BLOOD AND BONE MARROW IN INFECTIVE SUBACUTE AND CHRONIC ATROPHY OF THE LIVER

By E. MEULENGRACHT, M.D., AND H. GORMSEN, M.D.

INTRODUCTION

Numerous investigations have been made of the blood picture and bone marrow in hepatic diseases, particularly in cirrhosis of the liver. These have revealed that especially in cirrhosis of the liver, macrocytic anemia frequently develops. This can be accepted as a well-ascertained fact.4,5.21

It has further been shown that in liver diseases, especially in cirrhosis, changes also occur in the bone marrow. One sees rather frequently a slight or moderate increase in the number of erythroblasts, possibly a shift to the left in the erythroblasts, often a fair number of large forms, but no megaloblastic change in the marrow. There is also some increase in the plasma cells, and possibly also in the reticulum cells, with or without pigment. More rarely a slight myeloid hyperplasia or a slight eosinophilia are observed.6.7.12.13.15.20

As numerous cases of a special fatal form of subacute and chronic infective hepatitis have occurred in recent years and continue to do so here in Denmark, we have had the opportunity of examining the blood and bone marrow in this particular form of liver disease. The following notes deal with the nature of these cases.

In 1943 and 1944, subacute and chronic hepatitis began to occur in our clinic and in all other clinics in Denmark. Appearing suddenly, and so extensively and with such malignant symptoms, it produced the impression that an entirely new disease had arisen, and soon came to be known among both doctors and laity as "the malignant and dangerous jaundice." The most striking fact is that the patients were almost exclusively women of from 40 to 70 years of age.

The clinical picture is dominated in these cases by long-continued jaundice. It is, as a rule, only slight, but can be more intense or even severe. It varies somewhat in intensity, with a tendency gradually to subside, but in its place ascites and edema make their appearance in many cases in a few months' time, though usually in six months or a year, frequently at the stage when the jaundice has greatly decreased. After a longer or shorter interval, hepatic failure and death finally supervene. The prognosis is very grave, almost all the patients dying of the disease sooner or later.

At autopsy, a small contracted liver with isolated remains of liver tissue and an extensive development of coarse connective tissue is found, a picture previously described, among others, in 1930 during a Swedish epidemic.

Preliminary reports have been published on the numerous cases, and a more comprehensive joint report will appear in the near future. The disease must presumably be regarded as a form of acute infectious hepatitis, the extensive epidemic of which in Denmark reached its climax in 1944.

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Material

The material which we have ourselves intimately studied is based on 24 women with subacute and chronic infectious hepatitis of the type described above. Their ages varied from 40 to 80 years. The history of the disease, when the investigation took place, varied from 1–15 months' duration. In other words, such an interval had elapsed since the jaundice was first noticed, but as, in a number of these patients, the disease developed rather insidiously, it had probably lasted as a rule longer than the specified period. Some of the patients had ascites and edema at the time the investigations were made, others developed these symptoms later. Some died shortly after the investigation, but most died later on.

Besides the above material, we have had access to other cases of the disease,
approaching 150 in all, and from their records we have been able to collect further information about the blood changes.

**METHODS**

All the blood examinations were made by two well-trained technicians. The icteric index was determined by Meulengracht's method. The hemoglobin was estimated in an Autenrieth-Königsberger colorimeter, standardized to 100 per cent = 18.5 per cent oxygen-binding capacity determined by Van Slyke's method.

Dilution of the blood for counting was done according to Ellermann's principle with separate pipets and mixing tubes; the blood corpuscles were enumerated in Zeiss counting chambers. The color index determinations were reckoned on the assumption that 100 per cent Hb corresponds to 3,000,000 red blood cells. With these methods and standards we find the color index in normal persons to be about 1.1.

The mean diameter of the red blood cells was determined by Gram's method, where they are measured in their own serum, so that one can be sure of avoiding the changes which may occur on drying or washing in foreign media. A drop of blood is sucked up by capillary attraction into a 20 cm. long, thin glass tube (capillary tube of about 1 mm. thickness). The tube is sealed at both ends and put aside for some hours. The blood has then clotted, and the serum has separated at the side of the clot. In this serum, free blood corpuscles are present in sufficient quantities for measurement and in suitable numbers so that measurement is not impeded by the corpuscles lying too close to one another. After the ends have been broken off the tube a small drop of serum is blown out into a counting chamber of half the usual height and is covered with a thin cover glass. The measurements are made with the help of an ocular micrometer and immersion lens. The diameters of 100 blood corpuscles at random are measured and the mean diameter calculated. Mulberry-shaped, spherical and other abnormally shaped blood cells are avoided.

