

HEMOGLOBIN PRODUCTION FACTORS IN THE HUMAN LIVER  
ANEMIAS, HYPOPROTEINEMIA, CIRRHOSIS, PIGMENT ABNORMALITIES, AND  
PREGNANCY

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The most significant observations in the tables below relate to *hypoproteinemia*, pregnancy, and the postpartum state. With the reduction of protein stores which must accompany hypoproteinemia, we observe in practically all cases that the biological assay of these livers shows only  $\frac{1}{3}$  to  $\frac{1}{2}$  of the normal store of hemoglobin producing materials.

It is generally believed that the liver stores many factors essential for hemoglobin building—for example it holds the major stores of iron and the essential factor missing in pernicious anemia. We believe that the *liver stores some of all the factors essential for hemoglobin building* and it is not difficult to show that the building of hemoglobin can be limited by reducing *protein* stores and intake in experimental anemia (2, 6). This might indicate that the liver is concerned with the production and/or storage of *globin* or its precursors.

The *pigment radicle* of hemoglobin—the pyrrol aggregate—we know is discarded by the liver (bile pigment) and possibly the pigment radicle is made in the same organ. It does not seem possible to exhaust the capacity of the body to produce this pigment radicle—perhaps the mechanism being a ring closure of straight chain amino acids.

Some years ago we reported analyses of human livers (12). The iron content was determined and a *biological assay* of the hemoglobin-producing factors in human tissue was made by the utilization of carefully standardized anemic dogs. Our *control baseline* for the normal animal liver is that of the pig and we designate that as 100 per cent. Compared with this 100 per cent normal control we find that the *normal human liver* contains greater amounts of hemoglobin-producing factors—a ratio of 120 to 160 per cent to the control. There are considerable individual differences which we are inclined to relate to dietary habits, iron stores, and other unknown factors.

Subsequently we made biological assays upon the livers from common *domestic animals* (8). Compared with the hemoglobin production resulting from pig liver feeding as 100 per cent, we found beef liver rated at 70 per cent, rabbit liver 80 per cent, dog liver 100 per cent, and horse liver 130 per cent. *Fish* livers in contrast were relatively inert—perhaps 10 per cent being the upper limit for the various salt water fish tested (7, 10).

In our study of the biological assays of human livers (12) we observed that the usual acute and chronic infections showed about the same ratio as did the normal human group—average figures of 120 to 150 per cent as compared with 100 per cent for the normal control pig liver. *Cirrhosis* with no sign of liver insufficiency presented high normal values—160 per cent, but when there was evidence of a true hepatic insufficiency, the liver assay averaged only 50 per cent or  $\frac{1}{3}$  normal. The iron content was the same in both types of cirrhosis.

*Pernicious* and *aplastic anemias* showed large stores of iron and a high assay for hemoglobin production 220 to 200 per cent. The usual secondary anemias showed the biological assay to fall within normal range—130 per cent with low iron values.

Other experiments with *anemic horse liver* (14) show that it is not easy to exhaust the hemoglobin production factors from the liver by means of blood loss. Iron stores can be reduced very readily by blood removal. These and other experiments suggest that the important reserve stores for hemoglobin building are in part *protein* and are guarded jealously by the body even in the face of severe bleeding. We must diminish the protein intake to reduce this protein fraction of the hemoglobin production store (6).

Obviously a comprehensive understanding of hemoglobin fabrication within the body in health and disease calls for an analysis of the stores of hemoglobin-producing materials in animal and human livers. This knowledge may make for a better therapy in human disease.

#### *Methods*

*Methods* used have been described previously (15). The standard dogs are kept anemic at a level of 6 to 7 gm. hemoglobin per 100 cc. blood by bleeding and the hemoglobin removed is credited to that test period. During control periods the dogs are fed a complete diet—a salmon bread mixture (15) which is poor in iron but adequate for health and general nutritional maintenance indefinitely. The dogs are standardized by the feeding of pig liver at various times. Similar control tests are made by feeding iron during other test periods as described elsewhere (11). The average hemoglobin production due to pig liver feeding (300 gm. per day for 7 days) is 35 to 45 gm. When the human liver is tested, proper adjustment is made for the amount of liver used and this is then compared with the pig liver baseline as 100 per cent. The figures for this *ratio* are given in the last column in the tables.

In all these test experiments data on red cell counts, hemoglobin levels, blood volume, and animal condition are available in the histories of the various dogs but are not given in this report. These dogs were in a normal clinical state at all times.

