PERNICIOUS ANEMIA, NUTRITIONAL MACROCYTIC ANEMIA, AND TROPICAL SPRUE

A DISCUSSION

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The brilliant work of Minot and Murphy (1926 and 1927) on the curative action of liver in Addisonian pernicious anemia and the subsequent work of Castle and his colleagues (1929, 1930, 1931, 1936) on the mechanism of the formation of the liver principle opened up a vast field of research into the nature and the mode of action of this principle in pernicious anemia. It was a natural development to extend this field to include a study of other macrocytic megaloblastic anemias and that an explanation of the etiology of all these should be sought on the basis of Castle’s theory of an extrinsic-intrinsic factor reaction leading to the formation of the liver principle. Much experimental and clinical work was carried out on these lines, but the concentration on this one aspect of these diseases and recently on the effect of folic acid in the same anemias has, by thus limiting the field, led to a neglect of the study of the general pathology and the natural history of these conditions and perhaps to a too limited view of their etiology. In pernicious anemia, nutritional macrocytic anemia and tropical sprue, to limit the discussion to the three principle macrocytic anemias, anemia is only one aspect of each disease complex and the fundamental differences between the three disease entities have been overlooked in the light of the spectacular success of treatment with liver extracts and folic acid. It was attractive to fit these three diseases, or rather the anemia in each case, into the framework of Castle’s theory, according to which all three are due ultimately to a deficiency of the liver principle. Pernicious anemia thus would arise through a deficiency of the liver principle due to an absence from the stomach of the intrinsic factor, nutritional anemia to the same anemia arising from a lack of the extrinsic factor in the diet and the macrocytic anemia of sprue to a failure of absorption of the liver principle, in some cases associated with a reduction or absence of the intrinsic factor. Even had such an explanation in its simple form withstood the test of clinical trials and experimental work, it would not have explained the etiology of the pathologic change in the gastric mucosa in pernicious anemia, to which the absence of the intrinsic factor is attributed, nor that of the functional changes in the intestinal mucosa which lead to the failure of absorption in sprue. This is not to question the validity and applicability of Castle’s work; his experimental results belong to the group of “obstinate facts” that have to be reckoned with, but the simple explanation of the causes of the postulated deficiency in “the liver principle” in these diseases must be revised in view of certain experimental and clinical findings. Undoubted cases of macrocytic anemia due to a deficiency of the extrinsic factor do exist and respond to treatment with this factor (Moore et al., 1944, Watson and Castle, 1946). On the other hand

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in many areas where nutritional macrocytic anemia is endemic it has been shown that the anemia does not respond to treatment with purified liver extracts known to be active curatively in pernicious anemia even when these are given in doses equivalent to or much larger than those effective in pernicious anemia. However, in other areas cases have been shown to respond to enormous doses of purified extracts, when the question of traces of other factors possibly present in the extracts has to be considered (for review of literature see Watson and Castle, 1946). Animal experiments have also confirmed the inactivity of highly purified extracts in nutritional macrocytic anemia (Wills et al., 1937) and now modern work on the therapeutic activity of folic acid in macrocytic anemias has to be fitted into the picture.

A consideration of these findings necessitates a reorientation of our mode of approach to the study of these three diseases which does not imply a neglect of the study of the pharmacologic and physiologic action of the elusive liver factor or of that pleasantly concrete substance folic acid. But the approach should be widened to include the natural history of these conditions and a study of their general pathology. In these studies certain considerations should be borne in mind. It should be remembered that the response of the body to any one factor or deficiency varies with the past and present environmental condition, race, family, sex, climate, diet, illnesses, etc., all of which will have determined the physiologic and anatomic state of the body at any one moment of time. The presence or absence of one factor may condition the mode of action of another factor; starving animals on a rachitogenic diet fail to develop rickets. Metabolism proceeds by a series of chain reactions; this is beautifully seen in cellular metabolism where the members of the vitamin B2 complex play such an important role in the chain of oxidation reduction reactions. The links in these chains may be broken at many points, but the breakdowns thus produced may result in very similar pathologic states. It should also be stressed that a single symptom complex, such as that of a macrocytic hyperchromic anemia may be a part of many different disease entities. Much confusion has arisen for example by the attempt to bring all nutritional macrocytic anemias into one etiological group or to differentiate types on the basis of the serum bilirubin without due consideration of complicating factors. With these considerations in view an attempt will be made to review the natural history and to study the pathogenesis and interrelationships of these three clinical entities. In each case the discussion will be limited to the classic type of each disease, though mention will be made to the large group of ill defined and at present not worked out conditions which have many of the signs and symptoms of the clinical entities under consideration.

Geographic Distribution

The distribution of the three diseases varies widely. Pernicious anemia is mainly a disease of the temperate zones, the highest incidence being in the Nordic countries, the British Isles, Canada, and the northern portion of the United States of America. Nutritional macrocytic anemia in its endemic form is, broadly speaking, a disease of tropical and subtropical lands. Originally described as "pernicious ane-
Types of Macrocytic Anemia

Macroblobosity of pregnancy was first observed in India (Balfour, 1927, McSweeney, 1927, Wills and Mehta, 1930), it was later reported from British Guiana (Giglioli, 1934), East and West Africa, (Trowell, 1943), and probably from Puerto Rico, for though Castle and co-workers (1935) have described the cases of macrocytic anemia they studied there as sprue, it is probable that British workers would consider some, at least, of their cases as nutritional macrocytic anemia. Rodriguez-Molina (1939) has described typical cases of nutritional macrocytic anemia from the same island. A similar anemia, which will be discussed later, has been reported from Macedonia by Fairley and his colleagues (Fairley et al., 1933, Foy and Kondi, 1939). Sporadic cases, particularly among pregnant women, have been reported by many workers from Europe and America, but the relation of these cases to the endemic diseases is uncertain. Tropical sprue, as its name implies, occurs mainly in the tropics. It was first described in 1759 by Hillary in the West Indies and then rediscovered in the Far East (Manson, 1879-80), and has since been recognized in many parts of the world. Further, it has long been observed that certain districts and even houses in endemic areas have a high sprue incidence (Leishman, 1945, Keele, 1946).

Seasonal Incidence

Pernicious anemia is not a seasonal disease. The highest incidence of nutritional macrocytic anemia in Bombay is during the winter months and the lowest during the monsoon (Balfour, 1927). This variation may be related to humidity, temperature or changes in the small additions to the diet, which are of such importance in very deficient and monotonous diets. Marriage customs may also influence the incidence, as they give rise to a seasonal variation in the number of pregnant women in the community and thus to the number of cases of nutritional macrocytic anemia, as this is very prevalent among such women. Hare (1946) reports that in Assam the maximal incidence of anemia, including macrocytic anemia in pregnant women, is in the third quarter of the year, which is the rainy season there. At this time fresh green vegetables are not available and the home pounded rice normally eaten is replaced by milled rice. Napier (1941), in Calcutta found that the highest incidence in pregnant women occurred in the second half of the year, in which, owing to the hot weather and monsoon, less fresh vegetables and fish are taken. The onset of diarrhea in sprue is often associated with the hot weather, when in India the barometric pressure is lowest, but new cases arise at all times of the year.

