HEMOGLOBIN PRODUCTION FACTORS IN THE HUMAN LIVER

III. ANEMIAS—PRIMARY, APLASTIC AND SECONDARY—LEUKEMIAS

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The various anemias and leukemias with consequent anemia present material of especial interest for a study of this character. Biological assay of this human liver material shows high values for primary and aplastic anemias but low normal values for the secondary anemias due to loss of blood as well as for leukemias. A number of interesting points emerge from a study of this material and call for discussion.

Pernicious anemia at the present time is being subjected to intensive scrutiny by the physiologist, chemist and clinician and considerable progress is being made toward a more complete understanding of this disease. The findings tabulated below will be surprising or not, depending upon the conception which the reader may hold relating to the pathogenesis of pernicious anemia. On the basis of anatomical findings it was suggested (3) more than 10 years ago that pernicious anemia could be explained best as due to some lack of unknown material which might be responsible for red cell stroma production. It was pointed out that everywhere there was a large excess of pigment material in the red cells, body fluids and tissues, but this pigment material could not be used because of this lack of some essential factor. At the time this suggestion was made, the general belief in some unknown toxic substance responsible for the anemia was almost universal.

The tabulated data (Table 31) below give support to this hypothesis and show a definite excess of hemoglobin building factors stored in the liver in pernicious anemia in spite of the fact that death may be due to lack of red cells and hemoglobin. Surely there is a maximal stimulus for the production of red cells and hemoglobin but some essential keystone is missing and the other building stones cannot be
used. This also explains the well known fact that recovery and hemoglobin reconstruction are so spectacular in pernicious anemia when this missing factor is supplied—a large surplus of all other needed building materials being at hand. By contrast the recovery from secondary anemia is much slower as all these building materials must be produced within the body largely from food intake and this may consume a good deal of time.

Richter, Ivy and Kim (2) have made interesting observations relating to the liver in pernicious anemia. They find that the liver in an untreated case of pernicious anemia contained no factors which are abundant in the normal liver and promote a remission in human cases of pernicious anemia. Also the treated pernicious anemia case contains in its liver the material which promotes a remission in another case of pernicious anemia.

Aplastic anemia is due to lack of red bone marrow to furnish needed red cells. What causes the red marrow to shrivel to a mere remnant is not known. In this disease it is observed (Table 32) that the liver stores excess of hemoglobin building material and this is not surprising as there is no outlet for this material.

In secondary anemia we expected to find that these hemoglobin factors were much reduced if not almost completely exhausted. Much to our surprise it was found (Table 33) that in anemia due to loss of blood the liver contained a low normal concentration of these hemoglobin producing factors. This is true for man (Table 33) and for the horse (unpublished data) and therefore probably applies to other warm blooded animals.

Secondary anemia due to blood destruction within the body may present a different picture. The last case in Table 33 illustrates this point but more observations are needed. It is not surprising that the liver stores iron and hemoglobin producing factors when red cells are being destroyed in the body.

Leukemia is almost always associated with more or less anemia, the terminal hemoglobin figures being about 30–40 per cent of normal. Sometimes blood transfusions with or without bleeding will modify the picture. When one examines the bone marrow it is not difficult to visualize the anemia as due to marrow disturbance. The red cell producing chain of cells in the marrow is crowded by the great mass
of white cells and its function probably impaired. Biological assay shows that the leukemic liver and the liver of secondary anemia stand about on a par, estimated per gram gross liver weight. There is no evidence that these abnormal white cells which infiltrate the leukemic liver and increase its weight contribute any conspicuous amount of hemoglobin producing factors. If we say that these white cells "dilute" the potency of the liver cells, the figures for the leukemic liver potency would rise to normal.

The iron analyses deserve particular attention. The cases of secondary anemia due to bleeding show very low values (5.3 mg. per cent) as is to be expected and this is in harmony with the experiments in animals recently reported (1). Leukemia shows about the normal iron content but if we allow for the large size of the liver the actual liver parenchyma may contain a little more iron than the normal human control. The iron analyses for primary and aplastic anemias are very high (5–10 times normal) and we may believe this is a storage of material of use for hemoglobin construction but in these diseases there is no outlet for this material in normal hemoglobin production. The figures are higher in aplastic anemia than in primary anemia which is evidence against hemoglobin destruction being a large factor in primary anemia. The iron concentration in pernicious anemia drops rapidly with specific liver therapy.

Table 31. Diagnosis and histological description of liver.

Liver—autopsy weight 1050 gm.—laboratory specimen 900 gm.
Histological specimen—typical untreated pernicious anemia—yellow pigment very abundant in liver cells and Kupffer cells (much of this pigment contains iron). A little liver cell atrophy, no fat, slight portal fibrosis.

