

Observations on vitamin B₁₂ in serum and cerebrospinal fluid in multiple sclerosis

W. BASIL, J. K. BROWN, AND D. M. MATTHEWS

From the Department of Chemical Pathology, Institute of Neurology, the National Hospital, Queen Square, London

SYNOPSIS There are several claims that B₁₂ concentrations in serum and cerebrospinal fluid are grossly abnormal in multiple sclerosis, but results are conflicting. This paper reports measurements of these concentrations in 40 serum samples and 23 samples of lumbar cerebrospinal fluid from cases of multiple sclerosis, and in appropriate controls, using *Euglena gracilis*, z strain. The serum concentrations were found to be normal; the mean concentration in cerebrospinal fluid was slightly reduced, but all values were within the control range.

In both control samples and samples from cases of multiple sclerosis, the B₁₂ concentration in lumbar cerebrospinal fluid was correlated with the concentration in serum. There was no correlation between B₁₂ concentration and total protein in cerebrospinal fluid.

A number of estimations of serum B₁₂ were also made with *Lactobacillus leichmannii*, after extraction in the presence and absence of cyanide. These showed a difference between cases of multiple sclerosis and controls, one interpretation of which might be that the serum in multiple sclerosis contains an abnormally low concentration of hydroxocobalamin.

Several investigations have been made of the vitamin B₁₂ concentration in body fluids in multiple sclerosis, but the results are completely conflicting. Sobotka, Christoff, and Baker (1958) and Sobotka, Baker, Frank, Maker, and Margulies (1960), using *Ochromonas malhamensis*, found large increases in serum and cerebrospinal fluid B₁₂ in about a third of their cases. On the other hand O'Connor, Davis, Langworthy, and Chow (1960), using *Lactobacillus leichmannii*, reported that the serum B₁₂ was low in multiple sclerosis, half of their patients having concentrations within the pernicious anaemia range, and Bauer and Heinrich (1961), using *Euglena gracilis*, also reported very low serum values. Gjertsen and Schrupf (1962), using *Euglena gracilis*, found low values in cerebrospinal fluid. Grann and Glass (1961), using *Ochromonas malhamensis*, found normal serum B₁₂ concentrations in the majority of cases; three out of 21 cases had elevated concentrations, but two of these had been given parenteral B₁₂: one case had a subnormal concentration. The majority of their patients had normal B₁₂ absorption as estimated by hepatic uptake. Worm-Petersen (1962), using *Euglena gracilis*, z strain, found no significant difference in cerebrospinal fluid and serum B₁₂ concentrations

between cases of multiple sclerosis and controls. Kidd, Gould, and Thomas (1963), using *Euglena gracilis*, z strain, estimated B₁₂ in serum and cerebrospinal fluid in a variety of neurological disorders, including three cases of multiple sclerosis; in two of these the values were normal, though in one there was a slight rise in B₁₂ concentration in cerebrospinal fluid. The work reported here was undertaken in the hope of clarifying this confused position, and in order to gain information about the relationship of the B₁₂ concentration in cerebrospinal fluid to that in serum and to cerebrospinal fluid protein. The majority of the estimations were carried out with *Euglena gracilis*, z strain. In some cases, serum estimations were also made with *Lactobacillus leichmannii*, after extraction in the presence and absence of cyanide. This was done in view of the possibility that the difference between these two values indicates the relative proportions of cyanocobalamin and hydroxocobalamin in the serum and the suggestion that a disturbance of cyanide metabolism, involving an alteration in these proportions, might be concerned in the pathogenesis of demyelination (Smith, 1961). Since the present work was completed, Simpson (1964), using *Euglena gracilis*, z strain, has reported no significant dif-

ference between cerebrospinal fluid and serum B₁₂ concentrations in multiple sclerosis and control values.

MATERIALS AND METHODS

Estimations of levels of vitamin B₁₂ in serum and cerebrospinal fluid were made by a modification of the method of Hutner, Bach, and Ross (1956) with *Euglena gracilis*, z strain. The cerebrospinal fluid, which was not centrifuged, was usually assayed at a dilution of 1 in 6, though when the amount available was small, dilutions of 1 in 8 or 1 in 12 were used. Serum estimations with *Lactobacillus leichmannii* were made as described by Matthews (1962); each sample was divided into two parts, one of which was assayed after extraction by heating for 30 minutes with acetate buffer pH 4.6 containing 20 mg. NaCN per litre, and the other after extraction in the same way with buffer containing no cyanide.

