Magaloblastic and other macrocytic anaemias

Introduction to macrocytic anaemia

- Red cells are abnormally large (MCV >95 fL).
- Broadly subdivided into megaloblastic and nonmegaloblastic,
- based on the appearance of developing erythroblasts in the bone marrow.

Megaloblastic anaemias

- This is a group of anaemias in which the erythroblasts in the bone marrow show a characteristic abnormality i.e. maturation of the nucleus being delayed relative to that of the cytoplasm.
- Asynchronous maturation of the nucleus results defective DNA synthesis
- Usually caused by deficiency of vitamin Bl2 or folate.

Vitamin B12 (B12 **cobalamin)**

- This vitamin is synthesized in nature by microorganisms
- Animals acquire it by eating other animal foods, by internal production from intestinal bacteria (not in humans) or by eating bacterially contaminated foods.
- The vitamin consists of a small group of compounds, the cobalamins, which have the same basic structure
- with a cobalt atom at the center of a corrin ring which is attached to a nucleotide portion (Fig. 4.1).
- The vitamin is found in foods of animal origin such as liver, meat, fish and dairy produce but does not occur in fruit, cereals or vegetables.

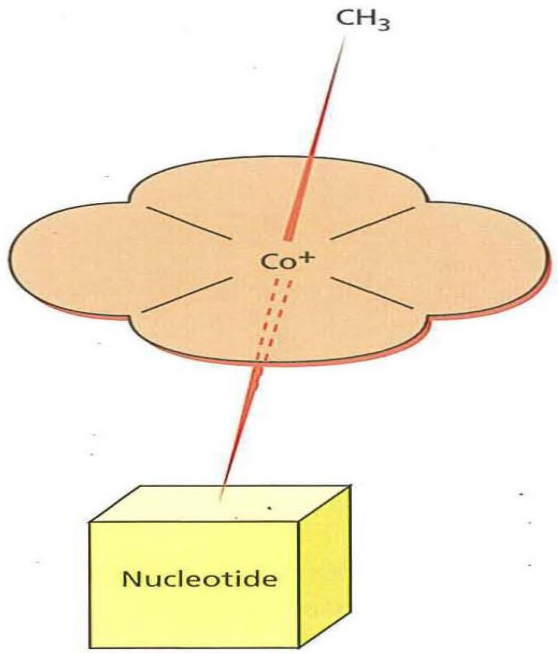


Fig. 4.1 The structure of methylcobalamin (methyl B_{12}), the main form of vitamin B_{12} in human plasma. Other forms include deoxyadenosylcobalamin (ado B_{12}), the main form in human tissues; hydroxocobalamin (hydroxo B_{12}), the main form in treatment; and cyanocobalamin (cyano B_{12}), the radioactively labelled (^{57}Co or ^{58}Co) form used to study vitamin B_{12} absorption or metabolism.

Absorption

- A normal diet contains a large excess of B12 compared with daily needs
- Bl2 is combined with the glycoprotein intrinsic factor (IF) synthesized by the gastric parietal cells.
- IF-B12 complex can then bind to a specific surface receptor for IF, cubilin, which then binds to a second protein, amnionless
- amnionless directs endocytosis of the cubilin IF-B12 complex in the distal ileum where Bl2 is absorbed and IF destroyed (Fig. 4.2).

