Pernicious Anemia and Liver Therapy

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I. ABSTRACT

PERNICIOUS ANEMIA AND LIVER THERAPY

C. J. Watson, and
O. P. Jones

Historical

Prior to 1926, although pernicious anemia was counted among the uniformly fatal diseases, spontaneous remissions were responsible for a variable duration of the disease. The observation of Minot and Murphy that adequate liver feeding induces and maintains remissions in pernicious anemia has lead to far reaching results. It is of great interest that this observation was based upon the work of Whipple and Robscheit-Robbins which first proved that anemia produced in dogs by hemorrhage was greatly aided by the addition of liver to the diet. Actually, as has now been clearly demonstrated, the effect of liver in post-hemorrhagic anemias is entirely unrelated to its effect in pernicious anemia; in fact, Whipple has shown that pernicious anemia liver is more potent in treating post-hemorrhagic anemia than is normal liver, although it is entirely inert in treating pernicious anemia (Wilkinson and Klein).

Pathogenesis

The investigations of Whipple, Minot and Murphy opened the way for even more important studies as regard the pathogenesis of pernicious anemia. The most outstanding contribution has been that of Castle and his co-workers. Castle found that various substances, notably beef, yeast and spleen pulp, after incubation with normal gastric juice, were capable of producing the same favorable effect in pernicious anemia as raw liver. The gastric juice from pernicious anemia patients when incubated with beef-steak and fed to other individuals with pernicious anemia was without effect. Because of these findings, Castle interpreted the antianemic material in the liver as being formed by the interaction of what he called the "extrinsic" factor, namely the substance derived from beef-steak, yeast, etc., with an "intrinsic" factor, a substance secreted into the gastric juice by the mucosa of the stomach, the lack of which causes Addison's anemia. Castle's original experiment has been simplified and improved and is now used as a test for the presence of the "intrinsic" factor in the gastric content. Further evidence that the "intrinsic" factor was formed in the gastric mucosa was supplied by Sturgis and Isaac who were able to successfully treat pernicious anemia patients with dried extract of hog's stomach mucosa. It now appears that the liver is simply a storehouse for this substance elaborated by the lining cells of the stomach after it has been acted upon by the "extrinsic" factor. As yet, relatively little is known of the chemical make-up of the active material in the liver. Recently, Subbarow, Jacobson and Fiske have isolated two reticulocytogenic substances from commercial liver extract. One of these appears to be closely related to tyrosin and the other has purine-like characteristics. The information regarding these substances is as yet very inadequate. Even less is known of the so-called "extrinsic" factor. For a time, it was believed that the latter might be identical with vitamin B_2 but further work has made this idea untenable since a number of investigators have failed to obtain responses on feeding various sources of vitamin B_2 mixed with gastric juice to patients having pernicious anemia. It appears probable, however, that in deficiency anemias, such as are encountered in sprue, the same "extrinsic" factor is lacking. Castle has clearly shown that the anemia of sprue is not necessarily associated with achlorhydria, also that the "intrinsic" factor is often, but not always, present in the gastric content. Improvement only occurs when sources of "extrinsic" factor have been added to the diet or have been supplied in the form of liver extract.

Recently, other methods of testing for the "intrinsic" factor have been described. Singer has observed that if neutralized normal gastric juice is injected into rats a reticulocyte response results within 4 to 5 days after the injection. However, if the normal gastric content is first boiled for from 5 to 10 minutes, no

reticulocyte response will result since Castle's "intrinsic" factor has been destroyed. Singer also found that neutralized gastric juice from pernicious anemia patients will not produce reticulocyte responses in rats, indicating the absence of the intrinsic principle. In all cases of anemia which he investigated apart from pernicious anemia, Singer was able to obtain a rat reticulocyte response, and he consequently maintains that this is a specific biological test for idiopathic pernicious anemia. In two instances of pernicious types of anemia, one in sprue, the other associated with duodenal stenosis, the "intrinsic" factor was found to be present with this method.

Gastrectomy

One of the most important experiments relating to the "intrinsic" factor and in fact to the pathogenesis of pernicious anemia in general was performed by Bence. It was first shown that extracts of hog's liver were entirely capable of producing remissions in patients with pernicious anemia. Hogs were then subjected to total gastrectomy, and after a period of 9 to 12 months, were sacrificed and the livers used to make liver extract as had been done previously with the normal animals. The liver extract from the gastrectomized hogs was entirely inactive in the treatment of pernicious anemia. This appears to be incontrovertible evidence that the stomach is necessary to the formation of the active principle. It is of great interest that the gastrectomized hogs regularly developed a microcytic, hypochromic anemia. This was quite comparable in morphologic appearance to the human form of idiopathic hypochromic anemia. The meaning of this result is by no means clear. However, the experiment would seem to indicate a closer fundamental relationship between pernicious anemia on the one hand and idiopathic hypochromic anemia on the other than has previously been assumed. The incidence and type of anemia after total gastrectomy in man has been of considerable interest, particularly since Castle's work demonstrated the important relationship between the stomach and blood formation. The time factor appears to be of considerable importance in this connection.