Iron analyses were made by the method described by Kennedy (3). These figures are somewhat higher than would be found in perfused livers which give values related to the *parenchyma* iron only. Significant clinical diagnoses and a brief description of the liver are given under the autopsy number.

## EXPERIMENTAL OBSERVATIONS

The experiments given in the tables below extend and confirm those previously reported (12). These data make for a clearer vision relative to hemoglobin production in various diseases.

TABLE 1  
*Pernicious Anemia*  
*Hemoglobin Production Factors Much Increased*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		gm.	mg. per cent	gm.	per cent
2100	Pernicious anemia, typical—no therapy	180	159	63	256
4609	Pernicious anemia, typical—no therapy	260	133	57	194
2262	Pernicious anemia, typical—no therapy	195	34	72	264
4394	Pernicious anemia—no therapy	270	164	73	253
6317	Pernicious anemia—slight therapy	166	95	47	257
3547	Pernicious anemia—slight therapy	175	21	56	192
5255	Pernicious anemia—slight therapy	132	23	53	300
6919	Pernicious anemia—slight therapy	154	57	48	254
4540	Pernicious anemia—good therapy	300	15	67	172
3447	Pernicious anemia—atypical	160	14	41	175
Average.....		199	72	58	232 (243)

Table 1. *Diagnosis and histological description of liver.*

2100. Pernicious anemia, typical—no liver therapy—terminal infection—hemoglobin 5 gm.—73 years.

Liver—autopsy weight 1500 gm.

Histological specimen—liver cells, in general, normal. They contain some fat droplets and many fine yellow pigment granules which give a positive stain for iron. Kupffer cells contain some of the same pigment. There are mononuclear cells in the periportal stroma—old cholangitis?

4609. Pernicious anemia, typical,—no liver therapy, hemoglobin 3.2 gm.—76 years.

Liver—autopsy weight 1870 gm.

Histological specimen—liver cells in center of lobules show fatty degeneration and considerable hyaline necrosis. Liver cells elsewhere are rich in fine granular pigment which gives a strong stain for iron. Kupffer cells show pigment.

2262. Pernicious anemia, typical,—no therapy—bronchopneumonia—hemoglobin 3.9 gm.—63 years.

Liver—autopsy weight 1610 gm.

Histological specimen—liver cells in general normal. Few granules of yellow pigment in hepatic epithelium.

4394. Pernicious anemia—transfusion reaction—no therapy—hemoglobin 3.4 gm.—64 years.

Liver—autopsy weight 2000 gm.

Histological specimen—liver cells excluding the large central necroses are normal. Pigment is abundant in liver cells and it gives a deep iron stain. Kupffer cells small and contain little pigment. Bone marrow is not hyperplastic but shows atrophy in ribs and vertebrae. The marrow which remains is compatible with the diagnosis of pernicious anemia.

6317. Pernicious anemia, typical,—occasional short liver therapy during 5 years—hemoglobin 4 gm.—63 years.

Liver—autopsy weight 1410 gm.

Histological specimen—liver cells in center of lobules show atrophy, some fatty degeneration and lipochrome pigment. Liver cells in margins of lobules show abundant iron staining pigment. Kupffer cells are filled with iron staining pigment. Bone marrow typical. Kidney epithelium in convoluted tubules contains iron staining pigment.

3547. Pernicious anemia—little therapy and no response—coronary occlusion—hemoglobin 5.7 gm.—59 years.

Liver—autopsy weight 1550 gm.

Histological specimen—liver cells normal. They contain many fine pigment granules some of which give a stain for iron. Kupffer cells contain some pigment. Scattered central hyaline necroses.

5255. Pernicious anemia—occasional liver therapy 2 years previously with relapse—moderate response to therapy in last week—arteriosclerosis—final cardiac death—hemoglobin 4.8 gm.—65 years.

Liver—autopsy weight 1030 gm.

Histological specimen—liver cells show atrophy and some fat droplets. The central liver cells show yellow pigment but the iron stain is faint. Kupffer cells inconspicuous and non-pigmented.

6919. Pernicious anemia—treatment 2 weeks “retigulogen”—pyelonephritis—bronchopneumonia—hemoglobin 7.3 gm.—red blood cells 1,700,000 to 3,000,000 under therapy—70 years.

Liver—autopsy weight 1430 gm.

Histological specimen—liver cells show fatty degeneration and some atrophy. Abundant *iron* staining pigment in liver cells and Kupffer cells. Kidney cells in convoluted tubules contain abundant iron staining pigment. Atrophic gastritis.

4540. Pernicious anemia—erysipelas—liver therapy for years—cirrhosis and cholelithiasis—hemoglobin 15 gm.—73 years.

Liver—autopsy weight 3080 gm.

