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Role of iron and protein in the treatment of hypochromic anemia

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THE ROLE OF IRON AND PROTEIN IN THE TREATMENT OF HYPOCHROMIC ANEMIA

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INTRODUCTION

It has been my observation, thus far in my infant medical career, that the treatment of hypochromic anemia is a controversial point among the members of the staff of this school. It is maintained by some that the administration of iron is all that is necessary for the correction of this abnormality. This group is, however, divided among itself in that one group believes massive therapy is essential to satisfactory hemoglobin regeneration. Others contend, however, that small doses are more effective than are massive doses.

Opposed to those of the iron therapy group are those who believe the dietary factor should receive more emphasis--particularly the protein element of the diet. In their minds, iron does not bear the significance as is commonly supposed.

There are still others who would choose a middle course, giving both liberal amounts of protein in the diet combined with varying amounts of iron--massive to small doses, that is.

Obviously, this leaves one in a quandry, since each group claim satisfactory response with their regime and cite cases to prove their point. Even so, one

wonders which is the most efficacious regime, and my interest has thus been aroused. Is iron sufficiently effective that it may be used to the exclusion of all other factors, or is it better to supplement this treatment with high protein feedings? Can iron be disregarded and the desired therapeutic effect attained by feeding diets high in protein content? It is, therefore, my purpose to show in the following pages that the treatment of hypochromic anemia involves more than the administration of iron alone.

HYPOCHROMIC ANEMIA DEFINED

Hypochromic anemia is an hematologic term applied to that large group of anemic states wherein the red blood cells contain insufficient hemoglobin. The hemoglobin content is, of course, an hematologic classification. Actually, the condition refers to a state of blood wherein the loss of hemoglobin has exceeded the loss of cells. Thus the color index is below one and each cell does not carry its quota of hemoglobin.

This type of anemia is frequently referred to as a secondary anemia, and rightfully so, since in the large majority of cases it is secondary to some other disease process or nutritional deficiency. Thus this abnormality becomes a finding, physically, or a symptom rather than a disease entity in itself. Inasmuch as so many diseases cast their reflections in the blood picture, this anemia becomes the commonest type of blood disorder found in medical practice.

As a general rule, the red cells themselves are smaller than normal, but not infrequently they are of normal size and shape. Regarding the number of red cells, they may be either in sufficient or insufficient quantities. It is not uncommon to find patients with normal counts displaying a profound anemia. On

the other hand, the red cell count may be found to be as low as two and one-half millions and the hemoglobin content at forty percent. In both cases, the degree of anemia is equal as regards oxygen-carrying capacity. Therefore, irrespective of the number of cells, the hemoglobin content is the most accurate and reliable criterion regarding the degree of anemia present. The number of red cells comes in for its importance in determining the type of anemia present (1).

ETIOLOGY OF HYPOCHROMIC ANEMIAS

As has been stated earlier, hypochromic anemias are due almost always to some other disease process going on in the body or to a nutritional deficiency (1).

The most common causes of this abnormality are:

1. Acute infections
2. Chronic infections
3. Nutritional deficiencies
4. Malignancies
5. Parasitic infestations
6. Pregnancy
7. Chronic blood loss

Acute infections of all types are usually accompanied by varying degrees of anemia (1). Especially is this true if the infection is widespread or systemic in character. The anemia here is caused by various modes, such as destructive effect on erythrocytes by the organisms and in some instances by the bacterial toxins. Thus Streptococcus hemolyticus is a prominent offender. Inhibition of normal bone marrow activity by the bacteria or their toxins also is productive of hypochromia. Generally, this anemia does not become marked unless the infection is of long duration or severe character.

Chronic infections produce anemia in much the same manner as described above for the mechanism of anemia

in acute infections. There is, however, another major factor in chronic infections not always present in acute infections, namely, impaired appetite and inadequate food intake. Thus anemia here may be due partly to bacterial action and partially to a nutritional deficiency.

Nutritional deficiencies constitute another very great factor in the production of anemias. Inadequate diets, according to Kracke (1), is the only factor causing anemia in a great multitude of cases. Usually these are of a mild character, however. It would seem from the literature that most of the anemias of infancy and childhood are mainly on a nutritional deficiency basis.

McKay (2), in her studies of nutritional anemias in infants, classifies them as due to iron deficiency. She contends that the anemia is more likely to occur after the fourth month of life at a time when the iron stored in the liver is beginning to be depleted. Thus she advises the early administration of iron to infants.

As will be shown later, protein limitation will definitely decrease the hemoglobin content of the blood. Kyes and Bethell (3) have produced anemia in pregnant rats being given a low protein diet. Drabkin and Miller (4) produced severe anemia in rats by feeding only milk and insufficient iron for hemoglobin production.

Warren and DuBois (5) state that adult women commonly become anemic because of insufficient iron intake to compensate for that lost by menstruation. Inasmuch as men normally do not undergo periodic blood loss, anemia should therefore be less common in the male sex than in women, and this is true.

Malignancies of all types are practically invariably characterized by an hypochromic anemia. This occurs even though the process is not grossly extensive and no bone marrow involvement has occurred (1). However, the anemia here is refractory to all forms of treatment, and therefore does not concern us here.

Hypochromic anemia is very commonly associated with infestations of intestinal parasites (1). Most of the symptoms of hookworm disease are, in fact, due to the anemia they produce. The mode of action here seems to be suppression of bone marrow activity by a substance elaborated by the parasites rather than hemorrhage or actual blood loss.

Two types of anemia occur in pregnancy, which are hypochromic microcytic and hypochromic macrocytic in character. The former is by far the more common, however. It must be borne in mind also that a physiologic anemia occurs in this condition brought about by an

increase of plasma without a relative increase in the formed elements. The causes of the pathologic hypochromic microcytic anemia may be stated as, first, and most important, the sudden and progressively increasing demand of the foetus for iron and other erythrocyte building material. Secondly, various gastro-intestinal disturbances as nausea, vomiting, etc., are not uncommon in pregnancy so that the woman may be unable to take in, retain, and utilize the normal amount of food. Thirdly, pregnancy may be accompanied by various manifestations of toxemia resulting in some instances in impaired liver function so that the liver is unable to properly store, metabolize, and dispense hematopoietic substances. Thus, any one or combination of these conditions, combined with the physiologic anemia, would result in a relatively severe anemia (1).

Patients, who are chronically losing blood as in menorrhagia, peptic ulcers, etc., present a combination blood picture brought about not only by blood loss but often times by actual deficient hematopoiesis, which in many instances is due to inhibition of bone marrow activity by sepsis, parasites, malnutrition, fever, etc. Both cell number and hemoglobin are decreased. The treatment here, certainly, concerns the eradication of

the primary lesion. Then the deficient hemoglobin may be treated by the most effective method (1).

