From the Department of Medicine, University of Utah, School of Medicine, Salt Lake City.

DIETARY FACTORS CONCERNED IN ERYTHROPOIESIS—Continued

By GEORGE E. CARTWRIGHT, M.D.

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IV. MINERALS—continued

In table 9 the average values of the iron content of various tissues under normal conditions, under conditions of prolonged iron deficiency, and following hemoglobin injections are given. These values are taken from the papers of the Rochester group.384

TABLE 9.—Distribution of Tissue Iron

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parenchyma (Iron Deficiency)</th>
<th>Normal (Standard Diet)</th>
<th>Following Hbg. Injections</th>
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<tr>
<td>Liver</td>
<td>2</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Spleen</td>
<td>6</td>
<td>46</td>
<td>105</td>
</tr>
<tr>
<td>Rib Marrow</td>
<td>12</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Heart</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Muscle</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Lungs</td>
<td>3</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are expressed in mg. per cent.

Little is known concerning the chemical nature of the stored iron. Whether the liver builds the simple precursors into compounds which are transferred to the bone marrow where they are assembled into hemoglobin or whether iron is sent to the bone marrow as a simple salt is not known. Recently several iron-containing compounds have been isolated from liver and spleen as indicated below. These may prove of great interest. Further work on these compounds may well elucidate the mechanism by which iron is incorporated into the hemoglobin molecule.

Approximately 60 per cent of the iron in thoroughly perfused liver is in protein combination, in so far as it is removable from solution by precipitation with trichloroacetic acid. McFarlane394 has shown that 43 to 60 per cent of the iron in perfused adult rat liver reacts directly with bipyridine in the presence of a reducing agent and must therefore be in some form of chemical combination other than that of the iron in hematin. About 60 per cent of this nonhematin fraction appears in

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the trichloroacetic acid precipitate. The total iron in a trichloroacetic acid filtrate reacts directly with potassium thiocyanate in acid solution after oxidation with hydrogen peroxide and this fraction accounts for about 40 per cent of the non-hematin iron. About 50 per cent of the total iron in the trichloroacetic acid filtrate is precipitated along with organic substances by normal lead acetate. The digestion of fetal calves liver, adult rat liver and muscle tissue, and beef spleen by pepsin at about pH 2 produces a five-fold increase in the iron content of the trichloroacetic acid filtrate. The autoproteolytic changes in the liver and spleen at pH 4.5 also include the decomposition of organic iron-containing compounds, presumably iron proteinates. This decomposition is accelerated by hydrogen sulfide and inhibited by copper.

With the knowledge available at the present time one may classify liver and spleen nonhematin iron compounds into the following: (a) ferritin, (b) non-crystallizable ferritins, (c) ferrin, (d) hemosiderin. The portion of the total non-hematin iron present in each of these four compounds is not known.

In 1937 Laufberger isolated the crystalline protein, ferritin, from horse spleen and demonstrated that it contained over 20 per cent by dry weight of iron. The protein was easily crystallized out as the cadmium salt and was found to be stable between pH 4 and 10. Kuhn, Sorensen, and Birkofer concluded that ferritin consists of 54.5 per cent protein, 12.1 per cent nucleic acid, and 35 per cent Fetet-OOH. Careful and thorough studies on the chemical, physical, crystalline, and magnetic properties of ferritin have been performed by Granick, Rothen, Michaelis, and Coryell and their results are reported in a series of papers. Horse spleen ferritin is a protein easily crystallizable as the cadmium salt and contains over 20 per cent iron. Ferritin can be freed from its iron by reduction to the ferrous state and removed by dialysis after combination with a-bipyridine. An iron-free, colorless protein solution results, from which the protein crystallizes in the presence of cadmium sulfate in the same crystal form as does ferritin. This colorless protein is designated as apoferritin and has been investigated in the ultracentrifuge and shown to be a very homogeneous protein with a molecular weight of 465,000. It was found by solubility studies, variation in the iron and phosphorus content, and by studies in the ultracentrifuge that ferritin is not a definite molecular species but consists of a mixture of a complex of apoferritin-iron hydroxide and about 25 per cent free apoferritin, the mass of these particles not being uniform. It was concluded that the iron of ferritin was most likely present in the form of micelles of ferric hydroxide interspersed in the apoferritin crystal lattice in the spaces between the protein molecules. The approximate composition of the iron-rich micelles of ferritin appears to be (FeOOH)₈ (FeO—OPO₃H₂). Magnetic measurements have shown that the iron in the micelles is present in the rarely occurring state of 3 unpaired electrons per iron atom. These workers were unable to confirm the presence of nucleic acid in ferritin.

Ferritin is widely distributed among mammals and the species from which it has been isolated are, in order of decreasing ferritin content, horse, man, dog, guinea pig, mouse, rat, pig, rabbit, and cat. The organs from which it has been isolated are spleen, liver, bone marrow, kidney, and testicle. No trace of ferritin
could be demonstrated in red or white blood cells or blood plasma. In man, ferritin has been found in the spleen, liver, and bone marrow. The total amount and concentration of ferritin in human liver is much greater than that in the spleen. It may be significant that extensive bleeding in horses lowers the ferritin and apo-ferritin content of the spleen. The decrease in the apo-ferritin content suggests a reutilization for blood production of the protein fraction of the ferritin as well as of the iron. That ferritin is found in high concentration in the red bone marrow is also suggestive of a role in blood formation. The function of ferritin as storage iron has been shown in dogs using the radioactive isotope. Radioactive iron in the form of ferric ammonium citrate when administered by vein is readily converted into ferritin iron in the liver. One hour after injection over 40 per cent of the injected iron was found to be present in the ferritin-rich fraction. In another dog, after 2 hours, 61 per cent of the injected iron was found in this fraction. It has also been demonstrated that iron liberated from destroyed red cells is used for the construction of new ferric hydroxide micelles of ferritin. The authors have therefore concluded that "ferritin iron acts in the capacity of storage iron in the animal body."

The in vitro conversion of radioactive iron to ferritin has been studied and it has been found that ferritin may take up almost 50 per cent of radioactive iron by mixture with ferric ammonium citrate. The incubation of guinea pig liver brei with apoferritin and radioactive ferric ammonium citrate, the subsequent isolation of crystalline ferritin, and the determination of its radioactivity indicate that iron micelles of ferritin are the result of metabolic synthesis, perhaps due to a specific enzyme.

After ferritin is removed by crystallization with cadmium sulfate, there remains a brown mother liquor which cannot be made to yield further crystals but contains a brown, colloidal, nondializable compound containing about 19.8 per cent iron and somewhat less nitrogen than ferritin although the relative nitrogen, phosphorus, and iron values do not differ appreciably from ferritin. It is thought that the iron is present as a colloidal ferric hydroxide, the micelles of which contain in part ionic constituents other than OH, and that these micelles are loosely attached to proteinaceous material, including some denatured apoferritin. This fraction is called "noncrystallizable ferritin" and its significance is unknown.

Libet and Elliott have described an iron-protein complex in liver which they have termed ferrin. This compound differs from ferritin in several respects. It precipitates rather than crystallizes on addition of cadmium sulfate, it precipitates at a lower concentration of ammonium sulfate than does ferritin, it is not present in appreciable quantities in the spleen, and the iron in ferrin is more active than the iron in ferritin in the catalysis of phospholipid oxidations. It is not known whether ferrin is a single and specific compound, nor is it known whether or not it represents a denatured product. The function of ferrin has not been studied.

The yellow brown granules seen in the tissues of most mammals have long been known as hemosiderin. The most unusual feature of this iron-containing compound is the appalling lack of knowledge concerning it. It is recognized that it is the iron-containing portion of hemoglobin which rests in the cells of most tissues but es-
Especially is it found in the spleen, liver, and kidney following the destruction of erythrocytes. Hemosiderin probably represents some stage or side reaction in the disintegration of hemoglobin into bile pigment but the exact nature of the reactions involved is not understood. There is little doubt that it functions as storage iron, for the granules are abundant following repeated injections of ferric chloride and disappear when the demands of the body for iron are great. The precise chemical nature is unknown. Cook in 1929 presented evidence that the iron-pigment consists of organic granules impregnated with ferric oxide. The iron can be removed by treatment with acid, leaving the substrate practically intact. This iron then reacts with thiocyanate and other substances in a manner which is not characteristic of ionic iron. Recent work by Michaelis and co-workers casts doubt on the homogeneity of hemosiderin granules. They conclude that the iron of hemosiderin is, "at least in the main part, in the same magnetic state as ferritin, and that some of its iron may be in a state of lower susceptibility." Hemosiderin granules isolated by differential centrifugation in a partial state of purity have an iron content of 8.19 per cent, a nitrogen content of 12.9 per cent, and a phosphorus content of 1.6 per cent.

Saha and Guha have obtained an iron-copper-nucleoprotein complex in a fairly pure state from fish. They suggest on a basis of the hemoglobin-regenerating potency of this compound in anemic rats that it may be a precursor of hemoglobin.

Anemia of iron deficiency.—The anemia of iron deficiency and its response to iron therapy are well recognized and have been described many times. Only certain features will be summarized here.

A hypochromic, microcytic anemia is characteristic of iron deficiency and has been described in many different species. In the advanced stages marked anisocytosis and poikilocytosis of the red cells are present. The mean diameter of the red cells is small and the mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin are reduced. The Price-Jones curves in severe cases show a marked shift to the left with a broad base. Reticulocytes, punctate basophilia, and nucleated red blood cells are occasionally seen. The white blood cells and platelets are usually unaffected.

In the absence of iron, hemoglobin cannot be synthesized and there is a maturation arrest at the normoblastic stage. The bone marrow is hyperplastic and shows a predominance of the more mature nucleated red blood cells, principally the polychromatophilic normoblasts. There is no evidence that there is an increased rate of destruction of red cells. The serum bilirubin and icterus index are either normal or diminished and hemoglobin destruction as measured by urobilinogen excretion is diminished.

