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.

I am grateful to Professor A. Kekwick and the members of the Clinical Research Committee, Institute of Clinical Research, Middlesex Hospital Medical School, for providing me with facilities to conduct much of this work. It was produced during the tenure of a Leverhulme Research Scholarship and a grant from the North-west Metropolitan Regional Board. I also thank my sponsors, Dr. Michael Kremer and Dr. R. J. Porter, for their continued advice and encouragement. Dr. George Discombe and Dr. C. C. Evans gave me considerable help with the haematological and electroencephalographic investigations, which proved invaluable in the assessment of these subjects. Finally, Dr. Frank Wokes enlisted the help of the vegan volunteers and gave me freely of his wide knowledge and advice.

REFERENCES

- Baxter, N., Horsford, J., Wokes, F., Norris, F. W., and Fernandes, S. J. G. (1953). *J. Pharm. Pharmacol.*, **5**, 723.
 Boxer, G. E., and Rickards, J. C. (1952). *Arch. Biochem.*, **39**, 7.
 Campbell, E. J. M., and Dickinson, C. J. (1960). *Clinical Physiology*, p. 333. Blackwell, Oxford.
 Cox, E. V., Meynell, M. J., Gaddie, R., and Cooke, W. T. (1959). *Lancet*, **2**, 998.
 Donath, W. F., Fischer, J. A., van der Meulen-van Eysbergen, H. C., and de Wijn, J. F. (1953). *Voeding*, **14**, 153.
 Freeman, A. G., and Heaton, J. M. (1961). *Lancet*, **1**, 908.
 Hardinge, M. G., and Stare, F. J. (1954). *J. clin. Nutr.*, **2**, 73.
 Heaton, J. M., McCormick, A. J. A., and Freeman, A. G. (1958). *Lancet*, **2**, 286.
 Hurst, E. W. (1942). *Aust. J. exp. Biol. med. Sci.*, **20**, 297.
 James, G. W., and Abbott, L. D. (1951). *Proc. Soc. exp. Biol. (N.Y.)*, **77**, 416.
 Lumsden, C. E. (1950). *J. Neurol. Neurosurg. Psychiat.*, **13**, 1.
 Matthews, D. M. (1961). *Lancet*, **1**, 1289.
 Mushett, C. W., Kelley, K. L., Boxer, G. E., and Rickards, J. C. (1952). *Proc. Soc. exp. Biol. (N.Y.)*, **81**, 234.
 Osborne, J. S., Adamek, S., and Hobbs, M. E. (1956). *Analyt. Chem.*, **28**, 211.
 Smith, A. D. M. (1960). *Brit. med. J.*, **2**, 1840.
 — (1961a). *Lancet*, **1**, 1001.
 — (1961b). *Ibid.*, **1**, 1346.
 Walton, J. N., Kiloh, L. G., Osselson, J. W., and Farrall, J. (1954). *Electroenceph. clin. Neurophysiol.*, **6**, 45.
 Wokes, F., Badenoch, J., and Sinclair, H. M. (1955). *Voeding*, **16**, 590.
 — and Smith, A. D. M. (1961). *Proceedings of 2nd European Symposium on Vitamin B₁₂ and Intrinsic Factor*. Hamburg.

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

vitamin B₁₂. The test was carried out in the puerperium after observation of the therapeutic response to treatment. The test was later repeated with the addition of 40 mg. of intrinsic factor preparation.

Twenty-four-hour stool collections were made for periods of 3 to 10 days while the patients were on a diet containing 40 to 80 g. of fat a day. Estimations of stool fat were carried out by the wet method of van de Kamer *et al.* (1949). (Normal daily fat excretion 5 g. or less.) Glucose-tolerance tests were performed, using a dose of 50 g. of glucose and estimating the rise in blood sugar in venous blood. (Normal maximum rise more than 25 mg./100 ml.: Rajan *et al.*, 1961.) Barium-meal examinations of the small intestine were made, using a non-flocculating medium (Paterson and Baker, 1958).