THE CHEMICAL COMPOSITION OF HEMOGLOBIN

At this point it is pertinent that we consider the composition of the material in question--hemoglobin. As described by Best and Taylor (6), hemoglobin is the coloring matter of the red blood cells, the chief function of which is to store and carry this pigment around in the circulation. This pigment, hemoglobin, is a conjugated protein consisting of an iron-containing portion combined with a protein of the histone class called globin. When this latter substance of the hemoglobin complex is in the natural state, it forms a loose combination with oxygen called oxyhemoglobin, the iron being in the ferrous form.

There are pigments called porphyrins which, alone or as a basis of more complex compounds, are found in all forms of plant and animal life. When, however, they are united with other compounds, these porphyrins are the basis of the blood and tissue pigments of various animals. The porphyrin molecule is composed of four purrol nuclei, each of which has the formula of C_4H_4N. The simplest porphyrin compound is that of chlorophyll. Protoporphyrin is the porphyrin of blood pigment.

Porphyrin compounds have the ability of uniting with various metals, the resulting compound then being

called a metalloporphyrin. Thus, protoporphyrin, combined with iron, forms the metalloporphyrin of blood pigment and is known as heme.

This compound, heme, has the further ability of combining with various proteins and nitrogenous compounds, which are then known as hemochromogens. When this protein is globin, we have formed the hemochromogen of vertebrate life.

Thus we see that hemoglobin is composed of iron plus a porphyrin, plus the protein globin. As regards the histone class of proteins, it might be well to add that they are water soluble and are precipitated by ammonia solutions and alkaloids. Also, they contain a large percentage of diamino acids, which are arginine and lysine.

THE PHYSIOLOGY OF IRON, ITS ABSORPTION, STORAGE, EXCRETION AND BODY NEEDS

No one denies the importance of iron in the human body. In view of this fact, it is essential that we consider the physiology of that element in the body.

The actual location and mechanism of iron absorption has not yet been completely settled. Robscheit-Robbins (7), after having done considerable work in the physiology of iron, states that varying portions of iron is absorbed. The site of absorption, she believes, is the entire gastro-intestinal tract, although the duodenum and a portion of the upper small intestine probably play the major role. The absorbed material then is carried by the blood, and to a lesser extent by the lymphatics, in the form of fine colloidal particles or in the dissolved form. Regarding the question of whether or not all iron compounds are absorbed similarly or differently, this worker quotes another experimenter as saying that all forms of iron have the same path of absorption, storage, and excretion. This has been supported by other investigators (8) as quoted by Hahn (9) who also states that a number of factors may conspire to make absorption difficult irrespective of the form in which the iron is given. One of the factors involves the hydrogen ion

concentration of the intestinal tract. For years, and still unsettled is the question of gastric acidity as concerns the absorption of iron. Robinson's work (10) indicates that the hydrogen ion concentration of the jejunum is higher than is usually suspected and that such a condition would be likely to facilitate the formation of insoluble basic iron compounds, thus interfering with absorption. This has been corroborated by Minot and Mettier (11). In their experiments to determine the role played by the hydrogen ion concentration of the gastro-intestinal tract, ten secondarily anemic patients were used. The hydrogen ion concentration of upper gastro-intestinal tracts of these people was held as nearly at a constant as possible during the experimentation. Diet was well controlled, and results were judged by reticulocyte responses. Ferric citrate was first given in small doses with an alkaline and then an acid beefsteak meal, followed by a four to twelvefold increase of the dose of iron. Reticulocyte responses of the patients to iron fed with beefsteak at an high hydrogen ion concentration were usually slightly less than those on iron with an acid beefsteak meal. This difference was accentuated when the dose of iron was increased. It was their conclusion, therefore, that an

acid medium does facilitate iron absorption. Hahn (9) is of the opinion on that basis that most of the iron is absorbed from the stomach, and possibly the upper duodenum.

Regarding the question of the importance of the hydrogen ion concentration of the gastric secretion, Drs. Warren and DuBois (5) state that hypochromic anemias associated with achlorhydria are not cured more readily, as a rule, by giving hydrochloric acid with meals, but that gastric acidity does favor the preservation and even the formation of ferrous irons and also prevents the formation of insoluble iron compounds, particularly ferric phosphate, all of which are conducive to more complete iron absorption.

It has been shown by Marlow and Taylor (12) that the oral administration of Ferric NH_4 Citrate caused no increase in plasma or urinary iron in either normal or anemic patients, but when the iron was given in acid-buffered medium, a slight increase in urinary iron occurred. Recently it has been shown (13) that an iron-rich diet has practically no effect on hemoglobin formation during periods of alkalinization, but that after alkalis were discontinued, there was a marked effect comparable to that obtained by adequate therapy with

inorganic iron. Thus, it would seem that gastric acidity does exert an influence upon iron absorption, and that the site of absorption is the stomach and duodenum.

Hahn, Bale, Lawrence, and Whipple (14), in their studies on iron metabolism using radioactive iron, found that where there is a need for iron, as in anemia, a fair quantity passes from the site of absorption to the blood stream. If no need of iron existed, very little was assimilated. Reasons for this phenomenon are not known, but it was postulated that possibly there were different "iron tensions." That speculation, however, was not held to be very probable inasmuch as the plasma of normal blood contains very little iron. They further contend that absorption is mainly in the small intestine.

Using the radioactive "tagged iron," peak absorption occurred in four to eight hours when the food is largely in the small intestine. At eighteen to twenty-four hours, the food is mainly in the colon and no appreciable amount of absorption was demonstrable. Once absorbed, plasma transports the iron from the gut to points of storage or utilization. In states of anemia, the appearance of iron in red blood cells is very early, as contrasted to neutral iron which is not

found in these cells before an interval of three to five days has elapsed. Radioactive iron, when given to anemic dogs, may be found in red blood cells within a few hours.

As stated by Hahn (9), storage of iron in the body is by no means well understood. When we consider the problem of iron storage in the body, we must begin by recognizing the different forms in which the element may occur in tissues. Iron is present in some form in all the tissues of the body. The bulk of the iron is present under normal conditions in the combined form of hemoglobin. Part of this hemoglobin iron is incorporated in the red cells and upon it is dependent the respiratory function of the cells. Normally, this fraction constitutes fifty-five percent of the body iron. It may, however, vary over a considerable range as in hypochromia and polycythemia. Another very important fraction of hemoglobin iron is found as the non-circulating compound in the striated muscles and is known as muscle hemoglobin. This compound is quite similar to blood hemoglobin and contains the same amount of iron. Prolonged severe anemia may slightly lower the amount of this hemoglobin, but exercise is more important than anemia in determining the level of this pigment (15).