Porphyrin metabolism has been inadequately studied in iron deficiency. Duesberg states that porphyrin excretion is diminished and on this basis suggests that iron may function in the preporphyrin stage of hemoglobin formation. Seggel has shown that erythrocyte protoporphyrin is increased in iron deficiency and this has been clearly confirmed by Watson et al. The normal erythrocyte protoporphyrin ranges between 20 and 45 gamma per 100 cc. of erythrocytes. In a woman with a marked hypochromic anemia due to chronic blood loss from bleeding...
hemorrhoids (case no. 12, Watson et al.) protoporphyrin was found to be as high as 613 γ. Following iron medication this fell to 40 γ. These authors assume that the increase is the result of cessation of hemoglobin formation at the porphyrin stage.

Moore, Doan, and Arrowsmith35 and others422 have demonstrated that the serum iron level is low in iron deficiency anemias of varied etiology. There is no consistent alteration of the "easily split-off" iron under these conditions. Under the influence of iron therapy, serum iron values return to normal as the mean corpuscular hemoglobin concentration increases to normal and as the state of iron deficiency is corrected.

6. Excretion.—It has long been claimed that iron is excreted by three routes—urine, bile, and the large intestine. The older methods of iron analysis were fraught with many difficulties. Since the use of radioactive iron much more reliable information is at hand and the older concepts will have to be altered.

It is now generally agreed that only a minimal amount of iron is excreted in the urine. It is questionable whether this small amount has any significance. It is probably derived from cellular debris. It has been shown that the urinary excretion of iron is rather constant in the same individual and that it is not related to iron intake. Barer and Fowler423 have summarized the literature and report the average value for 100 men to be 0.395 mg./day and for 100 women 0.489 mg./day. In rats Greenberg, Copp, and Cuthbertson424 were able to demonstrate that 1.5 per cent of the administered dose of radio-iron was excreted in the urine. Hahn and co-workers425 injected radio-iron intravenously in dogs and found that only small quantities were excreted by this route following iron injections. Later the excretion dropped to traces and even to zero. These workers suggested that the small quantities found immediately after the injection were derived from the diffusable fraction of plasma iron. Similar conclusions have been reached by Little, Power, and Wakefield.426

There is evidence that significant amounts of iron are eliminated in the bile under certain circumstances. However, under normal conditions iron is eliminated in the bile of dogs at the low but constant rate of 0.2 mg./day.427 The excretion is not increased by the injection of iron by vein. Confirmatory results have been obtained in the rat using the same method.424 Under conditions of increased blood destruction427 the elimination of biliary iron may increase ten-fold and parallels the increased output of bile pigment, but even then only 3 per cent of the released iron is eliminated in the bile.

Welch, Wakefield, and Adams428 were the first to challenge the older concept that the intestine holds the power to regulate the excretion of iron. They showed in a patient with an ileostomy that the excretion of iron into the colon was negligible. McCance and Widdowson439, 440 and Fowler and Barer425 by introducing iron parenterally came to the same conclusion. McCance and Widdowson440 further demonstrated that less than 0.5 per cent of the total amount of iron liberated by the destruction of red cells with acetyl-phenylhydrazine in a polycythemic patient was excreted. Recently these authors441 have studied iron excretion in a patient with a hemolytic anemia who received approximately 80 mg. of iron per day.
intravenously in the form of transfusions for 100 consecutive days. In addition to this, her daily dietary intake of iron was 5.6 mg. per day. On this regimen of 8.6 mg. intake of iron per day she excreted only 0.2 mg. total per day in urine and stools. From this the authors concluded that she was unable to excrete the large quantities of superfluous iron and that iron once absorbed remains in the body. Histologic study by Maddox and Heath of the gastrointestinal tract and of a colonic explant on the abdominal wall of dogs before and after the administration of iron revealed no evidence that iron can be observed in the process of excretion by these organs. Work with intravenously administered radioactive iron has confirmed this view. Hahn and co-workers found that in 5 dogs receiving 100 to 250 mg. of radio-iron the fecal excretion settled down to 0.05 to 0.4 mg. per day. Greenberg et al. in a similar experiment in rats found 1.9 per cent of the radio-iron in the gastrointestinal tract. Both groups of workers suggest that this small quantity may be derived from epithelial wastage.

In summary it may be said that small but insignificant amounts of iron (approximately 1 mg. per day) are excreted by the body through the urine, bile, and intestine. It would seem that the body controls the iron stores by controlling the absorption rather than its elimination and that once iron gains entrance into the body it remains there.

B. Copper.—There has been no subject in hematology more controversial than that of copper. The earlier literature concerned itself principally with the effectiveness or ineffectiveness of iron salts in the prevention and cure of the anemia produced in rats maintained on a milk diet. This controversy resulted in a voluminous literature. It has now been demonstrated many times in several different species that copper is needed in addition to iron in order either to prevent or to cure the anemia. This earlier literature has been reviewed by Elvehjem.

Unfortunately, so much emphasis has been placed on the value of copper in the treatment of experimental anemia in rats and in certain instances of iron deficiency anemia in human beings that, although the presence of copper in normal tissues has been recognized for a long time, little attention has been given to its function and metabolism. Since the original controversy interest in this element has waned. This is extremely unfortunate since an understanding of copper metabolism is essential to an understanding of the process of erythrogenesis.

Various reviews on the subject of copper are available. Copper deficiency in animals.—In 1924 a series of studies was begun at the University of Wisconsin which demonstrated that when rabbits or rats were raised on a diet consisting of whole milk an anemia developed. This anemia failed to respond to highly purified iron salts but did respond to the ash of liver, lettuce, or corn especially when supplemented with an iron salt. The pale blue color of the liver ash suggested that copper might be the active factor. It was soon discovered that the addition of 0.05 mg. of copper together with 0.5 mg. of iron to the whole milk diet produced an immediate and striking recovery. Iron alone failed to cause a reticulocytosis while copper alone produced a small prolonged response. When both iron and copper were given a reticulocytosis of 16 per cent developed in 4 days. The minimal daily requirements for the production of a typical reticu-
Dietary factors concerned in erythropoiesis

Locyte response in an anemic rat were found to be approximately 0.3 mg. of iron and 0.005 to 0.01 mg. of copper. Manganese as well as eleven other elements were found to be inactive. Underhill, Orten, Mugrage, and Lewis demonstrated that rats maintained for 667 days on a milk diet supplemented with iron and copper maintained a normal blood picture.

Smith and Medlicott have made a detailed morphological study of the red blood cells in rats deficient only in copper and have found that the anemia is microcytic and hypochromic and is accompanied by a moderate reticulocytosis of 8 per cent. Blood smears showed a microcytosis, hypochromia, and occasional basophilic red cells and poikilocytes. The microcytosis was not as marked as in iron deficiency anemia. The feeding of copper to rats deficient in both iron and copper produced a marked reticulocytosis, a rise in the erythrocyte count, and no change in hemoglobin. This work is summarized in Table 10.

Copper has been shown to be essential for erythropoiesis in dogs. Dogs made anemic by bleeding have abundant stores of copper in the liver and spleen and that the administration of copper has only a moderate or irregular effect on hemoglobin production. They state that they have no explanation for this indefinite and irregular response. Yet, they admit that the salmon bread diet used by them supplies at least 1 mg. of copper daily. Since copper is not a constituent of hemoglobin as is iron and whole blood contains only approximately 10 micrograms per 100 cc., phlebotomy draws only little on the copper stores. Therefore, there is no reason to expect that their dogs should have been copper deficient or that they should have responded to copper therapy. When growing dogs are maintained on a copper-low milk diet and then phlebotomized, it has been adequately demonstrated that they are copper deficient and consequently respond well to iron only after the addition of copper.

In copper deficiency anemia in dogs it has been reported that there is no significant alteration in the mean corpuscular volume or in the saturation index. However, during copper therapy the mean corpuscular volume increases inconstantly with the hemoglobin increase.

A condition called "enzootic ataxia" and variously known as "Gingin rickets" or "ataxia in young lambs" has been described in sheep in Western Australia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R.B.C. Millions per cu.mm.</th>
<th>Hbg. Gm. %</th>
<th>Ht. cc per 100 cc.</th>
<th>MCV cu.µ</th>
<th>MCH %</th>
<th>MCHC %</th>
<th>Retic. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.4</td>
<td>14.9</td>
<td>44.7</td>
<td>61</td>
<td>33</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Milk Anemia</td>
<td>3.2</td>
<td>3.4</td>
<td>11.5</td>
<td>37</td>
<td>28</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Fe—Fed</td>
<td>2.8</td>
<td>3.5</td>
<td>13.5</td>
<td>31</td>
<td>27</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Cu—Fed</td>
<td>4.2</td>
<td>3.4</td>
<td>14.6</td>
<td>35</td>
<td>23</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Modified from Smith and Medlicott. Average values are given; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.
Diseases of lambs with similar clinical features have been described from other parts of the world as "renguera," "paralysis of lambs," "sway-back," "swing-back," and "warfa." These conditions have been incompletely studied and their etiology is unknown.

The condition occurring in England and known as "sway-back" responds to copper treatment even though it has been demonstrated that the grasses in the affected areas and the livers of the affected animals are not deficient in copper. It has been suggested that the disease is due to a disturbance of copper metabolism.

