed a leukocyte count of 4,500 with 40 per cent polymorphonuclear neutrophils and 60 per cent lymphocytes; an erythrocyte sedimentation rate (Westergren) of 77 mm. in one hour; an agglutinin titer for brucella of 1 to 640; and Br. abortus isolated in pure culture. Intradermal tests with brucella antigens gave positive reactions. An aspiration of the sternal bone marrow was cultured for brucella, but no organisms were recovered. During his stay in the hospital, he had a febrile reaction for several days, reaching a maximum of 103 degrees. He was treated with a total of 12.6 grams of sulfadiazine over a period of 21 days. Following this therapy, his blood cultures remained sterile; he became afebrile; and he felt considerably improved.

Bone Marrow Pathology. Examination of marrow particles obtained by aspiration revealed only one small epithelioid nodule (fig. 13). This lesion was similar to the small epithelioid nodules seen in case 2.

CASE 4

This case is of particular interest because it represents a very serious complication of brucellosis.

Essential Clinical Data. K. E., U. H. no. 71570, a 33 year old white farmer entered the University Hospitals on October 11, 1946. Two years previously, he had had an episode of fever and weakness, and an agglutinin titer for brucella of 1 to 640. Following therapy with sulfonamides, he recovered completely within three months and was well until four months before entry. He then had a recurrence of fever, chilly sensations, weakness and sweats. On examination his temperature was 103°F, and pulse 100.
The outstanding findings were petechiae in both conjunctivae, evidence of aortic regurgitation, and splenomegaly. The laboratory data included a leukocyte count of 5,950 with 79 per cent polymorphonuclear neutrophils, 16 per cent lymphocytes, and 5 per cent monocytes; an erythrocyte sedimentation rate (Westergren) of 73 mm. in one hour; an agglutinin titer of 1 to 5,120 for brucella; Br. abortus in nine consecutive blood cultures; Br. abortus in a culture of urine; and Br. abortus in a culture of aspirated bone marrow. Intradermal tests with brucella antigen were negative. After the diagnosis of subacute bacterial endocarditis due to Br. abortus had been established, he was treated with a total of 118 grams of streptomycin over a period of 31 days. Coincident with this therapy, his temperature declined, but subsequent cultures of blood showed Br. abortus on two occasions.

Bone Marrow Pathology. The aspirated sternal bone marrow was hyperplastic, and accumulations of perivascular plasma cells and macrophages were prominent. One area, shown in figure 14, resembled portions of the various types of lesions encountered in case 2. It was not frankly epithelioid, but it did contain nuclei similar to those of epithelioid cells, and also numerous cells with contorted nuclei, lymphocytes, and a peripheral zone of increased numbers of eosinophils. These findings were considered representative of one type of change which may be encountered in brucellosis, but this lesion would have been extremely difficult to classify had it not been for extensive examination of material from the other cases.

In the remaining 5 cases, no lesions were demonstrated in the marrow. No particles for sectioning were obtained from the marrow of cases 5, 8 and 9. In case 6, the sections showed, for the most part, adipose tissue surrounded by erythrocytes, but in one part of the section a small area of red marrow was found containing one megakaryocyte. The fact that marrow was obtained only with difficulty in all but cases 1 and 4 should be noted. Only small quantities could be...
aspirated despite vigorous suction. It is possible that the size and the nature of the granulomatous lesions militate against a successful aspiration and that surgical biopsy might be desirable in cases from which no particulate marrow can be aspirated.

SUMMARY OF FINDINGS IN MARROW

In order to define more clearly and to correlate the findings in the marrow and the peripheral blood, complete examinations were carried out. A summary of these findings is presented in table 2.

The quantitative data obtained from the hematocrit readings indicate, when case 6 is excluded, fairly regular hyperplasia of the marrow. The normal myeloid-erythroid value in case 2 is the result of partial coagulation and the removal of a large mass for preparing sections. Examination of the sections showed the marrow to be hyperplastic. The normal myeloid-erythroid value in case 3 is apparently real since the sections revealed no hyperplasia. Cases 5 and 8 had myeloid-erythroid values in the upper range of normal variation.

The alterations in the cellular pattern of the sternal marrow of the 9 cases of brucellosis may be summarized as shown in table 3. A definitive picture of the histopathology of the bone marrow in human brucellosis can best be obtained by summarizing the data from the first 4 cases. This is presented in table 4.