A-1800. Pernicious anemia relapse—hemoglobin 20 per cent—60 yrs.
Liver—autopsy weight 1850 gm.—laboratory specimen 1760 gm.
Histological specimen—autopsy typical of pernicious anemia without liver treatment. Liver shows the usual pigment in gross and histologically. Iron staining pigment is abundant in the liver cells and also Kupffer cells. No liver necroses.

A-1045. Pernicious anemia—hemoglobin 30 per cent—pneumonia—during last 3 months had been taking liver irregularly—51 yrs.
Liver—autopsy weight 2150 gm.—laboratory specimen 1920 gm.
Histological specimen—the liver cells in general stain well and contain a finely granular yellow pigment which in part gives a positive stain for iron. This pigment is more conspicuous in the central part of the lobules. In a few lobules are noted small central necroses. The Kupffer cells show very little pigment.

X-2479. Pernicious anemia—pyelonephritis—no information about liver therapy—75 yrs.
Liver—autopsy weight 1500 gm.—laboratory specimen 1350 gm.
Histological specimen—central necroses are conspicuous and occupy perhaps 1/5 of the liver lobule. The liver cells in the peripheral 1/2 of the lobule are normal. There is abundant fine granular yellow pigment giving a strong iron stain in these liver cells. Kupffer cells contain no pigment.

Liver—autopsy weight 1260 gm.—laboratory specimen 1100 gm.
Histological specimen—liver cells almost normal. There is some finely granular yellow pigment but very little of this gives a stain for iron. Kupffer cells contain fine pigment granules which stain sharply for iron. This case had liver extract for 10 days with the usual blood improvement and this explains some of the pigment lack in liver and kidney.

A-425. Pernicious anemia—senility—hemoglobin 30 per cent—78 yrs.
Patient in Hospital May 25, 1927—hemoglobin 25 per cent—and improved on treatment. Left Hospital August 12, 1927—hemoglobin 65 per cent. Readmitted in moribund state and died in 24 hrs.—no therapy for over 1 mo.
Liver—laboratory specimen 1050 gm.
Histological specimen—liver cells in general normal. Few large fat droplets in periportal liver cells, some atrophy, lipochrome moderate. Kupffer cells numerous and full of iron-containing pigment.

Liver—autopsy weight 1000 gm.—laboratory specimen 930 gm.
Histological specimen—liver cells in general are normal except for fine granular yellow pigment in their protoplasm. Kupffer cells show similar pigment grains in small amount. Some of this pigment gives a stain for iron. A few liver cells show fat droplets. This case had been benefited by liver therapy and at death the hemoglobin was 80 per cent.

A-1173. Pernicious anemia—hemoglobin 25 per cent—bronchopneumonia—no liver therapy during last 6 months of life—24 hrs. in hospital—74 yrs.
Liver—laboratory specimen 1130 gm.
Histological specimen—liver cells in general are normal. There is abundant lipo-
ochrome pigment but no iron staining pigment within the liver epithelium. There
is iron staining pigment in the Kupffer cells. This lack of pigment is probably
due to preceding liver therapy and blood improvement 10 mos. before death.

Table 31 contains 8 cases of primary pernicious anemia in which
various amounts of liver therapy had been given during life. The
first case (A-371) is of especial significance as this man had had no liver
therapy at any time and presented the classical picture of pernicious
anemia which is very rarely seen today. The iron analyses show
maximal figures (162 mg. per cent) as does the biological assay for liver

TABLE 31

<table>
<thead>
<tr>
<th>Number</th>
<th>Cause of death</th>
<th>Iron content human liver</th>
<th>Liver intake per day</th>
<th>Hemoglobin output per 7 days feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fresh tissue</td>
<td>daily intake</td>
<td>Human</td>
</tr>
<tr>
<td>A-371</td>
<td>No therapy</td>
<td>162.0</td>
<td>208</td>
<td>129</td>
</tr>
<tr>
<td>A-1800</td>
<td>No therapy</td>
<td>36.7</td>
<td>92</td>
<td>239</td>
</tr>
<tr>
<td>A-1045</td>
<td>Sl. therapy</td>
<td>47.3</td>
<td>130</td>
<td>290</td>
</tr>
<tr>
<td>X-2479</td>
<td>Nephritis</td>
<td>36.5</td>
<td>70</td>
<td>190</td>
</tr>
<tr>
<td>A-1472</td>
<td>Sl. therapy</td>
<td>17.5</td>
<td>27</td>
<td>158</td>
</tr>
<tr>
<td>A-425</td>
<td>Sl. therapy</td>
<td>34.8</td>
<td>52</td>
<td>150</td>
</tr>
<tr>
<td>A-1122</td>
<td>Embolism</td>
<td>24.6</td>
<td>33</td>
<td>130</td>
</tr>
<tr>
<td>A-1173</td>
<td>No therapy</td>
<td>—</td>
<td>—</td>
<td>160</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>51.3</td>
<td>87</td>
<td>182</td>
</tr>
</tbody>
</table>

potency (420 per cent) and this great excess of iron is a factor in this
high figure for hemoglobin production. In the various tables given
in these papers, a biological assay for liver potency above 300 per cent
is unusual and figures above 400 per cent have been observed only
twice—one case of thyrotoxicosis (Table 4, Paper I) and this case
of primary anemia.