The control group for serum B₁₂ was made up of hospital in-patients and out-patients aged 18 to 75, none of whom were known to have any condition affecting the serum B₁₂ concentration. The group of cases of multiple sclerosis was made up of patients aged 17 to 57, in all of whom the diagnosis was firmly established. If the patients had received B₁₂, this was noted. Samples of lumbar cerebrospinal fluid were divided into three groups.

1 THE CONTROL GROUP In this, the cerebrospinal fluid protein concentration was not more than 50 mg. per 100 ml. and the cell count, Pandy Nonne-Apelt and Lange tests were normal, though many of the patients had organic neurological lesions.

2 A 'PATHOLOGICAL' GROUP In this, the cerebrospinal fluid protein concentration ranged from 55 to 500 mg. per 100 ml. and the white cell count up to 500 per c. mm., and in many cases there were other abnormal findings; the patients suffered from a variety of neurological disorders.

3 SAMPLES FROM CASES OF MULTIPLE SCLEROSIS

In addition, a small number of samples of ventricular cerebrospinal fluid were examined. These came from patients with a variety of neurological disorders; the protein concentration ranged up to 20 mg. per 100 ml.,

and in some cases there were other abnormal findings. None of the patients providing cerebrospinal fluid in groups 1 and 2 or ventricular cerebrospinal fluid were known to have any condition affecting the serum B₁₂ concentration.

STATISTICAL METHODS The distribution of B₁₂ concentrations in serum and cerebrospinal fluid fits a logarithmic distribution better than a normal one, as Worm-Petersen (1962) points out, and Simpson (1964) has used the logarithms of the observations for all calculations. We have also done this in addition to making calculations on the usual arithmetical basis. In the majority of instances the logarithmic method made no essential difference to the results of significance tests, and in such cases the logarithmic results are omitted for the sake of clarity. When there was an appreciable difference, logarithmic results are given. The significance of differences between means was assessed by the t test.

RESULTS

The B₁₂ concentrations obtained in serum and lumbar cerebrospinal fluid are shown in Table I. The mean concentration in 'pathological' cerebrospinal fluid was significantly higher than that in the control group ($P < 0.05 > 0.02$). In patients with multiple sclerosis, none of whom were known to have had B₁₂, the mean serum B₁₂ concentration was not significantly different from that in controls, but the mean concentration in cerebrospinal fluid was significantly lower (controls, mean of logs 1.33, S.E. 0.03; multiple sclerosis, mean of logs 1.18, S.E. 0.05; $P < 0.02 > 0.01$) and all the values except one were in the lower part of the control range (Fig. 1). In both control subjects and the cases of multiple sclerosis, there was a correlation between the B₁₂ concentration in serum and cerebrospinal fluid (Figs. 2 and 3). In controls the ratio of B₁₂ concentration in cerebrospinal fluid to serum concentration in paired samples was 5.6%, whereas in multiple sclerosis it was only 3.8%. Six other cases of multiple sclerosis were known to have been given parenteral B₁₂ within three months of the estimations. In these,

TABLE I

Group	B ₁₂ CONCENTRATIONS IN SERUM AND LUMBAR CEREBROSPINAL FLUID USING <i>EUGLENA GRACILIS</i> , Z STRAIN								
	Serum B ₁₂ (μg./ml.)				Cerebrospinal Fluid B ₁₂ (μg./ml.)				
	No. of Patients	Mean	S.E.	Range	No. of Patients	Mean	S.E.	Range	
1 Controls	85	432	21.4	150-960	50	24.2	1.9	6-68	
2 'Pathological' group	—	—	—	—	25	31.6	2.7	8-53	
3	Multiple sclerosis patients	34	397	25.5	150-820	17	17.2	2.3	6-45
		Multiple sclerosis patients given B ₁₂	6	—	—	715-2,000+	6	—	—

