LOCAL PROGRESSION WITH SPONTANEOUS REGRESSION OF TUBERCULOSIS IN THE BONE MARROW OF RABBITS, CORRELATED WITH A TRANSITORY ANEMIA AND LEUCOPENIA AFTER INTRAVENOUS INOCULATION.

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PLATES 8 TO 10.

(Received for publication, May 13, 1927.)

In following the blood of rabbits after an intravenous injection of 1 or 2 mg. of bovine tubercle bacilli, we have found that there develops a characteristic anemia combined with a fall of those white cells that normally arise in the bone marrow. In previous studies, Sabin, Doan and Cunningham (1) presented evidence indicating that the epithelioid cell and its derivative, the giant cell of the Langhans type, come from the monocyte of the tissues. Cunningham, Sabin, Sugiyama and Kindwall (2) then showed that the extension of a tubercular process in the tissues is reflected in the peripheral blood, which they interpreted to mean that from an advancing tubercular area with its massive increase in normal and abnormal monocytes there is a flooding of these cells into the blood stream. Moreover they demonstrated that the healing process involving the increase of lymphocytes around tubercular tissues is also reflected in the blood so that the ratio of monocytes to lymphocytes in the blood stream may be used as an index of the state of a tubercular process in the tissues.

About 2 weeks after an intravenous injection of bovine tubercle bacilli in doses of 1 mg., the percentage and the actual numbers of monocytes in the peripheral blood of rabbits rise. At the same time the monocytes show qualitative changes which involve some lessening of motility and the production of all the intermediate morphological stages between the normal monocyte and the epithelioid cell. The cytoplasmic changes consist in an increase in the number and a de-
crease in the size of bodies, probably vacuoles, that stain characteristically in neutral red in the living cell with a gradual accentuation of the rosette about the centrosphere. In a series of 53 rabbits, Cunningham, Sabin, et al., found that the percentage of monocytes in rabbits infected with bovine tuberculosis rose from an average of 8 per cent before infection to an average of 14 and a maximum of 52 per cent after infection. Their corresponding total numbers were from 943 monocytes per c.m.m. before infection to an average of 1455 and a maximum of 6348 after infection. There were no qualitative changes in the lymphocytes but their numbers were increased in rabbits with high resistance to tuberculosis. The normal ratio of monocytes to lymphocytes in the rabbit is 1 to 2.97, in round numbers 1 to 3; rabbits with high resistance developed a ratio of 1 to 3.56 while the animals with low resistance showed a ratio of 1 to 0.79.

The present series of 80 rabbits were infected with 1 or 2 mg. of bovine tubercle bacilli, injected intravenously. We have used the same strain of organisms as in the previous studies, namely Strain B1, which was isolated at Saranac but which we obtained from the Dows Laboratory of Tuberculosis, John Hopkins Hospital. Bovine Strain 214 E, secured from Dr. Paul A. Lewis, of the Department of Animal Pathology of The Rockefeller Institute, has given the same findings when used in comparable dosage in a few control animals. Beside studying the white cells in this group we have as a routine counted the red cells and taken the hemoglobin, making the studies on the average of every other day. For the counts of the red cells we have used pipettes and the Levy-Hausser counting chamber calibrated by the Bureau of Standards. The hemoglobin readings were made in a Duboscq colorimeter with the Newcomer standard about 2 weeks after the corresponding counts and by a different individual without knowledge of the totals of the red cells.

These studies have confirmed previous results (2) in demonstrating that in general the progress of the experimental tuberculosis in the tissues can be followed by the ratio of monocytes to lymphocytes in the blood. In studying animals which pass through the acute into the subacute and chronic phases of tuberculosis, it has been possible to follow further the extent of the pulmonary process, and the lesions in other organs, at various stages, and to correlate the changes with the
monocyte-lymphocyte ratio in the blood. In addition, we have found in this study that the curves of the platelets, the red cells, the hemoglobin and the granulocytic leucocytes can in turn be used as a general index of the progress of tuberculosis in the bone marrow.

In the analysis of this series, the animals may be considered in two sections: the first consists of the 12 rabbits studied for the early reaction at 24, 48, 72, 96 hours, and thereafter at intervals of 48 hours up to 18 days; the remaining 68 animals of the series fall naturally into three groups according to the phase of the anemia and leucopenia at the time of death. The progress and extent of the tuberculosis in the early reaction in organs other than the bone marrow are included in a following article (3). While the right femur was chosen for routine examination, these findings were determined to be representative of all the marrow by selected surveys covering both humeri, both femurs and the ribs.

**Early Reaction.**

During the first 6 days following the intravenous injection of tubercle bacilli, supravital studies of the bone marrow revealed no changes in the normal picture, except an increase, first noted at 48 hours, in the number (2 to 3 per oil immersion field) and activity of the clasmatocytes (3). Fat cells, megacaryocytes, myelocytes, erythroblasts and normoblasts were present in the usual percentage and distribution (11). Only the occasional mature monocyte, to be accounted for by the number to be found in the circulating blood, was seen. On the 6th day, however, there were found (R 217) for the first time, a few monocytes; and on the 8th day the supravital studies (R 218) showed two very definite and striking changes, undoubtedly correlated: (1) changes in the fat cells, and (2) the appearance of many young monocytes.

