THE MEGALOBLASTIC ANAEMIAS

With special reference to Treatment with Folic Acid and Vitamin B_{12}

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There are few facets of haematology of more interest to the clinician than the megaloblastic anaemias, ranging as they do from infancy to old age and embracing a number of conditions of widely differing aetiology. In some the pathogenesis of the anaemia is not understood fully, but in all there is a breakdown in the supply of substances which are essential for the normal maturation of the red blood cells.

The concept that megaloblastic change in the bone marrow was the result of a nutritional deficiency was established by the classical work of Minot and Murphy (1926) and Castle (1929). According to Castle, a substance in the food (extrinsic factor) combined with a factor present in normal gastric juice (intrinsic factor) and as a result of the interaction of the two the active haemopoietic principle was formed.

The isolation of vitamin B_{12} from liver (Smith, 1948; Rickes *et al.*, 1948) has not altered the fundamental nature of Castle's observations, for recent work has shown that vitamin B_{12} is probably identical with Castle's extrinsic factor (Berk *et al.*, 1948). Whether it is also identical with the haemopoietic principle is still not certain, for, although it is active when given parenterally, it may have to be altered in some way by a substance present in the body before it can become haemopoietically active.

The isolation of folic acid (Mitchell *et al.*, 1944) and the demonstration that it will restore normal erythropoiesis in all forms of megaloblastic anaemia has shown that a second dietary factor is necessary for normal blood formation. It is clear that a deficiency of either vitamin B_{12} or folic acid can lead to the development of a megaloblastic anaemia, but the exact way in which the two are related remains unknown.

The part played by a deficiency of either of these two vitamins in the pathogenesis of the megaloblastic anaemias is variable (Fig. 1). In true pernicious anaemia and after total gastrectomy lack of intrinsic factor leads to a deficiency of vitamin B_{12}. The fish-tapeworm anaemia probably is also the result of deprivation of this vitamin. All the other megaloblastic anaemias are usually the result of a deficiency of folic acid, but in any one of them there may be an associated deficiency of vitamin B_{12}.

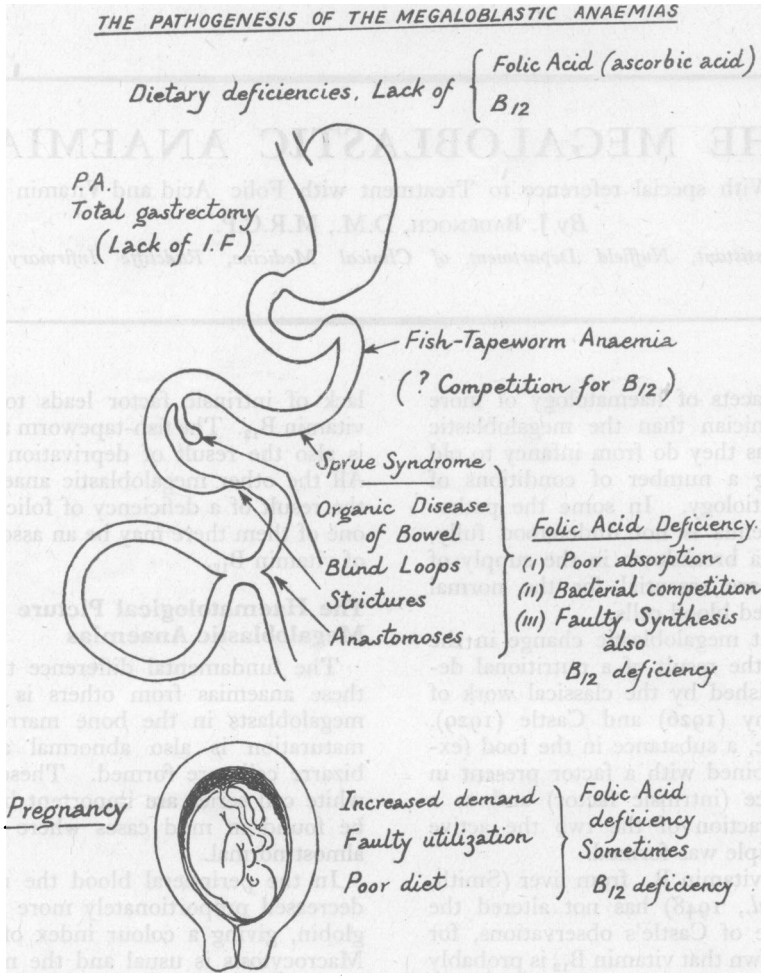
The Haematological Picture of the Megaloblastic Anaemias

The fundamental difference that distinguishes these anaemias from others is the presence of megaloblasts in the bone marrow. White cell maturation is also abnormal and many large bizarre cells are formed. These changes in the white cell series are important because they may be found in mild cases where erythropoiesis is almost normal.