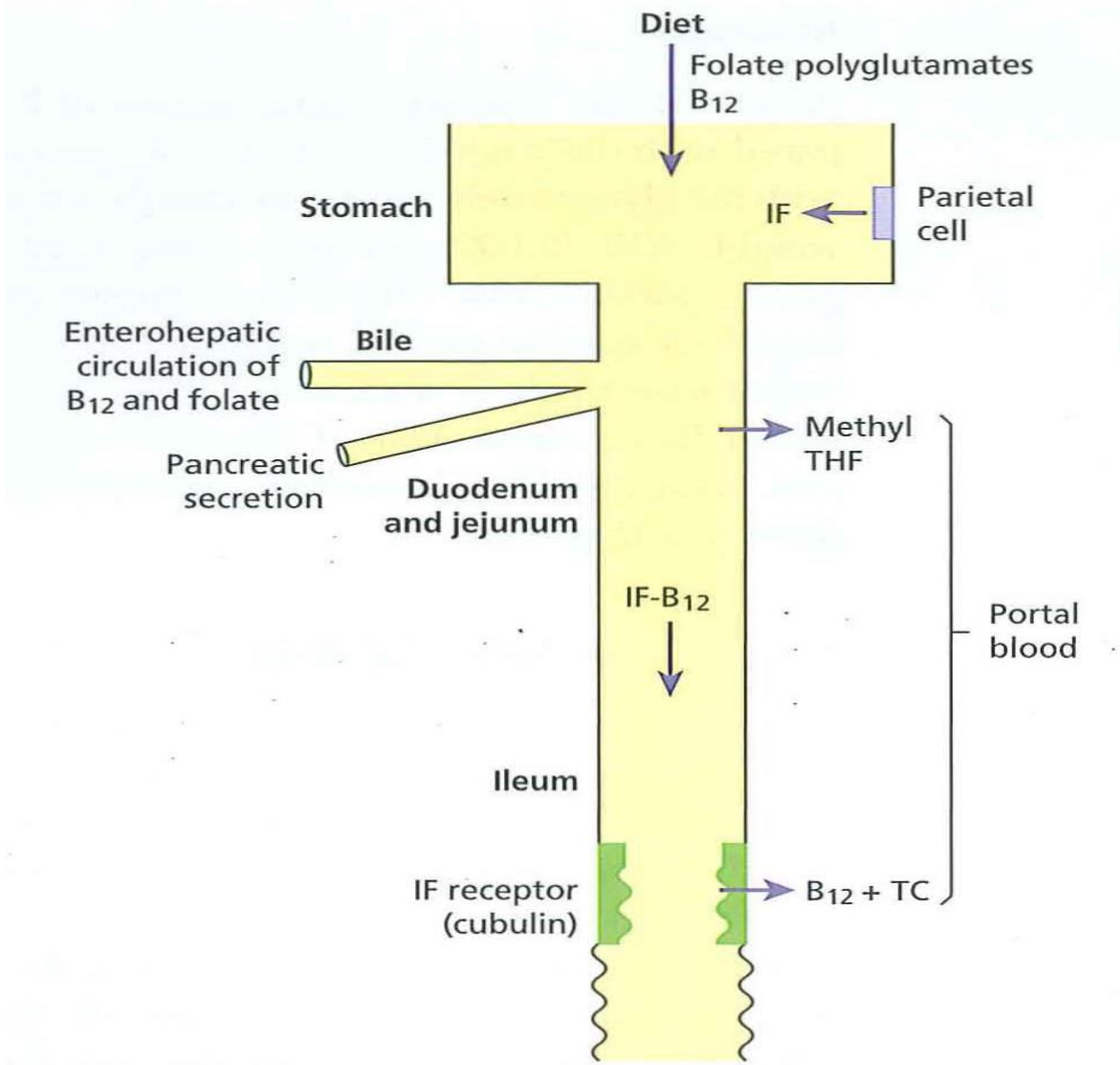


Fig. 4.2 The absorption of dietary vitamin B_{12} after combination with intrinsic factor (IF), through the ileum. Folate absorption occurs through the duodenum and jejunum after conversion of all dietary forms to methyltetrahydrofolate (methyl THF). TC, transcobalamin.