SUMMARY OF FINDINGS IN PERIPHERAL BLOOD

The peripheral blood picture requires some clarification. Erythrocytes, in all cases, showed minimal alterations, and there were no significant variations in the
## Table 1: Correlation of Findings in Bone Marrow and Peripheral Blood

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Case Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulomatous lesions</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>N.S.</td>
<td>A</td>
<td>N.S.</td>
<td>N.S.</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cc. marrow</td>
<td>1.00</td>
<td>0.37</td>
<td>0.70</td>
<td>0.91</td>
<td>0.24</td>
<td>0.80</td>
<td>0.54</td>
<td>0.45</td>
<td>0.40</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Fat, per cent</td>
<td>3.0</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>3.0</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, per cent</td>
<td>47.0</td>
<td>56.0</td>
<td>51.0</td>
<td>55.0</td>
<td>50.0</td>
<td>56.0</td>
<td>50.0</td>
<td>40.0</td>
<td>41.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloid-erythroid, per cent</td>
<td>13.0</td>
<td>3.5</td>
<td>6.0</td>
<td>11.0</td>
<td>8.0</td>
<td>2.0</td>
<td>11.0</td>
<td>8.0</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes, per cent</td>
<td>37.0</td>
<td>30.5</td>
<td>38.0</td>
<td>32.0</td>
<td>40.0</td>
<td>41.0</td>
<td>37.0</td>
<td>51.0</td>
<td>44.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>1.5</td>
<td>0.5</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>1.5</td>
<td>0.0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Leukoblasts</td>
<td>1.5</td>
<td>1.0</td>
<td>6.0</td>
<td>3.0</td>
<td>0.5</td>
<td>0.5</td>
<td>2.5</td>
<td>5.0</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Promyelocytes</td>
<td>3.0</td>
<td>4.5</td>
<td>2.0</td>
<td>4.0</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>0.0</td>
<td>10.5</td>
<td>9.5</td>
<td>13.0</td>
<td>4.5</td>
<td>5.5</td>
<td>6.0</td>
<td>6.0</td>
<td>9.5</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Bandforms</td>
<td>7.0</td>
<td>17.5</td>
<td>10.0</td>
<td>21.5</td>
<td>11.0</td>
<td>6.0</td>
<td>6.5</td>
<td>8.5</td>
<td>9.0</td>
<td>15.0</td>
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<tr>
<td>Eosinophils</td>
<td>11.0</td>
<td>9.0</td>
<td>8.0</td>
<td>14.5</td>
<td>16.0</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>12.0</td>
<td>16.0</td>
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</tr>
<tr>
<td>Eosinophils</td>
<td>17.5</td>
<td>12.5</td>
<td>7.0</td>
<td>12.0</td>
<td>23.0</td>
<td>15.0</td>
<td>19.5</td>
<td>10.5</td>
<td>9.0</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Normoblasts</td>
<td>Pronormoblasts</td>
<td>0.5</td>
<td>1.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Basophilia</td>
<td>1.0</td>
<td>1.5</td>
<td>3.0</td>
<td>2.0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Polychromat</td>
<td>39.0</td>
<td>23.5</td>
<td>36.0</td>
<td>16.0</td>
<td>15.0</td>
<td>1.5</td>
<td>15.0</td>
<td>40.0</td>
<td>30.0</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Orthochromat</td>
<td>3.0</td>
<td>0.0</td>
<td>3.0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.5</td>
<td>9.0</td>
<td>5.0</td>
<td>7.5</td>
<td>6.0</td>
<td>49.0</td>
<td>7.5</td>
<td>4.0</td>
<td>6.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Plasma cells</td>
<td>3.0</td>
<td>3.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>3.0</td>
<td>0.5</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.5</td>
<td>1.0</td>
<td>0.3</td>
<td>0.5</td>
<td>4.0</td>
<td>0.5</td>
<td>4.0</td>
<td>0.5</td>
<td>1.5</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Histiocytes and macrophages</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Epithelioid cells</td>
<td>P</td>
<td>P</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megakaryocytes per 18 x 18 sq. mm</td>
<td>820</td>
<td>80</td>
<td>90</td>
<td>86</td>
<td>112</td>
<td>0</td>
<td>278</td>
<td>40</td>
<td>358</td>
<td>50-75</td>
<td></td>
</tr>
</tbody>
</table>

### Blood

- **Neutrophils**: 53
- **Eosinophils**: 1
- **Basophils**: 0
- **Lymphocytes**: 38
- **Monocytes**: 8
- **Histiocytes**: A

