The Macrocytosis of Hepatic Disease.
I. Thin Macrocytosis

By John Bingham

The blood of patients with hepatic disease frequently contains macrocytic red blood cells. Since their recognition in 1883,1 numerous papers have been written about them, but there is still no agreement on the frequency, morphology, etiology or clinical significance of these large cells.

The purpose of this study was to re-examine this problem by studying a large group of patients suffering from the various types of hepatic and biliary tract disease in order to obtain information on the following: the frequency of macrocytosis in each type of hepatobiliary disease; the morphologic characteristics of macrocytosis; and the cause of macrocytosis.

Early in the study it became apparent that there was not one macrocyte but three macrocytes occurring singly or together in various proportions in the blood of these patients. These three types of macrocytes were termed thin, thick and target. Because there were fundamental differences in the morphologic characteristics and the etiology of these three types of macrocytes, they will be described separately. This paper will discuss in detail the thin macrocyte. It will also include a few observations of a general nature about the three types of macrocytes. The target and the thick macrocyte will be described in later publications.

Two hundred and twenty-two patients with various types of hepatic and biliary tract disease were studied. The various diseases from which they suffered are shown in table 1. The diagnosis was made at autopsy in 20 patients, by laparotomy in 42 patients, by needle biopsy of the liver in 18 patients and by clinical assessment in 142 patients.

Technical Procedures

The blood determinations were made on venous blood, collected in a bottle containing a mixture of potassium and ammonium oxalate as the anticoagulant. Two calibrated erythrocyte pipettes, each with a known correction factor, were used for the erythrocyte counts; two counting chambers were filled from each pipette and a total of four chambers counted. Hemoglobin was estimated as oxyhemoglobin in an Evelyn photoelectric colorimeter. The mean cell volume and mean cell hemoglobin concentration were calculated according to the method of Wintrrobe.2 Our normal results are shown in table 2.

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The blood films were made from the venous blood contained in the needle when it was withdrawn from the vein. A tourniquet was not used. Two blood films were prepared. One was stained in the usual way with Wright's stain. The other was stained for one minute with Jenner's stain and then gently rinsed in distilled water. When thoroughly dry, the second film was immersed for one minute in a jar containing aqueous eosin. The Jenner's stain was made by dissolving 0.5 Gm. of Jenner's powdered stain in 100 ml. of pure methyl alcohol. The aqueous eosin stain was made by dissolving 1 Gm. of eosin in 100 ml. of distilled water.

The mean cell diameter was estimated by projecting the erythrocytes of the specially stained venous blood smear on paper at a magnification of 2,000 diameters. The outlines of 200 cells, chosen at random, were traced on the paper and their diameters were measured. The mean of the diameters of the 200 cells and the standard deviation of the mean were calculated. The results obtained by measuring 200 cells were found to be almost as accurate as those obtained by measuring the 500 cells as advised by Price Jones. Our normal mean cell diameter was below 7.60 μ. Anisocytosis was expressed as the spread of individual cell diameters around the mean of all cell diameters of the individual blood film. Therefore anisocytosis was defined as the standard deviation of the mean diameter. The normal anisocytosis was between 0.34 and 0.56 μ.

The mean cell thickness was calculated according to the volume/area formula:

\[ \text{MCT} = \frac{\text{MCV}}{\pi \times \text{MCD}^2} \frac{4}{3} \]

Iliac marrow films were obtained in 84 patients of whom 27 had thin macrocytosis. The type of erythrocyte maturation was determined by direct inspection of the film and categorized, by nuclear structure, as normoblastic, macronormoblastic, atypical megaloblastic or megaloblastic. In 9 patients with thin macrocytosis, the size of the polychromatic-staining nucleated red cell and its nucleus were measured. The polychromatic nucleated red cell was chosen for special study because in normoblastic and macronormoblastic marrow films the earlier forms, basophilic and proablasts, are not always present in sufficient numbers to allow comparison of size or nuclear structure. However, in all bone marrow films regardless of the type of maturation, the polychromatic-staining nucleated red cells were present in adequate numbers for comparison.