Microcytic Precursor

F. C. Wood notes that of 67 instances of total gastrectomy 11 developed into pernicious types of anemia but only after an average duration of 6.1 years following the stomach operation. In this connection, it should be noted that two surviving gastrectomized hogs of Bence's above experiment have now, after 17 and 21 months, developed macrocytic anemia with an increased diameter of the erythrocytes up to 8.8 microns. It should be emphasized again that the anemia in these hogs was, at the onset, microcytic (3.8). It is noteworthy that the macrocytic anemia was accompanied by a decrease in the leucocytes and blood platelets and also an increase in the serum bilirubin. It is Bence's belief that pernicious anemia in man presents in the beginning stages a microcytic hypochromic anemia which, however, is not open to medical observation. Instances are on record, however, in which just such a transition was observed in man. These are noted by Scheumann and Saltzman and have also been observed by Downey. A patient recently observed on the medical service of the University of Minnesota Hospitals may have represented such a transition. The special features in this instance were as follows:

Female, 60. Smooth tongue, previously sore intermittently. Hb. 70%, color index 1.0. Feces urobilinogen 34 mg. per day. (This value is lower than normal and is of the magnitude frequently encountered in idiopathic hypochromic anemia, but not in pernicious anemia where values of over 300 mg. per day are regularly encountered (Watson)). Achlorhydria even after histamine.

In this instance, there was no response of reticulocytes or hemoglobin to liver therapy; however, on intensive iron therapy, the hemoglobin has increased to 74%, and the patient is being observed further. It is evident that some features of both pernicious and idiopathic hypochromic anemias are represented in this instance.

While there is no longer any doubt about the formation of "intrinsic" factor in the stomach, recent work by Greenspon suggests a possibility that Castle's work

will have to be interpreted in a different way. Greenspon believes that an "extrinsic" factor is unnecessary to explain Castle's results. He found that pepsin is definitely antagonistic to the antipernicious substance elaborated in the stomach. By inhibiting the activity of pepsin during the collection of gastric content, Greenspon claims to have obtained fully as good results with gastric juice alone, i.e. without the addition of meat or yeast, supposed to contain the "extrinsic" factor. Greenspon's results have not yet been confirmed or denied.

Tests

Means of testing antianemic material as to potency:

1. The lack of any chemical test for the active principle has considerably hindered research, both in regard to clinical responses and as to the chemical nature of substances present. As yet, it is still necessary to rely upon the effect of preparations by administering them to untreated cases of pernicious anemia. Minot and Castle have studied the reticulocyte response in considerable detail. They have shown that the response following an optimal amount of material (equivalent to 100 Gm. of raw liver daily) is inversely proportional to the initial red blood cell level. This fact has been utilized by the Council on Pharmacy and Chemistry of the American Medical Association to obtain a certain degree of uniformity in the various liver products on the market. The Council requires liver extracts and preparations to produce responses as follows:

Initial R.B.C. Level	Minimum Reticulocyte Response Necessary
1.0	30%
1.5	18
2.0	12
2.5	7
3.0	4

Minot and Castle place considerable stress upon what they call the double recitulocyte response. This means that if a small amount ("submaximal" dose) of active material is given only a relatively small reticulocyte response will occur. If potent material is now given in a larger amount, the reticulocyte response which occurs will be roughly proportional to the potency of the second substance given. In other words, if the second reticulocyte response is equal to or greater than the first, this indicates that the second material is at least equally as potent as the known active material used for the first response. Minot and Castle have emphasized the importance of infection in preventing reticulocyte responses. This is best illustrated in malaria where the use of quinine is followed by a prompt and often rather marked increase in reticulocytes and elevation of the level of the hemoglobin and red blood cells.

2. Jacobson has recently developed a method whereby guinea pig reticulocyte responses to various liver extracts were tested. Jacobson has standardized his method with what he calls the guinea pig unit (G.P.U.). The following table gives some idea of the amount of active principle which his method found in comparable amounts of different liver extracts and livers.

	G.P.U.
1. Commercial liver extract	164,000
2. Human liver	127,000
3. Human liver (pernicious anemia in partial remission)	47,000
4. Human liver (pernicious anemia in relapse)	650
5. Human liver (pernicious anemia in relapse)	348

It can be seen that the liver of the patient dying of pernicious anemia contains only a relatively negligible amount of material inducing reticulocytosis in the guinea pig. It is quite possible that this method of assay may prove to be very useful in determining potency of the substances effective in pernicious anemia. Nevertheless, it must be borne in mind that the underlying causes of reticulocyte response in the pernicious anemia patient and in the guinea pig are quite

different. Jacobson stated that the guinea pig marrow was quite similar to the marrow in human pernicious anemia. Jones has been unable to confirm this finding, noting that the pernicious anemia bone marrow is chiefly megaloblastic while that of the guinea pig is normoblastic in type.

