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Serum Pyridoxal, Folate, and Vitamin B_{12} Levels in Institutionalized Epileptics

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INTRODUCTION

Pyridoxal is known to be important in central nervous system metabolism and pyridoxine responsive convulsions in infants are widely recognized (Hunt et al., 1954; Scriver, 1960; Deneve and Jongbloet, 1961; Garty et al., 1962; O'Brien and Jensen, 1963; Gentz et al., 1967). Some success in the treatment of epilepsy with pyridoxine has also been reported. Ernsting and Ferwerda (1952) treated 14 patients aged 2 to 17 years, all of whom had petit mal epilepsy (and one grand mal as well); none had responded to conventional treatment. Pyridoxine was given in doses of 20 mg from three to six times daily. In five patients the attacks disappeared and three others showed improvement while in the remaining six, no decrease in the number of attacks was observed.

Hagberg et al. (1964) described three children with epilepsy of unknown origin and abnormal excretion of xanthurenic acid after a tryptophan loading test. In 1966 these authors studied a larger group of 43 children with cryptogenic epilepsy (Hagberg et al., 1966). They found no significant difference in the pyridoxal-5-phosphate values compared with a control group, but a significantly raised excretion of xanthurenic acid characterized the group with cryptogenic epilepsy. Twenty-six of the 43 patients excreted an excess of xanthurenic acid after a loading dose of tryptophan. Nineteen of these children were treated with 160 mg of pyridoxine daily, and in nine an improvement was noted. None of the patients with a normal tryptophan loading test responded to treatment with pyridoxine.

Livingstone et al. (1955) investigated vitamin B_6 deficiency in epileptics using the tryptophan loading test. No increase in the output of xanthurenic acid was found in five patients, but the sixth patient showed a marked elevation of xanthurenic acid excretion. These authors treated a total of 31 epileptic patients with pyridoxine using doses of up to 100 mg daily. The vitamin failed to control seizures in any of the patients. Fox and Tullidge (1946) reported that they had also found pyridoxal ineffective in controlling fits in epileptics using doses of up to 100 mg daily.

We report here the serum levels of pyridoxal, folate, and vitamin B_{12} as well as the red cell folate concentrations found in 68 institutionalized patients with severe epilepsy.

MATERIALS AND METHODS

Sixty-eight patients were available for study (29 males and 39 females), all confined to one institution and all suffering from severe epileptic seizures. Their ages ranged from 14 to 56 years (mean 25); 21 were 18 years of age or under. All were receiving various combinations of anticonvulsant drugs. A sample of clotted and EDTA anticoagulated blood was obtained from each patient. Serum was separated from the clotted samples within 2 hr of its collection and stored at -20°C until required for assay.

Serum pyridoxal-5-phosphate was first dephosphorylated using an acid phosphatase;

Key Words: *Serum pyridoxal — Serum folate — Serum vitamin B_{12} — Epilepsy — Institutionalized patients*

TABLE 1. *Reference ranges for serum pyridoxal*

Age (years)	Males (nmoles/liter)	Females (nmoles/liter)
10-19	30-96	28-96
20-29	26-96	25-90
30-39	25-85	24-60
40-49	25-66	22-54
50-59	23-59	22-52
60+	20-50	18-48

Derived from 371 observations.

the free pyridoxal was then assayed microbiologically using *Lactobacillus casei* as the test organism (Davis et al., 1973). The reference range for this vitamin is age and sex dependent and was constructed from the results obtained from 371 healthy volunteers (Table 1). Serum and red cell folate was measured by the method of Davis et al. (1970) and Millbank et al. (1970) using *L. casei* as the test organism. The reference range for serum is 2.7 to 18.5 μg/liter and for red cells 115 to 600 μg/liter. Vitamin B_{12} was assayed microbiologically using *Euglena gracilis* (Nicholas and Pitney, 1958), reference range 160 to 875 ng/liter. Standard techniques were used for all other measurements.

RESULTS

The mean age-corrected serum pyridoxal was 37.3 nmoles/liter SD 17.9 for males and 30.5 nmoles/liter SD 14.6 for females compared with a mean of 58.5 SD 16.5 for males and a mean of 49.5 SD 16.1 for females in a control group. Twenty-five of the 68 patients (37%) had a level below the lower limit of the reference range (Table 2).

The mean serum folate was 2.8 μg/liter SD 2.9. Thirty-three patients (48%) had a low serum folate, and 15 of these patients also had a low serum pyridoxal (Table 3).

To determine whether there was a relationship between serum pyridoxal and folate concentrations the correlation coefficient was calculated for the serum folate and age-corrected serum pyridoxal levels. No significant correlation was found; males $r = 0.1212$, females $r = 0.1144$.

