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Biochemical evidence for a deficiency of vitamin B_6 in the carpal tunnel syndrome based on a crossover clinical study

(pyridoxine)

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ABSTRACT **In a patient with severe carpal tunnel syndrome and a significant deficiency of vitamin B_6, the evidence for the deficiency was an extraordinarily low basal specific activity of the glutamic-oxaloacetic transaminase of the erythrocytes (EGOT). This enzyme was also deficient in pyridoxal phosphate. The patient was treated with the recommended dietary allowance of pyridoxine, 2 mg/day, for 11 weeks, then 100 mg/day for 12 weeks, a placebo for 9 weeks, and again pyridoxine at 100 mg/day for 11 weeks. Sixty-one monitorial assays of EGOT over 48 weeks supported the following interpretations. (*i*) His diet permitted the development of a debilitating carpal tunnel syndrome. (*ii*) Treatment with pyridoxine at 2 mg/day reduced the deficiency of EGOT activity from about 70% to 50%, maintained a deficiency of pyridoxal phosphate, and relieved but allowed a marginal syndrome. (*iii*) Treatment at 100 mg/day for 12 weeks nearly achieved a "ceiling" level of EGOT and eliminated the deficiency of pyridoxal phosphate. (*iv*) After placebo for 7 weeks, the deficiencies of EGOT activity and pyridoxal phosphate reappeared, and clinical symptoms become worse. (*v*) Retreatment at 100 mg/day reestablished a "ceiling" EGOT, with no deficiency of pyridoxal phosphate, and the patient was asymptomatic. These data also support the concept that a deficiency of vitamin B_6 is significant in the etiology of the carpal tunnel syndrome. Mechanistically, a state of deficiency of the coenzyme seems to lower the level of the apoenzyme; a state of no deficiency of the coenzyme regulates a ceiling level of the transaminase. The latter state is presumably desired for health.**

An exceptional study has been made of our 22nd patient who had a severe carpal tunnel syndrome. This study was cooperative and effectively combined both the biochemical and the clinical aspects. We describe herein the biochemical aspects which constitute evidence for an association of a deficiency of vitamin B_6 with the carpal tunnel syndrome as based on a crossover clinical study. The companion and detailed clinical aspects will be described elsewhere.

Ellis *et al.* (1) cooperatively found a deficiency of vitamin B_6, as pyridoxal phosphate, in a group of 10 patients who had a clinical status associated with the carpal tunnel syndrome. These patients showed a significant ($P < 0.001$) deficiency of vitamin B_6, as determined by the basal specific activities of the glutamic-oxaloacetic transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) of the erythrocytes (EGOT) in comparison with those of a control group. The detection and quantitation of the deficiency of pyridoxal phosphate was based upon the principle of unsaturation and saturation of a coenzyme-apoenzyme system—for example, EGOT.

These 10 patients were treated with pyridoxine. Disappearance of the deficiency of pyridoxal phosphate was observed and, notably, the level of EGOT activity increased by 55–68% during 2–4 weeks, respectively. Apparently, more apoenzyme had been biosynthesized, because the specific activities were significantly higher ($P < 0.001$ at 4 weeks) than before treatment. Because clinical evaluation showed an improvement after treatment with pyridoxine, it was concluded that the deficiency and the syndrome were related.

In a cooperative study (2), 11 additional patients were selected by clinical criteria as having the carpal tunnel defect, and they were also found to be deficient ($P < 0.001$) in vitamin B_6, by the same differential assay of EGOT.

The deficiencies of these patients without treatment over 6 weeks were essentially unchanged, and the deficiency had disappeared in a single patient. Seventeen clinical signs and symptoms, by scores, had disappeared or decreased ($0.001 < P < 0.01$) after 6 weeks of treatment; after 11 weeks, greater clinical improvement was evident ($P < 0.001$). Criteria included the use of the Preston pinch gauge for evaluation of hand performance, which was improved ($0.001 < P < 0.01$) after 11 weeks. Again, the deficiency appeared to be related to the neurological syndrome.