Results

Clinical Features.—Four of the nine women complained of symptoms referable to anaemia, such as breathlessness, pallor, palpitations, and oedema. All nine had existed throughout pregnancy on the usual diet of South Indian villagers, which is generally low in animal proteins, calories, vitamins, and other essentials (Rao and Rao, 1958). Six gave a history of disordered intestinal function suggestive of a malabsorption syndrome (Baker, 1957).

Haematology.—The results of the routine initial haematological examination, in each case made during labour, are set out in Table I, together with those of examinations made on the baby's (cord) blood in Cases 1 to 7. All the mothers were anaemic and had a megaloblastic bone-marrow, whereas the babies had no anaemia and, in all except Case 5, a normal bone-marrow. The bone-marrow of Case 5 showed abnormal developing red cells typical of "intermediate" megaloblasts. The child was given 50 µg. of vitamin B₁₂ by injection, and a bone-marrow examination five days later showed that the developing red cells were normoblastic.

Serum-vitamin-B₁₂ Levels.—These were measured in both mothers and babies. The results in Cases 1 to 7 are shown in Fig. 1. The maternal levels ranged from 20 to 90 µg./ml. (mean 49.7 µg./ml.), and the levels in the babies from 100 to 560 µg./ml. (mean 220 µg./ml.). In each case the level in the baby was much higher than that in the mother. The maternal serum-vitamin-B₁₂ levels in Cases 8 and 9 were 37 and 68 µg./ml. respectively.

Vitamin-B₁₂ Absorption.—In all cases the radioactive vitamin-B₁₂ excretion in the urine was 5% or less of the administered dose (Table II). A repeat test with the

administration of an active preparation of intrinsic factor did not produce any significant improvement in absorption.

Intestinal Function.—Studies of intestinal function were carried out on all nine women during the puerperium. The glucose-tolerance test was normal in three cases and "flat"—that is, a rise of less than 25 mg./100 ml.—in six cases (Rajan *et al.*, 1961). Seven patients had a steatorrhoea with excretion from 6 to 19 g. of fat a day. Two had no steatorrhoea (Table III). In Case 2 the barium meal was normal, but features of malabsorption syndrome (Paterson and Baker, 1958) were seen in the other eight.

Response to Treatment.—After investigation all cases were given 100 µg. of vitamin B₁₂ by injection. In eight cases (Nos. 2 to 9) this was followed by a reversion of red-cell development in the bone-marrow to normoblastic, a prompt reticulocytosis, and a subsequent satisfactory rise in haemoglobin and red cells. However, Case 1 did not respond either to 100 µg. or to 1,000 µg. of vitamin B₁₂, but there was subsequently a good response after folic acid administration (Fig. 2).

Breast Milk Vitamin B₁₂.—In Cases 1 to 7 samples of breast milk were collected on three consecutive days during the puerperium and before treatment with

FIG. 1.—Serum-vitamin-B₁₂ levels in mothers and in cord blood of infants.

TABLE II.—Results of Schilling Tests

Case No.	Percentage of Doses Excreted in Urine in 24 Hours	
	Dose Alone	Dose + 40 mg. I.F.
1	3.1	3.5
2	4.9	4.5
3	1.3	1.1
4	2.8	3.7
5	5.0	5.1
6	2.0	3.0
7	4.2	3.8
8	0.8	2.0
9	3.7	3.2

TABLE III.—Tests of "Intestinal Function"

Case No.	Glucose Tolerance, Maximum Rise in Blood Sugar (mg./100 ml.)	Mean Daily Fat Excretion (g./day)	Barium Meal
1	2	4.2	Abnormal
2	37	12.3	Normal
3	15	8.5	Abnormal
4	28	19.2	"
5	21	3.5	"
6	45	9.0	"
7	8	10.7	"
8	17	13.0	"
9	5	6.1	"

