Vitamin B₁₂ levels in the serum and cerebrospinal fluid in multiple sclerosis

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Vitamin B₁₂ has been widely used in the treatment of neurological disease, if only as a placebo. Whilst the results of a series of uncontrolled trials of its use in multiple sclerosis have not been impressive (Lereboullet, Pluvineau, and Coty, 1950; Schumacher, 1950; Simson, Herfort, Krim, and Meyer, 1950; Booth, Lawyer, and von Storch, 1951; Lereboullet and Pluvineau, 1952; Levin, 1955), there have been some reports of abnormalities both in the serum and cerebrospinal fluid levels of vitamin B₁₂ in multiple sclerosis. These, however, are not consistent amongst themselves. Thus low serum values (O'Connor, Davis, Langworthy, and Chow, 1960), elevated serum and cerebrospinal fluid values (Sobotka, Christoff, and Baker, 1958), and a low ratio of cerebrospinal fluid to serum vitamin B₁₂ (Gjertsen and Schrupf, 1962) have all been reported. Grann and Glass (1961) from their study of vitamin B₁₂ metabolism in 21 patients with multiple sclerosis concluded that it was probably normal (although they did not study cerebrospinal fluid). It was accordingly felt that a further investigation of vitamin B₁₂ in cerebrospinal fluid and serum was necessary, particularly as this department was carrying out a controlled trial of massive doses of hydroxocobalamin in the treatment of multiple sclerosis. Since commencing this investigation the work of Worm-Petersen (1962) has appeared and records no difference in the serum and cerebrospinal fluid vitamin B₁₂ levels between multiple sclerosis and other neurological disease. These discordant findings encouraged continuance of the present study.

METHODS

Specimens of blood (collected with disposable Mediplast syringes) and lumbar cerebrospinal fluid were collected at the same time into specially cleaned, sterile containers (Hutner, Bach, and Ross, 1956). Serum was separated aseptically and transferred to similarly treated containers. All samples were stored at -20°C. There is no appreci-

able loss of activity in specimens stored under these conditions for six to 24 months (Killander, 1957).

The specimens were assayed by the method of Hutner, Bach, and Ross (1956) with *Euglena gracilis*, Z strain, with the modification that hydroxocobalamin replaced cyanocobalamin. Total vitamin B₁₂ content was estimated after heating the assay mixture at 100°C. for 15 minutes before inoculation. In addition free vitamin B₁₂ was estimated in some specimens by the method of Ross (1950, 1952). Readings were made using a turbidimetric method in a spectrophotometer (Unicam SP 500) at 625 mμ.

RESULTS

Specimens were obtained from 46 patients (average age 39 years, range 22-55) in whom the diagnosis of multiple sclerosis was beyond all reasonable doubt. From these 46, five were taken during acute exacerbations. The remaining cases were all chronic; four chairbound, the rest ambulant and only mildly or moderately afflicted. The control group consisted of 23 patients (average age 48 years, range 30-77) with miscellaneous neurological and mental disorders, namely, cerebral tumour, 4; cervical spondylosis, 4; prolapsed lumbar disc, 3; motor neurone disease, 2; polyneuropathy, 2; cranial arteritis, 2; and one each of, pituitary tumour, headache, epilepsy, spinal artery thrombosis, obsessional neurosis, and hysteria. A truly 'normal' control group was considered to be unobtainable as there is little ethical justification for carrying out lumbar puncture on normal people. No patient had received previous treatment with vitamin B₁₂, and haemoglobin estimation, mean corpuscular haemoglobin concentration, and blood film examination were normal in all cases.

Since Worm-Petersen (1962) has shown that the distribution of vitamin B₁₂ levels fits a logarithmic distribution better than a normal one and the present results support this, the logarithms of the serum or cerebrospinal fluid levels of vitamin B₁₂ have been used for the calculations.