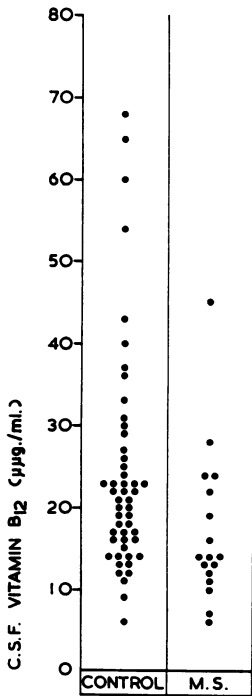


FIG. 1.

the serum and cerebrospinal fluid concentrations were high, five of the serum values and five of the cerebrospinal fluid values being above the upper limit of the control range. No correlation was found between the B₁₂ concentration in cerebrospinal fluid, and total protein or cell count, in any group. In ventricular cerebrospinal fluid, the mean B₁₂ concentration (8.4 µµg. per ml., S.E. 1.7, n = 10) was significantly lower than in lumbar control samples ($P < 0.001$).

SERUM B₁₂ CONCENTRATIONS OBTAINED WITH *Lactobacillus leichmannii* AFTER EXTRACTION IN PRESENCE AND ABSENCE OF CYANIDE The serum B₁₂ concentration obtained by this method in the presence of cyanide measures the total serum B₁₂. In 75 control patients the mean value was 463 µµg./ml. (range 115-950, S.E. 22). In 13 patients with multiple sclerosis, none of whom were known to have had B₁₂, the mean value was 481 µµg./ml. (range 220-700, S.E. 36); the difference is not significant. Values obtained in the absence of cyanide are lower than those in the presence of cyanide, for reasons discussed below. In 38 control sera, the mean value obtained without cyanide was 53.5% (range 28-75%, S.E. 2.0) of that obtained with cyanide. In the cases of multiple sclerosis (with a similar age and sex distribution),

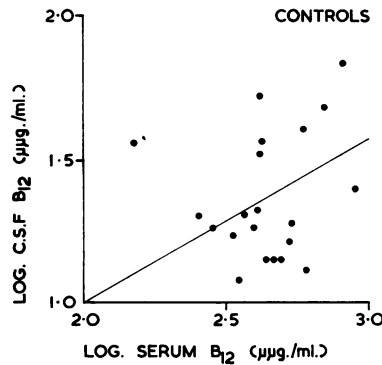


FIG. 2.

FIG. 1. Vitamin B₁₂ concentrations in lumbar cerebrospinal fluid in control samples and in multiple sclerosis.

FIG. 2. Correlation between log cerebrospinal fluid B₁₂ and log serum B₁₂ in controls. The calculated regression line is shown ($r^{12} = 0.46$; $P < 0.05 > 0.02$).

FIG. 3. Correlation between log cerebrospinal fluid B₁₂ and log serum B₁₂ in multiple sclerosis. The calculated regression line is shown ($r = 0.77$; $P < 0.01 > 0.001$).

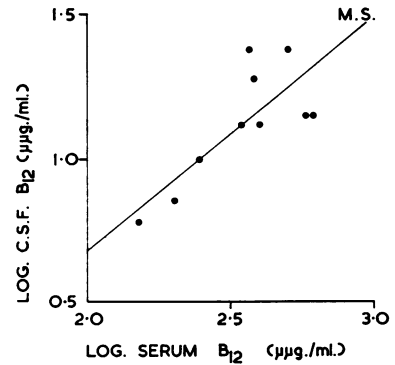


FIG. 3.

the mean value obtained without cyanide was 64.1% (range 51-75%) of that obtained with cyanide (Fig. 4). The difference is significant ($P < 0.01 > 0.001$).