TABLE I.—Haematological Findings in Mothers and Their Babies

Case No.	Mothers				Babies			
	Hb (g./100 ml.)	P.C.V. (%)	R.C.C. (mil./c.mm.)	Marrow	Hb (g./100 ml.)	P.C.V. (%)	R.C.C. (mil./c.mm.)	Marrow
1	6.6	24	2.1	Megaloblastic	21.2	62	5.8	Normoblastic
2	7.4	25	2.6	"	17.1	54	5.2	"
3	7.1	22	2.3	"	18.3	57	5.4	"
4	8.8	27	2.6	"	19.6	60	5.9	"
5	7.4	25	2.2	"	19.9	61	6.0	Intermediate megaloblasts
6	8.1	30	2.8	"	22.0	63	6.2	Normoblastic
7	4.8	16	1.4	"	17.6	55	5.4	"
8	5.9	20	2.0	"	—	—	—	—
9	5.8	18	1.9	"	—	—	—	—

vitamin B₁₂. The results are set out in Table IV, where the serum levels are also given for comparison. It will be seen that the vitamin-B₁₂ levels in the breast milk correspond closely with those of the serum in each case.

Accumulation of Vitamin B₁₂ in Foetal Livers.—The vitamin-B₁₂ content of the foetal livers is shown in Fig. 3. The total vitamin-B₁₂ content of the liver in control foetuses increases with increasing age, reaching a maximum of 20–25 μ g. at birth. The two stillborn foetuses from mothers with megaloblastic anaemia had a very greatly reduced vitamin-B₁₂ content, when compared with other foetuses at a similar stage of development.

Discussion

Several workers have reported a progressive lowering of serum-vitamin-B₁₂ levels during normal pregnancy, but this lowering is not normally of such a degree as to produce a frank deficiency state, and is not associated with any other detectable haematological change (Heinrich, 1954; Okuda *et al.*, 1956; Boger *et al.*, 1957; Lowenstein *et al.*, 1960).

Megaloblastic anaemia of pregnancy in the West is commonly associated with folic-acid deficiency (Chanarin *et al.*, 1959; Mollin, 1960). However, in India this condition has been shown to respond to vitamin-B₁₂ therapy (Patel and Kocher, 1950;

Chaudhuri, 1951), suggesting that it may be due to vitamin-B₁₂ deficiency. The patients included in the present study all had a small-intestinal disorder, a defect of vitamin-B₁₂ absorption, a low serum-vitamin-B₁₂ level, a megaloblastic bone-marrow, and in eight out of the nine cases a satisfactory response to the administration of vitamin B₁₂. In Case 1, which did not respond to vitamin-B₁₂ therapy, there was subsequently a response to folic-acid administration. In this case, however, there was also a low serum vitamin B₁₂ and a defect of vitamin-B₁₂ absorption, so it is probable that this patient had a combined deficiency of vitamin B₁₂ and folic acid. In the other eight cases a vitamin-B₁₂ deficiency appears to have been the predominant lesion, although no specific studies were made to determine the status of the patients in regard to folic acid.

It is of interest that in every case the serum-vitamin-B₁₂ levels in the cord blood were much higher than in the maternal blood. A similar finding in normal pregnancy has previously been reported by several workers (Okuda *et al.*, 1956; Dumont, 1957; Boger *et al.*, 1957; Dixit *et al.*, 1957). Baker *et al.* (1958), in a series of observations on mothers and babies, found three mothers with low serum-vitamin-B₁₂ levels (53, 80, and 100 μ g./ml.) whose babies all had cord blood levels of over 100 μ g./ml. (115, 310, and 330 μ g./ml. respectively). However, there is no mention of the haematological status of the mothers.

The finding of higher serum-vitamin-B₁₂ levels and a normal haemoglobin in these infants, in spite of low serum levels and moderately severe anaemia in the mothers, is further evidence that vitamin B₁₂ is preferentially transferred from mother to foetus. Nevertheless the newborn child may suffer from the effect of vitamin-B₁₂ deficiency, as shown by the finding of intermediate megaloblasts in the bone-marrow of baby No. 5.

The study of the accumulation of vitamin B₁₂ in foetal livers shows that the total body stores of the foetus increase throughout pregnancy. We have not been able to find records of a similar study in the literature. However, Ross and Mollin (1957) give a figure of 28 μ g. total liver vitamin B₁₂ for a stillborn child of 38 weeks' gestation, which is slightly higher than our figures though of the same order. The livers from the stillborn foetuses of the two women with megaloblastic anaemia had a much smaller content of

FIG. 2.—Haematological response in Case 1. ○—○, P.C.V. ●—●, Red-cell count. ×—×, Reticulocytes (same scale as P.C.V.).