Transport: the transcobalamins

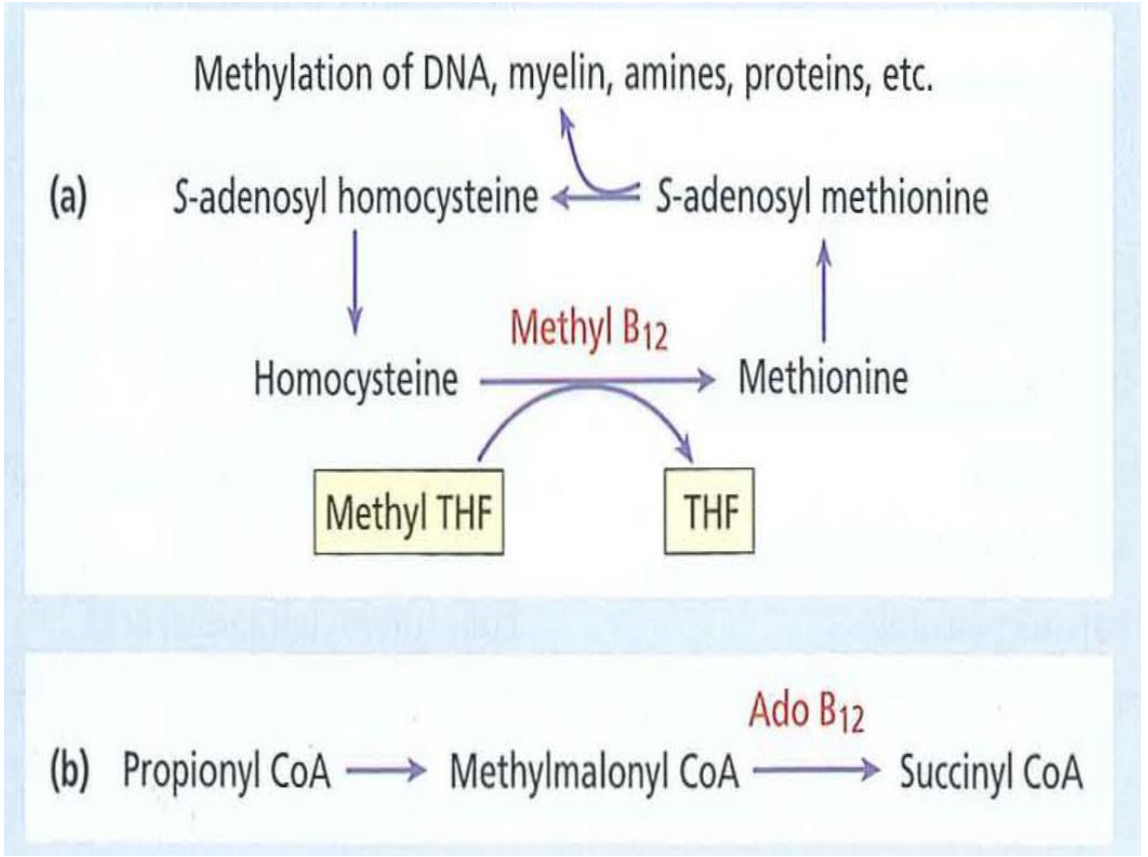
- Vitamin Bl2 is absorbed into portal blood where it becomes attached to the plasma-binding protein transcobalamin (TC, previously called transcobalamin II)
- TC delivers Bl2 to bone marrow and other tissues.
- The amount of Bl2 on TC is normally very low (<50 ng/L).
- TC deficiency causes megaloblastic anaemia because of failure of B12 to enter marrow (and other cells) from plasma.
- Serum B12 level in TC deficiency is normal because most B12 in plasma is bound to another transport protein, haptocorrin (previously called transcobalamin I).
- This is a glycoprotein largely synthesized by granulocytes and macrophages.
- B12 bound to haptocorrin does not transfer to marrow (functionally 'dead’).

Biochemical Function

Vitamin B12 is a coenzyme for two biochemical reactions in the body:

1. as methyl B12 it is a cofactor for methionine synthase, the enzyme responsible for methylation of homocysteine to methionine using methyl tetrahydrofolate (methyl THF) as methyl donor (Fig. 4.3a);

2. As deoxyadenosyl B12 (ado B12) it assists in conversion of methylmalonyl coenzyme A (CoA) to succinyl CoA (Fig. 4.3b).



Folate: Absorption, transport and function

- Folic (pteroylglutamic) acid is the parent compound of a large group of compounds, the folates(Fig. 4.4).
- Humans are unable to synthesize the folate structure and thus require preformed folate as a vitamin.

Absorption, transport and function

- Dietary folates are converted to methyl THF during absorption through the upper small intestine.
- Once inside the cell they are converted to folate polyglutamates (Fig. 4.5). Folate binding proteins are present on cell surfaces including the enterocyte and facilitate entry of reduced folates into cells.
- Folates are needed in a variety of biochemical reactions involving single carbon unit transfer, in (e.g. homocysteine conversion to methionine) (Fig. 4.5) and serine to glycine or in synthesis of purine precursors of DNA.