In the peripheral blood the red cell count is decreased proportionately more than the haemoglobin, giving a colour index of more than 1.0. Macrocytosis is usual and the mean cell volume (MCV) is increased (normal range 82 to 92μ^3). The amount of haemoglobin in each cell is increased, but, because the cells are large, the mean cellular haemoglobin concentration (MCHC) is no greater than it is in normal blood (32 to 36 per cent.). When the anaemia is severe megaloblasts may be found in the peripheral blood and leukopenia with thrombocytopenia is common. Many of the granulocytes may be abnormally large and show hypersegmentation of their nuclei.

Some patients with a megaloblastic anaemia have an associated deficiency of iron and this may alter the picture in the peripheral blood radically. The presence of numerous small cells, poorly filled with haemoglobin, may result in a colour index of less than 1.0. In these cases examination of the blood smear will reveal the presence of a double population of cells, some of which are macrocytes, and the true nature of the deficiency should be suspected.

Fig. 1



Macrocytosis itself, although common, is an unsafe criterion for the diagnosis of a megaloblastic anaemia, for red cells with a diameter of greater than 7.8μ are often seen in other types of anaemia. Such large cells may be present whenever erythropoiesis is abnormally active, as in haemolytic anaemia or during the recovery from an acute haemorrhage. They may occur in some cases of aplastic anaemia and in the anaemia of doubtful aetiology which accompanies chronic liver disease. Furthermore, it is possible for an acute megaloblastic anaemia to develop without any evidence of macrocytosis in the peripheral blood.

Pernicious Anaemia

True Addisonian anaemia is a disease of late middle life, characterized by a megaloblastic anaemia, achylia gastrica and signs of increased haemolysis. Neurological signs are common and treatment with vitamin B_{12} should produce rapid regression of the symptoms. The onset usually occurs after the age of forty and if symptoms appear in a younger person the diagnosis should be carefully confirmed. It is common in more than one member of a family and it is most often seen in temperate countries and in the white races. Many of the patients have prematurely grey hair and if the anaemia is severe the skin may assume a lemon yellow tint. There may be slight icterus of the sclerae. Lassitude, weakness, loss of appetite and breathlessness are common, but loss of weight is rare. Glossitis is often present, the tongue being either fiery red or glazed and pale. Splenomegaly is usual and the spleen is palpable in perhaps one-third of the cases. Looseness of the bowels and fever may suggest gastro-intestinal disease or an intercurrent infection. Symptoms

referable to the central nervous system occur in over 75 per cent. of patients with pernicious anaemia, and in many they may be the initial complaint. They vary from slight numbness and tingling of the extremities due to peripheral neuropathy, to frank subacute combined degeneration of the cord with inco-ordination, ataxia and spastic weakness. Some degree of mental disturbance is common in any anaemic patient, but occasionally the mental changes associated with pernicious anaemia are severe and require urgent treatment.

The diagnosis of pernicious anaemia is usually easy. The occurrence of a megaloblastic anaemia with achlorhydria, and a rise in the serum bilirubin in an elderly person, makes the diagnosis likely and this becomes almost a certainty if there is also evidence of subacute combined degeneration of the cord.

Megaloblastic Anaemia after Total Gastrectomy

Megaloblastic anaemia is rare after total gastrectomy, but the incidence increases if the patients survive the operation for more than a few years. MacDonald, Ingelfinger and Belding (1947) reported 46 patients who had survived total gastrectomy for more than three years and who apparently had not received prophylactic treatment with liver. In 12 macrocytic anaemia had developed, but it was rare for the anaemia to occur within two years of the operation. On the other hand, if treatment is stopped in true pernicious anaemia a relapse occurs in the first two years in 93 per cent. of the patients (Schwartz and Legere, 1944). In view of this, the length of time which may pass after total gastrectomy before megaloblastic anaemia occurs is surprising, if indeed the entire source of intrinsic factor has been removed. Byron Hall (1950) has suggested that some intrinsic factor may be formed in the intestine. However, the results of our own studies of intrinsic factor activity after total gastrectomy using radioactive vitamin B_{12} suggest that the removal of the whole stomach leads to a diminution of activity comparable to that found in true pernicious anaemia. Some of these patients have steatorrhoea and this may lead to a deficiency of folic acid as well as vitamin B_{12}.