3. As yet, only one chemical test has been devised for the active principle of liver extract. This was described by Duesberg and Koll, and depends upon methemoglobin formation by substances in the liver extract. Duesberg and Koll believe that methemoglobin formation was proportional to the amount of active antianemic substance present in the extract. However, this has not been confirmed. It is quite evident that this subject should receive further investigation since the possession of the chemical test for the active principle would be of the greatest value in further study of the disease.

Hemolysis?

Pernicious anemia is usually classified as a hemolytic anemia. This is true because of the regular increase of bilirubin in the plasma, the regular increase of urobilinogen in the feces, as well as the hemosiderosis of the organs. These findings have been generally assumed to indicate increased blood destruction; however, there is considerable reason to believe that the increased amounts of bilirubin and urobilinogen are in reality not coming from destroyed red blood cells but from materials which are not utilized in the formation of new hemoglobin. The chief fact favoring this concept is that patients are not infrequently seen with pernicious anemia whose hemoglobin and red cell level remains quite constant during a period in which there is increased bilirubin in the plasma and a marked increased excretion of urobilinogen in the feces. Since these individuals at the same time do not demonstrate an increased number of reticulocytes in the peripheral blood, it is extremely difficult to see how the increased pigment could represent destroyed red blood cells. The following case is an example of such an instance:

Male 72. Progressive weakness and slightly yellowish pallor 4 months. Smooth tongue. Very soft, smooth, thin skin. White hair, blue eyes. Hb. 28%, red blood cells 1,000,000, white blood cells 4,000, 55 to 60% lymphocytes; neutrophils show shift to right with frequent "P.A." neutrophils; marked macroanisocytosis and poikilocytosis. Icterus index 19.

Date	Hb.%	R.B.C.	Reticulocyte %	Feces Urobilinogen, mg. per day
4-11	27	1,040,000		
4-14	28	1,300,000	2.0	803.6
4-15				
4-17	28	- 20 cc. liver extract intramuscularly	1.5	
4-18	28		4.0	
4-20	30		31.0	
4-21	30		28.0	300.0
4-22	32		22.0	
4-23	36		16.0	
4-26	47		3.0	
5- 6		- 10 cc. liver extract intramuscularly		
5- 7	50	2,800,000	4.0	167.8
5-12			11.0	
5-14	54		3.8	

(It is interesting to note that the reticulocyte counts in this case illustrate Minot's "double recitulocyte response". After an optimal dose of liver extract has been given, the second response to additional liver extract, given some time later, is always smaller than the initial one.)

In this instance, it is clear that the hemoglobin and red count remained at the same level during the period in which the urobilinogen excretion was markedly elevated, in fact about four times the normal. If there had been an increased number of reticulocytes, one could readily have attributed the increased urobilinogen to increased blood destruction. This was not the case, however. It appears, therefore, that the increased excretion of pigment may actually have

represented failure in utilization. There is some suggestion that the same is true of iron metabolism in pernicious anemia; for instance, a very marked hemosiderosis of the liver and spleen is commonly observed, while this is usually not the case in ordinary hemolytic jaundice where it is evident that because of the very rapid rate of regeneration and high reticulocyte percentage the iron is being quickly reutilized. Such a failure in utilization of both pigment and iron may very well explain Whipple's finding that human pernicious anemia liver is twice as efficacious in treating post-hemorrhagic anemias of dogs, as is normal human liver. In the last analysis of this question, there are two more facts which should be mentioned and which in some degree point toward a hemolytic nature of the disease. Peabody noted that there was increased phagocytosis of red blood cells in the bone marrow in pernicious anemia during relapse. Mettier has shown a comparable increased phagocytosis in the Kupffer cells of the liver during relapse. It is difficult, however, to say whether these findings are sufficient to indicate that there is really an appreciable increase in blood destruction. One other feature which suggests but does not prove an increased hemolysis is the improvement which has been observed, particularly by Eppinger, following splenectomy. There is no doubt that some cases of pernicious anemia were markedly improved following splenectomy. This improvement is of variable duration, sometimes being very short, but, in certain instances, having lasted as long as three years. However, one need not ascribe such beneficial effect to a hemolytic activity of the spleen; a depressing effect on the liver is also possible.

Reports concerning reticulocytogenic effect of Congo red have recently appeared.

