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Treatment of pernicious anemia

Leo L. Diamond
University of Nebraska Medical Center

TREATMENT OF PERNICIOUS ANEMIA

BY

LEO L. DIAMOND

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INTRODUCTION

In recent years pernicious anemia has been held constantly before the attention of the investigator, the practitioner and even the layman by the many articles which have appeared in scientific journals. In no other clinical condition has our knowledge undergone more complete revision. The empiric and altogether unsatisfactory treatment of the past has been replaced by the therapeutic use of active agents, the effects of which may be predicted quite accurately. In contrast to the previous gloomy outlook with which the disease was regarded, both by physicians and the laity, the prognosis at present compares favorably with that of diabetes under insulin therapy.

In view of the fact that there is such a large amount of literature written on the subject of pernicious anemia all phases of the disease could not be considered in this paper. Both the etiology and treatment have been studied in great detail and as a result a sound theory for the etiology and a specific agent for the treatment have been found. In this paper the works of outstanding importance in the development of the present treatment of pernicious anemia have been considered in detail and a brief review of the more important literature which has lead to the formulation of the present concept of the etiology of the disease has been included.

HISTORY

Treatises on anemia have appeared from the earliest times, however, these descriptions were not sufficiently clear to enable more than a guess to be made as to whether the cases reported belonged to the category of pernicious anemia. In 1822 a Scottish physician, J.S. Combe (19), published in the Transactions of the Medico-Chirugical Society of Edinburgh an account of a case of very severe anemia in such detail that there can be no doubt whatever that the patient was suffering from pernicious anemia.

"The case was a man, aged 47, who had been born and had spent the greater part of his life in the country, where his duties were neither laborious nor unhealthy; who had led a regular and temperate life, and had enjoyed perfect health since childhood, and had never lost any blood. I was much struck by his peculiar appearance. He exactly resembled a person just recovering from an attack of syncope; his face, lips and the whole surface were of a deadly pale color; the whites of the eyes bluish; his motions and speech languid; he complained much of weakness; his respiration free when at rest, became hurried on the lightest exertion; pulse eighty and feeble; inner part of the lips and fauces nearly as colorless as the surface; bowels very irregular, generally lax, his stool very dark

and fetid; urine reported to be copius and very pale; appetite unimpaired; of late his stomach has rejected almost every kind of food; constant thirst; he has no pain referable to any part; and a minute examination could not detect any structural derangement of any organ.

"It was only about two months ago since he began to complain, but not until his friends had observed his altered complexion; he then lost strength and said his head troubled him. Of this last symptom he has no distinct recollection. His feet became edematous and his appetite failed him.

"My attention was drawn to the skin which was of waxen color, soft and delicate, the cellular tissue about the eyes and breast slightly distended with watery effusion. The pulse was feeble and easily excited by any emotion. A very minute examination of the case and a careful consideration of its history, scarcely solved, the nature of the affection; and its long continuance and inveteracy rendered our prognosis much more doubtful.

"He died six months after being first seen, from aggravation of all his symptoms, extension of the edema to the face and upper extremities, and evident marks of effusion into the chest. He died with all the symptoms usually attendant with hydrothorax. At first the treatment seemed to check the progress of the disease, but

latterly the stomach and bowels became so irritable as scarcely to admit of any medicine and only of the mildest diet."

Combe stressed the point that he was unable to find anything in the clinical or post-mortem findings to explain the condition. He suggested, however, that "it is probably owing to some disorder of the digestive and assimilative organs that its characteristic symptoms have their origin."

Thomas Addison (1) of Guy's Hospital first in 1849 and again in 1855 described in detail, as a preface to his work, "On Constitutional and Local Effects of Disease of the Suprarenal Capsule", the type of severe anemia which still bears his name. His description of the clinical and post-mortem findings was so similar to that given by Combe that it is not necessary to recapitulate it in full. He also strongly emphasized the fact that the anemia was found in cases in which there had been "no previous loss of blood, no exhausting diarrhea, no chlorosis, no purpura, no renal, splenic, miasmatic, glandular, strumous, or malignant disease." Accordingly he termed the anemia "idiopathic" to distinguish it from cases in which there existed more or less evidence of some of the usual causes, or concomitants, of the anemic state. He pointed out its mysterious insidious onset and fatal termination; its

occurrence in both sexes, generally but not exclusively subsequent to the middle period of life, and in persons whose frames were somewhat large and bulky. The only omissions worth noting were his failure to record the remarkable phases of remission and relapse, and to describe the symptoms caused by the degenerative changes in the central nervous system which are now recognized to be so characteristic of this disease. His only suggestion as to the etiology was that the disease was caused by some form of fatty degeneration. His description placed idiopathic anemia definitely among diseases which exist as separate clinical entities.

In 1867 and again in 1872 Professor Biermer of Zurick (2) published papers describing a very severe anemia which he named progressive pernicious anemia. His descriptions of the clinical and post-mortem appearances were almost identical with those of Addison, and he added little to our knowledge except by drawing attention to the tendency to small capillary hemorrhages, particularly in the retina, shown by persons suffering from the disease. Biermer noted that the disease appeared particularly in the poverty-stricken classes, and was associated with various causes, such as puerperal sepsis, unsuitable feeding, unhealthy surroundings, and particularly with chronic diarrhea. The disease was also found in

certain cases of cancer and Bothriocephalus infestation. In other words Biermer, under the title of progressive pernicious anemia, included a group of conditions of which one was the idiopathic variety described by Addison. The view that pernicious anemia was a symptom complex was hailed by Biermer's contemporaries and by the majority of later investigators, as the correct one, and in consequence has influenced the continental investigators up to the present time. (24)

William Hunter (43) was the first to realize that it was not merely a disease of the hemopoietic system, but that the gastro-intestinal and nervous systems were also involved. He strongly criticised Biermer's attempt to group several anemias, etiologically very different, under one heading, and maintained that Addisonian anemia was a single distinct entity. His studies of the glossitis and gastro-intestinal sepsis, which he stated were present in every case, and the remarkable siderosis which he found in the liver and which he believed to be due to intense portal hemolysis, were all arguments in favor of his theory that pernicious anemia was caused by absorption of some hemolytic substance from the intestinal tract. The blood picture, as a diagnostic criterion, was practically rejected by him.

In 1881 Ehrlich's (27) discovery of the value of

aniline dyes for the staining of blood films revolutionized the science of hematology. Pepper (65) in 1875 and Cohnheim (18) in 1876 were the first observers to describe the megaloblastic appearance of the bone marrow. They held that this change denoted a reversion to an embryonic type of blood formation. Ehrlich, however, stated that the megaloblastic bone marrow changes were degenerative and were the essential pathological and pathognomonic features of the disease. Muir (56) in 1894 described his extensive bone marrow investigations and came to the conclusion that these could be interpreted as compensatory to the portal blood destruction.

Further investigations by many workers brought the extremist views of Hunter and Ehrlich into closer relationship, and it was admitted that the condition of the gastrointestinal tract, and the evidences of hemolysis were as much a part of the anemic syndrome as were the bone marrow changes. (24)

During the past fifteen years various aspects of the disease have been more intensely considered. The presence of achlorhydria is now recognized to be one of the cardinal signs of the disease. Technical advances in bacteriology have resulted in a great increase in our knowledge of the inhabitants of the intestinal tract in health and disease. Pigment metablolism is now better

understood and recent work points to the importance of the part played by extra-hepatic structures. Blood formation and blood destruction are now viewed in a different light to that of twenty years ago. The relation of pernicious anemia to other deficiency diseases, and the constitutional element as a factor in its production, are at last being clearly recognized. Finally the value of liver and stomach as therapeutic agents has not only completely altered the hopeless prognosis which was the rule previously, but has helped to shed further light on the etiology of the disease. (24)

DISTRIBUTION AND INCIDENCE

Pernicious Anemia is said to occur mainly in countries of temperate climate and to be quite rare in tropical and subtropical regions. There has been no adequate explanation for this distribution but it appears reasonable that the Nordic and blonde peoples are quite rare in the tropical countries as compared to other races.(46) According to Cornell (20) it is more prevalent in northern Europe, North America, and the British Isles, than in any other part of the world. It is chiefly a disease of the white race and in particular of the Nordic types. It is doubtful if it ever occurs in a full blooded negro, but has been reported in mulatoes. (46) The disease appears to be very rare in Brazil; "Cheng has never seen a case among the natives of China." (24)

Friedlander (29) studied the racial factor in 500 cases of pernicious anemia. He found that it occurs in white races of the temperate zones and that of these the light-complexioned and fair-haired type are the most often affected with a high percentage in Nordics. He states that it is now a well known fact that dark skinned peoples, tropical residents, orientals and especially negroes seldom develop the disease. Of 8,527 negro patients admitted to the Morrisania Hospital only one case was

diagnosed as pernicious anemia. In case of dark-complexioned or heavily pigmented patients Friedlander obtained a history of light-complexioned ancestors in practically every case. The predisposition, therefore, would be racial, familial and constitutional, confined largely to those with a diathesis of fair complex, light hair, blue eyes and achlorhydria. "It is felt that these characteristics are definite components of the genotype in such individuals who under the so-called releasing factors in their environment as yet undetermined may eventually suffer a loss of intrinsic factor and develop pernicious anemia."

Draper (26) has studied very carefully the constitutional type of person that is the usual victim. He describes them as people with short, broad faces; large mandibular angles; very short noses; short but deep wide chests; and especially wide subcostal angles. He states that "the male of the pernicious anemia race is a medium to tall individual with short chest, high placed umbilicus, long abdomen, with relatively long lower extremities." They may show feminine tendencies as regards the secondary sex characteristics. Addison (1) in his original description, pointed out that the disease occurs in patients of a somewhat large and bulky frame and with a distinct tendency to fat formation.

Of scientific interest is the work of Smith (73) who determined the solar energy received per annum in various parts of the United States and on comparing this to the mortality rates of pernicious anemia in various parts of the country showed a significant relationship between relative lack of solar radiation and mortality from pernicious anemia in the United States prior to the introduction of liver therapy.

Cornell (20) collected statistics from many countries, which indicated that the death rate per 100,000 population ran usually from three to six. In the provinces of Canada, New Brunswick was high with nine and British Columbia from six to ten per 100,000 population. Ontario showed a yearly incidence of from fourteen to fifteen per 100,000 of population, the deaths from pernicious anemia actually constituting 1.28 to 1.31 per cent of the total deaths. Minot (51) states that in northern parts of the north temperate zone of America and Europe there are probably not less than thirty-five cases per 100,000 population.

In regard to sex incidence Minot (51) is of the opinion that the cases are equally divided between the two sexes. Others (24) found that at about the age of fifty, males are affected more frequently than females, whereas below fifty, females equally or even more frequently

exhibit the condition; still others (62) find that males are attacked more frequently in the United States and England, while more women have been reported with the disease in Germany and the Scandinavian countries.

Pernicious anemia is a disease of late adult life, being most frequent between the ages of forty and sixty. It is rare under thirty years, less than three per cent of the cases, and extremely unusual in children. It appears to make no distinction between rich and poor, city or country dweller, luxury or filth, occupation, overexertion or other similar factors, which are so important in the incidence of many infectious diseases.(62)

There is much evidence to indicate that there is a hereditary or family predisposition to the disease. Pernicious anemia has so often been observed in members of the same family and even in several generations of a family that a familial predisposition to this disease must be accepted as established. The occurence of pernicious anemia in twins has been recorded. It has been found that when this disease occurs in more than one member of a household it occurs among the blood relatives. The main factor must then be endogenous, not exogenous or environmental. This factor seems to be an inherited tendency to the development of gastric secretory deficiency because not only is achlorhydria

more common among relatives of these patients than in the general population, but its incidence in such relatives increases as their age advances.(62) Murphy (60) mentions many cases of two in a family and one with pernicious anemia was traced through five generations with identical symptoms of soreness of the tongue and hyperesthesia.