A preliminary biochemical report was made by Folkers *et al.* (3), at a symposium at Uppsala Universitet in Sweden in October 1977, on the 22nd patient with the carpal tunnel syndrome who was studied biochemically by monitoring the EGOT by 61 blood samples taken over 48 weeks; we now describe the details of this exceptional study of patient 22.

MATERIALS AND METHODS

The standard pharmaceutical quality of pyridoxine hydrochloride was used in a tablet which contained no other vitamin or essential mineral. The enzyme method of Kishi *et al.* (4, 5) was used. Specific activity was expressed as μmol of pyruvic acid formed per hr per 10^8 erythrocytes. The percentage deficiency of activity of EGOT was calculated by subtracting the basal specific activity from the specific activity determined in the presence of pyridoxal phosphate, dividing by the specific activity in the presence of pyridoxal phosphate, and multiplying by 100.

RESULTS AND DISCUSSION

The case history of patient 22, including his clinical signs and symptoms, during his initial dietary life style as a control period, during treatment with 2 mg of pyridoxine daily, during treatment with 100 mg of pyridoxine daily, during the administration of a placebo, and finally during treatment with 100 mg of pyridoxine daily will be summarized in a cooperative report elsewhere.

Brin *et al.* (6), in 1960, observed the effect of a deficiency of thiamin on the enzyme activity of a transketolase in erythrocyte hemolysates. Their report constituted, for the first time, a new principle for determining a vitamin deficiency which is based on the activity of an enzyme requiring the vitamin, or its coenzyme form, rather than on the determination of the vitamin as chemical substance. This new method has advantages,

Abbreviations: EGOT, erythrocyte glutamic-oxaloacetic transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1); RDA, recommended dietary allowance.

Table 1. EGOT data from patient 22

Date 1977	Specific activity Basal	Specific activity +PLP†	% Def.*
	Before treatment		
1/17	0.20	0.24	16
1/18	0.19	0.27	28
1/19	0.17	0.21	18
2/4	0.18	0.21	14
2/5	0.21	0.29	27
2/6	0.18	0.27	35
2/13	0.21	0.28	25
2/14	0.21	0.28	23
2/15	0.31	0.39	20
2/18	0.23	0.31	25
2/19	0.29	0.37	29
2/20	0.26	0.33	22
Mean	0.22	0.29	23
±SD	±0.04	±0.06	±6
	Treatment, PLP, 2 mg/day‡		
3/5	0.26	0.27	4
3/6	0.31	0.35	10
3/7	0.31	0.31	−1
3/18	0.30	0.30	3
3/19	0.25	0.27	6
3/20	0.34	0.37	8
3/31	0.33	0.41	20
4/1	0.30	0.36	16
4/2	0.35	0.43	20
4/20	0.31	0.39	21
4/22	0.30	0.38	21
4/23	0.31	0.37	14
5/3	0.32	0.37	14
5/6	0.35	0.45	22
5/7	0.36	0.47	24
Mean	0.31	0.37	14
±SD	±0.03	±0.06	±3
	P <0.001		P <0.01
	Treatment, PLP, 100 mg/day§		
6/2	0.37	0.37	1
6/3	0.38	0.40	6
6/4	0.36	0.39	9
6/16	0.50	0.52	6
6/17	0.53	0.52	−3
6/18	0.50	0.50	2
7/1	0.47	0.48	−9
7/2	0.41	0.38	−15
7/3	0.40	0.40	−1
7/14	0.57	0.55	−3
7/15	0.56	0.57	1
7/16	0.53	0.53	−1
7/29	0.55	0.58	5
7/30	0.65	0.60	10
7/31	0.53	0.51	−4
Mean	0.49	0.48	−1
±SD	±0.09	±0.08	±7
	P <0.001		P <0.001
	Placebo¶		
8/21	0.44	0.47	8
8/22	0.47	0.53	11
8/23	0.46	0.51	11
8/29	0.38	0.39	4
8/30	0.37	0.38	4
9/4	0.37	0.41	9
9/5	0.37	0.41	9
9/22	0.35	0.44	22
9/23	0.34	0.40	14
9/24	0.37	0.46	18
9/29	0.34	0.38	11
9/30	0.34	0.39	14
10/1	0.34	0.40	15
Mean	0.38	0.43	12
±SD	±0.05	±0.05	±5
	Treatment, PLP, 100 mg/day‖		
10/27	0.34	0.40	15
10/28	0.40	0.44	10
10/29	0.38	0.43	12
12/12	0.68	0.66	−2
12/13	0.53	0.56	5
12/14	0.66	0.63	−5
Mean	0.62	0.62	−1
±SD	±0.08	±0.05	±5
**	0.67	0.70	5