It has been shown by Hahn and Whipple (16) that only about one-half of the iron contained in perfused striated muscles can be accounted for as combined muscle hemoglobin. The remainder corresponds in amount to the iron of the body tissues following depletion of readily available stores. This has been called parenchyma iron, and varies in quantity from one to three milligrams per hundred grams of fresh tissue. Muscle hemoglobin and parenchyma iron are inviolate stores of iron, which are not drawn upon no matter how great the emergency due to anemia. Small quantities are found in the blood serum, the absolute amount of which is not known. It is thought that it is present to the extent of about one microgram per milliliter of serum.

Of particular interest is that important part of the body iron which may be designated as "available storage," available, that is, for the needs of the body, such as the production of new hemoglobin. It is generally conceded that the chief depots for the storage of iron include the liver, spleen, bone marrow, and, under some conditions, the kidneys (17) (18). Tissues vary widely in their capacity for storage. The spleen may hold more iron per unit of weight of the organ than any of the others but, in view of the

comparatively small bulk of tissue involved, cannot take up as large absolute amounts as the liver and marrow. There are probably storage places other than the liver, spleen, and marrow. Hahn and Whipple (16) have shown that only a little over one-half of intravenously injected colloidal iron can be accounted for in these perfused tissues, even though similar quantities would eventually be turned over to new hemoglobin if sufficient time were allowed to elapse. Therefore, although the iron was known not to have been eliminated, it escaped detection by the methods they employed. Thus, from the literature at hand, and the experimental work that has been done, it seems relatively well established that the liver, spleen, and bone marrow are the points of storage drawn upon when there becomes a need for iron by the body. There are "other" sites of storage which apparently are in existence but which have not as yet been definitely located. Eventually they will be accounted for, but at the present, we shall have to be satisfied with those storage points that have been definitely proved to exist.

Concerning the excretion of iron from the body, speculation, controversy, and uncertainty reigns here certainly. Judd and Dry (19) in their experiments

found that iron is eliminated in the bile but admitted that this was in all probability not the only path of excretion governing the body iron level under normal conditions. The kidneys play only a very small part, if any, in its excretion normally. It has long been postulated, states Hahn (9), that the large intestine is the chief excretory organ for the element since urinary iron is not readily changed by dietary factors, but recently Welch, Wakefield, and Adams (20), in studying the iron balance in a patient with an ileostomy stoma and an isolated colon, have shown that the iron excretion by the colon is negligible. And so it goes, nothing definite being known. There are as many experimental results to prove colonic excretion as there are to disprove it. As Hahn (9) states, "A complete knowledge of the transformations which iron takes in the animal body during its absorption, storage, fabrication into its several working forms, liberation, conservation, and excretion will probably not be forthcoming very soon. It is an exceedingly complex problem and will likely continue to offer a challenge to investigators for some years to come."

Brief mention might be made of the bodily needs of iron. Warren and DuBois (5) state that the total

iron content is four and one-half grams, and of this the circulating hemoglobin contains somewhat less than sixty-five percent; latent iron available for hemoglobin production and stored mainly in the liver, bone marrow and spleen comprises approximately thirty percent; and tissue iron, which is unavailable for hemoglobin formation, about five percent. They further state that iron requirements of an individual depends on two factors primarily, namely, age and sex. In infancy, after the first few months of life, the amount of iron that has to be added to the body decreases because growth is less rapid than initially, and at the age of about four years, it reaches a steady level. This level means not that there is no additional requirement but rather that the requirement is relatively constant at about a hundred milligrams a year. In adolescence, the growth rate increases rapidly, and during that period there is a large increase in the amount of iron necessary. This is agreed by almost everyone, including Heck (21) who says it is to be expected that logically one would expect anemia due to inadequate iron in infancy--especially in those babies kept on cow's or mother's milk for a long period of time--and at the time of puberty when the body's metabolism is

so markedly increased. Warren and DuBois (5) further state that with men who reach adult size, we may assume that the requirement approximates zero since they lose so little of the iron which has already been absorbed into the body. This applies to normal men. In women, conversely, the menstrual losses beginning in adolescence increase the requirement of iron needed to maintain normal hemoglobin levels. About twenty milligrams of iron is required for each menstrual period. Growth falls off, naturally, but the iron requirement owing to menstruation persists at a level of about three hundred milligrams a year until about forty-five years when it, too, will decrease nearly to zero. Each pregnancy will constitute a special demand on iron metabolism which, only partially compensated by the amenorrhea and a rise in the curve to nearly four hundred milligrams, may be expected. It is not generally conceded, however, that the menses in women do constitute a loss of iron sufficient to bring about an anemia. In some investigations, it was shown that there was no change in the hemoglobin values in normal women before and after the menstrual flow. There seems to be a preponderance of evidence, however, in favor of the hypothesis that there is a drain on the woman's system

by menstruation. Thus, from the foregoing it will be seen that one may expect to find the so-called iron deficiency anemias in early infancy--when the stores are being depleted--and adolescence and probably in women in the age group of from twenty to forty-five years, particularly if numerous pregnancies have drained their stores of iron.

In summary of the physiology of iron, the consensus of opinions is that absorption of iron occurs primarily in the upper gastro-intestinal tract. The precise location of this activity has not yet been determined, but studies concerning the hydrogen ion concentration of the gastro-intestinal tract indicate that absorption occurs most readily at those points where the hydrogen ion concentration is acid. Alkalinity seems to hinder, or at least does not enhance, absorption. This would lend support to those who believe the peak absorption to occur in the stomach and duodenum. The process of absorption occurs more readily in cases where there is a distinct need of iron and when radioactive preparations are used.

Iron is stored in several forms. Most of the element is stored in the combined form of hemoglobin. There seems to be two main types of iron storage, that

available for body needs and that not available. We are naturally concerned here with the available form. Those places of available iron storage, which have definitely been established, are the liver, spleen, and bone marrow.

Excretion is the most controversial point of all. Suffice it to say, nothing very definite has been established regarding exeretion. The amounts excreted are quite small, even in cases of large dose administrations.

That the body needs iron is beyond question. The amounts needed are dependent mainly on age and sex. Periods of rapid growth, as early infancy and puberty, greatly accentuate the body needs of iron. After having reached the age of twenty to twenty-four years, males require very minimal amounts normally while women, on the other hand, continue to have relatively much higher intakes of iron due to loss of this element through physiologic processes, as menstruation, pregnancy, lactation, ete. Eventually, as the menopause is reached, their requirements are quite minimal, being much the same as in males who have reached their growth.