The Takata reaction was performed by Jezler's modification. The blood sedimentation rate was determined by Westergren's method.

In the smear preparations of the sternal punctures, a differential count of 100 cells was made. Furthermore, in every case a section of the coagulum of the sternal puncture after embedding in paraffin was examined.

The investigations of the sternal punctures were all made by one of us (H. G.).

**RESULTS**

**Blood.** In table 1, the age, sex, duration of the disease and the results of the blood examinations are given for those patients we ourselves thoroughly investigated.

The icteric index varied from 7 to 135, it often became decreased as the cirrhosis progressed. The Takata reaction in most of the patients was strongly positive (+++), but in some it was weaker (+ or ++). In one case it was negative, but it is possible that the diagnosis was wrong. The blood sedimentation rate was increased in all the cases investigated.

The hemoglobin values varied from 50 to 105 per cent; red blood cells, from 2.17 millions to 4.65 millions. In most cases, there was some anemia. The color index varied from 0.8 to 1.36. It was usually around 1.1, which with our standards corresponds to the normal; in a few cases it was perceptibly diminished. On microscopic examination of the stained dry preparation, neither oval cells nor large ones of the nature of megalocytes were observed. From the other more extensive material we have had access to, we have collected 141 determinations in 75 patients. Hb varied from 58 to 104 per cent, R.B.C. from 2.5 millions to 5.1 millions, color index from 0.8 to 1.34. In figure 1 the Hb values are plotted against the red cell values, and the color index given. It will be seen that there is a marked tendency to a moderate degree of anemia, the bulk of the values lying between 60 and 80 per
cent Hb, and between 3 and 4 million red cells. The color indices are grouped around 1.1.

**Fig. 1. Hemoglobin Red Cells and Color Index in 141 Determinations in 75 Cases of Infective Subacute and Chronic Atrophy of the Liver.**

Lines are continuations from a common point representing zero for both hemoglobin and erythrocytes.

**Fig. 2. Mean Diameter of Red Cells in 64 Measurements in 52 Cases of Infective Subacute and Chronic Atrophy of the Liver**

In the closely studied cases, the mean diameter of the red blood cells varied from 7.64 μ to 9.0μ. From the other material we collected 64 measurements in 52 patients; there was about the same variation. The values are given in table 2 and figure 2.
It will be seen that over 50 per cent lie above 8 μ. With the same technic, Gram found values fluctuating between 7.7 μ and 8.0 μ in normal persons. Jørgensen and Warburg found roughly corresponding limits. In our laboratory also, the normal values found are almost identical with those of Gram. On the basis of these facts it can be asserted that there is a very decided tendency towards an increase in the mean diameter of the red cells in patients suffering from subacute or chronic atrophy of the liver; in No. 23 it reached as much as 9 μ.

### Table 2. Mean Diameter of Red Blood Cells in 64 Measurements in 52 Patients

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**Fig. 3. Mean Diameter Plotted against Icteric Index**

In figure 3, the mean diameter is plotted against the icteric index. It will be observed that there is no correlation between the two.

In figure 4, the mean diameter is plotted against the number of red cells. There is no correlation between the mean diameter and the degree of anemia.

In figure 5, the mean diameter is plotted against the color index. Again no correlation can be detected.

The number of white blood corpuscles varied between 1700 and 12700 in 129 counts in 81 patients (table 3 and figure 6). The last value was quite an isolated one. There was a distinct tendency to low counts, over 50 per cent of them being under 4000. Unfortunately, differential counts were done in only a few cases, but as far as they went no definite deviation from the normal was found.
The blood platelets were counted in only a few of the cases. There appeared to be a tendency towards low values.
1422. BLOOD AND BONE MARROW IN LIVER ATROPHY

The bone marrow. The results of the sternal punctures are given in table 4. As a basis for comparison, the normal values found by Gormsen7 in a material of 50 normal adults are given in the first column.

A comparison with the normal values indicates that in the neutrophile granular cell series there were no changes, no shift to the left, no myeloid hyperplasia and no eosinophilia. Hemocytoblasts, lymphocytes, megakaryocytes and reticulum cells exhibited normal appearances, but there were certain abnormalities in the erythroblasts and plasma cells.

Four patients had over 30 per cent erythroblasts, 8 patients had from 2.2. to 16 per cent, while the remaining 12, therefore half the patients, had below 20 per cent.