Populations Affected

A consideration of the racial and social groups who suffer from these three diseases is of considerable interest as they vary widely. Pernicious anemia appears to be par excellence a disease of people of European descent, particularly of the Nordic races, and is rare in Asiatic or Negro peoples (Friedlander, 1934). Within the racial group affected the disease is no respecter of persons, occurring in men and women of all social strata but showing a marked familial incidence. The individuals affected are characterized by or give a history in other members of the family of a fair complexion and hair and light colored eyes. The other two diseases are, however, class conscious. Endemic nutritional macrocytic anemia occurs in certain ill-
nourished populations, particularly among vegetarians (Taylor and Chhutani, 1945, Walters, 1947), the poverty-stricken poorer inhabitants of Indian cities (Wills and Mehta, 1930, Mudaliar, 1932, Napier, 1941) the East Indian laborer in Central America (Giglioli, 1934), Indian troops on active service (Marriott, 1945) or the returned Indian prisoner of war (Walters et al., 1947). Pregnant women are especially liable to develop the disease. Napier (1941) in Calcutta found that in pregnant women suffering from anemia there is a significant correlation between severe anemia and poverty, though very suggestive is not statistically significant. His results also suggested a correlation between vegetarianism and macrocytic anemia, but this finding was partly invalidated by the fact of a high positive correlation between vegetarianism and high economic status in the cases studied.

Tropical sprue, as originally described by Hillary (1759) in Barbados and as seen in India and the Dutch East Indies is, in contrast, not a disease of the ill-fed native resident, but of the relatively well-fed European or Anglo-Indian resident. A distinction must be made here apparently between sprue as originally described and seen typically in India and the Far East, and "sprue" as seen in Puerto Rico. In India, as mentioned above, the disease affects well-fed Europeans, in Puerto Rico the cases are reported (Castle et al., 1935, Rodriguez-Molina, 1939) as having subsisted for years on a very deficient diet, the intake of good biologic protein being particularly low. This is not to imply that in the established syndrome the diet is anything but deficient, for in the untreated cases it most certainly is, the patients limiting their own diets, but the disease originates in well-fed individuals. In this respect an outbreak of "acute" sprue that occurred among "the Chindits" in the Burma campaign is of considerable interest. These men, picked British troops, existed for weeks on a ration that was only meant for use in a short emergency. After a very short time, a matter of days, many of the men developed nausea and vomiting and practically all complete loathing of the ration and in a large proportion of the men the picture of advanced sprue developed in a matter of eight weeks (Keele and Bound, 1946). This history differs markedly from the usual one in a case of sprue and further work is necessary to fit these cases into the sprue picture. Many other cases were also reported from East Asia Command by these and other workers.

Clinical Features

It is not proposed to discuss these in detail but merely to point out the striking differences in the three conditions. A long experience of numerous cases of pernicious anemia, nutritional macrocytic anemia and tropical sprue as seen in India, makes it difficult to consider the three conditions as variants of the same disease entity. As already mentioned however, it must be borne in mind that the same pathogenic agent produces widely differing clinical pictures in individuals whose genetic makeup varies, who live under diverse conditions of climate, diet, housing, etc., and who have suffered from different stresses and strains all their lives. The disease entities may on the other hand be deceptively similar when one particular symptom, for example anemia, dominates the picture. Disregarding for the moment
the atypical cases, which may perhaps be the clue to the interrelationship of this group of clinical entities, let us consider typical examples of each disease and compare them one with another.

In pernicious anemia the patient usually presents as a well-covered, slightly lemon yellow colored, middle-aged or old individual of European descent, often already showing signs that the nervous system is involved. Very different is the picture in nutritional macrocytic anemia. The age of the patient varies from the late teens to relative old age; in India I never saw a case in a young child, but Giglioli (1934) reports 6 cases out of 51 cases below the age of 12 years, the youngest being 11 months old. The patient is commonly emaciated but in British Guiana, Macedonia and Calcutta, all areas where this anemia occurs in a population which has a high malaria rate, emaciation is not such a marked feature. Some degree of edema is common as in all severe anemias but it is sometimes extreme, occasionally from associated beri beri or hunger edema. As far as a dark skin will permit one to judge, the patient is not jaundiced, has clear conjunctivae and, with the exception of the prisoners of war, has no signs of associated disease of the central nervous system. In Bombay, where the nutritional anemia is extremely common, an occasional case may be frankly jaundiced but nearly always this was found to be associated with syphilis or malaria. In highly malarious areas such jaundiced cases are frequent. The sprue case, generally of European descent and frequently a middle-aged individual, is emaciated but with a distended abdomen often with visible peristalsis; the skin is greyish or a dirty yellow color. The signs and symptoms of other deficiencies such as tetany or purpuric manifestations may be present.

One sign which, together with the anemia, has been taken to indicate a close relationship between these conditions is diarrhea. This may occur in pernicious anemia, but is not a very constant finding and is often controlled by hydrochloric acid alone. In nutritional macrocytic anemia the occurrence of diarrhea is of considerable interest. In certain years in Bombay it was not a common complication though severe cases of nutritional macrocytic anemia occurred; in other years it was noted that diarrhea, often associated with a typhoid or hectic temperature which led to the isolation of the patient, was a very frequent complication. No specific organism could be isolated from the stool and the whole syndrome cleared on marmite or a crude liver extract and did not tend to relapse (Wills, unpublished). Giglioli (1934) reported diarrhea in only a few cases, whereas Napier (1941) reported a significant positive correlation between macrocytic anemia and diarrhea, an incidence of 42 per cent in 45 pregnant cases. A very similar sequence of events is seen in monkeys rendered anemic by faulty feeding; the anemia which is apparently the counterpart of the human condition might develop to an extreme degree without intestinal symptoms appearing or another time it might be associated with severe diarrhea which, with the anemia, improved immediately, as in the human cases, on treatment with active preparations of liver or yeast. In the animal cases too no specific organism could be isolated from the stools (Wills, unpublished). In returned Indian prisoners of war with a high incidence of nutritional macrocytic anemia, diarrhea was rarely complained of and was present in only 5 per cent of those requiring hospital treatment (Walters et al., 1947). In sprue,
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which commonly develops insidiously but may develop suddenly, sore tongue and diarrhea dominate the clinical picture. The type of stool is characteristically bulky, greasy, frothy and pale, differing from that seen in the diarrhea complicating nutritional macrocytic anemia of men and monkeys, where it is usually more watery and neither so pale or so frothy. But atypical enteric stools may occur in the acute phases of sprue. The sore tongue, which also occurs in pernicious anemia and nutritional macrocytic anemia is a far more constant feature in sprue.

