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Cyanide metabolism and vitamin B_{12} in multiple sclerosis

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In the course of an investigation of vitamin B_{12} concentrations in body fluids in multiple sclerosis (Basil, Brown, and Matthews, 1965), it was noted that the proportion of total B_{12} extractable from serum in the absence of added cyanide was higher in cases of multiple sclerosis than in a group of patients with other neurological disorders. This finding might mean that the serum in multiple sclerosis contained an excess of cyanide, or might represent a disturbance in the normal proportions of cyanocobalamin and hydroxocobalamin in the blood (Matthews, 1961, 1962, 1964; Smith, 1961; Anderson, 1964; Basil *et al.*, 1965), and thus suggested the possibility of some disturbance in cyanide metabolism. Chronic exposure to cyanide can produce lesions of the central nervous system, and possibly demyelination, in animals (*e.g.*, Hurst, 1942; Lumsden, 1950; Smith, Duckett, and Waters, 1963; Wilson, 1965), and it has been suggested that cyanide is the toxic factor in tobacco amblyopia, producing hydroxocobalamin deficiency by converting this substance to the cyano- form (Smith, 1961). Clinical and laboratory studies indicate that there is an abnormality in conversion of cyanide to thiocyanate in Leber's hereditary optic atrophy (Wilson, 1965). The present investigation was undertaken to see whether any abnormality of cyanide metabolism could be detected in multiple sclerosis, and whether there was any disturbance in the relationship between cyanide and vitamin B_{12} in this condition.

METHODS

The subjects of the present study comprised seven controls and six patients with multiple sclerosis, in-patients at The National Hospital, Queen Square.

The controls, four men and three women, were suffering from a variety of neurological disorders (idiopathic epilepsy, subarachnoid haemorrhage, chorea, meningioma, Arnold-Chiari malformation, acute infective polyneuritis and familial hypertrophic polyneuritis), and their ages ranged from 29 to 61 years (mean 46 years). The six patients with multiple sclerosis, four men and two women, were aged 18 to 51 years (mean 35 years).

Blood was drawn from each subject after an overnight fast. The specimens were drawn in pairs, one from the control group and one from a patient with multiple sclerosis, and the subsequent manipulations and analyses were carried through 'blind' on each pair. The controls were chosen as far as possible to resemble the patients with multiple sclerosis in weight, sex distribution, and degree of physical handicap. All patients were taking a normal ward diet, and none was bed-ridden. Seven patients with multiple sclerosis were originally included but one patient had to be excluded subsequently because she had been given vitamin B_{12} parenterally a few days before the blood specimen was taken. No other patient had received vitamin B_{12}.

All the patients were cigarette smokers, and smoking habits were very similar in both groups. To study the effect of smoking on cyanide metabolism, three additional patients, non-smokers, were investigated, but these were not included in the two main groups.

Plasma and urine cyanide and thiocyanate were estimated as described by Wilson (1965). Serum vitamin B_{12} was estimated with *Lactobacillus leichmannii* (Matthews, 1962); each sample was divided into two parts, one of which was assayed after extraction in the presence of cyanide (giving total serum B_{12}), and the other after extraction in the absence of cyanide.

The significance of differences between means was assessed by the t test.

RESULTS

The results in controls and patients with multiple sclerosis are compared in Table I. There was no significant difference between the two groups in plasma cyanide, plasma thiocyanate, urine thiocyanate, or serum vitamin B_{12}, though the urine cyanide was lower in the multiple sclerosis group ($P < 0{\cdot}05 > 0{\cdot}02$).

When the control patients, those with multiple

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TABLE I

PLASMA AND URINE CYANIDE AND THIOCYANATE AND SERUM VITAMIN B_{12} IN CONTROLS AND CASES OF MULTIPLE SCLEROSIS

	Controls	*Multiple Sclerosis*
Plasma $^-$CN (μM/100 ml.)	0·009 ± 0·003 (7)[1]	0·011 ± 0·004 (6)
Plasma $^-$SCN (μM/100 ml.)	10·8 ± 1·4 (7)	10·4 ± 2·5 (6)
Urine $^-$CN (μM/kg./24 hr.)	10·7 ± 1·9 (6)	5·7 ± 0·8 (6)
Urine $^-$SCN (μM/kg./24 hr.)	2·0 ± 0·3 (7)	2·4 ± 0·4 (6)
(a) Serum B_{12}—extracted with $^-$CN ($\mu\mu$g./ml.)	434 ± 23 (7)	410 ± 77 (6)
(b) Serum B_{12}—extracted without $^-$CN ($\mu\mu$g./ml.)	246 ± 30 (6)	267 ± 67 (6)
(b) as % of (a)	55 ± 5 (6)	61 ± 5 (6)
Mean daily cigarette consumption	14·6	15·8

[1]Mean ± S.E. Number of observations in parentheses

sclerosis, and the three non-smokers were grouped together, a very marked positive correlation between tobacco consumption, plasma thiocyanate concentration, and urinary thiocyanate excretion became apparent (Fig. 1) but there was no relationship between tobacco consumption and plasma or urine cyanide. No relationship was found between the proportion of serum B_{12} extractable in the absence of cyanide and tobacco consumption, or between this proportion and plasma or urine thiocyanate. There was no indication of a positive correlation between plasma cyanide and the proportion of serum B_{12} extractable without cyanide. The results did suggest, however, a negative correlation between plasma cyanide and total serum B_{12}. This correlation will be reported more fully after further investigation.

