The B-vitamins in malnutrition with alcoholism

A model of intervitamin relationships*

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1. The B-vitamin status of fifty-nine patients, mainly from the lower socio-economic classes in Bombay, with a history of chronic malnutrition, and of alcoholism of 1·5–20 years' duration, was studied before and during treatment, and in relation to their clinical, especially neurological, condition. These patients were divided into two neurological categories: (1) those with peripheral neuropathy (mainly sensory and distal) alone, (2) those with mental changes (mainly confusion and disorientation) also. Both categories frequently showed pellagrous pigmentation and mucocutaneous signs of B-vitamin deficiency.

2. Thiamin and erythrocyte transketolase (*EC* 2.2.1.1) activity, riboflavin, nicotinic acid, pantothenic acid, total and pyridoxal fraction of vitamin B_6, folate and total vitamin B_{12} were estimated in the blood and the cerebrospinal fluid (CSF) of these patients, and also in the blood of sixty-nine control subjects and in the CSF of some of them. The concentrations of all the vitamins, except vitamin B_{12}, were highly significantly lower in the blood of patients of category 1 compared to the controls, and erythrocyte transketolase activity and pyridoxal concentration in patients of category 2 were significantly lower than those of category 1 patients. Blood pantothenic acid and folate concentrations were reduced less consistently.

3. Serum vitamin B_{12} concentration was significantly increased (though within normal range) in the patients compared to the control group, probably because of the moderate hepatic insufficiency (as assessed by liver function tests) in the former.

4. The concentrations of thiamin, riboflavin, nicotinic acid and total vitamin B_6 were also highly significantly lower in the CSF in patients of category 1 compared with controls. Furthermore, thiamin, nicotinic acid and total vitamin B_6 concentrations were significantly lower in patients of category 2 than those of category 1 patients, indicating that CSF levels reflect better the neurological status of these patients.

5. There was a moderate increase in the blood concentration of all the vitamins tested, after a relatively poor hospital diet alone. There was a concurrent increase in the blood levels of thiamin, riboflavin, nicotinic acid and pantothenic acid after parenteral treatment with either thiamin or nicotinic acid. The administration of pyridoxine resulted in a significant increase in the blood levels of riboflavin and the pyridoxal fraction of vitamin B_6.

There is considerable information in the literature on the concentrations of various nutrients, including B-vitamins, in malnourished alcoholic patients (Fennelly, Frank,

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Baker & Leevy, 1964; Baker & Frank, 1968*a*), and on their neuropathological status (Victor, 1965). However, there is very little information on the levels in the cerebrospinal fluid (CSF), their correlation with those in the blood and with the neurological and mental status of the patients, and the intervitamin relationships, except in Wernicke's encephalopathy (Thompson, Frank, Baker & Leevy, 1971). Sobotka, Baker & Frank (1960) and Baker & Frank (1968*b*) estimated concentrations of some B-vitamins in the CSF of normal subjects. In alcoholism there is increased vitamin requirement to compensate for decreased hepatic storage and for cellular repair (Leevy, Thompson & Baker, 1970). In a small group of patients with alcoholism, Shaw (1971) did not find any significant reduction in the folate level of the CSF

Recently Pauling (1968) suggested that 'the cerebrospinal concentration of a vital substance may be grossly low' while that in the blood remains within normal limits, referring particularly to thiamin and nicotinic acid.

In view of the above considerations, as a part of our current multidisciplinary study of various nutritional disorders of the nervous system (Dastur, Wadia, Bharucha, Desai, Santhadevi, Quadros, Gagrat, Razzak, Avari & Irani, 1972), including malnutrition associated with alcoholism, simultaneous estimations of thiamin, riboflavin nicotinic acid, pantothenic acid, total vitamin B_6, folate and total vitamin B_{12} were done in the blood and the CSF; values obtained were correlated, in the present report, by sequential estimations, with the clinical status of these patients and with the effect of treatment with different B-vitamins by reference to the results from typical patients. The metabolic interrelationships between the B-vitamins are being stressed in the present report, with special reference to the peripheral neuropathy and mental changes. The relationship between the blood and CSF levels of some of these vitamins will also be discussed.

MATERIALS AND METHODS

Subjects

Patients attending the neurological out-patients' departments of the Sir Jamshetjee Jejeebhoy (JJ) Group of Hospitals and King Edward Memorial (KEM) Hospital, Bombay, and occasisionally from other hospitals, and presenting with the following clinical history or signs, were admitted as 'patients' under the project: criteria were (1) symptoms and signs of a peripheral neuropathy, with or without changes relating to the spinal cord or brain, in men or women between 20 and 50 years of age; (2) a history of alcoholism of more than 1·5 years' duration with a daily intake of about 0·3–1 l of a crudely distilled local brew which was mainly ethanol (370–490 g/l) and had no significant contamination by metals, methanol or neurotoxins, for example arsenic or lead; (3) overt evidence of malnutrition such as inanition or asthenia, or pellagroid pigmentation or mucosal change, or at least a prolonged history of an inadequate diet. The diet of all patients was inadequate, both qualitatively and quantitatively, for at least the duration of alcoholism but generally preceding it by several years, whether they were working or not; most of them were from a very low socio-economic group. There was no evidence of tuberculosis or any other major infectious disease.