THE EFFECTS OF IRON ADMINISTRATION UPON HEMOGLOBIN REGENERATION

"By far, the larger portion of the literature dealing with the treatment of anemia concerns a variety of drugs. A great multitude of medicinal preparations have been proposed, temporarily enthusiastically supported, their value then questioned, and finally their use abandoned" (7). In 1747, Menghini described the presence of iron in the blood, and ever since, this drug has been the subject of extensive usage. Iron has always enjoyed the greatest popularity among clinicians in the treatment of hypochromic anemia. The manner in which the drug is administered has changed rapidly over a period of time. Considerable debate has arisen as to the preparation to be given. Should organic or inorganic preparations be used? Soluble versus insoluble preparations have occupied the spotlight, also. Controversies regarding ferro over ferric salts have arisen, as well as they have over active magnetic forms in comparison to the inactive non-magnetic forms. Dosage has also come in for its share of discussion. The immensity of the problem becomes apparent.

Inasmuch as there has been much experimentation on dogs along this line, it might be wise to mention

the experimental setup for clarity. Whipple and Robscheit-Robbins (22), who have been especially prominent in this work, used standardized anemic dogs. These animals were carefully selected and given meticulous care. Their basal ration consisted of salmon bread. These animals were kept on this ration for long periods of time in order to determine a standard base line of hemoglobin production. Also, these dogs were kept at levels of forty to fifty percent of normal hemoglobin content by numerous frequent bleedings. Then, potent diet factors, iron preparations, etc., were administered and their effect on the hemoglobin production in these standardized animals checked. The net hemoglobin production for any period was calculated by taking the blood withdrawn to keep the dogs at a hemoglobin level of forty to fifty percent--even the small amounts for hematocrit readings--and subtracted from that the amount formed from the basal ration--their standard output, that is. Thus the net amount of hemoglobin produced by the administration of the iron preparation or diet factor was determined.

Using these standardized animals, Whipple and Robscheit-Robbins (16) set out upon an experiment using intravenous iron preparations. It was their finding

that intravenous iron, given in large or small doses, will be returned quantitatively in the proportion of ten milligrams of iron, producing three grams of new-formed hemoglobin. Given by mouth, the influence of the iron on hemoglobin formation is not proportional to the amount of iron given. Optimum doses of forty milligrams per day for two weeks gives a net output of fifty-five grams of hemoglobin, or thirty-five percent utilization. Larger doses of the iron preparation increased the hemoglobin output slightly, and with four hundred milligrams daily, the net output averaged ninety-five grams of hemoglobin, or five percent utilization. The iron of the basal ration, salmon bread, was found to be utilized up to forty percent. When liver was fed with the iron, there was a greatly accentuated hemoglobin response. It was the opinion of these workers that intravenous iron and liver represents the peak functional capacity of the dog to produce hemoglobin, which was approximately ten grams daily. It was their opinion, also, that iron salts are utilized with equal facility by dogs when given by mouth, that is, whether the salts were ferric, ferrous, or in the reduced state. The determining factor is the amount administered. In the discussion of their

findings, it was admitted that there is a great deal more to hemoglobin production than mere iron alone, which includes numerous other organic and inorganic factors. They were not willing to make any dogmatic statements regarding the iron in food.

Using the standardized anemic dogs again, Hahn and Whipple (16), working on iron metabolism, found that iron administered by mouth over short periods of time gave practically no storage of this element of the iron-depleted dogs. Given over longer periods, however, increased storage occurred in the dogs' tissues. The most conspicuous feature was the rapid formation of hemoglobin in these dogs, however. Intravenous iron in large doses was found to bring about marked storage of this element in the liver, spleen, and bone marrow. They were able to account for fifty-five to seventy percent of the total iron given intravenously in those tissues, but they were unable to say how much of the remaining iron was to be located in the body tissues or fluids, but which certainly was not eliminated.

From the foregoing, the importance of iron in hemoglobin production is obvious. However, these workers, Hahn and Whipple, were interested in the

formation of hemoglobin in standardized anemic dogs on a diet very low in protein (23). It was known to these men that the output of new hemoglobin in their anemic dogs could be kept at low levels by the limitation of iron intake. Inasmuch as hemoglobin is made of iron, a pigment radical, and a protein factor (globin), possibly the pigment and globin portions might be limiting factors in the production of hemoglobin in anemic dogs. By using anemic dogs with bile fistulas, they showed that the dog can produce practically limitless quantities of the pigment radical on any diet. Thus, the pigment radical, in all probability, does not serve as a limiting factor in hemoglobin production. They postulated that the dog can make pyrrol rings (pigment radical) by closure of straight chain compounds, since the diets given hardly contained the required material in the pyrrol ring form. The globin fraction constitutes ninety-five percent of the hemoglobin molecule. Therefore, hemoglobin regeneration would require protein, or protein split products certainly. Limitation of protein intake would thus make fabrication of new hemoglobin more difficult and if carried to a sufficient degree, should limit the regeneration in anemia in the face of excess iron intake. In one series of

dogs in this experiment, standardized anemic dogs were kept on a very low protein intake, plus massive doses of iron. From that procedure, it would seem that these animals should have considerable surplus iron in reserve stores but that there would be little or no protein-building material. These dogs were then fasted for three weeks, and it was found that they produced, on the average, fifty to seventy-five grams of new hemoglobin during the fast. Fasting dogs produce less new hemoglobin than the standard anemic dog which has been on salmon bread regime during one week of protein fasting. These investigators reasoned that the hemoglobin produced during the fast is due wholly to the conservation of nitrogenous factors related to protein wear and tear, because urinary nitrogen studies done on the fasting dogs show the urea-ammonia fraction to be greatly decreased, which suggests that the precursors of the urea-nitrogen material have been conserved. Since these anemic dogs can scarcely have any appreciable protein reserve stores, it may be properly assumed that the excess of new hemoglobin produced in the dogs on salmon bread regime is related to protein reserve stores retained during the salmon bread feeding periods. Iron in the colloidal form was given by vein

also in this experiment, and according to their calculations, the dogs should have produced ninety grams of hemoglobin on a standard salmon bread diet but, actually, only thirty-nine grams of hemoglobin was formed on the banana diet. They stated that the limitation here was due to the low protein diet.

It was concluded from this work that a low protein intake will cause limited hemoglobin production in the standard anemic dogs. From their work, it appeared to these men that the dog on a limited protein intake is unable to produce the usual amount of globin and therefore of hemoglobin, even in the presence of a large excess of iron. Iron by mouth, or by vein, gave the same result: the dog made anemic by blood withdrawal can not produce the expected new hemoglobin related to the iron intake when the protein intake is held at low levels. It was also the opinion of these men that liver potency is due to potent materials--probably proteins--rather than the contained iron.

