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# Pernicious anemia, particularly its etiology and treatment

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# PERNICIOUS ANEMIA,
# PARTICULARLY ITS
# ETIOLOGY AND TREATMENT

by

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I

1. Historical

2. Erythrogenesis

3. Pigment metabolism

In 1849 and again in 1855, as part of his work " On the Constitutional and Local Effects of Disease of the Supra-renal Capsules," Addison (1) described what we now know as Pernicious Anemia. Although Combe(15), in 1822, had reported a case that we can now recognize as probably the same disease, the former is usually credited with the first description.

In 1855, he writes (Quoted from (31)) :-

" For a long period I had from time to time met with a very remarkable form of general anaemia, occurring without any discoverable cause whatever; cases in which there had been no previous loss of blood, no exhausting diarrhoea, no chlorosis, no purpura, no renal, splenic, miasmatic, glandular, strumous, or malignant disease. Accordingly, in speaking of this form of anaemia in clinical lecture, I, perhaps with little propriety, applied to it the term 'idiopathic', to distinguish it from cases in which there existed more or less evidence of some of the usual causes or concomitants of the anaemic state.

" The disease presented in every instance the same general character, pursued a similar course, and, with scarcely a single exception, was followed, after a variable period, by the same fatal result. It occurs in both, sexes, generally, but not exclusively, beyond the middle period of life, and so far as I at present know, chiefly in persons of a somewhat large and bulky frame, and with a strongly-marked tendency to the formation of fat. It makes its approach in so slow and insidious a manner, that the patient can hardly fix a date to his earliest feeling of that languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted; the pulse perhaps large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement; there is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breath-

lessness on attempting it; the heart is readily made to palpate; the whole surface of the body presents a blanched, smooth and waxy appearance; the lips, gums and tongue seem bloodless; the flabbiness of the solids increases; the appetite fails; extreme languor and faintness supervene, breathlessness and palpitations being produced by the most trifling exertion or emotion; some slight oedema is probably perceived about the ankles; the debility becomes extreme, the patient can no longer rise from his bed, the mind occasionally wanders, he falls into a prostrate and half-torpid state, and at length expires; nevertheless, to the very last, and after a sickness of perhaps several months' duration, the bulkiness of the general frame and the amount of obesity often present a most striking contrast to the failure and exhaustion observable in every other respect.

" With, perhaps, a single exception, the disease, in my own experience, resisted all remedial efforts, and sooner or later terminated fatally. On examining the bodies of such patients after death, I have failed to discover any organic cause of such serious consequences. "

As can be seen, this description is so complete clinically that it may well have been written today. The only serious differences would be found in the failure to note the characteristic remissions and relapses and the symptoms arising from the pathology in the central nervous system. It is to be noted that he termed it an idiopathic anemia.

Apparently quite independently, Biermer(4) of Zurich, in 1867 and also in 1872, in his article " Form von progressiver, perniciöser Anämie mit Verfettungsvoängen in den Circulationswegen " described the same condition. But under his title he included not only this disease, but others which are now considered as being of a secondary nature, as well.

One other item of historical interest might be mentioned, and that is the discovery, by Ehrlich, in 1881, of the value of aniline dyes in the

staining of blood films. This became of value not only as an aid in the diagnosis of this disease, but also in the study of hematology in general.

The scope of this paper does not permit of discussion of the very interesting historical developements and controversies that followed these descriptions, except in so far as they may enter into the subsequent presentation.

Before proceeding, however, with the consideration of this disease, it should be stated etiology and treatment are the subjects that will mainly be dealt with. The other phases, symptomatology, clinical and laboratory findings, differential diagnosis, and diagnosis, will be mentioned only briefly, merely to give unity to the description of the disease.

Two matters fundamentally involved in this condition will be touched upon, before entering into the discussion proper. These are erythrogenesis and bile pigment metabolism. The questions concerning the place and mode of origin of the erythrocyte, of whether one should accept the Monophyletic Theory of the origin of the cells of the blood or not, do not concern us for the moment. The sequence of the maturation cycle is clear and only such phases will be mentioned as will enter into the treatment of the problem before us. The erythroblast, of endothelial origin, is the parent cell. It is a large cell with a deeply basophilic cytoplasm and a large nucleus. Successive generations of these cells become smaller and take up more hemoglobin, the nucleus becoming more dense and smaller as well. The stage of the normoblast is finally reached. At this stage, we have a cell containing a pycnotic nucleus with a solid mass of chromatin material and a cytoplasm like that of the mature erythrocyte. This cell now loses it nucleus, by extrusion or, more probably, by solution, and we finally have the mature erythrocyte. The nucleated cells are normally not found in the peripheral circulation, but exist only in the bone marrow. Only the non-nucleated mature erythrocytes are normally found in the peripheral circulation.

The megaloblast, a more primitive cell, belongs primarily to the bone marrow of embryonic life. The bone marrow of the normal adult contains only a few of these cells. Their presence here in large numbers as well as in the circulating blood is thought to be an indication of a degenerative process in the marrow.

The normal erythrocyte is thought to be acidophilic and homogeneous in its staining characteristics. The use of a so-called "vital" staining technic, however, will show that about one or two percent of normal cells have a basophilic reticulated structure. These are called reticulocytes. The presence of this skein of granular filaments may be considered as indicative of the maturity of the cells. As they become more mature, this basophilic material is, presumably, gradually lost. The greater the number of less mature cells in the circulation, the greater, therefore, will be the number of reticulocytes, or rather, the proportion of reticulocytes, found. This would suggest an increased production of erythrocytes by, or their more rapid liberation from, the bone marrow, or both.

It was previously thought that bilirubin was formed solely in the liver. The concensus of opinion of more recent investigators is, however, that this pigment is formed in the cells of the "reticulo-endothelial" system in general. Hemoglobin is broken up by these cells into hematin and the protein globin. Following this, the hematin is converted into bilirubin and iron compounds. The latter become available, at once or eventually, for the formation of new hemoglobin. The bilirubin is extracted from the blood by the liver cells and excreted by the bile capillaries as a constituent of the bile. We know, further, that this substance is changed in some way by this passage through the liver cells, since the Van den Bergh test enables us to distinguish bilirubin that has passed through the liver cells from that which has not.

## II

1. Symptoms

2. Findings

3. Pathology

Pernicious anemia may be defined as a recurring anemia caused, probably, by a deficiency of some specific substances or substances and characterized essentially by an embryonic type of hematopoiesis.

The complaints and findings can, perhaps, be best given in outline form.

Complaints:-

1. Insidious onset.
2. Pallor.
3. Loss of appetite.
4. Distaste for certain foods.
5. "Indigestion".
6. Nausea and vomiting, occasionally.
7. Sore tongue or mouth.
8. Weakness.
9. Dyspnea on exertion.
10. Faintness.
11. Palpitation.
12. Cardiac pain.
13. Amenorrhea in women.
14. Spastic or flaccid paralysis.
15. Tinnitus.
16. Dimness of vision or light flashes.
17. Loss of smell and taste sensations.
18. Numbness and tingling, pain, burning, coldness, etc. These start distally in the extremities and travel upward.
19. Loss of sensation, first of muscle sense and sense of position, then pain and temperature, and finally touch. These also start distally in the extremities and travel upward.

Findings:-

1. Pallor.
2. Peculiar icteric tint.
3. Slight edema.
4. Smooth, clean tongue.
5. Glossitis.
6. Stomatitis.
7. Hemic murmurs.
8. Rapid pulse, particularly on exertion.
9. Low blood pressure.
10. Enlarged heart, if there has been strain.
11. Paresthesias.
12. Loss of sensation (see 19 above).
13. Achlorhydria.
14. Blood changes.
15. Mental changes?

15. Motor changes. These may be either spastic or flaccid in type. There may be impairment, or loss, of control of both sphincters.

With regard to symptoms involving the central nervous system, it may be stated that they may be absent, slight, or severe. They may precede or accompany the anemia. They may continue unchecked by suitable therapy or they may even develope in a case under treatment. It should be borne in mind that paresthesias may accompany any severe anemia and their presence in 80% of cases of pernicious anemia is probably due to the anemia and the cardiac weakness and not to any cord lesion in many instances. They usually pass away as the anemia improves. The psychoses that, at times, accompany the disease are claimed by some to be due to the pernicious anemia and by others to be merely coincidental affairs (20)(28)(45)(84).