Nervous lesions other than signs of neuritis are lacking in both nutritional macrocytic anemia and tropical sprue. In Bombay in several hundred cases signs or symptoms of subacute combined degeneration were absent and Fairley (1936) reports the same absence in 450 cases of sprue seen by him personally. Ashford (1932) in a review of 3,000 cases of sprue does not mention any signs or symptoms of this complication (quoted by Fairley, 1936).

PATHOLOGY AND BIOCHEMICAL FINDINGS

Since the introduction of liver therapy the uncomplicated case of pernicious anemia seldom comes to postmortem, but such was not the case previously and both the older pathologists and the older literature can give a detailed account of the findings. Details of the pathology of nutritional macrocytic anemia are not available owing to the difficulty of obtaining permission for postmortems in such cases. It is also regrettable that most of the little material that is available came from cases living in areas where malaria is endemic and which were all examples of so-called “hemolytic nutritional macrocytic anemia” (Fairley, 1938); these cases differ in important respects from uncomplicated ones, which for clarity will be referred to as nonhemolytic nutritional macrocytic anemia.

At postmortem the body in a case of pernicious anemia is usually that of a well-nourished, middle-aged or elderly man or woman; the skin and sclerotics and particularly all the fatty tissues are a bright lemon yellow color and there is an excess of fat in and around the organs. In contrast in nonhemolytic nutritional macrocytic anemia and in sprue the body is usually emaciated, fat being conspicuously absent from all the organs and the characteristic lemon yellow color of pernicious anemia is also missing; all the organs are extremely pale. A further feature of these two diseases is the great reduction in the size and weight of the organs, especially the heart and liver, which is in contrast to the findings in pernicious anemia where the organ weight is not reduced and may be increased. Fairley (1930) thinks this decrease in organ weight may be of diagnostic significance. At the postmortem of a case of nonhemolytic nutritional macrocytic anemia, a male of about 23 years of age, the body weight was found to be under 6 stone, though he was of average height for an Indian and the heart weight was only 140 grams (Wills, unpublished). Mackie and Fairley (1929) report a heart weighing only 90 grams in a case of sprue that came to postmortem. In contrast are the findings in the hemolytic type of nutritional macrocytic anemia; in two incomplete postmortem examinations, on a pregnant woman and on a woman who had just been delivered respectively, (Fairley et al., 1938), the bodies were relatively well-nourished, subcutaneous fat was plentiful and of the same bright lemon yellow
TYPES OF MACROCYTIC ANEMIA

color as is seen in pernicious anemia. The organs, particularly the liver and spleen were enlarged and the heart, examined only in one case, showed some fatty degeneration.

The hemopoietic organs and the blood picture are of particular interest in the three diseases. The classic picture of a panhemopoietic dystrophy characterized by a megaloblastic erythropoiesis, a similar disturbance in the myeloid series with pathologic macro-myeloid cells and a reduction in number and abnormality in type of the thrombocytes, is present in all three conditions and the general opinion is that the changes in the cells in marrow and blood are identical in the three diseases. But both in sprue (Mackie and Fairley, 1929) and in nutritional macrocytic anemia (Balfour, 1927, Mitra, 1931, Wills, unpublished) examination of the tibia may show an aplastic marrow with a curious gelatinous appearance in the shaft, though in other cases the red marrow may extend from end to end of the bone. In nutritional macrocytic anemia in monkeys the tibial bone marrow may show similar red and gelatinous changes with a megaloblastic hyperplasia (Wills and Stewart, 1933). The detailed picture in the bone marrow revealed by sternal puncture preparations varies from case to case with the severity of the anemia and with complicating factors, but the essential pathology is the same and the changes resulting from adequate treatment with liver or folic acid are also the same. But there is a remarkable difference in the blood condition which has not been adequately stressed. In true pernicious anemia in relapse there is some factor constantly present which causes an increase in the serum bilirubin which gives rise to the characteristic coloring of the skin, sclerotics and of the body fat and also to an increased output of urobilin or urobilinogen in the urine and feces. Fairley (1941) has also shown the presence of methemalbumen in the plasma, which is taken as evidence of intravascular hemolysis. In uncomplicated cases of tropical nutritional macrocytic anemia in relatively nonmalarious areas and in uncomplicated cases of sprue the findings are in marked contrast to those in pernicious anemia in relapse and in the hemolytic type of nutritional macrocytic anemia as shown in table 1.

Earlier figures, lost during the blitz, from a larger series of cases of nonhemolytic nutritional macrocytic anemia gave similar findings, the mean figure for 50 cases being 0.33 mg. per 100 ml. Only 1 in 36 cases had urobilin or urobilinogen in excess in the urine. In the hemolytic type of nutritional macrocytic anemia seen in Macedonia the serum bilirubin in the cases reported by Fairley and colleagues (1938) was markedly raised, the mean figure being approximately double that of the author’s series of untreated cases of pernicious anemia (see table 1). In an earlier series of 48 cases of sprue seen in Bombay by Fairley only 3 had bilirubin values above 0.6 mg. per 100 ml. In a series of cases of ‘sprue’ seen in Puerto Rico (Castle et al., 1932) the icterus index was determined in 89 individuals; in 24 it was above 6 units, which was almost as great an incidence of a raised value as in the same authors’ series of cases of pernicious anemia. It is difficult to assess these figures as Puerto Rico is a malarious area. The difference in the figures for serum bilirubin and urinary urobilin or urobilinogen in the different clinical entities suggests that in untreated pernicious anemia and in the hemolytic type of nutritional macrocytic anemia there is a hemolytic factor which is absent in uncom-
plicated nutritional macrocytic anemia and sprue. The postmortem findings, though these are scanty in nutritional macrocytic anemia, are of interest in this connection.

The characteristic deposits of iron found in the liver, spleen, bone marrow and kidneys in pernicious anemia are not considered by most recent workers to be an index of increased hemolysis but rather of an inability of the blood forming organs to deal with the iron liberated by normal blood destruction (Minot and Strauss, 1943). But the iron in the kidneys is deposited within the cells of the excreting tubules, particularly in the proximal tubules, the glomeruli containing no iron pigment. Muir and Young (1941) have shown that large amounts of hemosiderin may be deposited in the cells of the tubules as a result of a hemolysis insufficient in degree to cause hemoglobinuria, and they suggest that these deposits in the kidney in pernicious anemia are due to such a hemolysis. Evidence in support of this view is the presence of methemalbumen in the plasma (Fairley, 1941). There are insufficient adequate postmortem studies to compare the hemosiderin deposits in complicated nutritional macrocytic anemia and sprue with those in pernicious anemia. The size of the liver and spleen are of some interest in this connection.