TABLE I
VITAMIN B₁₂ LEVELS IN SERUM AND CEREBROSPINAL FLUID ($\mu\mu\text{g./ml.}$)

	Control	Multiple Sclerosis	Significance
(a) Mean log. serum vitamin B ₁₂ and standard error (s.e.)	2.526 $\pm$ 0.046	2.427 $\pm$ 0.042	Difference of means $P > 0.05$
(b) Mean log. cerebrospinal fluid vitamin B ₁₂ and standard error (s.e.)	1.045 $\pm$ 0.011	0.947 $\pm$ 0.56	Difference of means $P > 0.1$
Antilog. (a) + 2 $\times$ s.e.	415	324	
(a) 336		267	
(a) - 2 $\times$ s.e.	271	220	
Antilog. (b) + 2 $\times$ s.e.	11.7	11.5	
(b) 11.1		8.9	
(b) - 2 $\times$ s.e.	10.5	6.8	
r	0.53	0.79	Control and multiple sclerosis $P < 0.005$
Slope of regression line	0.72	0.97	Difference between slopes $P > 0.4$ (Difference between intercepts $P > 0.4$)

Means of the log. vitamin B₁₂ values in serum and cerebrospinal fluid are given in Table I. The log. cerebrospinal fluid levels of vitamin B₁₂ have been plotted against the log. serum levels for both control and multiple sclerosis groups and the regression lines calculated (Figs. 1 and 2). The coefficients of correlation (r) and slopes are given in Table I.

It should be noted (Table II) that in six patients with multiple sclerosis the serum vitamin B₁₂ was less than 135 $\mu\mu\text{g./ml.}$ (the lowest control value) but this is not significant ($P > 0.7$). The serum and cerebrospinal fluid vitamin B₁₂ values for the five acute cases ranged from 428 (59) to 182 (10) $\mu\mu\text{g./ml.}$ which is comparable with results for chronic cases.

Free (uncombined) vitamin B₁₂ was measured in five control and 14 multiple sclerosis sera. A small quantity of free vitamin B₁₂ was detected in one

control and one multiple sclerosis serum. No difference between free and total vitamin B₁₂ levels occurred in 13 samples of cerebrospinal fluid but free vitamin B₁₂ was not detected in one control and five multiple sclerosis samples. Log. cerebrospinal fluid vitamin B₁₂ was plotted against log. cerebrospinal fluid protein (measured in the routine laboratory) and there was no correlation between the two.

DISCUSSION

Good correlation between cerebrospinal fluid and serum vitamin B₁₂ levels exists in both controls and patients with multiple sclerosis. This is contrary to the findings of Gjersten and Schrumpf (1962) on nine cases of multiple sclerosis. There is no signifi-

FIG. 1. In control patients, log. cerebrospinal fluid vitamin B₁₂ against log. serum vitamin B₁₂. The regression line is shown.

FIG. 2. In multiple sclerosis patients, log. cerebrospinal fluid vitamin B₁₂ against log. serum vitamin B₁₂. The regression line is shown.

TABLE II

DETAILS OF PATIENTS WITH LOW SERUM VITAMIN B₁₂ LEVELS

Serum Vitamin B ₁₂ Level (μg./ml.)	Diagnosis	Length of History (yr.)	Severity of Disease	Course of Disease	Physique
64	Multiple sclerosis	6	Severe	Progressive	Thin
94	Multiple sclerosis	6	Mild	Fluctuating	Thin
101	Multiple sclerosis	3	Mild	Fluctuating	Normal
103	Multiple sclerosis	2	Moderate	Progressive	Normal
108	Multiple sclerosis	2	Mild	Fluctuating	Thin
118	Multiple sclerosis	12	Mild	Static	Normal
135	Obsessional neurosis	—	—	—	Normal

cant difference in the cerebrospinal fluid levels of vitamin B₁₂ between multiple sclerosis and control groups, which is also contrary to the findings of Gjertsen and Schrumpf (1962), and to the raised values found by Sobotka *et al.* (1958). The latter, using *Ochromonas malhaemensis*, studied 20 cases of multiple sclerosis, of which 11 fell within the accepted normal range of cerebrospinal fluid vitamin B₁₂ (0.30 μg./ml.), six had high serum and cerebrospinal fluid levels, of which two were very high; and one had an abnormally low serum level.