As to the presence of complications, it might be concluded that, since pernicious anemia is usually a disease of later life, such conditions as occur in this period may accompany the anemia. Other diseases may, of course, also be found present. Complications involving infection, such as, for example, influenza or pneumonia, are not well tolerated.

The decrease in the red blood cell count and in the hemoglobin content will vary with the stage of the disease, but the relationship between the two will always be such as to give a color index greater than one. This high color index is not due to a greater concentration of hemoglobin in the cells, but rather to the fact that the cells are larger and, therefore, contain more hemoglobin than could be present in the ordinary cell(64). In other words, the saturation index is about normal. Poikilocytosis and anisocytosis are also present. The curves plotted by Price-Jones (in(20)) indicate this size variation in pernicious anemia as compared to other conditions.

The increase in size of the cells is most commonly indicated, practically, by the high values for the volume index. The megalocytosis is a

most characteristic and essential feature of the disease. The presence of megaloblasts is almost pathognomonic, but they may be hard to find. Study of the more detailed features of the cells reveals a punctate basophilia, reticulation, and nuclear particles such as Cabot ring bodies and Howell-Jolly bodies.

There is also a decrease in the white cell count. Most of this decrease is in the polymorphonuclear cells, so that a relative lymphocytosis is found. The lower the neutrophilic count, the more grave is the prognosis, as this indicates the approach to an aplastic state. There is also, usually, a shift to the right. The platelet count is also decreased.

The Van den Bergh reaction and the bilirubin content of the blood are also important diagnostic points. The former will show the positive delayed reaction indicative of a hemolytic jaundice. The bilirubin content, expressed either in terms of units or milligrams of pigment present or in terms of the icteric index, will be increased, but the amount of the increase will depend on the extent of the hemolysis.

It should, of course, be remembered that some of the symptoms and findings may be explained on the basis of the presence of some other disease. This should carefully be ruled out. Also, one should remember that all of the complaints and findings will not always be present. Enough of them, however, to suggest the diagnosis will be found. Ultimately, the diagnosis will depend on the laboratory findings (20)(60)(63)(64). This is finally confirmed, according to the opinion of some investigators (23)(56), by the response to liver or similar suitable therapy.

The matter of early diagnosis is important. Some borderline cases may be very difficult. As to differential diagnosis, only mention of those conditions that must be considered will be made here. These are:- Carcinoma of the stomach, Addison's disease, Chlorosis, Septic anemias, Chronic hemorrhagic anemias, Bothriocephalus latus infestation, Malaria, Sprue,

Leukemia, Acholuric jaundice, Aplastic anemia, Lymphadenoma. In fact, in such conditions as the fish tape-worm infestation, sprue, pregnancy, the picture is identically that of pernicious anemia and responds, as this does, to liver therapy.

The pathology of the disease can be divided into five groups. These features are associated with, (a) anemia, (b) abnormal blood formation, (c) excessive blood destruction, (d) the gastro-intestinal tract, and (e) the central nervous system.

All tissues, except the spleen and bone marrow and, at times, the voluntary muscles, show a marked pallor. Fatty degeneration is found in all organs, but is best seen in the heart. The bone marrow is soft and a dull red in color. It is in a state of active erythropoiesis, which is megaloblastic in type, and not normoblastic, as in secondary anemias. Leucocytes are present in larger number than would be expected from the leukopenia. The changes in the blood have already been mentioned. There is definite evidence of marked phagocytic activity in the cells of the reticulo-endothelial system. Siderosis is also marked, particularly in the liver, spleen, and kidney.

~~Practically~~ all cases show an achlorhydria. Glossitis is present in about 50% of the cases. The tongue is never coated. Some claim that there is an atrophy of the stomach and intestine, but this is disputed. There is, usually, a great increase in the number of the organisms that are normally found in the gastro-intestinal tract.

The cord is enlarged, due to edema and the lack of condensation resulting from the absence of newly formed neuroglial elements. Microscopically, only the white matter shows patches of degeneration. In the later stages, there may be secondary involvement of the gray matter as well. On section, one sees a ring of degenerated tissue surrounding the gray matter and the absence of neuroglial proliferation.

## III

1. Treatment

2. Etiology

3. Source and Nature of the Active Principle

No space will be given to the earlier attempts at treatment. They were, perhaps, as rational as the knowledge of the disease at any particular period permitted them to be. But they were, in general, unsuccessful. The effective treatment of pernicious anemia began, of course, with the work of Minot and Murphy, and their introduction of the liver diet. One type of diet or another had been previously used by a number of investigators, but none had produced the uniform results that are now obtained. In their first article (53), they call attention to the fact that McCollum had shown that liver and kidney furnish a high quality of protein and enhance the growth of animals. At this point it might be addeded that Goldberger (Quoted more recently by Sharp (90)) has stated that the quality and not the quantity of protein is important in pellagra. It was known too, that liver contained a substance that stimulated cell growth. And last, but not least, they call attention to the work of Whipple and Robscheit-Robbins and their co-workers (82)(83) on the influence of liver on hemoglobin formation in experimental anemias of animals. They thought that food itself may change the intestinal flora and thus decrease the bacterial toxemia, a possible etiological factor that is discussed elsewhere, or it may even influence the formation or destruction of the blood directly.

Without, therefore, any clear cut conception of the etiology, they started out with the use of liver. Their reasons for the dietary attack are best given in outline form, as they appeared in an article (54) published a year after the one mentioned above.

(a) The similarity of certain symptoms and signs of pernicious anemia to those of pellagra, sprue, and beriberi, diseases due to, or associated with, faulty diet.

(b) Patients with pernicious anemia usually have had an abnormal diet, usually containing an excess of fat and a deficiency of red meat. The blood

destroying properties of fats were known.

(c) The geographical distribution of the disease and the correlation of this with habits of diet and environment in different parts of the world.

(d) The knowledge that secondary anemia was occasionally associated with a faulty diet and the recognition of the importance of food in the alleviation of certain types of such anemia in man and animals.

(e) Patients with pernicious anemia were in better health on a balanced, high caloric diet rich in nitrogen.

(f) The nature of the disease.

Although liver was chosen for the reasons just given, it should be stated here that the fundamental work of Whipple and his students merely pointed to the importance of liver in the formation of hemoglobin. In pernicious anemia, the greater need is for stroma. Since this early work, however, Rioch and Robscheit-Robbins (71) have shown that the diets they used contained two factors, one apparently important in connection with hemoglobin formation and the other with the building of stroma.

Following the successful use of the liver diet, attention was turned to the production of an extract that might be used directly, on the assumption that the liver was effective because it contained some substance or substances that were directly the stimulators of the bone marrow. This culminated in the preparation, with the aid of Cohn (12)(13)(14), of the materials that are now available for the treatment of the disease orally, intramuscularly, or intravenously.

The ability to consistently produce remissions by means of these liver preparations enabled those interested to study carefully the changes produced by their administration.

The progress of the treatment is noted during the first two weeks by the change in number or percentage of reticulocytes, and after that by the change in the red cell count and hemoglobin content. These changes have been carefully worked out by Minot and his associates (13)(55)(56). The

percentage and number of reticulocytes at the height of their increase bear an inverse relationship to the initial red blood cell level. A mathematical expression has been worked out which enables them to calculate the expected response to therapy at any particular time. This states that

$$E_p \times R = \frac{E_0 \times R}{1 - R} = 0.73 - 0.2\ E_0$$

in which

$E_0$ = red blood cell level before treatment is begun.

$E_p$ = red blood cell level at the peak of the reticulocytosis.

R = maximum percentage of reticulocytes during treatment.

This expression is based on the assumption that

$$E_p \times R = E_p - E_0$$

and that the increase from $E_0$ to $E_p$ is due to the increment of reticulocytes. This last assumption is not entirely correct, however, since new, non-reticulated cells are undoubtedly produced during this period. As they put it,

" These conditions can only be expected to obtain provided there has been no significant destraction of red blood cells nor change in their concentration as the result of the redistribution of body fluids. In fact, not only must these conditions obtain, but during the length of time that these three equations yield identical results, there can have been no appreciable change of reticulocytes to adult cells in the blood, nor delivery of adult forms from the bone marrow. In the majority of cases, these quantities have not been identical, but their relations to each other are none the less significant. "

In other words, while the results are not always identical in the three equations, the percentage or number of reticulocytes can be estimated. The various processes involved must balance approximately, since any one equation holds with a fair degree of accuracy. This is of some importance, since the equations can be used to determine the efficacy of the treatment and the potency of the preparations used.