In pernicious anemia both organs are commonly moderately enlarged and show the characteristic deposits of hemosiderin and may show areas of extra medullary blood formation; fatty changes are very marked in the liver. In nutritional macrocytic anemia the size of the liver and spleen appears to vary with the geographic locality and the malarial infection rate. In Bombay, particularly in the male cases, these organs were rarely palpable and in the 2 postmortems the size of the organs was reduced; in one case the liver weighed only 630 grams and the spleen 220 grams; both organs gave a positive Prussion blue reaction. In some of the female patients both organs were just palpable. In Calcutta, British Guiana and Macedonia, all highly malarious areas, the findings differ markedly. Napier (1941) using pregnant cases, reports "a very marked association" between splenomegaly and severe macrocytic anemia, and in 6 out of 44 cases the spleen was below the navel.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No.</th>
<th>Mean mg. per 100 ml.</th>
<th>S.D.</th>
<th>C.V.</th>
<th>Range mg. per 100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal*</td>
<td>M &amp; F</td>
<td>100</td>
<td>0.539 ± 0.0247</td>
<td>0.247</td>
<td>45.9</td>
<td>0.1 – 1.7</td>
</tr>
<tr>
<td>Pernicious anemia in relapse†</td>
<td>M &amp; F</td>
<td>27</td>
<td>1.059 ± 0.1336</td>
<td>0.694</td>
<td>65.47</td>
<td>0.4 – 2.5</td>
</tr>
<tr>
<td>Nutritional macrocytic anemia</td>
<td>M &amp; F</td>
<td>42</td>
<td>0.516 ± 0.0375</td>
<td>0.243</td>
<td>47.1</td>
<td>0.1 – 1.0</td>
</tr>
<tr>
<td>Hemolytic N.M.A.§</td>
<td>F. pregnant</td>
<td>37</td>
<td>2.0</td>
<td></td>
<td></td>
<td>0.7 – 4.2</td>
</tr>
<tr>
<td>Tropical sprue‖</td>
<td>M &amp; F</td>
<td>20</td>
<td>0.37</td>
<td></td>
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† Wills, L.: 27 consecutive cases seen at the Royal Free Hospital (unpublished).
§ Fairley, N. H. et al.: 1938. Consecutive cases of Nutritional Macrocytic Anemia (hemolytic type) in pregnant women seen in Macedonia.

S.D. = Standard deviation. C. V. = Co-efficient of variation.
In most of the cases with enlarged spleens the liver was also enlarged and there was a definite correlation between a raised Van den Bergh and enlargement of these organs. There was also a strong suggestion that there is some association between a positive Wassermann reaction and a macrocytic anemia. Giglioli (1934) found the spleen enlarged, generally to below the umbilicus, in 94 per cent of his cases of macrocytic anemia and associated in the majority of cases with a raised serum urobilin. There was a marked positive correlation between the spleen and parasite rate and the incidence of the anemia, which suggests, he thinks, that chronic malarial infection is "a factor of very considerable importance" in the etiology of nutritional macrocytic anemia. In Macedonia the vast majority of the cases had grossly enlarged spleens.

In 2 cases of nutritional macrocytic anemia dying after parturition, postmortem examination (Balfour, 1927) showed very slightly enlarged spleens which, with the livers, gave a positive Prussian blue reaction. In the 2 cases of hemolytic nutritional macrocytic anemia in pregnant women examined postmortem by Fairley and colleagues the liver was very enlarged in 1 case with nutmeg changes from heart failure, and moderately enlarged in the other; both livers showed marked hypertrophy of the reticulo-endothelial cells with swollen Kupffer cells, phagocytosis and proliferation in the sinusoidal system. Malarial pigment was present in some of the Kupffer cells and hemosiderin in the liver cells, particularly in the outer zone of the lobules. The spleen was enlarged and hard in both cases (25 and 22.7 ounces respectively) and showed a hyperactive reticulo-endothelial system with some malarial pigment in the cells and a reduction in the lymphoid tissue; the hemosiderin was less than in the liver. The kidneys gave a negative Prussian blue reaction. Mitra (1931) found similar changes in material from cases in Calcutta which were probably hemolytic, but in his cases there was a fatty degeneration of the central part of the lobule with extravasation of blood.

The other systems of particular interest are the alimentary and nervous systems. In all three conditions the tongue may show characteristic changes but these may be absent and are not specific, as very similar changes occur in microcytic anemia and in pellagra. Abnormalities of the stomach and intestines may be present in all three diseases and are of fundamental importance in pernicious anemia. Many workers, particularly Castle et al., (1935) have compared the gut changes in pernicious anemia and "sprue" with those seen in pellagra and have stressed the atrophic tongue changes and the diarrhea, and the similar effect of treatment on these states in all three conditions. The dramatic effect of folic acid on the intestinal symptoms in pernicious anemia, nutritional macrocytic anemia and sprue also suggests that the lesions of the alimentary tract are similar in all three conditions. But though this may be true of the final state of the fully developed disease, the basic pathology would appear to be different in the three entities. The classic work of Magnus (1938) and Meulengracht (1939) has shown that the fundamental change in pernicious anemia is an atrophy, possibly genetic, of the mucosa of the fundus of the stomach and it is this lesion which appears to lead, by the production of an abnormal gastric juice, to a failure in the supplies of the liver factor or factors necessary for proper hemopoiesis and for the good health of the central nervous
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system. Jacobson (1939) would correlate the presence of similar hemopoietic properties to those of liver in dessicated stomach and small intestine to the presence of argentaffin cells, which are markedly reduced in pernicious anemia, both in the atrophic gastric mucosa and in the intestine. The characteristic changes in the gastric mucosa seen in all true cases of pernicious anemia are absent from the gastric mucosa of monkeys suffering from nutritional macrocytic anemia (Magnus—from a study of our material—unpublished) so presumably they are also absent from the stomach in human nutritional macrocytic anemia. The presence of free hydrochloric acid in normal amounts would also suggest that the mucosa is undamaged. Suitable material for the study of possible changes in the gut in nutritional macrocytic anemia is not available but from analogy with animal material there is probably a thinning of the gut wall due to the general emaciation and little else in the small intestine. Nonspecific ulceration of the large intestine was seen in one tropical case that came to postmortem (Wills, unpublished). The importance, in the examination of the intestines, of fixation immediately after death is well illustrated in the postmortem reports on cases of sprue. In 1929 Mackie and Fairley reported changes in the small intestine which they considered “begin as an inflammation but pass on to degenerative changes” (Fairley, 1930). More recent work (Mackie and Fairley, 1934) on material fixed immediately after death has failed to reveal any pathologic change except slight congestion of the margins of the valvulae conniventes and these authorities consider that the changes in the gut which result in such profound metabolic disturbances are functional and not pathologic. Hanes (1942) confirmed this absence of pathologic change, except extreme emaciation, in 4 fatal cases of sprue. Koppisch (Suarez et al., 1947) reported the post-mortem findings in 16 cases of “sprue” in Puerto Rico. He found evidence, but it may have been a postmortem artifact, of chronic gastritis in all but 3 cases and moderate atrophy of the gastric mucosa in half the cases. Gastroscopic examination confirmed the presence of atrophy of the gastric mucosa (Rodriguez-Olleros, 1938, Hernandez-Morales, 1944), but Rodriguez-Olleros considers that this atrophy results from the disease and does not precede it, as the atrophy was only found in fully developed cases. Hernandez-Morales found that after treatment the mucosa in many cases became normal again. Koppisch also reported a definite shortening and blunting of the villi of the small intestines, with an associated increase in the number of plasma cells in the tunica propria in half the cases examined. As in nutritional macrocytic anemia in monkeys and man, inflammation and nonspecific ulceration were found in the colon of the majority of the cases.