Congo-Red

Believing pernicious anemia to be a hemolytic anemia caused by over-activity on the part of the reticulo-endothelium, Massa and Zolezzi attempted to block this hemolysis by injecting Congo-red, intravenously, into 12 patients with pernicious anemia and 2 patients with a pernicious type of anemia. Clinical improvement and remission was obtained in 11 of these cases, while in 3 no reticulocyte response nor improvement was elicited. In some cases, the reticulocyte response was as high as 25%. Summary of their case 2 is as follows: Pt. Tondille, 31 years. Hemoglobin 62%, red blood cells 1,300,000, color index 2.38, reticulocytes 1%, white blood cells 3,800. After 17 injections (9-10cc.) of a 0.5% solution of Congo-red during the course of 29 days, the red blood cells were 4,500,000, hemoglobin 93%, color index 1.02, and bilirubin was almost normal. After the eighth injection, the reticulocytes increased to 15%.

Three of their cases were refractive and did not respond to Congo-red therapy while they did respond to liver therapy.

In this country, Mermod and Dock claim to have confirmed these results by obtaining reticulocyte responses in two patients after intravenous Congo-red therapy. One patient received 60 cc. of a 1.5% Condo-red 4_B in 6% dextrose in five days, the other 90 cc. in ten days. These patients had a rise in reticulocytes and fall in serum bilirubin comparable to that produced in similar cases by intramuscular injections of liver extract.

They also claim that daily intraperitoneal injections of Congo-red into guinea pigs will produce a reticulocyte response similar to that obtained by Jacobson after injecting liver extract into guinea pigs. Pointing to the neutralizing effect of Congo-red on such toxic substances as curare, strychnine, and certain bacterial toxins, Mermod and Dock believe that their results support the old theory regarding reabsorption of toxins, probably hemolytic substances from the gastro-intestinal tract.

Three typical cases of pernicious anemia in relapse, seen in the University of Minnesota Hospitals, have been given Congo-red intravenously in relatively large amounts (two of these cases actually became red in color without increase in hemoglobin, a phenomenon also noted by Bloomfield--personal communication). In

none of these three instances was any reticulocyte response observed, nor any increase in hemoglobin or erythrocytes. In each of the three parenteral liver extract effected prompt reticulocyte responses and subsequent remissions.

Toxic Factor

Wakerlin and Bruner, during the past two years, have collected a certain amount of evidence for the presence of a toxic factor in pernicious anemia. They used sterile urine collected from eight untreated pernicious anemia patients. For each urine studied, eight pigeons were used. One-half of the pigeons were injected, intramuscularly, with varying amounts of unheated urine for five days. The other pigeons received injections of urine previously heated at 100 degrees for two hours. Pigeons receiving the unheated urine showed a definite depression in the reticulocyte percentage. In some instances, the reticulocyte-depressing-principle was so toxic that it killed 15 out of 21 pigeons from one to eight days following the first injection. The reticulocyte decreasing substance is thermolabile, since none of the pigeons receiving heat-treated urine showed a depression in the reticulocytes. All of the pigeons receiving heat-treated pernicious anemia urine and those receiving heated and unheated normal human urines did not show any evidence of a reticulocyte decreasing effect.

"It is apparent, therefore, that urine from untreated patients with pernicious anemia contains both a thermolabile, comparatively toxic, reticulocyte decreasing factor and a partially thermostable, relatively non-toxic, reticulocyte stimulating principle for the pigeon. Normal human urine contains the latter but not the former, or at least not in the quantities of urine used.---If our impression that the principle acts through depressing erythrogenesis in the bone marrow should prove to be correct, the production of experimental pernicious anemia in animals by the separation and administration of sufficient quantities of the reticulocyte decreasing factor is not beyond the realm of possibility."

Bone Marrow Biopsies:

The bone marrow in pernicious anemia has always received a great deal of attention. As soon as reticulocyte counts were begun to be made, it became evident that the bone marrow in pernicious anemia, although actually hyperplastic, was not delivering young cells to the circulation at a rate any greater than the normal and often somewhat less.

In recent years, there has been quite an interest in the usage of bone marrow biopsies as an aid to the diagnosis and study of pernicious anemia. By far the greatest amount of work has been done by the Europeans, Segerdahl, Nordenson and Rohr, who altogether have examined 1,260 sternal punctures. These sternal punctures were made according to the technic first described by Arinkin, i.e., the aspiration of marrow from the sternum by means of a shortened spinal puncture needle. The resulting aspirated fluid consists of a mixture of bone marrow cells and blood which are subsequently spread upon a slide as in making a routine blood smear.