In this marrow of the 8th day were found, in the supravital studies, later confirmed in sections, a few clumps of fat cells showing the breaking up of the usually large, homogeneous fat globule into smaller droplets, with the beginning of the shrinking of individual cells. The demobilization of fat is obviously the principal mechanism of making room for new elements in bone marrow, as may be seen in the conversion from a fatty to a red marrow under many pathological condi-
ditions. That the fat is in an extremely labile form was demonstrated
by one of us (4) in studies in the experimental hypoplasia of avian
bone marrow. It was shown that in the pigeon the bone marrow of
the radius may be reduced to a completely hypoplastic or fatty state
during a fasting period in which there is a loss of from 100 to 150 gm.
in body weight. A biopsy at this stage was followed by the giving of
abundant food to the bird, with succeeding operative removals of
marrow at 24 hour intervals for comparison. In 24 hours there were
marked changes in the fat consisting in a regression of the fat cells
toward their embryonal state. It is well known that the developing
fat cell has the fat in small droplets, the nucleus being centrally
placed, the ultimate resting cell showing a flattened peripheral nucleus
with one, large, homogeneous fat globule within the stretched cell
membrane. The regressing fat cells of the pigeon's marrow showed
first a breaking up of the single, large, fat sphere into many smaller
droplets. This was followed by the shrinking of the individual fat
cells always toward the blood vessels to which they appeared to be
anchored, and into which the fat seemed to be passing for transport
from the marrow. In 48 hours all the fat had been removed from
the pigeon's radius coincident with the restoration of the marrow to
a rapidly regenerating hemopoiesis. It was the beginning of such a
process of fat demobilization which was evident in the marrow of
rabbits on the 8th day after a tubercular infection.

At the same time, the supravital studies showed marked evidence
of the development of young monocytes, many of them monoblasts,
with rosettes a single granule deep. No epithelioid cells were found.
The normal bone marrow does not contain monocytes younger than,
nor in numbers exceeding those of the circulating blood, but in this
instance as many as 20 per oil immersion field were counted repeatedly.
This observation with the living cells was confirmed in sections. In
the fixed sections stained with hematoxylin and eosin, it would not
have been possible to discriminate the individual monoblasts from
myeloblasts with such assurance without the aid of the preliminary
supravital studies. Nevertheless there were signs other than staining
reaction in the sections themselves which indicated the interpretation
to be correct. In normal bone marrow the number of myeloblasts is
exceedingly limited; most of the myeloid elements are in the stage of
the late myelocyte, with a full quota of granules, which have been designated Type C (5, 6). Moreover, in myeloid stimulation or in experimental depletion of the bone marrow, myeloblasts do not appear until the myelocytes, Type C, have been reduced and the marrow thrown back to the level of the earlier Types A and B; then such a marrow, if active regeneration is to occur, shows myeloblasts and early myelocytes in mitosis and in increased numbers. In contrast, this marrow of the rabbit 8 days after infection showed only the usual percentage of late myelocytes; thus the myeloid hemopoietic elements were as yet entirely unaffected and the peripheral blood at this stage indicated no change in those blood cells coming from the marrow. Yet in the sections there were large numbers of deeply basophilic, immature forms, with centrospheres easily seen as clear areas in the dense blue cytoplasm. These were the monoblasts and young monocytes which had been seen in the vital preparations. There was no indication that they arose elsewhere than locally in the bone marrow; at this stage there was no rise in monocytes in the blood stream to indicate their transportation through the blood into the marrow. We think that they arose in situ from the primitive undifferentiated mesenchymal stem cell which may give rise to any of the three strains of the white blood cells, the leucocyte, lymphocyte or monocyte (6); normally this stem cell, this undifferentiated mesenchyme or so called reticular cell, gives rise only to leucocytes in bone marrow, but, under the stimulus of a tubercular infection, and after the usual very definite latent period (7), the same stem cell may give rise to monocytes, preliminary to the development of the epithelioid cell typical of the specific pathology of the disease.

Chart 1 records the data gathered from the peripheral blood in counts taken before infection and just before autopsy in the 5 animals examined from the 10th to the 18th days of the disease. On the 10th day (R 227) after infection there was a definite decrease in the fat content of the marrow with more evidence of the nature of the demobilization in the number of cells showing the breaking up of the fat into many fine droplets. In addition to increased numbers of young monocytes, some with two nuclei indicating rapid multiplication, there were numerous mature monocytes and the beginning appearance of scattered typical epithelioid cells with a full quota of mitochondria. There was a definite depletion of the late Myelocytes C; Myelocytes B were obvious, but there were no increases in Myelocytes A or in myeloblasts. The red cell series showed a pre-
dominance of erythroblasts, with the appearance of megaloblasts and early erythroblasts. The clasmotocytes remained increased in number with their phagocytic activity stimulated above the normal for this tissue. The megacaryocytes, while not reduced in apparent number, showed here and there degenerating nuclei and cytoplasm.

Supravital studies on the 12th day (R 228) showed markedly decreased fat, many fields having none or only one greatly shrunken fat cell. Every field contained epithelioid cells, either singly or in small clumps; many cells had two nuclei. The myelocytes were thrown back to the B type, only a few C myelocytes being present. The neutrophilic leucocytes in the peripheral blood had fallen from an original count of 10,000 per c.mm. to 2800 (Chart 1). The red cells while showing a larger percentage of early erythroblasts than normal still contained normoblasts in appreciable numbers. The clasmotocytes showed engorgement with whole red blood cells. Many of the megacaryocytes appeared shrunken and dead with very little cytoplasm surrounding the nuclei. The platelets had fallen from an original count of 910,000 per c.mm. to 150,000 (Chart 1).

Rabbit 61 A (R 229) (14 days) again showed a bone marrow with the fat absent from many fields in supravital surveys, the presence of fine droplets indicating the continued transportation from the marrow of the final fat deposits. Numerous typical epithelioid cells, many young forms, singly and in clumps, were in every field of every preparation. A marked reduction in C myelocytes, with many
B types, but with the predominating blood cell the early erythroblast, indicated the gradual encroachment of the new growth on the blood-forming tissues. The red cells had fallen from an original level of 6,700,000 per c.mm. to 3,900,000, hemoglobin from 75 per cent to 42 per cent, and the leucocytes from 10,500 to 1580 (Chart 1). There were many clasmatocytes, actively phagocytic, with whole, nucleated and non-nucleated red blood cells and their fragments engulfed. Only two megacaryocytes, one without cytoplasm, were seen in a survey of many fields. The platelets had fallen from an original level of 640,000 per c.mm. to 180,000.