The hepatic vitamin B₁₂ content was estimated in 14 patients from liver tissue obtained at autopsy and compared with the presence and absence of macrocytosis in the patients. The estimation was performed by a microbiologic assay using Lactobacillus leichmannii.

Transfusion studies were carried out in 4 patients and 4 controls to determine whether transfused erythrocytes of normal size became macrocytic in the circulation of patients with macrocytosis. The donor erythrocytes were type O and the recipient's erythrocytes type A or B. At various periods after transfusion, the donor cells were recovered, counted and their diameters measured. The method of recovery was as follows:

Ten ml. of venous blood were collected without trauma and without a tourniquet. Five ml. were collected in an oxalate bottle for the Ashby count. Five ml. were used for cell measurement. The blood for cell measurement was placed in an Erlenmeyer flask containing 5 glass beads. The flask was gently rotated until a clot formed. Equal amounts of the defibrinated blood and of the appropriate antisera were centrifuged slowly for 10 minutes. The tube was then gently tapped to dislodge unagglutinated cells from the agglutinated cell mass. This centrifugation and tapping was repeated twice more. Drops of serum containing unagglutinated cells were smeared on glass slides, stained and the diameters of the cells measured as described above.

The mean cell diameter of the transfused cells was measured twice weekly from the time of the initial transfusion until the cells disappeared from the recipient's circulation, as determined by the Ashby counts.
The Three Types of Macrocytosis

Analysis of the blood films, indices and cell measurements of 222 patients showed:

1. The erythrocytes of 55 patients were of normal size; the mean cell diameter was below 7.60 μ and anisocytosis (standard deviation) was not greater than 0.56 μ.

2. Thirty patients had erythrocytes with a normal mean cell diameter but had increased anisocytosis ranging from 0.57 to 0.99 μ; this indicated that both macrocytes and microcytes were present.

3. One hundred and thirty seven patients or 62 per cent of the entire group had erythrocytes with a mean cell diameter of 7.60 μ or greater. These were said to have a macrocytic blood picture or macrocytosis.

4. The blood films of those patients with macrocytosis contained three different macrocytes; a thin macrocyte, a thick macrocyte and a target macrocyte. Just as leukocytosis might be described as neutrophilic, basophilic or eosinophilic, depending on the predominant leukocyte present, so macrocytosis has been defined according to the predominant macrocyte present. Thin macrocytosis is a blood condition in which all the macrocytes are thin. Thick macrocytosis is a condition in which both thick and thin macrocytes are present, the thick macrocytes being present in sufficient numbers to raise the mean cell volume to 110 μ³ or more. Target macrocytosis is a condition in which 10 per cent or more of the thin macrocytes have undergone target changes. The three types of macrocytosis are shown in figure 1.

The frequency of each type of macrocytosis varied with the type of hepatic disease (fig. 2 and table 1). The over-all frequency was as follows: Thin macrocytosis was found in 59 per cent of patients with macrocytosis (81 patients), target macrocytosis in 29 per cent (39 patients) and thick macrocytosis in 12 per cent (17 patients).

Thin Macrocytosis

Blood: In thin macrocytosis all the macrocytes are thin macrocytes. The thin macrocyte is an erythrocyte with an increased diameter (7.60 μ or greater), a decreased thickness and a normal volume (table 2). Compared to the normal erythrocyte it is a "flattened" cell. As the flattening becomes greater, the diameter of the cell increases (fig. 3). The shape of the thin macrocyte is round. The hemoglobin is distributed uniformly throughout the cell and the biconcave shape of the normal erythrocyte is not seen. The leukocytes and platelets are normal in patients with thin macrocytosis.