These above mentioned investigators claim that the diagnosis of pernicious anemia can be made from a sternal puncture by finding megaloblasts to be present. In their opinion, the presence of megaloblasts is pathognomonic for pernicious anemia. However, Tempka and Braun have reported the presence of megaloblasts in pernicious anemia of pregnancy and in cases of agranulocytosis following sepsis. They believe that the presence of megaloblasts alone does not establish the diagnosis of pernicious anemia, but that the presence of a regenerative-degenerative process of the large neutrophils and severe damage to the megakaryocytic system as being specific for pernicious anemia.

It has been our experience that megaloblasts are not specific for pernicious anemia since we have encountered them in pernicious types of anemia (pregnancy, tropical macrocytic anemias) and in one case of agranulocytosis. Although we have not had the opportunity to study all the various types of macrocytic hyper-

chromic anemia, it is our belief that they cannot be separated one from the other on the basis of bone marrow biopsies.

Considerable stress has been placed upon the peculiar type of neutrophil which is encountered in pernicious and pernicious types of anemias as an aid in diagnosing these conditions from the peripheral blood. It has been thought that these cells represented a functional alteration which took place in the blood stream, similar to the type of alteration which is encountered in the leucocytoid lymphocytes of infectious "mononucleosis". In our series of pernicious anemia bone marrows during relapse, we find that all stages of development have been considerably altered. This type of alteration is such that it has caused Tempka and Braun to believe these cells are undergoing a regenerative-degenerative change. The outstanding things present in this pathologically altered cell line are: the tendency to become hypersegmented commences before the cells have obtained any specific granulation. These cells are quite polymorphic in the promyelocyte stage and their nuclear structure is lighter than normal. True myelocyte and metamyelocyte stages are skipped in most instances. By means of peculiar invaginations and protrusions of the nuclear material, vacuole-like structures are formed in the nucleus. These holes in the nuclear material are circular to start with, but later they become irregularly shaped and in some instances may be drawn out into a narrow slit. It is by means of these vacuole-like structures and thinned out areas that the ultimate P.A. neutrophil nucleus becomes so bizarre. In addition to nuclear changes, there are changes in the cytoplasmic granules and in the size of the cell. These alterations are by no means degenerative but represent the changes which take place in a pathologic cell series under abnormal conditions.

Not only are the megaloblasts and P.A. neutrophils indicative of a pathologic condition existing in the bone marrow due to a lack of some principle in the liver, but the megakaryocytes are likewise altered. These cells are not affected quantitatively so much as they are qualitatively. In this case, it is found that some megakaryocytes show no evidence of platelet formation, their cytoplasm being very basophilic and devoid of granulation. This impairment of the megakaryocytic system is not the same in every case for in some there does not seem to be any change. It is believed that these alterations in the megakaryocytes are responsible for the decrease in the number of platelets in pernicious anemia during relapse.

According to Segerdahl's figures, all of the nucleated red cell elements in a normal human marrow constitute about 12% of the total marrow components. In pernicious anemia during relapse, the marrow contains approximately 36% of various hemoglobiniferous cells. Considering that the marrow is highly active and hyperplastic it is paradoxical that in the blood stream there should be an oligocythemia and leukopenia. Besides having something wrong with the maturation of the myeloid elements, there seems to be some disturbance in the delivery mechanism which releases cells into the peripheral blood.

After the administration of liver extract, the first effect seems to be upon the megaloblastic series. These cells gradually mature and disappear from the marrow 7 to 12 days after therapy During the disappearance of this series, the normoblasts are being formed rapidly. Segerdahl finds the marrow commences to return towards normal as early as 24 hours after therapy. The pernicious anemia neutrophils do not disappear as rapidly as the megaloblasts and may be extremely difficult to find at the end of two months according to Tempka and Braun. Somewhare in the neighborhood of 72 days after the commencement of therapy, the marrow is completely normal with regard to the red cells, neutrophils and megakaryocytes.

Deficiency Disease

From the foregoing, it is evident that pernicious anemia, at least insofar as the anemia is concerned, is in part, if not wholly, a deficiency disease in the sense that there is immediate produc-

tion of remission by feeding the active principle contained in liver and stomach mucosa. In this regard, one fact remains as yet entirely unexplained, namely spontaneous remissions which are known to occur in untreated instances. For the present, it must be assumed that for some reason not yet known the stomach is able to resume formation of the intrinsic factor, although this is entirely at variance with what is known about the complete atrophy of the mucosa so characteristic of pernicious anemia. In these spontaneous remissions, the possibility must always be borne in mind that the patient has received active antianemic substance in the diet in an unrecognized way. It has been shown, for instance, that both kidney and brain contain relatively large amounts of the antianemic principle. In fact, the concentration in kidney is almost as high as it is in the liver. With regard to the activity of the kidney, certain experments of Decostello should be mentioned. Decostello claims to have been able to effect reticulocyte responses in patients with pernicious anemia by means of urine enemas, the urine being obtained from other patients having pernicious anemia as well as from normal individuals. On the basis of these results, he believes is not, or at least not entirely, missing but most likely present in the blood as a precursor of the substance secreted into the stomach content. He considered that its lack in the gastric content in pernicious anemia might be due to a disturbance in secretion resulting from the atrophy of the mucous membrane. Naturally, these findings will have to be further studied and definitely confirmed before any great importance can be attributed to them. It should also be borne in mind in this connection that nonspecific reticulocyte responses may result from various irritative substances, although these responses are never as great as that obtained with specific antianemic material. It would seem quite likely that a urine enema, as employed by Decostello, would surely be productive of some kind of a revolution in the body which might or might not be associated with a reticulocyte response.