Methods

Over the 3·5-year period ending July 1973, fifty-nine patients were admitted to the project and intensively studied; full vitamin-status studies were done when sufficient amounts of samples were available, including the response to various therapeutic regimens, on forty-nine of these patients from June 1970 onwards. Examination of the peripheral blood and bone marrow, and augmented gastric analysis was done routinely. During the latter half of the project two general tests were done for intestinal absorption through the jejunum and ileum, using respectively: (*a*) D-xylose absorption by the method of Sammons, Morgan, Frazer, Montgomery, Philips & Philips (1967) and (*b*) [^{57}Co]cyanocobalamin absorption using the method of Armstrong & Woodliff (1970). Liver function tests included estimation of serum albumin and total protein levels by the Biuret method (Wootton, 1964), serum L-aspartate:2-oxoglutarate aminotransferase (*EC* 2.6.1.1) (AspOA) and serum L-alanine:2-oxoglutarate aminotransferase (*EC* 2.6.1.2) (AlaOA) by the method of Reitman & Frankel (1957). Electrodiagnostic tests and histopathological procedures were used to assess the status of nerve and muscle of all patients, but these will be reported in later communications.

Assays for the following seven vitamins were done using peripheral blood: microbiological assay methods of Baker & Frank (1968*b*) were used, *Ochromonas danica* for thiamin and riboflavin, *Tetrahymena pyriformis* for nicotinic acid, pantothenic acid and total vitamin B_6. The pyridoxal fraction was assayed separately using *Lactobacillus casei* (Anderson, Peart & Fullford-Jones, 1970). Folate was estimated microbiologically using *Lactobacillus casei*, essentially according to the method of Baker & Frank (1968*b*); and vitamin B_{12} by the isotopic dilution method of Matthews, Gunasegaram & Linnell (1967), using [^{57}Co]cyanocobalamin, including both the total cyanide-extractable and non-cyanide-extractable moieties (Dastur, Quadros, Wadia, Desai & Bharucha, 1972).

Using the same methods, these vitamins were also assayed, when sufficient amounts of samples were available, in the CSF of the patients and controls admitted after June 1971. Statistical evaluation of the mean values for the different groups was done using Student's *t* test.

In addition to the direct estimation of thiamin, its cofactor activity was studied in some of the patients presenting in the later stages of this study, and in control subjects, by the estimation of erythrocyte transketolase (*EC* 2.2.1.1) activity, and by the effect of further in vitro addition of thiamine pyrophosphate (TPP) by the method of Dreyfus (1962) for blood, and for erythrocytes by the procedure of P. M. Dreyfus (personal communication). Thiamin deficiency causes a decrease in transketolase activity with an increase in the TPP effect.

Identical vitamin assays were done for the various categories of asymptomatic, volunteer control subjects (*n* 69, age range 20–45 years) which included vegetarians and non-vegetarians, smokers and non-smokers, belonging to the medium and low socio-economic groups. Some of these subjects were occasional, moderate, social drinkers, and none of them was or had been addicted to alcohol.

Sequential estimations of B-vitamin concentrations in the blood were done on the patients while the following therapeutic schedule, with each treatment period lasting 10 d, was followed. (1) Alcohol was withdrawn for the total duration of the stay in hospital; patients were maintained on the hospital diet alone. (2) Patients with pellagroid skin changes received nicotinic acid (50 mg intramuscularly/d), while thiamin was administered (50 mg intramuscularly/d) to the other patients. (3) Hospital diet alone was given. (4) Another course of ten injections of nicotinic acid was given to those who had responded to it, while thiamin was given to the other patients. (5) Hospital diet alone was given. (6) Another course of ten injections of thiamin was given to those who had responded to it, while pyridoxine (50 mg intramuscularly/d) was given to the other patients. (7) Most patients left by this time, moderately or fully recovered. Those who did not were maintained on the hospital diet for 10 d followed by the administration of a B-vitamin complex for 10 d (1 ml containing 10 μg cyanocobalamin, 10 mg thiamin, 1 mg riboflavin, 50 mg nicotinamide, 5 mg pantothenyl alcohol, 3 mg pyridoxine/d).

RESULTS

Clinical observations. Results of clinical observations have been reported briefly elsewhere (Dastur, Wadia *et al.* 1972; Wadia, Bharucha, Desai, Irani, Santhadevi & Dastur, 1973). However, some clinical observations are relevant to the present study. There was a history of malnourishment accompanying and often preceding the duration of alcoholism (1·5–25 years) in the patient group. Symptoms (parasthesiae) and signs (loss of cutaneous sensations) of a peripheral sensory neuropathy, mainly in the lower limbs, were found in all patients except one. Other observations included (approximate proportion of patients (% total)) pellagroid pigmentation of the exposed skin (66), a loss of vibration (66), loss of postural sensation (33), depression or absence of ankle jerks (50), mild gastrointestinal symptoms (50), an enlarged liver (50), stomatitis or glossitis (25).

Six patients (two admissions in one case for similar complaints) showed mental changes in addition to the peripheral neuropathy. These consisted of confusion, loss of memory and disorientation in time and space, even while fully conscious. Florid confabulations and frightening hallucinations were not observed. On re-questioning, there was a history of more chronic and severe alcoholism in this group of patients compared to the other patients, but the background of malnourishment was as bad as in the other patients.

