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Manson House, Thursday, 16th February, 1978

Symposium on Tropical Diarrhoea

The President: Dr. S. G. BROWNE, *in the Chair*

Folate malnutrition in tropical diarrhoeas

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The development of folate deficiency in tropical malabsorbtion

There are many descriptions of folate deficiency developing in a variety of infective malabsorption syndromes (WELLCOME TRUST COLLABORATIVE STUDY, 1971). The nomenclature has been confusing but once parasitic infection, in particular giardiasis and strongyloidiasis, has been excluded it is customary to differentiate those severe cases with tropical sprue (malabsorption of two or more unrelated substances) from those with milder or subclinical features usually classified as tropical enteropathy. In villages in south India there are well defined epidemics of tropical sprue which occur against a background of levels of absorption in asymptomatic subjects which are abnormal when compared to U.K. residents. In natives of Puerto Rico and Haiti the comparison between patients with tropical sprue and control subjects is more distinct, both at a functional level as assessed by tests of absorption and at a morphological level according to appearances of the jejunal mucosa. The findings in expatriates developing tropical malabsorption are different again.

Diarrhoea is extremely common in expatriates whether volunteer workers (LINDENBAUM *et al.*, 1966), professionals, military personnel or travellers. Transient malabsorption is common during these episodes but, in most, lasts for a few weeks only (LINDENBAUM, 1965). A proportion, however, develop symptoms which persist for months or even years accompanied by nutritional deficiencies as described in British soldiers in Singapore, American servicemen in Vietnam and expatriates who were travelling or temporarily resident in the subcontinent of India (TOMKINS *et al.*, 1974). Folate deficiency is an important nutritional problem in many of these syndromes but there are striking and characteristic differences in frequency and severity of anaemia between the various clinical groups. These have been reviewed in detail by KLIPSTEIN (1967). Several recent studies have contributed considerably towards an increased understanding of how this deficiency develops.

For a long time it has been assumed that an inadequate diet is a major factor but accurate measurements of folate intake in communities where tropical malabsorption is common have been limited. This is partly due to the problems of obtaining reliable intake measurements among villagers but also because of a basic lack of information on the folate contents of foods, whether in the raw state or after cooking. Nevertheless O'Brien vividly describes the jettisoning of the almost inedible tinned rations issued to British servicemen in jungle conditions (WELLCOME TRUST COLLABORATIVE STUDY, 1971). The prohibitive cost of fresh vegetables together with absolute scarcity at certain seasons may be critical factors in limiting folate intake by the villager in developing communities such as in south India. The deleterious effect of prolonged soaking and cooking with resultant oxidation of dietary folate is a serious enough problem in developed countries. It is even worse in poorer communities where folate sources such as lentils require considerable cooking before they become digestible.

Dietary folate exists in the conjugated, polyglutamate form consisting of pteroylmonoglutamic acid joined with several glutamic acid moieties. Corcino and co-workers have prepared synthetic polyglutamate, radio-actively labelled within the pteridine ring in such a way that it was possible to measure digestion and absorption of folate in patients with tropical sprue in Puerto Rico (CORCINO *et al.*, 1976). They demonstrated impaired hydrolysis of the polyglutamate as well as less satisfactory absorption of its digestion product folic acid; digestion and absorption were both markedly improved when measured after a six-month course of tetracycline. Certain features remain unexplained; a particular problem is that many studies, including that of Corcino, have shown normal or even increased levels of the enzyme folate conjugase whether measured in the luminal fluid or in mucosal samples (KLIPSTEIN, 1967). The pH optimum of folate conjugase, 4·3, is not conducive to maximal activity within the lumen. It may be active intra-

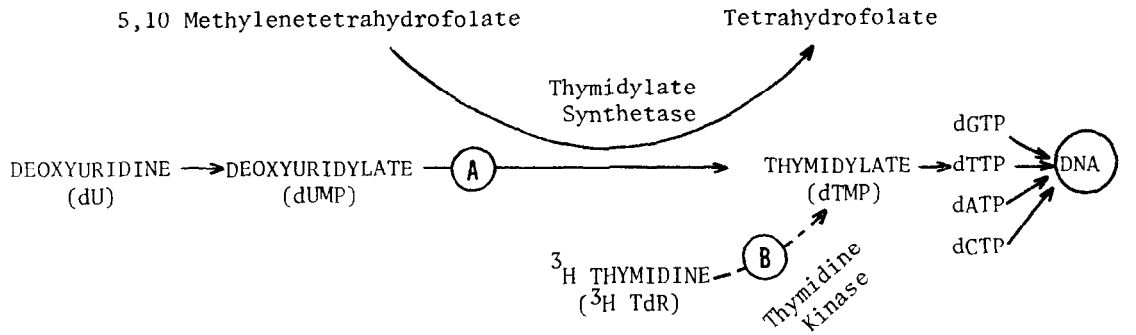

Fig. 1. Two pathways for DNA synthesis.
A. The conventional *de novo* pathway whereby deoxyuridine (dU) is converted to thymidylate (dTMP) by the folate dependent enzyme thymidylate synthetase.
B. The 'salvage' pathway whereby exogenous thymidine (TdR) is incorporated into dTMP independent of folate enzyme.

cellularly or, as suggested by BLAIR & MATTY (1974), it may be that an acid microclimate, immediately adjacent to the glycocalyx, is essential for its activity.