These authors have found that the number of reticulocytes at the peak of the increase is roughly proportional to the amount of active material administered, provided that sub-maximal amounts have been given in cases with less than 2,500,000 cells per cu. mm. And furthermore, that there is a direct relationship, up to a certain level, between the rate of increase of erythrocytes and the amount of potent material used. That is, if large amounts are used, both reticulocytes and erythrocytes will increase more rapidly. Ordinarily, the extract from 500 to 600 grams of liver is apparently enough to produce a maximum response in practically all cases. They feel that if the red cell level is less than 2,900,000, the increase in erythrocytes is due to the production of reticulocytes. If it is greater than 3,000,000, it is due to the liberation of mature cells from the bone marrow. Cases that start out with more than 3,000,000 cells at the beginning of treatment, never exhibit more than a slight response as far as reticulocytes are concerned. In all cases, however, adequate treatment causes the red cell level, as well as that of the other blood elements, to rise rapidly to normal.

They point out that the use of small amount of material or the presence of complications may cause only a slight reticulocytosis and that this may be delayed; and that a weak reticulocyte response may not be followed soon by a significant increase in the red cell count. And also, that if treatment is begun when there is a considerable spontaneous increase in reticulocytes, or when this has just occurred, no subsequent rise of these cells may follow.

The reticulocyte response discussed here takes place not only in pernicious anemia, but also in the anemia of pregnancy and some other unusual anemias, such as that associated with the fish tape-worm infestation; but it does not occur in the normal person nor in secondary anemias under the influence of liver therapy. Berglund et al (Quoted in(20)) also confirm the absence of the reticulocytosis in the normal person, although they add

that liver may cause an increase in red cell count to as high as 7,800,000. In view of this, Minot and his associates say that if one is dealing with a definite case of pernicious anemia, with a red cell count below 3,000,000, the absence of a response means that the extract is probably impotent. And, conversely, if the extract is known to be potent, the absence of a response means that there is probably a mistake in diagnosis and that the case is not one of pernicious anemia. Others, for example Fitz-Hugh (23), hold the same idea. The latter states that,

(a) An increase in the uric acid excretion in 48 to 72 hours, followed by a similar rise in the cholesterol content of the plasma;

(b) An increase in the reticulocytes, which reaches it peak within about one week;

(c) The disappearance of the leukopenia, thrombocytopenia, and bilirubinemia;

(d) An increase in the red cell count and hemoglobin percentage and the subsidence of the macrocytosis; and

(e) A return to the normal condition in about six to ten weeks following the institution of liver therapy;

indicate that the diagnosis of pernicious anemia is probably correct. The few exceptions are certain other conditions associated with anemia that give the same response. These are sprue, bothriocephalus latus infestation, and the pernicious anemia of pregnancy.

Not only do we find the characteristic reticulocytosis, but, as already suggested in the preceding paragraph, there are characteristic chemical changes as well. Muller et al (Quoted in(69)) state that there is an increase in both the lecithin and cholesterol content of blood plasma which accompanies the reticulocyte response during the early remission. In addition to this, Riddle (69) calls attention to the fact that the endogenous uric acid metabolism, which is intimately related to blood regeneration,

is also a reasonably reliable index of blood production during the early remission. The rise in concentration of the endogenous uric acid of the blood and its increased excretion in the urine have apparently the same significance as the increase in the number of reticulocytes and are detectable sooner, since the change in metabolism precedes by one or two days the similar changes in the reticulocyte concentration. It is estimated that an increase of 1,000,000 red cells per cu. mm. is accompanied by the excretion of approximately ten grams of uric acid during the early remission.

It is interesting to note that, according to Riddle (69), the rate of increase of the red cell concentration during the reticulocyte response, as well as the rate of liberation of reticulocytes into the blood during this response, both follow the general biologic law of growth and regeneration stated by Robertson (72).

In addition to the responses already indicated, the volume of the cell returns to normal as well. FitzHugh and Persons (22) have shown that the mean red cell diameter drops with the increase in reticulocytes, then rises to almost its original level, remains this way while the red count undergoes an increase of about 1,000,000, and then decreases again to about the normal level as the red count rises to more than 4,500,000 cells.

Accompanying the changes in the blood, there is a corresponding improvement in the clinical condition of the patient. The hydrochloric acid, however, does not return to the stomach, nor is there a change in the intestinal flora. The nervous symptoms may improve or they may not, depending on the severity of the lesion. If they are peripheral, they will improve, according to Baker et al (2), who state that large amounts of liver must be used over long periods of time before the improvement in the neurologic manifestations is noted. If they are of spinal cord origin, their cure by the use of liver, as stated by Fried (25), is impossible, because of the serious character, and the peculiarities of tissue repair of lesions of the central nervous system.

Although pernicious anemia has been questioned as being of etiological importance in the psychoses (28), Läufer (45) reports a case which was cured by the use of liver therapy.

The importance of the continued use of liver therapy has been urged by all those who have been mentioned thus far. It is also emphasized by Gibson and Fowler (27), who state that if it cannot be continued in the diet, a change should be made to one or the other of the various extracts. This is of particular importance because, as Isaacs (33) has pointed out, relapses may occur in one of the systems involved in the disease even during the use of liver. The blood picture, for example, may be normal, but there may be a relapse in one of the other systems. It is very important to maintain the therapy under these circumstances so that this type of abortive relapse may pass. If, let us say, therapy is not maintained during a period of anorexia, a blood relapse may follow. It is, therefore, essential that the use of liver be continued.

Another factor, according to Minot et al (56), is that of the well balanced diet. They state that the increase in hemoglobin is slower in cases treated with liver extract and a diet poor in sources of iron and foods building hemoglobin than in those in which the diet is well balanced and rich in such foods. Minot (57) also stresses the point of full adequate treatment of pernicious anemia. Enough liver should be given to produce the maximum clinical response as well as the return of the blood to a normal level. If there is a change from a liver diet to the use of a liver extract, allowance should be made for the fact that there is a certain loss of active principle in the preparation of the extract. Consequently, he states that if one wishes to administer about 660 grams of liver in the form of the extract, he should actually use an amount of extract equivalent to 1000 grams of liver in order to allow for this loss. In this way, one will not run the risk of inadvertently dropping the liver dosage to a point below the amount needed for adequate treatment.

Experiments have been carried out by Riddle and Sturgis (70) which show that the equivalent of approximately 3000 grams of liver given by mouth in a single dose in the form of Lilly's Extract 343 induces a maximum reticulocyte response which lasts for some period of time. Connery and Goldwater (19) have also used massive doses and state that the magnitude of this prompt response depends on the initial red cell level and on the presence or absence of complications.

Following the preparation of sufficiently pure materials for parenteral use, a number of investigators have reported on the use of liver subcutaneously, intramuscularly, and intravenously. Murphy (61), for example, reports that liver given by these methods produces prompt and striking effects. Strauss and Castle (77) state that the single dose of liver extract, given parenterally, required to produce a maximum effect, varies from a quantity obtained from 20 grams of liver to that obtained from 100 grams. Conner (17), too, states that the injection of an amount of extract obtained from ten to thirty grams of liver produces a similar effect to that produced by much larger quantities given orally. And the effect is produced much more rapidly.

Connery and Goldwater (18) give the following indications for the parenteral use of liver extract:-

(a) Nausea and vomiting which may prevent oral administration.
(b) The patient may be unwilling or unable to cooperate in oral therapy.
(c) Critical illness.
(d) Elimination of the possibility that the patient is not taking enough liver extract or liver in the diet.
(e) Those cases refractory to oral therapy.

Isaacs et al (34) state that the substances causing an undesirable reaction when liver extract is administered intravenously are removable by the use of permutit and acetone. The product obtained by this procedure produces a characteristic reticulocytosis with a peak which is, however, high-

er than that obtained by the use of forty times as much material, given orally, in divided doses, daily. They use the extract of about 100 to 125 grams liver per week until the blood count becomes normal. The extract is then administered once a month in order to maintain the normal level. Proper check-ups are needed to determine the maintenance dosage. The advantages of this procedure are:-

(a) Economy in liver.

(b) Freedom from daily medication.

(c) Non-limitation of the diet.

(d) Knowledge by the doctor that the patient is getting his dose.

(e) The response of some, who do not respond to liver or Ventriculin by mouth, to this procedure.

