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Vitamin Status in Patients on Chronic Anticonvulsant Therapy*

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Summary: Vitamin levels in blood were estimated in 146 epileptics, aged 20-40 years. Compared to healthy subjects, no higher risk rates of a vitamin deficiency were found in epileptics for vitamins B₁, B₁₂, A, C, E and β -carotene. α_{EGB} was elevated only in epileptic females, α_{EGOT} in epileptic males, indicating a higher risk of a vitamin B₂ and B₆ deficiency in these groups. Markedly reduced levels of folate, 25-hydroxy-cholecalciferol, and biotin were found in the epileptics; folate and biotin levels showed a significant negative correlation with the total amount and the average daily dose of anticonvulsants administered.

Introduction

The influence of a long-term treatment with anticonvulsants on the metabolism of some vitamins (folate, vitamin D) is known; for others, i. e. vitamin B₆ and vitamin E, such an effect is assumed, whereas for all other vitamins none was found or has not yet been investigated. The aim of this study was then to evaluate the vitamin status in epileptics on chronic anticonvulsant therapy and to compare their vitamin status with the results in healthy subjects.

* Parts of this paper have been presented as a poster on the Epilepsy International Congress, Kyoto, September 17-21, 1981.

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Patients and Methods

145 patients (96 males, 49 females) aged 20–40 years attending the Heidelberg out-patient clinic for epileptics were investigated. Most patients were treated with a combination of the following anticonvulsants for at least one year: phenytoin ($n=60$), primidone ($n=55$), carbamazepine ($n=42$), valproate sodium ($n=33$), phenobarbital ($n=14$), ethosuximide ($n=13$), CHP-phenobarbital (barbexaclone) ($n=12$), clonazepam, mesuximide, mephenytoin, sulthiam and trimethadione. The total amount and the mean daily dose administered was calculated using equivalent units⁵.

The results of the vitamin estimation in patients with epilepsy were compared to the results of the "Heidelberg Study" [1] in which the vitamin status of 20–40 years old healthy subjects in the region of Heidelberg had been evaluated. Only where no corresponding vitamin levels were determined in the "Heidelberg Study" the Swiss "Jura Study" [26] was used for comparison.

The supply situation of vitamin B₁ was evaluated measuring the erythrocyte transketolase activity (ETK) with (ETK₊) and without (ETK₀) added thiamin pyrophosphate under standardised condition. The technique is a modification of the methods of SCHOUTEN *et al.* [40] and DREYFUSS [11]. α_{ETK} (ETK₊/ETK₀) is a measure of the vitamin B₁ status. Values of $\alpha_{\text{ETK}} > 1.25$ correspond to the high risk category of a vitamin deficiency, values of $\alpha_{\text{ETK}} < 1.15$ to the low risk category; the intermediate range is marginal [5]. To assess the vitamin B₂ status the erythrocyte glutathione reductase (EGR) activity was determined according to GLATZLE *et al.* [14] without (EGR₀) added flavine adenine dinucleotide (FAD) and again after incubation with FAD (EGR₊). Values of $\alpha_{\text{EGR}} > 1.29$ belong to the high risk category, $\alpha_{\text{EGR}} < 1.20$ to the low risk category [5]. The status of vitamin B₆ was measured using the erythrocyte glutamate oxalacetate transaminase activation test (EGOT) as proposed by WEBER and WEGMAN [43] in which the apotransaminase is activated by pyridoxal-5'-phosphate. For higher workloads an automated version of the test according to TRINDER and KIRKLAND [42] was used. Subjects showing values of $\alpha_{\text{EGOT}} < 1.80$ with this test belong to the low risk category; subjects with $\alpha_{\text{EGOT}} > 2.0$ to the high risk category [5]. Biotin concentration in plasma was determined microbiologically using the technique of FRIGG and BRUBACHER [13]. Plasma biotin levels > 1.02 nmol/l are usually determined with this method [3]. Vitamin C was assayed fluorometrically [10] following the procedure described by BRUBACHER and VUILLEUMIER [7]. Plasma levels of vitamin C < 23 $\mu\text{mol/l}$ are considered to reflect inadequate supply [5]. The retinol-binding protein (RBP) was measured by radial immunodiffusion [23]. RBP-values of 2.4–4.8 $\mu\text{mol/l}$ seem to be normal. The molar ratio of retinol over RBP, which normally lays between 0.7 and 0.9, helps according to SMITH and GOODMAN [41] to identify cases of hypo- and hyper- vitaminosis A. In the "Heidelberg Study" and "Jura Study" vitamin A was analysed in plasma by fluorometry after chromatographic separation [6]. The procedure has been combined with the assay of β -carotene. Vitamin E was assayed by the colorimetric method proposed by HASHIM and SCHUTTRINGER [17]. In the study with epileptics the plasma concentrations of vitamin A, E, and β -carotene were determined by HPLC on an absorption mode silica column. Since a comparative study has shown no significant difference between the results of the two methods [J. P. VUILLEUMIER, personal communication] the values for the vitamins A, E, and β -carotene can be compared between the "Heidelberg Study" and the "Epileptic Study". The vitamin A level is somewhat higher in males than in females. Assay values < 1.05 $\mu\text{mol/l}$ (100 IU/100 ml) may be considered to reflect a marginal supply situation [5]. No threshold values for vitamin E can be given. In industrialized countries plasma levels around 23.2 $\mu\text{mol/l}$ and higher are found; values below 9.3 $\mu\text{mol/l}$ are likely to reflect low supply [5]. The values for β -carotene depend