The mean red cell folate was 234 μg/liter SD 77. Two patients had a low red cell folate of 48 and 81 μg/liter, and in both this was associated with a low serum folate.

The serum vitamin B_{12} levels ranged from 215 to 1,500 ng/liter mean 480, SD 149. There was no instance of a low serum vitamin B_{12} but in three patients levels of 1,500, 1,325 and 1,425 ng/liter were found. In one patient this was associated with a low serum folate and in another a low serum pyridoxal.

None of the patients was found to have a reduced level of hemoglobin although one female aged 23 years had a concentration of 113 g/liter which is the lower limit of the reference range. All patients had a normal mean corpuscular volume.

There was no clear relationship between

TABLE 2. *Serum pyridoxal levels in patients with epilepsy*

Age (years)	No.	Range (nmoles/liter)	Mean	No. low	Percentage low
Males:					
10-19	7	16-103	53	1	14
20-29	14	12-69	34	5	36
30-39	5	12-51	37	1	20
40-49	1		31	—	—
50-59	2	17-30	24	1	50
Total	29			8	28
Females:					
10-19	12	13-56	31	6	50
20-29	20	13-107	42	6	30
30-39	4	12-46	24	2	50
40-49	3	All 12	12	3	100
Total	39			17	44

TABLE 3. *Serum folate and pyridoxal concentrations in 68 patients receiving treatment with anticonvulsant drugs*

Drugs	No. of patients	Low folate	Low pyridoxal	Low folate and pyridoxal
Barbiturates	17	6	5	4
Barbiturates + phenothiazine	11	2	1	4
Phenothiazine	5	1	—	1
Carbamazepine Phenothiazine Diazepam	4	4	—	—
Barbiturates Carbamazepine Phenothiazine	6	1	—	1
Barbiturates Carbamazepine Chlorpromazine	3	1	1	1
Barbiturates + carbamazepine	7	—	2	3
Other combinations	15	3	1	1
Total	68	18	10	15

reduced serum vitamin levels and single or groups of anticonvulsive agents although the size of the groups was too small to permit a detailed study (Table 3).

DISCUSSION

The role of pyridoxal in the pathogenesis of epilepsy is controversial. In a study of 43 epileptics, Hagberg et al. (1966) found 26 to have a significantly raised excretion of xanthurenic acid after an oral tryptophan load. In another study no increase in xanthurenic acid excretion was found (Livingstone et al., 1955). These authors also found that the treatment of epileptics with pyridoxine failed to control seizures. Hagberg and his colleagues (1966) found no significant difference between the serum pyridoxal levels of epileptic patients and controls. However, they treated 26 of those in whom an abnormality of tryptophan metabolism had been demonstrated with pyridoxine and obtained an improvement in nine. It seems therefore that in some epileptics there may be an abnormality of pyridoxal metabolism the nature of which has not yet been clearly defined.

The purpose of the present study was to determine whether there was a relationship between pyridoxal levels and epilepsy and whether there was a correlation between a low serum pyridoxal level and a low serum folate rather than to attempt a clinical study on the effect of treatment of epileptics with pyridoxine. Twenty-five of the 68 patients (37%) in this series had a low serum pyridoxal. Thirty-three (48%) had a low serum folate and 15 of these patients also had a low serum pyridoxal. A reduced level of serum folate in patients receiving anticonvulsant drug therapy has been reported previously (Child et al., 1964; Klipstein, 1964; Malpas et al., 1966; Ibbotson et al., 1967; Davis and Woodliff, 1971). Recent experimental work has shown that at least three anticonvulsant drugs—diphenylhydantoin, primidone, and phenobarbitone—have a powerful antifolate action. They also reduce the cerebrospinal-fluid folate level, which is normal-

ly about three times higher than that found in serum (Reynolds, 1973). Although the serum folate level is depressed in many patients receiving treatment with anticonvulsant drugs, a macrocytic anemia is only seen occasionally. Treatment with folic acid may increase the fit frequency but this will occur only over a period of months because of the slow diffusion of folate past the blood brain barrier.

A reduced red cell folate level was found by Preece et al. (1971), but in the present study only two patients were found to have a low red cell folate and this accords with the infrequency with which a macrocytic anemia is seen in these patients.

If the depressed serum pyridoxal levels were due to the action of anticonvulsant drugs there should be a positive correlation coefficient between serum folate and pyridoxal levels. No significant correlation was found among males, $r = 0.1212$, or females, $r = 0.1144$.