Freund and Price (24) report a case that responded to the use of juice of whole liver that did not do so to other forms of therapy. Schulten (Quoted in (24)) and Berglund et al (Quoted in(24)) also report cases that did not respond to liver extract but did improve by the use of fresh whole liver. This is contrary to the experience of most authors, who state that cases will respond satisfactorily with any of the accepted methods of therapy. The results reported may have been due to the fact that the liver extract first used built up a sufficient concentration of the active principle and would, ultimately, have shown a response if the treatment had been continued. As it was, after the concentration had been built up, the addition of the whole liver was given credit for the result. Freund and Price also call attention to the fact that as the number of relapses increase, larger and larger doses are needed to maintain the red blood cell level.

It is not necessary to quote from all the investigators who have reported on the efficacy of liver, or of Ventriculin, in the treatment of pernicious anemia. Such articles can be found in the medical literature throughout the entire world.

The various references to the relationship of the stomach to pernicious

anemia and their association embryonically, both being derived from the fore-gut, suggested the possibility of the presence of the active principle in this organ. This led to the introduction, by Sturgis and Isaacs (78), of a desiccated, defatted whole stomach of hog, preparation, now known as Ventriculin. Ventriculin produces the same characteristic remissions as are produced by liver in any of its forms. Some claim that it has certain advantages, but the relative merits of liver and Ventriculin will not be discussed here. Presumably, it contains the same active substance found in liver.

Because of an interest in the questions of the source and nature of this active principle, which we may call an hemopoietic hormone, and also because of the value of finding other sources of supply from the medico-economic point of view, it occurred to the writer (3) that other organs, among them the spleen, might be of value in the treatment of the disease. Without going into the details of the earlier literature at present, suffice it to say that spleen had been tried by several investigators, with conflicting results. While the results obtained by us are not conclusive, they, nevertheless, seem to definitely indicate the presence of this hormone in this organ and suggest the possibility of its use as a commercial source. The details of its use will be discussed in connection with the case reports.

Although the pathology of the disease has already been mentioned, it was merely as a resume of the actual findings in the disease and did not include a consideration of the pathological processes involved. This problem, as well as that of etiology, will now be reviewed.

First, let it be stated that such matters as age, sex, geographical distribution of the disease, and constitutional or hereditary factors will not be taken up, because they seem to have but an indirect relationship to the disease. While the disease does seem to distribute itself in a certain fashion with respect to these factors, there is nothing in our present know-

ledge which will permit us to think of anything except some theoretical, indirect connection with pernicious anemia. They may in some way be associated with the cause, but the manner of the association can be only problematical or suggestive.

Nor will time be spent on some of the more unusual conditions associated with a picture of the type found in pernicious anemia. Just what it is in sprue or pregnancy, for example, that sets off a train of events that give us the same kind of anemia, is not known. Rowland (73) states that he thinks the mechanism of hematogenesis is over-strained by the load of the pregnancy or by infections, toxins, or nutritional deficiencies and that pernicious anemia is not a specific disease but a non-specific failure of hemopoiesis due to a variety of causes operating through the common mechanism of a deficiency or disorder of gastric function. In this, he is apparently combining the earlier continental view first expressed by Biermer and supported by many, including Ehrlich, with the more recent concept, propounded by Castle, of the importance of gastric function.

Much has been written on the etiology and pathogenesis of pernicious anemia. The battle has raged, so to speak, from earliest times, between those who have held that the essential factor was the megaloblastic marrow, indicative of a degenerative process, and that hemolysis was a secondary matter; and those who have contended that the essential factor was the hemolysis, due, probably, to the condition of the gastro-intestinal tract, and that the megaloblastic finding was secondary.

For a period of about twenty-five years, William Hunter studied and wrote about pernicious anemia. He held firmly to the view that two factors were fundamental. One was oral sepsis, which led to infection of the gastric and intestinal mucosa. The other was the portal hemolysis. He based his ideas on the presence of the glossitis and gastro-intestinal infection, and the marked siderosis of the liver. He felt that some hemolytic substance was absorbed from the gastro-intestinal tract. This caused the intense por-

tal hemolysis and the resulting siderosis in the liver.

Ehrlich (23), on the other hand, said that the finding of a megaloblastic bone marrow, first reported by Pepper in 1875 and Cohnheim in 1876, was indicative of a degenerative change, and that this was the essential pathological and pathognomonic finding. The real diagnostic criterion, therefore, was to be found in the blood smear. In his monograph, published in 1898 with Lazarus, he deals practically solely with megaloblastic blood formation and dismisses infection and hemolysis briefly.

Practically simultaneously, Hunter, in 1900, discusses blood formation in but a few paragraphs, barely referring to the idea which had, meanwhile, been accepted quite generally on the continent, and devotes practically his whole work to his thesis of gastro-intestinal sepsis and portal hemolysis. In his "Severest Anaemias" (32), published in 1909, he states that this was an intentional omission to show his disapproval of Ehrlich's views on the importance of megaloblastic marrow. He thinks that Biermer's introduction of the term "pernicious" had a great deal to do with the difficulty. To quote from his preface,

" The attention of the profession is invited more especially to the singular history of the erroneous and original identification of Addisonian anaemia with Biermer's "progressive pernicious anemia" - described in chapter X; to the character of the cases termed "pernicious" on which Professor Ehrlich made his first blood observations -- described on page 168; and to the singularly misleading etiological and hematological traditions, which in consequence of these observations, have for the past thirty to forty years gathered around the unhappy and unfortunate name of "pernicious anemia".

He found many adherents who, until at least very recently, have held to his views. Hurst (31), only a few years ago, in 1924, stated that Ehrlich's work " had the important effect of directing attention exclusively to

the blood picture in the diagnosis of the anaemias," but does not even mention megaloblastic marrow in his discussion of the causes of the anemia. He does say, however, that Hunter did important work which resulted in the definite proof that the anemia was caused by excessive blood destruction. Incidentally, he also objects to the term "pernicious" and feels that idiopathic or Addisonian anemia would be better. We find the same influence even more recently. Osler's " Principles and Practice of Medicine " (65), as late as 1925, states in the definition of the disease, " caused by hemolytic agents."

The evidence against the Hunterian concept is well summarized by Davidson and Gulland (20). This is as follows:-

(a) There is no evidence of the existence of intravascular hemolysis.

(b) The high iron content of the liver, kidney, or spleen may be the result of hemolysis anywhere, or of either intravascular or intracellular blood destruction.

(c) No specific hemolytic agent has ever been demonstrated.

(d) The fact that Bothriocephalus latus does contain a hemolysin is no proof that this is absorbed from the intestinal tract. Furthermore, not all anemias associated with this infestation are megaloblastic in type. Also, some cases of anemia in which this parasite has been present will undergo a relapse even after the worm has been removed. And lastly, liver produces remissions in these cases even if the parasite is not expelled.

(e) The injection of hemolytic agents does not produce a true pernicious anemia.

(f) The degree of hemolysis has been overestimated because too much stress has been placed on the importance of the siderosis. This phenomenon can be accounted for on the basis of increased storage due to the decreased demand by the bone marrow for material to make hemoglobin or on the basis of increased hemolysis, or both. The hemolysis is due,

however, not to an hemolysin, but to the fact that the marrow is producing abnormal cells, which are more easily destroyed.

(g) The estimation by Whipple (Quoted in(20))of the amounts of stercobilin excreted in the feces leads him to conclude that the deficient or abnormal blood formation is the essential fact.

As to the importance of the intestinal flora, it should be noted that, although there is a great increase in the number of organisms, no particular type of organism is related to the disease. Also, there is practically no change in their number under the influence of liver therapy; they remain practically as they were during the relapse. And furthermore, the degree of bilirubinemia is not related to the degree of intestinal sepsis at any time.

This does not mean, of course, that those who hold other views deny the existence of an increased hemolysis. They merely state that this is secondary and comes as the result of the fact that there is a disturbance in the process of red cell formation. As Riddle (69) puts it, there is a defect in hematopoiesis which lies in the inability of the primitive cells of the marrow to differentiate normally to the adult state. The hematopoietic tissue, therefore, becomes hyperplastic and crowded with megaloblasts.