Daft, Robscheit-Robbins, and Whipple (24), working earlier on protein-starved dogs receiving large doses of colloidal iron, believed that new hemoglobin can be produced from material derived from the dog's own body proteins. They, too, found that during protein

starvation, the urea and ammonia fraction was greatly decreased below the non-anemic control level, and they believed it logical to assume that that change was due to conservation of nitrogenous intermediates for use in new hemoglobin production. In their opinion, the new hemoglobin came from the animal's own body inasmuch as all reserves of protein had been quite well exhausted in their dogs by long periods of protein-deficient diets. In some of their dogs, during periods of iron and sugar intake, there was as much as one hundred and fifty grams of hemoglobin produced, amounting to about twenty-five and one-half grams of nitrogen. This must come from the dog's own body protein, and their experiments indicate that this nitrogen is derived from a more complete conservation of nitrogenous intermediate products as evidenced by a decrease of urinary urea ammonia fractions. In summary then, under conditions of protein starvation, the anemic dog can fabricate new hemoglobin from nitrogenous material derived from its own body proteins.

Beard and Myers (25) were interested in finding out whether supplements were needed with iron for hemoglobin regeneration, or whether iron alone was sufficiently effective. In their experiments, milk anemic

rats were used. Glass cages were used to prevent iron and copper contamination. The milk administered was never allowed to touch metal containers, thereby increasing its iron and copper contents. From their work, they concluded that milk, when given with as little as one-fourth milligram of iron daily, gave recovery of normal hemoglobin levels in six weeks. Two milligrams of iron daily gave faster recovery than any combination of iron with small additions of other elements, as copper. This same result was obtained by other workers, Mitchell and Smith, and Drabkin and Miller, as quoted by Beard and Meyers (25).

Alt (26) was interested in the effect of anemia in pregnancy upon the young. For his work he chose albino rats and placed them upon milk-powder diets and allowed them to become pregnant once or twice. He found that a single pregnancy did not cause anemia in the female rat but resulted in a marked depletion of iron content in the liver. The second pregnancy resulted in moderate anemia, though, due to iron deficiency. The first litter of rats of these anemic female mothers had normal hemoglobin levels at birth, but there was a considerable reduction in total iron content and, consequently, in the iron stores of these

animals. Second litter offspring of these female rats receiving iron deficient diets showed a definitely decreased hemoglobin level at birth, and the total iron content of these animals was one-fourth of normal values. Iron supplements were added to the mothers' diets and was found to completely protect the mother and offspring against iron deficiency anemia.

These findings are not directly applicable to man, naturally, due to shorter time of gestation and greater percent of mother's weight being represented by the litter in the rat. It may be said, however, that congenital iron deficiency is a biologic phenomenon, and it does occur in the human infant. Strauss (27) reported that infants born to mothers with anemia due to deficiency of iron had normal hemoglobin values at birth but showed the presence of anemia during the first year of life. He believed this to be indirect evidence of depletion of iron stores at birth, and to him it seemed quite advisable to give iron therapy during pregnancy to prohibit this anemia in early infancy.

McKay (2) in her studies has classified the nutritional anemias of infants as an iron deficiency state, and has recognized its analogy to the anemia

of experimental animals produced by a diet limited to milk. She states that this anemia is more likely to occur after the fourth month of life, at the time when the iron store in the liver is beginning to be depleted. If the diet contains inadequate iron at that time, as is the case in prolonged milk feeding, an iron deficiency will invariably develop. She has found that the majority of these cases of anemia respond very, very readily to iron therapy alone. Those not responding to iron alone will practically always do so by the addition of copper and an unknown factor found in yeast. In her opinion, the anemia of early infancy can be prevented by giving pregnant women adequate iron. She further advocates the administration of four and one-half grams of ferric ammonium citrate daily to the infant, beginning the second month.

Strauss and Corrigan (28) later studied the question of anemia in pregnancy further. Two hundred pregnant women were observed in the last four months of pregnancy. Every other woman was given one-half gram of ferrous sulfate daily, and the remainder were given placebos. Of the one hundred women on ferrous sulfate, none had hemoglobin below seventy percent postpartum, while of those getting the placebos,

twenty-four had hemoglobin levels well below seventy percent postpartum. On the basis of this, therefore, he advocated the administration of iron therapy during pregnancy.

Bethell, Gardiner, and MacKinnon (29), in their studies of the influence of iron and diet on the blood picture during pregnancy, fully appreciated that no wholly satisfactory standards for blood values in pregnancy have, as yet, been formulated. As they stated, what may be physiologic for one case may, for another, indicate pathology. They set tentative standards for average and minimum red blood cell and hemoglobin values in pregnancy on the basis of evidence of return to non-pregnant normal values within six weeks after delivery in the absence of medicinal or diet therapy. These standards, or minimums, were three and one-half millions of red blood cells and ten grams hemoglobin. It was their opinion that most anemias were of the deficiency group, but they did not believe that there was any great demand upon the maternal organisms' iron stores during pregnancy. The total additional requirement of iron incidental to pregnancy, they state, does not exceed two hundred and fifty milligrams--the amount of iron in five hundred cubic centimeters of blood.

Fifty-eight percent of their cases studied were anemic, according to their standards, and in their opinion this was the result of one or more of four primary etiologic factors, namely, low iron stores in the patient before becoming pregnant; restricted utilization of reserve iron in satisfying the maternal organisms' iron requirements; impaired absorption of dietary iron in pregnancy; low intake of food iron. It was their experience that many cases failed to respond to iron therapy, and the abnormality was corrected only by dietary adjustments which included, in some cases, heavy protein feedings. These men believed that the anemia associated with inadequate food intake is a true macrocytic type, and in its more severe forms is identical with the so-called "pernicious anemia" of pregnancy. They showed that, with a daily intake of protein over fifty grams, there were no macrocytic anemias, but with intakes of thirty to fifty grams, twenty-seven percent of their cases showed this anemia. Intakes of thirty grams, or less, was associated with anemia in forty percent of their cases. To test the efficacy of iron therapy, forty-two hypochromic anemic patients were divided into two groups wherein one group of nineteen was given thirty-two hundredths gram of ferrous

sulfate three times daily. The other group was given nothing. Their pregnancies were of five to eight months' duration when the observations were begun. At six weeks postpartum, the blood picture of those treated compared favorably with the same non-pregnant normal women. Those untreated patients averaged two and six-tenths grams of hemoglobin below the treated group.