---

**No.** = bone marrow differential—normal values

**A** = absent

**N.S.** = no sectionable particles aspirated

**V** = variable

**+** = increased

**++** = greatly increased

**+++** = very greatly increased

**-=** = no striking increase

**P** = present

*Note: After Scott,* corrected to nearest 0.5 per cent and for terminology of this report.*

*Schilling's hemogram.*
number of platelets as estimated from direct smears. Total leukocyte counts were normal or slightly below normal, but differential counts revealed the presence of

### Table 3.—Cellular Pattern in Bone Marrow

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Relative decrease in cells of the neutrophil leukocyte series</th>
<th>Relative and absolute marrow eosinophilia</th>
<th>Relative and absolute increase in developing erythrocytes</th>
<th>Relative and absolute increase in plasma cells</th>
<th>Relative and absolute increase in monocytes</th>
<th>Relative and absolute increase in histiocytes and macrophages</th>
<th>Presence of epithelioid cells in smears</th>
<th>Relative and absolute increase in megakaryocytes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 5, 6, 7, 8, 9</td>
<td>1, 5, 7, 9</td>
<td>1, 3, 7</td>
<td>1, 3, 8, 9</td>
<td>1, 2, 4, 8</td>
<td>1, 2, 5, 7, 9</td>
<td>1, 3, 4, 7</td>
<td>1, 2</td>
<td>All but 6 and 8</td>
</tr>
</tbody>
</table>

*Case 6: significance questionable. Case 8: probably real decrease, degenerate forms numerous.

In all cases megakaryocytes were phagocytic, and many extremely large forms were present.

### Table 4.—Summary of Histopathological Lesions in Bone Marrow of 4 Cases of Bruceiosis

<table>
<thead>
<tr>
<th>Case Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of marrow replaced by lesion</td>
<td>Very large</td>
<td>Very large</td>
<td>Small</td>
<td>Very small</td>
</tr>
<tr>
<td>Character of granulomatous lesions</td>
<td>Most diffuse. Some nodular &amp; small</td>
<td>Most nodular of variable size. Boundaries indistinct in many</td>
<td>Nodular &amp; small</td>
<td>Nodular &amp; small</td>
</tr>
<tr>
<td>Cell types comprising lesions:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelioid</td>
<td>Very numerous</td>
<td>Very numerous</td>
<td>Present</td>
<td>Questionable</td>
</tr>
<tr>
<td>Giant cells</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Langhans' Epithelioid Foreign body</td>
<td>Not found</td>
<td>Rare</td>
<td>More numerous</td>
<td>Rare</td>
</tr>
<tr>
<td>Lymphocytes &amp; other mononuclear cells</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Plasma cells &amp; eosinophils</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Evidence of cell disintegration &amp; presence of neutrophils:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>Slight</td>
<td>Slight</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Peripheral</td>
<td>Not marked</td>
<td>Marked</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Peripheral zone of increased eosinophils &amp; plasma cells</td>
<td>Present</td>
<td>Marked</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Fibrinoid material</td>
<td>Absent</td>
<td>Present (?)</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Megakaryocytes are not included under giant cells because the increase in megakaryocytes apparent in all these cases is generalized. Although many of the megakaryocytes are very large, no completely reliable criterion for distinguishing them from ordinary megakaryocytes could be found.

fairly regular relative lymphocytoses in combination with percentages of monocytes in or above the upper range of normal variation. Histiocytes were recognized in the blood of case 8. It is of interest that cultures of both the marrow and the
blood in this patient were positive for Br. abortus. In case 4, despite the persistent bacteremia and the presence of a bacterial endocarditis, no completely characteristic histiocytes could be found, but many large monocytes containing numerous vacuoles were present.

The lymphocytic reaction in the peripheral blood is extremely variable. It has been recognized as one which may be very similar to that of infectious mononucleosis.\textsuperscript{19} The cases included in this report showed evidence of relative lymphocytosis. All cases revealed the presence of occasional functionally altered or leukocytoïd lymphocytes,\textsuperscript{29} but only in cases 1 and 4 were these alterations present in the majority of the lymphocytes.

The lack of blood eosinophilia is of interest in view of the eosinophilia in the marrow. Only in case 2 were eosinophils increased in the blood. In this case, eosinophil metamyelocytes were found, which, in the absence of marked eosinophilia, is an extremely unusual finding.