*Deficiency.
† Plus pyridoxal phosphate.
‡ Begun on 2/20/77.
§ Begun on 5/7/77.
¶ Begun on 8/1/77.
‖ Begun on 10/1/77.
** In assay, PLP = 0.062 mg (half usual level).

(*continued*)

particularly in allowing a given individual to serve as his or her own control.

The principle, which is the basis of this new method for detection and quantitation of a vitamin deficiency in an individual, or in a group, was defined by Folkers (7) as follows: "The specific activity of a coenzyme-apoenzyme system is differentially assayed in the absence and in the presence of added coenzyme. A significant increase in the specific activity of the enzyme system in the presence of added coenzyme measures a deficiency of the coenzyme at the site or of the vitamin in the tissue." This principle was tentatively designated as the Coenzyme-Apoenzyme-System (CAS principle).

Subsequent to the report by Brin *et al.* (6), studies on vitamin deficiencies, based on this principle, by other investigators were summarized by Kishi *et al.* (4) for riboflavin, coenzyme Q_{10}, and vitamin B_6.

The EGOT data on patient 22 are in Table 1 and may be summarized as follows. Twelve blood samples were taken over 5 weeks for control determinations and were considered as adequately representative of his B_6 status on his dietary life style. It was not feasible to have a nutritional analysis of his diet which was that of a "country-family." It was important that, under his life-style, he had developed such a severe neurological disease that the symptoms strongly interfered with his work and with his day and night habits. The control mean (±SD) basal specific activity was 0.22 ± 0.04 ($n = 12$). This value is significantly lower ($P < 0.001$) than that of typical individuals who commonly serve as normal controls, which was 0.30 ± 0.05 ($n = 21$) (8). The mean control deficiency was $23 \pm 6\%$ which is significantly higher ($P < 0.05$) than the $18 \pm 5\%$ for a group of 21 control individuals (8). It was considered nutritionally sig-

nificant that patient 22 had such low control basal EGOT and that it appeared to correlate directly with his severe neurological syndrome. On the basis of his mean specific activity (0.22 ± 0.04) and the mean value of 0.69 ± 0.09 (n = 36) for individuals on a daily supplement of pyridoxine, the activity of this patient's EGOT was about 30%; it also was about 30% of what it became when he was treated finally with 100 mg of pyridoxine per day for 11 weeks (see also ref. 9).

During our research on human deficiencies of vitamin B_6, we have found that it may be as important or perhaps even more important to interpret low basal specific activities of an enzyme requiring pyridoxal phosphate than it is to consider percentage deficiencies.

For years, the recommended dietary allowance (RDA) of pyridoxine for adults has been 2 mg/day, according to the Food and Nutrition Board of the National Research Council (10). This RDA of 2 mg/day was seemingly based upon data of 1960–1969 including xanthurenic acid excretion after 10-g tryptophan load tests. It was important to determine the biochemical and clinical status of patient 22 when he was treated with the RDA (2 mg) of pyridoxine for 10–12 weeks. This period resulted from our study (9) which showed that periods of up to 12 weeks of supplementation with pyridoxine were necessary to reach a stable level ("ceiling") of the specific activity of EGOT, which was 0.69 ± 0.09. When patient 22 was treated with 2 mg of pyridoxine per day for 11 weeks, his mean basal specific activity was 0.31 ± 0.03 and his deficiency was 14 ± 8%. Biochemically, these two values are not significantly different from 0.30 ± 0.05 and 18 ± 5% for the basal specific activity and deficiency, respectively, for the group of 21 typical individuals who commonly serve as normal controls, only 6 of whom had a supplementation of pyridoxine ranging from 1 to 5 mg per day (8). Clinically, patient 22 showed definite improvement after 11 weeks of treatment with the RDA, but there still remained positive clinical evidence of the syndrome. Biochemically, the mean specific activity of 0.31 ± 0.03 (n = 15) was essentially unchanged for 9 weeks, and this level was barely 50% of the ceiling level. It is evident that the RDA of 2 mg per day was too low for this patient for his biochemical status and was submarginal for his clinical status.