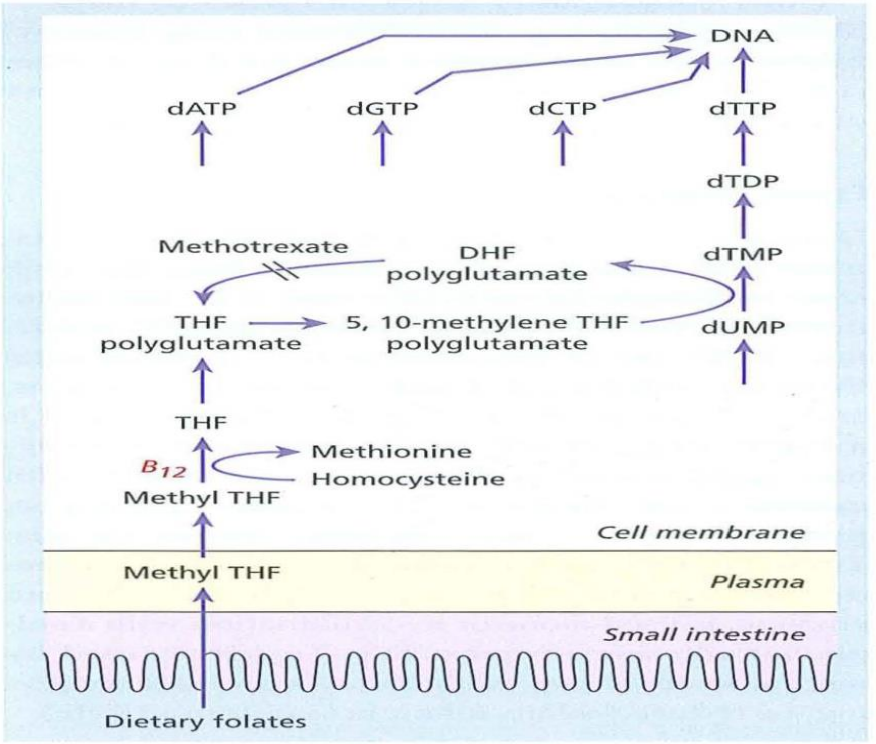


Fig. 4.5 The biochemical basis of megaloblastic anaemia caused by vitamin B_{12} or folate deficiency. Folate is required in one of its coenzyme forms, 5,10-methylene tetrahydrofolate (THF) polyglutamate, in the synthesis of thymidine monophosphate from its precursor deoxyuridine monophosphate. Vitamin B_{12} is needed to convert methyl THF, which enters the cells from plasma, to THF, from which polyglutamate forms of folate are synthesized. Dietary folates are all converted to methyl THF (a monoglutamate) by the small intestine. A, adenine; C, cytosine; d, deoxyribose; DHF, dihydrofolate; DP, diphosphate; G, guanine; MP, monophosphate; T, thymine; TP, triphosphate; U, uracil.

Biochemical basis for megaloblastic anaemia

- Folate deficiency causes megaloblastic anaemia by inhibiting thymidylate synthesis, a rate-limiting step in DNA synthesis in which thymidine monophosphate (dTMP) is synthesized.
- This reaction needs 5,l0-methylene THF polyglutamate as coenzyme.
- All body cells, including those of the bone marrow, receive folate from plasma as methyl THF.
- B12 is needed in the conversion of this methyl THF to THF, a reaction in which homocysteine is methylated to methionine.
- THF is a substrate for folate polyglutamate synthesis inside cells. THF polyglutamate, is the 'coenzyme form of folate involved in the thymidylate synthetase reaction (Fig. 4.5).
- Lack of *B12* prevents the demethylation of methyl THF, thus depriving cells of THF.
- Other congenital or acquired causes of megaloblastic anaemia (e.g. antimetabolite drug therapy) inhibit purine or pyrimidine synthesis at one or other step result in a reduced supply of one or other of the four precursors needed for DNA synthesis.