**COMPARISON OF LESIONS IN THE BONE MARROW WITH LESIONS IN OTHER TISSUES AND ORGANS AS REPORTED IN THE LITERATURE**

Although the granulomatous lesions in the bone marrow of proved cases of brucellosis have received but little attention, the granulomas as they occur in other organs of experimentally infected animals and of human beings have been described by many investigators. Schroeder\textsuperscript{21} first described the gross pathological changes in guinea pigs. In 1912, Smith and Fabyan\textsuperscript{2} and Fabyan\textsuperscript{1} presented the histopathological changes in the organs of guinea pigs inoculated with Br. abortus.

The lesions of human brucellosis described in the literature seem fundamentally similar in nature. Actual differences in type and degree of tissue reaction apparently occur, but most of the variability in the reported findings is probably the result of dissimilar nomenclature. In the lymphoid tissue, the lesions are found both within and outside of lymph nodules. The granulomas have been shown to vary in size from microscopic lesions to those 6 mm. in diameter.\textsuperscript{22} That the lesions are not always nodular is clearly evident from almost any of the descriptions in the literature as well as from the appearance of the lesions in the marrow of the present report. Wohlwill\textsuperscript{5} stressed the radiating character of the lesion in the marrow, and Rösle\textsuperscript{23} emphasized the lack of the nodular form in other organs.

That epithelioid cells are one of the most characteristic components of the cellular aggregates in any of the organs has been pointed out by Fabyan,\textsuperscript{4} Wohlwill,\textsuperscript{5} and Wegener.\textsuperscript{6} Gregersen and Lund\textsuperscript{24} claimed that the cells were not epithelioid cells, but simple fibroblasts. Smith\textsuperscript{25} stated that there "was a proliferation of a certain type of cell, which for convenience, is called endothelial." The cells have been described as reticulo-endothelial cells or as cells of the reticulo-endothelial system by many others.\textsuperscript{22, 5, 22, 3} Meyer\textsuperscript{9} believes the unquestioned precurors of the focal or diffuse lesions found in human brucellosis to be aggregates of monocytes, lymphocytes, and polyblasts.

The giant cells occurring in the lesions of brucellosis have been of interest since
the earlier studies with experimentally infected animals. In human lesions, Rabson found a few of the Langhans' type and others having the characteristics of epithelioid cells, but the most numerous giant cells were those suggestive of enlarged megakaryocytes. The foreign-body type and Langhans' giant cells have been reported by others. Löfler and von Albertini described formations similar to giant cells but believed them to be solid vascular capillaries.

Nodules showing central suppuration and, occasionally, necrosis have been described in material from infected animals. Suppuration has also been reported in human brucellosis. Destructive changes of the vertebrae, sometimes with suppuration, have been recorded. This has been observed in cases at the University of Minnesota Hospitals. However, no description of caseous necrosis in the granulomas of brucellosis has been found. Most authors are in agreement that when necrosis does occur in the central portions of the nodules, it is only partial. Löfler and von Albertini claimed that only pyknotic cell nuclei were found occasionally in the interior of the nodule, and Wohlwill described karyorrhectic disintegration of the large clear cells but could find no coherent necrosis.

The lymphoid reaction, interpreted by most authors as a chronic inflammatory change, is discussed repeatedly in the literature on brucellosis. The predominantly peripheral, but occasionally central, plasma cells were stressed by Löfler and von Albertini and by Wegener. Wohlwill emphasized the increase in eosinophils surrounding the nodule as a point of distinction from true tubercles, but he did not describe an increase in eosinophils surrounding the epithelioid cells in the bone marrow lesion.

A question of considerable practical importance is whether the granulomatous lesions or the tubercles of brucellosis may be distinguished from those of tuberculosis or of sarcoidosis. Askanazy described 2 cases of tuberculosis in which tubercles could be found in the bone marrow. He emphasized the fact that large reticular cells were increased at the periphery of the tubercles and stated that he could detect transitions between the reticulo-endothelial cells and the epithelioid cells. He also described an increase in eosinophils in the lymphocytic ring. In one of his cases, caseous necrosis was lacking. For this and other reasons, Stahel believed this case might have been sarcoidosis rather than tuberculosis.

Schleicher described 8 cases of miliary tuberculosis in which tubercles were found in sections made from aspirated sternal marrow. In all but 3 of these cases, the number of tubercles with caseation equaled or exceeded the number of tubercles with epithelioid cells and, in all the cases, caseated tubercles were identified. The epithelioid tubercles apparently may or may not contain giant cells.