ETIOLOGY

Until recent years the etiology of pernicious anemia was based on two entirely different theories. The first, originally propounded by Pepper (65) and Cohnheim (18), and later supported by Ehrlich (27) insisted that the primary pathological site of the disease was the bone marrow, which had reverted to an embryonic type, thus producing abnormal cells. Hemolysis was of secondary importance, since it was the result of phagocytosis of these abnormal circulating elements by the cells of the reticulo-endothelial system. No satisfactory explanation was given as to why this megaloblastic degeneration of the bone marrow occurred, unless the view was accepted that the developmental factor was of prime importance. The second view is associated with the name of Hunter (43), and has been subscribed to by the majority of English investigators. The primary site of the disease was held to be the gastrointestinal tract. The absorption of a hemolytic toxin was believed to produce great blood destruction in the portal circulation, and the marrow changes were interpreted as a secondary compensatory process. The nature of the hemolytic agent and its origin were never defined by Hunter, however.(24) Both these theories today take a very subsidiary place to the one formulated

by Castle.

The achylia gastrica, which is the most constant finding in pernicious anemia, should be considered first in discussing the etiology of this disease. It is claimed that there is a high incidence of this condition, possibly congenital, in supposedly healthy relatives of patients with pernicious anemia. Achylia gastrica has been found in certain instances to precede the onset of pernicious anemia by many years. Many people, however, with achylia gastrica never develop pernicious anemia. Hence, it cannot be the essential cause of pernicious anemia. It is an important confirmatory diagnostic feature, however, for it persists during all remissions.(63)

The true etiology of pernicious anemia was not determined until after the discovery of liver therapy, and the classification of this anemia as a deficiency disease. Whipple, Robsheit and their associates observed that the feeding of liver and kidney was effective in the treatment of certain types of anemia; however, it was not proved, until 1926 when Minot and Murphy (53) administered large quantities of liver to patients with pernicious anemia effecting a remarkable improvement, both clinically and hematologically. They found that the oral administration of liver elicited a response by stimulation of the bone marrow reflected in the peripheral blood by an

immediate reticulocytosis, followed by a steady rise of red cells to a normal level. In 1927 and 1928 Cohn and Minot prepared a concentrated liver extract which proved to be just as effective in the treatment of pernicious anemia as the amount of raw liver from which it was prepared. This was the first step in the determination of the deficient principle in pernicious anemia.

In 1928 and subsequently the experiments of Castle (6,7,8,9,10) did much to clarify the role of the stomach in pernicious anemia, and his results and conclusions have led to much work that has slowly resulted in a modern conception of the nature of the fundamental errors in the human body giving rise to pernicious anemia. The action of liver in benefitting cases of pernicious anemia suggested to Castle the possibility of a deficiency basis for the disease. The deficiency did not seem to be of the ordinary type, for liver is ordinarily absent from the diet of unaffected normal humans.

Castle in his experiments determined that when beef steak, which had previously been digested by a normal person's gastric juice, was given to a person suffering from pernicious anemia, a typical reticulocyte response could be elicited. Artificially pepsin digested beef-steak failed to evoke any reticulocyte response as a measure of hematopoietic stimulation. From these

experiments Castle concluded that it is possible that the achylia gastrica of pernicious anemia is operative in the production of a deficiency disease through a failure of the patients stomach to produce the substance apparently formed during digestion in the normal stomach.

Later to each of three cases of pernicious anemia between 200 and 300 gm. of beef muscle was given daily for a period of ten days without demonstrable effect on blood formation. Immediately thereafter to each of these patients and to seven others were given daily the incubated contents of a normal human stomach recovered after the ingestion of similar quantities of beef muscle. In each of the three patients first mentioned and in five of the seven others there appeared before the tenth day an increase of the immature red blood cells followed by a progressive improvement in the anemia similar to that obtained with liver therapy.

Castle consequently tested the hematopoietic effect produced in pernicious anemia patients by the administration of gastric juice alone and of beef muscle alone. Each of these substances alone proved inactive. However, to the patients previously tested there was administered the material resulting from the incubation of 300 c.c. of gastric juice from normal individuals with 200 gm. of ground beef at thirty-seven degrees

centigrade and at a pH of 2.5 to 3.5 and later neutralized to a pH of 5 prior to administration. In all but two of these cases there occurred a significant reticuloctosis and distinct clinical improvement.

As a result of these experiments Castle concluded that the interaction of beef and gastric juice produces some substance capable of causing a remission in certain cases of pernicious anemia. On the basis of these experiments Castle elaborated a theory of the existence of an "intrinsic" factor in the stomach or gastric juice and of an "extrinsic" factor in beef, which by their interaction, lead to the formation of a substance capable of stimulating bone marrow which he termed an "anti-anemic principle". Thus, all patients with Addison's anemia are unable to carry out this essential reaction.

He then conducted further experiments to substantiate his previous results and to attempt to ascertain the nature of the intrinsic and extrinsic factors. Incubation of beef muscle, first with human saliva and subsequently with duodenal contents free of gastric secretion, failed to elicit a hematopoietic response when administered to pernicious anemia patients in relapse. Incubation of beef with pepsin and hydrochloric acid yielded no response. Heating of the

material resulting from incubation of beef with normal gastric juice to a temperature of seventy degrees gave negative results when the material was administered to pernicious anemia patients in relapse. From these experiments Castle concluded that the active constituent of the normal human fasting gastric contents is secreted by the stomach; that the substance is organic, thermolabile, possibly an enzyme, capable of reacting with protein to produce a material having a marked hematopoietic effect in pernicious anemia patients; and that the lack of the intrinsic factor is probably the essential defect leading to the development of pernicious anemia.

Castle later showed that there may be a dissociation of gastric acidity and intrinsic factor. Cases of tropical sprue with normal stomach acid values may present an anemia which responds to liver therapy, while elderly individuals often have an achlorhydria but no anemia. Acid gastric juice from an anemic patient with sprue, when mixed with meat and fed to a patient with pernicious anemia, failed to induce a reticulocyte response while achlorhydric gastric juice from an elderly non-anemic individual was found to contain the intrinsic factor by the same test. Thus Castle believed that the intrinsic factor was absent from the otherwise

normal gastric contents of pernicious anemia patients and that it was present in the achlorhydric contents of patients without anemia. Some of the patients with pernicious anemia with apparent normal juice in which the intrinsic factor was shown to have been absent in relapse was later shown to be present after remissions induced by liver therapy.

Goldhamer and his associates (32,36) showed that the intrinsic factor may be present in patients with pernicious anemia in relapse. In an investigation of the question as to why any red blood cells mature at all in relapse, and why patients in relapse have different red blood cell levels, these observers collected gastric juice from twenty-six patients with pernicious anemia in relapse. The average secretion was 20 c.c. per hour as compared with 150 c.c. per hour in the normal individual. Incubation of this gastric juice with beef muscle and administration to patients with pernicious anemia gave responses which suggested that patients with pernicious anemia do secrete the hematopoietic factor but in deficient quantity. They also noted that the smaller the amount of gastric secretion the lower the red blood cell count.

In 1935 Castle (4) summarized the results of his work as follows: "It is believed that a physiological

mechanism exists in the normal individual which is usually absent in the patient with Addisonian pernicious anemia in relapse. Both the food and the stomach are involved in this process, which will obviously be initiated in the normal individual whenever extrinsic factor is taken in the food. It is logical to conclude that the integrity of this mechanism prevents the development of pernicious anemia in the normal individual; and that its absence leads to a deficiency of the active principle of liver and so to pernicious anemia. It is, however, evident that a deficiency of the active principle finally effective in the body may be brought about by at least three different types of derangement of this mechanism in the gastrointestinal tract. A lack of intrinsic factor in the stomach, a lack of extrinsic factor in the food, or a failure of the absorption or the destruction of these substances or their end products in the intestinal tract would have the same result. Furthermore, an interruption or inhibition within the body of any link in the chain of chemical events leading to the production of the end product active in the bone marrow would also have a similar effect. For the last supposition there is no detailed evidence at present except the variations in the effect of the parenteral administration of a given amount of the same

liver extract in different patients with pernicious anemia; and the fact that arteriosclerosis and infections appear to be common in patients reponding poorly to parenterally administered liver extract."

Castle and Ham have devised a schematic formula of the factors normally involved in the production of the active principle.

$$\frac{F \times G}{I} = L.E.$$

Here F stands for food, extrinsic factor, G for gastric, intrinsic factor, and I for intestinal impermeability or any defect causing malabsorption or destruction of those substances or a product of their effective interaction. L.E. stands for liver extract, the independently effective thermostable factor found in mammalian liver, kidney, and certain other organs. Probably in none of the anemias, that will respond to liver, is any factor on the left of the equation completely normal, and in every instance there is a variable participation of defects of one or both of the factors in the numerator or some increase of the denominator value. Any or all such changes will, however, result in a decrease of liver extract which, if sufficiently great, may allow the development of a macrocytic anemia which will respond to the parenteral

administration of liver extract derived from the liver of a normal animal.

Greenspon in 1936 (37) performed experiments which suggested a different interpretation of the observations of Castle. His theory was that normal gastric juice contains an antianemic factor and free pepsin. The pepsin has an antagonistic action toward the antianemic factor and renders it inert. When gastric juice is incubated with beef muscle the protein of the muscle interacts with the free pepsin to form bound pepsin, thereby permitting the antianemic factor to become active. From the experiments performed and certain theoretical considerations, a new method of making stomach preparations was suggested in which the antagonistic action of pepsin was eliminated.

Hanes and Hansen Prüss (40) and Ungley (79) treated a number of patients in accordance with the theories of Greenspon but they could not observe any evidence of improvement in the condition of the patients. Castle and Ham (5) repeated all Greenspon's experiments, reanalyzed Castle's previous results and conducted some new experiments. All this work tended to show that Greenspon had not adequately controlled the factor of food administration, and that an interaction between the intrinsic and extrinsic substances must have occurred

within the body. Castle concedes, however, that pepsin does have some inhibitory effect, especially when gastric juice digestion mixtures are allowed to stand for some time. Other facts not brought out by Greenspon are that the gastric juice in pernicious anemia is completely devoid of pepsin; that gastric juice alone has no effect and that ventriculin and gastric mucosa are not suitable for experimentation, since they contain not only intrinsic but extrinsic substances which by their interaction provide material that stimulates bone marrow.

The stomach defect had been considered the sole etiologic agent in pernicious anemia until the experiments of Meulengracht.(49) He demonstrated that desiccated material from the pyloric region of the stomach and duodenum of pigs was rich in the hematopoietic principle effective in pernicious anemia, and that the cardiac and fundic regions of the stomach were relatively inactive. This suggests that the glands of both the pylorus and the upper duodenum are non-functional in pernicious anemia, but in gastric resection the glands of the duodenum, in some cases, are capable of producing sufficient quantity of the intrinsic factor to prevent the development of macrocytic anemia. Cheney (15) confirmed the findings of Meulengracht by using scrapings of human duodenal mucosa incubated with liver and liver

extract in the treatment of patients with pernicious anemia. He suggested that the activity of the duodenal mucosa explains the absence of macrocytic anemia in some patients with gastric achylia and atrophy of the mucosa of the stomach, and also may account for failure of this type of anemia to follow gastrectomy in all instances.