Folate reduction

- During thymidylate synthesis, the folate polyglutamate coenzyme becomes oxidized from the THF state to dihydrofolate (DHF) (Fig. 4.5). Regeneration of active THF requires the enzyme DHF reductase.

- Inhibitors of this enzyme (e.g. methotrexate) therefore inhibit all folate coenzyme reactions, and so DNA synthesis (Fig. 4.5).

- Methotrexate is a useful drug, mainly in the treatment of malignant or inflammatory disease (e.g. of the skin) with excessive cell turnover.

- Toxicity caused by methotrexate or pyrimethamine is reversed by giving the reduced folate, folinic acid (5-formyl THF).

Vitamin B12 deficiency

- In Western countries, severe deficiency is usually caused by (Addisonian) pernicious anaemia.
- Less commonly, it may be caused by veganism in which the diet lacks *B12* (usually\ in Hindu Indians), gastrectomy or small intestinal lesions.
- There is no syndrome of *B12* deficiency as a result of increased utilization or loss of the vitamin. The deficiency takes at least 2 years to develop (i. e. the time needed for body stores to deplete at the rate of 1-2 μ*g1* day) when there is sevre malabsorption from the diet.
- Nitrous oxide, however, may
- rapidly inactivate body *B12*

Pernicious anaemia

- This is caused by autoimmune attack on the gastric mucosa leading to atrophy of the stomach.
- The wall of the stomach becomes thin, with a plasma cell and lymphoid infiltrate of the lamina propria.
- There is achlorhydria and secretion of IF is absent or almost absent.
- Serum gastrin levels are raised.
- *Helicobater pylori* infection may initiate an autoimmune gastritis which presents in younger subjects as iron deficiency and in the elderly as pernicious anaemia.
- More females than males are affected (1.6 : 1), with a peak occurrence at 60 years.
- The disease is found in all races but is most common in northern Europeans and tends to occur in families.
- There is also an increased incidence of carcinoma of the stomach (approximately 2-3% of all cases of pernicious anaemia).

Folate deficiency

- This is most often a result of a poor dietary intake of folate alone or in combination with a condition of increased folate utilization or malabsorption (Table 4.5).
- Excess cell turnover of any sort, including pregnancy, is the main cause of an increased need for folate, because the folate molecule becomes degraded when DNA synthesis is increased.
- Alcohol, sulfasalazine and other drugs may have multiple effects on folate metabolism.

Clinical features of megaloblastic anaemia

- The onset is usually insidious with gradually progressive symptoms and signs of anaemia.
- The patient may be mildly jaundiced (lemon yellow tint) because of the excess breakdown of haemoglobin resulting from increased ineffective erythropoiesis in the bone marrow.
- Glossitis (a beefy-red sore tongue) angular stomatitis and mild symptoms of malabsorption with loss of weight may be present because of the epithelial abnormality.
- Purpura as a result of thrombocytopenia and widespread melanin pigmentation are less frequent presenting features. Many asymptomatic patients are diagnosed when a blood count that has been performed for another reason reveals macrocytosis.