The condition occurring in Western Australia has been carefully studied and conclusively shown by Bennetts and Beck to be due to copper deficiency. A complete review of their work is worth while since it demonstrates in detail the manifestations of copper deficiency in sheep and shows so clearly the relationship of copper to hematopoiesis. Manifestations have been shown to be present in both pregnant or lambing ewes and in lambs. The disease occurs most commonly in lambs of 2 to 3 months of age. The first manifestations are "an appearance of unthriftiness" and retardation of growth. Incoordination of the gait affecting the hind limbs soon follows. The animals develop marked ataxia and eventually the forelegs are affected. They then remain in a position of decubitis and die in this condition in 3 to 4 weeks from malnutrition and intercurrent infections. The condition appears to be essentially one of ataxia without true paralysis. Pathologically the disease is characterized by demyelination of the nervous system, a typical degeneration of the myelin sheaths of nerve fibers in the cord being pathognomonic. Both motor and sensory fibers are involved. Extensive brain lesions especially in the medulla and cerebellum may occur. Hemosiderin was commonly seen in the livers and occasionally in the spleen and kidneys of the lambs. A definite hypochromic, microcytic anemia was frequently seen in the subacute disease. This is demonstrated in table 11. Blood smears showed anisocytosis and hypochromia. Stippled cells were rarely observed and were never numerous. The administration of copper to these animals completely alleviated the anemia. There was a striking correlation between the degree of anemia in the late stages of pregnancy or the early lactation period in the

<table>
<thead>
<tr>
<th>Subjects</th>
<th>R.B.C. X10^6 per cu. mm.</th>
<th>Hbg. Gm. ℓ/ℓ</th>
<th>Ht. cc. per 100 cc.</th>
<th>MCV cu. ℓ</th>
<th>MCH ℓ</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Healthy Lambs from &quot;Sound&quot; Localities</td>
<td>(14.0–16.9)</td>
<td>15.0</td>
<td>(13.2–17.6)</td>
<td>44</td>
<td>(9–11.0)</td>
<td>(19–43)</td>
</tr>
<tr>
<td>3 Ataxic Lambs</td>
<td>(9.4–17.1)</td>
<td>8.5</td>
<td>(5.9–13.2)</td>
<td>55</td>
<td>(6.0–8.8)</td>
<td>(30–35)</td>
</tr>
</tbody>
</table>

* Modified from Bennetts and Beck. MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration. Note: The MCH and MCHC were calculated from the data by the reviewer. Figures in parentheses represent range. Lower figures represent mean.
ewes and the development of ataxia in the lamb. A correlation was demonstrated between low blood copper in the mother and the occurrence of ataxia in the progeny. No definite relationship was found between the level of hemoglobin and blood copper in the lambs although low hemoglobin values were not noted until the blood copper had fallen to levels between 0.01 and 0.02 mg. per cent. In many cases the blood copper fell to these levels without a fall in the hemoglobin value. When the hemoglobin diminished it was usually some time after the copper level had fallen. Chemical analysis of the liver, blood, and milk of the mothers and of the liver and blood of the affected progeny demonstrated conclusively and constantly in all cases analyzed that a low copper status existed. Analyses of pastures in the affected districts were extremely low in copper as compared with pastures in nonaffected districts. The administration of copper to the mother during the gestation period prevented completely the occurrence of the disease. Copper administered to the lambs arrested the disease, even in the later stages, at which time the course was rapid in untreated controls. Treated animals, apart from persistence of some ataxia, recovered. Copper supplements in the form of a lick or as a top dressing prevented the occurrence of ataxia and promoted optimal growth in lambs.

The manifestations seen in adult sheep were somewhat different from those observed in lambs. "Stringiness" of the wool was observed in the animals after grazing a few months on copper-deficient pastures. Ataxia and pathological changes in the nervous system were not observed in adult sheep. There was, however, moderate hemosiderosis of the liver, kidneys, and spleen. The degree of hemosiderosis appeared to be related to the degree of anemia. The breeding ewes exhibited diarrhea and anemia. The anemia was severe. Values of as low as 2.7 Gm. per cent hemoglobin, 2.7 million red blood cells, and a volume of packed red blood cells of 15.5 cc. per 100 cc. were observed. The blood studies are summarized in table 12. In severe cases macrocytes were sufficiently numerous to raise the mean corpuscular volume significantly above normal. In spite of this slight macrocytosis a mild hypochromia existed. The authors state: "A classification of the anemia is not possible without further extensive investigations which are beyond the scope of the present inquiry." Examination of blood smears revealed marked anisocytosis, poikilocytosis, numerous macrocytes, stippled cells, and polychromatophilia. Howell-Jolly bodies and normoblasts were occasionally seen. In 1 case 49 normoblasts per 100 white blood cells were observed. In 2 cases "macroblasts" were seen. Reticulocytes were generally numerous in the more anemic animals (mean 4.2 per cent and maximum 11 per cent). As in the case of the lambs the anemia was associated with low copper values in the blood and liver. Very low blood and liver copper values were sometimes present in the absence of anemia and anemia generally developed several months after the blood copper had fallen to very low levels. After the completion of gestation and lactation periods the blood copper levels returned to normal earlier than the hemoglobin values, but low liver copper levels persisted. The anemia in all cases responded to "pure" copper salt supplements with a reticulocyte peak of approximately 10 per cent occurring on the 5th to the 7th day. Thereafter the blood picture steadily improved, reticulocytes and other abnormal cells disappeared from the circulation and by the fourth week the red
blood cell count, hemoglobin, and mean corpuscular volume approached normal values. During the same period control ewes became somewhat more anemic. The authors conclude, "It is clearly evident that in sheep as in other species copper is necessary for normal hemoglobin formation and erythropoiesis. It would appear, however, that this function may be carried out, in the sheep, under conditions of copper deficiency when the blood and liver copper values of the animal are very low, provided that it is not called upon to produce and rear progeny. In this event, owing to the drain on the mother's reserves for the embryo a breakdown may occur and anemia supervenes."

An enzootic disease of cattle is known to occur in the areas in which the enzootic disease in sheep is prevalent. This condition in cattle, known as "falling disease" because of its termination in sudden death, has been studied in detail and reported by Bennetts and his co-workers. Clinically the disease is characterized by a "rough staving coat," a depraved appetite, suppression of oestrus, anemia, and sudden death. Young animals show marked evidences of malnutrition and abnormal development; intermittent diarrhea and anemia are frequently present. Pathologically the disease is characterized by atrophy and scarring of the myocardium, marked hemosiderosis of the liver, spleen, and kidneys, and by an unusual type of glomerulonephritis in the fatal cases. The sudden death would appear to be a result of myocardial failure. The anemia has a seasonal incidence, being especially prevalent in September and October, it may be exceedingly severe, and it is reported to be definitely macrocytic and somewhat hypochromic. The degree of anemia correlates well with the degree of reduction of the blood copper. Anisocytosis, poikilocytesis, numerous macrocytes, polychromasia, punctate basophilia, and occasional Jolly bodies were seen in the blood smears. Reticulocytes were not numerous. Evidences of increased cell fragility or excessive hemolysis were not found. A low

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**Table 12.—Blood Values of "Normal" and Affected Sheep**

<table>
<thead>
<tr>
<th>Group</th>
<th>R.B.C. X106 per cu.mm.</th>
<th>Hbg. Gm. %</th>
<th>Ht. cc. per 100 cc.</th>
<th>MCV cu.m.</th>
<th>MCH µg</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Normal&quot;</td>
<td>(7.7-13.2)</td>
<td>(9.8-16.8)</td>
<td>(30.0-48.8)</td>
<td>36.5</td>
<td>(30-39)</td>
<td>(11-14)</td>
</tr>
<tr>
<td>&quot;Nonanemic&quot;</td>
<td>(6.7-13.1)</td>
<td>(8.3-15.8)</td>
<td>(20.0-42.5)</td>
<td>35.2</td>
<td>(19-42)</td>
<td>(10-14)</td>
</tr>
<tr>
<td>&quot;Anemic&quot;</td>
<td>(5.7-7.4)</td>
<td>(3.9-7.5)</td>
<td>(15.5-24.9)</td>
<td>20.9</td>
<td>(10-59)</td>
<td>(11-15)</td>
</tr>
</tbody>
</table>

* Summarized from Bennetts and Beck. MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration. Note: The values for MCH and MCHC were calculated by the reviewer from the data. The "normal" sheep consisted of animals taken from healthy areas. The "nonanemic" sheep were animals taken from disease-producing areas and received copper supplementation. The "anemic" animals were breeding ewes from a disease-producing area and had received no copper. Figures in parentheses represent range. Lower figures represent mean.
copper status in the liver and milk of the animals as well as a low copper content of the pastures has been demonstrated. Copper supplements prevented the appearance of the seasonal anemia as well as the other clinical signs of the deficiency and alleviated the anemia, promoting optimal health in affected animals maintained in affected areas. In an experimental dairy herd optimal response was obtained by the administration of pure copper supplements; the addition of other minerals had no appreciably beneficial effect. Thus it would appear that ‘falling disease’ in cattle is due to an uncomplicated copper deficiency.

In addition to rats, mice, rabbits, dogs, sheep, and cattle copper has been shown to be essential for erythropoiesis in chickens and pigs. Elvehjem has concluded “that copper is of fundamental importance in the formation of hemoglobin in all red-blooded animals.”

2. Copper deficiency in man.—In the treatment of nutritional anemia in infants it has often been demonstrated that when iron therapy is combined with copper therapy the rate of hemoglobin formation is more rapid than when the anemia is treated with iron alone. Josephs treated a large series of infants with iron and copper. The hemoglobin curves of the group treated with iron tended to flatten out at about 50 per cent, whereas the curves representing the infants on both iron and copper continued to rise steeply to about 70 per cent. Hutchinson treated anemic infants for periods of 1 to 5 weeks with ferrous sulfate. Medication was then discontinued for 1 to 6 weeks. At the end of this time the hemoglobin values had become stationary. Copper sulfate was then given. In every case there was a further rise in hemoglobin. Similar results have been reported in Parsons and Hawksley in 3 cases, by Elvehjem, Duckles, and Mendenhall in 6 cases, and by Lewis in 2.9 cases, as well as by others.

Usher, MacDermot, and Lozinski tested the efficacy of copper as a prophylactic agent against the anemia of infancy in institutionalized patients. They found that at the age of 1 year, the group receiving both copper and iron supplements had an average hemoglobin value of 19 per cent above that of the control group receiving no supplement, whereas the group receiving iron alone had an average hemoglobin value 15 per cent higher than that of the controls.