General investigations. In the patients, mild anaemia was present in about 50%, but megaloblastic marrow was detected in only one; free gastric acidity was absent in a few, but they did not have any haematological or neurological deficits other than those found in most other patients. Intestinal absorption was normal in all patients; the mean value for [^{57}Co]cyanocobalamin absorption, by determination of plasma radioactivity, was 0·95% administered dose/l, with a range of 0·48–1·30% administered dose/l at 8 h; the normal was more than 0·45% administered dose/l. Similarly, the D-xylose absorption test was normal; the 2 h excretion exceeded half the total 5 h excretion in all patients.

Vitamin status before treatment

Results obtained for the seven B-vitamins studied, for two categories of patients: (1) those with peripheral neuropathy (mainly sensory and distal) alone, (2) those with mental changes (mainly confusion and disorientation) also, and for control subjects, are given in Table 1.

Values for the blood concentrations of B-vitamins in the control subjects were significantly higher than those in the patients of category 1, except folate and vitamin B_{12}. Folate concentrations were slightly lower in patients of category 1, and vitamin B_{12} concentrations were significantly higher in the patients of both category 1 and 2 than those in the controls. For vitamin B_{12}, this applied equally to total cyanide-extractable as well as non-cyanide-extractable forms (Table 1); the latter possibly represents a dissociable, physiologically active form of vitamin B_{12} (Dastur, Quadros *et al.* 1972). However, values for patients of category 1 for blood thiamin and for the pyridoxal fraction of vitamin B_6 were lower and those for nicotinic acid and total vitamin B_6 were slightly significantly higher than those for patients of category 2; there was no significant difference between categories for riboflavin, pantothenic acid and folate concentrations.

CSF concentrations of thiamin, riboflavin, nicotinic acid and vitamin B_6 for control subjects were highly significantly higher than those in patients of category 1. There were no patients with CSF values in the normal range for any of these four vitamins. In contrast, the CSF concentration of pantothenic acid in many of the patients in category 1 was in the normal range. CSF concentrations of thiamin, nicotinic acid and vitamin B_6 only in patients in category 1 were significantly higher than those for patients in category 2 (Table 1; Fig. 1). Thus, the CSF concentrations of three of the six vitamins (excluding vitamin B_{12}) were significantly lower in patients with mental changes (category 2), compared with those in patients with peripheral neuropathy alone (category 1).

All patients had normal serum vitamin B_{12} levels, about 66% had normal blood folate or pantothenate levels, very few patients had normal blood levels for thiamin, riboflavin and nicotinic acid, and none had a normal total vitamin B_6 level. Moreover, none of the patients had normal CSF values for these four vitamins. In contrast, seven of the thirteen patients in whom the pantothenic acid concentration of CSF was estimated (Table 1), and three of the six patients in whom the folate concentration of CSF was estimated (Table 1), had values within the normal range. As we obtained no values for the folate concentration of CSF in controls, the normal range of 10–35 ng/ml given by Sobotka *et al.* (1960) was used. For the purposes of comparison the vitamin B_{12} concentration of CSF was not estimated as it is normally very low or may be undetectable, varying from 0 to 40 pg/ml, according to Basil, Brown & Matthews (1965) and Schrumpf & Bjelke (1970). Our own limited experience using a few normal subjects gave values in agreement with this range although the low levels present cannot be accurately estimated by the isotopic dilution method used.

There was no statistically significant difference in the erythrocyte transketolase activity between the control subjects and patients of category 1 (mean $\pm$ SE 158 $\pm$ 2 and

Table 1. *Concentrations of seven B-vitamins in blood and cerebrospinal fluid (CSF) of normal control subjects and two categories of patients with malnutrition associated with alcoholism*

(Mean values with their standard errors, no. of determinations in parentheses)

	Thiamin (ng/ml)				Riboflavin (ng/ml)			
	Blood		CSF		Blood		CSF	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control subjects† (normal, healthy volunteers)	39·0	0·76 (69)	12·9	0·73 (9)	272	5·1 (67)	130	19 (5)
		***		***		***		***
Patients:‡								
Category 1 (peripheral neuropathy only)	20·2	1·07 (38)	6·1	0·44 (21)	123	5·7 (34)	69	6·9 (12)
		***		***				
Category 2 (peripheral neuropathy and mental changes)	9·4	1·17 (7)	2·0	0·17 (6)	108	12 (6)	32 75 50	

	Nicotinic acid (μg/ml)				Pantothenic acid (ng/ml)			
	Blood		CSF		Blood		CSF	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control subjects† (normal, healthy volunteers)	3·63	0·06 (69)	2·08	0·10 (8)	277	4·9 (64)	228	15 (5)
		***		***		***		
Patients:‡								
Category 1 (peripheral neuropathy only)	2·18	0·11 (38)	1·45	0·04 (16)	185	9·2 (31)	176	17 (10)
		*		***				
Category 2 (peripheral neuropathy and mental changes)	1·56	0·19 (7)	0·84	0·01 (6)	216	33 (6)	330 150 150	

	Vitamin B_6 (ng/ml)					
	Total				Pyridoxal	
	Blood		CSF		Blood	
	Mean	SE	Mean	SE	Mean	SE
Control subjects† (normal, healthy volunteers)	37·2	0·72 (65)	6·4	0·45 (7)	4·02	0·24 (35)
		***		***		***
Patients:‡						
Category 1 (peripheral neuropathy only)	18·6	0·57 (40)	3·4	0·17 (15)	2·90	0·09 (20)
		*		***		***
Category 2 (peripheral neuropathy and mental changes)	15·0	1·7 (7)	1·65	0·13 (5)	1·71	0·19 (7)