No liver therapy in the final relapse is recorded in 2 other cases
(A-1800 and A-1173). In these cases liver therapy had been given in
earlier periods but not during the last few weeks or months preceding
death from a relapse. The liver analyses for iron exclusive of Case
A-371 show an average of 33 mg. per cent or about 3 times normal and the liver values for hemoglobin production factors are high. The lowest value in hemoglobin production factors appears in Case X-2479 (Table 31) which was complicated by a pyelonephritis which caused death. It is probable that this acute condition would reduce somewhat the content of hemoglobin producing factors in the liver—compare Tables 3 and 4 (Paper I).

In a comparative study of the cases in Table 31 we must keep in mind that all these cases show senile atrophy and the normal mean for this type of case is given in Table 2 (Paper I) as 117 per cent which contrasts with 218 per cent in Table 31. If we exclude the unusual figure in the first case, the average value for hemoglobin producing factors is 182 per cent. Even if we choose to explain some of this difference in potency on the basis of the contained iron we have an excess coming from unknown factors stored within the liver parenchyma cells. We assume that this excess represents building stones which are suitable for hemoglobin construction in the normal body but have no outlet in primary anemia.

Table 32. Diagnosis and histological description of liver.

Liver—laboratory specimen 1750 gm.
Histological specimen—swollen liver cells, some central liver cells are injured, few necroses. Portal liver cells full of yellow pigment. Few Kupffer cells show pigment. Both liver cells and Kupffer cells show iron-containing pigment.

A-1555. Aplastic anemia—purpura—transfusions—hemoglobin 40–60 per cent—57 yrs.
Liver—autopsy weight 1490 gm.—laboratory specimen 1300 gm.
Histological specimen—liver cells show many large and small fat droplets. They contain much yellow granular pigment much of which gives a stain for iron. Kupffer cells are large and contain iron staining pigment (transfusions). Bile ducts and stroma normal.

A-924. Aplastic anemia—hemoglobin 20 per cent—40 yrs.
Liver—laboratory specimen 1640 gm.
Histological specimen—the liver cells in general are normal and contain a little lipochrome pigment. There are scattered small central necroses. A moderate degree of fat deposit in liver cells in the mid zone is noted. No iron staining pigment found.
A-188. Aplastic anemia—terminal infection—4½ yrs. 
Liver—autopsy weight 910 gm.—laboratory specimen 900 gm. 
Histological specimen—liver cells normal. Lipochrome in liver cells. Large Kupffer cells are numerous and some contain iron staining pigment.

Table 32 shows 4 typical cases of aplastic anemia. Many transfusions had been given in the first 2 cases and this may explain in part the very high values for iron (78 and 105 mg. per cent). These high iron values will explain a part of the large excess of hemoglobin production observed in these cases. Moreover we should remember that this group represents a lower age period and consequently the normal base line should be 160 per cent (compare Table 1) for accurate control. When we make these allowances the aplastic anemias are not as much

<table>
<thead>
<tr>
<th>Number</th>
<th>Cause of death</th>
<th>Iron content liver</th>
<th>Liver intake per day</th>
<th>Hemoglobin output per 7 days feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fresh tissue mg. per cent</td>
<td>daily intake gm.</td>
<td>Human gm.</td>
</tr>
<tr>
<td>A-376</td>
<td>Bleeding</td>
<td>78.0</td>
<td>196</td>
<td>250</td>
</tr>
<tr>
<td>A-1555</td>
<td>Bleeding</td>
<td>105.0</td>
<td>195</td>
<td>185</td>
</tr>
<tr>
<td>A-924</td>
<td>Anemia</td>
<td>26.8</td>
<td>63</td>
<td>230</td>
</tr>
<tr>
<td>A-188</td>
<td>Infection</td>
<td>—</td>
<td>—</td>
<td>120</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>69.9</td>
<td>151</td>
<td></td>
</tr>
</tbody>
</table>

above normal by biological assay of the hemoglobin producing factors as is true for pernicious anemia. It may be argued that aplastic anemia does not last as long as primary anemia and that the liver therefore does not accumulate as large a surplus store as is true for pernicious anemia. In both cases there is no outlet for this hemoglobin production material—in primary anemia due to deficiency factors and in aplastic anemia due to lack of red marrow parent cells.

Table 33. Diagnosis and histological description of liver.

A-969. Anemia, secondary to carcinoma of cervix with bleeding—hemoglobin 25 per cent—43 yrs.
Liver—autopsy weight 1300 gm.—laboratory specimen 1030 gm.
Histological specimen—necrosis of central type involving 1/5 or less of liver parenchyma. Fat droplets numerous in mid zone. The liver cells in the peripheral half of lobules appear normal. Lipochrome pigment scanty.