An analysis of the livers of anemic infants has revealed a lower copper content than the livers of nonanemic controls. A reduced copper content of the livers of infants during the nursing period and an increase as soon as a mixed diet was supplied has been reported. The most convincing proof of the efficacy of copper is presented by Moore. Using the double reticulocyte method, he treated hypochromic anemia in infants with ferrous sulfate. After 2 weeks, copper sulfate was added to the iron. In several cases the copper initiated a second reticulocyte rise.

There is very little evidence to indicate that iron alone is as effective as combined iron and copper therapy in infants. That evidence which does exist is not convincing. Mackay found that iron and copper were no more effective in 6 cases with nutritional anemia than in 6 cases given iron alone. The iron preparation used in her series, as she admits, was not copper free. Lottrup obtained results similar to Mackay’s in 2 cases.

Elvehjem, Duckles, and Mendenhall make the statement: “Since milk is low
in both iron and copper, 1 liter of certified milk containing from 0.12 to 0.18 mg. of copper and approximately 0.50 mg. of iron, it is not surprising that the reserves of infants may become depleted and that anemia may result.

The value of copper in the treatment of hypochromic microcytic anemia in adults is controversial. Mills reported cases which did not respond satisfactorily to 15 to 60 grains of "Blaud's mass" daily for a period of one to several months but did respond rapidly when copper was added to 75 grains of Blaud's mass. Five cases which were treated initially with Blaud's mass containing copper responded both immediately and favorably. Adamson and Smith reported similar results in 10 cases. Dameshek found that about 75 per cent of the cases which he treated responded maximally to a copper-free iron preparation alone; in the remainder small doses of copper sulfate, given with iron, were effective in continuing the hemoglobin response. Waugh in referring to hypochromic microcytic anemia in adults makes the statement: "I am inclined to take the view that there is a more or less specific therapeutic value in the combination of copper with iron in the treatment for this condition," but he presents evidence in only 1 case. Gross treated 8 patients intravenously with a combination of iron cacodylate and copper formate and found this combination effective but observed no controls with the iron cacodylate alone. Jones states that he has encountered many patients who do not respond to either iron or iron and acid until copper is added, but he presents no data.

It is the general consensus of most hematologists that adult patients with hypochromic microcytic anemia, in the absence of infections, malignancy, and continued hemorrhage, will respond satisfactorily and rapidly to iron medication. Bethell et al. treated a series of patients with minimal amounts of purified iron in conjunction with a low copper diet and obtained a good rate of hemoglobin formation. The low copper intake apparently made no difference in the response. Fowler and Barer divided the treatment of 19 patients with hypochromic microcytic anemia into three periods. During the first iron was administered, during the second iron plus copper, and during the third the copper was omitted and the dose of iron increased. The average increase in hemoglobin in grams per cent during the first period was +0.415, during the second +0.233, and during the third +0.336. These authors concluded that copper sulfate did not increase the effectiveness of the iron.

From the data presented it is possible to draw certain general conclusions regarding the efficacy of copper in the treatment of microcytic hypochromic anemia in man. In evaluating the literature several factors must be considered. First, it is very probable that a few of the cases in which negative results were obtained were not actually cases of nutritional anemia. Secondly, there may have been factors present such as infections or malignancy which retarded or prevented a response. Thirdly, some of the negative results might be explained on the basis of contamination of the iron with copper or the hospital diets may have contained sufficient copper to make any beneficial effects from added copper difficult to detect. Admittedly, such experiments are difficult to control since the quantity of copper needed is so small. Fourthly, some of the cases presented as not responding to iron alone were probably not given a sufficiently long period of trial and when copper was added the response
may have been due to the iron alone. Reticulocyte counts were not done in any of
the cases recorded as not responding to iron.

In nutritional anemia in infants the rate of hemoglobin formation is accelerated
when copper is given in addition to iron. In adults supplemental copper therapy
may be of value in a few cases. These, if they occur, are rare. Most cases will respond
if adequate doses of iron are given. This does not necessarily indicate that copper is
not needed for hemoglobin formation or that it is not a dietary essential but rather
that the quantities needed are so small that sufficient copper is present in the body
stores in adult life, in the diet, or as a contaminant in the iron to supply the needs.
No uncomplicated or clearly defined case of copper deficiency in man has been
reported.

There is a need for careful studies on the treatment of hypochromic microcytic
anemia in adults in which the diet as well as iron used is free from copper and in
which use is made of the double reticulocyte method to determine the degree of
response to iron as well as the response to copper. Considering the amount of debate
which this controversy has caused it is amazing that such studies have not been
done. The opinions which have been expressed are based more upon general impres-
sions and poorly controlled experiments in a small number of cases than upon reli-
able experimental data.

3. The metabolism of copper in animals.—Copper is abundant in food and its
availability is not a problem. It has been shown that the copper in many natur-
ally occurring compounds is readily utilized by the body. Very little is known
concerning the mechanism or site of absorption. The absorption of copper from the
upper third of the jejunum has been demonstrated. When the copper stores of
rats are markedly depleted only about per cent of the dietary copper is re-
tained. This is more, however, than is retained by iron-deficient rats and is a
sufficient amount to give rise to maximal hemoglobin formation.

Following the ingestion of radioactive copper by the dog, the element appears
quickly in the plasma and continues to rise for 2 to 5 hours, after which it falls
abruptly. Sachs et al. have obtained similar results following the oral ad-
ministration of copper sulfate. Thus plasma is concerned with the transportation
of copper. The plasma copper is bound loosely to protein, principally the albumin
fraction. About two-thirds of it is split off by cold trichloroacetic acid and very
little of it is dialyzable, suggesting that it is in a unionizable form. Within
24 hours the radioactive copper can be demonstrated in the bone marrow as well as
in the circulating red cells. The amount in the red cells continues to
increase for 24 hours. The total activity in the blood represents about 45 to 65 per
cent of the total activity in the body. The relative retention of radio-copper is
greatest in the kidney and the liver but is present in almost all tissues. The feeding
of iron and copper for a short period of time to severely anemic animals deficient
in both iron and copper does not lead to an accumulation of copper in the bone
marrow but its presence there has been demonstrated. There is suggestive
evidence that in animals in which hematopoietic activity is accelerated the uptake
of copper by the red cell is somewhat increased.

The copper content of the whole blood of pigs as well as most other animals is
in the range of 100 to 180 micrograms per 100 cc. Copper is distributed approximately equally between the cells and plasma. In nutritional anemia in pigs the copper content of the whole blood falls to levels as low as 7 micrograms per cent and that of the plasma to 4 micrograms per cent. The feeding of small amounts of copper results in a very rapid rise. Schultze, Elvehjem, and Hart have suggested that in the pig rapid hematopoiesis cannot take place unless the copper content of the blood is maintained above 20 micrograms per 100 cc. of whole blood. Dogs made anemic by excluding iron and copper from their diet show a temporary increase in whole blood copper when iron alone is fed. The administration of copper alone is associated with a rise to normal of the blood copper level but the latter falls again in time if the administration is not continued. If then iron is given, there is a sharp rise in blood copper. Thus, when an acceleration of hematopoiesis takes place there is a mobilization of copper from the stores to the blood, provided such stores are available. This is in accord with the observations that following hemorrhage there is an increase in blood copper.

The storage of copper in the tissues, especially the liver, has been studied in detail by many investigators. The results of this work may be briefly summarized. During embryonal development there is a high concentration of copper in the liver. This increases until birth, at which time it is higher than any other time in the life of the animal. Since the copper content of milk is low the reserves of this element diminish during the nursing period. If the animals are then continued on a milk diet the copper content of the tissues is severely depleted. The feeding of copper results in the restoration of these reserves. It seems that in the stages when the liver is most actively engaged in hematopoiesis it contains the greatest amount of copper. The feeding of a high copper diet to mothers has very little effect on the fetal stores. Restriction of copper in the mother results in depletion of the fetal reserves.

There is very little information available regarding the excretion of copper. Using radioactive copper Schultz and Simmons found appreciable amounts in the urine. How much of this was due to contamination with the feces they were unable to state. Lindow, Peterson, and Steenbock detected copper in the urine of rats maintained on stock rations. When this ration was supplemented with copper the urinary excretion increased five-fold. However, 98 per cent of the added copper was excreted in the feces. Schubert and co-workers using the radioactive isotope report that the liver is the chief excretory organ.

Eden has studied the excretion of copper in the rabbit. Following oral administration he found that about 0.2 per cent was excreted in the urine. When the copper was placed directly into the stomach approximately 1 per cent appeared in the urine. After an intravenous injection the copper content of both the urine and the feces rose sharply. Sandberg and Perla splenectomized rats and noted an increased elimination of copper in the feces, which commenced two weeks after splenectomy. This was associated with a persistent negative nitrogen balance and they concluded that the spleen is essential for the utilization of copper.

4. Metabolism of copper in man.—The absorption and excretion of copper in man has been little studied. Tompsett noted a daily excretion of 0.63 mg. of copper in
2.70 DIETARY FACTORS CONCERNED IN ERYTHROPOIESIS

a patient receiving 0.21 mg. daily. Chou and Adolph47 studied the copper metabolism of 4 normal adults for periods of 1 to 3 days and found that equilibrium was reached on a daily intake of about 2.0 mg. of copper. Ohlsen and Daum414 found that when the intake of copper for 3 young women ranged from 0.96 mg. to 1.15 mg. the excretion exceeded the intake by amounts of from 0.08 to 0.40 mg. Leverton and Binkley515 found that the average daily intake of copper of 65 normal young women on self-chosen diets was 2.65 mg. The average daily retention by this group was 0.85 mg. As the copper intake increased the percentage retained increased. In general about 30 to 35 per cent of ingested copper is retained. It may be concluded from these balance studies that the daily requirement of copper for adults is 2.0 to 2.5 mg. This amount is easily obtained from normal diets.

In 3 normal preschool age boys Scoular516 has reported that only 15 to 58 per cent of the ingested copper is excreted by way of the alimentary tract and that the daily urinary copper excretion was fairly constant, averaging 4 per cent of the ingested copper. Daniels and Wright517 in similar subjects found a total excretion of 45 to 85 per cent.