In regard to the part played by the liver in pernicious anemia Goldhamer and his associates (35) have concluded from their observations that (1) the active principle may be absent from an inadequately treated case of pernicious anemia. (2) Active principle is present in liver in an adequately treated case of pernicious anemia. (3) A cirrhotic liver may not contain the active principle. (4) It is highly suggestive that a liver may be sufficiently damaged so that the active principle though present cannot be presented to the tissues for utilization. (5) A pernicious anemia-like blood picture may be present in a patient if the liver is so damaged that it cannot store the active principle or cannot present it to the body tissues in proper form for utilization. The demonstration that all livers do not contain active principle indicates it a storage product rather than an intrinsic part of the liver substance. Wintroube's (88) observations in a review

of 132 cases of various hepatic disorders support the conclusions of Goldhamer.

Castle (4) is not convinced that disease of the liver may specifically produce an interruption in the metabolism of the active principle of liver. The arguments in favor of this supposition neglect the fact that the animal kidney is as effective a source of the active principle of liver as is liver itself.

In studying the extrinsic factor Castle and Strauss (12) substituted various substances for the beefsteak of their original experiment and digested them with normal gastric juice. By this method the extrinsic factor has been found to be absent from casein, gluten, nucleoprotein from hen's blood and nucleic acid of yeast and of animal origin. Washed beef muscle protein contained moderate amounts and spleen pulp and autolyzed yeast (marmite) large amounts of the extrinsic factor. The further observations that the extrinsic factor in yeast was not destroyed by autoclaving and was soluble in eighty per cent alcohol led to the suggestion that it might be identical with or allied to vitamin B_2 (G). This has been disproven by Lassen and Lassen (47) who failed to obtain responses on feeding various sources of vitamin B_2 and gastric juice to patients having pernicious anemia. Also Brand

and his associates (3) have purified potent liver material until free from vitamin B_2 with no change of effect of the liver preparations on pernicious anemia.

TREATMENT

LIVER TREATMENT

The introduction of liver therapy has completely revolutionized the treatment of pernicious anemia. Previous to this discovery, innumerable methods of treatment existed, each claimed by their exponents to have some particular beneficial action. Their very multiplicity could be taken as direct evidence of their lack of specificity. Since pernicious anemia is essentially a three system disease, it is probable that in no other condition can so many different symptoms be present at the same time. This accounts for the introduction of the innumerable symptomatic remedies. Liver therapy, however, has greatly simplified matters, since the blood and gastrointestinal symptoms, and even some of the nervous symptoms when early diagnosis has been achieved, are all equally, efficiently and rapidly relieved. In liver we have a specific as opposed to a symptomatic remedy.(24)

Of the course of pernicious anemia, untreated or treated by measures in vogue previous to the liver era, much is known. The disease was always fatal, although characteristically masked by periods of great improvement or remission. Recovery has been reported

previous to Minot and Murphy's communication, but such instances are very doubtful. Occasionally the remission may last for several years, but usually it is limited to a period of a few months. In the great majority of cases death occurs within three years. The usual number of remissions in any given case is two, but there may be even four or five such periods of improvement. Remissions often occur spontaneously and have been observed after many and varied forms of treatment. In certain instances the patient may appear moribund, following which a remission may take place with astounding improvement. During this phase there may be all degrees of amelioration of symptoms, even to apparently complete restoration to health. Although there may be marked increase of hemoglobin and red blood cells up to nearly normal values, certain abnormal features persist in the blood picture. The red blood count rarely passes above 4,000,000 in a spontaneous remission, and a tendency to macrocytosis persists. The achylia gastrica and neurological symptoms, if severe, persist. In those cases in which remissions do not occur, the downward course is rapid, and death usually results in six to nine months.(63)

The discovery of the liver treatment followed the work of Whipple and Robscheit (85). These observers working with dogs, determined for each animal the

available red cell pigment by observations of the hemoglobin percentage and the blood volume. They then produced a uniform degree of anemia by the removal, on each of two successive days, of one-quarter of the already determined blood volume. The time taken for the available red cell pigment to return to its previous level was then observed, and the influence of a variety of dietetic factors noted. On an ordinary diet of mixed table scraps, the time taken for complete return to normal was four to seven weeks. On a liberal diet of meat and beef heart, however, the time was only three to four weeks while with cooked liver it was even less, two to four weeks.

In 1925 they (86) reported further experiments in which they produced a constantly-maintained severe secondary anemia, in place of the single post-hemorrhagic period previously described. The hemoglobin level was reduced to 40 to 50 per cent and maintained at this point by frequent bleedings of calculated amounts. Estimation of the total hemoglobin in the samples of blood, which it was found necessary to remove, gave a measure of the rate of blood regeneration. The maintenance of the constant hemoglobin level meant that the stimulus to regeneration, dependent on this factor, was fairly constant, so that the value of the diet factor could be

more properly appreciated. While in their first experiments, beef heart, muscle, and liver feeding were about equal in efficiency, they noted at this time that blood regeneration was more marked with liver feeding, beef heart and muscle being less effective. Their final conclusion was that liver feeding in these severe anemias remains the most potent factor for the sustained production of hemoglobin and red cells.

Treatment of anemia by diet, however, was not new before this work of Whipple and Robscheit was done. Davidson and Gulland (24) quote the review of J.G.McCrie on the early ideas on the place of diet in the treatment of pernicious anemia.

"Diet, as a factor in the treatment of pernicious anemia, has been referred to by most writers on the subject. Thus, in 1863, Habershon, describing what is apparently a case of this nature, writes that many patients at an early stage completely recover under the influence of bracing air, and a nutrient and stimulating diet. No remedy, however, he states later, is of any permanent benefit, and the patient very gradually, but with steady course, loses strength and power, till life ceases from simple exhaustion. What was appreciated at this time, evidently, was the desirability of prescribing easily-digested foods, in a condition known to have, as

one of its characteristic features, an unhealthy condition of the gastrointestinal tract. Pepper (1875) draws attention to the fatty degeneration of the liver and of the gastric mucosa, found post-mortem, the consequently impaired state of the digestion during life, and hence the necessity for careful attention to the nutrition, and the administration of food in the most digestible forms. The atrophic state of the mucous membrane of the stomach is also referred to by Fenwick (1877), and the subject of diet is spoken of as one of great difficulty, on account of the patient's lack of appetite. A hint of some special importance attaching to diet is, perhaps, given by Osler (1885), when he speaks of cases that appear to have gotten well with change of air and a better diet, after resisting all ordinary measures. It is natural that Hunter (1890), taking the view that pernicious anemia is a disease dependent on the presence, under certain favorable conditions, of organisms of a specific nature within the gastrointestinal tract, should attribute great importance to dieto-therapy in this condition. It is interesting to note that Hunter considered a farinaceous diet to be superior to a nitrogenous one. He lays stress on the fact that, while on an ordinary mixed diet the ratio of free sulfate in the urine to aromatic sulfate

was 9:1, on a farinaceous diet the ratio was 15:1 indicating a lessened production of supposedly toxic substances within the bowel. Of very great interest is a case recorded by Fraser in 1894. This patient went downhill under treatment with iron and arsenic, but remarkable improvement occurred when he was given bone-marrow. Soon after the administration of bone-marrow was commenced, the blood platelets, which had stood at a very low level, greatly increased in numbers, and the red blood corpuscles previously less than 1,000,000 per cubic millimeter rose in a short time to 4,000,000, and the hemoglobin percentage to 85, by which time the number of blood platelets had returned to about a normal level. The possibility of spontaneous natural remission cannot of course be excluded in this case, but it is striking that such improvement should have followed the exhibition of a substance so similar in nutritional composition to liver and kidney, the beneficial effects of which are dealt with subsequently. This clue does not appear to have been followed up, for, so late as 1917, Barker and Sprunt, in outlining a scheme of treatment for cases of pernicious anemia, discuss diet as relating simply to the general need of the patient, and to the state of his gastrointestinal tract. It is true, however, that in advising an abundant roborant diet, rich

in protein, they may, in the light of recent work, have been not so wide of the mark. The same applied to the statement by Fitch (1922) that the inclusion of bone-marrow, liver, and spleen in the dietary is to be advised on the ground that these foodstuffs are rich in nucleo-albumins containing iron. The idea that any specific treatment is here intended is, however, negatived by the subsequent statement that, though the life of the patient may be prolonged and a reasonable degree of comfort secured through careful supervision of digestion and elimination, there is no specific evidence that diet per se modifies the course of the disease.

"So far, then, it is evident that dietotherapy in pernicious anemia has been regarded chiefly as part of the general treatment of a patient suffering from a debilitating condition, and exhibiting in particular a state of hypofunction of the gastrointestinal tract. Diet, in fact has been largely of the nature of symptomatic treatment. A period now commences, however, in which diet begins to be studied with special reference to possible beneficial effects on the course of the disease itself. Thus Mosenthal (1918) states that, whilst a negative nitrogen balance has been thought to be characteristic of pernicious anemia, he has been able, by means of a forced diet, readily to produce a positive

balance, and that further, in each of the three cases observed, improvement in the blood picture is to be noted. A further important paper on metabolism in pernicious anemia is that of Gibson and Howard (1923). These observers tentatively suggest that an iron-rich and vitamin-adequate diet may be beneficial.

"A series of researches was carried out under the auspices of the Carnegie Institute of Washington (1919), in which the effect of pronounced restriction of diet was observed in the case of twenty-four normal healthy individuals. A slight anemia was produced in most of these cases, but the anemia was of the secondary type."

Although the treatment of pernicious anemia was placed on a dietetic basis, its importance was not recognized until Minot and Murphy in 1926 (53) reported their special diet. They reported observations on forty-five patients taking their special diet for pernicious anemia over a period of from six weeks to two years. This diet was composed especially of foods rich in complete proteins and iron, particularly liver and containing an abundance of fruits and fresh vegetables and relatively low in fat. The special diet used was made as palatable as possible and for each day was practically as follows:

1. From 120 to 244 gm. and sometimes even more of cooked calf's or beef liver. An equal quantity of lamb's kidney was substituted occasionally.

2. 120 gm. or more of beef or mutton muscle meat.

3. Not less than 300 gm. of vegetables containing 1 to 10 per cent of carbohydrate, especially lettuce and spinach.

4. From 250 to 500 gm. of fruit, especially peaches, apricots, strawberries, pineapple, oranges and grapefruit.

5. About 40 gm. of fat derived from butter and cream, to make the food attractive. However, animal fats and oils were excluded as much as possible.

6. If desired an egg and 240 gm. of milk.

7. In addition breads especially dry and crusty, potato, and cereals, to make the total intake of 2000 to 3000 calories, composed usually of about 340 gm. of carbohydrate, 135 gm. of protein, and not more than 70 gm. of fat. Grossly sweet foods were not given, but sugar was allowed very sparingly.

At the time the diet was advised for many of the patients, they were able to take only a small amount of food of any sort. Under these circumstances they were encouraged to take as much as possible of liver and fruits and at least some vegetables, while other sorts of food were not forced. During the first week of the diet, the

intake was often less than 1000 calories, but by the end of the first week they usually felt distinctly better and their appetite began to improve, and within two weeks they were taking the complete diet.