To test the efficacy of diet, twenty-five patients were divided into four groups. Six were given diet therapy and no iron; seven received no dietary instructions and no iron; seven received diet instructions and iron; and five were given iron but no dietary instructions. Evaluation of the results here was difficult because of variable adherence to diet instructions, but it was quite evident that improvement in diet, with particular respect to the intake of animal protein, was followed by a very significant increase in blood values. There was, however, a more uniform increase of both red blood cells and hemoglobin when iron was given in conjunction with the dietary instructions, which suggests that the metal may be of supplementary value in the treatment of anemias of pregnancy attributed to dietary deficiencies.

There are many cases of anemia outside of the realms of pediatrics and obstetrics. Anemia is commonly found in people otherwise apparently completely normal. The etiology is in most of these cases quite obscure and present for no apparent reason. Fowler and Barer (30), in their work on the hypochromic and generally microcytic anemia of the people, administered ferric ammonium citrate and found that excessive amounts by mouth gave more rapid hemoglobin production than did smaller, adequate doses. They offered no explanation for this reaction, but they found that of the amount given, only small amounts were utilized immediately. They reasoned that there should be a continued rise after large dosage administration because this iron excess was not excreted and, therefore, must be stored in the body somewhere. They were aware of the fact, though, that in many cases of anemia, it is necessary to continue the iron therapy after the cause is removed--if the cause is known. In their investigation of this question, apparently normal people with mild anemias were selected. The anemias in these cases were present for no apparent reasons, and no cases of hemorrhage infections were studied. These individuals were then given large doses of ferric ammonium citrate

for sixty days. The observation of these people continued then after the initial dose of iron for twenty-six weeks. They found that there was a gradual rise of hemoglobin content during the period when iron was given and for a short time after the iron had been stopped. The peak of hemoglobin regeneration came at about seventy days. Following this, there was a gradual decrease, so that the average values had returned to approximately the pre-treatment level by the twenty-sixth week.

Another group was given one gram of reduced iron per day for sixty days. This preparation contains more iron but it is less soluble. The peak of hemoglobin fabrication was reached at about the tenth week and then fell, after cessation of iron administration, to pre-treatment levels at about the twenty-sixth week. Apparently the fall was due to discontinuance of the iron therapy, and to settle this point, two other groups of anemic though otherwise normal people were given reduced iron and ferric ammonium citrate throughout the period of observation. Again, the peak of hemoglobin was reached at about the twelfth week, followed by a gradual diminution to a level only slightly above the pre-treatment levels at the end of the

twenty-sixth week. The graphic curve of the reaction of several of these people very closely simulated that of those cases where the iron therapy was discontinued at the end of sixty days, that is, peak regeneration at ten weeks followed by a decrease to pre-treatment levels. Generally speaking, however, the fall in those cases on continued iron therapy was not to as low levels as those in whom the iron was discontinued. The explanation offered for this observation was these people represent cases of low normal hemoglobin values, that is, these hemoglobin levels are normal for these people. It was reasoned in retrospect that the iron here was not acting only as a replacement but also, or solely, as a stimulant. Iron is quickly utilized in forming hemoglobin, so possibly the greater response to larger doses is due to availability of larger amounts of iron. But this was not the only explanation, they stated, because the serum iron drops rapidly to normal after a few hours, while hemoglobin formation goes on ten to fourteen days after the medication has been stopped.

Continuing their work to see if possibly iron did act mainly as a stimulant, iron was administered to individuals of high hemoglobin levels. They found that

the response was not as great, but the average values for the whole group did show an increase. The graphic curves in these cases simulated markedly those curves of the previous groups of anemic individuals. Thus, it appeared to these investigators that iron acts not only through replacement but also through stimulation.

In a paper on the treatment of hypochromic microcytic anemias by Heck (21), it is pointed out that well-balanced diets, with special reference to adequate protein intake, are quite essential to hemoglobin production. Iron is quite necessary, granted, but there are other factors equally or more important. Heck acknowledges slow hemoglobin formation on low protein intakes even in the face of excessive iron. He prefers to use ferrous rather than ferric salts, since it has been his observation that the latter preparation gives rise to numerous gastro-intestinal difficulties. In his practice, twenty to forty grains per day of ferrous sulfate has been adequate protein material. He stresses adequate amounts of iron rather than the type. If achlorhydria is present, the addition of hydrochloric acid may be of benefit. It is his contention that all diets contain sufficient copper that no additional supplement of this element is necessary.

Barer and Fowler (31) state that it was their finding that one-half gram of ferric ammonium citrate per day was too small an amount to be of value. One gram per day was found to be adequate for satisfactory hemoglobin response, and three grams per day gave no greater response. Reduced iron and ferric sodium citrate in doses of one gram per day gave practically the same response as did the one gram of ferric ammonium citrate. Ferrous sulfate in doses of thirty-six hundredths gram per day gave the same response as one gram of the other iron preparations and was better handled by the patient. Thus, he concluded that ferrous sulfate was the most satisfactory preparation to use in iron therapy.

The foregoing material has been given to bring out the fact that iron is quite essential for the production of hemoglobin, as is generally conceded by everyone. We have seen this element's importance in the anemias of early infancy and its role in prevention of this abnormality's occurrence in the infant by administration of adequate iron to the pregnant woman. That iron given during pregnancy protects the mother from becoming anemic herself seems quite well established. Experimental work has been presented to show that even on very low protein diets, dogs can, when

given large amounts of iron, continue to produce hemoglobin. However, in so doing, they use their own body proteins for this fabrication, as is indicated by studies of the urinary urea-ammonia fractions of these dogs. It is the conception of most people that iron, in whatever form it is given, acts through replacement, but investigations have been given to show that possibly the action of the element is partially or wholly one of stimulation. This question is far from settled at this time. However, in all this material showing the efficacy of iron in treating hypochromic anemias, there seems to be an undertone indicating that there is a great deal more to the treatment of this abnormality than just iron alone. All workers bring out the point that diet is a very great factor in this problem.

THE EFFECTS OF PROTEIN IN HEMOGLOBIN REGENERATION

The remainder of this treatise shall be directed at showing the importance of proteins and protein derivatives in the correction of hypochromic anemias and the effect of withholding proteins from the diet. The latter has been dealt with previously, however.

