blood coagulation were most active and platelet survival shortest when the diet was rich in dairy fats and eggs. When the diet contained little fat converse results were obtained. A diet rich in vegetable fat produced intermediate results. The serum cholesterol and phospholipid values were highest with the dairy fat and egg yolk diet. However, poor correlations were found between the level of the blood lipids and the coagulation tests. Thus, extended studies have shown that dietary control influences not only serum lipid values but also blood coagulation *in vitro* and platelet economy *in vivo*.

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VEGANISM: A CLINICAL SURVEY WITH OBSERVATIONS ON VITAMIN-B₁₂ METABOLISM

BY

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A vegan can be defined as an extreme vegetarian who excludes all animal protein from his diet. Dairy produce is acceptable to the ordinary lactovegetarian but not to the vegan, who may in certain cases feel that any animal contact is to be abhorred for ethical reasons and replace all articles of clothing and household equipment possessing an animal origin. The vast majority of these subjects neither smoke nor drink, and this renders them eminently suitable for clinical investigation, bearing in mind the possibility of vitamin-B₁₂ deficiency. Some previous observations from this viewpoint have already been published (Donath *et al.*, 1953; Hardinge and Stare, 1954; Wokes *et al.*, 1955). However, this previous work has been mainly preoccupied with the possible effects from various dietary deficiencies, and in the present paper it is hoped to offer a solution to the reverse of this context. The question seems to be, not why do some people on this form of diet develop vitamin-B₁₂ deficiency, but why many subjects do not. In an attempt to obtain an answer a small group of vegans were asked to co-operate in a clinical survey in the hope that some explanation of this apparent lack of deficiency might be forthcoming.

Material and Methods

Composition of Group.—Using a 100% criterion for veganism 12 subjects were found who agreed to the scheme of investigation proposed. They had lived on their rigorous diet for from 4 to 30 years, and their serum vitamin-B₁₂ levels, estimated by the *Lactobacillus leichmannii* method with cyanide extraction, ranged from 48 to 220 $\mu\mu\text{g./ml.}$ and averaged $128 \pm 15 \mu\mu\text{g./ml.}$ (normal range from conventional viewpoint, 140–900 $\mu\mu\text{g./ml.}$). This level compares closely with that obtained in the previous British series (Wokes *et al.*, 1955) and has been reported previously (Wokes and Smith, 1961). Apart from routine serum vitamin-B₁₂ assays the subjects were further analysed as follows.

History and Clinical Examination.—A detailed history was obtained from each volunteer. Particular attention was paid to symptoms that might have arisen from vitamin-B₁₂ deficiency, and other symptoms that were reported in the previous British series were also carefully inquired into. A full clinical examination, which included an entire neurological survey, was then made.

Ancillary Investigations.—Full blood count, serum iron, serum proteins, and electroencephalographic examination were performed on all subjects with two exceptions (see Table). In addition those with cardiac murmurs were further screened by chest x-ray and electrocardiographic examinations.

Results

In this small group the notable feature of the histories obtained lay in the paucity of the symptoms. Only

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Summary of Results on Vegans

Sex	Age	Years as Vegan	Hb %	Serum B ₁₂ (μg./ml.)	Serum Fe (μg./100 ml.)	E.E.G.
M	26	18*	94	220	200	—
M	57	15	92	190	83	0
M	56	5	88	176	30	+
M	12	12	100	160	50	+
M	56	22	90	142	16	+
M	45	17	98	130	147	+
F	29	13	88	120	67	+
F	53	4	80	90	Nil (?)	0
M	18	18	96	90	—	++
F	49	14	80	84	38	+
F	71	30	83	80	25	++
F	48	18	91	48	63	++

*Vegan until 12 years of age, then lactovegetarian until 20 years, then vegan again.

0=Normal. +=Slightly but definitely abnormal. ++=Markedly abnormal.

three subjects volunteered or admitted under questioning to any symptoms at all. On examination two of these were found to have unequivocal subacute combined degeneration of the cord. The third gave a complicated history suggestive of an onset due to psychogenic causes, but this was complicated by a family history of pernicious anaemia. This case is reported in detail below as it illustrates some features of importance. It was noticeable that oral symptoms—that is, sore tongue, etc.—amenorrhoea, and the so-called “vegan back” of the previous British series were completely absent in this group. Their heights and weights were within normal limits for age and sex.

Apart from the two vegans with subacute combined degeneration of the cord there were no abnormal physical signs of definite significance to be elicited in this series. Two had loud systolic murmurs at the apex which might have been compatible with an organic origin, but chest x-ray films and E.C.G.s were completely normal. The possible significance of this is discussed below.