In Rabbit 62 A (R 230) (16 days) supravital studies of the bone marrow revealed practically no fat. There were great numbers of monocytes, typical epithelioid cells and, for the first time, true giant cells of the Langhans type. There was a minimum of myeloid cells at any stage of maturity; the red cell series, largely erythroblastic with a few normoblasts, greatly predominated. The leucocytes had fallen from an original absolute number of 4800 per c.mm. in the peripheral blood to 372, while the monocytes had risen from 0 to 5084 (Chart 1). The clasmatocytes were still stimulated, and the megacaryocytes, though decreased in absolute numbers from the normal, were increasing over the percentage found at the immediately preceding stages.

At 18 days after infection (R 231) the only fat to be found in the bone marrow was an occasional fat globule the size of a red cell. There were many typical epithelioid cells, showing no tendency toward phagocytosis, in sharp morphological and functional distinction to clasmatocytes loaded with phagocytosed white and red blood cells. The monocytes rose from 650 per c.mm. to 3070 in the peripheral blood, and the clasmatocytes from 0 to 465 (Chart 1). There was striking limitation of the myeloid elements, baso-, pseudo- and eosinophilic myelocytes being all proportionately depressed. The red cells showed all stages of erythroblastic maturation, with very few megaloblasts and normoblasts. The red count had fallen from 6,350,000 per c.mm. to 3,030,000 and the hemoglobin from 76 per cent to 30 per cent, the leucocytes from 5000 to 1400. There was a striking excess of megacaryocytes in this marrow in contrast to those marrows of the period from 10 to 14 days. There were many, often nests of 5 per field, small, obviously young cells, with from one to three nuclei; and then, frequently, there would be found large, single, multinucleated cells covering half an oil immersion field. The platelets in the first count before infection in this animal had been 910,000 per c.mm. and in the final count they were 740,000. In the supravital preparation of the peripheral blood the platelets varied greatly in individual size, which variation was quite obvious in the counting chamber also. And this has been confirmed repeatedly in other animals in which the platelets have been followed (see for example Chart 9). During the period of low platelets in the peripheral blood there was a distinctly increased coagulation time for the blood. It will be readily seen from Charts 1 and 9, representative of 14 animals, that the platelets show a sharp decrease in the peripheral blood earlier than the
red or white cells, that the period of depression has a briefer duration (from the 9th to the 14th day after infection approximately) and that the return to the limits of normal comes often while the greatest depression of the other blood elements is being experienced. Coincident with these peripheral manifestations there may be correlated changes in the qualitative and quantitative characteristics of the megacaryocytes in the bone marrow. This evidence might be used in further presumptive corroboration of the direct relationship between the platelet of the blood and the megacaryocyte of the bone marrow, and, conversely, to question further the origin of platelets from the granulocytic and erythrocytic series.

The demonstration of the first appearance of young monocytes in the marrow on the 6th to 8th day after inoculation with massive intravenous doses of bacilli, no epithelioid cells, no tubercles yet having appeared, correlated with the constant development of marked tuberculosis of the marrow following close upon their appearance, is an added confirmation of the origin of the epithelioid cell from and through the monocyte; clasmocytes are present in increased numbers from 48 hours on. Since the development of an extensive tuberculosis of the marrow in from 12 to 21 days after infection has been a constant finding with two strains of bovine tubercle bacilli (B1 and 214 E) (see below), it is believed that the increase in young monocytes marks the onset of the local tubercular process. The fat is gradually depleted as the encroaching tubercular tissue advances and the anemia and leucopenia progress until the beginning spontaneous regression of the foreign tissue makes room within the rigid, bony confines of the hemopoietic organs for adequate blood formation.

The Later Reactions.

In the remaining 68 rabbits of this series, we have used the preliminary studies of the blood in each animal before infection with tubercle bacilli as the normal base line. The extent of the anemia and fall in neutrophilic leucocytes after infection are indicated in Table I. In this group, the onset of the fall of the red cells and hemoglobin has come on the average 11 days after the injection of tubercle bacilli. This average includes only 62 of the 68 rabbits, because to 6 of the animals had been given a hemolytic serum before the injection of the bacilli, thus introducing two factors in the production of the
anemia. In all 6 there was a further fall of the red cells after the injection of the bacilli but the time of onset was not so striking. In some instances the fall of the neutrophilic leucocytes (pseudo-eosinophilic in the rabbit) began on the same day as the fall in the red cells, but it occasionally preceded the latter, so that the average initiation of the fall in white cells was on the 9th day. The onset of the appearance of this effect on the bone marrow has been quite constant with reference to the subculture used; for example in one instance 12 animals injected from a given subculture all showed the onset of the anemia on the same day; in every group of animals in-

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<td>R.B.C. 5,425,697</td>
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<td>Hgb. 62 per cent (Newcomer)</td>
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<th>TABLE II.</th>
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<td>Age of Cultures of Tubercle Bacilli, Strain B1, When Used for Inoculation, with Average Period before Onset of Anemia.</td>
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fected from a given subculture 2 or more have developed the anemia simultaneously (Chart 2). There has, however, been no constant relation between the onset of the anemia and the period of incubation of the subculture (Table II); in each instance the culture chosen for inoculation has shown an excellent growth of bacilli and the effective range of incubation has been from 9 to 38 days. With a culture of 9 days the average onset of the anemia was on the 12th day, while with one of 38 days the average onset was 11 days after infection; with one old culture of 56 days there was no manifestation of the development

of an acute phase of the tuberculosis after 30 days in the 6 rabbits inoculated (Chart 5). We have realized that to get the exact time of the onset of the anemia and leucopenia it would be necessary to have counts of the blood every day which was not done in the original series, but the general period of depression has been remarkably constant. In 2 animals of a new series counted every day the onset of the anemia was on the 9th day in both instances and the onset of the leucopenia on the 5th day.