By the end of the first week of treatment the reticulated red cells of the blood had risen markedly, returning to a normal level by the end of the second week, by which time the red cell count and the hemoglobin percentage were beginning to show noticeable improvement. The rate of improvement was greatest in those cases who had the lowest initial counts. Four patients died, but all of these were so ill from the beginning that they could take no liver. The remainder all improved, irrespective of the length of time the disease had lasted before the inception of treatment, and all were in good health at the time of publication, though it was thought that patients who had had several previous relapses took on the whole longer to improve. After four to six months of treatment no patient had less than 3,500,000 red cells, eighty-one per cent had 4,000,000 or more, and thirty per cent had over 5,000,000. The icterus index had usually fallen to below normal by the time the red cell count had reached 2,500,000. Those patients who were most careful in taking the full amount of liver improved quicker than the more careless. Three

patients relapsed, but this was evidently due to their not taking the diet satisfactorily, and they quickly improved when this was corrected, showing apparently that the diet must be continued indefinitely if the patient is to remain well.

In 1927 Murphy (61) published the special details of ten of the cases reported in the original paper of Minot and Murphy noting particularly the prompt reticulocyte increase, followed by an increase of red cells and hemoglobin, a fall in the icterus index, the return of the morphology of the red cells to normal with a fall of the color index to one or less, and an increase in the protein of the corpuscles which was in almost direct proportion to the increasing hemoglobin concentration.

The discovery of the effectiveness of liver in the treatment of pernicious anemia naturally raised the question of the nature of the substance or substances which were thus effective, as well as that of the character of the disturbed physiological processes which were modified by the addition of liver to the diet.

Accordingly, the chemical dissection of the liver was undertaken by Cohn, Minot and their associates in 1927 (16) as a means of eliminating, one by one, those of its constituents not involved in producing the prompt acceleration of blood formation. Their resultant product

was given the name "Fraction G" which was at least as effective as whole liver in the treatment of pernicious anemia. This fraction in daily doses of 9 to 14 gm. was fed to three patients who promptly and rapidly improved. Although this fraction had been prepared free from iron, proteins and lipods, and although it was considered satisfactory from a therapeutic standpoint, it was still a relatively crude extract. Accordingly they attempted further fractionations in 1928 (17) from which they concluded that the active principle was a nitrogenous base or a polypeptid. This fraction contained the active principle in sufficient concentration such that 6 gm. of the material a day was sufficient to produce a pronounced response of reticulated red blood cells in a patient with pernicious anemia.

Following the introduction of the liver diet and an active liver extract, other sources for the potent material and other methods of preparation have been discovered.

Castle in 1929 demonstrated that stomach of normal persons secreted a substance which could develop a blood maturing principle from meat.

Sharp (70), on the basis of Castle's work, suggested that the feeding of stomach should have the same effect on patients with pernicious anemia as liver.

His theory was that there is a defective liver function as well as an abnormal gastric condition in the predisposed person. It appeared more rational to him to assume that liver or liver extracts supply an essential substance, readily synthesized from ordinary food in normal gastric conditions, but imperfectly or scantily converted in achylous digestion.

On the basis of these criteria and the fact that the anlages of the stomach and liver are analogous in origin, being derived from the embryonic foregut, he believed that stomach tissue might contain the same antianemic factor abundant in liver. Moreover, if achylia gastrica is caused by an acquired or congenital defect of the glandular portion of the gastric mucosa, it seemed reasonable to believe that tissues of the stomach might be therapeutically applicable from the standpoint of organ specificity.

Working with Sharp on the basis of his theory Sturgis and Isaacs (78) prepared an extract of desiccated hog's stomach (ventriculin) free from fat of which 30 gm. of the final material represented 218 gm. of fresh tissue.

Sturgis and Isaacs administered 15 to 30 gm. of this defatted and desiccated hog's stomach to three patients with typical pernicious anemia, all three of

which showed a prompt and very active response characterized by an increase of reticulocytes, which was comparable with, if not greater than, the response to feeding a commercial extract of 300 to 600 gm. of fresh liver. The feeding of desiccated whole hog's stomach produced a complete remission and indicated that stomach tissue contains a red blood cell maturing substance. It appears that patients with pernicious anemia have lost, or never had, the ability to secrete a substance in their stomachs which has the power to produce a blood-maturing material from food. They also concluded from the above findings that stomach tissue, per gram of fresh material, is more active than liver, as a smaller amount of the former is required to induce a remission.

Renshaw (68) recorded a case in which liver and various liver extracts had failed, and in which administration of 7.5 to 20 gm. of ventriculin caused remission. Treatment with the same preparation kept the patient in good health although the red corpuscles did not reach normal numbers.

Morris and his associates (55) reported two cases in which single doses of concentrated gastric juice from swine produced a complete remission in previously untreated cases of pernicious anemia.

The first case was a white male, age 65, who entered the Cincinnati General Hospital, May 23, 1932, with a red cell count of 1,400,000, a hemoglobin of 47 per cent, and a reticulocyte count of 0.6 to 1.3 per cent. No nucleated red cells were seen. June 7, 5 c.c. of concentrated gastric juice was given intramuscularly. On June 8, 4000 nucleated red cells per c.c. were found. The reticulocytes rose steadily from 4 per cent on June 8 to 40.1 per cent on June 13, and remained between 25 and 42 per cent until July 9. The reticulocytosis ended July 13 after a period of 34 days. On June 28 the red blood count and hemoglobin started to rise and continued steadily until September 21, when examination showed 4,500,000 red cells and 93 per cent hemoglobin. On September 21 another 5 c.c. was injected as a maintenance dose. The other case showed a similar response. Marked subjective improvement was noted in both cases within a few days.

From this experience it seemed probable that a product could be obtained from the gastric juice of such potency that a single intramuscular injection would be sufficient to bring about a complete remission in pernicious anemia. Should this be true one may predict that one injection of potent material at intervals of

two or more months may be sufficient to maintain the red cell count and hemoglobin.

The potency of a liver extract has been demonstrated to be definitely increased by incubation with normal human gastric juice. Walden and Clowes (83) have reported a marked increase in potency of liver extracts incubated with unheated stomach tissue, suggesting that liver contains extrinsic factor which is activated by gastric intrinsic factor, in addition to the finished anti-pernicious anemia material. Helmer, Fouts, and Zerfas (41) fed patients with pernicious anemia in relapse a daily subminimal dose of liver extract, that amount derived from 100 gm. of liver, which had previously been incubated at forty degrees centigrade for four hours with 100 c.c. of normal human gastric juice obtained after histamine stimulation. During the control and test periods the diets contained no meat or eggs. This preparation produced maximal reticulocyte responses and marked clinical improvement when administered daily by mouth to patients with pernicious anemia in relapse. These results were in harmony with and lend confirmation to the observations of Walden and Clowes. They state, however, that it still remains to be determined whether the enhanced activity which they observed is due to an increase in the active principle originally

present in the liver or to an effect of the type described by Castle and his associates when human gastric juice was allowed to act on beef muscle, beef muscle extracts and yeast.

Herron and McEllroy in 1933 (42) reported a marked increase in the anti-pernicious anemic potency of liver autolyzed for several days in weak acid, suggesting the presence of both intrinsic and extrinsic factors in liver itself. They suggested that autolyzed liver given by mouth has an effect approximating that of other liver preparations when given intramuscularly or intravenously. Given intramuscularly, autolyzed liver is stated by these authors to be ten times as effective as other preparations obtained from the same original amount of liver.

This work was contradicted by Castle and Strauss (13). Using experimental and commercial autolysates of liver, made according to the directions of Herron and McEllroy, they concluded that it has less hematopoietic activity in the treatment of pernicious anemia than the amounts of liver from which they were derived.

Their method of testing involved making a comparison of the reticulocyte response produced by the daily administration of a uniform submaximal dose of the material to be assayed with the reticulocyte

response obtained from a known source of active material similarly administered during an immediately succeeding period. Minot (52) has shown that if a second reticulocyte peak occurs under these conditions, it indicates that the potency of the known substance administered in the second period is greater than that of the unknown substance first administered. Castle and Strauss obtained this second reticulocyte peak following the use of a known source of active material.

Richter and his associates (69) prepared an extract of horse liver, of which 1.8 gm. was derived from 100 gm. of raw liver, and which on oral administration induced a complete remission in pernicious anemia. They treated seven patients with cattle liver extract and ten patients with their horse liver extract and obtained a quicker and better response with horse liver extract. The extracts were prepared from the same amounts of raw liver.

From the results obtained by use of horse liver in the treatment of their patients they concluded that the concentration may be greater in the liver of the horse than in liver of cattle.

The use of parenteral injections of liver extracts was first reported by Ganssleín in 1930. (30) He obtained satisfactory results in the treatment of pernicious anemia by daily intramuscular injections of

an extract derived from 5 gm. of liver, but failed to publish the details of his method of preparation.

In 1931 Castle (14) prepared an extract of liver based on the work of Cohn which on intravenous administration proved to be highly potent in pernicious anemia. Maximal reticulocyte responses were obtained from a single intravenous injection of the amount of extract derived from 100 gm. of liver. One of the patients so treated had failed to respond satisfactorily to the previous oral administration of a similar extract derived from 3000 gm. of liver given within a period of ten days. Though he considered the intravenous treatment of life-saving value to the very sick patient he did not recommend its use because of the reactions which in some patients may become more serious than the disease itself.

Castle (76) later prepared an extract which was a modification of the intravenous extract of which 10 c.c. contained the extract derived from 50 gm. of liver. Injections of 2 c.c. daily were used in the treatment without local or systemic reaction and were not painful. The clinical results were very satisfactory; the patient felt better within two days, within three days increases in reticulocytes were obtained and maximum production of reticulocytes

was reached within from five to seven days.

The response was just as rapid as with the intravenous route and indicates that the intramuscular injection is as efficient as the intravenous route in treatment of severely sick patients, and is far more satisfactory because of the total absence of undesirable immediate or delayed reactions.

In the treatment of thirty patients with pernicious anemia by means of liver extract parenterally, Murphy found improvement in the blood to be more rapid and striking than that to be expected from the ingestion of much larger doses of liver or potent liver extract. Treatment was followed by an increase in reticulocytes generally within a shorter period than occurs after treatment by mouth.(57)

Goldhamer and his associates (34) reported two cases of pernicious anemia treated with a non-purified liver extract intravenously in which a maximum reticulocyte response was produced in a shorter time than orally with a dosage which would have been wholly inadequate if taken by mouth. A definite response to therapy as evidenced by an increase in the reticulocyte percentage was noted at the end of twenty-four hours. In one case the maximum reticulocyte response of 49.5 per cent was reached in 108 hours and in the second

case a maximum reticulocyte response of 52.8 per cent in 88 hours. These responses are significant when one considers that only slightly more than the liver extract made from 100 gm. of fresh liver was given, which amount has little or no value when administered orally. In the first case there occurred an increase per cu. mm. of 1,000,000 red blood cells in ten days and 2,000,000 red blood cells in fifteen days. In the same period of time the hemoglobin percentage increased 24 per cent and 27 per cent above its original level. In the second patient there occurred an increase of 1,000,000 red blood cells within a ten day period with a hemoglobin increase of 28 per cent. The usual increase of appetite, strength and sense of well being, as well as the decrease of pallor and icterus, took place much more rapidly with intravenous therapy. In these cases evidence of clinical improvement was noted within forty-eight hours whereas in oral administration of liver extract clinical improvement is usually seen in seventy-two to ninety-six hours.

The reactions noted in these patients, however, contraindicate the use of liver extract by the intravenous method. The picture during and immediately following the injection is characteristic of a systemic reaction to histamine or choline. The patients described a sensation of warmth over the entire body, especially the face.