The haemoglobin values ranged from 80 to 100%, and averaged $90 \pm 2\%$ of the standard. This compares with an average of 97% in the previous British series. On the other hand, the average serum iron level dropped from 91 ± 38 μg./100 ml. at a serum vitamin-B₁₂ level of 187 μg./ml. to 42 ± 11 μg./100 ml. at 71 μg. vitamin B₁₂/ml. The serum proteins were normal.

An E.E.G. was obtained from 11 subjects. It was normal in only two; these had an average serum vitamin-B₁₂ level of 140 μg./ml. The recording was slightly but definitely abnormal in six, with an average serum vitamin-B₁₂ level of 136 ± 14 μg./ml., and markedly abnormal in three, with an average serum vitamin-B₁₂ level of 73 ± 13 μg./ml. The assessment of the E.E.G.s was made by an independent observer who was unaware of the clinical status or vitamin-B₁₂ levels. The E.E.G. abnormalities were of a diffuse nature and compatible with a generalized metabolic defect with the exception of one case (see below).

The two subjects with subacute combined degeneration of the cord had serum vitamin-B₁₂ levels of 142 μg./ml. and 176 μg./ml. respectively. The five oldest vegans, aged 53, 56, 56, 57, and 71 years, had serum vitamin-B₁₂ levels of 90, 142, 176, 190, and 80 μg./ml. respectively. There was no evidence, therefore, that the low average vegan level was due to a high proportion of old people.

Case in Detail

A vegan woman aged 48 had been adhering strictly to this form of diet for 18 years. She gave a strong family

history of pernicious anaemia—three close blood relatives had suffered from this disease. In addition her serum vitamin-B₁₂ level was 48 μg./ml. and the E.E.G. showed a marked diffuse generalized abnormality with a suggestion of a left temporal lobe focus. Full blood count was normal, with a haemoglobin of 91% and no evidence of macrocytosis. The serum iron was 63 μg./100 ml. She complained of increasing tiredness interspersed with many typically hysterical complaints. Physical examination was negative. It was noticeable that this woman thought her son aged 18 years (vegan since birth) had been more irritable and “not as well as before” since taking up smoking two years previously. The son denied these symptoms emphatically, and, in any case, they would have been difficult to evaluate as he appeared to have certain difficulties connected with his personal life. It must be admitted, however, that his serum vitamin-B₁₂ level was low at 90 μg./ml. and that the E.E.G. was also markedly abnormal.

In view of the family history of pernicious anaemia it was obviously desirable to exclude this as an additional

Normal gastric biopsy from a vegan subject whose serum vitamin-B₁₂ level was known to be below 100 μg./ml. for at least six years. ($\times 78$.)

factor in the production of her low vitamin-B₁₂ and abnormal E.E.G. Sternal puncture was refused, but she willingly agreed to an augmented histamine test meal and a gastric biopsy. The stomach was found to contain free acid, and the biopsy (Dr. Margot Shiner) was thought to be within normal limits (Dr. R. Drury) (see Fig.). Two injections of cytamen (1,000 μg. each) failed to alter the pattern of the E.E.G. or relieve her symptoms. It was found out later that this particular patient had been seen at the Hammersmith Hospital six years previously, when her serum vitamin-B₁₂ level had been estimated by the *Euglena gracilis* method and found to be 60 μg./ml. (Dr. D. L. Mollin, personal communication).

Discussion

The main interest in this small clinical survey lies in the absence of manifest signs and symptoms due to vitamin-B₁₂ deficiency in 9 of the 12 subjects examined. This is in spite of vitamin levels in some cases compatible with a diagnosis of pernicious anaemia or subacute combined degeneration. Our thinking with respect to vitamin-B₁₂ deficiency has been conditioned by the presence or absence of these two clinical conditions, but, as stated before (Smith, 1961a), it seems necessary to reorientate our clinical and biochemical approach if we are to diagnose the wider concept of this metabolic disorder.

The paradox of veganism may be contrasted with tobacco amblyopia. Here you have a condition which responds to cytamen injections in the face of normal vitamin-B₁₂ levels. Prior to this line of therapy it was essential that the patients discontinued smoking if improvement was to be effected. Heaton *et al.* (1958) showed that a series of cases with this condition had a mean average vitamin-B₁₂ level of 218 $\mu\text{g./ml.}$ and yet responded to cytamen injections while continuing to smoke. Furthermore, they claimed (Freeman and Heaton, 1961) that the retrobulbar neuritis of pernicious anaemia occurred only in smokers and could be attributed, like tobacco amblyopia, to a "toxin" in tobacco.