Pernicious Types of Anemia

Considerable interest has recently centered about the macrocytic anemias which occur with disease of the liver. Higgins and Stasney have produced liver disease (cirrhosis) in animals with carbon tetrachloride, Wintrobe with cincophen. Macrocytic anemia resulted with considerable regularity in the instances having advanced degrees of cirrhosis ofthe liver. In 1933, Wintrobe and Schumacker collected 43 cases of macrocytic anemia associated with liver disease from the literature and added 11 of their own. In one-half of the instances studied by Wintrobe and Schumacker, free hydrochloric acid was found in the stomach content. One of their cases was an instance of acute catarrhal jaundice. Spontaneous recovery occurred within a period of 24 days. About one year ago, we had an opportunity to study an individual with a much more prolonged jaundice who also developed macrocytic anemia during the period of observation. The important features in this case were as follows:

Female 31. Deep jaundice and pruritis of 2 months duration, preceded by several days of anorexia and weakness. No pain. Liver and spleen enlarged. Subsequent findings:

Date	Icterus Index	Hemoglobin	Red Blood Cells	Reticulocytes	Urobilinogen in mgs. per day	
					Urine	Feces
10-15-34	224	89%				
10-16	179	85				
10-20						241.0
10-21					56.8	
10-24						803.3
10-28					224.2	1,257.0
11- 1	104	65	2,680,000			
11- 3						461.0
11- 3						990.0
11-12	43			15%		
11-16	35					
11-19		78	3,300,000			146.2
11-23					23.6	
11-27	25	80	3,700,000		35.5	
*12-4-34				2%	3.8	68.4

*This patient has now been in good health for nearly 2 years. The spleen is no longer palpable, the liver barely so.

It will be observed that during the period of anemia, the reticulocyte count was high in the above instance. This was also true in a similar case of Van den Bergh and Kamerling, which, however, terminated fatally and at autopsy it was found that there was an advanced cirrhosis of the liver. The importance of the reticulocyte count in studying these macrocytic types of anemias cannot be over-emphasized. If it is very much elevated prior to liver therapy, the diagnosis of true pernicious anemia may be excluded with a fair degree of certainty. Macrocytic anemias have often been observed in association with cancer in the gastro-intestinal tract, particularly cancer of the stomach. Schauman and Saltzman point out that the cancers in these instances are often relatively small. It is certain that the degree of involvement bears no relation to the development of macrocytic anemia. A patient studied recently in the University of Minnesota Hospitals with a very diffuse and extensive linitis plastica involving not only the stomach but the duodenum presented no anemia whatever. Pernicious types of anemia have been observed with cancer of the cecum, and also of the small bowel. In the light of our present knowledge of the site of formation of the "intrinsic" factor, it is rather difficult to explain the mode of origin of these macrocytic anemias unless one assumes a secondary effect on the liver via the portal circulation. Erhard-Bock has recently studied macrocytic anemias with carcinoma of the stomach and compared them with pernicious anemia and has found that the average size of the red blood cells in the former was uniformly less than is seen in true Addison's anemia. The average in 17 cases of carcinoma of the stomach was 7.9 microns and in 28 cases of pernicious anemia 8.3 microns, the normal value varying between 7.1 and 7.6. For these measurements, Erhard-Bock used what he called an erythrocytometer which permits direct measurements of the average diameter of the erythrocytes. Such an apparatus has now been in use in the University of Minnesota Hospitals for about one year. While it is of distinct value in determining whether anemias are macrocytic or microcytic, it is considered extremely doubtful that the cause of macrocytic anemias can be determined by its use as Erhard-Bock has stated.

In the differential diagnosis of pernicious types of anemia associated with gastro-intestinal cancer from true pernicious anemia, the presence of occult blood in the feces together with an elevated reticulocyte count prior to liver therapy should make one exceedingly suspicious of the former diagnosis.