Accompanying this was a choking sensation, a feeling of air hunger or a sense of marked pressure over the chest. There was a throbbing sensation in the head, the face became flushed and large beads of perspiration were observed over the forehead and about the mouth. These symptoms were transient and apparently could be somewhat influenced by the rate of injection, and disappeared entirely with cessation of injection. However, within forty-five minutes the patients were seized with a severe chill. The temperature gradually mounted to 104 degrees in one patient and 102 degrees in the other. Accompanying the chill one patient had a severe vomiting spell, became cyanotic and complained of numbness of fingers and hands. Within twenty-four hours all symptoms disappeared. Both patients showed a marked drop in systolic and diastolic blood pressure which did not return to normal for forty-eight hours.

The results obtained after 1000 intravenous injections of purified liver extract in 140 patients were reviewed by Isaacs and his associates.(45) The injections were given at the rate of 20 c.c. in from one to twenty minutes. They found that the most convenient dosage by this method was 20 c.c., obtained from 100 to 125 gm. of liver, once a week until the blood count reached normal, then once every month. This

dosage was varied somewhat depending on the response of the various patients. The extract used was treated with permutite and acetone which removed the impurities producing the reactions described above. With the intravenous liver extract the reticulocytes started to increase in number from twelve to twenty-four hours and reached their maximum percentage in from sixty-eight to 108 hours. Compared to the maximum reticulocye percentage reached with six vials daily of potent liver extract by mouth, the total of the average maximums was over two-fifths higher than the totals of the averages calculated for this series. This was interesting when it is remembered that the response here is the result of giving liver extract made from about 125 gm. of fresh liver as compared with that from 4200 to 4800 gm. by the oral method. It is evident that much of the active substance is destroyed or not absorbed when the extract is taken by mouth. The advantages of this route of treatment are the same as those of the intramuscular method. Because of the long intervals between the doses and the lack of discomfort, they found that many patients preferred the intravenous method to the intramuscular route.

Strauss (74) advocates the use of intramuscular therapy and outlines a course of treatment. Ten units

of a purified solution of liver extract should be injected immediately following diagnosis. This should be repeated on the next two days and then at weekly intervals until the blood values are normal. Larger doses are required to raise the red cell count to 5,000,000 per cu. mm. Frequently after twelve months of normal blood counts it is possible to increase the interval between injections to two weeks. Strauss advocated larger doses of liver extract to arrest completely all progress of degeneration of the spinal cord. "It has been unequivocally established", he said, "that such arrest can be achieved if sufficient amounts of liver extract are employed." This would require thirty-five units intramuscularly during the first week and at least ten units a week thereafter and double the dose if the neurologic lesions progress. This treatment must be continued for at least a year and reduced later with caution.

The unit mentioned is the amount of material which when given daily will produce a satisfactory rise in the reticulocyte count and an adequate increase in the number of erythrocytes and the percentage of hemoglobin in selected patients with Addisonian pernicious anemia.(74)

In a large series of cases Sturgis (77) used an

intramuscular preparation standardized on the basis of 2 c.c. being equal to 10 gm. of liver. He gave an initial dose of 12 to 16 c.c. weekly, 4 c.c. at a time until the blood was normal and then 4 c.c. weekly at one dose.

Fouts (28) gives daily intramuscular injections for three to four days, then weekly injections. The amount of the injections range from 1 to 10 c.c. depending upon the concentration of the extract. He obtains a reticulocyte response in a few days reaching a peak on the fifth to the seventh day, the red cell count returning to 4,000,000 in one month, and to 5,000,000 in one and a half to two months in most of the cases.

Young (89) advocated the use of 3 c.c. of a liver extract, derived from 100 gm. of liver, on the first, second, and fourth days followed by the injection of 1 c.c. weekly until the blood count reached the normal level (4,500,000 in women, 5,000,000 in men); and as a maintenance dose one injection every two to four weeks, depending upon how well the patient's blood is maintained. When a cold or other infections occur, the dose should be increased to one and a half times the regular dose.

Dakin and West (22) have prepared a more

concentrated solution of liver extract anahaemin, from commercial liver extract. Their method is based on the removal of much relatively inactive material by precipitation with alcoholic calcium acetate, followed by a precipitation of the active material with Reinecke acid. Subsequently purification was affected by salting out the active material with ammonium sulfate and later by the use of either magnesium sulfate or sodium chloride. Thirty mgm. of the product caused a perceptible reticulocyte response in suitable pernicious anemia patients, while 80 mgm. have given a maximal response. On hydrolysis they found that it yielded an aminohexose and a number of amino acids, namely lysine, arginine, glycine, leucine, hydroxyproline, and aspartic acid.

The results of Ungley and his associates (80) in the treatment of thirty-six patients with Dakin and West's liver fraction indicate that anahaemin is highly active for blood regeneration in pernicious anemia. Total quantities of 1 to 6 c.c. (100 to 600 mgm, average 359 mgm.) administered usually in divided doses to eleven cases with initial red blood cell counts below 2,000,000 per cu. mm. were sufficient to cause an average increase in erythrocyte count amounting to 2,300,000 in forty days. Good responses followed the

administration of amounts sometimes as small as 10 mgm. daily or 100 to 200 mgm. as a single dose. For maximal reticulocyte responses and for the production of red blood cells at a maximal rate, larger doses were usually required. There is not sufficient data to assess quantitatively the potency of anahaemin as compared with other liver extracts but in their experience no other liver extract given in the small amounts used in their investigation has produced such striking results.

Wilkinson (87) has confirmed the work of Dakin and West. He prepared products of which 58 mgm. produced a maximal reticulocyte response and a rapid remission in a patient with pernicious anemia. By applying this method to other methods of separation a further increase in hemopoietic potency was secured so that as little as 18 mgm. of the product was sufficient to initiate a maximal reticulocyte response and rapid remissions in pernicious anemia.

The use of concentrated liver extracts intramuscularly have many advantages over that of whole liver or potent substances by mouth or less concentrated solutions for intramuscular injection. The dosage is under absolute control of the physician. Since the patient must return for further treatment, the physician will see the patient at frequent intervals, thereby

insuring him of the best results. Its striking rapid effect makes it the treatment of choice for the patient in severe relapse. The first maximum effects are evident many hours before these effects may be observed following the administration of liver or liver substitutes by mouth. It is valuable in patients in whom it has been found difficult to maintain an essentially normal condition of the blood. This apparent resistance to satisfactory improvement may be due in some instances, when liver or liver extract is given by mouth, to an inability on the part of the patient to obtain, to take or to utilize an adequate amount of the material in liver which is effective in producing blood.(77,59) Since control of the conditions associated with spinal cord or peripheral nerve damage depends primarily upon the utilization of optimal amounts of the active substance in liver, the concentrated solution given intramuscularly is the treatment of choice. The certainty of the dosage and the convenience of its use, because of the relative infrequency of administration in uncomplicated cases particularly, is generally appealing to the patient. The cost of the concentrated products is small in comparison with that of liver or its substitutes for oral use. It is often difficult for the patient traveling to obtain good liver along the

route and the substitutes for injection are bulky to carry. Intensive intramuscular therapy prior to the trip will be sufficient for several weeks without further treatment, or a few vials may be easily carried and injected as desired enroute.(89,59) There are no local or general reactions following its use as in intravenous injection.(77)

The fundamental point in the treatment, however, is quantitative and is not the choice of a special preparation of liver or its mode of administration. One must make certain that for the case under treatment enough potent material is given and that this is absorbed.(63,75)

In reference to this statement the Council on Pharmacy and Chemistry in 1935 (21) published the standards that would thereafter be required of preparations made for the treatment of pernicious anemia. Their specifications were that a liver preparation must be of such potency as to produce the response as set forth by them in the following table.

Initial Red Blood Count Million per Cu. Mm.	Minimum Reticulocyte Response Per Cent
1.0	30
1.5	18
2.0	12
2.5	7
3.0	4

The figures given have been obtained by the daily oral administration of material derived from 300 to 400 gm. of liver, or of 30 to 40 gm. of desiccated stomach, or by the daily parenteral injection of material derived from 10 to 15 gm. of liver. The Council will require that all preparations designed for use in the treatment of pernicious anemia be manufactured by a satisfactory method and that they be labeled with the amount of the contained material which will produce the standard rise of reticulocytes when assayed in the manner defined by them.

Since 1935 the standardization of anti-anemia preparations has been placed in the hands of the U.S. Pharmacopeia Anti-Anemia Preparations Advisory Board which just recently, March 1938, stipulated that liver and stomach preparations will now be labeled in units and that the patient should receive for maintenance dosage an average of about one unit each day. Under the rules of the Board the amount of liver supposed to be represented in the product may not be mentioned on the label, as it is likely to be misleading; a product derived from 100 gm. of liver is not necessarily twice as potent as one made from 50 gm. The unit, as mentioned previously (74) is the amount of material which when given daily will produce a satisfactory

rise in the reticulocyte count and an adequate increase in the number of erythrocytes and the percentage of hemoglobin in selected patients with Addisonian pernicious anemia.

From the practical standpoint the difference between liver and stomach preparations should also be pointed out. The essential principle in liver is thermostable, or heat resistant, while that in stomach preparations is destroyed if heated above sixty-five degrees. Therefore stomach preparations are prepared at a low temperature and are available only in dry powdered form, from which no products suitable for injection have been made available. Also, no stomach products in aqueous solution are available because of the danger of bacterial contamination. On the other hand, liver preparations are available in the form of dry powdered extracts for oral use, and concentrates prepared from them for purposes of injection. (46)

The following table taken from Castle and Minot (11), shows the available preparations of liver and stomach, the amounts of fresh material from which they are derived, and the method of use, each amount being that necessary to produce a maximal reticulocyte response in pernicious anemia.

Substance	Original Weight (Grams)	Prepared Weight or Volume	Route of Administration
Liver or kidney	500	400 Gm.	Oral
Desiccated hog stomach (Ventriculin)	250	30 Gm.	Oral
Liver extracts			
Aqueous concentrate	500	65 cc.	Oral
Precipatate 95 per cent alcohol fraction "G"	600	27 Gm.	Oral
Dilute solution fraction "G"	10	2 cc.	Intram. Inj.
Concentrated solution fraction "G"	20	0.6 cc.	Intram. Inj.
Liver stomach preparation	20	4.5 Gm.	Oral

A study of the above table shows that for a patient to obtain a maximum reticulocyte response he can eat 400 gm. of liver, or 30 gm. of desiccated hog stomach, or drink 65 c.c. of aqueous solution of liver concentrate, or be injected with as little as 0.6 c.c. of concentrated liver extract derived from 20 gm. of fresh liver. The extracts have been further refined so that the patient can be injected with one c.c. concentrated from 100 gm. of fresh liver, which on the basis of the figures in the above table, should be five times the amount necessary to produce a maximum reticulocyte response. (46)

The effectiveness of liver preparations when injected, is about sixty times of that taken by mouth. Therefore, the injection of 1 c.c. derived from 100 gm. of fresh liver should be equivalent to the effects obtained from the oral ingestion of about 6000 gm. of

fresh liver. It seems evident, therefore, that a patient can be more efficiently and economically treated by injections of liver, rather than by the oral administration of it.(46) Furthermore it has the advantages mentioned previously.