The diet offered to these patients was adequate and well balanced although it is possible that some of the patients rejected food from time to time. However, it is unlikely that 37% of the patients would have rejected food without it coming to the attention of those caring for the patients. A possible explanation for the low serum pyridoxal levels is that of increased demand. Using the ratio of 3-hydroxykynurenine to 3-hydroxyanthranilic acid excreted after an oral dose of tryptophan, French et al. (1962) found two of five patients tested to be pyridoxal-deficient. These patients had normal dietary histories but nevertheless responded to treatment with vitamin B_6 with better seizure control and possible acceleration of motor development. Hellström and Vassella (1962) found an increased excretion of xanthurenic acid in a number of children with infantile spasms and concluded that the increase represented a general trend and was not related to the marked abnormality that is to be expected in patients with an inborn error of metabolism. These authors felt that the increased excretion of tryptophan metabolites reflected a relative deficiency of vitamin B_6 which could be caused by an increased demand for pyridoxal-5-phosphate in the brain where it plays an important role in the regulation of γ-aminobutyric acid. The results from this study give some support for this hypothesis. It is clear that the relationship between pyridoxal and epilepsy is not a simple one and requires further study.

SUMMARY

Serum pyridoxal, folate, and vitamin B_{12} concentrations were measured in 68 institutionalized patients with severe epilepsy. Twenty-five patients had a reduced level of pyridoxal and thirty-three a reduced level of folate. There was no instance of a low serum vitamin B_{12} although in three patients the levels were found to be abnormally high. Fifteen patients had both a low serum pyridoxal and a low serum folate but there was no significant correlation. All patients had a normal hemoglobin concentration and a normal mean corpuscular volume.

There was no close relationship between reduced serum vitamin levels and single or groups of anticonvulsant agents, although the size of the groups was too small to permit a detailed study.

RÉSUMÉ

Les concentrations sériques de pyridoxal, de folate et de vitamine B_{12} ont été mesurées chez 68 patients en institution présentant une épilepsie sévère. 25 patients avaient un taux bas de pyridoxal et 33 un taux bas de folate. On n'a jamais trouvé de taux bas de vitamine B_{12} sérique, bien que chez 3 patients on ait trouvé des taux anormalement élevés. 15 patients avaient à la fois un taux bas de pyridoxal et un taux bas de folate sérique mais il n'y avait pas de corrélation significative. Tous les patients avaient une concentration en hémoglobine et un volume corpusculaire normaux. Il n'y avait pas de relation étroite entre la diminution des taux sériques de vitamines et un agent ou des agents anticonvulsivants bien que les groupes fussent trop petits pour permettre une étude détaillée.

(C. A. Tassinari, *Marseilles*)

RESUMEN

Se han determinado las concentraciones séricas de piridoxal, folato y Vit. B_{12} en 68 enfermos institucionalizados con severa epilepsia. Se hallaron cifras reducidas de piridoxal en 25 enfermos y 33 mostraron una reducción de folato. No se encontraron reducciones de la Vit. B_{12} sérica, aunque en 3 casos los niveles séricos eran anormalmente altos. En 15

enfermos se encontraron cifras séricas bajas de folato y piridoxal sin que existiera una correlación significativa. Todos los enfermos mostaron valores normales de hemoglobina y de volumen corpuscular. No se observó ningun tipo de relación entre la reducción de niveles sericos de vitaminas y la medicación o combinación de medicaciones administradas. Sin embargo los grupos de enfermos estudiados en este aspecto eran demasiado reducidos para permitir un análisis detallado.

(Alberto Portera Sanchez, *Madrid*)

ZUSAMMENFASSUNG

Die Serumkonzentrationen von Pyridoxal, Folat und Vitamin B_{12} wurden bei 68 institutionalisierten Patienten mit schwerer Epilepsie gemessen. 25 Patienten hatten einen reduzierten Spiegel des Pyridoxal und 33 einen solchen des Folats. In keinem Fall trat ein niedriger Vitamin B_{12}-Spiegel im Serum auf, hingegen waren die Spiegel bei 3 Patienten abnorm hoch. 15 Patienten hatten sowohl einen niedrigen Serumsspiegel von Pyridoxal als auch von Folat, zwischen beiden bestand aber keine signifikante Abhängigkeit. Alle Patienten hatten normale Hämoglobinkonzentrationen und ein normales mittleres Blutzellvolumen. Es bestand keine enge Beziehung zwischen der Reduktion der Vitaminspiegel im Serum und einzelnen oder kombiniert angewandten Antikonvulsiva; allerdings war die Gruppergrösse zu klein, um eine detaillierte Untersuchung zu erlauben.

(D. Scheffner, *Heidelberg*)

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