There is little information about the metabolism of folate once it is in the epithelial cell and few studies have attempted to determine the folate status of the intestinal mucosa. In weanling animals fed a folate-deficient diet to which sulphadimidine has been added to inhibit folate synthesis by intestinal bacteria, it has been possible to prepare mucosal samples and assay the folate which is stimulatory to the growth of *Lactobacillus casei* (KLIPSTEIN *et al.*, 1973). This is in general an appropriate assay to use because it measures 5-methyl tetrahydrofolate, the metabolically active form in most tissues. However, recent work in mammalian intestine has shown the considerable variety of folate analogues that are present (BROWN *et al.*, 1974). A further problem is that actively proliferating cells have greater concentrations than more slowly metabolizing cells, a factor which makes it difficult to interpret the results of analysis of a mixture of crypt and villus cells as, for example, in the small piece of tissue sampled at jejunal biopsy.

An alternative approach is to assess the functional folate status of the mucosa, concentrating particularly on the activity of the folate-dependent enzymes necessary for synthesis of DNA for cell replication. The conventional *de novo* pathway for thymidylate (precursor of DNA) synthesis is by the conversion of deoxyuridylate→deoxyuridine monophosphate (Fig. 1). This reaction depends on adequate supply of folate for the activity of the key enzyme in this conversion, thymidylate synthetase. In the presence of inhibitors of this enzyme or in nutritional folate deficiency the alternative, 'salvage' pathway is utilized whereby preformed thymidine is incorporated (TOMKINS *et al.*, 1976). Indeed, in experimental folate deficiency, thymidine kinase (the enzyme associated with the salvage pathway) activity of isolated crypt cells is five times higher than in controls (BADCOCK & TOMKINS, 1978).

The addition of large amounts of unlabelled deoxyuridine (dU) in substrate amounts will

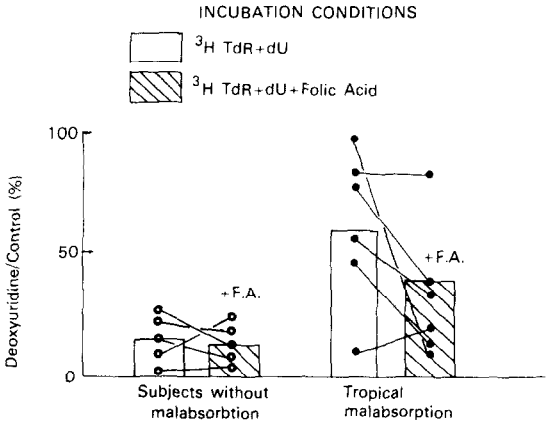

Fig. 2. The Deoxyuridine Suppression Test on incubated jejunal biopsy samples showing impaired 'suppression' (indicating mucosal folate deficiency) in tropical malabsorption.

'suppress' the uptake of radio-actively labelled thymidine (^{3}H TdR) into DNA (via pathway B in Fig. 1) provided there is adequate folate to permit the activity of thymidylate synthetase (pathway A, Fig. 1). In 'control' subjects, those with diarrhoea but without malabsorption or folate deficiency, there is suppression to approximately 20% by the addition of dU (Fig. 2). The addition of folic acid to the incubation medium makes no difference to the degree of suppression, provided folate status of the tissue is normal. By contrast, in patients with tropical malabsorption, the addition of dU fails to 'suppress' the uptake of ^{3}H TdR. The improvement in 'suppression' by *in vitro* addition of folic acid indicates a functional mucosal folate deficiency. This occurred in five of the six patients with tropical malabsorption in Fig. 2. Only two of these patients, however, had megaloblastic anaemia, the remainder had normal bone marrow morphology and red blood cell folate concentrations suggesting that mucosal malnutrition occurs in the absence of systemic

deficiency. The jejunal epithelium has very high folate requirements because of the demand for DNA synthesis by the crypt cells. They probably rely to a considerable degree on intraluminal supply and any disturbance in intraluminal metabolism, perhaps due to bacteria adherent to the mucosa (TOMKINS *et al.*, 1975), could affect mucosal nutrient status.