Van Ravesteyn518 fed 150 mg. of copper sulfate per day for 3 days to adults. Approximately 65 to 75 per cent of the copper was recovered from the feces over a period of 6 to 9 days. The copper content of the bile was more than doubled but the excretion of copper in the urine was not affected. Following the intravenous injection of copper the blood level rose to 300 to 400 micrograms per cent and during the course of the next 2 to 4 hours gradually returned to normal. The excretion in the bile and urine rose temporarily and the fecal copper increased markedly. This increase was greater than could be accounted for by the excretion through the bile. Rabinowitch519 has reported that the copper content of normal urine ranges from traces to 0.7 mg. per 24 hours. Tompsett513 found 0.08 to 0.48 mg. per liter. Chou and Adolph47 obtained an average value of 0.25 mg. per day and found that the excretion did not vary appreciably with changes in copper intake.

It has been estimated that the adult body contains between 100 and 150 mg. of copper.471 During embryonic development copper becomes concentrated in the liver and reaches a maximum concentration at term.472, 509 There is then a sharp decline after the second month.521 The distribution of copper in human tissues has been reported in detail.471, 521–523 The copper content of the liver has been noted to be increased in Mediterranean anemia,522, 524 hemochromatosis,524 cirrhosis of the liver,525, 527 acute yellow atrophy of the liver,528 tuberculosis,521 and carcinoma.521, 525 Sandberg, Gross, and Holly524 have made a study of the copper content of the liver, spleen, and stomach in a large series of cases of severe chronic disease accompanied by secondary anemia and have reported that there is a huge storage of copper in the depot organs. In cancer accompanied by anemia the marked increase in copper storage was out of proportion to the anemia and even took place in several cases in the absence of anemia. The retention was significantly higher in cases with extensive metastasis. Buchwald and Hudson589 found that the copper content was high in the liver and bile, intermediate in the kidney, heart, and pancreas, and low in the tumor tissue and spleen in cases of malignancy.

There was little agreement in the earlier literature as to the amount of copper in the blood.208, 482, 498 There was even less agreement as to the ratio of its distribu-
tion between the cells and serum. These disagreements were most likely the result of poor methods. It is now generally agreed that the range for whole blood copper in normal adult males is approximately 90 to 150 micrograms per cent while that for normal adult females is approximately 100 to 160 micrograms per cent. Sachs, Levine, Hill, and Hughes recently reported an average value of 102 micrograms per cent for whole blood copper in adult males and 107 micrograms per cent for adult females. Normal females have been persistently found to have slightly higher values than males.

It is now generally agreed that copper is distributed approximately equally between the cells and serum. Sachs and his co-workers have reported serum values for normal males between 70 and 132 micrograms per cent and for normal females between 78 and 124 micrograms per cent. Cartwright, Jones, and Wintrobe determined the copper content of the serum of 25 healthy adult males and 25 healthy adult females. The average value for the males was 116 micrograms per cent. The lowest value obtained was 92 and the highest 134 micrograms per cent. For the females the average was somewhat higher, 131 micrograms per cent with values ranging between 103 and 159 micrograms per cent. Nielsen reports the serum copper for normal males as 100 ± 12.5 micrograms per cent and for normal females ± 16.5 micrograms per cent.

Sachs and his group have made studies on whole blood copper as well as iron at various ages. At birth there is an increase in iron and a decrease in copper. During the first 2 months of life there is a sharp and pronounced drop in iron and a sharp rise in copper. From 2 months until 1 year of age there is a gradual rise in both iron and copper. During the age period of 2 to 12 years the values for both iron and copper tend to remain stationary although the values for iron are lower than those found in adults and the values for copper are somewhat higher. At the onset of puberty the iron gradually increases until it settles in the adult range and the copper decreases until it reaches the normal. Thus from infancy throughout life there is an inverse relationship of the copper and iron of whole blood.

Many investigators have reported the presence of increased blood copper during pregnancy. In spite of this, blood taken from the umbilical cord has a low copper content. In hypochromic microcytic anemia in infants and adults the whole blood copper is markedly diminished. The copper content of whole blood has been reported as increased in pernicious anemia, anemia of sepsis, anemia associated with Addison's disease, sickle cell anemia, Banti's disease, malaria, myelogenous leukemia, arsenic poisoning, and carcinoma. In no case or condition has a hypocupremia been reported. In all of the conditions studied by Sachs and his group there has been an inverse relationship between the copper and iron content. As the iron falls the copper content tends to rise. Hypercupremia is the usual response to hypoferremia. This has been clearly illustrated in cases of polycythemia vera treated with phenylhydrazine. As anemia developed the iron content of the blood diminished and the copper content increased. Then as polycythemia reappeared the iron rose and the copper fell to normal.

It must be pointed out that the above studies were done on whole blood. Since
blood iron is present principally in the hemoglobin molecule it would be expected that whenever anemia is present the total blood iron would diminish. It does not necessarily follow that there is a reciprocal relationship between serum iron and copper. Such studies would be most desirable. The iron content of the serum is known to be elevated in pernicious anemia. Sachs has reported that in this condition the whole blood copper is increased. This must mean that the copper content of red cells is greatly increased or that there is both a hypercupremia and hyperferremia in the serum. Simultaneous studies on cellular iron and copper and serum iron as well as copper in a variety of conditions, might uncover important facts pertinent to the metabolism of these two elements and to their relationship to erythropoiesis.

Elevated serum copper values have been reported in pregnancy and in infections accompanied by anemia. In both of these conditions there is a low serum iron. The serum copper content is subject to diurnal variations but is not affected by menstruation.

5. The function of copper.—The precise manner in which copper is related to the formation of red cells is not understood. It is not a constituent of the hemoglobin molecule. Since the administration of copper to anemic rats is followed by a rise in the erythrocyte count without a rise in hemoglobin it has been postulated that copper is essential for stroma formation of the cell or for the release of erythrocytes from the bone marrow rather than for hemoglobinogenesis. Schultze, Elvehjem, and Hart state that it is not possible to assign to copper a specific function for formation of either erythrocytes or hemoglobin, as the two processes are interdependent.

When iron is fed to iron- and copper-deficient animals the total iron content of the liver and spleen increases in proportion to the amount of iron fed. Thus in the absence of copper iron is absorbed and stored normally. If copper is then fed to these animals in place of iron, hemoglobin formation takes place and the iron content of the liver and tissues is reduced to a level of that found in severely anemic animals. From this it has been concluded, and generally accepted, that copper is essential for the mobilization of iron from the tissues and for its conversion into hemoglobin; or, stated in another manner, copper acts as a "catalyst" for the transformation of inorganic iron into hemoglobin.

In 1934 Cohen and Elvehjem reported a marked decrease in the cytochrome content of the heart, liver, and brain of rats with nutritional anemia. The feeding of small amounts of copper restored the cytochrome spectrum, while the feeding of iron was ineffective. With this lead, Schultze studied the cytochrome oxidase activity in the liver, heart, and bone marrow of copper-deficient rats and found that the activity of this enzyme was markedly diminished. Copper therapy initiated an immediate increase in cytochrome oxidase activity in the bone marrow. Maximal activity was approached within 24 hours. Iron, manganese, and cobalt did not affect the oxidase activity. Schultze and Kuiken have shown that the catalase activity of the liver, kidney, and blood of copper-deficient rats is markedly diminished. When copper was given the catalase activity returned rapidly to normal.

Thus it has been shown that copper is essential for the activity of at least three enzymes. The common denominator between these three enzymes and hemoglobin...
is protoporphyrin type III no. 9 since it is a constituent of each of these compounds. The interesting question arises, Is copper essential for the formation of protoporphyrin? Schultze attempted to answer this by determining whether or not protoporphyrin is excreted by anemic copper-deficient rats. He was able to isolate protoporphyrin type III no. 9 from the feces of such animals but unfortunately was not able to rule out the possibility of synthesis by intestinal flora. The question is, therefore, unanswered.

A second possibility is that copper is a structural component of cytochrome oxidase and catalase. If this should be true the effects of copper on the blood could be due directly to its effect on cytochrome oxidase since it has been shown that a high activity of this enzyme in the bone marrow is intimately associated with hematopoiesis. It is unlikely, however, that these enzymes contain copper as a structural component.

A third possibility suggested by Schultze is that the effect of copper on hematopoiensis is dual, first on cytochrome oxidase activity of the bone marrow and second on the synthesis of hemoglobin.

There are numerous other recognized functions of copper. The oxidation of crystalline glutathione is accelerated in the presence of small amounts of copper and it has been frequently observed that during recovery from hemorrhagic anemia the glutathione content of the blood is increased. The catalytic effect of copper on the oxidation of cysteine as well as ascorbic acid has been reported. It has been stated that the addition of copper to a scorbutic diet prevents the appearance of signs of scurvy in guinea pigs and causes their recession when incorporated after the appearance of scurvy. Glycolysis is known to be activated by copper and accelerated in anemia. What relationship, if any, these functions may have to the formation of red cells is now only speculative.

C. Cobalt.—The role of cobalt in erythropoiesis is unique. A deficiency results in anemia. The administration of small amounts to normal animals produces erythrocytosis, whereas the administration of large amounts depresses erythropoiesis.

The enzootic occurrence of cobalt deficiency has been reported from several regions of the world. The soil and herbage in these areas have been shown to be deficient in cobalt. Anemia is present, oftentimes severe, but its morphological characteristics have not been carefully studied. The disease caused by a deficiency of cobalt is known by various names in different parts of the world.