Nickerson examined the bone marrows from 5 cases of sarcoidosis which came to autopsy. Lesions were found in the vertebral marrow in 3 cases, and in 1 case the femoral marrow revealed a few solitary lesions in an inactive marrow among groups of fat cells. There were sparse but usually conglomerate lesions.
BONE MARROW OF PATIENTS HAVING ACTIVE BRUCELLOSIS

present in a mass of hematopoietic tissue in the vertebral marrow. Each lesion was surrounded by a dense rim of lymphocytes and eosinophils. A minimal amount of collagen was present. Nickerson claimed that the giant cells of sarcoidosis differed from those of tuberculosis in that they showed moderate variation in size; they were usually much larger and contained many more nuclei, often as many as 25 to 30; and the nuclei were evenly distributed throughout the cell, seldom being arranged in the elliptical manner characteristic of tuberculous giant cells. Unlike tuberculosis, the lesions of sarcoidosis did not show the presence of necrosis.

Stahel32 recovered granulomatous lesions from the bone marrow of a case of miliary tuberculosis and of a case of sarcoidosis by means of sternal aspiration. The tubercle of miliary tuberculosis showed caseation necrosis and was surrounded by only a small wall of lymphocytes. Eosinophils were increased in the periphery of the nodule and in the uninvolved portions of the marrow. "Reticular plasma cells" as well as cells with long oval nuclei, believed to be reticular elements, were also increased. He was impressed by the minimal reaction of the tissue in the marrow surrounding the tubercles, but examination of his figure 1 suggests that this reaction was probably no less pronounced than that seen in the periphery of the granulomas of brucellosis. Stahel also emphasized the presence of marrow eosinophilia not accompanied by blood eosinophilia in tuberculosis. The nodules of Stahel's case of sarcoidosis lacked caseation necrosis but contained epithelioid cells, between which were a few cells with dark nuclei and rare giant cells containing up to ten nuclei. The periphery of the nodules contained thickly crowded epithelioid cells with long dark nuclei. These cells formed a sharp limit between normal marrow and the nodule, but eosinophils were markedly increased. The uninvolved marrow was hyperplastic and the megakaryocytes described as about normal. He particularly emphasized the lack of peripheral reaction in sarcoidosis in contrast to the definite though minor peripheral reaction manifested in tuberculosis. However, as Nickerson34 pointed out, the peripheral reaction in sarcoidosis may be extensive. Stahel32 also considered the relatively greater increase in eosinophils in sarcoidosis to be important, and it is interesting to note that this marrow eosinophilia was accompanied by 9 per cent eosinophils in the peripheral blood.

It is evident that it is difficult to differentiate miliary tuberculosis, sarcoidosis, and brucellosis as they occur in the bone marrow. It would appear that the distinction is particularly difficult between sarcoidosis and brucellosis. As a basis for further studies, the following comparative data on the bone marrow are presented.

1. Caseous necrosis is absent in the lesions of brucellosis, and it is obviously present in most of the tubercles encountered in miliary tuberculosis. (2) Both Langhans' and foreign-body giant cells were present in case 2 with proved brucellosis. This has not been described for either tuberculosis or sarcoidosis of the bone marrow. (3) No peripheral limit of crowded and elongated epithelioid cells as seen in Stahel's32 figure 3 of a sarcoid nodule was identifiable in the lesions of brucellosis. (4) The lesions of brucellosis are often surrounded peripherally by small numbers of degenerating marrow cells and neutrophils. This has not been described
for the lesions of tuberculosis or sarcoidosis, but one may very well question whether the lack of description can establish its absence. (5) Peripherally, the lesions of brucellosis are generally surrounded by a moderate increase in eosinophils and plasma cells. This is apparently minimal in tuberculosis, but the eosinophilia is pronounced in sarcoidosis. (6) The cases of brucellosis included in this report show marrow eosinophilia with little or no evidence of blood eosinophilia. Stahel's(32) report would indicate similar findings in miliary tuberculosis. (7) The increase in megakaryocytes in brucellosis may be of aid in distinguishing it from sarcoidosis, but data are incomplete. Neither Stahel nor Schleicher(33) discussed the megakaryocytes in miliary tuberculosis, but we have found megakaryocytosis in cases of miliary tuberculosis in which we were unable to demonstrate tubercles in sections of the marrow.