According to Heilmeyer (29), pernicious anemia is characterized by a combination of a disturbance in maturation and a blocking of the marrow with an extraordinary increase in hemolysis. Along with a change from normoblastic to megaloblastic type of regeneration, there is a change in the blood pigment metabolism of the cells concerned. A further characteristic of the disease is the liver effect. While liver produces in other hemolytic anemias a simultaneous increase in both blood forming and destroying processes, in Morbus Biermer the hemolytic process is specifically arrested. At the same time the marrow blockade is raised and the disturbance in maturation eliminated. The red blood cells again mature in the marrow to the physiologically mature form before they enter the peripheral blood. The elevation of the red cell count is due to the establishment of normal hem-

atogenesis and raising of the blockade of the marrow as well as by lessening the destruction, which may be considered as having been the result of the abnormal youthfulness of the newly formed cells. Of all anemias, only pernicious anemia shows this characteristic behavior. On these grounds, he thinks it impossible to consider the pathogenesis of this disease, alone or predominantly, from the point of view of hemolysis. Pernicious anemia is not primarily a hemolytic anemia. The most prominant feature is the disturbance in marrow function, which manifests itself by the blocking of the marrow and the disturbance in maturation, by the morphological changes in the cells, and by the changes in the pigment metabolism of these cells.

According to Peabody (65), the myeloid hyperplasia is most marked during the relapse. During the remission, the marrow has a tendency to its normal structure. He finds that during the relapse, the essential histologic lesion is the picture which comes as the result of the rapid and extensive proliferation of the primitive cells, the megaloblasts, with a relatively diminished tendency to the differentiation of mature red blood cells. The marrow shows cellular hyperplasia, but is functionally inefficient. The remission is characterized by the presence of but few megaloblasts and a great relative increase of normoblasts and mature cells in the bone marrow. He explains the anemia of a relapse as being due to a functional ineffectiveness of the bone marrow, resulting from the failure of megaloblasts to differentiate to mature erythrocytes. The blood picture of a remission is explained by the resumption of the more normal type of cell developement, with increased production of normoblasts and erythrocytes. He states that the effect of liver is not to stop hemolysis, but rather to stimulate the maturation of the immature cells crowding the bone marrow. He suggests that this effect may be due to some factor which affects cell metabolism and promotes the developement and differentiation of mature red blood cells. Minot et al (56) also state that the active principle in liver seems particularly to stimulate the formation of red blood cells.

One of the interesting theories of etiology is that advanced by Macht (51), who finds that the blood of the pernicious anemia patient contains a toxin. He tests this by comparing the growth of a plant exposed to normal serum with one exposed to the serum from the pernicious anemia case. This toxin is susceptible to ultra-violet rays, since the blood from the anemia case loses its toxicity when irradiated. Irradiation of patients, which would, presumably, destroy the toxin in their blood, causes a return of the red count and hemoglobin content to normal and an improvement in symptoms. His findings find a certain amount of support in the work of Seyderhelm and Kreitmair (74), who show that ultra-violet irradiation of the blood produces an anti-anemic substance effective in anemias due to intoxication. This substance causes a marked stimulation of erythropoiesis. It was isolated in a solution and used for intramuscular injections. They give it the name " Cytagenin."

Among the earliest attempts at the formation of a theory of the cause of the disease were those which linked the gastro-intestinal tract with the anemia. Even though these may have failed in so far as they led to the assumption of toxins from the digestive system, nevertheless, many authors have pointed to the relationship between pernicious anemia and achlorhydria or the matter of gastric secretion in general. Combe (15), as early as 1822, even though he was not discussing the disease as we now know it, first suggested the pathology in the gastro-intestinal tract as the etiological factor, when he wrote "it is probably owing to some disorder of the digestive and assimilative organs that its characteristic symptoms have their origin."

Hartman, Moynihan, Morawitz, and Poole and Foster are all referred to by Friedenwald and Morrison (26) as having stated that pernicious anemia has followed gastrectomies. Ivy (35) also calls attention to the same fact. Walters (79), however, reports a case in which there is no developement of pernicious anemia two years after a total gastrectomy. And Mann and Graham (52) also state that there is no change in the blood of the dog, after

gastrectomy, which simulates that of pernicious anemia. But, they add that pernicious anemia has never been observed in the dog. A considerable number of gastrectomies, nevertheless, have been reported in which pernicious anemia did develope.

Hurst (32) calls attention to the fact that, although the absence of free hydrochloric acid from the gastric contents of cases of pernicious or Addisonian anaemia was first noted by Martius in 1897, little was thought of it until the past ten or so years. In this past few years, however, many authors have discussed this subject and stated that achlorhydria is practically a constant finding in pernicious anemia. Ivy (35) and Friedenwald and Morrison (26) both also point out that an achylia or achlorhydria always precede the anemia.

All of these associations have led to the developement, by Castle and his co-workers (5)(6)(7)(8)(9), of a most interesting and comprehensive theory as to the etiology of pernicious anemia and its relation to the gastro-intestinal tract. It followed the studies made by Minot and others on the response of the blood to an apparently specific hematopoietuc stimulant, for it was not until this work was published that there were any methods available, except an indefinite wait for ultimate improvement of the condition, to determine the activity of any such substance. And in addition, of course, this work stimulated a great deal of interest in the problem.

On the basis of facts which follow, relating the gastro-intestinal tract to pernicious anemia, he postulates the theory that the anemia is due to the absence of some essential factor from the gastric juice which acts upon or reacts with some other factor in the diet to form the substance important for blood formation. Some of the facts which follow have already been mentioned. The facts are:-

(a) The inability of the patient with pernicious anemia to secrete hydrochloric acid even with histamine stimulation.

(b) The familial nature of the disease and its association in these families with individuals that have either low gastric acidities or a complete absence of acid.

(c) The fact that an achlorhydria precedes for many years the symptoms of pernicious anemia.

(d) The occasional occurrence of pernicious anemia in chronic alcoholics, where it is known that the alcoholism produces a temporary, and in some cases a permanent, achlorhydria.

(e) The association of pernicious anemia with cancer in which there was an achlorhydria.

(f) The association, in a girl, of pernicious anemia with an achylia due to obstruction caused by tuberculous glands about the pylorus.

(g) The occurrence of pernicious anemia in those who have undergone gastrectomies.

Castle, therefore, makes the disease a deficiency disease, something which had already been suggested. But he says that this is a "conditioned deficiency" disease. In other words, the disease is due, not directly to a deficiency of some substance, but to a lack which depends on a deficiency or disturbance of gastric function.

He feels that the possibility of a vitamin deficiency, which will be discussed later, has not sufficient support. Two important arguments that might be brought in questioning his hypothesis, intestinal sepsis and the fact that not all gastrectomies develope pernicious anemia, are answered by calling attention to the fact, first, that the intestinal flora undergoes no change during a remission, a statement previously made, and, second, that some of the total gastrectomies do not develope the disease because they may get sufficient quantities of the hemopoietic stimulant in the diet or because they may not really be total gastrectomies. In connection with the second possibility, he points out that, in view of the fact that the cardiac end of the stomach is richly supplied with glandular tissue, to be considered

"total", the division must be above the cardia. The first possibility finds an adherent in Kern (38), who also feels that some potential pernicious anemias do not develope the disease because their diets contain the hemopoietic stimulant. Deprivation of these foods precipitates the anemia. Kern points out that Germany suffered the greatest food lack after the war and during the financial crash of 1923. Protein food was particularly scarce. And in 1923, the Göttingen clinic had seven times as many pernicious anemia cases as in 1908 and three times as many as in 1926.

In support of his concepts, Castle offers the fact that when individuals with pernicious anemia are fed with raw beef muscle, there is no hematopoietic response as measured by our modern methods. But when these individuals are fed with raw beef muscle which has been incubated in the normal human stomach, a characteristic remission, similar to the one produced by liver, is produced. A number of other substances were found to be also ineffective when merely fed as such. These were gastric juice and splenic pulp. An indifferent protein incubated with normal gastric juice obtained by histamine stimulation was also not active. But beef muscle, incubated with the same sort of gastric juice, did give positive results. Accordingly, therefore, there must be a substance, which he calls the "intrinsic factor", present in the gastric juice. This acts upon a second substance, which he calls the "extrinsic factor", that must be present in the material entering the stomach. The reaction between these two produces the hematopoietic stimulant. The disease, therefore, is a deficiency state in the sense that the absence of the intrinsic factor conditions the deficiency of the material needed for stimulation of the bone marrow.

The intrinsic factor is not the acid itself, nor is it one of the enzymes, pepsin or rennim. He proves this by incubating beef muscle with the gastric juice of two pernicious anemia cases in relapse that is normal in every respect except with regard to the content of this factor and finding that the feeding of the product obtained causes no remission. On the other

he incubates beef muscle with an achylic gastric juice from one patient without an anemia and from three patients suffering from a hyperchromic anemia and finds that this product does cause remissions. So that there is no relation between the intrinsic factor and the substances mentioned.