The patients with thin macrocytosis did not have a severe anemia. In 31 patients the erythrocyte count was greater than 4 million, in 38 patients it was between 3 and 4 million, and in only 12 patients was it less than 3 million erythrocytes per cu. mm.

The degree of anisocytosis varied independently of the degree of macrocytosis. Twenty-five patients had erythrocytes which were uniformly enlarged and had anisocytosis of normal degree. Fifty-six patients had anisocytosis of varying degrees.
FIG. 1.—Normal erythrocytes and the three types of macrocytosis occurring in hepatic disease.
(a) Normal erythrocytes. R.B.C. count 5,120,000; MCD 7.16 microns; anisocytosis 0.49 microns; MCV 96 cubic microns.
(b) Thin macrocytosis. R.B.C. count 4,650,000; MCD 8.86 microns; anisocytosis 0.69 microns; MCV 101 cubic microns.
(c) Target macrocytosis. R.B.C. count 4,400,000; MCD 8.00 microns; anisocytosis 0.82 microns; MCV 101 cubic microns.
(d) Thick macrocytosis. R.B.C. count 1,600,000; MCD 8.41 microns; anisocytosis 0.82 microns; MCV 131 cubic microns.

Bone Marrow: The bone marrow films of 27 patients with thin macrocytosis were examined. Twenty-six films showed macronormoblastic maturation of erythrocytes and one an atypical megaloblastic maturation.

Macronormoblastic maturation differs from normoblastic or normal maturation in both the size and structure of the cell. At all stages of development both the cell and its nucleus are larger than normal. The structure of the nucleus is similar to the nucleus of normoblastic maturation but is less compact. This difference in compactness is best seen in the polychromatic-staining cells in which the chromatin is in distinct and widely separated wedges in contrast to the pyknotic nucleus of normal maturation.
One patient had maturation of the atypical megaloblastic type which has been described by Jones and which lies midway between macronormoblastic and megaloblastic maturation.

The mean diameter of the polychromatic-staining nucleated cells of nine patients with macronormoblastic maturation was 11.06 μ. The mean diameter of the polychromatic-staining cells of three patients who had no liver disease and normoblastic marrows was 9.73 μ. Thus, when macrocytosis is present in the peripheral blood, the cells in the marrow are also macrocytic.

**Etiology of Thin Macrocytosis**

**Transfusion Studies:** Transfusion studies were carried out in four patients with thin macrocytosis and in four control patients to determine whether normal size donor erythrocytes might change shape and become macrocytic while circulating in the blood of patients with macrocytosis. The results are shown in table 3. In no instance did the normal size donor erythrocytes become macrocytic. This observation indicated that there was no factor in the peripheral circulation of patients with macrocytosis capable of causing macrocytosis by altering the shape of normal erythrocytes.

**The Type of Hepatic Disease:** Thin macrocytosis was found in the blood of patients suffering from all types of hepatic disease (fig. 2 and table 1). The
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Table 1.—Hepatic Disease and the Three Types of Macrocytosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No. of Patients</th>
<th>No. Macrocytosis</th>
<th>Thin Macrocytosis</th>
<th>Target Macrocytosis</th>
<th>Thick Macrocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laennec's Cirrhosis</td>
<td>25 (a) Alcoholic</td>
<td>96</td>
<td>29</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(b) Nonalcoholic</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Acute Hepatitis</td>
<td>10</td>
<td>37</td>
<td>24</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Subacute Yellow Atrophy</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>1*</td>
</tr>
<tr>
<td>Biliary Cirrhosis</td>
<td>14</td>
<td>14</td>
<td>5</td>
<td>4</td>
<td>5†</td>
</tr>
<tr>
<td>Carcinoma of Liver, Pancreas or Gall Bladder</td>
<td>38</td>
<td>38</td>
<td>10</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Choledocholithiasis</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Other Benign Obstruction</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous†</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>222</td>
<td>222</td>
<td>85</td>
<td>81</td>
<td>39</td>
</tr>
</tbody>
</table>

*Target and thick types combined in 1 patient.
†Target and thick types combined in 3 patients.
Includes hepatic lobatum, 2 patients; hemochromatosis, 2 patients; primary amyloidosis, polyserositis, undiagnosed granulomata, 1 patient each.