The so-called pernicious anemia of pregnancy has been a subject of considerable study in the last two years. Perhaps the most important contribution to this subject is that by Wintrobe and Schumacker who discuss the factors which they consider of importance in the development of this type of anemia. They point out that the fetus in the latter months of pregnancy has a mild macrocytic anemia comparable in every respect to a pernicious anemia in partial remission. This finding, combined with the fact that pernicious anemia in the mother only develops in the latter part of pregnancy, disappears after temination of the pregnancy, not to recur even if liver therapy is discontinued, suggests that in these instances the mother is simply unable to manufacture enough active principle to take care of herself and the fetus. Other findings which they considered of importance were liver insufficiency or damage associated with toxemia of pregnancy, also a decreased gastric activity. In this regard, they point to Strauss' work which showed that pernicious types of anemia of pregnant women often occurred in those having a low gastric secretion and often in individuals who were on a faulty diet as regarded intake of animal protein.

Pernicious types of anemia are known to occur in a variety of other diseases among which may be mentioned fish tapeworm infestation, leukemia, syphilis, myxedema and sprue. The presence of the "intrinsic" factor and often of hydrochloric acid in the gastric content in the latter disease has already been mentioned (Castle, Rhoads, Lawson, Payne). It is of interest that the pernicious type of anemia occasionally seen with Bothriocephalus latus infestation has been found to yield to liver therapy without the removal of the worm. It is surprising that this form of anemia appears to be excessively rare in Minnesota, a locality where fish tapeworm infestation is supposed to be relatively common.

One of us (C.J.W.) has observed two instances of lymphatic leukemia with very typical pernicious types of anemia. In one of these, in fact, the pernicious anemia was observed for a considerable period prior to the development of leukemia and the patient had already responded on one occasion to liver therapy.

The association of tertiary syphilis and pernicious anemia has been mentioned on a number of occasions in the past. Schaumann and Saltzman have discussed the question of this relationship at some length and conclude that there is no proof whatever of any causal relationship between syphilis and pernicious anemia. One of us (C.J.W.) observed a patient at the Minneapolis General Hospital two years ago who had syphilitic aortic regurgitation and pernicious anemia. The Wassermann reaction was positive. It is worthy of note that this patient's anemia responded promptly to adequate liver therapy although no adequate antiluetic therapy had been given in this period.

Results of Liver Therapy

Little need be said about the effect of adequate liver therapy upon the blood findings in pernicious anemia. It has now become evident that unless the patient is moribund before liver therapy is begun a prompt remission will result and can be maintained by further administration of adequate amounts of liver. At present, the most acceptable and probably the cheapest method of administering liver therapy is to give the equivalent of at least 500 grams of raw liver in the form of an intramuscular extract. With a potent liver extract, this amount will be sufficient to induce a reticulocyte response of the proper magnitude. A maintenance dose of 10 cc. equivalent to 300 grams of raw liver given every 3 or 4 weeks usually proves sufficient to maintain the hemoglobin and red blood cells at a normal level.

The question as to the effect upon the associated subacute combined degeneration of the spinal cord has been somewhat more in doubt. Probably the most careful and extensive study in this regard was reported in 1935 by Strauss, Solomon, Schneider and Patek. These investigators reported the results of 300 neurologic examinations made on 26 cases having clear-cut evidence of subacute combined degeneration of the cord. In this study, the average period of time elapsing before the final evaluation of

neurologic signs was 34 months, varying from 14 to 46 months. The results of these 300 examinations are tabulated in their report. Three main points stand out clearly in this tabulation.

"First and of greatest significance is that in no instance did a single objective neurologic sign become more marked during the period of treatment nor did an abnormal sign not previously present appear. Complete objective arrest of the lesion has occurred in every case.

"Second, subjective improvement of a greater or less degree appeared in every patient. In two patients, this was very slight. In others, it enabled previously bedridden patients to return to their usual occupation. Nevertheless, such changes may be due entirely to re-education and training achieved in the presence of a definite but completely arrested spinal cord lesion. It is to be emphasized again that the tremendous gains of strength and activity often observed in such cases are not necessarily evidence of regeneration of the spinal cord fibers or cells.

"Third, excluding changes in gait and paresthesias, approximately 58% of all the abnormal signs encountered in the 26 patients remained objectively unchanged."

Impressions

1. Prior to 1926, pernicious anemia was apparently a uniformly fatal disease with spontaneous remissions causing variations in its course.

2. The obeervation of Minot and Murphy that adequate liver feeding induces and maintains remissions in pernicious anemia has lead to far reaching results.

3. The use of liver in pernicious anemia is based upon the work of Whipple and Robscheit-Robbins who proved that experimental anemia in dogs was greatly aided by the addition of liver to the diet.

4. The effect of liver in post-hemorrhagic anemia is entirely unrelated to its effect in pernicious anemia.

5. According to Castle, the antianemic factor in liver is formed by the interaction of an "extrinsic" factor derived from beef-steak, yeast, etc. with an "intrinsic" factor formed by the mucosa of the stomach.

