Thiocyanate Metabolism in Human Vitamin B₁₂ Deficiency

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Summary

Patients with subacute combined degeneration who smoked had significantly lowered plasma thiocyanate levels than control smokers, but plasma thiocyanate levels in non-smoking patients with neurological disease due to vitamin B₁₂ deficiency were not significantly different from control values. The results provide no support for the hypothesis that chronic cyanide intoxication is responsible for the occurrence of neurological disease in a minority of patients with vitamin B₁₂ deficiency, although they do not conclusively exclude this possibility. The association between smoking and subacute combined degeneration of the cord has been confirmed in this study but it remains unexplained.

Introduction

Interest in cyanide and thiocyanate metabolism in vitamin B₁₂ deficiency stems from the evidence suggesting that vitamin B₁₂ is an important intermediary in one of the pathways of detoxication of cyanide, through its ready conversion from hydroxocobalamin to cyanocobalamin (Boxer and Rickards, 1952). It has been suggested that in conditions of relative or absolute vitamin B₁₂ deficiency heavy cyanide exposure, as from, for example, tobacco-smoking, might lead to amblyopia either from chronic cyanide toxicity (Wokes, 1958) or from inactivation of vitamin B₁₂ (Smith, 1961).

It was shown that in healthy subjects plasma concentrations of cyanide and thiocyanate tended to vary inversely with vitamin B₁₂ concentrations (Wilson and Matthews, 1966), and extrapolating from this data Wilson and Langman (1966) proposed a more general hypothesis which would account for the occurrence of neurological complications in a minority of patients with vitamin B₁₂ deficiency.

This hypothesis can be summarized thus. Cyanide and thiocyanate are in metabolic equilibrium, cyanide concentrations being determined (a) by conversion to thiocyanate through the activity of the enzyme rhodanese (Lang, 1933; Sörbo, 1953, 1955, 1957), present in, for example, liver and kidney; (b) by incorporation of cyanide into the 1-C metabolic pool through the intermediary action of vitamin B₁₂ (Boxer and Rickards, 1952); (c) by exposure to exogenous sources of cyanide or cyanogens—namely, smoking, infections, and diet; and (d) by the endogenous formation of cyanide from thiocyanate by an enzyme “thiocyanate oxidase” present in red blood cells (Goldstein and Rieders, 1951, 1953.)

Dietary intake of preformed thiocyanate as from, for example, brassicae or milk would also therefore indirectly affect thiocyanate levels. The scheme can be represented as shown in the Chart.

An important rate-limiting factor in this scheme is the red

cell mass since this determines “thiocyanate oxidase” activity. It was suggested that this alone might explain the well-known inverse relation between the occurrence of neurological complications and the degree of anaemia in Addisonian pernicious anaemia—that is, anaemia has a neuroprotective effect. It was further suggested that the anorexia which is a prominent feature of vitamin B₁₂ deficiency might not only exaggerate abnormalities of folate metabolism by depressing dietary intake of folate, but also diminish thiocyanate levels in body fluids by diminishing the dietary intake of thiocyanate. Thus anorexia and dietary habits might also be subsidiary rate-limiting factors.

If thiocyanate or cyanide intake are important factors in the development of neurological complications in vitamin B₁₂ deficiency then differences might be expected in the plasma thiocyanate concentrations of patients who have and who have not developed neurological disease. We have therefore made such a comparison in an unselected group of patients with vitamin B₁₂ deficiency. The results of this study are discussed in relation to more recent studies characterizing the nature of “thiocyanate oxidase” activity.

Subjects and Methods

Heparinized venous blood was obtained from patients who were placed in the following groups relating to their conditions: (1) uncomplicated pernicious anaemia; (2) vitamin B₁₂ deficiency and dementia, separately identified because of their different presentation from groups 1 and 3; (3) subacute combined degeneration of the cord; (4) nutritional folate deficient megaloblastic anaemia; and (5) miscellaneous medical and surgical conditions in patients of 60 years and over. These patients were attending the following hospitals: Central Middlesex Hospital (groups 1 and 2), Epsom District Hospital (groups 1, 2, 3, 4, and 5), and Nottingham General Hospital (groups 1 and 3), National Hospital for Nervous Diseases, Queen Square, London (group 3), St. Bartholomew's Hospital, London (groups 1 and 3), and Royal Marsden Hospital (group 5).

Because the normal controls for human thiocyanate studies (Wilson, 1965) were drawn from healthy and relatively young volunteers living in London, it was recognized that they might not be valid for comparison with predominantly older patients. Through the kind collaboration of Dr. P. C. Elwood, of the M.R.C. Epidemiological Research Unit in Cardiff, venous blood samples were obtained from older males and controls participating in a study of coronary thrombosis in South Wales. They make up two groups: S. Wales coronary thrombosis (group 6) and S. Wales Controls (group 7).