Table 2.—Measurements of Erythrocytes from Normal People, from Patients with Hepatic Disease and No Macrocytosis and from Patients with Hepatic Disease and Thin Macrocytosis

<table>
<thead>
<tr>
<th></th>
<th>Normal Erythrocytes (22 Healthy People)</th>
<th>Normal Erythrocytes (81 Patients with Hepatic Disease but No Macrocytosis)</th>
<th>Thin Macrocytosis (81 Patients with Hepatic Disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Cell Diameter (Microns)</td>
<td>7.14 (6.80-7.49)</td>
<td>7.24 (6.60-7.59)</td>
<td>7.94 (7.60-8.90)</td>
</tr>
<tr>
<td>Mean Cell Thickness (Microns)</td>
<td>2.30 (1.9-2.6)</td>
<td>2.30 (1.8-3.0)</td>
<td>2.01 (1.6-2.3)</td>
</tr>
<tr>
<td>Anisocytosis (Microns)</td>
<td>0.45 (0.34-0.56)</td>
<td>0.53 (0.35-0.99)</td>
<td>0.60 (0.40-1.07)</td>
</tr>
<tr>
<td>Mean Cell Volume (Cubic Microns)</td>
<td>95 (86-100)</td>
<td>97 (75-113)</td>
<td>99 (73-110)</td>
</tr>
<tr>
<td>Mean Cell Hemoglobin Concentration (Per Cent)</td>
<td>31 (28-34)</td>
<td>30 (21-36)</td>
<td>30 (22-36)</td>
</tr>
<tr>
<td>Erythrocyte Count (Millions)</td>
<td>4.8 (4.10-5.60)</td>
<td>3.86 (2.10-5.86)</td>
<td>3.62 (1.60-5.40)</td>
</tr>
</tbody>
</table>

From the similarity of these figures it would appear that the type of hepatic disease was not of major importance in the etiology of thin macrocytosis. Further studies revealed that patients with fatty infiltration of the liver or with obstructive jaundice of short duration—for example, in patients who had a stone—did not have thin macrocytosis. In both of these conditions there was no parenchymal cell damage. Thus the important etiologic factor appeared to be damage to the hepatic parenchymal cells irrespective of the cause.
Severity of Hepatic Disease: An attempt was made to determine whether thin macrocytosis was related to the severity of hepatic disease as measured by physical abnormalities, abnormal biochemical tests of liver function and the number of deaths.

(a) Physical abnormalities: The five physical abnormalities chosen for the clinical assessment of the severity of hepatic disease were: jaundice, spider naevi, hepatomegaly, splenomegaly, ascites and/or edema. As patients with the more severe forms of hepatic disease usually have more of these abnormalities, a separate study was made in these cases. There was no relationship between the number of abnormalities and macrocytosis. However, when the relationship of each individual abnormality was considered with the presence or absence of thin macrocytosis (table 4), the results showed that thin macrocytosis was found more frequently in patients with ascites and/or edema than in those without this abnormality. There was no relationship to other abnormalities.

(b) Liver function tests: Each of eight biochemical tests shown in figure 4 were plotted against the mean diameter of the erythrocytes of the patients without macrocytosis and with thin macrocytosis. There was no relationship between them.