Table 1 (*cont.*)

	Folate (ng/ml)				Vitamin B_{12} (pg/ml blood)			
	Blood		CSF		Total cyanide-extractable		Non-cyanide-extractable	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control subjects† (normal, healthy volunteers)	5·45	0·38 (52)	—	—	292	20 (69)	130	21 (61)
		*				***		***
Patients‡								
Category 1 (peripheral neuropathy only)	3·87	0·51 (46)	13·6	6·4 (5)	540	44 (46)	246	22 (46)
Category 2 (peripheral neuropathy and mental changes)	5·58	0·94 (6)	7·00	—	514	119 (6)	244	58 (6)

Statistical significance of difference between mean values (*a*) for control subjects and category 1 patients, (*b*) for category 1 and category 2 patients: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

† For details, see p. 145.

‡ For details, see p. 146.

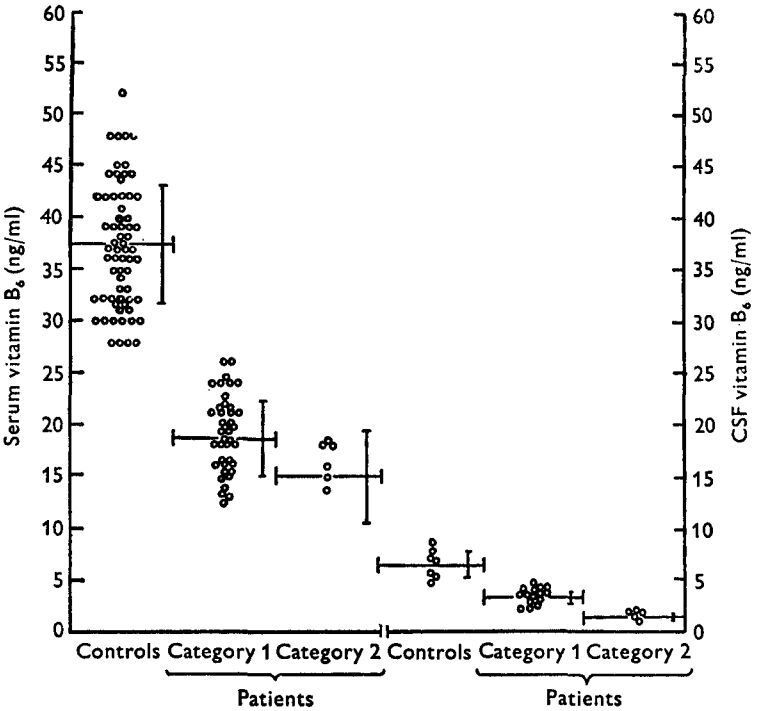

Fig. 1. Total vitamin B_6 concentrations in the serum and cerebrospinal fluid (CSF) for normal control subjects and for two categories of patients with malnutrition associated with alcoholism: (1) those with peripheral neuropathy (mainly sensory and distal) alone, (2) those with mental changes (mainly confusion and disorientation) also. Mean values, represented by horizontal bars, and standard deviations, represented by vertical bars. The difference between mean values for the following groups were statistically significant (no. of determinations in parentheses): serum vitamin B_6: control subjects (65) and category 1 patients (40) $P < 0{\cdot}001$, category 1 and category 2 (7) patients $P < 0{\cdot}05$; CSF: control subjects (7) and category 1 patients (15) $P < 0{\cdot}001$, category 1 and category 2 (5) patients $P < 0{\cdot}001$. For details of control subjects, see p.145, and of patients, see p. 146 and Table 1.

143 ± 5 μg sedoheptulose-7-phosphate formed/ml per h respectively). However, the TPP effect was statistically significantly ($P < 0.01$) higher in category 1 patients as compared to the controls (mean ± SE 26 ± 0·52 and 11·8 ± 2·6 stimulation respectively). In three patients of category 2 the transketolase activity was even lower (mean 60 μg sedoheptulose-7-phosphate formed/ml per h) and the TPP effect further increased (mean 67% stimulation).

Therefore, the trend for blood levels of thiamin and the pyridoxal fraction of vitamin B_6 was: control subjects > patients of category 1 > patients of category 2 (Table 1). A similar trend was found in the CSF levels of thiamin, nicotinic acid and total vitamin B_6 (Table 1).

Biochemical tests related to liver function

The results for patients, and for control subjects of a similar age-range, are summarized in Table 2. Comparing total values for the patients with those of the controls the mean serum albumin level and albumin:globulin (A:G) ratio for the patients were significantly lower, but the serum AspOA and AlaOA levels were not significantly different (range 4–54 and 0–154 μmol pyruvate formed/min per l respectively). It was then decided to determine the number of the patients who had laboratory evidence of hepatic insufficiency: patients with (1) A:G ratio less than 1·1, (2) serum AspOA activity of more than 23 μmol pyruvate formed/min per l or (3) serum AlaOA activity of more than 38 μmol pyruvate formed/min per l were considered to have hepatic insufficiency. Thirty-eight of the forty-five patients studied had laboratory evidence of hepatic insufficiency. Serum AlaOA activity was significantly higher in this subgroup, while it was significantly lower in the subgroup without hepatic insufficiency, compared with the controls. The relationship between these results and serum vitamin B_6 concentrations will be discussed later. Mild to moderate liver enlargement was found more frequently in the subgroup with hepatic insufficiency.