⁵ 1 equivalent unit = 50 mg phenytoin, 30 mg phenobarbital, 125 mg primidone, 50 mg CHP-phenobarbital, 200 mg carbamazepine, 50 mg mephenytoin, 250 mg ethosuximide, 300 mg valproate sodium, 2 mg clonazepam, 300 mg mesuximide, 100 mg sulthiam, or 250 mg trimethadione.

on diet and can only be used for the overall assessment of the nutritional status; no threshold values are known.

Vitamin B₁₂ and folate were assayed by a simultaneous radioassay according to GUTCHO and MANSBACH [15] with minor modifications. Release of bound vitamins from their endogenous binders and the destruction of these binders are effected by a heating step at pH 9.3. The subsequent binding reactions with hog intrinsic factor and milk binder protein proceed simultaneously in the same tube at pH 9.3. Complete separation of bound radioactivities, ⁵⁷Co-cyanocobalamin and ¹²⁵I-labeled pteroylmonoglutamic acid-histamide is obtained in a dual channel gamma counter. Vitamin B₁₂ values ≤ 150 pmol/l and folate values ≤ 3 nmol/l seem to reflect a low supply [1]. 25-Hydroxycholecalciferol was determined by a radioassay in a modification of the method described by BELSEY *et al.* [2]. 25-Hydroxycholecalciferol standards were prepared in vitamin D-deficient, charcoal treated serum instead of ethanol as described by the authors. The normal range is 50 to 300 nmol/l with marked seasonal variation.

Vitamin B₁₂, folate, and 25-hydroxy-cholecalciferol determinations have been carried out at the Department of Surgery, University of Heidelberg, the others by F. Hoffmann-La Roche & Co., Basle.

Results

The mean values of the vitamin determinations and their distributions are given in table I and figures 1–6, respectively, both for male and female epileptic patients and their controls of the same age. The distributions are compiled only

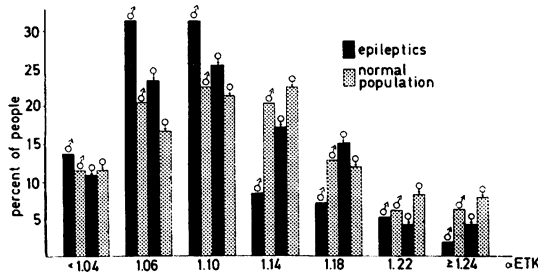


Fig. 1: Distribution of α_{ETK} -values in epileptics and normal population.

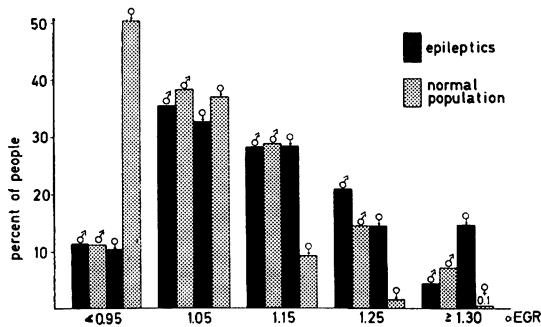


Fig. 2: Distribution of α_{EGR} -values in epileptics and normal population.