The lowest red count in the series was 1,410,000 cells and the lowest hemoglobin was 21 per cent. There were several instances of an
extreme fall in neutrophilic leucocytes to percentages under 10 and to total numbers ranging from 100 to 200 cells per c.mm. There was one count of 100 white cells with no neutrophilic leucocytes present. The other types of granulocytes, the eosinophilic and basophilic leucocytes, have also shown changes. The eosinophilic leucocytes normally occur in small numbers in the rabbit, ranging from 1 to 2 per cent, and they disappear entirely from the peripheral blood during the leucopenic phase of the disease; the basophilic cells, normally ranging from 8 to 10 per cent, do not decrease so markedly during the leucopenic phase but, as will be shown, they are subsequently increased during the compensatory leucocytosis.

In the analysis of this latter series the animals fall naturally into three groups, according to the phase of the anemia and leucopenia at the time of death. Most of the animals died spontaneously of the disease, but a few were killed when it was estimated that they might not live through the night and supravital studies of the fresh tissues were desired. The first group, 30 animals, showed falling red cells and granulocytes at the time of death, and all died acutely during the 1st month after infection. Of this group 22 died and 8 were killed. The average length of life was 22 days, the extremes being 15 and 34 days. Only 2 of the animals lived longer than 30 days, one 31 days, the other 34 days, and neither of the 2 had a blood count for 4 days preceding death, so that the red cells may have begun to rise in them before death.

The second group consists of 17 animals which showed the beginning of recovery of the bone marrow as reflected by the onset of a rise in red cells, hemoglobin and granulocytes. Of these animals 14 died and 3 were killed. The fatalities came approximately during the 2nd month, with an average length of life of 35 days and a range of from 18 to 60 days after infection; there were 3 animals with a duration of life under 30 days; 1 was killed at 18 days, 1 died at 19 and 1 at 20 days. This group shows the remarkable fact of animals dying with an advancing pulmonary lesion while the bone marrow was recovering from tuberculosis.

The third group consists of 21 animals, in all of which the peripheral blood indicated a restoration of the bone marrow, in that the red cells, the hemoglobin and the granulocytes had either returned to or
exceeded the original normal level. The average length of life in this group was 150+ days, the range of life being from 59 to 247 days, not including 1 rabbit which was infected March 22, 1926, and is still alive after 1 year. Of these animals 14 died, 5 were killed when it was estimated that they might not have lived through the night, 1 was killed while still in good condition and 1 is still living.

On Chart 3 (R 19) is shown the record of the peripheral blood of 1 of the animals representative of the first group. The red cells and hemoglobin are shown in the upper section of the chart, the neutrophilic leucocytes (pseudoeosinophilic in the rabbit), the lymphocytes and monocytes in the lower section. Before infection the white cells ranged from 5300 on the 6th of November to 11,500 on the 13th, the increase being due to lymphocytes, not an unusual differential curve in certain normal rabbits; the monocytes were consistently below 1000. As will be seen on the chart, there was a rise in monocytes, which were entirely normal, qualitatively, on the 2nd day after infection. We have found this transitory rise in, usually normal, monocytes on the 2nd or 3rd day after infection in many of the animals of this series. This rise may be due to a division of the monocytes already in the blood stream or to the entry of new cells into the vessels from the tissues, but the survey of the general body tissues during this period (3) reveals primarily a clasmatocyte reaction, so that it would appear more probable as a reaction closely related to the blood itself. From the 1st of December there was a phenomenal rise in monocytes, a large proportion of which were abnormal with lessened motility and with markedly increased bodies staining in neutral red. There were also some typical epithelioid cells. From the 1st to the 4th of December, there was a marked rise in desquamated endothelial cells, clasmatocytes, ranging from 4 to 17 per cent of the white cells in the blood. Two of the endothelial cells from this animal have been illustrated in a preceding paper (9). 1 On December 4th, just before the animal was killed there were several sheets of endothelial cells, one of which contained 19 cells, seen in the peripheral blood, and similar sheets were seen in supravital preparations of the spleen immediately afterward. With the extreme rise in monocytes during the last 3 days, there was a fall in lymphocytes, the final ratio of monocytes to lymphocytes being 75.5 to 14 in percentage and 9475 to 1757 in numbers.

The lines of monocytes and lymphocytes on the chart show the progress of the tuberculosis in the general tissues, while the three lines of red cells, hemoglobin and neutrophilic leucocytes indicate the progress of the lesion in the bone marrow. The abrupt fall in the curve of the red cells came between the counts recorded for the 25th and the 30th, making the drop of the red cells, hemoglobin and the leucocytes come between the 9th and 14th days after infection. In the animals on which a count has been made every day for a comparable period

1 Sabin and Doan (9), Figs. 5 and 11.
the fall has been a gradual one. The curve of the hemoglobin acts as a check on
the red cell counts, the close paralleling of the two records indicating the relative
accuracy of our present technical methods for ascertaining comparative values
at least.

At autopsy the lungs of this animal showed diffuse generalized tuberculosis of
so marked a grade that distinct tubercles were not seen in the gross; microscopic
sections showed an extreme grade of the disease with only small areas with patent
air sacs; there was very little caseation. The spleen was markedly enlarged,
weighing 17 gm., the normal average weight being 0.9 gm. according to Rous and
Robertson (10); it showed an extreme dilatation of the sinuses and extensive
tuberculosis. The free cells obtained by gentle scraping of a freshly cut surface
showed some epithelioid cells, large numbers of clasmatoocytes with ingested red
cells and many sheets of endothelial cells. On section the spleen showed extreme
tuberculosis and markedly dilated sinuses. Our impression is that the marked
acute splenic tumor present in every animal of the series that died during the
1st month was due to the dilatation of the sinuses. The lymph glands likewise
showed marked tuberculosis with caseation; also dilatation of the lymphatic
sinuses. Both glands of the hilum of the lung and the mesenteric lymph nodes
were studied. There were a few small tubercles in the liver; the adrenals were
negative except for an occasional single epithelioid cell.