Kyes and Bethell (32), in their investigation concerning the role of protein in the prevention of anemia in pregnancy, used rats in their studies and diets containing varying amounts of protein were administered to these experimental animals. The effects of these diets were measured by the number of red blood cells, percental volume of the erythrocytes, and the amount of hemoglobin. Diets of either naturally occurring foods or purified proteins were fed to female rats for at least seven days prior to breeding and throughout their pregnancies. It was found that those diets containing ten percent or less protein led to a variable reduction of the number of erythrocytes, a decrease of hemoglobin content, and an increase in the size of the erythrocytes. Generally, a relatively severe macrocytic anemia developed in these animals. In control groups on high protein diets, the number of

erythrocytes ranged from seven and three-tenths millions to ten and one-half millions. The hemoglobin in these latter animals averaged thirteen and seven-tenths grams per hundred cubic centimeters of blood. The animals being fed on low protein diets had an average number of five and six-tenths millions of erythrocytes and eleven and seven-tenths grams of hemoglobin. Obviously, this is quite a marked difference in blood pictures.

Since it had been shown that proteins were so essential to the maintenance of normal hemoglobin levels, interest arose in feeding pure amino acids to anemic experimental animals to test the efficacy of these preparations in the correction of that abnormality.

Drabkin and Miller (33) used milk anemic rats in their experiments. When the anemia in these animals became quite severe, five and four-tenths milligrams of ferrous sulfate was added per quart, which furnished two-tenths milligram of iron per rat per day. Each animal consumed about thirty-five cubic centimeters per day, and it was found that this amount was insufficient to cure their anemia, but it was found that a synthetic diet containing that much iron was curative. Copper-free water was used to dissolve the amino acids, and for drinking water. The amino acids were added in

molecular equivalents to portions of the same milk iron mixture.

In the first series of experiments, arginine monohydrochloride was used. Prior to the administration of this acid, the progressive anemia could not be stopped by the addition of larger amounts of iron. Then, a hundred milligrams of pure arginine monohydrochloride was added to the diet, and a definite increase of hemoglobin concentration resulted. The effect was no greater with two hundred milligrams than with one hundred milligrams.

Alanine, glutamic acid, and proline were then tried in the second series of experiments. Alanine was found to be quite ineffective, so much so that the animals died. Strikingly opposite were the effects of glutamic acid. Proline was effective also, but less so than the glutamic acid.

In the third series, arginine, tryptophane, sodium hydrogen aspartate, pyrrolidonecarboxylic acid, hydrochloric acid, histidine dihydrochloride, alanine hydrochloride, and sodium hydrogen glutamate were used. These acids were fed over longer periods of time than were the preparations in the previous series. The results obtained from this series showed that, upon

arginine and sodium hydrogen glutamate, the recovery from severe anemia is notable and continuous. Animals receiving hydrochloric acid, histidine dehydrochloride, and alanine hydrochloride, plus the usual milk-iron mixture, failed to regain health and eventually died. Some of these animals were changed to sodium glutamate at a time when they were practically in a state of collapse, with the result of a striking hemoglobin regeneration and growth. Tryptophane, sodium hydrogen aspartate, and pyrrolidonecarboxylic acids produced the same result as those effective acids of the first and second series. However, three of the four rats receiving the sodium hydrogen aspartate became again severely anemic following the initial hemoglobin rise. This did not occur with any of the other preparations.

All these acids, with the exception of glutamic acid, contained traces of iron, but the amounts were so small that the results can not possibly be attributed to that metal. Regarding the copper content, those acids found to be effective were copper free, as shown by very delicate qualitative reactions.

It was therefore concluded from these experiments that amino acid feedings are effective in hemoglobin

regeneration, and that copper is not an essential for hemoglobin fabrication. The experiments were very carefully done and well handled. Numerous problems arose but were controlled as well as possible in order that the true efficacy of the amino acids might be established.

Further investigations, concerning the effect of amino acid feedings upon hypochromic anemia, have been carried on by Whipple and Robscheit-Robbins (34). In their work, standardized anemic dogs, previously described, were used and were fed various doses of amino acids. It was found that one gram per day was the optimum intake of amino acids over a two-week period. Rarely, did a larger amount have any added effect, but smaller doses than one gram per day did, in some cases, bring about less hemoglobin regeneration.

According to Schmidt, quoted by Robscheit-Robbins and Whipple (34), hemoglobin contains fifteen amino acids, but this analysis accounts for only seventy-one percent of protein. Of these fifteen acids, seven are said to be essential to growth. These seven essential acids are leucine, isoleucine, histidine, lysine, phenylalanine, tryptophane, and arginine. Experimentation has shown that these essential acids are

no more potent in hemoglobin regeneration than the others.

Hemoglobin (34) is a peculiar basic protein, and possibly its formation in the body differs from that of plasma protein and cell proteins. Its disintegration may differ also, very little being known regarding the fate of the globin fraction following erythrocytic destruction. The plasma-depleted dog can not use globin to make new plasma protein, nor is the globin conserved for the protein needs of the body in fasting experiments, except to produce new hemoglobin. Plasma proteins, however, can supply the protein needs of the fasting dog.

Globin can contribute to the building of hemoglobin in the standard anemic dog, and as horse globin can be utilized, it seems safe to assume that the globin is broken down slightly before contributing to the regeneration of canine hemoglobin. Globin is very well utilized to form hemoglobin. From the feeding of one hundred grams of globin, thirty to forty grams of hemoglobin is formed in the standardized anemic dog.

The results of these experiments showed that certain amino acids, when fed to anemic dogs, do produce a definite increase in hemoglobin production. The

potency of the acids vary as do combinations of these compounds. It was conceded that iron must be available also for hemoglobin regeneration, as well as the pigment radical and protein component globin.

Later work (35) revealed that amino acids in doses of one to five grams per day, plus the basal salmon bread ration, are practically all absorbed, and that practically any amino acid given over a two-week period will bring about a rise of hemoglobin concentration in the blood. The other needed proteins, it was stated, are derived from the diet protein, protein stores, and protein wear and tear in the body. It was their opinion that when an amino acid fails to bring about a satisfactory response, some other essential supplement necessary for the fabrication of hemoglobin was not available at that time.

In the experiments where dogs were used and fed the various amino acids, objections were raised by some who said the results were misleading because of the added protein in the basal ration. Whipple (36) holds, however, that this objection is not valid because the amino acid being administered is only one of a great complex, and the response of hemoglobin regeneration is very much greater in most all cases when amino acids

are fed than has ever been noted by the administration of the basal ration alone. He points out further that, as has been shown earlier, dogs on a complete fast with no food intake at all do continue to produce new hemoglobin, and if other proteins were not obtained in the diet intake, they would be taken from the dog's own body, as is the case in fasting experiments.

Further work by Whipple and Robscheit-Robbins (37), using valine and iso-valeric acids, showed these to be well utilized in hemoglobin regeneration, as a general rule. There were, however, cases with completely negative results which were not explained further than that one or more other supplements essential for hemoglobin formation were not present.