Megaloblastic Anaemia in the Sprue Syndrome

In recent years there have been several full accounts of the changes in the blood in patients with steatorrhoea (Thaysen, 1932; Innes, 1948; Cooke *et al.*, 1948) and there is general agreement that the anaemia can vary in type. It may be frankly megaloblastic with macrocytosis, leukopenia and thrombocytopenia. In other patients hypochromic anaemia occurs and in a third group, probably the largest, examination of the blood reveals the presence of a dual deficiency.

The megaloblastic anaemia of steatorrhoea has more in common with the nutritional megaloblastic anaemias than with true pernicious anaemia. In the sprue syndrome the serum bilirubin is seldom raised, free acid may be present in the gastric juice, and treatment with folic acid may induce a remission when vitamin B_{12} has failed (Israels and Sharp, 1950). Although peripheral neuropathy is common, subacute combined degeneration of the cord is very rare in patients with steatorrhoea (Woltman and Heck, 1937).

Uncomplicated megaloblastic anaemia is usual in tropical sprue, but in idiopathic steatorrhoea it is often accompanied by a deficiency of iron. In coeliac disease in children the iron deficiency dominates the picture and a megaloblastic anaemia is rare.

In the sprue syndrome megaloblastic anaemia probably results from a failure of absorption of folic acid (Girdwood, 1953), although faulty synthesis and bacterial competition for the available supplies of the vitamin within the lumen of the intestine may be partly responsible.

In some patients lack of vitamin B_{12} also occurs. Intrinsic factor may be absent from the gastric juice of a patient with sprue in relapse, although the ability to secrete it may return during a remission (Castle *et al.*, 1935). Moreover, folic acid will not restore normal erythropoiesis in every case (Ferguson and Calder, 1948) and prolonged treatment with folic acid alone may fail to maintain a normal blood count (Badenoch, 1952).

Megaloblastic Anaemia associated with Organic Disease of the Gastro-Intestinal Tract

The occurrence of megaloblastic anaemia in association with organic disease of the gastro-intestinal tract has been recognized for many years (Faber, 1897). In a recent review Cameron, Watson and Witts (1949) collected 60 cases from the literature and added one of their own in which a blind loop of intestine was found to be present. Strictures of the bowel had occurred in 37 patients in this series and abnormal anastomoses were found in 23. The symptoms resembled either pernicious anaemia or steatorrhoea. The average age of the patients was less than that found in true pernicious anaemia and the incidence of neurological symptoms was high. Free acid was found in the gastric juice in about 50 per cent.

Many of these patients suffer from Crohn's disease, tuberculosis of the intestine or other forms of organic disease of the bowel, and steatorrhoea is common. Steatorrhoea also occurs where there is an anastomosis between the colon and the stomach or the upper intestine, but the failure to

absorb fat and the disorganization of intestinal function which accompanies it cannot explain the occurrence of the anaemia in every case. Many of the patients with intestinal strictures do not have steatorrhoea and in them the cause of the anaemia remains unknown. The strictures interfere with peristalsis and produce stagnation of the contents of the bowel. It is thought that bacteria proliferate in the stagnant loops and substances are formed which interfere with the absorption or utilization of factors that are necessary for normal erythropoiesis.

Nutritional Megaloblastic Anaemia

Megaloblastic anaemia of dietary origin is common in the tropics, but is extremely rare in other parts of the world. The tropical form of the disease is most common in pregnant women, but it occurs in both sexes and at all ages. The symptoms may resemble pernicious anaemia, but diarrhoea is often severe and loss of weight is common, while subacute combined degeneration of the cord is almost unknown. Although the marrow is megaloblastic, many of the patients also suffer from iron deficiency and the peripheral blood picture is sometimes predominantly hypochromic. Free acid is often present in the gastric juice and the serum bilirubin is seldom raised.

The exact nature of the dietary deficiency has not been fully explained. In most areas where the disease is common the diet consists largely of carbohydrate with little animal protein and the incidence among vegetarians is high (Taylor and Chhuttani, 1945). The anaemia can be relieved by treatment with yeast, Marmite, crude liver extract or folic acid, but it may fail to respond to refined extracts of liver (Wills and Evans, 1938). The response to vitamin B_{12} is usually poor, but good results have been claimed in some cases (Patel, 1948). It is probable that tropical megaloblastic anaemia has a complex aetiology and that a deficiency of both folic acid and vitamin B_{12} and possibly other factors may be responsible.