Histological specimen—liver cells in margin of lobules show a good deal of fat but little if any pigment. There is much periportal stroma which is rich in mononuclear cells—the cirrhosis is probably related to a cholangitis. Liver lobules are distorted but liver cells are in good condition. The pernicious anemia was under control.

3447. Anemia (pernicious?)—some reticulocyte response to liver therapy—coronary occlusion with infarct—hemoglobin 8 gm.—74 years.

Liver—autopsy weight 1200 gm.

Histological specimen—liver cells show atrophy. There are scattered small liver necroses. A few granules of iron staining pigment are found in liver cells and Kupffer cells. Marrow shows no typical hyperplasia. If this is pernicious anemia it must be a very early stage. The picture is not that of the ordinary secondary anemia.

Table 1 presents 10 cases of pernicious anemia, 8 of which are typical and had received no therapy or inadequate therapy. If we exclude the last 2 cases in Table 1, we obtain an average ratio of 243 per cent, which means that the pernicious anemia liver contains unusually large stores of hemoglobin-building factors. The missing substance X present in liver extracts, liver, stomach, and kidney tissue, is lacking and these unused substances pile up in reserve. When liver therapy is effective these liver stores decrease to normal and ratios close to the normal are observed (case 4540, Table 1 above) *as new red cells* are formed in great numbers.

The last 2 cases in Table 1 show high normal ratios for the hemoglobin-producing factors. In 4540 the therapy had been adequate and there was no anemia at time of death. In case 3447 the anemia was not severe and the findings suggest an early stage of pernicious anemia.

Table 1 is very like the similar report on pernicious anemia ((13) Table 31). Average ratios for hemoglobin-producing factors in the liver previously reported were 218 per cent.

TABLE 2  
*Anemia—Aplastic and Secondary*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		gm.	mg. per cent	gm.	per cent
4395	Aplastic anemia	205	244	65	280
6690	Aplastic anemia—benzol?	200	89	38	154
3958	Secondary anemia (macrocytic)	215	16	38	161
2817	Secondary anemia—nephritis	165	28	52	207
2433	Secondary anemia—cancer of cervix	125	4	21	125
4948	Secondary anemia—myocarditis	150	4	21	89
5407	Secondary anemia—therapy	180	7	15	60

Table 2. *Diagnosis and histological description* of liver.

4395. Aplastic anemia—transfusions—hemoglobin 2.5 gm.—white blood cells 500—no pigmentation—53 years.

Liver—autopsy weight 1570 gm.

Histological specimen—liver cells in general are normal. There are some fat vacuoles and scattered central hyaline necroses. Fine pigment granules which give an iron stain are abundant in liver and Kupffer cells.

6690. Aplastic anemia (benzol?)—bleeding—albumin 3.1 gm.—globulin 2.4 gm.—fibrinogen 533 mg.—duration 2 months—hemoglobin 2 gm.—73 years.

Liver—autopsy weight 1570 gm.

Histological specimen—there are small scattered central hyaline necroses. Pigment is scant in liver cells but obvious in Kupffer cells. Marrow shows few cells and those are immature.

3958. Anemia (macrocytic)—senility—cachexia—bronchopneumonia—hemoglobin 9.5 gm.—84 years.

Liver—autopsy weight 1500 gm.

Histological specimen—there are mononuclear cells in the periportal stroma. Liver cells show lipochrome pigment but no iron staining pigment. Bone marrow does not suggest primary anemia.

2817. Anemia—chronic nephritis—death uremia—hemoglobin 5.2 gm.—27 years.

Liver—autopsy weight 1400 gm.

Histological specimen—liver cells show some small fat droplets and lipochrome pigment but no iron staining pigment. Marrow shows no hyperplasia. Evidently in this type of anemia the iron and other hemoglobin-building factors are not depleted from the liver—perhaps the mechanism of hemoglobin production is disturbed by the nephritis.

2433. Anemia secondary to cancer of cervix—red blood cells 1,000,000—hemoglobin 2 gm.—38 years.

Liver—autopsy weight 1250 gm.

Histological specimen—liver shows fatty degeneration of cells in the centers of lobules. Other liver cells show cloudy swelling. Scanty lipochrome pigment.

4948. Anemia, secondary—myocarditis—old gastroenterostomy—hemoglobin 4 to 6 gm.—72 years.

Liver—autopsy weight 1800 gm.

Histological specimen—liver is negative except for some lipochrome pigment in the centers of the lobules. Bone marrow shows little if any hyperplasia. Iron stores obviously have been depleted.