If it is true that mucosal folate status is determined more by intestinal than systemic events, then the question remains—what events? A number of observations appear relevant. It is now well established that ethanol, a metabolic product of the abnormal intestinal microflora that we (TOMKINS *et al.*, 1975) and others (KLIPSTEIN *et al.*, 1973) have demonstrated in jejunal fluid from patients with tropical sprue, has a deleterious effect on intestinal enzyme activity (GREENE *et al.*, 1974). This effect may be ameliorated if folic acid is given at the same time as the ethanol. The demonstration of an increase in glycolytic enzyme activity of the mucosa obtained from volunteers shortly after taking folic acid infers that folate may be important in pathways of protein synthesis. The requirements for new protein synthesis in jejunal mucosa are extremely high, 146% of the protein (MCNURLAN *et al.*, 1978) content being resynthesized every day. There is no direct information on the effect of folate deficiency on protein turnover in gut but in a study of patients with severe megaloblastic anaemia in Nigeria the authors suggested that the raised plasma amino-acid levels were the result of failure of methylation and formylation of RNA due to the folate deficiency (JACOBS & FLEMING, 1970).

The effects of folate deficiency on intestinal mucosa

It has been difficult to be certain of the effects of a folate deficiency alone upon the intestinal mucosa in man because most cases of severe folate deficiency with megaloblastic anaemia have been in patients with malabsorption or in alcoholics; both conditions in which mucosal lesions occur unrelated to any malnutrition. Absorption studies in patients with megaloblastic anaemia have shown variable results; nevertheless, the striking precipitation of mucosal atrophy and malabsorption during the folate deficiency of pregnancy in wives of British servicemen in Singapore and Bantu women in South Africa would support a role for folate in the regulation of the intestinal mucosa (WELLCOME TRUST COLLABORATIVE STUDY, 1971). Earlier reports of rapid improvement of diarrhoea in some patients with tropical sprue who were treated with a variety of foods containing haematinics have been followed by more objective assessment of changes measured on repeated jejunal biopsy specimens. The megaloblastic appearance of nuclei of crypt cells changes markedly within two days of commencement of folic acid therapy, such changes being accompanied by some improvement in villus morphology.

Recently COOK (1976) has studied absorption using intestinal perfusion techniques in patients with severe megaloblastic anaemia of primary nutritional origin in Africa. There was no impairment of absorption of glucose, xylose or glycine when compared with African control subjects; similar results were obtained by GOETSCH & KLIPSTEIN (1977) in folate deficient animals. Despite these results there were discernible morphological abnormalities of the jejunal mucosa. At first sight it seems anomalous that such a mucosa with shortened villi and biochemical evidence of reduction of cell numbers at all levels of the villi is capable of absorbing certain nutrients quite normally. However, a consideration of the biochemical characteristics of the different cells on the villi reveals striking differences, particularly apparent in folate deficiency.

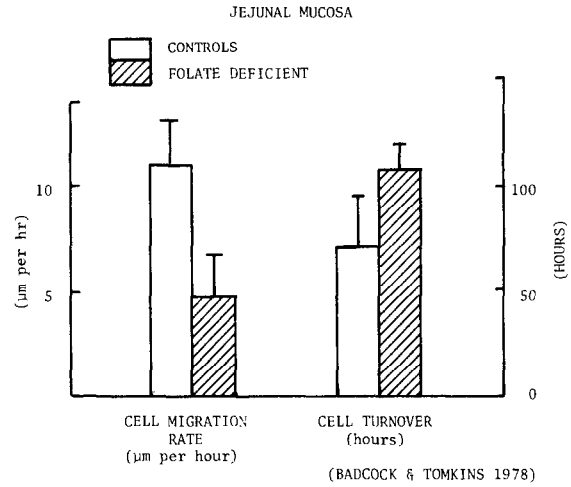

Fig. 3. Abnormal replacement of epithelial cells in experimental mucosal folate deficiency shown as slow migration rate and increase in cell turnover time.

The normal immature crypt cell has low levels of the digestive enzymes, such as sucrase, and acquires these enzymes as it migrates up the villus to become a mature absorptive cell. In folate deficiency there is a slowing of the transit time (Fig. 3) with the result that villus tip cells become elderly in functional terms. In fact the specific activity of sucrase of villus tip cells in folate deficiency is one and a half times as high as villus tip cells from well nourished animals (BADCOCK & TOMKINS, 1978). This is sufficient to maintain enzyme activity of the whole intestine and absorption, of disaccharides at least, is not compromised. It may be argued that this is an adaptive response but the protection afforded is rather tenuous as any mucosal damage cannot be repaired because of the constraint on cellular renewal.