A-1333. Anemia secondary to bladder carcinoma—hemoglobin 15 per cent—51 yrs.
Liver—autopsy weight 1220 gm.—laboratory specimen 1030 gm.
Histological specimen—liver cells in general normal—no pigment seen. Fatty degeneration is found in the centers of lobules. The portal tissue is increased and filled with mononuclears. The casts in some of the bile canaliculi are very dark and of some duration, probably related to the cholelithiasis and an earlier cholangitis.

A-1461. Anemia secondary to cancer of stomach—cirrhosis—hemoglobin 40 per cent—47 yrs.
Liver—autopsy weight 1800 gm.—laboratory specimen 1700 gm.
Histological specimen—liver lobules are relatively normal and hepatic epithelium not disturbed and practically normal but for some fatty degeneration. An annular type of portal cirrhosis is observed but the connective tissue is not dense and the distortion but slight. This scar tissue contains many mononuclears but few bile duct sprouts. No pigment is observed anywhere.

A-1971. Anemia secondary to carcinoma of stomach—hemoglobin 50 per cent—64 yrs.
Liver—autopsy weight 1200 gm.—laboratory specimen 1175 gm.
Histological specimen—liver cells in peripheral half of lobule show large fat vacuoles. Liver cells in center of lobule normal. Lipochrome scanty. Portal tissue slightly increased and infiltrated with mononuclears.

A-1375. Anemia secondary to carcinoma of stomach—60 yrs.
Liver—autopsy weight 1750 gm.—laboratory specimen 1600 gm.
Histological specimen—the liver cells are practically normal except for rather abundant lipochrome pigment. Kupffer cells normal.

A-1372. Anemia secondary to carcinoma of kidney—hemoglobin 50 per cent—57 yrs.
Liver—autopsy weight 2160 gm.—laboratory specimen 2000 gm.
Histological specimen—liver lobules show central congestion and cell atrophy with abundant lipochrome pigment. Marginal half of lobules essentially normal.

A-1081. Anemia secondary to hypernephroma—hemoglobin 50 per cent—60 yrs.
Liver—autopsy weight 1850 gm.—laboratory specimen 1540 gm.
Histological specimen—practically normal hepatic epithelium. Lipochrome pig-
ment is fairly abundant, particularly in the central portion of the lobules. Kupffer cells show no pigment.

A-1192. Anemia secondary to carcinoma of prostate—hemoglobin 40 per cent—uremia—68 yrs.
Liver—autopsy weight 1680 gm.—laboratory specimen 1650 gm.
Histological specimen—liver cells in general are normal. Lipochrome pigment noted in center of lobules where also liver cells show a few fat droplets. Kupffer cells normal.

A-1625. Anemia secondary to gastric carcinoma—hemoglobin 58 per cent—61 yrs.
Liver—autopsy weight 1550 gm.—laboratory specimen 1480 gm.

| TABLE 33
Secondary Anemia |
|------------------|

<table>
<thead>
<tr>
<th>Number</th>
<th>Cause of death</th>
<th>From content human liver</th>
<th>Liver intake per day</th>
<th>Hemoglobin output per 7 days feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-969</td>
<td>Ca. cervix</td>
<td>2.9 mg. 4 gm. 150 gm. 300 gm. 26 gm. 52 gm. 100 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1333</td>
<td>Ca. bladder</td>
<td>3.3 mg. 5 gm. 145 gm. 300 gm. 35 gm. 52 gm. 140 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1461</td>
<td>Ca. stomach</td>
<td>4.0 mg. 10 gm. 240 gm. 300 gm. 30 gm. 38 gm. 100 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1971</td>
<td>Ca. stomach</td>
<td>4.6 mg. 8 gm. 167 gm. 300 gm. 46 gm. 58 gm. 144 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1375</td>
<td>Ca. stomach</td>
<td>2.8 mg. 6 gm. 225 gm. 300 gm. 23 gm. 45 gm. 68 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1372</td>
<td>Ca. kidney</td>
<td>7.7 mg. 23 gm. 300 gm. 300 gm. 53 gm. 35 gm. 152 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1081</td>
<td>Hypernephroma</td>
<td>— mg. — gm. 220 gm. 300 gm. 52 gm. 35 gm. 200 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1192</td>
<td>Ca. prostate</td>
<td>10.6 mg. 25 gm. 220 gm. 300 gm. 22 gm. 30 gm. 100 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1625</td>
<td>Ca. stomach</td>
<td>6.7 mg. 14 gm. 210 gm. 300 gm. 44 gm. 49 gm. 129 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-1618</td>
<td>Endocarditis</td>
<td>23.2 mg. 29 gm. 125 gm. 300 gm. 65 gm. 72 gm. 217 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>................</td>
<td>7.3 mg. 14 gm. 200 gm.</td>
<td></td>
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</tr>
</tbody>
</table>

Histological specimen—liver lobules show a central hyaline necrosis involving perhaps 10–20 per cent of the hepatic epithelium. Liver cells elsewhere show a little fat and lipochrome pigment. Kupffer cells, stroma and bile ducts normal.