6. It is the absence of the "intrinsic" factor which causes pernicious anemia according to Castle. The liver stores the substance elaborated by the stomach after it has been acted upon by "extrinsic" factors.

7. The chemical structure of the active principle of liver is not yet known. One is closely related tyrosin and the other has purine-like characteristics. The "extrinsic" factor was thought to be identical with vitamin B_2, but this is not likely in spite of the fact that the "extrinsic" factor is apparently lacking in sprue.

8. The liver principle causes a reticulocyte response. Tests on rats suggest that they may be used for determining the presence of the "intrinsic" factor in the gastric juice.

9. After gastrectomy in hogs, the active principle in the liver disappears. These animals develop a microcytic hypochromatic anemia, indicating a possible relationship between this form of anemia and macrocytic hyperchromatic anemia. Two animals allowed to survive apparently developed a macrocytic hyperchromatic anemia.

10. Some gastrectomized humans also develop a pernicious type of anemia some time after the operation.

11. Bence believes that there is a possible preliminary phase in pernicious anemia in which the anemia is of the microcytic hypochromatic variety. Mixed varieties have been observed in our hospitals.

12. Greenspon believes that Castle's work may be explained on the basis of the

antagonism of pepsin to the "intrinsic" factor in the stomach. Gastric juice with inhibited pepsin content acts the same as Castle's meat, yeast, etc. gastric juice combination.

13. The antianemic principle in liver has been standardized on the basis of its reticulogenic potency in untreated cases in man. Normal guinea pigs have also been used.

14. Infections may inhibit the response of reticulocytes.

15. Only one chemical test has been devised for the active principle in liver extract. This depends upon methemoglobin formation but has not yet been confirmed.

16. Although pernicious anemia is considered a form of hemolytic anemia, studies indicate that it may be an increased excretion of pigment due to failure of utilization. The same is also true of iron metabolism as pernicious anemia differs from all other forms of hemolytic anemia in the amount of iron deposited in the tissues. The effect of splenectomy and evidence of phagocytosis support the hemolytic theory?

17. Giving Congo-red may or may not interfere with the reticulocyte response. It was done with the idea of inhibiting the activity of the reticulo-endothelium which was thought to be a factor in the genesis of pernicious anemia.

18. A thermolabile toxic factor for pigeons has been found in urine of pernicious anemia patients, which depresses the reticulocyte count; a reticulocyte stimulating agent is present in normal urine which is thermostabile.

19. Even though megaloblasts are obtained in bone marrow biopsies, pernicious anemia is not necessarily present.

20. Nuclear changes in the developing neutrophil can be observed in bone marrow biopsies (pathologic regeneration?). Qualitative changes in the megakaryocytes are also present. At the same time, the marrow is very hyperplastic and contains a very high percentage of developing red cells.

21. After effective liver therapy, the megaloblasts disappear from the marrow and there is a rapid return to normal red cell formation. The neutrophils are slower to change.

22. Pernicious anemia is essentially a deficiency disease but this does not explain why remissions occur in untreated cases unless the stomach has the capacity to resume normal function for a time. There are also sources of "liver" principle outside of the liver.

23. Urine enemas from normals and pernicious anemia patients produce a reticulocyte and hemoglobin response in pernicious anemia patients.

24. Other varieties of macrocytic anemia are described. They may be due to liver disease, cancer of the stomach, bowel, pregnancy and other diseases.

25. It is possible to measure the red cell size by the erythrocytometer. While this is of assistance in distinguishing between microcytic and macrocytic anemia, it does not help in finding the cause of macrocytic anemias.

26. Unless the patient is moribund before liver therapy is begun, prompt remission will result from its administration and this can be maintained by adequate amounts.

27. The cheapest and best method of administering liver is to give the equivalent of 500 grams (15-20 cc.) of raw liver in the form of an intramuscular injection. The maintenance dose is the equivalent of 300 grams of raw liver every 3 or 4 weeks (10 cc.).

28. The effect of liver on the associated changes in the spinal cord have been studied. The objective neurological signs do not become more marked under treatment, other abnormal signs do not appear and complete objective arrest occurs. Subjective improvement occurs in almost every patient. Excluding changes in gait and paresthesias, 58% of all abnormal signs in 26 patients remained unchanged (no progression).

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II. LAST WEEK

Date: May 14, 1936

Place: Recreation Room, Nurses' Hall

Time: 12:15 - 1:10

Program: Movie: Sunny Worthersee
Lymphogranuloma Inguinale

Present: 95

Discussion: J. W. Tedder
M. H. Manson
K. W. Stenstrom
R. W. Cranston
N. L. Leven

Gertrude Gunn,
Record Librarian

III. MOVIE

Title: Towards Unity

Released by: Garrison Film Co.