Enzootic marasmus is a disease of cattle and sheep occurring in a localized area of about 9000 acres in the Denmark district near the southeastern coast of Western Australia. Clinically the disease is characterized by progressive emaciation, weakness, a rough coat, and pallor of the mucous membranes. The animals develop a craving for harsh fibrous brush and in the late stages anorexia is pronounced. Diarrhea is often seen in young calves. In cows lactation is diminished, oestrus rarely occurs, and breeding is seldom successful. Abortions are common. Death occurs in from 6 weeks to 2 years after symptoms are first noticed, the shorter duration being usually observed in the young animals. Pathologically the most significant finding is hemosiderosis of the spleen, liver, and kidneys. Fatty infiltrations in the liver are frequently seen. Blood studies reveal a marked anemia which in
lambs is normocytic hypochromic and in calves is either normocytic or slightly microcytic and hypochromic. The reticulocytes are reduced. Anisocytosis and poikilocytosis are common. There is a hypoplasia of erythrogenic tissue in the bone marrow. A megaloblastic or erythroblastic bone marrow has not been reported. There is no evidence of increased blood destruction. The administration of 0.1 mg. of cobalt daily to sheep or a dose of 0.3 to 1.0 mg. daily to cattle is capable of both preventing and curing the disease. Animals so treated have been maintained in good health for periods up to 12 months while still grazing on affected land. The administration of copper has no beneficial effect although the copper stores are often low. Low blood copper values have not been found. Following the administration of cobalt the hemoglobin rises initially and then falls sharply. After this the blood returns gradually to normal. It has been suggested that the temporary fall in hemoglobin is due to the fact that the iron stores, even though excessive, are rapidly used and finally depleted and that the hematopoietic system is temporarily unable to support the strain placed on it by excessive growth. The hemosiderosis has been observed to disappear during cobalt therapy. Reticulocytosis following therapy has not been observed. Traces of nickel have been found to increase the action of suboptimal doses of cobalt. Liver cures as well as prevents the disease and its action is not thought to be due to its cobalt content since this is low. It has been suggested that the potency of liver may be due to the presence of a stored factor and that cobalt may function through the production of this factor in the body.

A disease similar to enzootic marasmus has been observed in sheep grazing in certain coastal regions in Southern Australia. This has been termed "coast disease" and is characterized clinically by listlessness, lethargy, anorexia, weakness, pallor, and finally death. Wool growth is affected and the fleece is dull. Edema is oftentimes present. At autopsy marked hemosiderosis of the pancreas and liver and to a lesser extent the spleen is observed. The total blood volume appears to be reduced although determinations have not been made. A severe anemia is present. It is stated that the anemia is microcytic and normochromic. Blood smears show anisocytosis, some polychromatophilia, and an occasional nucleated red blood cell. Treatment with copper is ineffective. Treatment with cobalt alone permits growth but fails to restore the hemoglobin entirely to normal. Treatment with both copper and cobalt prevents the disease and once the disease is manifest restores the animals rapidly to normal. The average hemoglobin content in volumes percent of oxygen of untreated animals was 2.4, in copper-treated animals 4.7, in animals treated with cobalt 9.9, and in animals treated with copper and cobalt 13.8. In an additional group of animals treated with iron, nickel, manganese, and zinc in addition to copper and cobalt the final hemoglobin value was the same as in the group treated with only cobalt and copper. Copper analyses revealed that the content of the tissue was reduced although there was no significant alteration from normal in the amount of copper in the blood. It would appear that "coast disease" is due in most cases to a deficiency of both cobalt and copper but the occurrence of uncomplicated cobalt deficiency in sheep in Southern Australia has been noted by McDonald. Hematological examination in these animals revealed a marked variability in the degree of anemia present. Some animals even when moribund
showed almost normal blood hemoglobin levels, whereas in others the hemoglobin was extremely low. The anemia was normocytic and blood smears showed only slight poikilocytosis. General observations at postmortem suggested that one of the most striking features of the anemia was a gross reduction in blood volume, but no direct measurements were made.

A disease of sheep similar to "enzootic marasmus" and "coast disease" known as "bush sickness" or "Morton Mains disease" has been reported from New Zealand. This condition likewise responds to cobalt therapy. Anemia exists but has not been studied. A similar condition has been reported as occurring in sheep in Canada. A disease of sheep known as "pine disease," "pining," "border pine," "Cheviot pine," or "Northumbrian pine" occurring in Scotland, characterized by emaciation, lethargy, retardation of growth, unthriftiness, anemia, and a fatal termination, has been reported. The effect of cobalt on this condition is in dispute.

Cobalt deficiency in cattle has been reported as occurring in certain regions of the United States. Geyer, Rupel, and Hart have reported a condition in cattle in the northeastern region of Wisconsin characterized by unthriftiness and anemia, which responds to 3 mg. of cobalt per animal per day. A condition known as "Grand Traverse disease" or "Lake Shore disease" and characterized by unthriftiness, anorexia, depraved appetite, emaciation, anemia, and finally death has been observed in Michigan. Following the administration of cobalt the animals exhibit a spectacular return of appetite and a progressive improvement in their condition. The hemoglobin shows an initial decrease of from 10 to 20 per cent and is then followed by a gradual return to normal. A disease in Florida cattle known as "hill sick" or "salt sick" has been reported by Neal and Ahmann. The affected animals showed a rough coat, scaliness of the skin, listlessness, retarded sexual characteristics, anorexia, emaciation, and muscular atrophy. Anemia, microcytic and hypochromic in type, accompanied by a reduction in reticulocytes, anisocytosis, poikilocytosis, and a lymphocytosis, was observed. Pathologically myocardial degeneration, a decrease in splenic pulp, liver degeneration, and hemosiderosis of the liver and spleen were found. The condition was found to be aggravated by iron and copper but responded rapidly to cobalt therapy.

An experimental anemia due to cobalt deficiency has not been produced in rats and dogs. Underwood and Elvehjem were unable to demonstrate a cobalt deficiency in rats on a milk diet. Frost, Elvehjem, and Hart have reported that in most dogs the addition of iron and copper to milk results in normal hemoglobin building. Small amounts of cobalt in addition to iron and copper therapy stimulated hematopoiesis in certain dogs in which the rate of blood formation appeared unusually slow. In a later paper from the same laboratory rapid increases in hemoglobin, volume of packed red cells and red cell counts to normal values were noted in all animals when iron and copper together were fed to dogs maintained on milk and rendered anemic by bleeding.

Studies in man are too few to warrant conclusions. In general, as one would expect, cobalt has not been found to be of value in the treatment of anemias. Whether or not there is a human requirement for cobalt cannot be stated. It may be
significant that iron compounds used therapeutically contain small amounts of cobalt.606

The administration of small amounts of cobalt to normal rats,606,609 dogs,607-609 guinea pigs,610 frogs,611 mice,610 rabbits,606,611-613 chickens,205 pigs,605 and ducks614 produces a definite and marked polycythemia which is accompanied by a reticulocytosis, hyperplasia of the bone marrow and an increased erythropoietic activity of the spleen and liver. Following the subcutaneous injection of cobalt into rabbits there is a marked reticulocytosis, normoblasts appear in the circulating blood, there is anisocytosis and polychromasia of the red cells, an eosinophilia and transient lymphocytosis.612 The polycythemia is a true one and is not due to a decreased blood volume.615 The mechanism by which cobalt produces such a polycythemia is not understood.616 The respiratory activity of the bone marrow is not impaired.616 Furthermore, such a polycythemia increased work performance under conditions of reduced oxygen tension.617 Cobalt does not produce polycythemia in splenectomized rats620 or when the diet is deficient in iron or in copper.606 However, cobalt produces a polycythemia in low-protein rats at approximately the same rate and to about the same degree as in normal rats.616 Ascorbic acid,616 manganese,613 concentrated liver extract,611 ventriculin,601 whole beef,608 and whole liver615 have all been reported to counteract the effects of cobalt. Frost, Spitzer, Elvehjem, and Hart620 observed an inhibition of the normal hematopoietic response to iron and copper feedings in dogs made anemic by hemorrhage and fed cobalt prior to the addition of iron and copper. Hematopoietic activity was resumed on the feeding of whole dry liver or liver extract. It is of interest that large doses of cobalt depress erythropoiesis.508,616,620

Studies on the metabolism of cobalt in rats have been made by Greenberg and his group using the radioactive isotope.424 Within 72 hours following the injection of 0.1 mg. of the isotope 3.5 per cent was excreted in the bile, approximately 65 per cent in the urine, and 5 per cent in the feces. Two and one-half per cent was demonstrable in the liver. During the same interval of time following the oral administration of a similar dose 2 per cent was excreted in the bile, 20 per cent in the urine, and 40 per cent in the feces. Three and one-half per cent was demonstrable in the liver. Small amounts were also found in other organs including the bone marrow. After 4 days 95 per cent of the radio-cobalt given by either route had been excreted. These results are in accord with the work of others in animals622,623 and are in sharp contrast to iron since only 2 to 8 per cent of this element when given parenterally is excreted in a comparable period. Evidently the body retains very little cobalt and the requirement must be extremely small. The bodies of rats on a normal diet contain only about 5 micrograms of cobalt.621

In human beings Penati and Ruata625 found that when cobalt was given orally only 3.5 per cent appeared in the urine in 24 hours. Le Goff626 injected 24 mg. of cobalt chloride intramuscularly into a man and recovered 28 per cent of the salt in the urine within the next 18 hours. Kent and McCance627 after the intravenous injection of cobalt into a man found that 22 per cent was excreted in 1 week. Seventy-four per cent of this amount was excreted by the kidneys. They concluded
that in man once cobalt reached the tissues, the process of elimination was very slow.

V. DISCUSSION

It is the ultimate goal of one of the phases of research in hematology to write the precise chemical reactions, step by step, for the formation of the red cell. It would be desirable in this review to follow the description of the factors concerned in erythropoiesis by a complete discussion of their interrelationships and of the manner in which they are concerned with erythrogenesis. Unfortunately, with the knowledge now available this is not possible with any degree of completeness or accuracy.

The factors concerned in erythropoiesis might be classified in several ways. In this review a classification based on the chemical nature of the substances has been used. Another classification might be as follows:

I. Formation of the red cell stroma
   A. Substances used for the construction of the stroma
   B. Substances essential for the formation of the stroma but which are not included in the stroma

II. Formation of the hemoglobin molecule
   A. Substances used in the construction of hemoglobin
   B. Substances essential for the construction of hemoglobin but which are not included in the molecule

The factors known to be used for the construction of red cells are presented schematically in figure 2.