The bone marrow of this animal (R 19) is shown in Fig. 1. In the gross the
marrow was dark red, elastic, not gelatinous. The striking points in the section
are the complete elimination of the fat cells, the extensive tuberculosis and the
reduction of the marrow to an early erythroblastic level. The supravital prepara-
tions showed no fat, but there were epithelioid cells as evidence of the tuberculosis
and early erythroblasts with an occasional megaloblast. In contrast to normal
bone marrow, there was a great reduction in myelocytes. A few myelocytes
are seen in a gray tone across the artery in the center of the section. The edge
of the marrow, which is usually marked by a prominent zone of mature myelo-
cytes, will be seen in this section to be markedly depleted. The section shows
two types of areas, the paler zones which are tubercular tissue, the darker zones
which are masses of early erythroblasts. There are some megacaryocytes, but
our impression is that they are reduced in number. Such a bone marrow cor-
responds with the peripheral blood on the last day of Chart 3; the leucocytes
were reduced to 2.5 per cent with 3,13 as the total number; the red cells were
2,040,000 and the hemoglobin 27 per cent. In the normal marrow there are
many times more myelocytes of Type C (6) than nucleated red cells; in this
marrow the supravital studies and the section show vastly more erythroblasts
than myelocytes but the erythroblasts were for the most part too young to have
been ready for the peripheral blood. Thus this marrow had been depleted of its
fat to make room for the tubercular tissue and at the same time the marrow had
been depleted of most of its store of myelocytes and normoblasts: it had been
thrown back to the level of the early erythroblasts with no sign whatever of a
stimulation of them into the late erythroblasts and normoblasts. Moreover, there was a tendency toward the formation of myeloblasts to indicate a regeneration of the white series. From such a marrow the anemia and the low level of granulocytes in the peripheral blood are readily understood. This marrow stained for tubercle bacilli showed often as many as 10 or 12 acid-fast organisms in the occasional epithelioid cell.

On Chart 4 is shown the blood of a second rabbit (R 18) of the first group. It shows the same points except that the terminal rise in monocytes was not quite so marked though the animal died with the monocytes exceeding the lymphocytes in the peripheral blood. These two charts are entirely representative of the complete group of 30 animals that died in the acute phase of the disease.

In every instance there has been extreme or miliary tuberculosis of the lungs; acute splenic tumor with marked tuberculosis; involvement of the lymph glands and extensive tuberculosis of the bone marrow. All the charts of the peripheral blood of this group are practically identical: they show a fall in red cells, hemoglobin and granulocytes which has been correlated at autopsy with the demonstration of a marked tuberculosis of the bone marrow; as a sign of the general tuberculosis, there has usually been the transitory rise in monocytes on the 2nd day, with a marked rise in abnormal monocytes in the 3rd week and a corresponding fall in lymphocytes.

The second group of rabbits, the 17 that survived the first acute hemopoietic depression from the infection, yet died for the most part
in the 2nd month, all showed the beginning of a recovery of the bone marrow.

On Chart 6 (R 80) is the record of the peripheral blood of an animal showing the beginning change from the reaction which occurred in the first group. Before infection this animal received two doses of a hemolytic serum which caused some fall in both red cells and hemoglobin. The blood was counted twice on the day of the injection of the bacilli. At 9.00 a.m. the total white count was 6150; the bacilli were given at 9.30; by 1.30 the white cells numbered 16,500, and the chart shows that the rise was due to a transient outpouring of the neutrophilic leucocytes. The onset of the anemia and the fall in leucocytes secondary to the tuberculosis began on the 9th day. The interesting point in the chart is that there was a slight rise in the red cells, more marked in the hemoglobin just before the death of the animal. A rise in hemoglobin accompanied or followed by a rise in the red cells is constant for the entire group. The terminal rise in leucocytes shown on this chart is unusual for animals dying at this stage. At autopsy, this animal showed extensive tubercles of the lungs, some of which were caseated and surrounded by lymphocytes; this is in contrast to the extreme diffuse reaction of the first group. The spleen had marked dilatation of the sinuses, extensive tuberculosis of the pulp, with some involvement of the follicles. The marrow was most interesting (Figs. 4 and 5). With the low power, the marrow is not far different from that of Fig. 1; there is the same extensive tuberculosis, the same
complete elimination of fat and the same reduction of the marrow to an early erythroblastic state. The distribution of the epithelioid cells is rather more diffuse, and this is characteristic of most of the marrows in the early stage; it indicates that there is no reaction whatever on the part of the connective tissue framework in the marrow. The differences between this marrow and that of Fig. 1 are shown in the higher power of Fig. 5 and consist (1) in the signs of degeneration of the single epithelioid cells and (2) in the presence of normoblasts. The large pale epithelioid cells are very clear with the reduced and altered chromatin of the nuclei and the vacuolated cytoplasm. In both marrows there are certain pale areas, from which the epithelioid cells seem to have disappeared entirely as if bone marrow was unfavorable soil for their persistence. There are no signs of any degeneration of the white cells, but in Fig. 5 there are two swollen endothelial cells, which have been shown (4, 11) to mark the onset of the regeneration of red cells, and certain lines of normoblasts are quite clear along the left border of the section. It is interesting to note that so slight a change in the red cells as is shown in the chart is readily found in the bone marrow. When this marrow was stained for tubercle bacilli the results were most interesting. Whole bacilli in epithelioid cells were rare. On the other hand the vessels were outlined by cells filled with acid-fast granules in the Ziehl-Neelsen technique. These cells occur along the large venous sinuses, the smaller veins and capillaries and even along the collapsed capillaries which Doan (4) first described as passing between individual fat cells. They make the vascular pattern almost as plain as an in-
jection. The cells with the granules are the clasmatocytes, the so-called adventitial cells of Marchand, and occasionally it appears as if the endothelium itself might contain some of the granules. Evidence is presented in a following paper (3) indicating that the clasmatocyte fragments tubercle bacilli in this manner while the epithelioid cell retains them intact.