CLINICAL CHANGES AFTER LIVER TREATMENT

The patient with adequate therapy shows marked improvement of symptoms. Subjective improvement is very definitely apparent even before gross changes can be noted in the blood. There is an increase in the appetite and a general sense of well-being.(46,53,63) An abnormal redness of the skin of the face has frequently been noted after two or three weeks treatment, giving the patient an appearance of improved health which is not borne out by the still relatively low red blood count.(54,63) The glossitis, dyspepia, and diarrhea usually disappear within the first two weeks.(54,46) The dyspnea on exertion rapidly disappears as the blood improves; the general weakness and edema of the ankles are somewhat more persistent, but vanish as the blood approaches normal. Hemic cardiac murmurs slowly disappear, and normal sounds are heard.(24) The rather uncommon symptom of cardiac pain disappears early.(54) Those cases who had a low

blood pressure before treatment attained a normal level; hypertension was not observed to develop in any case.(48) The early loss of weight, from the disappearance of edema, is soon counteracted by a rapid gain with an increased appetite.(54) Sexual activities, usually lost during relapse, return to normal with the disappearance of the anemia.(63) Usually the achlorhydria persists even after prolonged treatment.(24) The appearance of the intrinsic factor was noted by Castle (10) during remission in one case treated with liver extract. Davidson (23) reports a case with all the features of pernicious anemia, in which the stomach gained the power to secrete hydrochloric acid and pepsin after treatment with fish liver extract for ten days.

The writers have more or less agreed on the clinical changes occurring following adequate treatment of pernicious anemia in all the symptoms and signs with the exception of the neurologic changes. There is some variation of opinion in this regard and a large part of the literature on pernicious anemia has been devoted to this subject as a result.

In Garvey's (31) series of forty-seven cases of pernicious anemia who received uninterrupted treatment with liver for an average period of twenty-five months, forty cases presented signs of cord degeneration. In

the majority of these the spinal cord degeneration progressed in spite of the treatment; the rapidity of progression was variable. In some patients the degenerative process seemed to progress relentlessly, while in others, perhaps the majority, the progress of the neural changes was retarded. "That improvement in the cord changes is unusual is not surprising when one considers the inability of axis cylinders once destroyed to regenerate. While both the axis cylinder and myelin sheath may eventually be destroyed, some investigators believe that the myelin sheath is affected first. If this is correct, institution of treatment may lead to functional restoration of the partly damaged nerve fiber."

Garvey and his associates feel that treatment begun before degenerative changes in the cord are well established is of value, in the majority of patients, in the prevention of serious cord lesions. Certain patients, however, fail to respond in a favorable manner; in these the cord degeneration progresses in spite of persistent treatment. Continued treatment appears advisable, as its interruption in several cases has been followed by aggravation of signs and symptoms referable to the nervous system. In one of their cases after liver was discontinued for a period of four months, the patient

returned with more pronounced neurologic symptoms and signs although the red blood cell count was 5,400,000 and the hemoglobin 105 per cent.

Grinker and Kandel (38) state that liver therapy is not efficacious in improving or preventing degeneration in the central nervous system complicating pernicious anemia. Liver improves the general strength of the patient with combined degeneration of the cord by increasing the number of red cells in the circulating blood or by some obscure direct action. Weakness may closely imitate the effects of damage to the spinal cord. The peripheral nerve complications of this disease consisting of dysesthesia, atrophic changes in the skin, and perhaps other dissociated sensory defects as well as mental symptoms may be alleviated by the recession of the anemia. The majority of cases of combined degeneration of the cord develop rapidly at the onset and then progress slowly, no matter what therapy is employed. Gradual progression of signs may be falsely interpreted as signifying improvement. Combined degeneration develops not infrequently before the anemia and should be diagnosed by the character of the cord syndrome, the achlorhydria, glossitis, and blood smear. Liver therapy in pernicious anemia should be controlled by the height of the blood count. In their opinion

quantities of liver in excess of that necessary to maintain a normal blood level are wasted.

In their own series of cases Grinker and Kandel were unable to discover, even in patients who ingested a pound of liver a day for two years, any improvement in the organic neurologic signs or symptoms.

In discussing the pathology they point out that the entire neuron is destroyed in small foci, often in perivascular areas throughout the cord, but most markedly in the posterior and posterolateral columns. They disagree with the theory that a selective demyelinization takes place; but believe that the whole structure is destroyed, including most of the glial stroma. In their opinion the possibility of such a lesion undergoing improvement is unthinkable. "Gerard and Grinker from work of their own have shown that even in immature mammals under ideal circumstances regeneration cannot take place."

A series of 461 cases of pernicious anemia was studied by Goldhamer (33) to determine the occurrence of neurologic manifestations and to observe the effect of various types of antianemic therapy on them. They found clinical evidence of spinal cord changes in 89.2 per cent of the cases and cerebral symptoms in 64 per cent. Regardless of the type of adequate therapy

improvement in symptoms of the central nervous system was observed in less than 50 per cent of the cases and improvement in signs in about 2 per cent of the cases. Goldhamer concluded from this series of patients that antianemic therapy when given in sufficient amounts does not have a specific curative effect on spinal cord degeneration, but contributes only indirectly to the improvement of the manifestations of the central nervous system. Whereas younger individuals appear to have a better prognosis, sex and duration of the disease do not appear to be significant factors. Great individual variation was noted in the progress of the disease in different individuals, and clinically there are slow and rapid types.

In a histopathologic study of the cords of seventeen cases of pernicious anemia with subacute combined degeneration Davidson (25) found progressive glial changes in the seven cases which were treated with liver. The untreated cases observed prior to the institution of liver treatment failed to show this glial reaction. The myelin sheaths and axis cylinders were not influenced by the liver therapy. Clinically there was apparent improvement in the neurologic signs and symptoms in only two of the seventeen treated patients.

In support of the argument that there is a beneficial effect of liver on cord lesions Mills (50) discusses the effect of therapy upon central nervous system degeneration as observed in forty-five patients under treatment. For comparison the cases are divided into two groups. The cases who received continuous and adequate therapy are compared with the remainder who through apathy, financial, or other reasons failed to receive more than sporadic periods of treatment. He describes adequate therapy as that preventing numerical and particularly morphologic blood changes and preventing the progress of spinal cord degeneration. Of these forty-five cases twenty-eight were adequately treated cases, seventeen were inadequately treated cases. Approximately forty-seven per cent of the adequately treated cases showed improvement in the subacute combined degeneration and an additional forty per cent showed no tendency to progress. On the other hand in the group of inadequately treated only seventeen per cent showed improvement while eighty-two per cent showed progressive degeneration. In the former group only three of twenty-eight cases showed functional disability, while in the second group six are dead, three totally disabled, five partially disabled, and only three admitted feeling physically fit.

The results obtained in one of Mills' cases are as follows: The onset of the disease was in 1924 with severe anemia and incoordination in leg movements. He was treated for tabes in 1930 by a general practitioner because of absent tendon reflexes, a positive Babinski, and a positive Rhomberg. In may 1930, patient was discharged from the hospital on a wheel chair on a diet of raw liver pulp. In July 1930, he reported on crutches, being unable to walk; his blood count was normal. In October he had discarded the crutches and was walking with the aid of a cane and ankle supports; in January 1931, he discarded the cane; by July 1931, he was able to play a few holes of golf; and in September 1932, he walked to and from the office daily, could dance, and play eighteen holes of golf. It is of interest that the knee jerks returned though the positive Babinski persisted.

"The improvement which may occur in cases of subacute combined degeneration of long duration, after six years as in the case quoted, is hard to explain satisfactorily, but the fact remains that it does occur not once or twice but in a considerable proportion of all cases. Not only are the patients subjectively improved and able to perform their daily duties more efficiently, but objectively the concomitant, pathologic signs improve or even disappear. Thus in six cases the

knee jerks returned after having been absent for months or years, and in four cases plantar flexion replaced a Babinski elicited on many occasions by different observers. Such improvement is unexpected and can be explained only on the basis of degeneration of nerve tissue without actual destruction. That such degeneration may persist for long periods and yet eventually respond to therapy is to say the least, surprising; but the fact remains it does occur. The physician should set no limits to the improvement which may occur in the disease if its victim possesses courage and a determination to get well." (50)

After experience with six hundred cases Sturgis (77) states that cord changes were often found strikingly improved subjectively, but objective evidences of improvement occurred only in a small percentage. He reports a case with striking improvement following treatment: A woman, sixty-one years of age with a red blood count of 1,000,000, confined to bed with well advanced combined degeneration of the cord, intense infection of the urinary tract, extensive decubitus ulcers, and incontinence of theurine and feces, now walks unassisted and does all her own housework including the family washing. She has been on a maintenance dose for four years.

Murphy (60) also mentions cases that had progressed to the point where they were incapacitated for years with severe spinal cord findings, who following treatment were able economically to support themselves and proceed with their same work and hobbies as before their illness.

Evaluation of the contradictory reports is difficult in view of the fact that they are submitted by men recognized as authorities in the field of hematology. However, there are a few fundamental factors relevant to the spinal cord symptomotology that must be considered before making any conclusions.

These were discussed by Strauss and his associates in 1935.(75) The first of these is the nature of the pathologic lesions. He quotes Fried's summation: "Indeed, the pathologic process, like the clinical picture, passes through a series of nuances from barely demonstrable degenerative changes to complete degeneration of nerve cells and axis cylinders followed by a gliosis." Later he states: "Whereas in viceral organs degenerative cellular changes are often followed by a 'restitutio ad integrum', analagous processes of regeneration of nerve cells or nerve fibers in the central nervous system have never been observed." Regeneration of completely destroyed nerve cells and fibers of the central nervous system following any therapy cannot be

expected in subacute combined degeneration of the spinal cord any more than in tabes dorsalis or paralytic poliomyelitis. It is true, however, that in both the latter conditions, provided renewed injury is prevented, much improvement may be expected over a long period of time through re-education, and because it is true that unaffected cells and fibers may take over functions previously mediated by destroyed pathways. These considerations lead to the conclusion that the effectiveness of therapy on subacute combined degeneration of the spinal cord must be determined not by the degree of improvement but by the evidence of arrest of the process.

Secondly, apparent improvement in the neurologic condition may be entirely due to betterment of general health and strength, re-education and training, and improved circulation and subsidence of edema in the spinal cord. Furthermore, since peripheral nerves are capable of complete regeneration as long as the cell body has not degenerated, signs and symptoms referable to such peripheral nerve injury may completely disappear and lead to the impression that marked regeneration has occurred in the spinal cord.

The third point to be borne in mind is that sepsis has a deleterious effect on many kinds of spinal cord lesions and may inhibit the effect of liver therapy

in pernicious anemia not only on the spinal cord but on the blood. The fact that such patients do not do well is not to be construed as meaning that liver therapy has no effect on the spinal cord but rather that it has no effect on sepsis.

The fourth and most important consideration is the fact that there is no standard dose of liver, stomach or their products, nor is the usual dose adequate for many patients.

Ordway, Gorham, and Isaacs (63) have summarized the more or less accepted treatment of central nervous system changes in pernicious anemia. They believe that adequate dosage of potent material will often arrest and sometimes actually improve serious lesions of subacute combined sclerosis. Occasionally the disease progresses in spite of all therapeutic effort. Secondly, adequate dosage will as a rule prevent the development of cord lesions. They do not advise the confinement of patients with advanced neurologic involvement to bed, for they are likely to become worse. Patients with retention of urine due to cord changes may often be trained to void every three or four hours and hence retain control of the bladder and avoid repeated catheterization and its only too frequent subsequent cystitis. Control of defecation is more difficult to regain, but careful

attention to regularity of bowel movements is helpful. Great precaution in avoiding bed-sores is necessary as in any disease of the nervous system in which pressure, incontinence, infection and possible trophic influences are troublesome factors. However, large and deep ulcers may heal by the usual attention to relief of pressure and infection, meticulous cleanliness, mild antisepsis and exposure to air and light, daylight or ultraviolet light. Appropriate exercises for those cases with signs of persistent cord degeneration should be recommended as soon as the patient is able to be up and about. Re-education is just as important and valuable as in locomotor ataxia. Passive and active exercise, massage and dry heat are helpful.

