Vitamin B6 Nutritional Status of a Psychiatric Outpatient Population

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Abstract

The B6 status of 232 psychiatric outpatients was evaluated using a modification of the erythrocyte oxaloacetic glutamic transaminase (E-GOT) assay of Kishi and Folkers. On a control group this assay procedure was compared with plasma B6 levels and dietary intake. A good correlation was found between dietary intake, plasma B6 levels and the degree of deficiency measured on the basis of pyridoxal phosphate stimulation of the E-GOT assay.

The patient population fell into three groups on the basis of their B6 intake. One group was not taking any supplemental B6, a second group was taking supplements up to 10 mgs per day while the third group was taking mega dose amounts of B6. Basal E-GOT levels for the three groups were 58.3, 73.9, and 110.0;uM/hr/ml RBC respectively. The mean percent deficiency of the group not taking any B6 was 17.7 percent while the group taking supplemental levels of B6 had deficiencies of 7.2 percent. B6 deficiency was more prominent in young adults and in the aged. The E-GOT assay involving stimulation by pyridoxal phosphate appears to be a useful procedure for the evaluation of the B6 status of individuals and for following a therapeutic regime involving vitamin B6

Introduction

Deficiencies of water soluble vitamins in psychiatric patients have **been reported by** numerous investigators. B12 and folic acid deficiencies in such patients were noted by Carney (1979a), pyridoxine by Nubbs (1974), and thiamine in regard to mental aberrations in alcoholics by Riding (1975). Mental depression was noted in subjects on a synthetic diet deficient in B6 (Hawkins and Barsky, 1948). This was the earliest reported study of human B6 deficiency induced by having individuals on a synthetic B6 deficient diet for a 25 day period. No definitive symptoms of B6 deficiency appeared except for the mental depression. Sauberlich et al. (1970) have reported that subjects on a B6 deficient diet develop personality changes characterized by depression, irritability and a loss of sense of responsibility. Carney et al. (1979b) reported that pyridoxine malnutrition may play a role in endogenous depression.

Some investigators have proposed a significant role for water soluble vitamins in the treatment of mental illness (Hoffer et al., 1957; Pfeiffer, 1974). For over a decade vitamins have been employed as part of the treatment regimen of the Brain Bio Center in the treatment of various psychiatric and metabolic disorders with little evidence of any undesirable side effects.

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The B6 nutritional status of the psychiatric patients would be useful in an evaluation of whether supplementation with this vitamin might be indicated and what dose level might be employed.

Several approaches have been used in the evaluation of B6 status. Direct methods of determining the levels of the vitamin in the blood have not been altogether satisfactory as they are complicated by the multiplicity of chemical forms of B6 (Loo and Cort. 1974), or else bioassay procedures, with their inherent drawbacks, are used (Sauber-lich, 1967). Of the indirect methods, the most promising for use in evaluating deficiency is the in vitro assay of B6 dependent enzymes and their stimulation by the addition of pyridoxal phosphate (PALP) (Kishi and Folkers, 1976).

Glutamic oxaloacetic amino transferase activity (E-GOT) and the in vitro stimulation of this system by the addition of pyridoxal phosphate have been shown to be useful indices of vitamin B6 nutritional status. Stimulation of this enzyme system by vitamin B6 as a measurement in the evaluation of B6 nutritional status was originally described by Raica and Sauberlich (1970), and has recently been employed extensively by Folkers et al. (1978).

The assay of Kishi and Folkers (1976) is based on the principle that in B6 deficiency the apoenzyme is not saturated with the co-factor and, therefore, the in vitro addition of pyridoxal phosphate will stimulate the system. The amount of stimulation is an indication of the degree of deficiency. If the B6 nutriture is adequate no stimulation occurs in the in vitro test as the apoenzyme is saturated with the cofactor.

Several studies have shown that a dietary deprivation of B6 results in a decrease in E-GOT activity and a decrease in the degree of saturation of the apoenzyme. Dietary pyridoxine supplements have been shown to give the reverse effects.

Materials and Methods

Two hundred and thirty two new patients of the Brain Bio Center were evaluated on their initial visit. In addition to a regular workup which consists of Metpath Chem
Screen and Hemogram, analysis of blood for polyamines, lead, manganese, analysis of serum for iron, zinc and copper levels and urinalysis for protein and pyrroles; blood was drawn into an EDTA containing vacu-tainer for the assay of glutamic oxaloacetic amino transferase activity (E-GOT). The sample was prepared and assayed essentially as described by Kishi and Folkers except that incubation was carried out at 37° for 30'. We found the assay procedure was more reproducible at the higher incubation temperature. The assays were run blind with the analysts having no knowledge as to whether the subject was or was not taking B6. Plasma B6 was determined by the Tet-rahymena bioassay of Baker and Frank (1968).