The same processes, carried a little farther, are shown on Chart 7 (R 71). The corresponding bone marrow is shown in Figs. 6 and 7. The animal lived for 31 days after inoculation and the chart shows a definite rise in hemoglobin, and the very beginning of a rise in the red cells. The lungs had an extreme tubercular pneumonia and no lymphoid reaction. The spleen showed dilatation of the sinuses and tuberculosis of the follicles. In the bone marrow (Fig. 6), it is plain that there are two different types of areas: first, the large tubercular zones such as the one on the left with a few fat cells in the center; second, zones such as the center of Fig. 6 and nearly all of Fig. 7 (higher power) from which the epithelioid cells have disappeared entirely, leaving the normal reticular framework of the marrow. In these latter areas the clumps and lines of developing red cells with their deeply staining nuclei are obvious. Beside the red cells there are small clumps of early myeloid elements, myelocytes, Types A and B, much too young to be giving rise to leucocytes for the blood stream. Again the more advanced regeneration of the marrow is in the red elements as is obvious in Fig. 7, but neither red cells nor white cells are sufficiently advanced to have made a marked change in the blood.

The comparison of Figs. 5 and 7 brings out what happens to tubercular tissue in the bone marrow: the individual epithelioid cells degenerate as shown in Fig. 5 without any evidence of caseation; the debris is quickly cared for as formed and there are left the zones so obvious in Fig. 7 which reveal the reticular framework quite unchanged. In all the marrows, the early erythroblastic group has persisted throughout the acute phase of the disease, only a few megaloblasts having been encountered, that is to say the production of red cells has only rarely been completely thrown back to the level of the parent endothelium, a stage produced in the pigeon by underfeeding (4), and described for the human by Peabody (12). In general, in the animals of our series, the first step in regeneration (excluding the platelets) has been a rise in the hemoglobin, showing that the first compensatory mechanism is an increase in the amount of hemoglobin per cell; the next step is the increase in the number of the red cells and the last the rise in leucocytes. This relative reaction time of red cell granulocyte follows both the embryonic and the regenerative potentialities of these respective cell types as shown by Sabin (13) and Doan (4, 11).

Further stages in the recovery of the marrow are shown on Charts 8 (R 20), 9 (R 196) and 10 (R 12). On Chart 8 the rise in hemoglobin and red cells is now definite; the leucocytes have stopped falling and are perhaps just beginning to return to the blood. Chart 9 depicts a further recovery in red cells and hemoglobin, with definite recovery in the neutrophilic leucocytes while the animal
was dying of acute pulmonary tuberculosis. This graph is representative of the
group of animals in which findings with reference to blood platelets and tempera-
ture were studied. In 14 animals in which the platelet count was followed there
was uniformly an earlier (average 7 days) and more sudden depression of these
elements in the peripheral blood than of the other bone marrow derivatives,
though the period of greatest depression usually coincided with a falling red and
neutrophil count. The recovery of the platelets both as observed in the periph-
eral blood and as correlated with the disintegration and regeneration of mega-
caryocytes in the bone marrow (see Chart 1) indicates a more sensitive mechanism
here in its response to the general depression of the disease with a more vigorous
and immediate readjustment to the new conditions. In this respect it will be

noted (Chart 9) that the temperature response coincides with the sharp fall in
platelets and neutrophils while the red cells are only beginning to show a more
gradual decline. The elevation of temperature shows the higher range during
the gradual depression of the elements from the bone marrow in the peripheral
blood, a period corresponding to the rapid development and extension of the
tubercular process in the other organs and tissues of the body as well. Chart 5
(R 193) indicates the lack of elevation of temperature and the uniform blood
picture relative to all the bone marrow elements after a 1 mg. injection of bacilli
from a culture 56 days old. A reinfection with 2 mg. of a young virulent cul-
ture of the same organism promptly initiated the usual changes in the blood with
an immediate fall in platelets and a rise in temperature. The clasmaticocytic
shower on the day following the reinfection (March 22) is represented by the cell reproduced in another paper (3). This animal died during the acute progression of the tubercular process in the bone marrow. The other 5 animals of this experiment all died acutely within 12 hours after the second reinfection, obviously being in an allergic state even though the blood picture had remained within normal limits and the tubercular process relatively quiescent.

These changes both in the red cells and in the leucocytes are well marked on Chart 10, where the red cells are practically at the original level of between 4000 and 5000. The bone marrow corresponding to Chart 10 is shown in Fig. 2. This animal was in very poor condition, was losing weight and had very sluggish circulation, so it was killed. The lungs showed large and small tubercles sur-

rounded by lymphocytes. There was very little caseation; no pneumonia. The spleen had very few tubercles; in supravital films most of the free cells were of the clasmatocyte type with but few monocytes. The bone marrow in supravital studies showed the return of the fat; there were large areas of mature myelocytes, some zones of the primitive reticular cells and myeloblasts. Among the red cells were many normoblasts. There is an oblique line across the section to the left of which is a depleted zone from which it is probable that epithelioid cells have just disappeared. There are a few tubercles in the marrow, none showing in this photograph. To the right of the oblique line is an area in which the

\[10^3\] \[10^4\] \[10^5\]

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2 Sabin and Doan (3), Fig. 5.
myelocytes predominate over the clumps of nucleated red cells as in normal bone marrow; the area is, however, hyperplastic. To the left of the oblique line the marrow is still considerably depleted and there are patches of younger myelocytes, Types A and B. In both R 20 and R 12 the tuberculosis of the lung was in the form of tubercles rather than the diffuse reaction. The spleen of the animal from which Chart 8 was taken was still enlarged; the one corresponding to Chart 10 was of normal size and contained only a small amount of tubercular tissue.