5407. Anemia—gastric resection, subtotal, 7 years previously—hematuria—recovery from anemia with liver and iron therapy—death from meningitis—last hemoglobin 13 gm.—63 years.

Liver—autopsy weight 1540 gm.

Histological specimen—liver cells essentially normal and contain a few grains of lipochrome pigment. Marrow is normal. The reserve of iron and other hemoglobin-building materials in the liver has been exhausted.

Table 2 presents various types of anemia and the contrasts are striking not only in the iron stores but in other hemoglobin-producing factors. In the usual type of secondary anemia due to blood loss we expect to find very low iron stores and a low normal figure for hemoglobin production (135 per cent in a previous publication—Table 33 (13)). The last 3 cases in Table 2 illustrate well the low iron stores and also the decreased stores of hemoglobin-producing factors.

In contrast to these stands *aplastic anemia* (4395—Table 2), a case with very

large stores of iron and hemoglobin-producing factors. The other aplastic anemia (6690) possibly due to benzol shows quite large iron stores but only average stores of hemoglobin-producing factors. This unexpected low value may be explained in part by a definite hypoproteinemia which tends to reduce hemoglobin-producing materials in contrast to aplastic anemia which tends to increase these stores. In part these large stores of iron and hemoglobin-producing factors may be related to transfusions. The liver probably stores these essential hemoglobin-producing factors because the bone marrow is aplastic and unable to use the stored material in spite of the demand due to the anemia.

Two cases (3958 and 2817 in Table 2) are not easy to understand. In spite of severe anemia of considerable duration, the liver stores of iron and hemoglobin-producing factors are large. In advanced nephritis in dogs there may be a lack of ability to make new hemoglobin under controlled experimental conditions (16). The supplies are adequate but the mechanism for hemoglobin production is disturbed just as may happen due to an infection (9). Perhaps this explains the observed stores (autopsy 2817—Table 2) and their non-utilization by a bone marrow which appears normal or hyperplastic. The other case of macrocytic anemia (3958) was observed in a male of 84 and here cachexia may have been a factor.

TABLE 3  
*Hypoproteinemia—Cirrhosis*  
*Hemoglobin-Producing Factors Greatly Decreased*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		gm.	mg. per cent	gm.	per cent
6630	Carcinoma of stomach	200	4	4	17
6406	Gastric ulcer	160	5	12	61
6543	Lymphoma	350	10	29	52
3572	Tuberculosis	230	3	33	94
6892	Hepatitis—icterus	200	13	10	46
2237	Hepatic insufficiency	230	3	19	64
2824	Cirrhosis	245	2	37	90
5436	Pyelonephritis, ? pellagra	134	13	43	240
Average.....		219	7	23	83 (61)

Table 3. *Diagnosis and histological description of liver.*

6630. Cancer of stomach—hypoproteinemia—peritonitis—considerable weight loss in 6 months—albumin 3.4 gm., globulin 2.5 gm.—hemoglobin 12.8 gm.—67 years.

Liver—autopsy weight 1400 gm.

Histological specimen—liver cells show atrophy and scattered fat droplets.  
Lipochrome present in moderate amount.

6406. Gastric ulcer—anemia—hypoproteinemia—pyloric stenosis—phlegmonous gastritis—bronchopneumonia—pulmonary infarcts—bleeding ulcer for 4 years—total plasma proteins low = 3.8 to 6.0 gm. per cent—hemoglobin 9 gm.—35 years.

Liver—autopsy weight 1250 gm.

Histological specimen—liver cells show hyaline necrosis in the centers of all lobules. The midzone shows fatty degeneration. Lipochrome pigment easily seen.

6543. Lymphoma—hypoproteinemia—infiltration of ileum—bronchopneumonia with abscesses—total plasma proteins 6.1 gm. to 4.3 gm. per cent.—hemoglobin 12.8 to 8.5 gm.—53 years.

Liver—autopsy weight 2950 gm.

Histological specimen—liver cells show fatty degeneration, especially in the periportal areas. There is some cell atrophy in the centers of the lobules.

3572. Tuberculosis, disseminated—anemia, macrocytic—hypoproteinemia—ascites—albumin 2.2 gm., globulin 2.0 gm.—hemoglobin 10 gm.—61 years.

Liver—autopsy weight 1700 gm.

Histological specimen—liver cells show advanced fatty degeneration. No tubercles. Marrow shows some hyperplasia but the cell elements are normal.