A-1618. Subacute rheumatic endocarditis—hemoglobin 35 per cent—34 yrs.
Liver—autopsy weight 900 gm.—laboratory specimen 880 gm.
Histological specimen—liver cells show atrophy and many fat droplets but very little pigment. Kupffer cells show large amounts of pigment and are greatly enlarged. This pigment gives heavy stain for iron. The stroma and bile ducts normal. Anemia due to red blood cell destruction in body, not loss of hemoglobin from body.
Table 33 shows 9 cases of severe secondary anemia due to loss of blood and one case due to blood destruction within the body. Biological assay of the hemoglobin production factors in these 10 cases shows low normal values (135 per cent) and if we exclude the single case of hemolytic anemia, the average for the 9 cases of anemia due to loss of blood from the body is 126 per cent. This shows how tenaciously the liver cell holds to these hemoglobin production factors even in the face of a long continued severe anemia due to loss of blood.

In contrast the iron analyses show that the normal iron store is considerably reduced by this type of anemia. The normal iron value (12 mg. per cent) contrasts with 5.3 mg. per cent, the average for 9 cases of secondary anemia (Table 33) due to loss of blood. These figures are not quite accurate because of blood contained in the capillaries and do not measure accurately the iron contained within the liver cells. They are in general accord with accurate figures obtained from blood free liver tissue in dogs (1) which show that about 10 weeks of severe anemia due to bleeding will reduce liver parenchyma iron to an irreducible minimum of 4–5 mg. per cent.

The case of hemolytic anemia due to rheumatism—A-1618, Table 33—as expected shows a high figure for iron (23 mg. per cent) and for liver content of hemoglobin producing factors 217 per cent. The red cells were destroyed within the body and this salvaged material stored in part in the liver.

Table 34. Diagnosis and histological description of liver.

A-1769. Acute leukemia—hemoglobin 80 to 35 per cent 3 wks.—46 yrs.
Liver—autopsy weight 1510 gm.—laboratory specimen 1320 gm.
Histological specimen—liver sections show typical leukemic infiltration. Liver cells show some atrophy and a few small fat droplets—also some lipochrome pigment. Kupffer cells are large but show no pigment. No iron staining pigment observed.

Liver—autopsy weight 1950 gm.—laboratory specimen 1675 gm.
Histological specimen—much degeneration of liver cells which are very granular and swollen. Small fat droplets numerous. Central atrophy plus lipoehrome. Many scattered large hyaline necroses of liver parenchyma.
A-1383. Acute leukemia—hemoglobin 40 per cent—61 yrs.
Liver—autopsy weight 3500 gm.—laboratory specimen 3300 gm.
Histological specimen—advanced leukemic infiltration of liver with white cells.
Liver capillaries very rich in red cells. Liver cells show no pigment and a moderate grade of atrophy but in general are normal.

A-1231. Acute leukemia—hemoglobin 50 to 20 per cent—transfusions—terminal infection—13 yrs.
Liver—autopsy weight 1470 gm.—laboratory specimen 1420 gm.
Histological specimen—liver shows the usual leukemic infiltration in portal tissue and between the liver strands. There are small central hyaline liver necroses. Some liver cells show fat droplets. Kupffer cells show a yellow granular pigment which only occasionally gives a positive iron stain.

Liver—autopsy weight 1140 gm.—laboratory specimen 1100 gm.
Histological specimen—liver cells are normal. White cells are very numerous in all liver capillaries and there is conspicuous infiltration of the periportal stroma. Very little pigment is found anywhere. Kupffer cells are large. Occasional pigment granules within the liver cells give a positive stain for iron.

A-1688. Acute leukemia—hemoglobin 28 per cent—3 yrs.
Liver—autopsy weight 1020 gm.—laboratory specimen 920 gm.
Histological specimen—liver shows a typical white cell infiltration especially marked about the portal spaces. The liver cells show some fat droplets in the central portion of the lobules. Otherwise normal.

A-436. Acute leukemia—hemoglobin 60 per cent—42 yrs.
Liver—laboratory specimen 2300 gm.
Histological specimen—premyelocytes fill capillaries. No eosinophiles. Central 1/2 of liver lobules shows much fatty degeneration. Outer 1/2 of lobule normal. Kupffer cells large and often show yellow granular pigment. Lipochrome scarce. Kupffer cells full of iron-containing pigment; hepatic cells contain no iron staining material.