Plasma was separated promptly from all subjects and stored at -20°C until the thiocyanate determinations were performed by the method of Aldridge (1945) after deproteinization with

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Mean Plasma Thiocyanate and Haemoglobin Concentrations

Clinical Group	Sex		Smokers or Non-smokers	Mean Plasma Thiocyanate Concentration in $\mu\text{mole}/100\text{ ml}$ ($\pm$ S.D.)	Mean Haemoglobin Concentration in $\text{g}/100\text{ ml}$ ($\pm$ S.D.)
	M.	F.			
1 Uncomplicated pernicious anaemia	9	28	N.S.	3.9 $\pm$ 1.9	6.9 $\pm$ 2.2
2 Dementia and vitamin B ₁₂ deficiency	5	6	S.	7.9 $\pm$ 5.0	7.4 $\pm$ 1.3
3 Subacute combined degeneration	4	8	N.S.	5.4 $\pm$ 2.0	9.3 $\pm$ 2.8
4 Folate deficiency	3	7	N.S.	5.0 $\pm$ 1.8	11.3 $\pm$ 2.5
5 Elderly medical controls (group 5)	5	6	S.	6.0 $\pm$ 3.3	10.6 $\pm$ 2.9
6 Coronary thrombosis patients (group 6) ..	0	10	N.S.	2.4 $\pm$ 1.2	7.0 $\pm$ 2.6
7 Normal individuals (group 7)	25	8	N.S.	4.4 $\pm$ 1.7	
8 Combined controls (groups 5, 6, and 7)* ..	34	0	S.	9.9 $\pm$ 4.0	

*Mean plasma thiocyanate concentrations respectively 4.2, 4.6, and 4.3 $\mu\text{mole}/100\text{ ml}$ in non-smoking normal controls (group 7), coronary thrombosis patients (group 6), and elderly medical controls (group 5). Concentrations respectively 10.1, 9.6, and 9.9 $\mu\text{mole}/100\text{ ml}$ for smokers.

Statistical comparison of mean thiocyanate concentrations (*t* test).

Group 8 (N.S.) v. group 4 (N.S.) $P < 0.001$; group 8 (S.) v. group 1 (S.) $P > 0.05$; group 8 (S.) v. group 3 (S.) $P < 0.01$; group 1 (N.S.) v. group 3 (N.S.) $P > 0.05$.

10% trichloroacetic acid. Haemoglobin was estimated at referring laboratories by the cyanmethaemoglobin spectrophotometric method.

Results

The mean plasma thiocyanate concentrations found in the various groups with the numbers of men and women studied, their smoking habits, and their mean haemoglobin concentrations are given in the Table.

Plasma thiocyanate concentrations were almost the same in the three control groups studied (elderly medical controls, patients with coronary thrombosis, and normal individuals) with uniformly higher levels in smokers than in non-smokers, and comparisons were therefore made between the combined control group and the other clinical groups. The similarity between the results in the control groups and the findings in a previously published study of an entirely different group of healthy and relatively young subjects living in London (Wilson, 1965) suggests they constitute valid controls. Thiocyanate concentrations were lowest in patients with folate deficiency, all of whom were non-smokers, and the difference from the control was statistically significant ($P < 0.001$). Concentrations were also slightly lower in non-smoking pernicious anaemia patients than in controls but the difference was not statistically significant ($P < 0.05$). By contrast non-smoking patients with dementia, and those with subacute combined degeneration on average had raised plasma thiocyanate concentrations but the differences were not statistically significant. As found previously haemoglobin levels tended to be high in patients with subacute combined degeneration.

In smokers thiocyanate concentrations tended to be lower in those with vitamin B₁₂ deficiency than in the controls, irrespective of the clinical presentation, levels being significantly low in patients with subacute combined degeneration ($P < 0.01$). Patients with the latter disorder also differed from anaemic patients in the relative proportions of men and women and of smokers and non-smokers. There were more men and more smokers among patients with subacute combined degeneration than in those presenting with anaemia (male to female sex ratios 1:1.6 and 1:2.4 respectively, and ratios of smokers to non-smokers 1:0.9 and 1:3.4 respectively) and the difference in proportions of smokers and non-smokers was statistically significant ($P < 0.02$).

Discussion

The present findings of a differing sex ratio in patients with subacute combined degeneration and anaemia are less pronounced than those which we had noted previously (Wilson and

Langman, 1966). The trend is the same, however, and is in agreement with trends observed in the Registrar General's mortality data for pernicious anaemia and subacute combined degeneration in the years before effective treatment became available (M. J. S. Langman, unpublished observations).

The association between smoking and the presence of the disease has emerged more clearly than the differing sex ratio and gives confirmatory support to previous similar findings when we suggested that the varying sex ratio is a secondary expression of a primary association between smoking habits and liability to neurological disease or anaemia. The tendency for plasma thiocyanate concentrations to be lower in patients with pernicious anaemia than in those with subacute combined degeneration, after taking account of smoking habits, is consistent with the suggestion that dietary intake of thiocyanate is reduced in pernicious anaemia. Poor intake may also explain the low levels noted in patients with folate deficiency.