Tab. I: Vitamin status in epileptics and controls

Vitamins	Epileptics		Controls	
	male	female	male	female
B ₁ (α_{ETK})	1.10 \pm 0.06 (n = 95)	1.11 \pm 0.06 (n = 47)	1.12 \pm 0.07 (n = 636)	1.13 \pm 0.09 (n = 744)
B ₂ (α_{EGR})	1.12 \pm 0.11 (n = 96)	1.16 \pm 0.23 (n = 49)	1.11 \pm 0.11 (n = 633)	1.00 \pm 0.07 (n = 749)
B ₆ (α_{EGOT})	1.86 \pm 0.23 (n = 96)	1.80 \pm 0.26 (n = 49)	1.76 \pm 0.20 (n = 629)	1.81 \pm 0.26 (n = 748)
B ₁₂ (pmol/l)	335 \pm 112 (n = 96)	347 \pm 123 (n = 49)	570 \pm 332 (n = 727)	400 \pm 264 (n = 712)
Biotin nmol/l	0.92 \pm 0.34 (n = 75)	0.88 \pm 0.22 (n = 42)	1.81 \pm 0.73 ² (n = 57)	1.88 \pm 0.95 ² (n = 36)
Folate (nmol/l)	4.4 \pm 3.1 (n = 94)	5.0 \pm 3.2 (n = 49)	16.0 \pm 16.0 (n = 624)	7.0 \pm 8.0 (n = 714)
C ($\mu\text{mol/l}$)	35.8 \pm 16.5 (n = 96)	43.8 \pm 22.7 (n = 49)	33.5 \pm 23.9 ¹ (n = 488)	28.4 \pm 21.0 (n = 355)
β -Carotene ($\mu\text{mol/l}$)	0.61 \pm 0.39 (n = 95)	0.79 \pm 0.76 (n = 49)	0.59 \pm 0.29 ³ (n = 75)	0.65 \pm 0.35 (n = 582)
A ($\mu\text{mol/l}$)	2.27 \pm 0.51 (n = 96)	1.84 \pm 0.52 (n = 49)	2.78 \pm 0.64 ¹ (n = 487)	1.74 \pm 0.45 (n = 582)
Retinol binding protein $\mu\text{mol/l}$	3.04 \pm 0.76 (n = 96)	2.57 \pm 0.67 (n = 49)	3.14 \pm 0.57 ³ (n = 75)	2.95 \pm 0.57 ³ (n = 25)
A/RBP (molar ratio)	0.75 \pm 0.12 (n = 96)	0.72 \pm 0.05 (n = 49)	0.74 \pm 0.05 ³ (n = 75)	0.73 \pm 0.04 ³ (n = 25)
D (nmol/l)	103 \pm 106 (n = 95)	90 \pm 74 (n = 49)	140 \pm 110 (n = 728)	163 \pm 71 (n = 690)
E ($\mu\text{mol/}$)	24.8 \pm 6.9 (n = 95)	24.4 \pm 4.4 (n = 49)	29.2 \pm 9.0 ¹ (n = 486)	22.3 \pm 4.4 (n = 632)

¹ Jura-study [26].² Blood donors and patients with dermatological disease [3].³ Blood donors (H. E. KELLER and J. P. VUILLEUMIER, personal communication).

for those vitamins for which the values for both sexes were available from the "Heidelberg Study". The percentage of patients and controls falling into the high risk category of a vitamin deficiency can be found in table II.

When the correlation between vitamin levels and either the total amount or the daily dose of anticonvulsants administered was investigated by linear regression a negative correlation ($p < 0.05$) was found for folate (figure 7) and biotin [20].

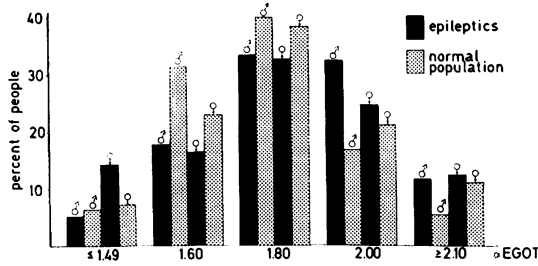


Fig. 3: Distribution of α_{EGOT} -values in epileptics and normal population.