The dried substance of the stroma is composed of protein (40 to 60 per cent), lipids (10 to 12 per cent), inorganic salts, and enzymes. The amino acids include arginine, lysine, histidine, tyrosine, tryptophan, cystine, and methionine. The lipid fraction is chiefly in the form of phospholipids (cephalin, sphingomyelin, lecithin) (58 to 72 per cent) but in addition there are free cholesterol and cholesterol esters as well as neutral fat. Adenine-ribose-nucleotide, glyoxalase, and certainly other enzymes are present. The various mineral elements present include sodium, potassium, magnesium, phosphorus, copper, and probably calcium as well as others.

Although copper is a constituent of the stroma its function is not known. It has been suggested that copper is essential for the mobilization of iron from the tissues and for its conversion into hemoglobin. It is true that when copper is administered to a copper-deficient animal iron is mobilized. This would be true, however, simply because erythrogenesis proceeds. A direct relation between iron and copper metabolism need not be implied.

The constituents of hemoglobin are heme and globin. Heme consists of the union of iron and protoporphyrin. The protoporphyrin is attached to the globin molecule through the two carboxyl (propionyl) groups. Very little is known concerning the precursors of protoporphyrin. As far as is known the animal does not depend upon a dietary supply of pyrrole or its derivatives. It is generally assumed, although
DIETARY FACTORS CONCERNED IN ERYTHROPOIESIS

- Erythrocytes
  - Amino acids
    - Arginine
    - Lysine
    - Histidine
    - Threonine
    - Tyrosine
    - Methionine
    - Others
  - Enzymes
    - Adenine-ribosenucleotide
    - Glyoxalase
    - Others
  - Minerals
    - Sodium
    - Potassium
    - Magnesium
    - Phosphorus
    - copper
    - Others
  - Lipids
    - Cephalin
    - sphingomyelin
    - Leithin
    - Cholesterol
    - Cholesterol esters
    - Neutral fats
    - Others

- Hemoglobin
  - Globin
    - Apodoerin
  - Feritin
  - Iron

- Protoporphyrin
  - Pyrrole methine
  - Pyroles
  - Acetoacetic esters
  - Ammonia
  - Knoer synthesis

Figure 1. A Schematic Diagram of Erythropoiesis.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Species</th>
<th>Type of Anemia</th>
<th>Bone Marrow</th>
<th>Leukopenia</th>
<th>Total Leukocytes</th>
<th>Thrombocyto-</th>
<th>Reticulocy-</th>
<th>Serum Iron</th>
<th>Hemoglobin</th>
<th>Hematologic Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>Rat, dog, pig, monkey</td>
<td>Normocytic</td>
<td>±</td>
<td>0</td>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Dog</td>
<td>Normocytic or macrocytic</td>
<td>Hypoplastic</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>Chick, rat, dog, pig</td>
<td>Microcytic, hypochromic</td>
<td>Hypoplastic</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>H</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Rat</td>
<td>Normocytic, (?), normochromic</td>
<td>Variable</td>
<td>+</td>
<td>+</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>casei factor</td>
<td>Man (pernicious anemia)</td>
<td>Macrocytic, normochromic</td>
<td>Megaloblastic</td>
<td>+</td>
<td>+</td>
<td>H</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_6$</td>
<td>Chick</td>
<td>Macrocytic, hypochromic</td>
<td>Variable</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin M</td>
<td>Monkey</td>
<td>Normocytic</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrinsic factor</td>
<td>Man</td>
<td>Macrocytic, normochromic</td>
<td>Megaloblastic</td>
<td>+</td>
<td>+</td>
<td>N, L</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>Pig, (?) rat</td>
<td>Normocytic, normochromic</td>
<td></td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>0</td>
<td>+</td>
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<tr>
<td>Tryptophan</td>
<td>Rat, pig</td>
<td>Normocytic, normochromic</td>
<td>Normoplastic</td>
<td>±</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>Rat</td>
<td>(?), Macrocytic</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phenylalanine</td>
<td>Rat</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>Rat</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Iron</td>
<td>Many species including</td>
<td>Microcytic, hypochromic</td>
<td>Hyperplastic</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>L</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Copper</td>
<td>Chicken, sheep, cattle,</td>
<td>Microcytic, hypochromic</td>
<td></td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
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<td></td>
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<tr>
<td></td>
<td>rabbit, rat, dog, pig,</td>
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<tr>
<td></td>
<td>mice, (?) man</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td>Cattle, sheep</td>
<td>Normocytic or microcytic</td>
<td>Hypoplastic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ present; 0, absent; N, normal; H, high; L, low.

There is not a great amount of evidence that protoporphyrin is an intermediate in the synthesis of hemoglobin and that substituted pyroles are intermediates in the synthesis of protoporphyrin. It was through these intermediates that it was possible for Fischer to synthesize heme in vitro. That such steps take place in vivo is
only speculation. It is true that protoporphyrin is normally present in erythrocytes and especially in young red cells and is present in relatively large amounts in the developing chick embryo.

Recently, the identities of two precursors of protoporphyrin, a derivative of acetic acid and glycine have been established.297, 631 Since condensations involving acetoacetic esters and ammonia are known to yield pyrroles in vivo (Knorr synthesis)632 and since Fischer and Fink633 have found that pyrroles are formed by condensing formylacetone with glycine the new in vivo findings are exceedingly interesting. Thus the precursors of protoporphyrin may be very simple substances and this may help to explain the ease with which the body can synthesize protoporphyrin, why the body can afford to discard the bile pigments rather than conserve them, and why anemia due to a failure to synthesize protoporphyrin is difficult to produce. The possibility must be kept in mind, however, that the hemoglobin molecule may be constructed around the iron and that true porphyrins and pyrroles are not present at any time during the synthesis except in small quantities produced in a side reaction. It has been suggested that the pyrrole rings of tryptophan, proline, and hydroxyproline might be utilized in the synthesis of protoporphyrin (figure 2). Direct evidence for this is completely lacking.

Globin is undoubtedly formed from various combinations of amino acids. Very little is known of the steps of this synthesis, of the nature of the intermediate compounds, or of the site or sites at which the synthesis takes place in the body. It is interesting, and may or may not be significant, that extensive bleeding in horses lowers the apoferritin content of the spleen. This suggests that this substance may be utilized directly for hemoglobin synthesis. It is even possible, although there is no evidence at present to demonstrate it, that ferritin as such may be utilized in hemoglobin synthesis.

The hematological manifestations of the various known deficiency syndromes are summarized in table 13. These syndromes have been inadequately studied. In each of the experimental anemias it would be desirable to have data, for example, concerning the erythrocyte protoporphyrin, porphyrin excretion, serum copper, serum cobalt, various bone marrow enzymes, and so forth. As such information is obtained it may be expected that the relationships between the various factors will be elucidated.

VI. SUMMARY

Riboflavin is essential for normal erythropoiesis in rats, dogs, pigs, and monkeys. There is no evidence that this vitamin is required for normal erythropoiesis in man. The anemia in swine is normocytic.

Nicotinic acid deficiency is accompanied by a severe anemia in dogs. The type of anemia produced is normochromic and may be either macrocytic or normocytic and is associated with a mild reticulocytosis. Limited observations indicate that the bone marrow is hypoplastic and that erythropoiesis stops at the erythroblastic level. An anemia due to a deficiency of this vitamin has not been demonstrated in other species nor in man.

Pyridoxine is essential for normal erythropoiesis in chicks, rats, dogs, and pigs.
The anemia is microcytic and slightly hypochromic in type. Anisocytosis, microcytosis, polychromatophilia, and normoblasts can be seen in the blood smear. An irregular reticulocytosis is present. The bone marrow is hyperplastic and there is an increase in the nucleated red blood cells. The anemia is accompanied by hemosiderosis of the tissues, an elevated serum iron level, and degeneration in the nervous system. There is no evidence of an increased rate of hemolysis. No relationship between pyridoxine and erythropoiesis has been demonstrated in man.

The "Lactobacillus casei group" includes the norite eluate factor, the L. casei factor from liver, folic acid, the Streptococcus lactis R factor of Kefesztesy et al., the yeast factor of Stokstad, the factor of Hutchings et al., vitamin M₁₅, xanthopterin, vitamin B₆, vitamin B₉ conjugate, vitamins B₁₀ and B₁₁, and pyracin.

The L. casei factor from liver has been identified as pteroylglutamic acid. The available evidence indicates that the norite eluate factor, folic acid, vitamin M₁₅, vitamin B₆, vitamin B₉, and vitamin B₁₁ are identical with pteroylglutamic acid. The Streptococcus lactis R factor of Kefesztesy et al. may be pteroic acid. The yeast factor of Stokstad is unidentified. The fermentation factor of Hutchings et al. has been identified as pteroyltriglutamic acid. Vitamin B₁₀ conjugate is now known to be pteroylheptaglutamic acid. Thus the various members of this group are closely related chemically and represent minor alterations of a basic structure. The corresponding deficiency syndromes are probably identical. In the rat the deficiency is manifested by severe normocytic anemia, severe granulocytopenia, leukopenia, and thrombocytopenia. Nucleated red cells appear in the peripheral blood. Bone marrow studies suggest a maturation arrest in the early stage of development of all three of the cellular elements of the blood. The manifestations of the deficiency in the chick are macrocytic anemia, leukopenia, and thrombocytopenia. Again immature red cells are present in the peripheral blood. In the monkey the manifestations of the deficiency are normocytic anemia, leukopenia, and thrombocytopenia. In human beings the synthetic L. casei factor from liver (pteroylglutamic acid) has been shown to be effective in the treatment of various types of macrocytic anemia including pernicious anemia and sprue. The relation of this substance to the antiperinocic anemia substance in liver remains to be determined.