If a high dietary thiocyanate intake or high cyanide intake by smoking predispose to subacute combined degeneration then it might be expected that patients with the disorder would have high plasma thiocyanate levels relative to those with pernicious anaemia and perhaps relative to controls. The data obtained in non-smokers, with slightly though not significantly higher plasma thiocyanate concentrations in patients with neurological disease and low figures in those with pernicious anaemia, support such a concept. However, the unduly low thiocyanate concentrations in smokers with subacute combined degeneration counterbalance these results. A block in conversion of cyanide to thiocyanate in vitamin B₁₂ deficiency has been suggested (Wokes, 1958) and would explain the anomalously low thiocyanate levels in smoking patients with the disorder, but the difference in thiocyanate levels between smokers and non-smokers with pernicious anaemia, a variation similar to that observed in normal people, fails to support this suggestion. The low thiocyanate concentrations observed in smokers with the disease could also be explained by a relatively low tobacco consumption.

The importance or otherwise of cyanide in the genesis of subacute combined degeneration is likely to be determined only by direct assessments of cyanide metabolism or toxicity. Present methods of measuring free cyanide levels in plasma are inadequate to cope with the small amounts normally circulating, and toxicological experiments are now in progress to try to elucidate the role of cyanide in the development of subacute combined degeneration and, more importantly on a world scale, of tropical neuropathy.

The thiocyanate determinations were performed by Mr. T. Basu and Mrs. M. Barker at the former M.R.C. Clinical Genetics Research Unit at the Institute of Child Health and Institute of Neurology, London, of which one of us (J. W.) was formerly a member. The co-operation of physicians who referred patients under their care for inclusion in this study is greatly acknowledged.

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MEDICAL MEMORANDA

Phenytoin Treatment of Thalamic Pain

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Treatment of the painful, burning dysaesthesias which occasionally occur after thalamic infarction has been a particularly vexing problem. Administration of a variety of psychotropic and analgesic drugs has yielded variable but generally impersistent and ineffective results. The improvement noted in some cases after stereotaxic lesions in thalamic nuclei or other parts of the sensory pathway has often been transient. The following two cases illustrate successful treatment of thalamic pain with phenytoin.

Case Reports

Case 1.—A 56-year-old man with hypertensive cardiovascular disease abruptly developed tingling paraesthesias in his left fingers which progressed over 24 hours to involve the arm and leg. In the next few weeks he developed progressively severe, persistent, painful dysaesthesias of the left hand and foot aggravated by touch. Examination of these areas showed a modest rise in pain threshold. Strong painful dysaesthesias occurred with tactile or pin stimuli. During his second week of treatment with 300 mg of phenytoin daily there was a progressive decrease of the dysaesthesias culminating in their disappearance. Phenytoin was discontinued one month later because of toxicity. Over the next 10 days the dysaesthesias progressively recurred as the toxic signs diminished. On 150 mg of phenytoin daily he noted no toxicity and a tolerable reduction of dysaesthesias.

Case 2.—A 51-year-old man with hypertensive and atherosclerotic cardiovascular disease awoke with persistent tingling of the left face, hand, and foot. During the next few days the foot became normal. Increasingly severe burning sensations developed in the hand and face. Examination showed an increased threshold to pin

and touch stimuli on the face, and forearm. Tactile and pin stimulation evoked burning, dysaesthetic pain. Progressive improvement began on the sixth day of phenytoin treatment. One year later the dysaesthesias recurred when phenytoin was stopped. Reinstitution of treatment again caused alleviation of pain.

Comment

The abrupt onset and progression of symptoms, the lack of E.E.G. or brain scan abnormalities, and the presence of chronic hypertension are consistent with the diagnosis of a small lacunar thalamic infarction (Fisher, 1965; Caplan and Young, 1970).

Effective relief of severe, persistent pain by phenytoin has been reported in cases of trigeminal neuralgia, sphenopalatine neuralgia, tabes, and peripheral neuropathy (Green, 1961; Boshes and Arief, 1968; Meyer, *et al.*, 1970). Experimentally, phenytoin stabilizes electrolyte transfer across neuronal membranes and increases the neuronal threshold to repetitive stimulation. Hyperexcitability is lessened by a reduction of both post-tetanic facilitation and the duration of the after-discharge occurring after supra-threshold stimulation (Domino, 1964; Schmidt and Wilder, 1968). Cortical responses to single repetitive thalamic stimulation are also reduced by phenytoin (Herman and Bignall, 1967). In light of these experimental results it is reasonable to postulate that phenytoin may be effective in thalamic pain by reducing the spread of abnormally excessive excitatory discharges resulting from the thalamic lesion.

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