The population under study was heterogeneous consisting primarily of individuals with psychiatric complaints but it also included individuals with metabolic disorders and apparently healthy individuals. The group surveyed consisted of 113 males and 119 females. The males ranged in age from 7 to 83 years with a mean of 34.4 ± 17.1 while the females ranged from 9 to 79 years with a mean of 40.9 ± 17.2 years. The following psychiatric symptoms were reported in the group which was not taking any supplemental B6: depression 66 percent, anxiety 59 percent, compulsions 19 percent, mind racing 47 percent, memory loss 36 percent, thought disorder 27 percent, paranoia 27 percent and hallucinations 23 percent. During the taking of their case histories, they were questioned as to how much B6 they were taking on a regular basis and for how long.

New patients were employed because we were interested in evaluating the B6 status of the patient population coming to the Brain Bio Center. In addition to the E-GOT test we ascertained whether the persons were taking any B6 and how much they were taking daily. Of the group studied, 44 percent were not taking any B6 while 29 percent were taking vitamin supplements containing less than 10 mgs B6. The remainder were already taking mega levels of B6 ranging from 50 to 1250 mg/day. We, therefore, had three groups who were taking

significantly different amounts of B6.

Results and Discussion Pilot Study Control Group

In order to evaluate whether the E-GOT assay reflects the B6 status of an individual a pilot study was run on a group of healthy individuals who were not taking any supplemental B6. The dietary B6 intake was estimated on the basis of a food journal kept for three days. The daily B6 intake is the mean intake for a day based on this journal. The intake estimate is probably on the low side. The results (Table 1) indicate that some of the control subjects were not getting minimum recommended daily dietary allowance of 2.0 mg per day. Three male subjects who had a significant deficiency had a rather limited diet. The females' diet, with the exception of one who had just started a fad diet, had a more varied food intake. This is reflected in the plasma B6 level and deficiency measured by the E-GOT assay.

Although theoretically the apoenzyme should be saturated with coenzyme if the diet is adequate in B6 this is apparently not completely the case. In the assay there is invariably a small amount of stimulation with added pyridoxal phosphate indicating either that normally the apoenzyme is not completely saturated or else it has been slightly dissociated in the course of sample preparation. The percent deficiency is based on the amount of stimulation of enzyme activity observed in the presence of added pyridoxal phosphate as compared to basal enzyme activity. Some investigators have expressed the results as a ratio of saturated enzyme activity to basal activity. The individuals on multivitamin B6 level < 10 mg per day have a deficiency of 7.2 percent and a mean ratio of 1.07 even though it would appear they are getting adequate amounts of B6.

A deficiency of less than 25 percent or a ratio of PALP stimulated to basal activity of < 1.25 we believe is not significant in considering an individual to be deficient.

Kishi and Folker have used the assay on several control populations. For a group of football players, on a training table diet they found a mean deficiency of 13 percent with a standard deviation of ± 9 percent. None of these individuals would be considered significantly deficient by our criteria.

Williams (1976) used the assay to evaluate the B6 status of a group of normals. He reported deficiencies of 0 to 20 percent or an activation coefficient of 1.00 to 1.20. The data agree well with our own and we feel that our criteria of >25 percent deficiency or an activity coefficient of >1.25 is conservative. A high correlation was observed ($r= 0.795$ $p <.01$) between dietary intake and the E-GOT assay. No significant negative correlation was observed between plasma B6 levels and basal E-GOT activity.

Solomon and Hillman (1979) have presented evidence that the basal E-GOT activity reflects the long term B6 nutriture while the stimulation of activity by added pyridoxal phosphate reflects the immediate plasma B6 level. Induction of GOT apoenzyme is found in young erythroid cells. In response to changes in B6 level the basal E-GOT level varies with the age of the erythroid cell population.

Survey of Psychiatric Outpatients

Basal (E-GOT) levels were found to be significantly different in the groups taking different levels of B6 (Table 2). The E-GOT level for the group not taking any B6 was 58.3 ± 19.0 uM/hr/ml RBC as compared to 73.9 ± 24.7 for the multivitamin level group and 110.0 ± 27.3 for the mega-vitamin group.

Table 3 lists the degree of deficiency as measured by the amount of stimulation upon addition of pyridoxal phosphate. The mean percent deficiency was 17.7 for the group not taking any B6 and 51.4 percent of this population had a ratio (basal saturated activity to enzyme activity) of greater than 1.25. The group taking multivitamin levels of B6 had a mean deficiency of 7.25 percent and 17.6 percent of the population had a ratio greater than 1.25.