There is one particular drawback to the complete acceptance of thie entire view. And that is the fact that Morris and those associated with him (58)(59) have reported that a normal gastric juice concentrate, in itself, produces typical remissions when injected intramuscularly. Either, therefore, there is no necessity for both an extrinsic and an intrinsic factor, or the gastric juice, administered parenterally, reacts with something acting as an extrinsic factor that is found in the system. It seems more reasonable to accept the first possibility and assume that some specific substance, of apparently wide distribution, is responsible for the results obtained.

The concept of a deficiency of some sort is not a new one. It was the thought that led Minot and Murphy to the use of liver. It was also held by others who thought, however, that the disease involved a vitamin deficiency. Koessler and his associates (39), in calling attention to the prominence of the gastro-intestinal symptoms as well as to the findings in the blood in pernicious anemia, suggest that, in view of the fact that a vitamin A deficiency leads to intestinal atrophy, it may also produce atrophy of the gastric glands and the resulting achylia. It is known, furthermore, that vitamin A is important in connection with the formation of blood. Blood regeneration cannot take place in the absence of vitamin A. The addition of vitamin A to the diet of an animal whose store of vitamin A has been depleted causes a rapid formation of new blood cells. A properly regulated vitamin A deficiency causes, in animals, a condition similar to human pernicious anemia. The rate and intensity of blood regeneration in these animals is a function of the quantity of vitamin A added to their diets. In 1927, they (40) state the theory that

(a) Changes in the bone marrow, blood, and intestinal tract are due to a chronic deficiency or imbalance of the fat-soluble vitamins.

(b) The lesions and symptoms involving the nervous system are so similar to the ones in pellagra that there may be a relative chronic deficiency of vitamin B.

(c) The tendency to hemorrhage, retinal or otherwise, may be due to a long-standing deficiency in vitamin C.

So they propose the use of a high caloric, rationally balanced diet, rich in vitamins A, B, and C, for the treatment of pernicious anemia. The diets they used did prove effective. Unfortunately, from the standpoint of substantiation of their claims, they contained liver or kidney, or other substances which may contain the active principle assumed to be present by those who favor the view of the existence of a specific hemopoietic stimulant.

Conner (16) also uses a diet based on the presence of a vitamin deficiency and also obtains good results. He too points out the resemblance of pernicious anemia to sprue and pellagra in certain respects and states that liver, contrary to the earlier opinion of Minot, contains both vitamin $B_1$ and $B_2$.

It is interesting to note that these authors obtain remissions with smaller quantities of liver than the ones that Minot and others claim to be necessary.

This brings us to the question of the source and nature of the substance effective in the treatment of pernicious anemia, whether it be the extrinsic or intrinsic factor of Castle or something which is acting directly. There are certain possibilities that present themselves as to the origin of this hormone, if we may call it that. It may be secreted by a certain type of cell. It may be secreted by all cells, which is not very likely, if we accept it as a hormone and consider that hormones are usually thought of as substances secreted by only certain cells. If the former is true, the absorption of the hormone from the blood would account for its presence in various organs. Or, it may not even be a secretory product. It may be some substance which the body can get only from the outside, or else one of the products of cell metabolism in general. One can readily see that, at the present time, the possibilities are rather complicated.

The experiments of many to the contrary notwithstanding, the active principle, as has been suggested by some, seems to have a more or less ubiquitous distribution in the body. Furthermore, it is not limited to mammals. Conner (16) and Jones, Phillips, and Larsell (37) have shown that aqueous extracts of fish livers also contain the material.

Its wide distribution in the body is demonstrated by the reports of many investigators. According to Larsell and Jones et al (36)(37)(41)(42)(43)(44), a hemopoietic stimulant is present in tissues with a high nuclear content in general. They find it in the nucleated red cells of the fowl; in liver, spleen, pancreas, and heart of beef; in calf thymus; and in the kidney, liver, and stomach of the hog.

There are many other reports of its distribution, its presence in organs other than liver. The work of Morris and Schiff et al (58)(59) and that of Sturgis and Isaacs (78) is definite proof of the presence of the principle active in pernicious anemia, in the stomach. As was suggested by Minot, Murphy, and Cohn (loc. cit.) and also according to McCann (49) the stimulant is found in the kidney.

Cheney and Niemand (11) suggest that the intrinsic factor of Castle may be the trypsin of the pancreas. The details of their work and other considerations which cannot be discussed in detail here, however, do not give this much support.

The paper by Seyderhelm and Kreitmair (74) indicates that there may be something in whole blood after ultra-violet irradiation. Although just what the irradiation may do to the blood in this respect is not known.

Charlton (10) thinks that all organs rich in reticulo-endothelial cells contain a substance, which he calls " Reticulin," capable, because of its hemolytic powers, of stimulating blood formation. An extract of this material, obtained from omentum, will cause an increase in the red cell count in pernicious anemia, but not in secondary anemia, as is the case with liver, unless iron is added. It is in this connection that the work of McMaster and Haessler (50) is significant. These observers mention the fact that others have noted that blood regeneration takes place less rapidly after hemorrhage than it does when a destruction of cells has occurred within the body. They then proceed to show that the repair of the blood loss in chronic anemia, induced in rabbits by bleeding, takes place more rapidly when the animals receive subcutaneous injections of hemoglobin than when they do not. That this is a case of stimulation of blood forming activity, rather than merely a matter of furnishing hemoglobin for cells, is indicated by a greater demand for stroma in the injected animals than in the uninjected controls. In other words, blood formation is predicated on blood destruction. And hemoglobin itself, or some product of its breakdown or metabolism, stimulates the bone marrow.

The spleen and bone marrow have also bee considered by some as a source of an active erythropoietic substance. The position of the spleen with respect to blood formation has been a more or less controversial one. It still is in dispute. Even today we find Naegeli(62), for example, thinking that the spleen inhibits bone marrow activity, while Eppinger (Quoted in (62))

says that it stimulates it. Despite these contradictory views, the fact remains that Danilewsky (Quoted in(67)) and Silvestri (Quoted in(67)) have both found a hemopoietic stimulant in splenic extracts which they used parenterally in normal and anemic dogs. The anemia was a dietary one.

While this work and that of Leake (46)(47)(48) does not indicate that this is necessarily the same one as that effective in pernicious anemia, Jones and Larsell et al (36)(43) and the author (3) have found that spleen or splenic extracts are able to produce remissions in the disease.

This rather wide distribution, in itself, suggests that if there is a specific hormone, it is probably secreted in one organ and then stored in various tissues, perhaps more or less selectively. This thought finds a certain substantiation in the report of Richter, Ivy, and Kim (68), who obtained extracts of the livers of two patients who had pernicious anemia and died. One of the patients had had liver therapy and the extract from his liver had hemopoietic activity when used in another case of pernicious anemia. The other patient had had no treatment and the extract from this liver was able to produce no response in another pernicious anemia case.

Very little can be said at the present time as to the nature of the material that seems to stimulate the bone marrow. It may be that we are dealing, not with a specific substance, but rather with the non-specific effect of a number of substances. The work thus far probably favors the view of a specific factor being involved in production of remissions in pernicious anemia. If it is a specific compound, the answer to question of its nature will come when it is isolated in pure form, analyzed, and then synthesized.

Up to the present time certain ideas have been presented. Larsell and Jones (loc.cit.) believe that this substance is of nuclear origin and that it is probably a nucleic acid or its salt. West and Howe (80) thought that it was an amino acid derivative, but later (81) withdrew this idea. On the basis of the work of Charlton (10), one might consider it to be hemoglobin

or one of its derivatives.

It is interesting to note, in view of the earlier claims of the association of the disease with a vitamin deficiency, that Strauss and Castle (76) suggest that their extrinsic factor is not a nucleoprotein or nucleic acid but is something closely related to vitamin $B_2$, and it may even be this vitamin itself. They base this hypothesis on experiments in which they incubated normal gastric juice, containing the intrinsic factor, with various substances. They found that, while beef spleen, which is a rich source of nucleoprotein, and other materials are without effect, autolyzed yeast, by this procedure, does produce the principle capable of effecting remissions.

Mention should also be made, although it is highly questionable, of the intimation that the intrinsic factor may be trypsin (11).