(c) Deaths: The number of deaths in patients with Laennec's cirrhosis during the hospitalization period was counted. There was no difference in the death rate between patients with and without thin macrocytosis.
Table 3.—Absence of Any Change in the Size of Normal Erythrocytes Transfused into the Circulation of Patients with Thin Macrocytosis of Hepatic Disease

<table>
<thead>
<tr>
<th>Type of Liver Disease</th>
<th>R.B.C. Diam. of Recipient's Cells</th>
<th>R.B.C. Diam. of Donor Cells Before Transfusion</th>
<th>R.B.C. Diam. of Donor Cells After Transfusion</th>
<th>No. of Days After Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mrs. A.B. Normal</td>
<td>6.99</td>
<td>6.87</td>
<td>6.82</td>
<td>70</td>
</tr>
<tr>
<td>Mrs. A.R. Untreated pernicious anemia</td>
<td>8.24</td>
<td>6.69</td>
<td>6.60</td>
<td>90</td>
</tr>
<tr>
<td>No. 72 Biliary cirrhosis (without macrocytosis)</td>
<td>7.47</td>
<td>6.87</td>
<td>6.67</td>
<td>47</td>
</tr>
<tr>
<td>No. 265 Laennec's Cirrhosis (Alcoholic—without Macrocytosis)</td>
<td>7.42</td>
<td>6.85</td>
<td>7.00</td>
<td>47</td>
</tr>
<tr>
<td>Macrocytosis Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 43 Subacute Yellow Atrophy</td>
<td>8.13</td>
<td>7.15</td>
<td>7.16</td>
<td>9</td>
</tr>
<tr>
<td>No. 91 Laennec's Cirrhosis (Alcoholic)</td>
<td>8.10</td>
<td>6.59</td>
<td>6.74</td>
<td>32</td>
</tr>
<tr>
<td>No. 171 Ca. head pancreas metastasis in liver</td>
<td>8.00</td>
<td>7.24</td>
<td>7.48</td>
<td>25</td>
</tr>
<tr>
<td>No. 223 Biliary Cirrhosis</td>
<td>7.86</td>
<td>6.77</td>
<td>6.80</td>
<td>36</td>
</tr>
</tbody>
</table>

Table 4.—Per Cent of Patients with Laennec's Cirrhosis With and Without Thin Macrocytosis Showing Physical Abnormalities With Chi Square and Probability Values for the Differences Between the Two Groups in Number Showing Each Abnormality

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>With Thin Macrocytosis %</th>
<th>Without Macrocytosis %</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>40.4 (47)</td>
<td>51.5 (33)</td>
<td>0.97</td>
<td>&gt;0.30</td>
</tr>
<tr>
<td>Spider Naevi</td>
<td>53.3 (45)</td>
<td>34.5 (29)</td>
<td>2.51</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>87.8 (41)</td>
<td>83.7 (29)</td>
<td>0.02*</td>
<td>&gt;0.80</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>28.9 (38)</td>
<td>34.5 (29)</td>
<td>0.23</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Ascites and/or Edema</td>
<td>66.7 (45)</td>
<td>33.3 (30)</td>
<td>8.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Corrected for continuity.

Nutritional Deficiency: Studies were conducted to determine if thin macrocytosis might be caused by a nutritional deficiency.

(a) Diets: The diet eaten in the several weeks preceding hospitalization by patients with Laennec's cirrhosis were analyzed. The results showed no relationship between a dietary deficiency and thin macrocytosis. Many patients with good diets had thin macrocytosis. Furthermore, other patients developed thin macrocytosis before any nutritional deficiency could possibly have occurred, for example, in patients with acute infectious hepatitis.

Peripheral neuritis was taken as evidence of vitamin deficiency. In patients with Laennec's cirrhosis, peripheral neuritis occurred no more frequently in those with thin macrocytosis than in those without thin macrocytosis.

(b) Treatment with folic acid, liver and vitamin B12: Sixteen patients received subcutaneously one of the following: vitamin B12 (100 µg. or more weekly), concentrated liver extract (40 units or more weekly) or folic acid
Fig. 4.—Erythrocyte size plotted against each of 8 biochemical tests of liver function. Each dot represents one patient. Note the absence of any correlation between cell size and degree of abnormality of each test.