Effect of treatment on blood vitamin levels

All the patients were receiving a 'non-vegetarian' diet, which included an occasional (twice/week) small portion of meat and egg in addition to the daily quota of cereals, pulses, vegetables and a small quantity of milk. Alcohol was totally withdrawn except for one patient. The results of an analysis for vitamin content of a 3 d pooled diet from the JJ Hospital kitchen, in 1973, indicated that it provided less than the minimal daily requirement (Gopalan, Ramasastri & Balasubramanian, 1971)) in respect of thiamin, riboflavin, nicotinic acid and pyridoxine (Santhadevi, 1974). Moreover, even the total quantity of food was found insufficient by many patients, from 1972 onwards. This was due to financial stringency.

Of the fifty-nine patients studied ten patients absconded before they had received adequate treatment. Of the remaining forty-nine, forty-five improved their clinical status: six patients showed improvement with hospital diet alone, given for 3–6 weeks, and in the number of patients indicated in parentheses the following treatments brought about improvement: nicotinic acid (18), thiamin (13), calcium pantothenate (1), pyridoxine (1), thiamin + pyridoxine (2), thiamin + nicotinic acid (1), thiamin +

Table 2. *Biochemical tests of liver function for normal control subjects and for patients with malnutrition associated with alcoholism, with and without hepatic insufficiency*

(Mean values with their standard errors; no. of determinations in parentheses)

	Total proteins (g/l)		Albumin (A) (g/l)		Globulin (G) (g/l)		A:G		Serum AspOA (μmol pyruvate formed/min per l)		Serum AlaOA (μmol pyruvate formed/min per l)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control subjects† (normal healthy volunteers)	75·1	1·27 (10)	43·9	1·68 (10) ***	31·2	0·25 (10) *	1·42	0·01 (10) ***	11	0·6 (10)	28	2·9 (10)
Patients with malnutrition and alcoholic neuropathy:‡ Total	71·5	0·55 (45)	34·5	0·85 (45)	36·7	1·09 (45)	1·00	0·05 (45)	19	2·6 (43)	44	6·9 (43)
Without hepatic insufficiency	70·3	2·57 (7)	39·5	1·81 (7)	30·8	1·74 (7)	1·30	0·64 (7)	10	1·1 (7)	14	1·5 (7)
With hepatic insufficiency	71·7	0·42 (38)	33·7	0·97 (38)	37·8	0·39 (38)	0·94	0·05 (38)	20	3·0 (36)	50	6·8 (36)

For serum AlaOA, mean values for control subjects and patients with hepatic insufficiency, and those for patients with and patients without hepatic insufficiency were significantly different: $P < 0.05$; and those for control subjects and patients without hepatic insufficiency were significantly different: $P < 0.001$.

AspOA, L-aspartate:2-oxoglutarate aminotransferase (*EC* 2.6.1.1); AlaOA, L-alanine:2-oxoglutarate aminotransferrase (*EC* 2.6.1.2).

Statistical significance of difference between mean values (*a*) for control subjects and patients, (*b*) for patients with and patients without hepatic insufficiency: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details, see p. 145.

‡ For details, see p. 146.

Table 3. *Changes in B-vitamin concentrations in blood, and clinical response to diet and B-vitamin therapy* in a patient (B.D.) who presented in 1971 with peripheral neuropathy*

Date	Treatment given during previous 10 d period	Thiamin (ng/ml)	Riboflavin (ng/ml)	Nicotinic acid (μg/ml)	Pentothenic acid (ng/ml)	Vitamin B_6 (ng/ml)		Folate (ng/ml)	Vitamin B_{12}		Clinical assessment
						Total	Pyridoxal		Total (pg/ml)	% Total	
13 May	(Admission)	14·0	88	1·35	325	18·0	3·20	1·75	382	31	Pellagrous skin; mild peripheral sensory loss; no symptoms; AJ absent
28 May	Hospital diet	27·0	—	2·88	—	25·0	4·80	—	—	—	Skin returned to normal
10 June	Nicotinic acid (50 mg/d)	38·0	248	5·40	300	26·0	4·80	2·50	336	60	Cutaneous sensation; normal UL; reduced LL level
21 June	Hospital diet	28·0	208	3·60	270	24·0	—	—	—	—	No change
2 July	Thiamin (50 mg/d)	65·0	320	4·24	310	26·0	5·00	—	—	—	No change
13 July	Hospital diet	41·0	270	3·72	300	24·0	5·50	—	—	—	Further improvement in sensation in lower limbs
29 July	Pyridoxine (50 mg/d)	42·0	330	3·20	330	46·0	—	—	—	—	Paraesthesiae for the first time, otherwise no change
7 Aug.	Hospital diet	42·0	280	3·44	350	35·4	—	—	—	—	Paraesthesiae disappeared
19 Aug.	Nicotinic acid (50 mg/d) (Discharged)	—	—	—	—	—	—	—	—	—	Much improved; sensory loss on toes only; AJ persistently absent

AJ, ankle jerk; UL, upper limbs; LL, lower limbs. * For details, see pp. 146 and 153.

nicotinic acid + pyridoxine (1), B-vitamin complex (2). One patient who was minimally affected showed complete recovery within 1 week on hospital diet alone, without parenteral administration of vitamins. The improvement was in a remission of the signs and symptoms of neuropathy and also in a subjective feeling of well-being. Of patients with neuropathy and mental changes, two showed marked improvement with hospital diet and initial injections of nicotinic acid, and two showed improvement with diet and initial thiamin therapy. Two of these patients did not improve appreciably.