The last animal of the second group to be illustrated is shown on Chart 11 (R 68), with the corresponding bone marrow in Fig. 3. In this animal it is very clear that the hemoglobin started to rise before the red cells themselves. At the time of death, which was 59 days after infection, the red cells were back to

\[ \text{CHART 10.} \]

the original level, while the neutrophilic leucocytes were varying around 6000 in contrast to the original base line of 3000. It will be noted that after the marked rise of monocytes in the 3rd week, they remained high and the lymphocytes dropped, indicating low resistance, and at autopsy both lungs were riddled with tubercles, with large areas of caseation and cavitation. The spleen was of normal size; there were caseous tubercles in the kidneys. On the chart has been added the line of the desquamated endothelial cells, or clasmatocytes. It will be noted that on April 12 the clasmatocytes rose together with the characteristic increase in monocytes of the 3rd week of the disease and then gradually fell to normal numbers. This period of the first great rise in monocytes after massive infection coincides with the period of the extreme diffuse lesions in the lung and
with the acute splenic tumor. At that time there is always the rise both in monocytes and clasmatocytes, together with degenerating types that cannot be analyzed and much cellular debris in the peripheral blood. This is the period of the anemia and the supravitral films of the blood always show increased fragmentation of the red cells even when the preparation is looked at immediately and when the temperature of the warm box is carefully regulated so that it does not exceed 37° (14). Beside the free fragments of the red cells in the films of blood, we frequently find some of the clasmatocytes of the blood stream filled with them. Films of the lungs and spleen at this stage always contain clasmatocytes filled with fragments of red cells, the possible stimulus to their increase.

The bone marrow of this animal (R 68), as will be seen in Fig. 3, is hyperplastic. In the center of the photograph is a small tubercle; the rest of the section shows the gray masses of myelocytes, many of which are still of the younger Types A and B, and the normal proportion of groups of erythroblastic cells. The interesting point in this second group of animals is the universal tendency to a spontaneous healing of the bone marrow of rabbits, irrespective of the eventual fatal progress of the disease in the animal body elsewhere.

When the rabbits survive beyond the 2nd month into the more chronic phase of the disease, the bone marrow passes through the hyperplastic state to the normal. This phase is illustrated in Chart 12 (R 11), and Fig. 8 (R 11), Fig. 9 (R 97) and Fig. 10 (R 95). On Chart 12 is shown a typical picture of the peripheral blood in a rabbit of good resistance; the animal was killed 110 days after
inoculation while still in good condition. In regard to the indications on the chart of the general tubercular infection, there was the usual sharp rise in monocytes on the 2nd day after infection; the monocytes then fell to normal until the 1st of December when there was the characteristic rise of the 3rd week. From the 6th to the 16th of December, the period of greatest leucopenia, it will be noted that the monocytes were again normal and the ratio of monocytes to lymphocytes was entirely normal; this is the period of recovery from the first extreme reaction of the lungs. Then follows the record of a slowly progressing tuberculosis of the lungs in the gradually rising monocytic curve. At autopsy the left lung was largely air-containing; the right was riddled with tubercles, and in sections it appeared that the center of each tubercle was filled with leucocytes instead of showing the usual caseation. The liver and spleen did not show any tubercles; there was some myeloid reaction in the spleen. The mesenteric lymph glands were loaded with clasmataocytes filled with brown pigment but no tubercles were found. In the bone marrow no tubercles were found in sections and no epithelioid cells in the fresh films.

As a record of the functional activity of the bone marrow, it will be seen on the chart that following the period of the anemia and fall of the leucocytes, the marrow recovered its hemopoietic activity and passed into a stage of hyperactivity which was still present when the animal was killed. The lowest line of the chart represents the basophilic leucocytes and shows that though they do not disappear as do the eosinophils during the period of the leucopenia, they, with
the eosinophils, increase during the general hyperplasia of the marrow. In the
section of the marrow, Fig. 8, it will be noted that there is a return of the fat
cells, though not yet to their normal numbers. No tubercles were found in the
sections; the marrow is hyperplastic in still more marked grade than was shown
in Fig. 3. There are vast gray areas of myelocytes, largely of Type C with many
metamyelocytes. Among the red cells the predominating type was the late
erthroblast with normoblasts. The final stage of the disease as far as the
bone marrow is concerned involves the complete return to the normal structure
with a normal supply of cells to the peripheral blood.

The marrow of Fig. 9 is from a rabbit that lived 135 days and of Fig. 10 from
an animal that lived 153 days. In both animals the findings in September after
the summer interval showed that the marrow was giving a normal output of red
cells and granulocytes to the blood. The rabbit (R 97), from which the mar-
row of Fig. 9 was taken, had 4,970,000 red cells and a hemoglobin of 61 per cent.
The white cells were 11,500, of which the neutrophils were 55 per cent, the lympho-
ocytes 16 per cent and the monocytes 29 per cent. The reversal of ratio of mono-
cytes and lymphocytes is the striking feature. The animal was gaining in
weight. The autopsy showed restricted tuberculosis of the lungs but well
marked active renal lesions. In all the sections of the bone marrow we found
only one tubercle, which is shown in Fig. 9; all the rest of the marrow was en-
tirely normal in appearance. However, in the Ziehl-Neelsen stain the clasmatoc-
cytes showed the same acid-fast fragments, though in decreased numbers, as
have been described for the early stage.

The rabbit from which the marrow of Fig. 10 was taken also showed a normal
output of cells from the bone marrow. The red cells were approximately
6,000,000, the hemoglobin 57 per cent; the neutrophilic leucocytes 47 to 57 per
cent; the lymphocytes 9 to 15 per cent and the monocytes 25 to 30 per cent, again
a striking reversal of lymphocytes and monocytes. The animal was killed on
account of marked tuberculosis of the eyes. The bone marrow appeared en-
tirely normal in the gross and supravital studies, and the sections confirmed this.
In this marrow there were still a few clasmatocytes along the vessels containing
acid-fast granules.

SUMMARY.