(15 mg, three times weekly.)* In some patients macrocytosis disappeared, in some it decreased and in others it persisted.

There was no correlation between the drug given and the response of the patient. The improvement in blood counts and the decrease in macrocytosis,

*The vitamin B₁₂ and the folic acid were kindly supplied by the Lederle Company.
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TABLE 5—Absence of Any Correlation Between Hepatic Vitamin B\(_{12}\) Content and Macrocytosis. Figures Refer to Number of Patients With and Without Macrocytosis

<table>
<thead>
<tr>
<th>V/Gm. B(_{12}) in liver</th>
<th>No. without Macrocytosis</th>
<th>No. with Macrocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above 500</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>400-500</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Below 400</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

where present, was no greater than we have observed in similar patients who did not receive these drugs. The improvement was due to improved hepatic function rather than to the drugs received. Where there was no improvement in hepatic function there was no decrease in macrocytosis.

(c) **Hepatic vitamin B\(_{12}\):** Measurements were made of the total vitamin B\(_{12}\) content of livers, obtained at autopsy, from six patients without macrocytosis and from eight patients with thin macrocytosis (table 5). The measurements were kindly made by Dr. H. D. Bett of the Connaught Laboratories. No significant correlation was observed between the presence of thin macrocytosis and a low hepatic vitamin B\(_{12}\). Thin macrocytosis occurred in some patients with a high hepatic vitamin B\(_{12}\) content (above 500 \(\mu\)g.) and was absent in other patients with a low hepatic vitamin B\(_{12}\) content (below 400 \(\mu\)g.).

**Miscellaneous Observations**

(a) **Anemia:** The presence and the degree of thin macrocytosis was found to vary independently of the erythrocyte count. The concentration of hemoglobin (MCHC) in patients with thin macrocytosis did not differ from that of patients without macrocytosis.

(b) **Reticulocyte count:** Because of the possibility that the macrocytes might actually be reticulocytes, reticulocyte counts were done in all patients. These counts were unrelated to the presence or absence of thin macrocytosis.

(c) **Gastric hydrochloric acid:** Free gastric hydrochloric acid was present in seven of nine patients with thin macrocytosis. Achlorhydria, therefore, was not a causative factor in the development of thin macrocytosis.

(d) **Plasma volume:** The plasma volume of 12 patients with and without thin macrocytosis was measured by the Evans Blue dye technic. The plasma volume was elevated in four of five patients with thin macrocytosis and in five of seven patients without thin macrocytosis. Since no significant difference was observed between the two groups, it was believed that changes in the plasma volume did not contribute to the development of thin macrocytosis.

**Discussion**

The frequency of macrocytosis in hepatic disease reported in the literature varies greatly: Wintrobe, 32.6 per cent\(^2\); Cheney, 36.8 per cent\(^5\); Meulengracht, 56 per cent\(^8\); Larsen, 59 per cent\(^5\); Jarrold, 66 per cent\(^10\); Berman, 75 per cent\(^11\); and Rosenberg, 91 per cent\(^12\). These differences are partly due to the type of hepatic disease chosen for the study but principally to the methods used for measuring cell size. All the workers except Meulengracht and Lar-
sen used either the mean cell volume or halometry to measure cell size. Their results varied widely. When the size of the cell was directly measured, as in Meulengracht's and Larsen's method and in the present series, the results were similar—56, 59 and 62 per cent, respectively.

Since the first recognition of macrocytosis in hepatic disease, its cause has been the subject of much speculation. The various theories which have arisen might be divided into two groups: "central" and "peripheral" (Larsen). These theories have been based on studies in which macrocytosis was not subdivided into the three types. However, from inspection of the reports it would appear that the macrocytosis was predominately of the thin type. Those workers who supported the "central" theory believed that macrocytes arose from defective maturation of erythrocytes in the bone marrow. This might occur either early or late in the process of cell development. A variant of this theory was that the macrocytosis was merely the result of an outpouring of large reticulocytes. Those workers who favored the "peripheral" theory believed that erythrocytes were normal when delivered from the bone marrow but that they underwent swelling, or flattening, in the peripheral circulation and thus became macrocytic.