An increase in the blood levels of some of the B-vitamins other than the one which was administered therapeutically, was an important finding of the present study. Thus, the administration of thiamin alone invariably brought about a concurrent increase in the blood levels of riboflavin, nicotinic acid and pantothenic acid. Similarly, with the administration of nicotinic acid alone, there was a concurrent increase in the blood levels of thiamin, riboflavin and pantothenic acid. The administration of pyridoxine was followed by a concurrent and significant increase in the blood level of riboflavin only. Assays done in our laboratory indicated that the vitamin preparations used (obtained from Glaxo Laboratories (India) Ltd, Bombay, India) were not contaminated by any other vitamin.

The detailed schedule of the biochemical and clinical response to successive treatment with one or more of the B-vitamins, for one patient with neuropathy alone (category 1) and one patient with mental changes as well (category 2), as an example of the response of two typical patients to therapy, is given in Tables 3 and 4 respectively. Patient B.D. (Table 3), a man aged 45 years, had a predominantly sensory neuropathy as indicated by clinical, electrodiagnostic and histopathological studies. The important finding was the increase, from initial abnormally low levels in the blood, of thiamin, nicotinic acid and vitamin B_6 (total and pyridoxal) concentrations when given the hospital diet only, and a further increase in thiamin, riboflavin and nicotinic acid concentrations after administration of nicotinic acid alone, and then thiamin alone; however, there was no further increase in vitamin B_6. On administration of pyridoxine, the patient showed paraesthesiae for the first time, which cleared after a second course of nicotinic acid.

Patient C.P. (Table 4), a man aged 26 years, was totally disoriented and confused on admission and had to be forcibly fed during the first week in hospital. In addition to very low blood levels of all B-vitamins studied, except folate and vitamin B_{12}, the CSF levels were also very low. The patient was considered to have either a severe form of pellagra with mental changes, or a thiamin deficiency condition bordering on Wernicke's encephalopathy. However, he had no oculomotor signs, and after the first intramuscular injection of 100 mg thiamin he improved remarkably, responding to questions and starting to eat on his own. After five thiamin injections he was mentally fully recovered, sensory testing became possible, and he complained for the first time of tingling and numbness. Although the levels of thiamin, riboflavin, nicotinic acid and pantothenic acid were increased with this treatment, the level of vitamin B_6 increased while he was on hospital diet only. The signs and symptoms of peripheral neuropathy appeared to improve after nicotinic acid treatment, and he was discharged totally asymptomatic within 6 weeks of admission, although his nerve biopsy specimen had

Table 4. *Changes in B-vitamin concentrations in blood and clinical response to diet and B-vitamin therapy* in a patient (C.P.) who presented in 1971 with peripheral neuropathy and mental changes*

Date	Treatment given during previous 10 d period	Thiamin (ng/ml)	Riboflavin (ng/ml)	Nicotinic acid (μg/ml)	Pantothenic acid (ng/ml)	Vitamin B_6 (ng/ml)		Folate (ng/ml)	Vitamin B_{12}		Clinical assessment
						Total	Pyridoxal		Total (pg/ml)	% Total	
16 Dec.	Force-feeding in hospital (1 week)	7·0	135	2·20	175	16·0	2·50	6·75	644	46	Confused and restless; pellagroid skin changes; all reflexes preserved
3 Jan.	Thiamin (100 mg/d on alternate days i.e. five doses)	35·0	– 275	3·76	250	17·0	2·40	—	—	—	Well oriented in time and space but withdrawn and uncooperative; paraesthesiae up to ankles were complained of for the first time 2 d later
10 Jan.	Hospital diet	28·0	220	3·00	200	28·8	4·00	—	—	—	Skin normal; mental functions normal; other findings same
20 Jan.	Nicotinic acid (50 mg/d)	49·0	330	4·30	300	28·0	3·80	—	—	—	Marked improvement in paraesthesiae and thermal appreciation
29 Jan.	Hospital diet	36·5	275	4·40	240	37·6	4·60	—	—	—	Asymptomatic; no neurological deficit
CSF levels on admission		2·25		1·14		1·72					

D-xylose absorption was normal: 2 h output 0·75 g, 5 h output 1·08 g; [^{57}Co]cyanocobalamin absorption 0·74 % administered dose/l. plasma; nerve thaimin concentration 1·99 ng/mg.

CSF, cerebrospinal fluid.

* For details, see pp. 146 and 153.

shown mild chronic degenerative changes and the thiamin concentration in the nerve sample was very low (Table 4) compared to the level in control subjects (above 5 ng mg). The levels of some of the B-vitamins in the peripheral nerve will be reported elsewhere.