6892. Cirrhosis—hepatitis—liver insufficiency—coma—icterus, severe—hemoglobin 13 gm.—39 years.

Fibrinogen 360 mg. per cent—albumin 1.6 gm. per cent—globulin 2.3 gm. per cent—N.P.N. 45 to 130 mg. per cent.

Liver—autopsy weight 1800 gm.

Histological specimen—typical portal type of cirrhosis with much scar tissue and new bile ducts. Little fatty degeneration but *considerable* areas of necrosis. Many polymorphonuclear leucocytes are observed in lobules and in cellular portal tissue. Larger bile passages are clear. Bile canaliculi are distended with brown colloid casts. Serious parenchyma injury.

2237. Cirrhosis—hemorrhages—anemia—hepatic insufficiency—alcoholism and syphilis—no blood clots—bleeding into tissues—icterus—hemoglobin 10.6 gm.—44 years.

Liver—autopsy weight 1690 gm.

Histological specimen—typical portal type of cirrhosis with much scar tissue and new bile ducts. There is much fatty degeneration. Practically no pigment seen. Marrow shows some hyperplasia.

2824. Cirrhosis—icterus—bronchopneumonia—alcoholism—hemoglobin 10.2 gm. Albumin 2.1 gm., globulin 4.0 gm.—fibrinogen 335 mg.—61 years.

Liver—autopsy weight 1930 gm.

Histological specimen—liver cells show advanced fatty degeneration. Lipochrome pigment is abundant. Bile canaliculi are distended with yellow-brown casts. The lobulation is irregular due to dense bands of scar tissue in which are seen many mononuclears and immature bile ducts. The liver is poor in iron and other hemoglobin-building material.

5436. Hypoproteinemia (albumin 2.1 gm. and globulin 2.6 gm.)—hyperchromic anemia—pyelonephritis—pellagra?—restricted, inadequate diet months before death—hemoglobin 5.5 gm.—52 years.

Liver—autopsy weight 1740 gm.

Histological specimen—liver cells show much fatty degeneration, especially in the centers of lobules—pigment very inconspicuous. There are a few small focal necroses. Kupffer cells show an occasional grain of pigment.

Table 3 is probably the most significant in this paper. With *hypoproteinemia* in all cases but one there is a sharp drop in the content of hemoglobin-producing factors. In fact if we exclude case 5436 from the group we find an average ratio of 61 per cent or about 40 per cent of the normal human liver content of hemoglobin production factors. Anemia is not prominent (excluding 5436) and the iron stores are depleted. The normal iron values for this type of human liver material is 12 mg. per cent. We were surprised to note this uniformly low value for the hemoglobin-producing factors and one can scarcely escape the conclusion that protein factors (perhaps precursors of *globin*) are depleted by the hypoproteinemia whether associated with hepatitis or not.

Case 5436, Table 3, does show a surplus of hemoglobin-building stores and a normal store of iron in spite of an anemia (hemoglobin 5.5 gm.) plus a severe hypoproteinemia (4.7 gm. per cent) preceded by a long period of inadequate diet (pellagra?) and pyelonephritis. We have no adequate explanation but note a pyelonephritis which like the nephritis in Table 2 may prevent the utilization of the hemoglobin-producing stores.

TABLE 4  
*Pigment Metabolism Abnormal*  
*Hemolytic Icterus, Hemochromatosis, Erythroblastic Anemia*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		gm.	mg. per cent	gm.	per cent
5340	Hemolytic icterus	250	80	50	177
3891	Hemolytic icterus	240	37	55	177
4226	Hemochromatosis	200	644	35	98
5812	Mediterranean anemia	240	418	50	155
2414	Mediterranean anemia	115	292	64	398

Table 4. *Diagnosis and histological description of liver.*

5340. Hemolytic icterus—splenectomy—thrombosis femoral veins—pulmonary embolism—icterus—pigmentation of liver and kidneys—32 years.

Liver—autopsy weight 1800 gm.

Histological specimen—liver shows recent hyaline central necrosis and many areas which show active repair of this injury. Pigment is abundant in the liver cells, phagocytes, and Kupffer cells, also in renal tubular epithelium. Marrow shows very active hyperplasia.

3891. Hemolytic icterus—splenectomy—pericarditis—pachymeningitis—pigmentation of liver, kidneys, and intestines—red blood cells 1,610,000—reticulocytes 10 per cent—hemoglobin 4.5 gm.—50 years.

Liver—autopsy weight 1800 gm.

Histological specimen—specimens of liver show advanced postmortem autolysis. Considerable iron staining pigment is observed in liver cells, Kupffer cells, and renal tubular epithelium. Marrow shows hyperplasia.