Nutritional megaloblastic anaemia is very rare in this country and occurs only in people who live on very abnormal diets. Strict vegetarians, 'Vegans,' are liable to develop a deficiency of vitamin B_{12}, but as their diet contains plenty of folic acid signs of involvement of the central nervous system may precede the development of megaloblastic anaemia. In one such patient, a vegetarian boy of 16 years of age (Raffan, H. M., personal communication), treatment with vitamin B_{12} was effective. Intrinsic factor was present in his gastric juice in normal amounts and there was little doubt that his symptoms were the result of a pure dietary deficiency of the vitamin.

Megaloblastic anaemia of dietary origin is sometimes seen in infants in temperate countries and for a time the disease was quite common in the United States of America (Zuelzer and Ogden, 1946). Most of the children who developed the anaemia were between 9 and 12 months old and many had been fed on a proprietary milk of low protein content. More recently May, Nelson and Salmon (1949) have shown that a similar anaemia can be produced in monkeys by feeding them on a comparable diet, but that the anaemia does not occur if the diet is supplemented with ascorbic acid. From the results of their experiments it appears that megaloblastic anaemia in infancy results from a deficiency of folic acid and that this deficiency is more prone to occur if the diet is low in vitamin C.

Pernicious Anaemia of Pregnancy

Pernicious anaemia of pregnancy is rare, but it should be suspected whenever a severe anaemia develops either during the third trimester or during the puerperium. The onset may be sudden and often appears to have been precipitated by an infection. Gastro-intestinal symptoms are common and the signs of toxaemia of pregnancy are often present. Jaundice may occur, but it is rare, and purpura and haemorrhages have been described. The spleen is palpable in about one-third of the cases. The blood picture may resemble true pernicious anaemia, but if the anaemia has developed rapidly there may be no macrocytosis (Callender, 1944). Hypochromic anaemia is often present and may make diagnosis difficult unless the marrow is examined, and this should always be done when a severe anaemia develops in pregnancy. Free acid is usually present in the gastric juice and rarely there may be considerable haemolysis with a raised serum bilirubin and an increased excretion of urobilinogen in urine and faeces.

The results of treatment with folic acid are good and suggest that the anaemia must be due to a lack of the vitamin or to a fault in its utilization. In pregnancy the demand for folic acid is probably increased, for the anaemia is prone to occur in twin pregnancies and when pregnancies follow in rapid succession. In some women this increase in demand may not be met because their diet is inadequate, or because recurrent vomiting or diarrhoea interfere with absorption. In others, however, the diet is excellent and no adequate cause for the deficiency can be found.

Whether the anaemia is the result of an increased demand for folic acid, or the result of some breakdown in its metabolism, there is no doubt that it is intimately related to the pregnancy. Once the puerperium is passed treatment with folic acid can be discontinued without fear of relapse, although the patient should be watched in any subsequent pregnancies, for a second attack sometimes occurs.

On the other hand, true pernicious anaemia may have its onset during pregnancy and in a few women with latent steatorrhoea the demands of pregnancy may unmask a megaloblastic anaemia for the first time. In both of these conditions treatment will have to be continued indefinitely if the patient is to remain well.

Fish-Tapeworm Anaemia

Infestation with the fish-tapeworm *Diphyllobothrium latum* may lead to the development of a megaloblastic anaemia similar in all clinical respects to true pernicious anaemia, although free acid may be present in the gastric juice. In some countries, notably Scandinavia, Switzerland and Japan, infestation with the worm is common, but only a small proportion of those infested develop a megaloblastic anaemia. When the anaemia occurs the worm is found to be lodged in the upper part of the small intestine. The anaemia responds to treatment with vitamin B_{12} or folic acid or to expulsion of the worm, but as long as the worm remains in the upper bowel treatment has to be continued indefinitely. Von Bonsdorff has shown that the fish-tapeworm itself contains large amounts of vitamin B_{12} (von Bonsdorff and Gordin, 1952). Presumably the intact worm competes with the host for the available supplies of vitamin B_{12} and this results in a deficiency of the vitamin.