Although the difference is not significant there is a suggestion that serum vitamin B₁₂ levels may be abnormally low in some patients with multiple sclerosis. Six of these patients had serum vitamin B₁₂ levels which lay below the lowest control value and only one of these could be considered severely afflicted and possibly malnourished (Table II). Grann and Glass (1961) found one of 21 cases of multiple sclerosis with a subnormal serum vitamin B₁₂ level. Intestinal absorption of Co⁶⁰-labelled vitamin B₁₂ in all their cases was normal. One control, two patients with migraine, and five of the nine cases of multiple sclerosis of Gjertsen and Schrumpf (1962) had serum vitamin B₁₂ values of 100 μg./ml. or below (by *Euglena gracilis* method). O'Connor *et al.* (1960) found that where the mean serum vitamin B₁₂ in 360 control subjects was expressed at 100%, the mean in 21 cases of probable, long-standing multiple sclerosis was only 50%. Whilst they do not quote the mean levels, they claim that 10 of the 21 patients with multiple sclerosis (only three of whom were severe and not ambulant) had readings comparable with those for pernicious anaemia (50 μg./ml., assayed using *L. leichmanii*). They further found that absorption of Co⁶⁰-labelled vitamin B₁₂ was normal but that serum-binding capacity for vitamin B₁₂ was lower in multiple sclerosis, and urinary excretion higher, than in the controls. From this they deduced that tissue retention of vitamin B₁₂ was impaired in some cases of multiple sclerosis.

The results of the present investigation are in agreement with those of Worm-Petersen (1962) who also found that Ross's (1950, 1952) method of esti-

imating free vitamin B₁₂ showed no free vitamin B₁₂ in the serum whereas the cerebrospinal fluid contained roughly equal quantities of free and total vitamin B₁₂. He pointed out, however, that using dialysis there was no free vitamin B₁₂ in the serum or cerebrospinal fluid, and that *Euglena gracilis*, Z strain can utilize non-dialysable vitamin B₁₂ (Hoff-Jørgensen and Worm-Petersen, 1962). This factor may account for the observations in cerebrospinal fluid but does not appear to operate in the serum. The ratio of serum protein to cerebrospinal fluid protein (200 : 1) is very different from the ratio serum vitamin B₁₂ to cerebrospinal fluid vitamin B₁₂ (25 : 1). Both these differences suggest that vitamin B₁₂ binding in serum may differ from that in cerebrospinal fluid.

Meyer, Bertcher, and Mulzac (1959) found dialysis experiments with cerebrospinal fluid and radioactive vitamin B₁₂ that the ratio of radioactive vitamin binding capacity to protein concentration was higher for cerebrospinal fluid than for serum. They concluded that in cerebrospinal fluid vitamin B₁₂ was not bound to protein or that this protein was different from that in serum.

Normally vitamin B₁₂ is bound in both serum and cerebrospinal fluid to an immunologically indistinguishable alpha-1-globulin and it is suggested that the greater binding capacity of cerebrospinal fluid protein may be associated with some physicochemical difference in this protein (Clausen and Munkner, 1962).

There is probably a difference between vitamin B₁₂ binding in serum and in cerebrospinal fluid, but there is not yet any convincing proof of abnormal vitamin B₁₂ metabolism in multiple sclerosis. Although the present work offers no rational basis for the use of vitamin B₁₂ in the treatment of multiple sclerosis, there is still a place for placebo therapy and the psychosomatic effect of an injection is greater than that of a tablet. As some patients with multiple sclerosis have low serum vitamin B₁₂ levels, there may be something to be said for the administration of vitamin B₁₂ in this disease but the results of a controlled trial are awaited.

SUMMARY

Serum and cerebrospinal fluid vitamin B₁₂ levels were estimated in 46 patients with multiple sclerosis and 23 patients with miscellaneous disorders were used as a control group.

No significant difference between multiple sclerosis and control groups could be found either in the absolute values of serum or cerebrospinal fluid vitamin B₁₂ or in the serum: cerebrospinal fluid ratio. There was a significant correlation between serum and cerebrospinal fluid vitamin B₁₂ levels but not between vitamin B₁₂ and protein concentrations in cerebrospinal fluid. Vitamin B₁₂ binding in serum and cerebrospinal fluid is discussed. It was noted that a few patients with multiple sclerosis in this and previous reports have abnormally low serum vitamin B₁₂ levels although this is not statistically significant.

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