The extrinsic factor of Castle is still unidentified. It now seems reasonable that it is related in some way to pteroylglutamic acid. It is unlikely that it is identical since the synthetic L. casei factor is effective even in the absence of normal gastric juice. A deficiency of the extrinsic factor in man results in an anemia which is identical with pernicious anemia and the bone marrow is cytologically indistinguishable. An accompanying neutropenia and thrombocytopenia are also frequently seen. The anemia responds rapidly to the parenteral administration of highly purified antiperinocic anemia liver extracts and to pteroylglutamic (folic) acid. Achlorhydria is generally not present. Macrocytic anemia of nutritional origin occurring in the tropics varies from this anemia in one important aspect. It fails to respond to highly purified liver extracts. This strongly suggests that the factor responsible for the deficiency is distinct from that of the extrinsic factor of Castle. A deficiency of this factor has been produced in monkeys and the deficiency syndrome consists of a macrocytic anemia with a megaloblastic bone marrow. The
anemia fails to respond to highly purified liver extracts which are effective in the treatment of pernicious anemia but does respond to crude liver extracts and to marmite, an autolyzed yeast extract. The relation between this factor and the \textit{L. casei} factor has not been investigated.

The role of ascorbic acid in erythropoiesis is not clear. Although the scorbutic state in both guinea pigs and human beings is frequently accompanied by anemia it is questionable whether the anemia is due specifically to a deficiency of ascorbic acid. Much of the animal experimentation is inconclusive because pure ascorbic acid supplements were not used. Further work in animals is needed. In man it has been both asserted and denied that synthetic ascorbic acid is effective in relieving the anemia. It would seem, however, that there are some scorbutic patients who respond specifically to pure ascorbic acid. The anemia accompanying scurvy has been reported as macrocytic, normocytic, and microcytic. An induced, uncomplicated ascorbic acid deficiency in a human being did not result in anemia.

Pantothenic acid deficiency results in a normocytic anemia of moderate degree in pigs in about two-thirds of the animals. There is evidence which suggests that a deficiency of this vitamin in rats may result in anemia, granulocytopenia, and bone marrow hypoplasia. Not all animals show these changes and pantothenic acid, although completely preventive, does not exert a curative action in all animals. There seems to be a relation between pantothenic acid deficiency and a deficiency of the \textit{L. casei} factor in the rat.

Choline deficiency in dogs results in a severe anemia. In many animals this change is irreversible. This may be explained by the irreversible liver damage which is present.

Biotin is necessary for the production of hemoglobin values greater than 14 grams per cent in dogs maintained on a highly purified ration. There is no evidence that biotin has an effect on erythropoiesis in other species.

In addition to the factors described above it has been shown that monkeys, pigeons, and guinea pigs require at least one more additional factor for normal erythropoiesis.

There is no evidence that thiamine, p-aminobenzoic acid, and inositol are concerned in erythropoiesis in any species.

Considering the relative size of the globin fraction of the hemoglobin molecule it is understandable that a deficiency of protein results in anemia. This has been demonstrated in rats and dogs. It has been pointed out that because of a marked reduction in the total blood volume only when the total circulating hemoglobin is determined and adjusted to a unit of surface can the true severity of the anemia be appreciated. Equine globin contains all ten of the 'essential' amino acids and at least nine 'nonessential' amino acids. Human globin has not been so extensively studied. It would be expected that a deficiency of any one of the 'essential' amino acids would give rise to anemia. Actually, specific deficiencies of tryptophan, lysine, phenylalanine, and isoleucine have been produced in the rat and anemia developed in each instance. The morphological characteristics of these anemias have not been carefully investigated. The anemia due to tryptophan deficiency in the rat has been stated to be normocytic and normochromic. An anemia probably
due to a lack of tryptophan has been produced in pigs. This anemia is normocytic, normochromic, and accompanied by a hypoplastic or normoplastic bone marrow and a normal level of iron in the serum. No increase of hemosiderin in the tissues has been noted. Whether the anemia produced in rats by feeding deaminized casein is due to a toxic substance rather than a deficiency of lysine is unsettled although large amounts of lysine prevent its development. Evidence that glycine is utilized in the synthesis of the pyrrole rings of protoporphyrin has been obtained by labeling this amino acid with N\textsuperscript{15} and feeding the labeled compound to rats. Pyrroles have also been synthesized in vitro from glycine. Similar evidence is available to indicate that acetic acid, or a derivative of it, is utilized for porphyrin synthesis.

Three mineral elements, iron, copper, and cobalt, have been shown to be essential for normal erythropoiesis in at least one species each. Iron is probably required for erythropoiesis in all mammals. A deficiency results, at least in the chronic stages, in a microcytic hypochromic anemia and is accompanied by a normoblastic, hyperplastic bone marrow and a low serum iron level, an increased amount of protoporphyrin in the erythrocytes, and an elevated serum copper level. Nucleated red blood cells are occasionally seen in the peripheral blood and the reticulocytes are increased.

The fundamental concepts of iron metabolism have changed greatly in recent years. These may be summarized. Iron is absorbed chiefly in the duodenum. In man it is absorbed principally as ferrous iron. Dogs absorb both valence forms well although some animals absorb the ferrous form more readily than the ferric form. Rats absorb both forms equally well. The absorption of iron is also dependent upon the concentration of the iron in the intestine, upon the solubility of the iron salt, and in the human being at least upon the presence of reducing substances in the diet as well as the reducing action of the gastric hydrochloric acid. In addition to these factors the need of the body for iron may determine, to a certain degree, the amount absorbed. This is known as the "selective absorption" theory. Recently it has been suggested that apoferritin acts as a receptor compound in the mucosal cell. As the concentration of the plasma iron falls, ferrous iron is removed from the mucosal cell resulting in a diminution of ferritin in the mucosa. When the ferritin has diminished to a point where the cell is no longer saturated with respect to ferrous iron, more iron is absorbed into the mucosal cell. Once absorbed the iron is transported in the plasma to the tissues where it is stored to a great extent as ferritin, a protein-iron complex. The iron is then used over and over again for hemoglobin synthesis. Iron is excreted from the body in only insignificant quantities. This theory requires substantiation.

Copper has been shown to be essential for normal erythropoiesis in chickens, mice, rats, rabbits, dogs, pigs, sheep, cattle, and infants. A deficiency of this mineral in rats is manifested by a microcytic hypochromic anemia and a moderate reticulocytosis. A condition due to a deficiency of copper, known as "enzootic ataxia," occurs in sheep in Western Australia. Anemia may be severe. In young lambs it is microcytic and hypochromic and is accompanied by demyelination of the nervous system and hemosiderosis of the tissues. In adult sheep the anemia is slightly macrocytic and hypochromic. Blood smears reveal anisocytosis, poiki-
Dietary Factors Concerned in Erythropoiesis

...locytosis, Howell-Jolly bodies, normoblasts, numerous macrocytes, stippling, and polychromatophilia. Similar blood changes have been reported in copper-deficient cattle in Western Australia. In nutritional anemia in infants the rate of erythropoiesis is accelerated when copper is given in addition to iron. In adults supplemental copper therapy may be of value in a few cases. Such cases, if they occur, are rare. Most cases will respond if adequate doses of iron are given. This does not necessarily indicate that copper is not needed for erythropoiesis or that it is not a dietary essential but rather that the quantities needed are so small that sufficient copper is present in the body stores in adult life, in the diet, or as a contaminant in the iron used therapeutically to supply the needs. No case of uncomplicated copper deficiency has been reported in man. The manner in which copper is related to the formation of red cells is not understood.

The role of cobalt in erythropoiesis is unique. A deficiency results in anemia. The administration of small amounts to normal animals produces a polycythemia, whereas the administration of large amounts depresses erythropoiesis. The enzootic occurrence of cobalt deficiency in sheep and cattle has been reported from various regions of the world. Anemia is present and is oftentimes severe. The anemia is either normocytic or microcytic and usually hypochromic. Blood smears reveal anisocytosis and poikilocytosis. There is a hypoplasia of erythrogenic tissue in the bone marrow, hemosiderosis of the tissues and a reduction in reticulocytes in the blood. An experimental anemia due to cobalt deficiency has not been produced in either rats or dogs. There is no substantial or convincing evidence that cobalt is needed by human beings for normal erythropoiesis. The administration of small amounts of cobalt to normal rats, dogs, guinea pigs, frogs, mice, rabbits, chickens, pigs, and ducks produces a marked polycythemia which is accompanied by a reticulocytosis, hyperplasia of the bone marrow, and an increased erythropoietic activity in the spleen and liver. Larger doses of cobalt inhibit erythropoiesis. The metabolism of cobalt is unlike that of iron. The excretion of cobalt from the body once it is absorbed is exceedingly rapid and is principally through the kidneys.

In conclusion, certain vitamins, namely, riboflavin, nicotinic acid, pyridoxine, “folic acid,” and the extrinsic factor, have been shown to be essential for normal erythropoiesis in at least one species each. It has been claimed that ascorbic acid, pantothenic acid, choline, and biotin play a role in erythropoiesis but these claims need substantiation. There is no substantial evidence that thiamine or inositol is concerned in red cell formation. The significance of p-aminobenzoic acid has yet to be determined. Protein is essential for normal red blood cell formation. The globin fraction of the hemoglobin molecule contains all of the “essential” amino acids as well as many of the “nonessential” ones. The stroma of the red cells also contains amino acids. It is logical, therefore, to assume that in the absence of any one of the so-called essential amino acids hemoglobin formation cannot take place normally. Actually specific deficiencies of tryptophan, lysine, phenylalanine, and isoleucine have been produced in the rat and anemia has developed in each instance. There is evidence to show that glycine and acetic acid, or a derivative of it, are utilized in the synthesis of the pyrrole rings of protoporphyrin. Three mineral elements, iron, copper, and cobalt, have been shown to be essential for normal erythropoiesis.
I gratefully acknowledge my indebtedness to Dr. Maxwell M. Wintrobe for his kind advice and aid in the preparation of this review as well as for the liberal use of his extensive reprint file.

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