The best analyses on a highly purified liver preparation have been made by Cohn (12)(13)(14). On the basis of certain chemical reactions, the details of which need not be covered here, he thinks that the active principle is either a polypeptid or a nitrogenous base. It may be significant, in view of what has been said about hemeglobin and its derivatives, that his analyses show that the nitrogen content of this principle is approximately that found in compounds that are closely related to hemoglobin.

# IV

# Case Reports

Case 1. Hospital No. 41355. Miss M. B., an American, white, single woman, aged 21, entered the University Hospital on 11-19-32 for the second time complaining of (1) weakness, (2) a sense of fullness in the left side, and (3) loss of appetite.

She had been well until January 1929. At that time she came down with influenza and stayed at home for two weeks. On her return to school she was tired, weak, and "became sick at her stomach." She gradually grew worse and in June went to a hospital where she was treated for pernicious anemia with iron, liver, hydrochloric acid, and sodium cacodylate. She went home in July feeling well. She again became ill and went to the hospital in February 1930 and stayed until May. This time she was not as well on her return as before. She was hospitalized again in September 1930 and stayed till Christmas. She entered the University Hospital for the first time in February 1932 with the above complaints. She improved under liver therapy and was dismissed in March. She was well until July and then again relapsed because of her inability to eat liver or to buy extract to take its place.

Examination reveals a sallow, pale skin; an enlarged heart, liver, and spleen; absent knee jerks; and impairment of motor power. The heart rate was 100 and the blood pressure 105/60. There was a systolic murmur.

The gastric contents showed no free hydrochloric acid, either with or without histamine stimulation. The hemoglobin was 22%(Sahli), the red cell count was 1,360,000 cells per cu. mm., and the white cell count 2,800 cells per cu. mm. The blood smear showed marked variation in size but less of shape of the cells. There were a number of large oval forms. There was some achromia.

In view of the past history and the present findings, a diagnosis of pernicious anemia was made.

One dose of Extralin had been given on 11-20-32, but spleen was, nevertheless, started on 11-21-32 in small quantities. For about a week it was possible to administer satisfactory doses, but after that the patient would

not cooperate and treatment with spleen was discontinued. On 11-22-32 the reticulocytes were 2%; on 11-26-32 they were 3.5%; and on 11-29-32 they were 5%. Although this indicates a response to the administration of spleen, there was no appreciable change in the blood count during this time.

Liver therapy was started on 12-1-32 with a dose of Extralin. On 12-2-32 intramuscular injections were started, 2 cc. being used daily from this time to the date of her dismissal. On 12-9-32 the reticulocytosis reached its peak, with a value, however, of only 7%. The blood picture and clinical condition of the patient gradually improved and she was dismissed on 12-23-32 with a count of 3,150,000 cells per cu. mm.

This patient demonstrates, even though treatment was not continued, the presence of a bone marrow stimulant in the spleen. The lack of a marked response and the relative slowness with which it was obtained can reasonably be accounted for on the basis of the fact that those cases which have had previous relapses never respond as well to ordinary doses. They usually require much more material than is otherwise needed. In this case, even 2 cc. of liver extract given intramuscularly, which is ordinarily sufficient to produce a good response, caused a reticulocytosis with a peak of only 7%.

Better cooperation, that would have made administration of sufficient quantities of spleen possible, might have produced a different result. The response obtained, however, was sufficient to justify further trial.

This case is from the Service of Dr. Frank Conlin. It is a pleasure to acknowledge his cooperation and courtesy in permitting its use for these experiments.

Note:-The statement that the urine and Wassermann were negative was inadvertently omitted.

Case 2. Hospital No. 41971. Mrs. C. M., an American, white, married woman, aged 56, entered the University Hospital on 1-4-33 for the first time complaining of (1) loss of forty pounds in weight since last July, (2) weakness, (3) dull soreness in right epigastrium, (4) yellow color which has been getting worse since last October, (5) loss of appetite, and (6) numbness and tingling in hands and feet.

The patient has always been well except for a period twelve years ago, at which time she menstruated for eight months. She was treated with Radium at Rochester (Mayo Clinic?) and had been well since, except for her present condition. Her past history is of no significance except for the fact that she once burned the back of her hand and felt no pain.

Examination reveals a woman of about the stated age showing a certain degree of emaciation and a yellowish tinge to the skin. Other than a slight enlargement of the heart, a loud systolic murmur, and a tongue which is somewhat smooth at the edges, the physical examination is not remarkable. This should be amended by adding that the liver extends about 5 cm. below the costal margin.

Urinary findings are negative. The blood Wassermann is negative.

The gastric contents show no free hydrochloric acid either with or without histamine stimulation.

Examination of the blood shows 29% hemoglobin (Sahli), 1,670,000 red and 2,800 white cells per cu. mm. The smear shows considerable change in size and shape of the cells, with a tendency to macrocytosis. There is poikilocytosis. There is a moderately positive indirect Van den Bergh reaction. Quantitatively, the blood contains 23.2 mgm. bilirubin per liter.

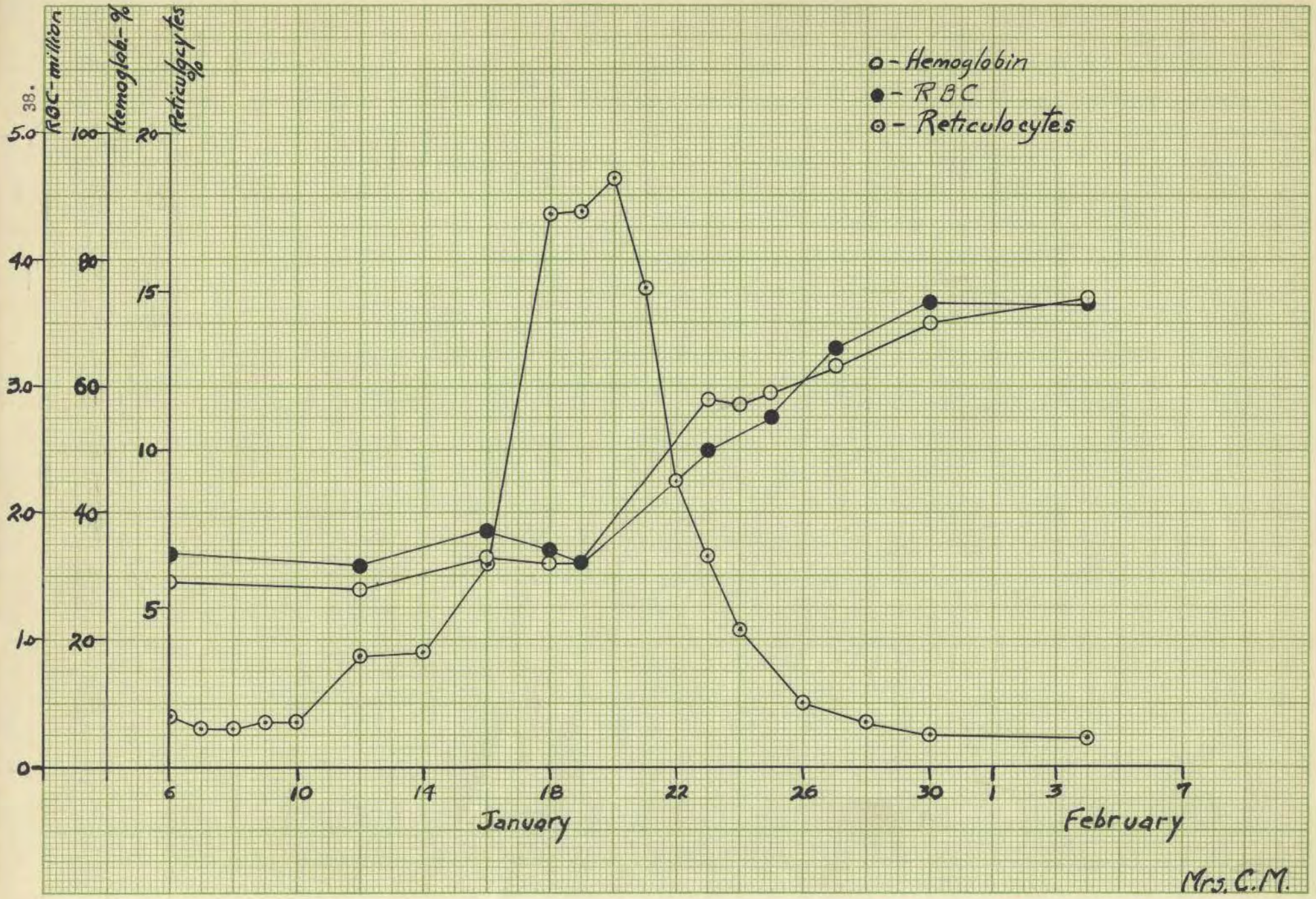
On the basis of symptoms, gastric, and hematologic findings, a diagnosis of pernicious anemia was made.