Evidence has been offered (Smith, 1961a) that the response of tobacco amblyopia to cytamen injections could be explained by adopting the concept that hydroxocobalamin is the potent physiological analogue of vitamin B₁₂. A logical conclusion from this argument is that the serum and tissue hydroxocobalamin levels are the real index of vitamin-B₁₂ deficiency and that until this estimation can be accurately performed we shall remain at a clinical disadvantage. Cyanide in tobacco smoke (Osborne *et al.*, 1956) can be incriminated on experimental grounds as a demyelinating agent (Hurst, 1942; Lumsden, 1950), and it is debatable whether pure cyanocobalamin has ever been given to sufferers from pernicious anaemia or subacute combined degeneration of the cord.

The initial spectrophotometric screening of ampoules of vitamin B₁₂ is not sufficient to justify the conclusion that they will contain 100% cyanocobalamin, as the action of photolysis on this substance is well known, with the subsequent production of hydroxocobalamin (Baxter *et al.*, 1953). In any case, the haematological deficit in pernicious anaemia will respond to artificially prepared cobalamins as well as to folic acid and cortisone. In this context it is interesting to note that the injection of the thiocyanate analogue in pernicious anaemia with subacute combined degeneration has been shown to produce a prompt haematological response, but that the neurological response is disappointing (James and Abbott, 1951). Although small doses of the therapeutic agent were used in this experiment it nevertheless again suggests that the haematological deficit requires the cobalamin portion of the molecule for correction, whereas the more generalized effects of vitamin-B₁₂ deficiency (including involvement of the central nervous system) require hydroxocobalamin.

Vegans (with rare exceptions) neither smoke nor drink and their exogenous cyanide intake is therefore at a minimum. The limited amount of serum vitamin B₁₂ which they possess is almost certainly mainly in the hydroxo form and able to protect their central nervous system from manifest deficiency. However, the E.E.G. evidence in this series supports the view that this might be occult in nature, although in the case described in detail it was a little unusual to find the abnormal pattern uninfluenced by cytamen injections if it was, in fact, due to vitamin-B₁₂ deficiency. The presence of a query focus in the left temporal region in this case raised the possibility of an area of demyelination due to hypovitaminosis-B₁₂, but the lack of response of the generalized abnormality was not in keeping with previous observations (Walton *et al.*, 1954; Smith, 1960). The exact aetiology in this particular case must therefore remain in doubt. The two subjects with subacute com-

bined degeneration of the cord were both smokers and their exogenous cyanide intake was therefore at a level to justify the assumption that their limited amount of available hydroxocobalamin would be exhausted under these circumstances. We know that hydroxocobalamin plays an important part in the detoxification of cyanide (Mushett *et al.*, 1952; Boxer and Rickards, 1952), and its availability in these people may be of crucial importance. Furthermore, in the present state of our knowledge the occurrence of hypovitaminosis B₁₂ with so-called normal serum levels (as in tobacco amblyopia and the vegans with cord and peripheral nerve damage) can only be explained by the thesis of hydroxocobalamin availability.

Another puzzling feature was the finding of subnormal serum iron levels without hypochromic anaemia. Failure of iron absorption is probably due to the considerable quantity of phytic acid in the wholemeal bread of the vegan diet. The absence of macrocytic anaemia due to the low vitamin-B₁₂ levels can be readily explained by the fact that these subjects consume a surfeit of folic acid (Wokes and Smith, 1961), but the absence of hypochromia remains unexplained. It has previously been shown (Cox *et al.*, 1959) that iron-deficiency anaemia is often associated with low vitamin-B₁₂ levels and that the vitamin-B₁₂ level rises when the anaemia is corrected with iron alone. However, this work was performed using evidence for serum vitamin-B₁₂ levels produced by *L. leichmannii* in the absence of cyanide in the extraction process. It has been demonstrated quite clearly that this is an unsatisfactory method of estimating serum vitamin-B₁₂ levels with this organism (Matthews, 1961; Smith, 1961b) if one wishes to obtain results comparable to those found with *E. gracilis*. Other workers have encountered a similar situation and reverted to cyanide extraction. However, it must again be pointed out that this unsatisfactory state of affairs is applicable only if one wishes to obtain the higher result for the reasons given above, and extraction without cyanide may provide a measure of the hydroxocobalamin in the serum (Smith, 1961a). In any case, the metabolic effects of vitamin B₁₂ are so wide and varied that an influence on iron metabolism seems highly probable.