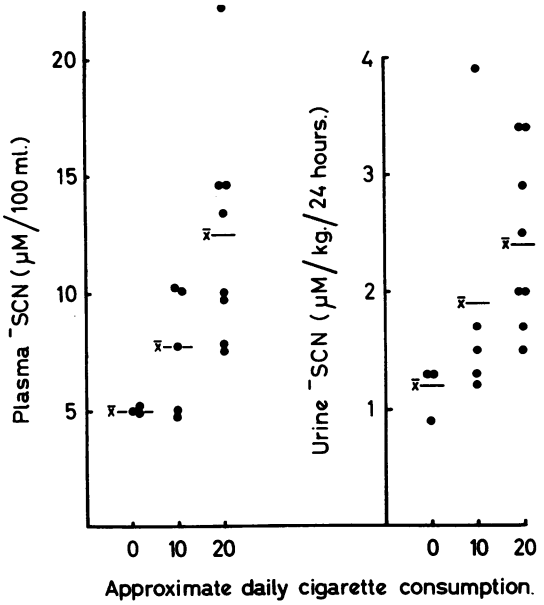

FIG. 1. *Plasma and urine thiocyanate in relation to smoking habits.*

DISCUSSION

The present results do not support the hypothesis that there is an abnormality of cyanide metabolism in multiple sclerosis. Smoking results in an increased cyanide load (Osborne, Adamek, and Hobbs, 1956), and one might expect any abnormality of cyanide metabolism to be accentuated by this, yet in these patients, all of whom were smokers, the values for plasma cyanide and thiocyanate, and for urine thiocyanate, were very similar in the control and multiple sclerosis groups. The values obtained in the two groups were also similar to those found in a larger group of normal smokers (Wilson, 1965). Though the urine cyanide was lower in multiple sclerosis than in controls, the significance of this is doubtful, since values for urinary cyanide can be markedly affected by the presence of bacteria (especially *E. coli*) and are therefore a relatively unreliable index of endogenous cyanide metabolism.

The serum B_{12} values obtained in the present work are essentially similar to those previously reported (Basil *et al.*, 1965). The total serum B_{12} in multiple sclerosis was completely normal. Though the proportion of B_{12} extractable without cyanide was apparently slightly higher in multiple sclerosis than in controls, the difference was not statistically significant in this small series. Even if this difference is real, its biological significance is uncertain. Recent work (Lindstrand and Ståhlberg, 1963; Lindstrand, 1964) suggests that B_{12} exists in blood in several forms and that hydroxocobalamin and cyanocobalamin are present only in relatively small amounts. Consequently the original hypotheses about the relationship between the proportion of B_{12} extractable from serum in the absence of added cyanide (Matthews, 1961; Smith, 1961) and the proportions of hydroxocobalamin and cyanocobalamin in blood may require modification. The possibility that these proportions are related primarily to the B_{12}-binding capacity of the plasma proteins has not yet been entirely excluded, and it has been claimed that B_{12}-binding capacity is reduced in multiple sclerosis (O'Connor, Davis, Langworthy, and Chow, 1960). Work on these problems is now in progress.

One relationship which emerges very clearly in this work is that between tobacco consumption and plasma and urine thiocyanate. This relationship is to be expected, since thiocyanate is probably the major detoxication product of cyanide (*e.g.*, Himwich and Saunders, 1948; Boxer and Rickards, 1952). It is clearly essential to ensure that smoking habits

are closely similar in test and control groups in any study of this type.

In conclusion, it may be pointed out that to produce neurological lesions in animals, it is necessary to give relatively large doses of cyanide over long periods, and that in some cases repeated sublethal doses have been used. This suggests that if abnormalities of cyanide metabolism were concerned in the aetiology of multiple sclerosis, they would be readily detectable. Consequently, though the present series of cases is small, the negative results suggest that further enquiry along these lines is unlikely to be fruitful.

SUMMARY

In view of observations suggesting that there might be an abnormality of cyanide/B_{12} metabolism in multiple sclerosis, plasma and urine cyanide and thiocyanate were measured in a group of patients with multiple sclerosis and in a control group with other neurological disorders. Total serum B_{12}, and serum B_{12} extractable without added cyanide, were also estimated using *Lactobacillus leichmannii*. The results did not indicate any abnormality of cyanide metabolism in multiple sclerosis. Total serum B_{12} concentrations were normal in this condition, and though there may be a slight increase in the proportion of serum B_{12} extractable without cyanide in multiple sclerosis, the significance of this is uncertain.

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