Treatment with spleen was started, essentially, on 1-10-33. The response, as can be seen in the accompanying chart, was very satisfactory. The patient showed improvement not only in her blood picture, but clinical-

ly as well. She became stronger, her appetite increased, and she gained in weight. The temperature, which is usually slightly elevated in these cases, returned to normal. There was improvement also as far as the numbness and tingling were concerned.

She was dismissed 2-4-33 with a count of 3,660,000 red and 5,400 white cells per cu. mm. and 74% hemoglobon (Sahli). Her treatment outside will, of course, have to be carried on with liver.

This case is from the Service of Dr. George Pratt. It is also a pleasure to acknowledge his cooperation and courtesy in permitting its use for these experiments.



Case 3. Hospital No. 42088. Mrs. A. N., an American, white, married woman, aged 62, entered the University Hospital on 1-13-33 for the first time complaining of (1) asthenia, (2) loss of weight, (3) occasional nausea and vomiting, (4) nervousness, and (5) gas and belching.

The details of her past until history up to a year ago are irrelevant as far as her present condition is concerned. At that time she took some "Crazy Crystals". It was then that she noticed her loss of weight, irritability, and an inflamed and painful mouth. Although always quite well and energetic, she now began to notice dyspnea, palpitation, and a fluttering of her heart on exertion. Her gastric symptoms began at this time too. Previously a good eater, she now became of aware of a loss of appetite.

She was jaundice five years ago. There were no stool abnormalities.

Examination reveals a well developed and well nourished woman, showing evidence of a moderate weight loss and a pale, sallow skin with a yellowish tinge. The mucous membranes and the nail bed are pale. Ophthalmoscopic examination shows some retinal atrophy. The retina is pale and there are flame shaped areas of hemorrhage. There is a systolic blow heard best over the apex. There is accentuation of the first sound over the tricuspid area and at the base. Blood pressure is 140/75.

Urinary findings are negative. The blood Wassermann is negative. Kidney function is essentially normal. Occult blood is present in the stool on a meat free diet. There is no free hydrochloric acid in the gastric contents. Hydrochloric acid medication had been started, so this test was not repeated with a histamine injection.

X-ray examination revealed arthritic hip joints, a slight increase in density of the right frontal and maxillary sinuses, a probable cholecystitis and gall-bladder calculi, but no signs of malignancy of the gastro-intestinal tract.

Examination of the blood showed 35% hemoglobin(Sahli), 1,550,000 red and 4,600 white cells per cu. mm. There were poikilocytosis, anisocytosis,

macrocytosis, stippling, and polychromasia. The volume index was 1.1. The Van den Bergh showed a faint delayed direct reaction. The bilirubin content was 4.5 mgm. per liter.

In view of the fact that there was no evidence of malignancy of the gastro-intestinal tract and on the basis of symptoms and findings, a tentative diagnosis of pernicious anemia was made. The patient was started on spleen therapy on 1-23-33. She showed no response whatever. Spleen therapy was, therefore, discontinued on 2-7-33.

Intramuscular injections of liver extract were begun on 2-9-33, 4 cc. b. i. d. being administered. On 2-15-33, this was changed to 5 cc. once a day and on 3-4-33, it was again changed, to 4 cc. daily. On this treatment she showed a tremendous response, the reticulocytosis reaching a peak of 59.9% within about five days. She rapidly improved and left the hospital with a hemoglobin of 61%(Sahli) and a red count of 3,400,000 cells per. cu. mm.

The only comment that could be made with regard to the use of spleen here is that it was not successful because insufficient quantities may have been used. This possibility is based on the necessity for the large quantity of liver that was administered to produce the response. This is, of course, merely a suggestion. It may, just as well, be due to the inefficacy of spleen entirely. The question of absorption may also have entered.

This case was admitted on the Service of Dr. Howard B. Hunt and was later transferred to that of Dr. John F. Allen. I am grateful for the cooperation and courtesy which permitted its use for these experiments.

Note:- I am indebted also to Miss Angeline Phillips and to Miss Helen Wyandt for their kindness in connection with arrangement of diets and carrying out of laboratory determinations, respectively.

Case 4. (78) Reported by Sturgis and Isaacs. F. J. W., a man, aged 57, had been treated with a preparation of liver extract about two years previously, receiving six vials daily, representing the extract from 600 grams fresh liver. At that time his initial red blood cell count varied between 890,000 and 1,000,000 per cu. mm., and his maximum reticulocyte count was 16% on the tenth day of treatment. He developed a complete and perfect remission, but later deiscontinued the medication and had a complete relapse. With 30 grams of dried hog's stomach, an initial red blood cell count of 1,120,000 per cu. mm., and a hemoglobin of 29%(Sahli), the maximum rise in the percentage of reticulated red blood cells was 18.8% on the seventh day. He was not given any hydrochloric acid with his meals. The reticulocytes had dropped to their normal percentage, about 1%, by the twenty-fourth day. On the forty-sixth day the red blood cell count had risen to 4,430,000 per cu. mm. and the hemoglobin to 76%(Sahli). The leucocytes had increased from 2,400 to 5,400 per cu. mm. There was a marked subjective and objective improvement, which began early in the course of treatment.

This case demonstrates the result obtained with Ventriculin.

Case 5. (34) Reported by Isaacs, Sturgis, et al. A man, aged 22, entered the hospital in his fifth relapse, his disease having dated back four years. He had had remissions induced by liver and by liver extract. In the previous relapse he had responded only to the juice of from 1 to 3 pounds of liver a day, desiccated hog stomach in doses up to 150 gm. daily for eighteen days having proved ineffectual. It required from 6 to 10 vials of Lilly's liver extract (343 N. N. R.) to maintain his blood count at a normal level. When this was discontinued a relapse set in within a month. He was then given desiccated hog stomach, from 40 to 50 gm. a day, with a markedly submaximal response in both reticulocyte and red blood cell production. He was then given liver intravenously and responded perfectly. Defective absorption of the active material from the gastro-intestinal tract was the probable mechanism of the failure of medication by mouth, as nausea,

vomiting and diarrhea were clinical features until the remission was induced.

Case 6. (59) Reported by Morris, Schiff, et al. C. S., a white man, aged 65, was admitted to the Cincinnati General Hospital, 5-23-32. Examination of the blood showed 1.4 million red cells, from 0.6 to 1.3 percent reticulocytes, 47% hemoglobin (Sahli) and 5,300 leucocytes. No nucleated red cells were seen in smears made on four different days prior to treatment. On 6-7-32, an intramuscular injection of 5 cc. gastric juice was given. This represented material obtained from the concentration and purification of 3,200 cc. of native swine juice (32 units). A remarkable hematologic response set in the following day. On 6-8-32, about twenty hours following the injection, there were 4,000 nucleated red cells per cu. mm. The blood crisis persisted for twelve days. Coincident with the crisis, there appeared in the blood large numbers of erythrocytes containing nuclear particles. These were usually in polychromatophilic cells and were minute, i. e., pin-point in size and , as a rule, single, resembling in all respects Howell-Jolly bodies, except for their smaller size. They reached their maximum on 6-17-32, when they numbered 19,000 per cu. mm. The reticulocytes rose steadily from 4.0% on 6-8-32 to 40.1% on 6-13-32. Instead of the anticipated decrease, however, they remained between 25 and 42.9 percent (red blood cells 1,500,000) continuously (daily counts) until 7-9-32. By 7-13-32, the reticulocytosis, which lasted over a period of thirty-four days, was ended. There was no noteworthy change in the red cell count until 6-28-32, two weeks after the injection, when they numbered 2.0 million; the hemoglobin was then 56 per cent. Thereafter there was a steady rise in red count and hemoglobin till 9-21-32, when examination of the blood showed 4.5 million red cells and 93% hemoglobin. Since the original injection on 6-8-32, the patient has received no further treatment. A few days after the onset of the reticulocytosis, there was a marked subjective improvement. A maintenance dose (30 units) was injected intramuscularly

9-21-32. There was no reaction, indicating that anaphylaxis is not produced by concentrated gastric juice of swine, when injected intramuscularly.

# V

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