Our results indicate that thin macrocytes are "born abnormal" and are the result of an abnormal maturation process. The statement is based on the following evidence:

1. When thin macrocytosis is present in the peripheral blood the nucleated red cells in the bone marrow are macrocytic. The changes in cell size and nuclear structure of the nucleated red cells indicate a new pattern of cell development, the result of the same flattening process evident in the mature cell.

2. Erythrocytes of normal size, transfused into the circulation of patients with macrocytosis, do not themselves become macrocytic.

3. In the majority of our patients with macrocytosis, anisocytosis is increased. If the thin macrocytes are caused by some factor operating in the peripheral circulation that alters the shape of normal erythrocytes, all the erythrocytes will be affected equally and anisocytosis will not be increased. As the course of macrocytosis was followed in the individual patient it was apparent that the degree of anisocytosis varied. As macrocytosis developed, anisocytosis increased. The peak degree of anisocytosis was reached when half the erythrocytes were macrocytes and half normocytes. When all the cells were macrocytes, anisocytosis disappeared. As the macrocytes disappeared and were replaced by normocytes, anisocytosis again increased. It is difficult to explain these changes in anisocytosis on a peripherally acting factor which alters the shape of cells.

4. The shape of the macrocytes is not caused by swelling or imbibition of fluid. Macrocytes of the thin type have been shown to be thin cells. Furthermore, normal erythrocytes exposed to hypotonic salt solutions do not develop increased diameter; their volume increases but their diameter remains unchanged.
(5) The macrocytes are not reticulocytes and do not show the characteristic reticulum when they are stained with vital stain. In our patients there is no relationship between the reticulocyte count and the degree of macrocytosis.

For these reasons we believe that the thin macrocyte is an abnormal erythrocyte produced in the bone marrow by a macronormoblastic (or rarely atypical megaloblastic) marrow.

The cause of this change in bone marrow maturation is not a deficiency of any known hematopoietic substance. There is no evidence of a nutritional or a vitamin deficiency. The pre-hospitalization diets, the frequency of peripheral neuritis, the iron content of the erythrocytes, and the hepatic vitamin $B_{12}$ level are the same in patients without macrocytosis as in those with thin macrocytosis. The parenteral administration of liver, folic acid or vitamin $B_{12}$ does not correct thin macrocytosis.

Thin macrocytosis does not appear to be due to any specific hepatic disease. It may follow all types of hepatic disease providing hepatic parenchymal cells are damaged. If there is no damage to hepatic parenchymal cells, as, for example, in fatty infiltration of the liver or obstructive jaundice of short duration, such as for a stone removed in a week or two, thin macrocytosis does not occur.

The severity of the hepatic parenchymal cell disease process judged clinically and by liver function tests and by the death rate appears to be unrelated to the presence or absence of thin macrocytosis.

For these reasons thin macrocytosis is regarded as a specific response to nonspecific hepatic disease. The link between the hepatic disease and the bone marrow changes giving rise to thin macrocytosis is unknown. Also unknown is the explanation why some but not all patients with hepatic disease develop thin macrocytosis.

A thought-provoking suggestion is that of Berman and his co-workers. They attribute macrocytosis to chronic hemolysis, which in turn is due to hypersplenism. In support of this theory is the frequency with which increased destruction of erythrocytes accompanies hepatic disease. However, it is difficult to determine which is the “cart” and which is the “horse.” Does chronic hemolysis stimulate a macronormoblastic reaction in the marrow and thin macrocytosis or does hemolysis occur because the thin macrocytes are abnormal cells and therefore destroyed prematurely? Does hemolysis cause macrocytosis or does macrocytosis cause hemolysis?