In this series of rabbits it was found that the rabbits dying during
the 1st month after an injection of 1 or 2 mg. of bovine tubercle bacilli
show the same conditions: extreme tuberculosis of the lungs, acute
splenic tumor with tuberculosis, involvement of the lymph glands, an
occasional small tubercle in the liver and extensive tuberculosis of the
bone marrow. The peripheral blood has shown a sharp fall in the
platelet count, an anemia and a fall in the granulocytic strain of white
cells, and these changes have been correlated with the condition of the
bone marrow. There has also been a rise in monocytes and a fall in lymphocytes, to a reversal of the normal ratio.

When the rabbits have survived the first acute phase of the disease longer than 3 to 4 weeks, there have been signs in the peripheral blood of a recovery of the bone marrow; the first indication of this has been an increase in platelets, then a rise in hemoglobin followed in 1 or 2 days by a rise in red cells and later a return of the three strains of granulocytes. The bone marrow has shown a rapid spontaneous disintegration of the epithelioid cells correlated with the appearance of increased evidence of acid-fast debris in clasmocytes, especially clear in those that lie along the vessels.

The animals that have survived into the 3rd month have all shown a hyperplastic phase of the healing marrow, both the red cells and all types of the granulocytes appearing in the peripheral blood in numbers above the normal. The epithelioid cells originally containing many bacilli all disappear from the marrow and the only sign left, possibly suggestive of the tuberculosis, is the acid-fast granules in the clasmocytes. Finally, the marrow becomes entirely normal, giving the normal number of red cells and granulocytes to the blood. Thus, bone marrow in the rabbit has become involved in every instance with the injection of massive doses of viable bacilli. The findings at autopsy in those animals followed during the early reaction to infection confirm this directly and, since the curves of the cells in the peripheral blood of the more chronic animals were the same during the early stages of the disease as in those that died, the same conclusion seems justified from indirect inference for them. The method of healing has been a rapid disintegration of the epithelioid cells without caseation. The bone marrow heals itself entirely regardless of the progress of the disease elsewhere, so that one sees the remarkable condition of an animal recovering from the anemia and leucopenia while dying of tuberculosis elsewhere. The spleen also shows a tendency toward spontaneous healing. In the animals that have lived beyond 100 days there has been some gradual lessening of the diffuse distribution and extent of pulmonary lesions with the development of cavitation together with a marked involvement of the kidneys and lesions in the eyes.
CONCLUSIONS.

1. With massive intravenous injections (1 to 2 mg.) of bovine tubercle bacilli in rabbits there is a marked involvement of the bone marrow in the early acute phase. This reaction is initiated on the 8th to 10th days by the development of large numbers of young monocytes in situ.

2. From the 12th to the 20th day, approximately, there is an increasing development in bone marrow of typical tubercular tissue, epithelioid cells and giant cells of the Langhans type, many showing tubercle bacilli. This new growth eliminates the normal fat cells and encroaches upon and depresses the hemopoietic foci.

3. The bone marrow always tends toward spontaneous healing provided the animals survive the first acute reaction sufficiently long.

4. The method of healing involves a rapid disintegration of the epithelioid cells without caseation and the phagocytosis of debris by the clasmatocytes.

5. The extent and progress of the tuberculosis of the marrow are accurately reflected in the peripheral blood by a decrease of platelets, an anemia and a fall in the granulocytic leucocytes.

6. The onset of the recovery is initiated by the return of the platelets to normal, by a rise in hemoglobin, followed quickly by a rise in red cells, and by a more gradual increase in the granulocytes.

7. During the 3rd month, and after, there is a hyperplasia of the blood-forming elements in the bone marrow with a rise in the peripheral blood of the red cells, hemoglobin and the granulocytes above their original levels.

8. The bone marrow becomes entirely normal when the animal survives beyond 100 days, regardless of a steadily progressing, extensive tuberculosis elsewhere.

9. The varying length of survival in this series of rabbits under uniform environmental conditions, and infected with the same dosage of the same strain of organism, tends to emphasize the importance of the factor of individual resistance of the host in susceptibility to infectious disease.

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EXPLANATION OF PLATES.

PLATE 8.

Fig. 1. R 19, hematoxylin and eosin, × about 120. Length of life 18 days. Bone marrow showing extensive, diffuse tuberculosis. Note absence of normal content of fat and myeloid depression.

Fig. 2. R 12, hematoxylin and eosin, × about 120. Length of life 44 days. Bone marrow with returning fat cells after regression of the local tubercular process, with hemopoietic hyperplasia.

Fig. 3. R 68, hematoxylin and eosin, × about 120. Length of life 59 days. Bone marrow showing one remaining tubercle, without surrounding cellular reaction; marked hyperplasia of myeloid and erythroid foci and beginning reappearance of fat cells.

PLATE 9.

Fig. 4. R 80, hematoxylin and eosin, × about 120. Length of life 20 days. Bone marrow with absence of fat cells and depression of hemopoiesis by invading tubercular tissue.

Fig. 5. R 80, hematoxylin and eosin, × about 875. Detail of Fig. 4. Beginning regression of the tubercular invasion, showing partition of chromatin in disintegrating nuclei and vacuolated cytoplasm of the epithelioid cells.

Fig. 6. R 71, hematoxylin and eosin, × about 120. Length of life 31 days. Bone marrow with local regression of the epithelioid cells showing open areas of regenerating red cells and returning fat even in the midst of intact tubercular areas.

Fig. 7. R 71, hematoxylin and eosin, × about 260. Detail of open area in Fig. 6, showing intravascular limitation of developing erythroblasts, a noncellular matrix marking the former site of invasion of the epithelioid cells.

PLATE 10.

Fig. 8. R 11, hematoxylin and eosin, × about 120. Length of life 110 days. Bone marrow hyperplasia of blood-forming elements, no evidence of tuberculosis remaining locally.

Fig. 9. R 97, hematoxylin and eosin, × about 120. Length of life 135 days. One tubercle with lymphoid focus in otherwise essentially normal bone marrow.

Fig. 10. R 95, hematoxylin and eosin, × about 120. Length of life 153 days. Return of bone marrow to normal.