DISCUSSION

Comparing our study with those done in Western countries (e.g. Victor & Adams, 1953; Leevy & Baker, 1963), it would appear that our patients suffered from a more chronic history of dietary insufficiency and a less severe form of alcoholism; most of them were of low or almost penurious economic status. Fennelly *et al.* (1964) concluded from their study of alcoholics that hypovitaminaemia was not correlated with alcohol consumption but rather with previous dietary habits; to our knowledge they are the only other group which has estimated B-vitamin concentrations. They found the most consistent reduction was in thiamin, followed by folate, whereas we found that total vitamin B_6 was most consistently reduced; no patient had a value in the normal range. We found that thiamin, riboflavin and nicotinic acid were the next most frequently reduced blood vitamins; pantothenic acid and folate were least frequently affected. While intestinal absorption, as assessed by D-xylose and [^{57}Co]cyanocobalamin absorption tests, were unimpaired in all patients alcohol is known to interfere selectively with the absorption of thiamin even in the absence of a malabsorption syndrome (Thompson, Baker & Leevy, 1970). Normally, too, the maximum absorptive capacity for thiamin in man appears very limited (Matthews, 1967). Our results for blood concentrations of B-vitamins for control subjects are similar to values for thiamin, riboflavin, nicotinic acid and vitamin B_6 reported by Baker & Frank (1968*b*), those for total cyanide and non-cyanide-extractable vitamin B_{12} reported by Matthews *et al.* (1967), and those for folate reported by Forshaw & Harwood (1971).

The five important findings are: (1) patients of category 1 had reduced blood levels of all the vitamins (except vitamin B_{12}); (2) patients of category 2 (with mental changes) had a significantly lower blood level of thiamin and pyridoxal fraction of vitamin B_6 and lower transketolase activity; (3) patients of category 2 had lower B-vitamin levels in the CSF, comparable to differences found in blood, particularly of thiamin, nicotinic acid and total vitamin B_6; (4) there was an interrelationship between many of the B-vitamins, particularly noticeable during treatment; there was an increase in thiamin, riboflavin, nicotinic acid and pantothenic acid concentrations when thiamin or nicotinic acid were administered alone; (5) the serum vitamin B_{12} level was not reduced in any of the patients but was actually increased in the patient group compared to the controls.

The metabolic implications of these findings, particularly the interrelationships between some of the B-vatamins will be considered. According to McIlwain & Bachelard (1971), thiamin, nicotinic acid, pyridoxine and vitamin B_{12}, in that descending order, are metabolically more significant for the brain. The role of thiamin in the intermediary metabolism of the brain is well known, particularly as the cofactor TPP. If, in thiamin deficiency, the reduction in oxidative metabolism is severe, over-all cerebral dysfunction would be expected (cf. patients of category 2). However, this would appear to have been a reversible biochemical lesion only, as most of them

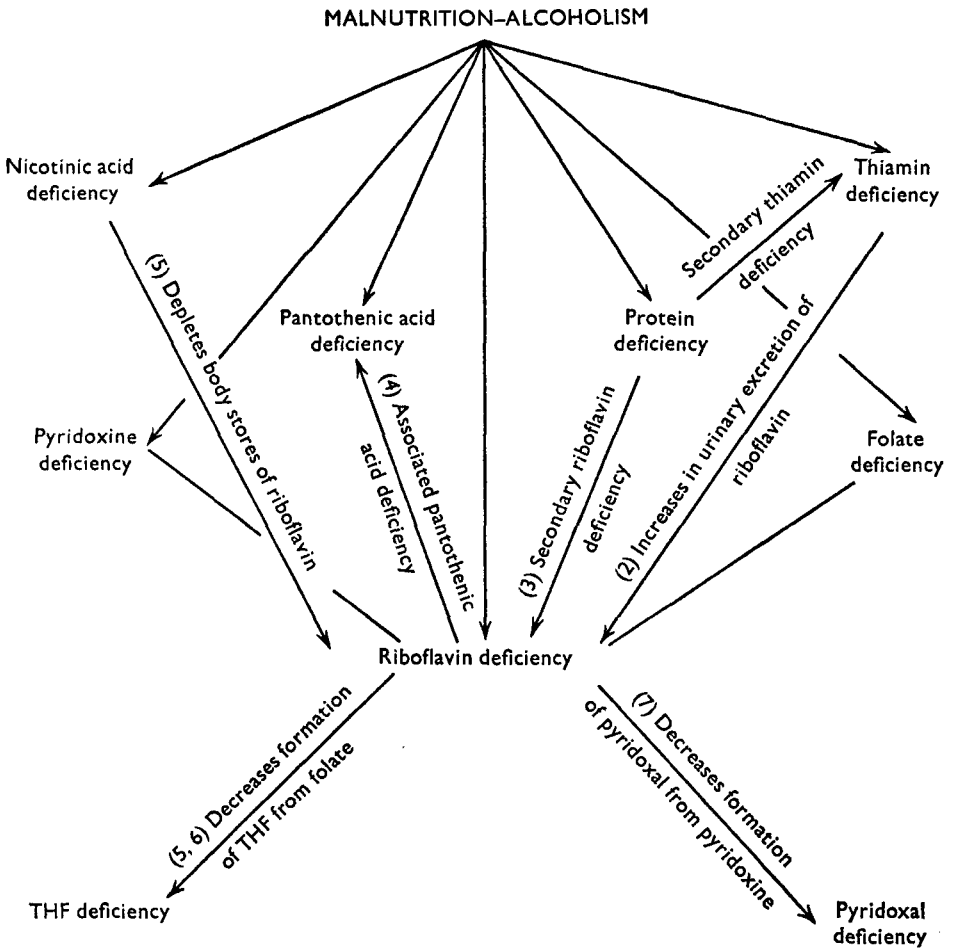

Fig. 2. Scheme of interrelationships between individual B-vitamins as indicated by the findings of the present study and those of reported studies. (1) Baker, & Frank (1968*a*); (2) Sure (1944); (3) Rasmussen (1958); (4) Spies *et al.* (1940); (5) Tamburro *et al.* (1971); (*b*) Bovina, Landi, Pasquali & Marchetti (1969); (7) Wada & Snell (1961). THF, tetrahydrofolate.