The nervous lesions of subacute combined degeneration of the cord, so characteristic of pernicious anemia, are rarely, if ever, seen in true tropical sprue or nutritional macrocytic anemia. Peripheral neuritis may occur in all three conditions but is an inconstant finding due to an associated dietary deficiency in the vitamin B complex in the nutritional cases and probably to conditioned deficiency in the case of sprue.

Chemical examination of the gastric juice in the three conditions shows a complete and persistent histamine resistant achlorhydria associated with achylia and absence of the intrinsic factor in true pernicious anemia; in nutritional macrocytic
anemia and sprue the gastric acid varies from hyperchlorhydria to hypochlorhydria and achlorhydria, the last being more frequent in cases of sprue than in cases of nutritional macrocytic anemia. The amount of intrinsic factor present in the gastric juice of such cases is also variable, being absent in some cases of sprue (Castle et al., 1935) and in certain cases of nutritional macrocytic anemia associated with pellagra (Moore et al., 1944). It has not been possible to test the gastric juice of cases of uncomplicated tropical nutritional macrocytic anemia, as no cases of true pernicious anemia were available in Bombay for test purposes.

Various biochemical tests are now used for the diagnosis of sprue, the most important being the fat content of the stool, the fat absorption test and blood lipid curves and the oral glucose tolerance test. It is doubtful whether any of these can be considered to give specific diagnostic results as similar findings to those usually obtained in sprue cases are also found in other conditions, such as multiple vitamin B complex deficiencies, but in conjunction with a typical history and clinical picture these tests can confirm the diagnosis. In sprue the stools are typically bulky, pale and fermenting, the color being commonly pale but dark colored stools being not uncommon (Black, 1945). The stools contain an excess of fat, the greater part of which is split. Fat balance experiments show a decreased absorption and though after treatment the diarrhea may be controlled and the percentage of fat in the stools decreased, fat absorption is still defective and it may be some considerable time before it improves (Black et al., 1946, Davidson et al., 1947). In nutritional macrocytic anemia the stools frequently appear normal (Wills, unpublished, Walters et al., 1947). Fairley and colleagues (1938) give figures showing a low fat content with normal ratio of split to unsplit fat. Such values would be expected in most of the cases as poverty limits the fat intake. A dietetic survey in Bombay among the families of patients with nutritional macrocytic anemia showed that the average daily consumption per adult was 45 Gm., of which 20 Gm. was animal fat (Wills and Talpade, 1930). Cook (1944) and Chandhuri (1944) have described a spruelike condition in the civil population in India associated with macrocytic anemia and diarrhea but the diarrhea was watery and not fatty in both series. Walters (1947) and Girdwood (Davidson et al., 1947) however, describe a steatorrhea in a deficiency syndrome resembling sprue in Indian soldiers. In certain of these cases the diarrhea improved with nicotinic acid and in many sulphaguanidine controlled it, suggesting an underlying infective condition (Marriott, 1945, Chandhuri and Chandhuri, 1944).

The typical flat oral glucose curves that occur in sprue are not found in the uncomplicated case of nutritional macrocytic anemia (Fairley et al., 1938) but in those cases associated with a spruelike syndrome flat curves are found in a few instances (Chandhuri and Chandhuri, 1944).

**Treatment**

Since the epoch making discovery of Minot and Murphy of the therapeutic activity of liver in pernicious anemia, treatment of this and other allied macrocytic anemias has involved the use of different liver extracts, with stomach preparations, and in the nutritional cases with various so-called sources of Castle's extrinsic
factor. But until recently, when the discovery of the hemopoietic activity of folic acid and its conjugated forms at last gave workers a chemically pure active substance, no pure substance with similar activity was available, the various factors postulated in Castle’s theory of the formation of the liver principle and the principle itself having remained elusive. This fact makes the interpretation of the activity or inactivity of different so-called purified preparations difficult, as varying doses mean varying amounts of substances other than the liver principle which, when massive doses are given, may be present in large enough amounts to be active. This may explain certain of the contradictory results reported in the treatment of nutritional macrocytic anemia with some of the more highly purified extracts.

It is not proposed to go into the vast literature on the therapeutic use of liver and stomach extracts and of folic acid but only to deal with those aspects of this work which have a bearing on the etiology of the three conditions under consideration. In the early days of crude extracts, pernicious anemia, nutritional macrocytic anemia and the macrocytic anemia of sprue all responded well to liver preparations. It was originally thought that nutritional macrocytic anemia was due to a lack of Castle’s “extrinsic” factor but doubt was thrown on this explanation when it was found that relatively purified extracts, known to be potent in cases of pernicious anemia in relapse, were completely inactive in the same or larger doses in the nutritional macrocytic anemia of monkeys, though campolon, a very crude liver extract was active curatively in relatively small doses (Wills et al., 1937). This work was confirmed by the same authors (1938) in a series of cases of nutritional macrocytic anemia in Bombay, by Napier (1939) in Calcutta, though he found that large doses of the same “purified” extract as that used by Wills and co-workers was active curatively in a few cases, and by Giglioli in Central America (personal communication). Various workers in different parts of the world have shown that “purified” extracts may be active in certain types of nutritional macrocytic anemia, as for example that occurring as a complication of pellagra (Moore et al., 1944) and in enormous doses in the hemolytic type in women, particularly in pregnant women in Macedonia but not in men in the same area (Fairley et al., 1938, Foy, 1939). Cases of nutritional macrocytic anemia also respond to marmite (autolysed yeast) and other so-called good sources of the extrinsic factor when given by mouth. Recently Castle and co-workers (Watson and Castle, 1946) have shown that more than one type of nutritional macrocytic anemia occurs: one that responds as pernicious anemia does to the highly purified liver extracts given parenterally in normal doses; another that responds to an unknown factor, “Wills’ factor” as Castle calls it, present in crude liver extracts and yeast, given either orally or parenterally, but not to purified liver extracts given parenterally; and finally one that responds to “purified” liver extracts given parenterally when the dose is increased tenfold. Such an enormous dose might contain sufficient “Wills’ factor” to produce a remission.