A-385. Acute leukemia—hemoglobin 50 per cent—terminal infection—33 yrs.
Liver—laboratory specimen 2300 gm.
Histological specimen—central liver cells are much injured with fine fat droplets and poor staining. Some focal areas of necrosis in mid zone. Kupffer cells large and phagocytic. Many myeloblasts. Outer half of liver lobule well preserved and normal.
A-377. Acute leukemia—hemoglobin 70 per cent—90,000–150,000 white blood cells—56 yrs.
Liver—laboratory specimen 2650 gm.
Histological specimen—liver cells swollen, very little fat, central liver cells show much yellow pigment (lipochrome). No iron staining granules. Lymphocytes abundant especially about the portal tissue. Kupffer cells large and some show little pigment but red blood cell and white blood cell inclusions.

Liver—autopsy weight 1750 gm.—laboratory specimen 1560 gm.
Histological specimen—liver lobules contain many white cells in the capillaries and in the periportal stroma but there is no conspicuous infiltration. There are scattered small hyaline necroses. The liver cells contain some fat droplets and lipochrome pigment. Kupffer cells are large and contain some yellow granular pigment which stains for iron. Fine pigment grains in the liver cells also give a stain for iron.

A-1349. Leukemia—myeloid—hemoglobin 35 per cent—65 yrs.
Liver—autopsy weight 1775 gm.—laboratory specimen 1550 gm.
Histological specimen—central hyaline necrosis is conspicuous and possibly involves 1/10 of the liver cells. Cell infiltration of the lobules is not conspicuous. Pigment within the liver cells is abundant; that in the center of the lobules gives no iron stain (lipochrome) but some pigment grains in the periphery of the lobules do give a faint iron stain. Kupffer cells are conspicuous and some contain pigment which gives no iron stain.

A-316. Myeloid leukemia—hemoglobin 60 per cent down to 40 per cent—bleeding—71 yrs.
Liver—laboratory specimen 1980 gm.
Histological specimen—great numbers of myelocytes everywhere, perhaps making up 1/4 of the tissue or more. Liver cells are granular and rich in pigment which gives a strong stain for iron. No fat droplets. Kupffer cells not conspicuous and contain no pigment.

A-1828. Leukemia chronic lymphoid—hemoglobin 30 per cent—59 yrs.
Liver—autopsy weight 2700 gm.—laboratory specimen 2480 gm.
Histological specimen—there is only a slight leukemic infiltration of the portal tissue and liver lobules. There is abundant lipochrome pigment in the liver cells especially in the central part of the lobule. Kupffer cells show no pigment.

A-1943. Chronic lymphatic leukemia—hemoglobin 55 per cent—66 yrs.
Liver—autopsy weight 3360 gm.—laboratory specimen 3180 gm.
Histological specimen—the liver lobules show extreme infiltration with lympho-
cytes, most marked in the periportal tissue. The liver cells in general look normal but contain a few yellow pigment grains which stain for iron. Most of this pigment is in the margin of the lobule. Kupffer cells are large but contain no pigment.

A-77. Myelocytoma, subacute nephritis—hemoglobin 60 per cent—53 yrs.
Liver—laboratory specimen 1830 gm.
Histological specimen—few focal liver necroses, little central fat. Lipochrome moderate in amount, stroma normal. Congestion marked. No tumor in liver.

### TABLE 34

#### Leukemias

<table>
<thead>
<tr>
<th>Number</th>
<th>Cause of death</th>
<th>Iron content human liver</th>
<th>Liver intake per day</th>
<th>Hemoglobin output per 7 days feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[mg. per cent] [mg. gm.]</td>
<td>Human Control</td>
<td>Human Control Human Control ratio Human to Control</td>
</tr>
<tr>
<td>A-1769</td>
<td>Acute Leuk.</td>
<td>11.4 21</td>
<td>180 300</td>
<td>48 58 137</td>
</tr>
<tr>
<td>A-476</td>
<td>Acute Leuk.</td>
<td>24.5 59</td>
<td>225 300</td>
<td>57 54 139</td>
</tr>
<tr>
<td>A-1383</td>
<td>Acute Leuk.</td>
<td>13.5 62</td>
<td>470 300</td>
<td>118 56 134</td>
</tr>
<tr>
<td>A-1231</td>
<td>Acute Leuk.</td>
<td>10.9 22</td>
<td>200 200</td>
<td>61 62 98</td>
</tr>
<tr>
<td>A-1956</td>
<td>Acute Leuk.</td>
<td>9.4 14</td>
<td>157 300</td>
<td>45 60 87</td>
</tr>
<tr>
<td>A-1688</td>
<td>Acute Leuk.</td>
<td>8.6 11</td>
<td>130 300</td>
<td>53 72 171</td>
</tr>
<tr>
<td>A-436</td>
<td>Acute Leuk.</td>
<td>——</td>
<td>328 300</td>
<td>51 43 109</td>
</tr>
<tr>
<td>A-385</td>
<td>Acute Leuk.</td>
<td>——</td>
<td>329 300</td>
<td>47 34 127</td>
</tr>
<tr>
<td>A-377</td>
<td>Acute Leuk.</td>
<td>——</td>
<td>379 300</td>
<td>87 53 130</td>
</tr>
<tr>
<td>A-1963</td>
<td>Acute Leuk.</td>
<td>19.2 42</td>
<td>220 300</td>
<td>84 61 187</td>
</tr>
<tr>
<td>A-1349</td>
<td>Myeloid Leuk.</td>
<td>11.7 26</td>
<td>220 300</td>
<td>47 35 181</td>
</tr>
<tr>
<td>A-316</td>
<td>Myeloid Leuk.</td>
<td>——</td>
<td>283 225</td>
<td>46 30 121</td>
</tr>
<tr>
<td>A-1828</td>
<td>Lymphoid Leuk.</td>
<td>3.6 13</td>
<td>350 300</td>
<td>39 58 57</td>
</tr>
<tr>
<td>A-1943</td>
<td>Lymphoid Leuk.</td>
<td>16.4 74</td>
<td>450 300</td>
<td>101 81 84</td>
</tr>
<tr>
<td>A-77</td>
<td>Myeloma</td>
<td>——</td>
<td>261 300</td>
<td>50 43 135</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>12.9 34</td>
<td>279</td>
<td></td>
</tr>
</tbody>
</table>