It was stated by these workers than an added specific amino acid enhances the flow of other acids in the direction of globin formation which, it is assumed, is the limiting factor of hemoglobin regeneration in many cases. They further contended that, if a dog can break up certain amino acids and recombine these to form other amino acids and body proteins, there then should be no reason why the same reaction could not occur in the rapid production of hemoglobin protein in experimental anemias.

Guerrant and Hogan (38), in their investigations of the effect of amino acids on anemia caused by deaminized caesin, found that deaminized caesin, as a sole source of nitrogen, was quite inadequate. When combined with other proteins (39) (40) as gliadin, or gelatin, or a laboratory preparation of lactalbumin, and fed to rats, they became very anemic, failed to grow, and eventually died. But if deaminized caesin were added to normal caesin, these abnormalities did not arise. They found, also, that if caesin were hydrolyzed and the hydrolysate combined with the anemia-producing ration, the animals recover from the anemia and grow normally. It was assumed, therefore, that the antianemic factor or activity of the hydrolyzed caesin was explained by the amino acid content.

These investigators continued their work (41) on the anemia caused by deaminized caesin, using various individual amino acids and found that lysine was the antianemic factor in the deaminized caesin syndrome. Earlier work, using this substance, failed because insufficient quantities of the substance were fed. The finding that rather large amounts of lysine must be fed to be effective is in keeping with the work of other investigators, as Rose (42) who states that the

ration should have one percent lysine if recovery is to occur. Why such large doses are needed is only speculative. Possibly, since deaminized caesin contains a toxin, lysine may act as a detoxifier, but this is not likely because lysine could not be found in the urine of the animals by delicate analyses.

Muller, quoted by Hogan, Powell, and Guerrant (41), demonstrated a marked reticulocyte response with lysine injections. It was his contention that that response is due to "...stimulation and proliferation of red blood cells, and an extenstion of blood-forming tissues".

As stated by Guerrant, Powell, and Hogan (41), hemoglobin contains lysine, and a lysine deficiency might hinder hemoglobin fabrication. Also, heme itself might be synthesized, presumably from amino acids, and possibly lysine is a precursor of this compound.

In their earlier work, the antianemic activity of various proteins was unknown, but from these later experiments, this factor appeared to these investigators to be lysine. Autoclaving destroyed this activity apparently by damage to the lysine. Failure of lactalbumin preparations was attributed to thermal damage to the lysine. Lack of antianemic activity of corn and

wheat glutens was explained by their low lysine content.

All the above emphasizes the importance of suitable dietary protein in restoring normal hemoglobin levels in the body.

The work of Whipple, Robscheit-Robbins, and their co-workers, seemed to show that almost any amino acid fed in sufficient quantities would produce satisfactory hemoglobin regeneration. The work of Hogan, Guerrant, and fellow workers does not support this view, but possibly some of this inconsistency may be due to the difference of experimental animals. At any rate, the efficacy of amino acids in hemoglobin regeneration seems quite well established. Iron has not been used in most of these experiments and hemoglobin response has been equally if not greater than in those experiments where the emphasis has been on the iron factor.

Having shown quite definitely the importance of proteins in the restoration of normal hemoglobin levels, Robscheit-Robbins and Whipple (43) studied next the potency of various protein foods. Liver, heart muscle, and striated skeletal muscle were all studied, and it was found that liver feeding is the most potent factor for sustained production of hemoglobin and red blood cells. Its efficacy was invariable in all

experimental animals. This was attributed to storage in liver of parent pigment substances, and indicates that the liver is quite intimately concerned with pigment construction. Whether the "parent pigment substances" were proteins, metals, or what, was not stated; in other work, the effectiveness of liver has been attributed to the protein content. Striated muscle did not bring about the same uniform favorable reaction as did liver. In some cases of severe anemia, muscle feeding brought about a most favorable reaction, while only a moderate reaction occurred in other cases. Beef heart muscle was found to be less effective than liver, but more effective than beef striated muscle. Explanations for the difference of potencies are not well understood.

Regarding the clinical application of protein feeding for the alleviation of hypochromia, very little has been done. True, well-balanced diets are stressed, but iron continues to hold the spotlight in the treatment of hypochromia. Generally speaking, proteins are not recognized as being of major importance in the restoration of normal hemoglobin levels. McAlpine (44), in his discussion of the management of nutritional anemias of infancy, feels that iron therapy is the only

way to prevent or cure anemia in early infancy. It is his contention, further, that early feeding in infancy plays no part in prevention of hypochromia, and that any favorable influence that early feeding of vegetables, etc., has must be attributed to other factors as vitamins, minerals, and other unknown factors. No mention is made of protein as possibly being important. But, even though proteins have not yet been generally recognized as being essential in the fabrication of new hemoglobin clinically, considerable experimental evidence does indicate that the administration of high protein diets is effective in restoring a normal blood picture. Whether the result is due to any one specific amino acid, or whether all amino acids are equally effective, remains to be shown.

CONCLUSIONS

1. Hypochromic anemia is a very common disorder of the human body.

2. Hemoglobin is a very complex compound, being composed of protein material largely, plus the inorganic element iron.

3. If iron is to be given, the most practical and efficacious preparation seems to be ferrous sulfate.

4. Iron administration does bring about an increase in hemoglobin concentration of the blood. This is true even though the experimental animals are on a starvation diet of sugar-water and massive doses of iron. When, however, studies are made on the urinary ammonia-nitrogen fraction, a definite decrease is found of these substances, which indicates that the precursors of the urea-nitrogen material have been conserved. This, and other works, supports the view that in hemoglobin formation, if there are not sufficient protein materials for the fabrication, they will be taken from the body, which certainly is not desirable.

5. Iron therapy is probably the most effective means of alleviating the anemia of early infancy and pregnancy if the diets are adequate. It seems likely

that early administration of ferrous sulfate to the infant and pregnant woman is advisable.

6. The mechanism of iron therapy may be not only replacement but stimulative as well, inasmuch as iron administered to persons with high hemoglobin levels will increase their hemoglobin values.

7. The basic etiologic factor in a great number of hypochromic anemias is a protein deficiency, and the administration of liberal protein diets or pure amino acids will effect a cure in these cases. This is particularly true in adults.

8. Lysine, thus far in experimental work, has been found to be particularly effective in hemoglobin production, and this might be expected since globin is composed mainly of the class of diamino acids of which lysine is a member.

9. The general conclusion that has been reached from this work is that hypochromic anemias can be cured by liberal protein administration. For maximum hemoglobin response, iron, in small doses and of the ferrous type, should supplement the high protein feedings.

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