The Diagnosis of the Megaloblastic Anaemias

If the anaemia is the result of a deficiency of vitamin B_{12} or folic acid examination of the marrow will reveal the presence of megaloblasts. These are larger than normal erythroblasts and the chromatin of the nucleus is arranged in a fine reticular network which persists with little tendency to clump as the cell matures. In typical cases the presence in the peripheral blood of macrocytes and large polymorphonuclear granulocytes with hypersegmented nuclei will suggest the diagnosis. However, if there is an associated hypochromic anaemia the true nature of the deficiency may be missed unless the marrow is examined.

Investigation should not end with the demonstration of megaloblasts in the bone marrow, for if treatment is to be effective the cause of the anaemia must be found. If the patient is elderly, if there is achlorhydria and a raised serum bilirubin, and if subacute combined degeneration of the cord accompanies the anaemia, the diagnosis of true pernicious anaemia can be made with confidence. Even when the diagnosis of pernicious anaemia has been made a barium meal should be carried out to exclude a gastric carcinoma, for the incidence of cancer of the stomach is higher in patients with pernicious anaemia than in the general population.

In younger persons, in those in whom free acid is found in the gastric juice, or where there is an associated hypochromic anaemia, some other cause for the anaemia must be considered. In these a full X-ray examination of the gastro-intestinal tract may reveal the presence of organic disease of the bowel, and, if none is found, a fat balance should be carried out to exclude steatorrhoea.

In many patients with gastro-intestinal disease or with steatorrhoea, diarrhoea may be minimal and careful questioning will be necessary to elicit a history of dyspepsia.

Nutritional megaloblastic anaemia is rare in this country, but it should be considered whenever the patient has lived on a very abnormal diet. In pernicious anaemia of pregnancy the peripheral blood picture is often misleading and the diagnosis may be missed unless the marrow is examined.

Finally, diagnosis may be very difficult in patients with subacute combined degeneration of the cord without anaemia, or in those with true pernicious anaemia in whom partial treatment has restored the marrow to normal. Here, histological examination of a fragment of the gastric mucosa obtained with Wood's flexible gastric biopsy tube (Wood *et al.*, 1949) or the assay of intrinsic factor by measurement of the absorption of radioactive vitamin B_{12} may make diagnosis possible when other methods have failed.

The Treatment of the Megaloblastic Anaemias

Since the introduction of liver therapy by Minot and Murphy in 1926 great strides have been made in the treatment of the megaloblastic anaemias. Vitamin B_{12} has largely taken the place of the more expensive and sometimes unreliable liver extracts in pernicious anaemia, and the isolation and synthesis of folic acid has made it possible to restore normal erythropoiesis in all the other forms of megaloblastic anaemia. Oral preparations of liver which probably acted by virtue of their folic acid content are not used extensively today, except in the nutritional anaemias. New oral preparations are becoming available in which vitamin B_{12} is combined with a source of intrinsic factor to ensure its absorption, but there is little doubt that for the routine management of patients with pernicious anaemia regular injections of vitamin B_{12} will remain the treatment of choice.

Our knowledge of pathogenesis has increased more slowly, but enough progress has been made to provide a rational basis for treatment. In true pernicious anaemia, after total gastrectomy, and in the fish-tapeworm anaemia, treatment with vitamin B_{12} is necessary. In all other forms of megaloblastic anaemia folic acid is more certain in its action, although a response to vitamin B_{12} may occur. Whenever there are symptoms of subacute combined degeneration of the cord it is essential to give vitamin B_{12}.

There is some evidence that prolonged treatment with folic acid, in the presence of intestinal disease or steatorrhoea, may lead to a deficiency of vitamin B_{12} and it is wise to add small supplements of this vitamin whenever folic acid is used as the main treatment.

The length of time for which treatment will have to be continued varies from one form of megaloblastic anaemia to another. In the nutritional anaemias and in most cases of tropical sprue once folic acid has produced a cure it can be discontinued. In pernicious anaemia of pregnancy also there is no need to continue treatment once the puerperium is passed. On the other hand, in pernicious anaemia, in idiopathic steatorrhoea and in organic disease of the bowel where surgical cure is impossible treatment will have to be continued indefinitely if the patient is to remain well.

In all forms of megaloblastic anaemia it is important that the patient's diet should be generous and contain plenty of protein, and that iron should be added to the treatment whenever there is an associated hypochromic anaemia.

The Dosage of Vitamin B_{12} and Folic Acid

The minimal effective dose of vitamin B_{12} is in the neighbourhood of 1 μg. daily by intramuscular injection. In a severe case of pernicious anaemia in relapse it is usual to start with a dose of 100 μg. each week until a maximal response has been obtained, and then to continue with 20 to 50 μg. every two to four weeks. The requirements of individual patients vary considerably and in some it may be necessary to give larger doses.