The case described in detail is of theoretical interest because it is known that the subject had been in possession of a very low serum vitamin-B₁₂ level for at least six years. In spite of this the gastric biopsy was normal and conflicts with the suggestion that the low vitamin-B₁₂ level leads to changes in the mucosa (Campbell and Dickinson, 1960). However, it does not entirely exclude this possibility, as a longer period of time may be necessary to produce gastric atrophy.

It has been noted (Smith, 1961, unpublished data) that the so-called haemic murmurs of pernicious anaemia can also occur in sufferers from subacute combined degeneration of the cord without anaemia. Furthermore, this murmur disappeared dramatically on institution of therapy, which suggests that it might be due to a cardiomyopathy secondary to the deficiency. The normal E.C.G.s and chest x-ray films in the two vegans with cardiac murmurs in this series are evidence against the latter being due to a cardiomyopathy, but unfortunately the subjects concerned refused cytamen injections with subsequent auscultatory examination.

Veganism therefore produces a biochemical and metabolic state which lends itself to many questions of

practical interest. However, for various social and ethical reasons, the subjects felt unable to co-operate further and other lines of investigational approach had to be abandoned.

Summary

A series of 12 vegans have been investigated clinically from the aspect of vitamin-B₁₂ metabolism and deficiency. It is suggested that their comparative freedom from manifest signs and symptoms in the central nervous system is due to their having the major portion of their low serum vitamin B₁₂ in the hydroxo form.

A comparison is made between veganism and tobacco amblyopia, and attention is drawn to cyanide metabolism. The possibility of endogenous and exogenous factors interfering with this and producing subsequent demyelination is discussed. Although most of these subjects were clinically normal, the electroencephalographic evidence suggests that occult damage to the central nervous system is taking place and that this form of diet may produce subtle cerebral changes that are unrecognizable by normal clinical methods.

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The recently published *Register of Research in the Human Sciences 1960-61* gives details of over 300 research investigations and the people engaged in them. The register includes work done in the fields of anatomy, medical sociology and social medicine, physiological psychology, and criminology. It has been compiled by Warren Spring Laboratory, Department of Scientific and Industrial Research. (H.M.S.O., price 10s., postage 5d.)

VITAMIN-B₁₂ DEFICIENCY IN PREGNANCY AND THE PUERPERIUM

BY

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Among the non-pregnant population of South India megaloblastic anaemia due to vitamin-B₁₂ deficiency occurs frequently, and is often associated with tropical sprue or tropical malabsorption syndrome (Baker, 1957, 1958). Some women with a mild malabsorption syndrome become pregnant and, like so many others, come to hospital only at term or at the onset of labour. This has given us an opportunity to study some of the effects of maternal vitamin-B₁₂ deficiency; these are reported in this paper.

Materials and Methods

The patients studied were drawn from the obstetric service of the Christian Medical College Hospital, Vellore. Seven patients were admitted at full term and were delivered of apparently normal babies. Two were admitted in labour at 28 and 32 weeks respectively and gave birth to stillborn foetuses.

Initial blood counts, serum for vitamin-B₁₂ estimations, and bone-marrow specimens were taken at the time of admission and repeated at intervals thereafter. All patients included in this study had evidence of a malabsorption syndrome, a megaloblastic anaemia, and a low serum-vitamin-B₁₂ level at the time of delivery. Blood counts and serum-vitamin-B₁₂ estimations were done on cord-blood specimens from each of the seven full-term babies. Estimations of vitamin-B₁₂ levels in breast milk were made at intervals during the puerperium.

The livers were removed from the two stillborn foetuses, stored at -20° C., and subsequently assayed for vitamin-B₁₂ content. The accumulation of vitamin B₁₂ in normal foetuses was also studied by obtaining livers from stillborn foetuses of different ages from mothers with normoblastic bone-marrow and normal serum-vitamin-B₁₂ levels, and estimating the total liver vitamin-B₁₂ content.

The haematological techniques employed were those described by Dacie (1956). Vitamin-B₁₂ estimations were carried out using *Euglena gracilis*, Z strain, as the test organism (Ross *et al.*, 1957). Vitamin B₁₂ in serum was estimated as "total" vitamin B₁₂ (both bound and free). The liver specimens were homogenized in acetate buffer at pH 4.6 and then heated at 100° C. for 30 minutes; each specimen was extracted three times, and the values for each extraction were added together (Ross and Mollin, 1957). Vitamin B₁₂ in breast milk was assayed after papain digestion. Vitamin-B₁₂ absorption was tested by the urinary excretion method of Schilling (1953), using a 1-μg. test dose of ⁵⁷Co-labelled vitamin B₁₂, and a flushing dose of 1,000 μg. of unlabelled