TABLE 3.—Number of Leukocytes in 129 Counts in 81 Patients

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In none was the number of erythroblasts found to be lowered. In one patient only (No. 22) was there a shift to the left an the erythroblasts, but it was very pronounced. No definite macrocytosis of the erythroblasts could be detected in any of the cases. Micrometric investigations of the size of the erythroblasts, which normally vary considerably, were not undertaken. Megaloblasts were not observed in any of the cases.

With regard to the plasma cells, they were 4 per cent in 3 patients, 3 per cent in 6 and below 3 per cent in the other 15; that is to say, in 9 of the patients there was a slight increase in the plasma cells, since the maximal value of 3 per cent for the normal number of plasma cells is very high.

Microscopic sections of the bone marrow in 8 patients (Nos. 1, 2, 3, 8, 11, 12, 17, 18) showed slight to moderate hyperplasia of the marrow, but in the others the cell content was normal. The hyperplasia seemed to depend in part on a striking increase in the erythroblasts, and also on a slight augmentation of all the other cell...
forms, so that the state of the marrow seemed to point to a slight increase in the functioning of the bone marrow with greatest emphasis on erythropoiesis.

**Table 4. — Results of Sternal Puncture in 24 Cases of Infective Subacute and Chronic Atrophy of the Liver**

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**Comment**

In the blood examinations, i.e., determination of Hb per cent, R.B.C., color index and direct microscopy, no changes such as those found in pernicious anemia.
were observed. In conformity with this no sign of megaloblastic transformation of the red bone marrow was seen in any of the bone marrow punctures. We have in some cases attempted to influence the anemia by liver injections, but there was no reticulocyte reaction and no rise in the number of red blood cells.

On the other hand, a marked tendency to an increase in the mean diameter of the red blood cells was observed, which in a few cases assumed very high proportions. Gram, in 1883, in his measurements of the diameter of the red cells in various diseases demonstrated that the mean diameter in "ikterus catarrhalis," that is, hepatitis, is increased. Determined in their own serum, the values lay between 8 µ and 8.5 µ; in one case with cirrhotic changes it was 8.9 µ. This finding was later confirmed by others using the same method. The increase in the mean diameter in such cases of liver disease has been attributed to changes in the plasma, possibly to the presence of the salts of bile acids. The fact that in our bone marrow investigations only doubtful macrocytosis of the erythroblasts was demonstrated, and at all events no megaloblastic change in the bone marrow, seems to support the theory that the macrocytosis may at least partly be due to a change in the plasma with consequent swelling of the blood corpuscles. Since there is no correlation between the degree of jaundice and the increase in the mean diameter, the latter cannot depend upon the bilirubinemia but must be caused by other substances in the plasma (bile acid salts?). The question needs a more elaborate investigation.

The tendency to an increase in the plasma cells of the bone marrow must undoubtedly be connected with the plasma protein changes causing the positive Takata reaction, which are characterized by an alteration in the albumin/globulin ratio, decrease in the albumin fraction and increase in the globulin fraction.

Conclusions

In the observed cases of subacute and chronic infectious atrophy of the liver, of the special type which occurs at the present time in Denmark, a moderate degree of anemia of a hypochromic, normochromic or hyperchromic type was found in the majority of the cases. The color index showed a fairly uniform distribution around the normal value. There was nothing in the blood picture that resembled true pernicious anemia.

Measurements of red blood cells in their own serum revealed an increase in the mean diameter, often considerable, in over half the cases.

The blood picture with respect to the white corpuscles showed a pronounced tendency in the direction of leukopenia.

In some of the cases, sternal punctures showed the presence of a more or less advanced erythroblastosis, but no really definite macrocytosis of the erythroblasts was observed, and megaloblastic erythropoiesis occurred in none of the cases. A slight increase in the plasma cells was seen in some cases. Sections of the bone marrow, in a number of cases, showed moderate hyperplasia of the marrow.

In no case, either in the blood or bone marrow, could changes be demonstrated which would suggest nonstorage of the material necessary for the maturation of the red blood cells. The increase in the mean diameter of the red blood cells measured in their own serum, which was observed in some cases, is not due to megalob-
blastic erythropoiesis, but possibly to the serum content in substances which affect
the osmotic condition of the blood corpuscles.

The plasma cell increase in the bone marrow that was found in some of the cases,
is presumably connected with the hyperglobulinemia which is a very common
accompaniment of chronic hepatitis.

The blood and bone marrow changes in our patients thus do not seem to differ
from the blood and marrow changes hitherto recognized in chronic hepatitis and
liver cirrhosis.

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