The observation of Hall is of interest in this respect. Using chromium-tagged erythrocytes, he found a shortened erythrocyte life span in one half of 14 patients with hepatic disease, but no correlation between the rate of erythropoiesis and the mean cell diameter. This observation suggests that the macrocytes are not caused by increased cell destruction. While chronic hemolysis may play a part in the etiology of thin macrocytosis there would seem to be other more important factors operating. Further work should be directed to the type of hemoglobin present in thin macrocytosis and to the explanation of why thin macrocytosis is more common in patients with ascites and edema.
A macrocytic blood picture was present in 62 per cent of 222 patients with various types of hepatic disease.

Three different types of macrocytes were present in the blood films: a thin macrocyte, a target macrocyte and a thick macrocyte. Just as leukocytosis might be described as neutrophilic, basophilic or eosinophilic according to the predominate leukocyte present, so macrocytosis has been defined according to the predominate macrocyte present: thin macrocytosis, target macrocytosis and thick macrocytosis. This report deals with the first type, thin macrocytosis.

1. Thin macrocytosis is, by definition, the type of macrocytosis in which all the macrocytes are thin macrocytes. It is the commonest type of macrocytosis (59 per cent of all types of macrocytosis).

2. The thin macrocyte is a flattened erythrocyte. It has the same volume as the normal erythrocyte but is broader and thinner. As the diameter of the cell increases its thickness decreases.

3. Thin macrocytosis is caused by an alteration in erythropoiesis in the bone marrow and not by a flattening or swelling of normal erythrocytes in the peripheral circulation, as suggested by various workers, because:
   (a) Normal size erythrocytes transfused into the circulation of patients with thin macrocytosis do not become macrocytic.
   (b) Anisocytosis is increased.
   (c) The nucleated red cells in the marrow are macrocytic.

4. The thin macrocyte is produced by a macronormoblastic (or rarely atypical megaloblastic) type of maturation in the bone marrow.

5. This altered erythrocyte maturation which gives rise to thin macrocytosis is a specific response to nonspecific hepatic parenchymal cell damage. It occurs in a significant percentage of patients suffering from all types of parenchymal and obstructive hepatic disease. It does not occur in patients with simple fatty liver or obstructive jaundice of short duration where hepatic cells are not damaged. A deficiency of any known hematopoietic factor plays no part in the etiology of this disorder.
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volumine como le erythrocyto normal, sed illo es plus large e plus tenue. In tanto que le diametro del cellula se augmenta, assi etiam su spissitate se reduce.

3. Leptomacrococytosis es causate per un alteration del erythropoiese in le medulla ossee e non per un applattation o expansion de erythrocytos normal in le circulation peripheric. Iste ultime interpretation, acceptate per plure recercatores, non es correcte proque (a) erythrocytos de dimensiones normal, quando transmunitionate in le circulation de patientes con leptomacrococytosis, non deveni macrocytic, (b) le anisocytosis es augmentate, e (c) le erythrocytos nucleate in le medulla es macrocytic.

4. Le macrocyto tenue es producite per un typo macronormoblastic o, in casos infrequente, atypicamente megaloblastic de maturation in le medulla ossee.

5. Iste alterate maturation del erythrocytos, le qual resulta in leptomacrococytosis, es un responsa specific a nonspecific lesions of cellulas hepatocparenchymal. Illo occurre in un procentage significative de patientes qui sufre del un o del altere typo de morbo hepatic parenchymal o obstructive. Illo non occurre in patientes con simple hepate grasse o con jalnessa obstrucrive quando le cellulas hepatic non es lesionate. Carentia de un del cognoscite factores hematopoietic non pote esser incriminate in le etiologia de iste disordine.

REFERENCES

The Macrocytosis of Hepatic Disease. I. Thin Macrocytosis

JOHN BINGHAM