In the presence of subacute combined degeneration of the cord or if the blood count is not maintained at an optimal level it is preferable to increase the frequency of injections rather than raise the size of the dose, because a large proportion of each dose is excreted in the urine.

Untoward reactions are uncommon after treatment with B_{12} and when they occur they are due to the presence of impurities. In such patients crystalline vitamin B_{12} can be used.

Folic acid is effective when given by mouth. The dose is 5 to 20 mg. daily. It is water soluble and in most cases is freely absorbed, but it can be given parenterally if the patient is vomiting or in severe cases of steatorrhoea where absorption may be impaired.

In appropriate cases improvement with vitamin B_{12} or folic acid is extremely rapid and even in the severely anaemic patient it is seldom necessary to resort to blood transfusion. Arteriosclerosis, the presence of an infection or an associated deficiency of iron may delay improvement, but the most common cause of failure is inaccurate diagnosis. When pernicious anaemia is suspected the indiscriminate use of ' shot-gun ' remedies before the diagnosis is made adds nothing to our knowledge and may condemn the patient to treatment for life without good reason.

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BIBLIOGRAPHY

BADENOCH, J. (1952), ' D.M. Thesis,' Oxford.
BERK, L., CASTLE, W. B., WELCH, A. D., HEINLE, R. W., ANKER, R., and EPSTEIN, M. (1948), *New Engl. J. Med.*, **239**, 911.
CALLENDER, S. T. E. (1944), *Quart. J. Med.*, **37**, 75.
CAMERON, D. G., WATSON, G. M., and WITTS, L. J. (1949), *Blood*, **4**, 793.
CASTLE, W. B. (1929), *Amer. J. med. Sci.*, **178**, 748.
CASTLE, W. B., RHOADS, C. P., LAWSON, H. A., and PAYNE, G. C. (1935), *Arch. intern. Med.*, **56**, 627.
COOKE, T. W., FRAZER, A. C., PEENEY, A. L. P., SAMMONS, H. G., and THOMPSON, M. D. (1948), *Quart. J. Med.*, **41**, 9.
FABER, K. (1897), *Berl. klin. Wschr.*, **34**, 643.
FERGUSON, J. W., and CALDER, E. (1948), *Glasg. med. J.*, **29**, 341.
GIRDWOOD, R. H. (1953), *Lancet*, **ii**, 53.
HALL, B. E. (1950), *Brit. med. J.*, **ii**, 585.
INNES, E. M. (1948), *Edinb. med. J.*, **55**, 282.
ISRAELS, M. C. G., and SHARP, J. (1950), *Lancet*, **i**, 752.
MACDONALD, R. M., INGELFINGER, F. J., and BELDING, H. W. (1947), *New Engl. J. Med.*, **237**, 887.
MAY, C. D., NELSON, E. N., and SALMON, R. J. (1949), *J. Lab. clin. Med.*, **34**, 1724.
MINOT, G. R., and MURPHY, W. P. (1926), *J. Amer. med. Ass.*, **87**, 470.
MITCHELL, H. K., SNELL, E. E., and WILLIAMS, R. J. (1944) *J. Amer. chem. Soc.*, **66**, 267.
PATEL, J. C. (1948), *Brit. med. J.*, **ii**, 934.
RICKES, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., and FOLKERS, K. (1948), *Science*, **107**, 396.
SCHWARTZ, S. O., and LEGERE, H. (1944), *J. Amer. med. Ass.*, **124**, 637.
SMITH, E. L. (1948), *Nature*, **161**, 638.
TAYLOR, G. F., and CHHUTTANI, P. N. (1945), *Brit. med. J.*, **i**, 800.
THAYSEN, TH. E. H. (1932), ' Non-Tropical Sprue,' Oxford.
VON BONSDORFF, B., and GORDIN, R. (1952), *Acta med. Scand.*, **142**, *suppl.*, **266**, 283.
WILLS, L., and EVANS, B. D. F. (1938), *Lancet*, **ii**, 416.
WOLTMAN, H. W., and HECK, F. J. (1937), *Arch. intern. Med.*, **60**, 272.
WOOD, I. J., DOIG, R. K., MOTTERAM, R., and HUGHES, A. (1949), *Lancet*, **i**, 18.
ZUELZER, W. W., and OGDEN, F. N. (1946), *Amer. J. Dis. Child.*, **71**, 211.