In sprue the macrocytic anemia has been shown to respond to marmite by mouth, to crude liver extracts by mouth and parenterally and also in many cases to purified liver extracts parenterally. A high protein diet increases the hemopoietic effect.

Finally folic acid has been shown to produce remarkable hemopoietic responses
in all three diseases. In pernicious anemia folic acid, either parenterally or orally, induces in the vast majority of cases a maximal reticulocyte response followed by an immediate rise in the red and white cell counts. The dose necessary to produce this effect is 5 to 10 mg. daily or a single dose of 100 mg. Jacobson (1947) by incubating folic acid with the enzyme xanthopterase, has thereby enormously enhanced the hemopoietic activity of the folic acid, and he suggests that by this means folic acid has either been converted into Castle liver principle itself or into another compound with great hemopoietic activity. It is of interest in this connection to note that both the cases of pernicious anemia treated with the incubated material showed a steady rise in the red cell count and hemoglobin percentage to normal levels, the count reaching the 5.0 million level and the hemoglobin a corresponding one. This is in contrast to most workers' experience with folic acid; they find that often, after an excellent initial response, it is impossible, even with increasing doses, to get or maintain the blood at really optimal levels (Wilkinson, 1947, Davidson and Girdwood, 1947, Goldsmith, 1947, Meyer, 1947).

Folic acid produces in all three of the anemias under consideration an immediate sense of well-being, of the same order as that produced by an active liver extract. But again in the treatment of nutritional macrocytic anemia the blood fails to reach normal values and macrocytosis persists; this is particularly so in cases of nutritional macrocytic anemia with diarrhea and also in cases of sprue, though the general clinical improvement is remarkable (Davidson et al., 1947, Morrison and Johnston, 1947, Suarez et al., 1947). It also has a miraculous effect in controlling the diarrhea of sprue and nutritional macrocytic anemia, though analyses have shown that in spite of the steatorrhea being decreased there is no immediate alteration in fat absorption (Suarez, Spies and Suarez, 1947, Davidson et al., 1947, personal cases).

In contrast to the general dramatic improvement is the complete ineffectiveness of folic acid treatment in arresting or preventing the development of symptoms of subacute combined degeneration (Spies and Stone, 1947, Wilkinson, 1947). The significance of these findings will be reviewed in the discussion on etiology of these three diseases.

In brief it can be said that pernicious anemia, including the symptoms of subacute combined degeneration can be successfully treated and health maintained with crude or purified liver extracts given parenterally or orally and proteolysed liver extract by mouth, by different preparations of hog's stomach by mouth and by digests of beef muscle or autolysed yeast with normal human gastric juice. Folic acid and its conjugated forms (Spies et al., 1947) produces a remission, which is often suboptimal, of the hematologic symptoms, an immediate sense of well-being but no effect on the nervous symptoms; the hematologic effect is said to be enhanced by incubation with xanthopterase.

Nutritional macrocytic anemia, both the nonhemolytic and hemolytic types seen in endemic form in many tropical countries, responds to crude liver extracts parenterally or by mouth and to autolysed yeast extracts (Wills, 1938, Napier, 1939). Relapses do not take place after cessation of treatment if the diet is improved. Folic acid has, in the few cases reported, the same action as in pernicious
anemia (das Gupta and Chatterjee, 1946).* The hemolytic type seen in Macedonia is very resistant to treatment but some cases respond to enormous doses of both crude and purified liver extracts and to very large oral doses of marmite (Fairley et al., 1938, Foy and Kondi, 1939).

The treatment of sprue has been studied by Fairley (1936) in cases largely from India and the Far East, by a group of workers in Cuba and Puerto Rico (Spies et al., 1946-47) and by the service authorities in India and the Far East where the disease was of relatively rapid onset (Leishman, 1945, Maegraith et al., 1945, Keele and Bound, 1946). All agree that for optimal improvement a high protein diet with liver extract or folic acid are required. In the critically ill, blood transfusion may be necessary and in the service cases sulphaguanidine often controlled the diarrhea. The high protein diet leads to an improved nutrition, as the absorption of protein does not appear to be affected.

**Pathogenesis and Discussion**

Our present knowledge of the three clinical entities described supports, in the author's opinion, the view that they are three distinct separate diseases, with essentially different natural histories and pathological pictures. It is now proposed to discuss the evidence for this belief in the light of the facts set out in the previous paragraphs. Authorities will not be quoted when they have already been given. It is proposed to limit this discussion to the classic conditions as generally understood. Pernicious anemia is a well recognized disease in which a persistent achylia gastrica is a diagnostic feature. The title nutritional macrocytic anemia is limited to the disease as seen in endemic areas but will include sporadic cases occurring in other parts of the world, but to simplify the discussion the sporadic cases of 'pernicious' or 'macrocytic' anemia of pregnancy will not be considered, as it is felt that this group probably includes several different entities. Tropical sprue is less well defined and recent experience in the services has led to the inclusion under this title of certain relatively acute conditions closely resembling sprue, but not yet sufficiently worked out to be definitely included under the title of "tropical sprue," which for the purposes of this discussion will be limited to the classic disease as described by workers from Hillary in 1759 to Fairley in 1938. The service cases and 'sprue' as seen in Puerto Rico will be discussed in relation to the classic picture.

A consideration of the geographic, ethnic, and social distribution of these three diseases leads to the conclusion that they are three separate entities. Pernicious anemia is a familial disease of persons of European descent, with certain very definite genetic characteristics, the distribution of the disease corresponding to that of the racial groups affected, chiefly fair and Nordic peoples. Individuals of all classes are affected. In marked contrast is the distribution of nutritional macrocytic anemia. This disease occurs mainly in tropical and subtropical lands, but the