The third group which was on mega dose levels exhibited an apparent negative inhibition of 11 percent with only 1.6 percent of the population having a ratio of greater than 1.25. This apparent negative inhibition has been observed when pyridoxal phosphate is added to cells already saturated with the co-

enzyme. Kishi et al. (1979) reported inhibition of the in vitro E-GOT assay upon addition of pyridoxal phosphate to cells from patients on intravenous hyperalimentation of a preparation giving 100 mgs of B6 per day. Folkers et al. (1978) reported the same effect in subjects receiving large amounts of B6. A possible explanation for the phenomenon is that excessive amounts of pyridoxal phosphate may compete with pyruvic acid formed in the assay for the available phenylhydrazine resulting in a decrease in the amount of pyruvate phenylhydrazone formed giving the apparent inhibition.

Table 4 compared E-GOT assay results with plasma B6 levels determined by bio-assay in subjects taking different levels of B6. These results indicate that the E-GOT assay is a good measure of the B6 status of the individual and can be of use in following the patient clinically.

Plasma B6 levels of greater than 500 ng/ ml were found in individuals who were taking 1000 mgs of B6. Since the assay was run blind we were not able to determine an exact value as the limit of the assay was 500 ng/ml.

The group that was not taking any B6 was analyzed on the basis of age (Table 5). B6 deficiency was most prevalent in individuals less than 20 years old and in the elderly. The less than 20 year old group excreted larger amounts of urinary pyrrole as evidenced by the presence of larger amounts of Ehrlich reacting chromogens. Pfeiffer et al. (1974) have described a syndrome in young adults which is characterized by psychosis and a stress induced pyro-luria. The condition responds to treatment with B6 and Zn. B6 is believed to form a complex with the pyrrole making the vitamin unavailable. The basal E-GOT level is lowest for the group over 59 years of age. The group also has the greatest percent deficiency as reflected by stimulation of the enzyme system upon addition of pyridoxal phosphate. The prevalence of B6 deficiency in the elderly confirms several earlier reports on the subject. B6 deficiency in the elderly was first reported by Hamfelt (1964) and confirmed by others. Baker et al. (1979) have recently shown the vitamin most lacking in the elderly population is B6. Aging appears to be associated with depressed vitamin B6 levels even when the diet is presumably adequate.

TABLE 1

SUBJECT	SEX	ESTIMATED DIETARY INTAKE OF B6	E-GOT (uM/hr/ml/RBC) BASAL	E-GOT (uM/hr/ml/RBC) SATURATED	PERCENT DEFICIENCY	PLASMA B6(ng/ml)
S.M.	F	1.85	60.7	63.4	4.3	120
D.C.	F	0.71	57.4	66.4	13.5	61
B.A.	F	1.16	58.4	67.8	16.2	46
K.J. (diet)	F	0.06	58.1	75.0	22.5	24
P.S.	M	1.05	58.0	79.6	27.0	44
J.W.	M	0.98	52.5	75.2	30.1	44
E.W.	M	0.57	50.1	68.8	27.2	39
T.C.	M	1.41	65.8	85.9	23.4	60

TABLE 2

BASAL E-GOT LEVELS (uM / HR / ML RBC)

		A NO B6	B MULTIVITAMIN LEVEL		C MEGAVITAMIN LEVEL
n		103	68		61
E-GOT RANGE		31.1-129.3	39.6 - 137.6		63.1-205.0
E-GOT x±S		58.3 ±19.0	73.9 ±24.7		110.0 ±27.5
	A vs B t=4.627 P<.00l			B vs C t = 7.794 P<.001	

TABLE 3

MEAN % DEFICIENCY	NO B6 B6 DEF1CIE	NCY MULTIVITAMIN LEVEL	MEGAVITAMIN LEVEL
	17.7 ±13.1	7.2 ±13.9	-11.1 ±12.1
MEAN RATIO	1.22 ±0.22	1.07 ±0.23	0.91 ±0.11
% of POPULATION DEFICIENT (RATIO >1.25)	51.4	17.6	1.6

TABLE 4

SUBJECT	E-GOT	PERCENT	PLASMA	B6 DAILY B6 ng/ml	
1 2 3 4 5 6	112.0	DEFICIENCY	DOSAGE BIOASSAY		
	116.4	-9.2 -17.5 -14.0		>500	1000 mgs
	127.2	-6.4 + 17.8 + 6.8		>500	1000 mgs
	88.4			>500	1000 mgs
	63.8			93	25-50 mgs
	47.5			38	No B6
				62	No B6

TABLE 5

AGE AND B6 STATUS IN THE NO SUPPLEMENTAL B6 GROUP

					EHRLICH REACTING CHROMOGENS PERCENT OF POP-
AGE GROUP	■	uM/hr/ml/RBC	PERCENT DEFICIENCY	uG/DL	ULATION DEFICIENT
20	16	59.3	21.2	61.3	62
20-29	30	57.8	17.2	52.6	50
30-39	19	56.6	17.0	38.0	47
40-49	14	67.8	13.1	30.2	43
50-59	9	56.3	17.0	32.8	44
59	11	51.7	22.4	33.6	54

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