CHANGES IN BLOOD AFTER LIVER TREATMENT

The most significant and earliest change which occurs in the blood following treatment with liver, liver extract, or other potent preparations is an increase in the number of reticulocytes. The onset, duration, course, and amount of the reticulocyte reaction, according to Minot and Castle (52), depend particularly upon five factors.

(1) The initial red blood cell level. - If a large uniform daily dose of active

material is given to each of several patients with pernicious anemia an inverse relationship between the peak value of the reticulocytes and the initial red cell count is observed. In patients with over 3,000,000 red cells per cu. mm. the reticulocyte responses become variable and may not appear despite a significant effect upon the total red cell count. It is probable that under these circumstances the demand of the anemia upon the bone marrow is not sufficient to cause delivery of immature cells into the blood stream, or that the proliferation of the primitive red cells in the bone marrow is not sufficient to provide a large amount of tissue for rapid maturation.

(2) The amount of active material given.- If to each of several patients with pernicious anemia and the same initial red cell level, various amounts of effective material are administered in uniform daily dosage, it appears that there is a direct relationship between the amount of material given and the peak of the reticulocyte value. This effect approaches a maximum, however, at a decreasing rate as the amount of active material is increased.

(3) The portal of entry to the body.- The effectiveness of liver extract when rectally administered is very slight compared with its activity upon oral administration. Likewise, if the same amount of a given active material is administered in uniform daily doses by mouth and by intramuscular injection in patients with comparable degrees of anemia striking differences in the effectiveness of the material are observed. The most accurate comparisons have been made between the effectiveness of the daily oral administration of the liver fraction "G" of Cohn and of the daily intramuscular injection of a dilute

aqueous solution of the same material. Although the data are scanty, it is evident that the effect of the daily oral administration of this extract derived from 600 gm. of liver is usually no greater than the effect of the daily parenteral administration of the same material derived from 10 gm. of liver.

(4) The rate at which the material enters the body.- In general the effect of a single massive dose of orally or parenterally administered material is to accelerate the appearance of increased numbers of reticulocytes in the blood stream and to increase the peak value. The tendency for large uniform daily doses of material to cause earlier reticulocyte responses than smaller doses also acts in a similar way. On the contrary, the daily administration of small doses of active principle tends to produce a prolonged low curve with a delayed and not very acute apex.

(5) The reactive state of the bone marrow.- It is necessary to recognize the fact that certain patients are more or less able than the average subject to develop a reticulocyte response. Thus patients, whose initial level of reticulocytes is in the vicinity of 1 to 8 instead of the usual 1 to 3 per cent, may be expected to respond more readily but to give less marked increases at the peak. In general, older persons and those with arteriosclerosis respond less readily than younger ones. Extensive neurological involvement is often associated with slightly lower reticulocyte responses. Severe damage to the vital organs, such as the liver, or infection of a variety of types, may greatly inhibit reticulocyte responses.

These factors brought out by Minot and Castle are supported by results obtained by various men in their

treatment of patients. In a case of Morris's (55), age sixty-five, who entered the hospital with an initial red cell count of 1,400,000 and a reticulocyte count of 0.6 to 1.3 per cent, the reticulocytes started to increase in number the following day reaching a peak of 43.1 per cent on the sixth day, and in somewhat over three months, the red cell count was 4,500,000 and the hemoglobin was 93 per cent. This response followed a single dose of 5 c.c. of concentrated gastric juice from swine. By the intravenous route still higher and more rapid responses were noted (34), a maximum reticulocyte response of 52.8 per cent in 88 hours was obtained in one case, and 49.5 per cent in 108 hours in a second case. However, in these cases the initial red cell counts were low.

In the average patient treated by the intramuscular route the reticulocytes are increased in forty-eight hours and reach the peak in four to seven days. During this time they may rise from less than 1 per cent to as high as 50 per cent.(46) By the oral route the response occurs later, the reticulocytes rising to 5 or 6 per cent within from three to five days, reaching a maximum of from 10 to 30 per cent in about ten days and falling again by the fifteenth to the twentieth day. Responses up to 50 per cent have been obtained by the oral

administration of liver extract. After the initial rise, the number of reticulocytes falls rapidly, so that within two to three weeks it reaches about 2 per cent and then steadily falls to the normal level of 1 per cent or less. (24,63)

The red blood cells show a prompt rise, in some instances reaching a level of 4,000,000 per cu. mm. within a month, although there is a difference in the individual cases as to the rate of increase. The increase ranges between 50,000 and 125,000 cells per cu. mm. per day and usually there is complete restoration of the cellular values to normal within two to three months. Examination of the blood shows gradual disappearance of the atypical microcytes, and macrocytes, the bizarre forms, and other evidences of the anemic state, and after four to six weeks the picture on the stained smear looks fairly normal. The hemoglobin percentage tends to rise somewhat more slowly, and as a result the color index falls to 1 or slightly below. (24,46,63)

The icterus index, which ranges from 6 to 20 in the average cases (64) but may rise as high as 40, begins to fall at the end of the first week of treatment and usually reaches the normal value of 4 to 6 within from three to four weeks. The fall of the icterus index

is accompanied by the disappearance of the peculiar yellow color of the patient's skin.(63)

The morphology of the red cells gradually returns to normal as the macrocytes, microcytes, poikilocytes and polychromatophilia disappear. In a typical case of pernicious anemia the progressive decrease in the mean corpuscular volume and the mean corpuscular hemoglobin may be followed as the red count and hemoglobin percentage rise.(63)

Accompanying the increase of the red blood cells, the white blood count rises, polymorphonuclear leukocytes and eosinophiles appearing in great numbers. The eosinophiles may rise to 20 to 40 per cent after one month of liver diet. "Goldhamer has found no constant eosinophilia developing after the use of desiccated stomach preparations." The platelet count also increases to normal figures.(63) Murphy (57) obtained a prompt increase in the number of white blood cells and blood platelets within a few hours of the beginning of parenteral treatment and a continuance of a normal or slightly elevated level during the course of treatment.

EXPLANATION OF FAILURE OF LIVER TREATMENT

It is well recognized that an occasional person is refractory to the use of adequate amounts of liver extract.

In such cases many factors must be considered as a possible cause. (24,63)

1. Infectious processes and other diseases as arteriosclerosis, diabetes, hyperthyroidism and more frequently hypothyroidism, and cancer occasionally occur, and may seriously impair the effectiveness of liver administration. About 20 per cent of cases have gall bladder infection or disorder (89).

2. The giving of insufficient amounts of liver or its extract may be the cause of failure to obtain satisfactory results. The importance of insisting upon the administration of the optimal amount of potent material to each individual case cannot be over-emphasized.

3. Failures may occur in cases incorrectly diagnosed as pernicious anemia. The pain, weakness and other symptoms of rheumatoid arthritis have lead to a mistaken diagnosis of subacute combined degeneration of the cord. Hyperchromic megalocytic anemia is sometimes associated with myxedema and when thyroid is prescribed the anemia will disappear (82). Sharpe and Bisgard (72) have recently reported the development of a macrocytic anemia following complete thyroidectomy in rabbits.

4. The use of impotent liver extrats will give a poor response to treatment. Only those of proven

efficiency and prepared under the most strict and careful supervision should be employed.

5. Response to liver diet has been reported unsatisfactory following multiple transfusions. The suggestion is made that perhaps the bone marrow has in some way been injured or rendered inactive by numerous transfusions of blood. There are, however, many examples which demonstrate that in spite of multiple transfusions, the patients have responded in a satisfactory manner to liver treatment.

6. Certain cases apparently do not attain a satisfactory level of red blood cells and hemoglobin until large doses of iron are given in addition to liver.

7. In the very exceptional case there apparently occurs a failure which cannot be explained with entire satisfaction. It may possibly be due to a failure of absorption or to a lack of reactive power on the part of the bone marrow. These instances are so exceptionally rare, that one should not conclude that he is dealing with such a case until months of persistent use of a potent liver extract by the intramuscular route has been diligently tried.

MAINTENANCE DOSAGE

An important feature of the treatment and control

of the patient is the establishment and regulation of the amount of liver necessary to maintain him free from symptoms and hematologic changes. This can be done only by the trial and error method. After the patient has been restored to normal from a state of relapse and is in complete remission, the amount of liver required is not considerable. However, if this treatment is given up, or if too small an amount of liver is given, a relapse invariably will follow. (46,63) Therefore, to succeed the patient must appreciate that therapy controls and does not eliminate the disease, and because of this, treatment and observation by the physician must be carried on throughout the remainder of the patient's life. It must also be remembered that the amount of liver or liver substance required to keep the blood at a satisfactory level varies in every case, and it is essential that each individual's requirements be ascertained by examining the blood at intervals. (31,24) In the case of relapse, first the hemoglobin and red cells decrease, and unless active treatment is resumed, symptoms of the disease return. Therefore, for the purpose of determining the maintenance dose, the patient should report at monthly intervals for hemoglobin estimation and a complete blood count, so that this point may be established individually for each case. At.

the same time careful notes may be made as to the presence of any symptoms or physical signs which might possibly indicate the approach of a relapse. When the patient's symptoms have disappeared, and the red blood count is normal, usually in two to four months, a reduction in the dosage may be permitted.(63)

This reduction in treatment may be done in two ways, by decreasing the amount of each dose, or by increasing the interval between doses. The first decrease advised is from one-half pound daily to one-half pound five times a week, or from the extract of 400 gm. of liver to that of 300 gm. A further reduction may be made in the intake to one-half pound of liver three times a week or to the extract of 200 gm. of liver daily, which is, as a rule, the minimum. The great danger is the giving of too little rather than too much potent material. The exact dosage must be determined for each individual case, as stated above. When the intramuscular method is used it is customary gradually to increase the time interval between injections by two to three days. The period between is found to vary from one to six weeks. In more recent reports we find that in the average patient concentrated liver extract, given at weekly intervals, was sufficient to maintain a remission (28,60) while others have been just as successful with

injections at three to four week intervals.(46,77,84,89) Murphy (60) stresses the importance of the use of concentrated liver extracts in that both inconvenience and expense are spared the patient. He obtained the same results with the injection of concentrated liver extracts at three to four week intervals as he did with the injection of weaker extracts daily, but the expense of the former course of treatment was obtained at a cost as low as fifteen dollars per year, whereas in the latter course of treatment the cost ranged between four hundred and five hundred dollars per year.

At the recommended monthly follow-up visits, it is important to obtain data concerning the relief or prevention of the more severe neurological symptoms and signs, due to involvement of the spinal cord. There has been considerable doubt regarding the effectiveness of liver treatment upon neurological symptoms, but evidence is accumulating to show that large doses of potent material over long periods are often of definite value.(63)

The importance of a proper maintenance dose and the value of the intramuscular method of administering liver extract can be most effectively illustrated by a case report, given in the Oxford Monographs on Diagnosis and Treatment (63), which was followed for a period of seven years. The patient, age 42, was first seen in

November, 1927. A diagnosis of pernicious anemia was made, the red cell count being 1,400,000. A rapid response followed with the use of liver extract by mouth, the count reaching 5,800,000. The patient then reduced the intake of liver extract to one-half and then gave up the use of liver extract entirely because of the expense, with a resultant sharp fall in the red cell count to 4,800,000. Raw liver 85 to 100 gm. was substituted for the liver extract but the count continued to drop to below 4,000,000 and a sore tongue developed. With the level of red cells approaching 3,000,000 the raw liver intake was doubled, but numbness of the legs, loss of vibration sense, difficulty in walking, and a positive Babinski reaction developed, nevertheless, in January, 1933. At this time liver extract (3 c.c. representing 100 gm. of liver) was given intramuscularly every four to seven days with remarkable improvement in neurological symptoms, the red count rising sharply to 5,500,000. This patient could not be made to take sufficient liver or extract by mouth or to report regularly for financial reasons. When the seriousness of her cord lesion became apparent, she was convinced of the necessity of proper treatment and since has been maintained in good health by weekly intramuscular injections of liver extract.