4226. Hemochromatosis—endocarditis—typical picture of this disease with pigmentation of skin, liver, pancreas, and other tissues—cirrhosis—55 years.

Liver—autopsy weight 2350 gm.

Histological specimen—liver shows an advanced type of cirrhosis. The liver cells are filled with granular yellow iron-containing pigment. The same pigment is abundant in phagocytes and in the scar tissue within the liver. Bone marrow normal.

5812. Thalassemia (Mediterranean anemia)—hemoglobin 3 to 4 gm.—7 years.

Liver—autopsy weight 2550 gm.

Histological specimen—the picture is typical of this disease (17). Liver cells are filled with granular yellow pigment which gives a strong stain for iron. Kupffer cells are rich in the same pigment. There is slight increase in stroma but no real cirrhosis.

2414. Thalassemia (Mediterranean anemia)—liver rich in iron pigment—marrow hyperplasia—hemoglobin 3.7 gm.—albumin 3.3 gm., globulin 3.4 gm.—typical disease picture as described by Cooley—5 years.

Liver—autopsy weight 810 gm.

Histological specimen—liver cells very rich in yellow granular pigment staining for iron. There is central fatty degeneration—no increase in stroma. Kupffer cells numerous, large, and full of pigment.

Pigment metabolism is seriously disturbed in the cases listed in Table 4. *Hemochromatosis* shows very heavy iron deposits in the liver, no anemia, and a normal marrow. The biological test of this liver is subnormal in spite of the very large amount of iron in the liver tissue. The iron alone if available for absorption should give a response above that recorded. This suggests that the iron is in a compound (like hemoglobin) not readily available for absorption.

Erythroblastic anemia (Cooley) or Mediterranean anemia or Thalassemia shows heavy iron deposits, severe anemia, and a very hyperplastic marrow. One case shows only a normal ratio and another in Table 4 is more than twice normal. Another case reported previously ((13) Table 35), shows a normal content of hemoglobin building factors.

Hemolytic icterus presents a pigment disturbance of another type. Blood destruction is a factor and iron is overabundant in the liver tissue. The biological assay gives ratio figures somewhat above normal.

TABLE 5  
*Polycythemia—Leukemia*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		<i>gm.</i>	<i>mg. per cent</i>	<i>gm.</i>	<i>per cent</i>
5266	Polycythemia	200	4	24	86
2380	Leukemia—acute	160	102	55	195
3634	Leukemia—myeloid	285	223	71	153
4193	Leukemia—myeloid	320	33	62	116
4283	Leukemia—myeloid—chronic	165	44	78	431

Table 5. *Diagnosis and histological description of liver.*

5266. Polycythemia—arteriosclerosis—cerebral thrombosis—hydrazine therapy here 14 years—hemoglobin 20 to 15 gm.—76 years.

Liver—autopsy weight 1550 gm.

Histological specimen—liver cells are normal. There is lipochrome pigment in the liver cells near the central vein. Kupffer cells are numerous but free of pigment. Kidney shows iron staining pigment in epithelium of convoluted tubules and this is surely related to the hydrazine therapy.

2380. Leukemia, acute, typical acute myeloid type—anemia—hemoglobin 2 gm.—12 years.

Liver—autopsy weight 1280 gm.

Histological specimen—liver shows infiltration with white cells of myeloid type. There is central fatty degeneration. Pigment is abundant in liver cells and in some Kupffer cells. Marrow shows great hyperplasia of the white cell type and red cells are few in number.

3634. Leukemia, myeloid, 1 year—anemia—hemoglobin 7.6 gm.—38 years.

Liver—autopsy weight 2700 gm.

Histological specimen—liver shows abundant iron staining pigment in liver and Kupffer cells. Fat droplets appear in liver cells. Infiltration with myeloid cells is conspicuous.

4193. Myeloid leukemia—bleeding—white blood cells 39,000—hemoglobin 7.2 gm.—53 years.

Liver—autopsy weight 3050 gm.

Histological specimen—liver cells in the center of lobules show fatty degeneration and occasional focal hyaline necroses. Portal tissues are filled with myelocytes which are abundant in all tissues. Marrow is typical.

4283. Chronic myeloid leukemia—pericarditis and bronchopneumonia—anemia—70 years.

Liver—autopsy weight 1170 gm.

Histological specimen—liver cells show a good deal of fatty degeneration in the centers of lobules where focal necroses are numerous. Lipochrome pigment is abundant. Kupffer cells inconspicuous. Leukemic infiltration of liver is absent. Marrow is typical.