Table 34 shows 15 cases of leukemia representing all types of the disease. There is one case of myeloma which perhaps does not belong in this group but at least the biological assay of liver potency falls close to the general average so that no harm is done. Biological assay of the hemoglobin producing factors shows a low normal figure (126 per cent) which corresponds to the assay of cases of secondary anemia (Table 33). The great majority of these cases are acute with large
numbers of parent or undifferentiated cells in the blood, marrow and viscera. Two cases of chronic lymphoid leukemia show low values by biological assay (57 and 84 per cent). We have no explanation for these low figures.

The anemia in leukemia would seem to depend upon marrow insufficiency due to encroachment of the white cells on the red cell chain. There is little or no evidence for blood destruction as the iron analyses are close to normal.

Table 35. Diagnosis and histological description of liver.

A-1367. Familial anemia (Cooley)—siderosis of viscera—hemoglobin 50 per cent—5 yrs.
Liver—autopsy weight 860 gm.—laboratory specimen 740 gm.
Histological specimen—the liver cells show large deposits of iron staining pigment. Kupffer cells contain much pigment and some stains for iron. The liver cells are relatively normal but for the pigment. There is a little central fatty degeneration.

A-1819. Hemochromatosis—cancer of stomach—hemoglobin 78 per cent—57 yrs.
Liver—autopsy weight 2170 gm.—laboratory specimen 1980 gm.
Histological specimen—typical picture of moderately advanced case of hemochromatosis with annular cirrhosis. There is little new bile duct proliferation. Iron staining pigment is abundant in liver epithelium and especially in phagocytes in stroma about the portal areas. Kupffer cells are rich in the same pigment. There are calcium deposits in portal stroma.

A-526. Peculiar leukemia with marrow aplasia—liver pigment rich in iron—44 yrs.
Red blood cells 640,000; white blood cells 1600; hemoglobin 10 per cent. Differential 80 per cent lymphocytes. Platelets numerous.
Liver—laboratory specimen 2150 gm.
Histological specimen—central necroses involve about 1/4-1/3 of liver lobules. Many mononuclears here. Much pigment in liver cells and some gives an iron stain. A little fat in mild zone.

Liver—autopsy weight 1560 gm.—laboratory specimen 1460 gm.
Histological specimen—liver sections show not a single normal liver cell. There is much necrosis of the central liver cells but remaining liver cells may show a pale nucleus and a mass of fat droplets replacing the protoplasm. Typical picture of
extreme chloroform injury. No blood clots at autopsy indicate a serious liver insufficiency.

A-62. Eclampsia in last part of pregnancy (7 mos.). Child \textit{in utero} at autopsy. 25 + yrs.
Liver—laboratory specimen 1725 gm.
Histological specimen—typical hemorrhagic periportal necroses of eclampsia.
Parenchyma elsewhere normal. Possibly 1/5 of liver cells or less are injured.

A-1261. Pulmonary embolism in normal puerperium—nursing—30 yrs.
Liver—autopsy weight 1700 gm.—laboratory specimen 1450 gm.
Histological specimen—liver in all respects normal.