DISCUSSION

The published values for B₁₂ concentration in lumbar cerebrospinal fluid vary very widely (*e.g.* Ross, 1950; Sobotka, Baker, and Frank, 1960; Bauer and Heinrich, 1961; Yavorkovsky and Mai, 1961; Gjersten and Schrupf, 1962) but the control values we have found agree well with those of Herbert and Zalusky (1961), Worm-Petersen (1962), and Kidd, *et al.* (1963). The finding of a correlation between B₁₂ concentrations in serum and cerebrospinal fluid confirms the reports of Worm-Petersen (1962) and of Simpson (1964). We also agree with these authors on the lack of correlation between B₁₂ concentration and total protein in cerebrospinal fluid, though this conflicts with the report of Yavorkovsky and Mai (1961). Values for B₁₂ in ventricular cerebrospinal fluid have not been reported previously as far as we know. It is not obvious why the B₁₂ concentration is lower in ventricular than in lumbar fluid. The slight increase in B₁₂ concentration in pathological lumbar cerebrospinal fluid (excluding samples from multiple

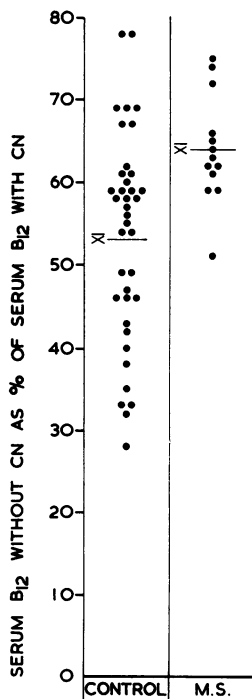


FIG. 4. Control sera and sera from multiple sclerosis: the B_{12} value obtained after extraction without cyanide as a percentage of that obtained after extraction with cyanide (*Lactobacillus leichmannii*).

sclerosis) may reflect an increase in the permeability of the blood-brain barrier.

The present work has produced no evidence of major alterations in B_{12} in serum and cerebrospinal fluid in multiple sclerosis, but it does suggest that there are minor abnormalities. High values were only found when the patients were known to have received B_{12} . Excluding these cases, the total serum B_{12} was found to be normal using two different assay organisms, and the cerebrospinal fluid concentrations were all within the control range, though the mean was significantly lower than in controls. The tendency for levels of B_{12} in cerebrospinal fluid to be reduced in multiple sclerosis contrasts with the tendency for it to be increased in the group of miscellaneous neurological disorders. The results suggest that at a given concentration of serum B_{12} the concentration of B_{12} in cerebrospinal fluid is slightly lower in multiple sclerosis than in controls. No explanation for this can be suggested without further investigation, and it is unlikely to be explained until the factors governing the concentration of B_{12} in cerebrospinal fluid are better understood. These probably include not only the concentration of B_{12} in serum, but the permeability of the blood-brain barrier, the B_{12} -binding capacity of the cerebrospinal fluid (Meyer, Bertcher, and Mulzac, 1959; Clausen and Munkner, 1962; Worm-Petersen, 1962) and perhaps utilization by the brain. Our finding of

normal concentrations of total serum B_{12} in multiple sclerosis confirms the reports of Grann and Glass (1961) and Worm-Petersen (1962). Simpson (1964) also found that the mean serum concentration was not significantly different from that in controls, but in his series there were several values below the lower limit of the control range. There is no major disagreement between the present finding of a moderate reduction in cerebrospinal fluid B_{12} concentration and the results of Worm-Petersen and of Simpson, for both these authors found that the mean cerebrospinal fluid B_{12} concentration in multiple sclerosis was slightly lower than in controls, though the difference was not statistically significant. We cannot confirm the reports of very low serum and cerebrospinal fluid B_{12} concentrations. The reports of high concentrations may well be due to the administration of B_{12} , as the investigators concerned do not state whether or not this had been given.

The results of the measurements of serum B_{12} with *Lactobacillus leichmannii* after extraction without cyanide show a small but significant difference between cases of multiple sclerosis and controls. It has been shown that extraction of serum in the absence of cyanide causes a large though variable loss of B_{12} in association with the protein precipitate, which may be in the form of hydroxocobalamin (Matthews 1961; 1962), and it is possible that the serum B_{12} value obtained in the absence of cyanide represents serum cyanocobalamin while the additional B_{12} obtained in the presence of cyanide represents serum hydroxocobalamin. This hypothesis, which has recently received some further experimental support (Anderson, 1964), is energetically advocated by Smith (1961) who claims that patients with tobacco amblyopia have an abnormally low proportion of hydroxocobalamin in the blood. Interpreted in this way, the present results would indicate that in multiple sclerosis the blood tends to contain less hydroxocobalamin and more cyanocobalamin than normal. Unfortunately, it is not yet possible to interpret the results with certainty, as the forms in which B_{12} exists in the blood are unknown. If the bulk of it is in the coenzyme form, the value obtained in the absence of cyanide may reflect not the concentration of cyanocobalamin in the blood but the concentration of cyanide or thiocyanate. However, the finding of the difference that has been described between patients with multiple sclerosis and control subjects indicates the desirability of further investigation.