The possible etiological relationship between brucellosis and Hodgkin's disease has been reported.35, 36 Despite the many types of giant cells seen in the lesions of the marrow in the present report, none of the giant cells showed the characteristic morphology of the Sternberg or Dorothy Reed giant cell.

**COMMENT**

As stated previously, the present investigation is part of a general study to define the pathogenesis of brucellosis. It has been known for several years that brucella tend to localize in organs containing abundant reticulo-endothelial tissue, namely, the bone marrow, liver, spleen, and lymph nodes. Furthermore, the granuloma is a characteristic type of tissue reaction to the bacterial invasion. Since hypersensitivity of the tissues to a product or products of brucella is one of the outstanding features of active brucellosis, the question arises as to whether the granulomatous lesion is a manifestation of tissue hypersensitivity. It is of interest that 8 of the 9 patients in the present study possessed tissue sensitivity to products of the brucella as judged by the intradermal response to injected antigens. Although case 4, having subacute bacterial endocarditis, did not show evidence of such hypersensitivity, study of the bone marrow revealed the basic pattern of the granulomatous lesion. The failure of patients with subacute bacterial endocarditis due to Br. abortus to exhibit tissue hypersensitivity has been commented on in previous studies.37, 38 It would appear that hypersensitivity to brucella is not essential for the development of the granuloma. This is in accord with the conclusions of Rich(39) with respect to the genesis of granulomatous lesions caused by Mycobacterium tuberculosis.

The disease brucellosis is noted for its chronicity, and one might question whether the granuloma harbors viable brucella. It is of importance that in this study cultures of the bone marrow did not reveal the presence of brucella when simultaneous cultures of venous blood remained sterile. Similar studies, to be reported elsewhere,10, 40 revealed the presence of granulomatous lesions in the liver and spleen of living subjects, and in no instance were brucella recovered from these tissues. Although the problem of the pathogenesis of the granulomatous lesions is being studied further in experimental animals, as far as human brucellosis
BONE MARROW OF PATIENTS HAVING ACTIVE BRUCELLOSIS

is concerned this lesion represents a response of the tissue to brucella; it is not necessarily associated with tissue hypersensitivity to a product or products of brucella; and viable brucella cannot be consistently cultured from tissues with granuloma.

When this study was initiated, it was hoped that the histological pattern of the bone marrow in active brucellosis might be of some diagnostic aid. This hope has only been partially realized. The presence of granulomatous lesions in the marrow in combination with the alterations in the cellular pattern of the bone marrow and the peripheral lymphocytosis and mononcytosis strongly suggest the possibility of brucellosis. The alterations in the cellular pattern of the bone marrow are not considered to be specific at the present time; they are detailed in this report only because it is felt that further investigations might prove that this pattern is sufficiently consistent to be of some diagnostic value. If groups of epithelioid cells are present in the smears, it can be assumed that sections would contain granulomatous foci.

It is recognized that it is the chronic case of human brucellosis which affords the clinician the greatest difficulty in arriving at a correct diagnosis. In such a patient, the symptomatology is frequently vague, and no abnormal signs are apparent. The finding of dermal sensitivity to brucella antigens and a low titer of agglutinins very often only serves to confuse the picture. It is in cases of this type that a study of the bone marrow might prove of extreme value. Certainly the recognition of the granulomatous lesions in the marrow could be considered confirmatory evidence of brucellosis. Finally, aspirated sternal marrow from suspected cases of active brucellosis should be cultured for viable brucella in a larger number of cases.

SUMMARY

1. Aspirated sternal bone marrow was studied in 9 patients with active brucellosis. Eight of the 9 patients had a demonstrable bacteremia due to Br. abortus.
2. Particles of marrow in 4 of the 9 patients revealed the presence of granulomatous lesions. In 1 case, the presence of a relatively large lesion permitted the study of the lesion in detail by means of serial sections. The alterations in the cellular pattern of the marrow and of the peripheral blood of the 9 patients were described.
3. The histopathology of the marrow in active brucellosis has been compared with the alterations in the other organs and tissues as reported in the literature. Comparisons have also been made between the granulomatous lesions in the marrow of brucellosis and those found in tuberculosis and sarcoidosis.
4. Aspirated sternal marrow for cultural and histological purposes merits further consideration as a diagnostic aid in active brucellosis.

We are indebted to Henry Morris for the photomicrographs.

REFERENCES

R. DOROTHY SUNDBERG AND WESLEY W. SPINK

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