Clinical Features of Megaloblastic anaemia

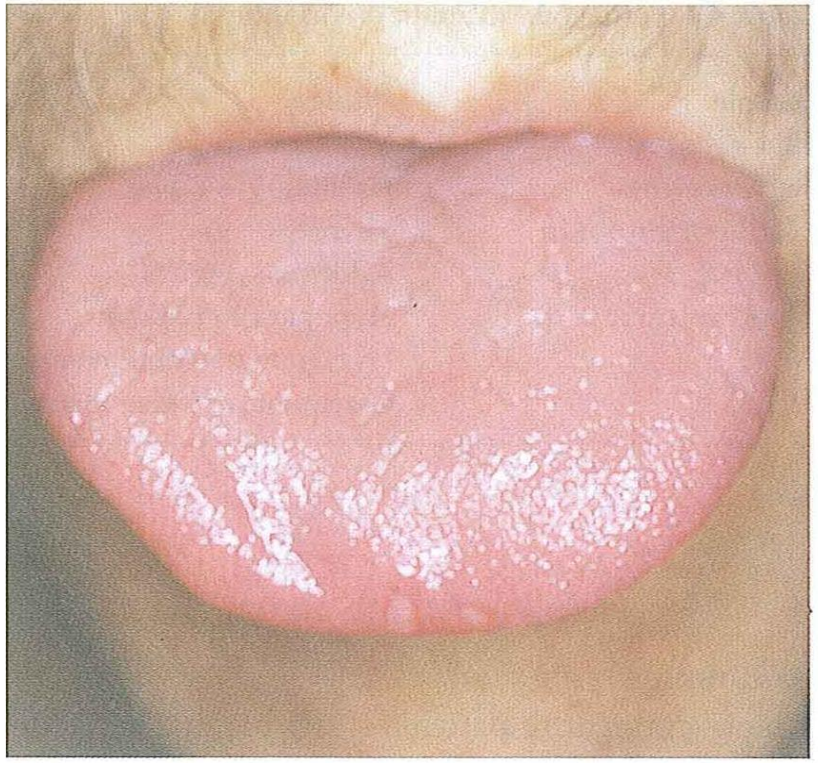


Fig. 4.7 Megaloblastic anaemia: glossitis—the tongue is beefy-red and painful.

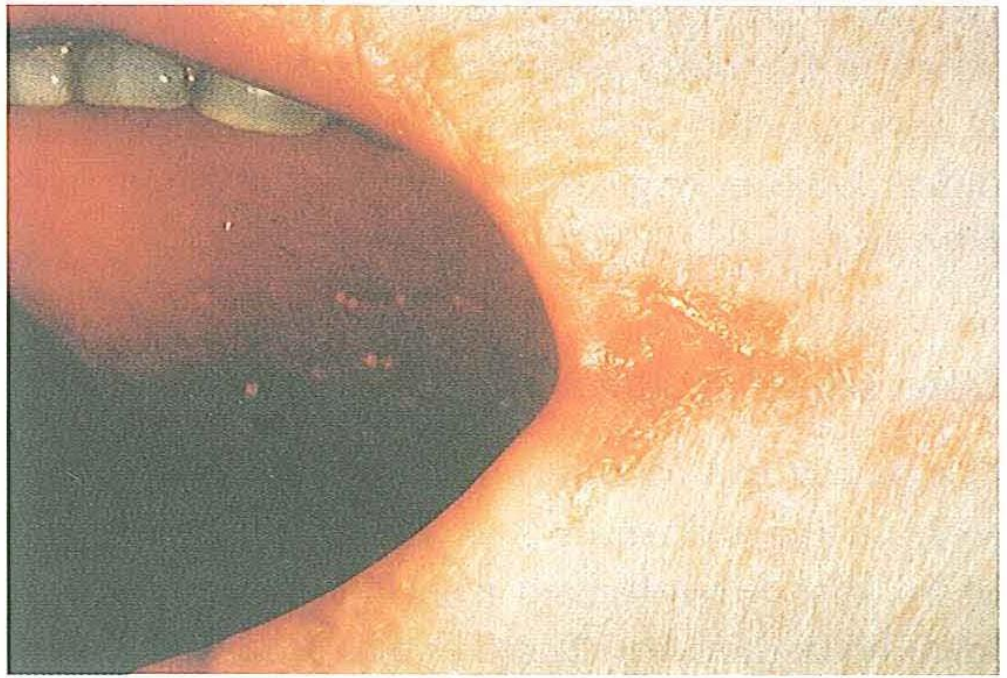


Fig. 4.8 Megaloblastic anaemia: angular cheilosis (stomatitis).

Vitamin B12 neuropathy (subacute combined degeneration of the cord)

- Severe B12 deficiency can cause a progressive neuropathy affecting the peripheral sensory nerves and posterior and lateral colunms.
- The neuropathy is symmetrical and affects the lower limbs more than the upper limbs, tingling in the feet, difficulty in walking and may fall over in the dark.
- Rarely, optic atrophy or severe psychiatric symptoms are present.
- The cause of the neuropathy is related to the accumulation of S-adenosyl homocysteine and reduced levels of S-adenosyl methionine in nervous tissue
- Results of defective methylation of myelin and other substrates.
- Folate deficiency in the adult can cause neuropathy and some data suggesting it causes psychiatric changes.