Though B_{12} is often given in multiple sclerosis, there is little to suggest that it influences the course of the disease (Simpson, 1964), and the present work has produced no evidence of overt B_{12} deficiency in the condition. If the vitamin is to be given, there is

a case for using hydroxocobalamin, which is retained in the body longer than cyanocobalamin. The present results might be regarded as additional justification for preferring hydroxocobalamin to the cyano-form.

We wish to thank members of the staff of the National Hospital for allowing us to study patients under their care, and Dr. P. K. Thomas for providing some of the specimens. We are also grateful to Mrs. V. Newbery for technical assistance, and to Miss June Quick for preparation of the figures.

ADDENDUM

Since this paper was submitted for publication, it has been shown (Lindstrand, 1964) that the bulk of the B₁₂ in normal blood is in the form of methylcobalamin, and this finding must be taken into consideration in interpreting the present results. Further work on the inter-relationships between cyanide and B₁₂ in controls and patients with multiple sclerosis is to be published elsewhere (Matthews, Wilson, and Zilkha, 1965).

REFERENCES

- Anderson, B. B. (1964). *J. clin. Path.*, **17**, 14.
 Bauer, H., and Heinrich, H. C. (1961). In *Chemical Pathology of the Nervous System*, edited by J. Folch-Pi, p. 120. Pergamon Press, Oxford.
 Clausen, J., and Munkner, T. (1962). *Acta path. microbiol. scand.*, **54**, 129.
 Gjertsen, F. A., and Schrupf (1962). *Nord. Med.*, **67**, 162.
 Grann, V., and Glass, G. B. J. (1961). *J. Lab. clin. Med.*, **57**, 562.
 Herbert, V., and Zalusky, R. (1961). *Fed. Proc.*, **20**, 453.
 Hutner, S. H., Bach, M. K., and Ross, G. I. M. (1956). *J. Protozool.*, **3**, 101.
 Kidd, H. M., Gould, C. E. G., and Thomas, J. W. (1963). *Canad. med. Ass. J.*, **88**, 876.
 Lindstrand, K. (1964). *Nature (Lond.)*, **204**, 188.
 Matthews, D. M. (1961). *Lancet*, **1**, 1289.
 — (1962). *Clin. Sci.*, **22**, 101.
 —, Wilson, J., and Zilkha, K. J. (1965). *J. Neurol. Neurosurg. Psychiat.* In press.
 Meyer, L. M., Bertcher, R. W., and Mulzac, C. (1959). *Proc. Soc. exp. Biol. (N.Y.)*, **100**, 607.
 O'Connor, J. S., Davis, R. L., Langworthy, O. R., and Chow, B. F. (1960). *Ibid.*, **103**, 180.
 Ross, G. I. M. (1950). *Nature (Lond.)*, **166**, 270.
 Simpson, C. A. (1964). *J. Neurol. Neurosurg. Psychiat.*, **27**, 174.
 Smith, A. D. M. (1961). *Lancet*, **1**, 1001.
 Sobotka, H., Baker, H., and Frank, O. (1960). *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 801.
 —, Christoff, N., and Baker, H. (1958). *Ibid.*, **98**, 534.
 —, Baker, H., Frank, O., Maker, H., and Margulies, M. E. (1960). *J. Mt Sinai Hosp.*, **27**, 618.
 Worm-Petersen, J. (1962). *Acta neurol. scand.*, **38**, 241.
 Yavorkovsky, L. I., and Mai, L. A. (1961). *Vop. med. Khim.*, **7**, 25.
 Quoted by Kidd *et al.*, (1963).