* Since the writing of this paper, Kemp (Lancet 2: 351, 1947) has reported 3 cases of nutritional macrocytic anemia who showed remarkable improvement with folic acid but were not studied long enough to show whether the blood level would have reached completely normal values and whether the macrocytosis, still present with red cell counts at the 4 million level, would have disappeared.
distribution is associated with poverty, a low calory, largely or entirely vegetarian diet, with pregnancy and lactation and also with certain diseases such as syphilis and particularly chronic malaria, which result in a hypertrophied reticulo-endothelial system. It also occurred in vegetarian Indian troops under the stress of service conditions and in Indian prisoners of war. An anemia considered the animal counterpart of the human disease can be produced in monkeys by feeding with a diet based on one in common use among sufferers from this disease. Pregnancy and lactation are known to increase the maternal requirements and these conditions will convert a latent deficiency into an overt one. The relation of chronic malaria to this anemia will be discussed later. These findings strongly suggest a direct nutritional origin, in other words that this anemia is an unconditioned deficiency state. The fact that this anemic syndrome is often associated with other symptoms including diarrhea, often watery but sometimes fatty, referable to a vitamin B2 deficiency supports the view that the anemia is a deficiency state. The geographic, ethnic, and social distribution of tropical sprue presents a much more difficult problem. Classic sprue has generally a gradual onset and is associated with residence in a warm climate but affects Europeans rather than pure Indians or Negroes. The nature of the illness, which is generally afebrile throughout, throws little light on the etiology but the result of treatment suggests a deficiency state. Though this certainly exists in the fully developed syndrome there is little evidence for a nutritional origin for the altered intestinal absorption which conditions the deficiency. A more detailed examination of the distribution of the condition, the heavy incidence in certain areas in the large endemic zones and even in certain houses, combined with the fact that very many sprue patients come from the ranks of the well-fed, suggests a possible infective agent as the primary cause but this is purely conjectural. A consideration of the outbreaks of acute sprue in the service is of interest in this connection, though further study is necessary before these can be definitely considered the same clinical entity as classic “tropical” sprue. Sprue in service personnel showed the same concentration in certain districts and sometimes in certain camps; explosive outbreaks which assumed epidemic proportions in certain areas also occurred. Leishman (1945) reports from Chittagong that nine separate units were affected, some with a 50 per cent attack rate and one R.A.F. unit had 10 per cent of its personnel down with diarrhea three weeks after its arrival in India, which diarrhea rapidly turned to the sprue syndrome. These findings are very strongly suggestive of an infective origin for this type of sprue.

A study of the pathologic and biochemical findings in these diseases supports this idea of their essential individuality. The pertinent findings in pernicious anemia are the atrophic changes in the fundus and cardia of the stomach, with its associated achylia gastrica, the increased plasma bilirubin, the presence of methemalbumen in the plasma, the distribution of iron in the tissues and other evidence of a hemolytic factor in the anemia and the changes in the central nervous system characteristic of subacute combined degeneration of the cord. The atrophy of the gastric mucosa would appear to be the basic defect; it involves all the coats of the stomach wall and results in a complete histamine resistant achlorhydria and an associated achylia. There is no evidence of preceding inflammatory processes and
Magnus (1938) thinks the evidence points to the change being the final stage of an atrophic process, the cause of which is unknown but might be 'the end result of some endocrine or nutritional deficiency or might even be congenital in origin.' The evidence for the genetic factor is the familial and racial incidence and the lack of any indications of an infective, nutritional or endocrine origin. Idiopathic hypochromic anemia, an iron deficiency anemia associated with achylia gastrica also occurs in families subject to pernicious anemia (Wintrobe and Beebe, 1933). In sprue an atrophy of the gastric mucosa has been reported by the Puerto Rico workers but the evidence points to it being secondary to the disease, as it occurs only in the fully developed syndrome. Mackie and Fairley, from a study of specially fixed material, report a normal mucosa. In the nutritional macrocytic anemia of monkeys there is no significant change in the gastric mucosa and presumably the mucosa is normal in the corresponding human conditions. Test meal findings confirm the essential normality of the gastric mucosa in most cases of sprue and nutritional macrocytic anemia. The defect in the gastric mucosa seen in pernicious anemia according to Castle's well known theory produces a lack of his intrinsic factor, a substance with enzymic properties, and it is this deficiency that leads to the failure of the formation of the liver principle. Castle's work has shown the mode of action of this intrinsic factor, but neither it nor the extrinsic factor have been isolated, any more than the liver principle itself.

This defect in the gastric secretion, with its interference with the formation of the liver principle and possibly with another principle essential for the proper functioning of the nervous system, appears to be the basic lesion in pernicious anemia, but unless we accept the view that a certain variable time is necessary for the postulated genetic factor to bring about this gastric atrophy it is necessary to look further for a factor producing this atrophy; from the evidence it seems that there is little to suggest an inflammatory one. This same time factor in the development of the symptoms of pernicious anemia appears to operate in those cases which develop the disease after total gastrectomy, a period as long as ten to fifteen years occurring between the time of operation and the time of development of symptoms (Meyer et al., 1941). This time lag is also unexplained. In this connection Rhoads's (1933) experiments on the production of a syndrome resembling pernicious anemia in hogs by feeding modified black tongue diets are of interest. The deficiency not only produced tongue changes, a macrocytic anemia and nerve lesions, but a histamine resistant achlorhydria; this syndrome, though it resembled that of pernicious anemia, differs from it in that the gastric changes are reversible and the condition could be cured, whereas in true pernicious anemia replacement therapy is always necessary, the primary lesion being irreversible.

Another experiment of interest in this connection is that of Petri (1944) and co-workers; these authors have shown in experiments on dogs and swine that total gastrectomy produces signs of pellagra, due apparently to interference with absorption of nicotinic acid. Furthermore the livers from such pigs were ineffective in the treatment of pernicious anemia. However, nicotinic acid by the parenteral route compensated in these animals for the absence of gastric secretion, the livers from gastrectomized pigs receiving parenteral nicotinic acid being fully effective.
in pernicious anemia in relapse. This experiment indicates one of the factors that may influence the formation of the liver principle and may explain the pathogenesis of those macrocytic anemias which respond to nicotinic acid (Cook, 1944).

The hemolytic process seen in pernicious anemia is of great importance both in a consideration of the essential pathology of the disease and in considering its relationship to nutritional macrocytic anemia and sprue. There is little to explain the increased serum bilirubin and other evidence of increased red cell destruction except that it ceases under appropriate treatment with liver, hog's stomach or folic acid and might, therefore, be thought to be due to the nature of the macrocytes. There is, however, no increased fragility of the red cells and no hemolysis has been demonstrated in the blood stream but there is evidence of active phagocytosis of the red cells in the reticulo-endothelial cells of the bone marrow, liver and spleen, which might account for some of the increased bilirubin in the circulation. But the presence of methemalbuminemia and a positive Schumm test would suggest some additional intravascular hemolysis (Fairley, 1941). With certain exceptions already mentioned, there is no evidence of hemolysis in either nutritional macrocytic anemia or sprue and as the megaloblastic reaction is generally considered identical in all these anemias, it is unlikely that the hemolysis in pernicious anemia is due entirely to the nature of the cell. It would appear more likely that it is due to an association of an overactive reticulo-endothelial system with abnormal cells, though the cause of this hypothetical increased activity is unknown. The evidence of increased hemolysis seen in cases of nutritional macrocytic anemia occurring in areas of endemic malaria supports this view.