recovered on a modest hospital diet supplemented by injections of thiamin or nicotinic acid alone. Dreyfus & Victor (1961) reported severe neurological deficits in thiamin-deficient rats only when the brain thiamin level decreased to 20% of the normal value. The only available report on tissue thiamin concentrations in subjects with undernutrition due to fever or alcoholism is that of Ferrebee, Weissman, Parker & Owen (1943), who found low levels in brain, liver, kidney, heart and skeletal muscle, compared to control subjects. Two indirect biochemical findings suggest cerebral involvement in patients of category 2. First, both the TPP effect and the erythrocyte transketolase activity were affected; secondly, their CSF levels of nicotinic acid, pyridoxine as well as thiamin were reduced. Dreyfus (1962) and Truswell, Hansen & Konno (1972) have stressed the significance of an increased TPP effect in detecting thiamin deficiency in Wernicke's encephalopathy.

While riboflavin and nicotinic acid are required in enzymic hydrogenation of ethanol, nicotinic acid appears to be more essential (Gellene, Cherrick & Leevy, 1966). In patients of category 2 the Wernicke-Korsakoff type of encephalopathy was not evident, neither did they have full-blown pellagra. Recently, Shah, Panday & Rathi (1972) reported that nineteen of their twenty-two maize-eating pellagra patients showed psychiatric changes including depression, anxiety, schizophreniform psychosis, confusion and dementia, all of which remitted completely with nicotinic acid therapy, and Lehmann (1952) reported improvement in the mental disorder of apparently non-pellagrous patients given nicotinic acid. On the other hand Lewy, Spies & Aring (1940) successively treated neuritis of overt pellagra by the administration of thiamin alone, a feature stressed recently by Grinker & Sachs (1967). In our study too, of those patients, of the total of six in category 2, who improved on treatment two had been given thiamin and two had been given nicotinic acid initially.

A dietary protein intake of less than 38 g/d may also lead to a deficiency of vitamins such as thiamin, biotin, and ascorbic acid (Baker & Frank, 1968*a*), poor utilization of riboflavin (Rasmussen, 1958) and poor retention of riboflavin in the liver (Sarett & Perlzweig, 1943). A secondary deficiency of riboflavin may result from its increased excretion, which has been reported in states of thiamin deficiency (Sure, 1944) (Fig. 2). Nicotinic acid deficiency can also reduce riboflavin stores; lack of riboflavin then reduces the biosynthesis of tissue folate (Tamburro, Frank, Thompson, Sorrell & Baker, 1971) (Fig. 2). In our patients restoration of blood riboflavin levels was found consistently after the administration of thiamin or nicotinic acid alone (Tables 3 and 4). Riboflavin deficiency, in turn, can lead to pantothenate deficiency (Spies, Stanberry, Williams, Jukes & Babcock, 1940) (Fig. 2), which was restored by the administration of riboflavin.

Coursin (1961) has reviewed the metabolic implications and clinical manifestations, of vitamin B_6 deficiency. Riboflavin is a cofactor in the synthesis of pyridoxal phosphate (Wada & Snell, 1961; and Fig. 2). It is also possible that the common biochemical denominator for the oral lesions in states of vitamin B depletion is the lack of pyridoxal phosphate, either directly due to deficiency of pyridoxine or indirectly, conditioned by riboflavin deficiency (Bamji, 1972). Lakshmi & Bamji (1974) have confirmed that the oral lesions in man respond to treatment with either riboflavin or pyridoxine. In the present study we found that levels of riboflavin and pyridoxal were restored to normal on administration of pyridoxine (Table 3). Although most patients with liver insufficiency had high aminotransferase activities (Table 2), the markedly low serum AlaOA activity in patients without evidence of liver insufficiency, was apparently related to the low total vitamin B_6 levels. Ning, Baker & Leevy (1966) also reported low transaminase activity in malnourished alcoholics and attributed it to vitamin B_6 deficiency. There is experimental evidence of a severe reduction in the serum AlaOA activity in rats subjected either to a dietary deficiency of vitamin B_6 or to a competitive deficiency produced by the administration of deoxypyridoxine (Talageri, 1961).

The mean serum vitamin B_{12} concentration for the two categories of patients was significantly higher than that for the control subjects (Table 1). A moderate extent of liver dysfunction was found for the patient group as a whole (Table 2). This could have

resulted in a reduction in the storage capacity of the liver for this vitamin (Kato, Narita & Kamohara, 1959). In contrast, both total cyanide-extractable and non-cyanide-extractable serum vitamin B_{12} levels were markedly lower in non-alcoholic patients with megaloblastosis and neuromyelopathy (Dastur, Santhadevi, Quadros, Gagrat, Wadia, Desai, Singhal & Bharucha, 1975).

A close correlation between serum and CSF folate has been reported (Reynolds, Gallagher, Mattson, Bowers & Johnson, 1972), and similar trends in serum and CSF folate levels were also found by us. Six patients with low CSF values (five of category 1 and one of category 2) had low serum folate levels. There was no evidence of either organic brain syndrome or pyramidal track damage, such as has been attributed recently to a deficiency of folate (Reynolds, Rothfeld & Pincus, 1973).

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