\textbf{TABLE 35}
\textit{Miscellaneous}

<table>
<thead>
<tr>
<th>Number</th>
<th>Cause of death</th>
<th>Iron content human liver</th>
<th>Liver intake per day</th>
<th>Hemoglobin output per 7 days feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fresh tissue</td>
<td>daily intake</td>
<td>Human</td>
</tr>
<tr>
<td>A-1367</td>
<td>Thalasemia</td>
<td>123.0</td>
<td>130</td>
<td>105</td>
</tr>
<tr>
<td>A-1819</td>
<td>Hemochromatosis</td>
<td>96.0</td>
<td>271</td>
<td>280</td>
</tr>
<tr>
<td>A-526</td>
<td>Marrow aplasia</td>
<td>68.0</td>
<td>208</td>
<td>300</td>
</tr>
<tr>
<td>A-1785</td>
<td>Chloroform</td>
<td>6.0</td>
<td>13</td>
<td>205</td>
</tr>
<tr>
<td>A-62</td>
<td>Eclampsia</td>
<td>——</td>
<td>——</td>
<td>150</td>
</tr>
<tr>
<td>A-1261</td>
<td>Embolism</td>
<td>5.9</td>
<td>12</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Lactation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 35 contains cases of unusual interest which do not seem to fit in any of the tables given above. Each case deserves a special note. Thalasemia (A-1367) is a term which we have used to designate a peculiar familial anemia found in races about the Mediterranean first described adequately by Cooley and recently from this laboratory (4). This disease presents some features resembling pernicious anemia and hemochromatosis. There are large deposits of iron-containing pigments in the liver, pancreas, viscera and ductless glands. It is not surprising that there should be a high potency for hemoglobin production in the liver assay—308 per cent. One should however contrast a typical case of \textit{hemochromatosis} (A-1819) with more than twice the
daily iron intake and half the hemoglobin output. Here is a conspi-

cuous dissociation of the iron factors and the hemoglobin production

factors in two livers which have many similarities.

The third case (A-526) presented diagnostic difficulties. At first it
was classed as a chronic lymphatic leukemia of atypical type but sub-
sequently there was evidence of marrow aplasia which was confirmed
at autopsy. The biological assay of hemoglobin producing factors
(65 per cent) would put it in the group of chronic leukemias whereas
the iron analyses (68 mg. per cent) suggest aplastic anemia. We pre-
fer not to attempt to classify this case at the present time.

Chloroform poisoning (4th case, A-1785) is unusual in many ways.
We expected a very low value for the hemoglobin producing factors as
the patient died from chloroform injury of liver with low fibrinogen,
bleeding and all the essential features of this condition. Moreover
the liver shows histological injury of extreme grade. Yet there is
abundant material in the abnormal liver tissue from which the normal
amount of hemoglobin is produced (159 per cent). It is suggested
that these unknown factors remain even in these dead and injured
cells as the process was acute and the injured cells have not yet auto-
lyzed and been removed as follows repair in chloroform poisoning.
The acuteness of the injury differentiates this type of case from the
other type of hepatic insufficiency and liver injury given in Table
25 (Paper II).

Eclampsia presents an unusual observation (A-62, Table 35). Only
one other case (Table 21, Paper II) with severe passive congestion gives
zero value for hemoglobin production factors. We cannot correlate
these two observations but subsequent findings in eclampsia would be
of interest.

The last case (A-1261, Table 35) is of considerable interest because
it suggests that lactation may reduce the store of hemoglobin produc-
ing factors in the normal liver. There is some evidence that material
which can be used to build hemoglobin in an emergency (anemia) may
be used to build up and replace plasma protein or tissue protein when
needed. The production of milk protein might come into this same
group. We have long suspected that it might be possible to show
experimentally that building materials coming to the liver might be
used for one important product (hemoglobin) at one time and again for
another different but related substance under different demand or emergency conditions. We hope to obtain further information about lactation and the storage of these potent hemoglobin producing factors in the liver.

SUMMARY

Biological assay of the human liver in various types of anemia shows conspicuous differences in the concentration of hemoglobin producing factors.

Pernicious anemia shows very high values and the liver in untreated cases may show maximal storage of the hemoglobin producing factors. Liver therapy reduces this store as the missing factor is supplied and new hemoglobin and red cells can be turned out by the marrow.

Aplastic anemia likewise shows high concentration of hemoglobin producing factors as there is no outlet for this material through the red marrow.

Secondary anemia due to loss of blood will show low normal values but even long standing severe anemia will not seriously deplete this store of hemoglobin producing factors in the liver.

Secondary anemia due to blood destruction within the body shows higher values and some excess store of hemoglobin producing factors and iron.

Leukemia gives a biological assay like secondary anemia due to blood loss and always presents definite anemia.

Iron analyses show conspicuous differences and iron concentration within the liver parenchyma does not in any way parallel the concentration of hemoglobin producing factors. The highest values for iron concentration are found in aplastic anemia (70 mg. per cent)—high values in pernicious anemia (51 mg. per cent)—normal values in leukemia (13 mg. per cent)—and low values in anemia due to loss of blood (5.3 mg. per cent).

These findings should aid in a more complete understanding of the pathogenesis and internal metabolism of various anemias.

BIBLIOGRAPHY