Patient 22 was then treated with 100 mg of pyridoxine daily for 12 weeks. As expected, the basal specific activity increased over the first 6 weeks and then became reasonably stabilized. The mean specific activity for the entire 12-week period was 0.49 ± 0.09, which was about 70% of the ceiling level but, notably, the deficiency had disappeared as shown by the value −1 ± 7%. The difference between the basal specific activity and the deficiency values for the treatments at 2 mg and 100 mg per day are all significant ($P < 0.01$ and $P < 0.001$). We believe that, had patient 22 been maintained on 100 mg/day for longer than 12 weeks, his basal specific activity would have leveled off closer to 0.69, because a value of 0.65 was observed once after 12 weeks; his specific activity did reach the ceiling in the final treatment period after the placebo.

Clinically, there was improvement on the 100 mg/day regimen over that on the RDA (2 mg), and two orthopedic surgeons independently concluded that "surgery of the carpal tunnel was unnecessary."

The patient was then placed on a matching placebo for 9 weeks. After 7 weeks, his biochemical status had deteriorated as evidenced by a mean specific activity of 0.35 ± 0.01 and a deficiency of 16 ± 4%. Clinically, his status had also deteriorated, and signs of the syndrome had reappeared. Even the patient had become apprehensive about his clinical deterioration, and it was necessary to treat him again with pyridoxine; a dosage of 100 mg/day was prescribed.

After 11 weeks, the mean basal specific activity was 0.62 ± 0.08 and the deficiency was −1 ± 5%. The ceiling activity of EGOT had been established, the deficiency had been corrected, and clinically the patient was asymptomatic.

This 40-year-old man had probably been deficient in vitamin B_6 for a decade or longer. Such long-standing deficiency could slowly lead to overt signs of the carpal tunnel syndrome. His diet was common, country-style, and there was no vitamin supplement; he developed such a debilitating syndrome that his working and private functions were severely harmed. Treatment with the RDA (2 mg) only partially corrected deficiencies of EGOT and pyridoxal phosphate. Although the RDA improved his clinical status, signs of the disease unambiguously remained. The RDA was too low for this individual. Seeking his adequate RDA was not our objective; rather, it was our purpose to normalize his EGOT, eliminate the deficiency of pyridoxal phosphate, and cause him to be asymptomatic. A level (100 mg), which is undoubtedly higher than that of an adequate but unknown RDA, was administered; his biochemical status was normalized, and overt symptoms disappeared. Treatment with a matching placebo allowed reappearance of a deficiency of both the apoenzyme and coenzyme, and clinical symptoms reappeared. Even the patient became apprehensive on the placebo (unknown to him) and it was necessary to retreat with pyridoxine. A dosage of 100 mg/day again corrected the biochemical and clinical status.

This study shows again (9) that the administration of pyridoxine (to increase pyridoxal phosphate) in the presence of a diminished level of an apoenzyme, which is deficient in pyridoxal phosphate, somehow regulates a mechanism that brings about an increased level of the apoenzyme having its full complement of the coenzyme to constitute the transaminase. It appears that a diminished level of the apoenzyme results from a deficiency of the coenzyme, or that a biochemical state of no deficiency of the coenzyme is associated with the ceiling level of the transaminase; this latter state is presumably the one desired for health.

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