Tropical sprue is a dramatic disease in which spectacular pathologic changes in the tissues might be expected but postmortem examinations have failed to reveal any anatomic changes except those in the bone marrow and those of extreme inanition, the loss of fat and shrunken organs. There are no lesions of the nervous system as in pernicious anemia. Histologic examination of material from the gastrointestinal tract fixed immediately after death has shown essentially normal structures. The changes in the gut are functional and not anatomic. In a brilliant review of recent work Stannus (1942) has marshalled the evidence for a failure of phosphorylation of fatty acids, glycerol and glucose being the basic lesion, the point of functional breakdown. There is much evidence in support of this view. Maegraith and colleagues (1945) have shown in cases of spruelike conditions that in the active phase the absorption of glucose is grossly impaired though that of fructose is not, suggesting that there is an impairment of phosphorylation of glucose, although the diffusion of sugars across the membrane is unaffected. Leishman (1945) suggests that the vitamins riboflavin and nicotinic acid are concerned in this process of phosphorylation, in their phosphorylated forms as co-enzyme I and yellow oxidase they take part in cellular metabolism, acting as H acceptors or rejectors and Leishman thinks it is possible that they may catalyse the process of phosphorylation. It is possible that some other member of the vitamin B complex takes part in this process. But if the failure in phosphorylation is due to deficiency of any B vitamin how does this deficiency arise in well-fed people and why did it not occur among prisoners of war in Japanese hands, who suffered so badly from
deficiencies of these vitamins. Leishman points out in this connection that the B vitamins are synthesized in very appreciable amounts in the gut, and that a change of diet or dysentery might alter the balance of the intestinal flora and hence of vitamin synthesis. Work along these lines might be illuminating.

Pathologic and biochemical observations throw little light on the essential pathology in nutritional macrocytic anemia. As in pernicious anemia and sprue the hemopoietic organs show the changes characteristic of a macrocytic anemia but both in nutritional macrocytic anemia and in sprue the bone marrow from the tibia (the bone examined) may show gelatinous changes as well as the extension of the red marrow characteristic of pernicious anemia. Beyond these changes and those due to aninition there are no obvious pathologic lesions. Magnus has shown the presence of a normal gastric mucosa in material from monkeys suffering from nutritional macrocytic anemia. In man the gastric acidity and the blood sugar levels after oral glucose fall within normal limits. There is no evidence of increased hemolysis, except in areas with a high incidence of malaria. Fairley and Foy have given detailed accounts of this type in which the increased serum bilirubin values, a positive Schumm test, the increased urobilin output, the yellow color of skin and body fat are all indications of an increased hemolysis. The spleen is enormously increased in size, frequently the liver also and postmortem material has shown a hyperactive reticulo-endothelial system. Fairley sums up the picture thus: "A reticulo-endothelial system irritated, activated and hypertrophied as a result of repeated malarial infections, phagocytoses these non-parasitized, abnormal corpuscles in considerable numbers, producing a haemolytic anaemia."

The corpuscles are abnormal macrocytes, the result apparently of a marrow rendered megaloblastic by a deficiency of the same nature as that operation in uncomplicated nutritional macrocytic anemia.

It has been suggested in the preceding paragraphs that these three diseases all exhibiting an apparently identical panhemopoietic dystrophy are distinct clinical entities. It can be postulated that this dyshemopoietic anemia probably results from the breakdown of some intracellular enzyme system in which the liver principle plays an important part. The relation of folic acid to this system still awaits solution but the fact that it has such remarkable, if limited, hemopoietic activity in all three conditions, as well as a dramatic effect on the well-being of the patient and on diarrhea if present, shows that it plays an all important role in rectifying the faulty cellular metabolism. The liver principle is active curatively in pernicious anemia but does not seem to be the missing factor in nutritional macrocytic anemia as it has been shown to be inactive in this anemia in man and monkeys in doses equivalent to or greater than those giving maximal responses in cases of pernicious anemia with similar initial blood levels. That this factor, "Wills' factor" as Castle calls it, is not Castle's extrinsic factor follows from the fact that the "liver principle" in purified preparations is inactive in nutritional macrocytic anemia. Recent work on the relationship of folic acid to vitamin M deficiency in monkeys (Day, et al., 1945 and 1946, Wilson, et al., 1946) suggests that possibly folic acid is the missing factor. It is possible that "Wills' factor" is an activator or co-enzyme in an enzyme system in which the liver principle plays the important part.
Other factors may also be concerned and a deficiency in any one of these might produce a similar failure in the function of the liver principle.

Finally it is interesting to consider these three entities from the point of view of preventive medicine. If a genetic defect is the ultimate cause of pernicious anemia then only selective breeding, at present a Utopian and risky measure, can eradicate it. If, as seems highly probable, nutritional macrocytic anemia is a deficiency disease due to a lack in the diet of some factor associated with good biologic protein, then improved economic conditions with the improved diet that always goes with them should eradicate the disease, except in those cases where religion limits the diet when only a new revelation can assist. But the problem of sprue awaits further work, as until we know the nature of the original cause of the intestinal breakdown it is impossible to take preventive measures.

SUMMARY

The following tentative conclusions as to the relationship of pernicious anemia, nutritional macrocytic anemia and tropical sprue to one another and their pathogenesis are drawn from a study of the literature and from unpublished work:

1. That these three clinical conditions are three distinct entities possessing a common characteristic in the presence of a panhemopoietic dystrophy characterized by a megaloblastic erythropoiesis and corresponding changes in the myeloid cells and platelets.

2. That this panhemopoietic dystrophy possibly results from the breakdown of an intracellular enzyme system but that the deficiencies causing the breakdown differ; in pernicious anemia the liver principle is apparently at fault, in endemic nutritional macrocytic anemia another unidentified factor is missing, in sprue either or both may be at fault.

3. Folic acid is active therapeutically in all three diseases, but in all it generally fails to restore completely normal blood levels.

4. Pernicious anemia is probably due to a genetic defect which produces an atrophy of the gastric mucosa. As a consequence of this interference with gastric function there is a failure in the formation or absorption of the liver factor and possibly of another neurotrophic factor, which failure results in the development of a macrocytic megaloblastic anemia and the characteristic changes in the nervous system. Indefinite replacement therapy is necessary as the changes in the gastric mucosa are irreversible. The cause of the increased hemolysis is unknown.

5. Endemic nutritional macrocytic anemia is an unconditioned food deficiency, the deficiency being in a factor other than the liver principle, possibly a co-enzyme present in or associated with good biologic protein and the vitamin B<sub>12</sub> complex. There are no characteristic pathologic changes except those of the hemopoietic organs which are not specific to the disease. A hemolytic type of the disease occurs in areas of high malarial incidence. After successful treatment the disease does not relapse if the diet is satisfactory. Pregnant women are particularly liable to develop the disease.

6. Tropical sprue is due to a functional disorder of the intestine, possibly primarily a failure in phosphorylation of fatty acids, glycerol and glucose. Diarrhea
with characteristic stools and a macrocytic anemia are characteristic findings. The macrocytic anemia may be due to a failure in absorption of one or more essential hemopoietic factors or to a lack of Castle's intrinsic factor. The cause of the functional breakdown is unknown. Treatment is with a high protein diet and liver extracts. Relapses are common.

My thanks are due to my colleagues for carrying on my work while I wrote this paper.

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PERNICIOUS ANEMIA, NUTRITIONAL MACROCYTIC ANEMIA, AND TROPICAL SPRUE: A DISCUSSION

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