Neural tube defect

- Folate or B12 deficiency in the mother predisposes to neural tube defect (NTD) (anencephaly, spina bifida or encephalocoele) in the fetus. The lower the maternal serum or red cell folate or serum B12 levels the higher the incidence of NTDs.
- Supplementation of the diet with folic acid at the time of conception and in early pregnancy reduces the incidence of NTD by 75%.
- Mechanism is thought to be related to build-up of homocysteine and S-adenosyl homocysteine in the fetus which may impair methylation of various proteins and lipids.
- Polymorphism in 5,10-methylene tetrahydrofolate reductase (5,10-MTHFR) results in higher serum homocysteine and lower serum and red cell folate levels compared with controls.
- The incidence of the mutation is higher in the parents and fetus with NTD than in controls and in the sera of women who have a baby with NTD.

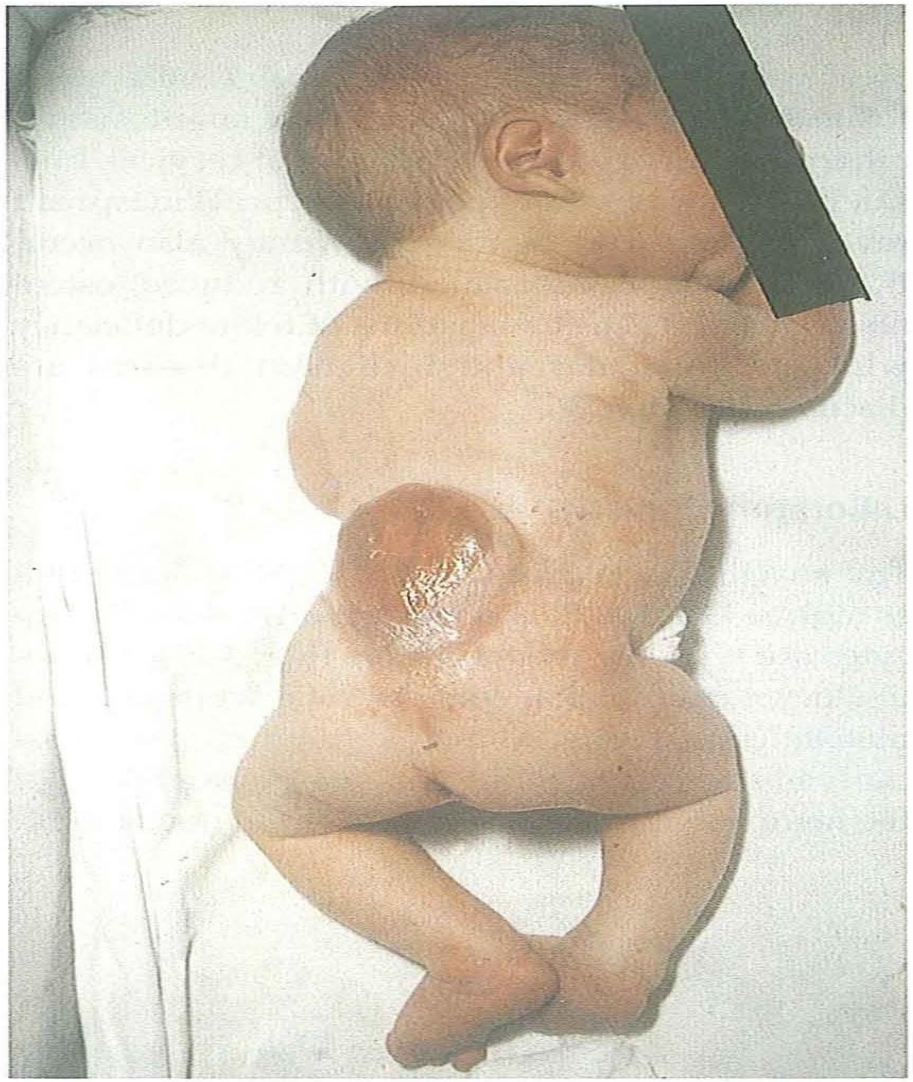


Fig. 4.10 A baby with neural tube defect (spina bifida). (Courtesy of Professor C.J. Schorah)