TABLE IV.—Serum and Breast Milk Vitamin B₁₂ in the Puerperium Before Treatment

Case No.	Vitamin B ₁₂ in Serum (μ g./ml.)	Vitamin B ₁₂ in Breast Milk (μ g./ml.)
1	84	90
2	70	65
3	50	52
4	48	48
5	38	45
6	32	41
7	20	20

FIG. 3.—Total liver vitamin B₁₂ (in micrograms) in foetuses of different ages. ●, Livers from foetuses from normal mothers. ○, Livers from foetuses from mothers with vitamin-B₁₂ deficiency.

vitamin B₁₂ than the control fetuses, showing that vitamin-B₁₂ deficiency in the mother may seriously affect the vitamin-B₁₂ stores of the foetus. It seems probable, therefore, that infants born of vitamin-B₁₂-deficient mothers will start life with defective body stores of this vitamin, even though cord blood levels may not be very low.

As was to be expected the levels in the breast milk followed closely the serum levels. Therefore if vitamin-B₁₂ deficiency in the mother persists the breast-fed child will have a lower intake of vitamin B₁₂ than children suckled by normal mothers.

The daily requirements of the infant for vitamin B₁₂ are not known. However, if the daily requirement of an adult weighing 50 kg. is taken at 1 µg. a day, the requirement of a baby weighing 3 kg. will, on a weight-for-weight basis, be approximately 60 mµg. a day. If the normal quantity of breast milk secreted in 24 hours is taken as 500 ml., then in order to supply the infant's needs the minimum concentration of vitamin B₁₂ in the breast milk should be in the region of 120 µµg./ml., and anything below this concentration may lead ultimately to vitamin-B₁₂ deficiency. When this defective vitamin-B₁₂ intake is coupled with low body vitamin-B₁₂ stores, then the infant may develop an overt vitamin-B₁₂ deficiency, with megaloblastic anaemia and neurological changes (Jahdav *et al.*, 1962). It may therefore be wise to treat all children born of vitamin-B₁₂-deficient mothers with prophylactic parenteral vitamin B₁₂.

Animals deficient in vitamin B₁₂ may give birth to young with skeletal and other abnormalities (O'Dell *et al.*, 1951; Grainger *et al.*, 1954). No such abnormalities were seen in the seven live children in this series, and macroscopic dissection of the two stillborn fetuses did not reveal any abnormality. It is not known whether the vitamin-B₁₂ deficiency played any part in the foetal death *in utero* and premature delivery in Cases 8 and 9.

Summary

Seven women with tropical malabsorption syndrome who had megaloblastic anaemia of pregnancy associated with vitamin-B₁₂ deficiency were investigated, together with their babies.

The women had a moderate to severe anaemia and low serum-vitamin-B₁₂ levels. Breast milk vitamin-B₁₂ levels in the puerperium were similar to serum-vitamin-B₁₂ levels. The babies had no anaemia, and had much higher serum-vitamin-B₁₂ levels than their mothers. One baby had "intermediate" megaloblasts in its bone-marrow.

The accumulation of vitamin B₁₂ in foetal livers was studied. This reached a maximum of 20 to 25 µg. of total liver vitamin B₁₂ at birth. Two stillborn fetuses from vitamin-B₁₂-deficient mothers had greatly reduced liver vitamin-B₁₂ stores.

Babies born of and suckled by vitamin-B₁₂-deficient mothers may have lower body vitamin-B₁₂ stores, may receive less vitamin B₁₂ in breast-milk feeds, and may be in danger of developing frank vitamin-B₁₂ deficiency.

We thank Professors M. Manuel and D. Paranjothy, of the Department of Obstetrics, and Dr. M. D. Graham, of the Department of Paediatrics, for allowing us access to these cases, and for their help and encouragement; and Dr. W. L. Williams, of Lederle Laboratories, New York, for the gift of intrinsic factor concentrate used in this study. This work was in part carried out under a research grant from Glaxo Laboratories India Limited, to whom we express our thanks.