In recent years there has been increasing interest in the immunocytes of the lamina propria. Selective staining and other techniques have shown them to be a mixture of T and B lymphocytes. There is evidence of functional impairment in protein energy malnutrition of even marginal type (TOMKINS *et al.*, 1977); the rejection response to experimental infection with the intestinal nematode *Nippostrongylus brasiliensis* is delayed. It is uncertain whether similar problems occur in folate deficiency but circulating lymphocytes are markedly abnormal in megaloblastic anaemia (DAS & HOFFBRAND, 1970) and preliminary studies in animals show impairment of mast cell responses and IgE production in folate-deficient animals receiving an inoculation of intestinal nematodes.

Table I—Viruses in faecal specimens from asymptomatic subjects in rural northern Nigeria

		Breast fed babies age 2-4 months	Weaned children 2-4 years	Adults
Virus culture	Adenovirus	3/18	4/14	1/13
	Enterovirus	0/18	3/14	1/13
Negative Stain Electron Microscopy	Coronavirus-like particles	12/18	13/14	12/13

Folate deficiency in paediatric gastro-enteritis

The close association of gastro-enteritis and malnutrition, particularly in pre-school children in developing countries has been confirmed repeatedly since the classic studies of GORDON *et al.* (1964). The fairly consistent pattern of frequent and serious diarrhoea around the time of weaning has focussed attention on the significance of deficiencies of energy and protein in the pathogenesis of this weanling diarrhoea, but other nutrients have received less attention. Recent studies have contributed towards an understanding of both the infectious and nutritional component of this syndrome.

The high incidence of gastro-enteritis may be due to many environmental factors including temperature, humidity, abundant surface water, inadequate sanitation and poor personal hygiene. Such conditions favour the proliferation of enteropathogenic bacteria but viruses may also be important. There are now frequent reports of virus particles (BISHOP *et al.*, 1973) in faecal specimens and even jejunal mucosa of children with gastro-enteritis in both developed and developing countries. Virus particles are uncommon in healthy children in developed communities. This contrast with the findings in developing areas, such as south India, where many asymptomatic children have virus-like particles in faecal specimens (MATHAN *et al.*, 1975).

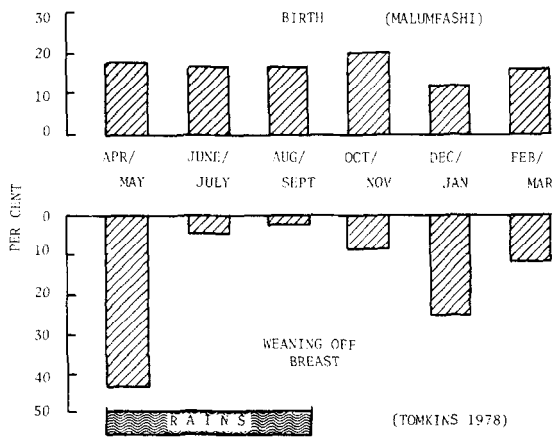

Fig. 4. Seasonal pattern of weaning off breast in rural northern Nigeria indicating risk of seasonal folate deficiency.

Our own studies in Nigeria also suggest that these are acquired in very early life (Table I), being present in a high proportion of breast fed infants, without diarrhoea, during the first year of life.

A marked decrease in nutrient intake is also an important component of paediatric gastro-enteritis in developing communities. In some areas the decrease in quantity of breast milk available is probably secondary to maternal malnutrition developing as a result of the continuation of peak physical activity of farming during the rains and the severe over-all food shortage at this season. There are also characteristic increases in the incidence of gastro-enteritis and malnutrition during the rainy season in areas such as northern Nigeria (DOSSETOR, 1975), where careful seasonal studies show no evidence of limitation of energy or protein intakes (SIMMONDS, 1971), in the adults at least. A striking feature here, however, is that nearly half the infants are abruptly weaned off the breast during the early months of the rains, possibly as a result of the pressures on women in certain areas to work on the fields (Fig. 4). Apart from the problem of energy and protein deficiency as a result of this rapid weaning there is a drastic reduction in folate supply. Breast milk is the major source of dietary folate, especially important during the rainy season when the only other source of folate (fresh vegetables) is particularly scarce. A study from another area in Nigeria has recently emphasized the close association of megaloblastic anaemia with protein energy malnutrition (AKINKUGBE, 1977) clearly showing that anaemia is most common in the season with most gastro-enteritis and malnutrition. It is still not clear how much of the growth failure in such cases can be attributed to folate deficiency but Gandy (GANDY & JACOBSON, 1977) has demonstrated impressive improvement in weight gain of folate-deficient children following oral folic acid therapy alone. However, despite the widespread occurrence of folate deficiency in many children in developing countries and the impression among many paediatricians that folic acid therapy improves malnourished children with chronic diarrhoea, there is little published information. As diarrhoea and malnutrition continue to be major causes of morbidity in many developing countries it is important to develop our understanding of these problems more fully. The relevance of folate deficiency in these clinical syndromes requires further consideration.

Acknowledgements

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