Polycythemia (Table 5) shows a *low biological assay* and a subnormal iron store. These figures are significant and suggest that the iron and other hemoglobin-producing factors are turned over very rapidly to form new red cells to maintain the high red cell counts so characteristic of this disease. An *over-absorption* of red cell and hemoglobin-producing factors would not seem to be responsible for this disease picture. This case had been under observation and treatment with hydrazine in the Strong Memorial Hospital for 14 years. The presence of *iron* in the convoluted tubular epithelium of the *kidney* is related to the hydrazine therapy and emphasizes the fact observed in dogs (1) and human beings that the *kidney* is an organ concerned at times with *iron conservation*. Compare also the iron-containing pigment of the renal epithelium in hemolytic icterus cases—Table 4.

Leukemia (Table 5) presents an interesting picture. We reported a similar biological assay on 14 cases ((13) Table 34). In that report the iron content was about normal and the biological assay showed a ratio of 120 per cent or a low normal value. There are several factors which may influence the hemoglobin-producing stores in the liver in this disease. There may be bleeding which would deplete these liver stores but usually the iron stores are normal or above (Table 5) indicating that the drain due to bleeding is not serious. The marrow may be so choked with white cells that it can turn out too few red cells and anemia results. Meanwhile the stores of iron and hemoglobin-producing factors may heap up in the liver (case 4283—Table 5). Infiltration of the liver with white cells and associated liver degeneration may militate against the storage of protein hemoglobin-producing factors. The iron stores in the cases of leukemia (Table 5) run from 3 to 20 times normal.

TABLE 6  
*Eclampsia—Lactation—Controls*

No.	Diagnosis	Liver intake per day	Liver iron content	Hemoglobin production from 7 day feeding of liver	Ratio Human liver to control liver Hb. production
		gm.	mg. per cent	gm.	per cent
4494	Eclampsia—7 mos.	225	5	29	72
3694	Eclampsia—8½ mos.	143	9	14	58
6369	Lactation—4 wks.	223	4	16	60
4297	Thyroid storm	165	13	25	112
4148	Pneumonia—senile	128	8	30	163
6728	Hydrocephalus—youth	230	5	29	109

Table 6. *Diagnosis and histological description of liver.*

4494. Eclampsia—7 months pregnancy—hypoproteinemia (albumin 1.4 and globulin 3.7 gm. per cent)—39 years.

Liver—autopsy weight 1810 gm.

Histological specimen—there are numerous typical hyaline liver necroses with hemorrhage in the periphery of many lobules. The remaining liver cells show cloudy swelling and a few small fat droplets.

3694. Eclampsia—8½ months pregnancy—convulsions—coma—blood pressure 190—albumin and globulin 6 gm.—hemoglobin 11.7 gm.—25 years.

Liver—autopsy weight 1370 gm.

Histological specimen—liver is normal in gross as well as under the microscope. Many sections examined but none shows any periportal or other necrosis. Kidneys show lesions typical of eclampsia. Possibly the normal plasma proteins gave some protection against liver injury.

6369. Postpartum (4 weeks) lactation—pulmonary embolism—hemoglobin 10.2 gm.—23 years.

Liver—autopsy weight 2050 gm.

Histological specimen—liver is normal. Cells contain a few small fat globules and glycogen granules.

4297. Thyrotoxicosis—postoperative bronchopneumonia—hypertension, 200/80—red blood cells 2,800,000—hemoglobin 7 gm.—67 years.

Liver—autopsy weight 1170 gm.

Histological specimen—liver cells show atrophy, a little fat infiltration, and some lipochrome pigment; normal for an elderly female.

4148. Pneumonia—senile—hemoglobin 12.6 gm.—80 years.

Liver—autopsy weight 1800 gm.

Histological specimen—liver cells show cloudy swelling and small amount of lipochrome pigment.

6728. Hydrocephalus—meningitis—normal organs except brain—well developed—hemoglobin 16 gm.—16 years.

Liver—autopsy weight 1790 gm.

Histological specimen—liver cells quite normal.

Table 6 presents 3 important cases related to *pregnancy*. The evidence is clear from these and other cases reported previously ((13) Table 35), that the iron and protein stores are very low in late pregnancy. Case 4494 eclampsia shows hypoproteinemia—compare Table 3. Demands coming from the fetus are probably largely responsible but the needs for protein due to *lactation* are real and deplete protein stores in the liver which otherwise might go to form plasma protein or hemoglobin.