The development of infection, arteriosclerosis, or any of the other diseases previously mentioned should be watched for and the dose of liver extract increased. (46,63,71,89)

TRANSFUSION

Transfusion, as a curative measure, has consistently failed, since the rejuvenation of blood by repeated transfusion never alters the prime pathological cause, namely, abnormal blood formation in the bone-marrow. As a temporary measure, however, transfusion was in the past and still is, a very valuable procedure. If early diagnosis and efficient liver therapy were carried out in every case of pernicious anemia, transfusion would cease to have a place in our armamentarium, but unfortunately this desirable state of affairs is far from being attained at present. The question whether transfusion of blood should be carried out or not is often a difficult one. The answer must depend on the clinical state of the patient more than on the degree of anemia present. Cases have been seen with counts of 700,000 red cells per cu. mm. that responded to liver therapy at once, while others with a count of 1,000,000 or more would have died before the liver extract could have acted. (24) There is, therefore, a restricted group of cases where it may be indicated as a single immediate life-saving measure.

We refer to those cases in severe relapse in whom death is imminent, because of nausea, vomiting, diarrhea, and stupor, with the red cells often reduced far below 1,000,000 per cu. mm. Under such circumstances, transfusion with 500 c.c. of carefully matched blood may be indicated as a temporary measure until intramuscular injections of liver extract have had time to act. (24,46,63,74)

Davidson and Gulland in 1930 (24) advocated the combined use of transfusion and liver therapy in cases with a red cell count under 1,000,000. With this combined treatment they obtained reticulocyte responses as high as 52 per cent, whereas with liver therapy alone they rarely obtained a retic ulocyte response over 20 per cent. However, their treatment consisted only of whole liver and liver extract by mouth. Because of this one can readily see that in a patient in severe relapse with gastrointestinal disturbances, the physician would have poor co-operation on the part of the patient in administering enough potent substance to obtain a significant response. Transfusion under such conditions would probably be of some value. However, at the present time with the highly concentrated preparations and by intramuscular injection one can obviate these difficulties and obtain a good response within twenty-four to forty-eight

hours with reticulocyte peaks of forty to fifty per cent. (46,63)

Minot and his associates (53) are of the opinion that the addition of blood by transfusion adds an extra burden to the bone marrow and renders its response to liver feeding much less satisfactory.

It is obvious, therefore, from the above data that transfusion is indicated only as an emergency measure in severe relapse, and excepting for such cases is entirely unnecessary.

REST

When the patient first comes under medical care, which usually is during a relapse, absolute rest in bed and freedom from all responsibilities are indicated. During severe exacerbation it is, of course, obvious that rest in bed is necessary, yet many patients are slow to realize this, even though the degree of anemia present may be so severe as to cause dyspnea, palpitation and marked dizziness. In such cases insecurity of gait and station may lead to injuries from falling. The utmost precaution must be taken to prevent such accidents. Since fever often is present, and the hemoglobin and red cells are markedly lowered, the rest treatment should be continued until the temperature falls to normal, and

until the hemoglobin and red cells show a pronounced rise. When the hemoglobin is approximately 60 per cent and the red cells are about 3,000,000 per cu. mm., the patient is allowed to sit up and gradually get about, but the increase in physical activity should be held well within the limits of fatigue.(63)

HYDROCLORIC ACID

Before the advent of liver therapy the use of hydrochloric acid was recommended by many in the treatment of pernicious anemia. Hurst's (44) claims regarding its gastric antiseptic properties have greatly increased its therapeutic use. In 1923 he reported the presence of streptococcus longus in the duodenal contents of nine patients with pernicious anemia and in four cases of subacute combined degeneration of the cord, while in four normal individuals and thirty-seven patients suffering from other diseases streptococcus longus was found only in four cases. He concluded from this that infection by the streptococcus longus is an essential factor in production of pernicious anemia. Hurst, therefore, treated patients from the standpoint of removing the infection. Since the achlorhydria is the consistent finding in these cases he recommended the use of hydrochloric acid for treatment, with the removal of

foci of infection especially if present in the teeth and tonsils which may be the source of the organism. They treated their cases with a dram and a half of dilute hydrochloric acid diluted in four ounces of water to be taken during the course of each meal.

Pitzman (67) reported persistent remissions in cases treated with relatively intensive hydrochloric acid treatment. He administered 15 to 30 drops of hydrochloric acid in water at half hour intervals for a total of 3 to 5 doses after each meal. He noted immediate improvement of the digestion of all non-moribund cases and progressive improvement of the blood picture and all other minor signs and symptoms.

No definite explanation for the response to hydrochloric acid has been given other than a general improvement in gastrointestinal symptoms and an increased appetite. Gastrointestinal symptoms, even when severe, have been found to clear up promptly under proper liver therapy without hydrochloric acid or other drugs.(63) However, there are many, at the present time, who recommend the use of large doses of hydrochloric acid, two to four cubic centimeters well diluted in water or fruit juices with each meal for the relief of diarrhea and other gastrointestinal symptoms.(46,51,77)

ARSENIC

Before the advent of liver therapy arsenic was advocated in the treatment of pernicious anemia because of its stimulating effect on the bone marrow. The best results were obtained by giving arsenic in solution either as Fowler's solution or as the hydrochloric solution. The dose was regulated by the individual tolerance, and it was found that those who could take larger doses had a better prognosis. Under such a regime a fair proportion of cases responded well, but eventually a relapse occurred, and with each succeeding relapse the beneficial action of the drug was correspondingly decreased.(24) Piney (66) found, in addition to the increased reticulocytes in the circulation following arsenic, that the amount of hemolysis was also reduced, as evidenced by the disappearance of bilirubin from the blood serum. However, he is of the opinion that the hematopoiesis is not due to a stimulation of the bone marrow but that it is possibly due to the stimulation of the megaloblastic tissue in the liver.

In regard to the reticulocyte response, Minot and Castle (52) noted that the reticulocyte curve obtained with arsenic is different from that obtained with liver; that the reticulocytes do not undergo a progressive alteration toward more mature forms but

continue to be the same; that the total red cell count does not increase appreciably or may rise only after a period of two to four weeks; and finally that the administration of liver extract will produce a typical reticulocyte response as soon as the response to arsenic has subsided. It is their opinion, therefore, that arsenic is more or less an indirect stimulus to hematopoiesis, rather than a replacement of a specific nutritional substance necessary for bone marrow stimulation.

It has been mentioned that arsenic may be a blood irritant, and that it may even produce an aplastic type of anemia. Its prolonged use may cause symptoms closely simulating true pernicious anemia, and the resulting anemia and the pigmentation may be very confusing. (63) Since the administration of liver substance or extract is followed by the complete maturation of the bone marrow cells, and since it is so much more certain and satisfactory, arsenic should be discarded in the treatment of pernicious anemia. (46,63)

IRON

The use of iron is usually unnecessary in the treatment of pernicious anemia. The body is already "saturated" with iron-containing pigment in the form of hemosiderin. (24,63) The use of potent hematopoietic

material in the form of liver extract is usually sufficient for the restoration of the red cells, but the rate of cell production may become so rapid that there will develop the so called "hemoglobin lag" with the color index falling below one due evidently, in spite of the fact mentioned above, to a temporary iron deficiency. In such instances it is necessary to give suitable iron preparations to avoid a transient hemoglobin deficiency and a resulting hypochromic type of anemia. Preparations that are commonly used are iron ammonium citrate and reduced iron in daily doses of 1.5 to 2 gm. The use of iron may be discontinued after the red cell count and hemoglobin have reached the normal level.(46,60,77,82,84,89) Fouts (28) noted in a few cases that red cell counts above 3,500,000 and 4,000,000 could not be obtained without the addition of an iron preparation. It was also found that clinical improvement in patients whose red cell counts had reached a level of about 4,500,000 and did not rise higher was often striking if iron was then given in addition to liver.(82) Murphy (58) believes that all patients should receive maximum doses of iron as well as liver treatment from the onset, and claims that if this is done there is a greater and more rapid improvement in both red cells and hemoglobin.

SPLENECTOMY

Removal of the spleen in cases of pernicious anemia, a dangerous procedure and never of proven value, is now only of historical interest. Before the advent of liver therapy such operations were advocated on the supposition that over-activity of the spleen was a factor in the pathogenesis of the disease, manifesting itself by the development of a hemolytic toxin and active endothelial cell phagocytosis of red blood cells. The establishment of the fact that pernicious anemia is a deficiency disease, and the uniformly splendid results of liver therapy have made further consideration of splenectomy unnecessary. (63)

SURGICAL PROCEDURES IN PERNICIOUS ANEMIA

The only surgical procedures which should be undertaken are those necessary for the eradication of septic foci. These should be carried out as efficiently and completely as possible, and at the earliest opportunity. However, under no condition should any surgical procedure take place until liver therapy has been given a thorough trial. Although, in the majority of cases, septic foci definitely retard remissions, large doses of liver or liver extract will elicit a response. Some react slowly and only to a moderate degree, while others, in spite of

marked sepsis, respond in a miraculous way. Since all, however, respond to some degree, surgical treatment should be delayed until treatment has been administered.(24)

In thirty-two patients operated upon Hahn (39) obtained the impression that surgical operations have a definite tendency to precipitate or increase the development of neurologic symptoms. Therefore, he advocates intensive liver therapy before and after operation. He is of the opinion that patients with pernicious anemia treated intensively before and after operation not only tolerate operations well but are good surgical risks.

SUMMARY

There is general agreement that the normal maturation of red cells is governed by the action of a so-called hematopoietic factor, sometimes called the "antianemic factor". The absence or deficiency of this factor in the bone marrow results in the arrest of maturation of erythrocytes at the megaloblastic level. There is further agreement that the hematopoietic factor is formed by the union or interaction of a substance present in the diet known as the extrinsic factor, with a principle produced by the glands of the normal stomach and upper duodenum, known as the intrinsic factor. After formation of the hematopoietic factor in the stomach, it is supposed to be absorbed, then stored in the liver, kidneys, and other tissues. Presumably it is released by these tissues and utilized by the bone marrow for production of sufficient number of erythrocytes to maintain the normal level.

In regard to the treatment the administration of adequate amounts of liver or potent liver extract, particularly by the intramuscular route, if persistently and continuously followed, seems to be a specific for pernicious anemia. If there is careful instruction of the patient, intelligent follow-up treatment and co-operation of the patient with his physician, in each

instance the symptomatic cure may, in a great majority of cases, become complete.

The improvement of the patient's condition may be followed by periodic observation of clinical changes, and changes in the blood. A correct diagnosis before starting the treatment, and the removal of foci of infection are important factors in the successful treatment of the pernicious anemia patient. The use of transfusion in patients in severe relapse, in whom death is imminent may be indicated as a temporary measure until intramuscular injections of liver extract have had time to act. Hydrochloric acid is advocated by many for the relief of diarrhea and other gastrointestinal symptoms. Also iron should be administered in cases where the increase in hemoglobin percentage tends to lag behind the increase in the number of red cells. The use of arsenic and splenectomy no longer has a place in the treatment of pernicious anemia.

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