REFERENCES

- Baker, H., Ziffer, H., Pasher, I., and Sobotka, H. (1958). *Brit. med. J.*, **1**, 978.
 Baker, S. J. (1957). *Indian J. med. Sci.*, **11**, 687.
 — (1958). *Indian J. Path. Bact.*, **1**, 11.
 Boger, W. P., Wright, L. D., and Bayne, G. M. (1957). Vitamin B₁₂ and Intrinsic Factor, 1. Europaisches Symposion, Hamburg, 1956. Ferdinand Enke, Stuttgart.
 Chanarin, I., MacGibbon, B. M., O'Sullivan, W. J., and Mollin, D. L. (1959). *Lancet*, **2**, 634.
 Chaudhuri, S. (1951). *Brit. med. J.*, **2**, 825.
 Dacie, J. V. (1956). *Practical Haematology*, 2nd ed. Churchill, London.
 Dixit, C. H., Mody, B. N., Jhala, H. I., Parekh, J. G., and Ramasarma, G. B. (1957). *Indian J. med. Sci.*, **11**, 145.
 Dumont, M. (1957). *Presse méd.*, **65**, 601.
 Grainger, R. B., O'Dell, B. L., and Hogan, A. G. (1954). *J. Nutr.*, **54**, 33.
 Heinrich, H. C. (1954). *Klin. Wschr.*, **32**, 205.
 Jahdav, M., Webb, J. K. G., Vaishnava, S., and Baker, S. J. (1962). To be published.
 Kamer, J. H. van de, Humink, H. ten B., and Weyers, H. A. (1949). *J. biol. Chem.*, **177**, 347.
 Lowenstein, L., Lalonde, M., Deschenes, E. B., and Shapiro, L. (1960). *Amer. J. clin. Nutr.*, **8**, 265.
 Mollin, D. L. (1960). *Ann. Rev. Med.*, **11**, 333.
 O'Dell, B. L., Whitley, J. R., and Hogan, A. G. (1951). *Proc. Soc. exp. Biol. (N.Y.)*, **76**, 349.
 Okuda, K., Helliger, A. E., and Chow, B. F. (1956). *Amer. J. clin. Nutr.*, **4**, 440.
 Patel, J. C., and Kocher, B. R. (1950). *Brit. med. J.*, **1**, 1924.
 Paterson, D. E., and Baker, S. J. (1958). *J. Fac. Radiol. (Lond.)*, **9**, 183.
 Rajan, K. T., Rao, P. S. S., Ponnusamy, I., and Baker, S. J. (1961). *Brit. med. J.*, **1**, 29.
 Rao, B. R. H., and Rao, P. S. S. (1958). *Indian J. med. Sci.*, **12**, 726.
 Ross, G. I. M., Hutner, S. H., and Bach, M. K. (1957). Vitamin B₁₂ and Intrinsic Factor, 1. Europaisches Symposion, Hamburg, 1956. Ferdinand Enke, Stuttgart.
 — and Mollin, D. L. (1957). *Ibid.*
 Schilling, R. F. (1953). *J. Lab. clin. Med.*, **42**, 860.

ANCYLOSTOMIASIS IN ENGLAND

BY

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The arrival in England of large numbers of immigrants from tropical countries where hookworm infection is widespread prompted a study of such infections in this country. The occurrence of severe anaemia due to hookworm infection in several in-patients of this hospital further stimulated interest in the incidence of symptoms in this condition.

The study is divided into two parts. In the first part the incidence of hookworm infection in 99 apparently healthy immigrants selected for employment in a large London organization was ascertained by examination of their stools; the presence of eosinophilia and anaemia in these subjects was also recorded. The second part consisted of the analysis of the symptoms and effects of hookworm infection in cases diagnosed at this hospital.

Method.—Stool specimens were examined by the zinc sulphate flotation method (Otto *et al.*, 1941) within 12 hours of being passed, after being stored as soon as possible at 0° C. Two 7/8-in. (22-mm.) square coverslip preparations were scanned under the 16-mm. objective, and suspicious objects were examined under the 4-mm. objective. The haemoglobin level and the total and differential white-cell counts were estimated in all subjects. Occult blood was examined by the haematest