These observed facts should direct the attention of the clinician to adequate intake of iron and proteins for the woman in late pregnancy and during lactation. Whether these depleted protein reserves are in any way related to the state of eclampsia is not known but protein depletion is known to favor liver injury (5) and impair the defense against infection (4).

The last three cases in Table 6 show control values which are in line with many others reported earlier (12). Thyrotoxicosis does not cause any change

in the iron content or the biological assay of the liver. Pneumonia likewise presents figures within the normal range. The last case (hydrocephalus) was a healthy, well developed male of 16 years—death due to meningitis. The figures for liver iron are low but the circulating hemoglobin was normal. Biological assay of the liver shows hemoglobin-producing factors to be below normal possibly due to some diet limitations.

#### DISCUSSION

The anemia of leukemia has received a good deal of attention, is a well recognized fact, and might be explained in various ways. Loss of blood is often a diagnostic factor in leukemia but the observations in Table 5 indicate that in many cases it is not responsible for the anemia. There are large iron stores and normal or above normal reserves of hemoglobin-producing factors in the liver. *White cell infiltration* of the marrow and liver is probably responsible for the values recorded in Table 5. When the marrow is stuffed with white cells the *red cell* elements can not function properly and there is a tendency toward anemia with some overaccumulation of hemoglobin-producing factors in the liver. There is no evidence of lack of absorption of iron or protein factors. Infiltration of the liver by the leukemic cells may "dilute" the potency of the liver cells as measured by biological assay. Mechanical or toxic injury due to infiltration may be inflicted upon the liver cells and check the storage of proteins in these cells.

*Hypoproteinemia* is viewed with disfavor by the clinician with good reason. That edema may develop needs no debate but there are degrees of hypoproteinemia not sufficient to produce edema yet adequate to lower the body defense against infection (4) and toxic liver injury (5). It is probable that such degrees of hypoproteinemia are more common than is generally appreciated. The drain coming from the fetus or lactation may cause such *plasma protein depletion* and therefore deserves the attention of the obstetrician. It should not be difficult by adequate diet to replete the protein reserve stores. In rare cases of vomiting plasma could be given by vein to replete these important stores. It is at least possible that one factor in precipitating the toxic condition designated *eclampsia* may be the serious depletion of body protein reserve stores. The severity of depletion of the protein stores may determine whether the clinical case of eclampsia does or does not present widespread hyaline liver necroses.

*Hepatitis* with jaundice often shows low stores of hemoglobin-producing factors—both iron and protein (Table 3). Low protein stores and hypoproteinemia favor liver injury—a vicious circle—as liver injury tends to slow up protein production. Obviously a correction of this state is greatly to be desired and if proteins cannot be given by mouth then plasma protein can be given by vein or peritoneum. Because methionine has a specific protective effect against

liver injury due to certain poisons (5), it deserves a clinical test where we suspect a continuing liver injury. Protein by mouth or plasma by vein should also furnish material needed for the prompt repair of injured liver or other tissues.

#### SUMMARY

Human liver tissue has been assayed to determine the amount of hemoglobin production factors in normal and abnormal states. Standardized dogs made anemic by blood removal have been used in this biological assay. Normal animal liver as control is rated as 100 per cent.

Normal human liver tissue as compared with the normal animal control contains more of these hemoglobin production factors—a biological assay ratio of 120 to 160 per cent. Infections, acute and chronic, do not appear to modify these values, the concentration of hemoglobin-producing factors falling within the normal range.

Pernicious anemia and aplastic anemia both show large liver stores of hemoglobin-producing factors—a biological assay ratio of 200 to 240 per cent. Therapy in pernicious anemia reduces these liver stores as new red cells are formed.

Secondary anemia presents a low normal or subnormal liver store of hemoglobin-producing factors—an assay of 60 to 130 per cent.

Hemochromatosis, erythroblastic anemia, and hemolytic icterus in spite of large iron deposits in the liver usually show a biological assay which is normal or close to normal.

Polycythemia shows low reserve stores of hemoglobin-producing factors. Leukemias present a wide range of values discussed above.

Hypoproteinemia almost always is associated with low reserve stores of hemoglobin-producing factors in the liver—biological assays of 60 to 80 per cent. Hypoproteinemia means a depletion of body protein reserve stores including the labile protein liver reserves—a strong indication that the prehemoglobin material (or globin) is related to these liver stores.

Pregnancy, eclampsia, and lactation all may present subnormal liver stores of hemoglobin-producing factors. Exhaustion of protein stores lowers the barrier to infection and renders the liver very susceptible to many toxic substances. It should not be difficult to correct hypoproteinemia under these conditions and thus relieve the patient of a real hazard.

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