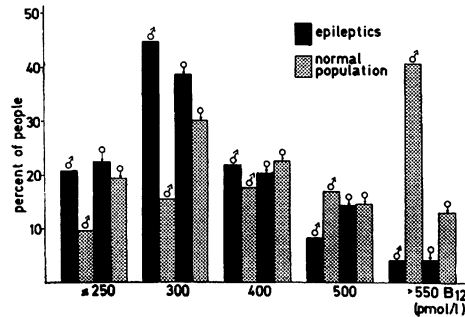


Fig. 4: Distribution of plasma levels of vitamin B₁₂ in epileptics and normal population.

Tab. II: Percentage of epileptics and controls with a high risk of a vitamin deficiency

Vitamins	Epileptics		Controls	
	male	female	male	female
B ₁ $\alpha_{ETK} (\ge 1.25)$	2	4	6	7
B ₂ $\alpha_{ETK} (\ge 1.29)$	4	15	7	0.1
B ₆ $\alpha_{EGOT} (\ge 2.0)$	24	18	11	18
B ₁₂ ($\ge 150 \mu\text{mol/l}$)	1	2	3	2
Biotin ($\ge 3 \text{ nmol/l}$)	83	81	2 ²	0 ²
Folate ($\le 3 \text{ nmol/l}$)	46	37	5	27
C ($\le 11.4 \mu\text{mol/l}$)	6	8	13 ¹	10
A ($< 1.05 \mu\text{mol/l}$)	0	2	0	2
D ($< 50 \text{ nmol/l}$)	43	35	15	1
E ($< 9.3 \mu\text{mol/l}$)	0	0	0 ¹	0

¹ Jura-study [26].

² Blood donors and patients with dermatological diseases [3].

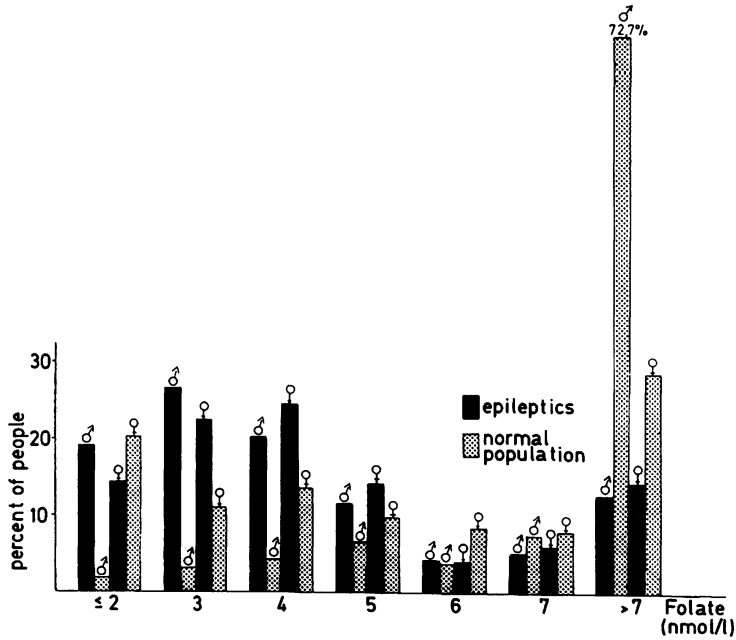


Fig. 5: Distribution of plasma levels of folate in epileptics and normal population.

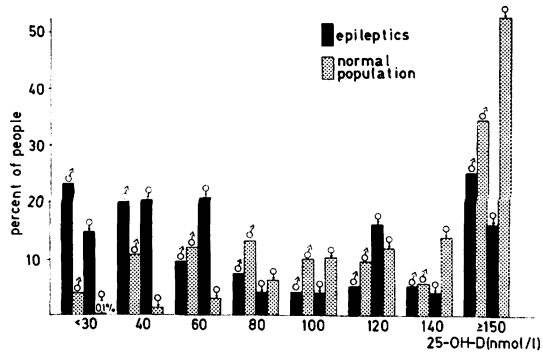


Fig. 6: Distribution of plasma levels of 25-OH-cholecalciferol in epileptics and normal population.

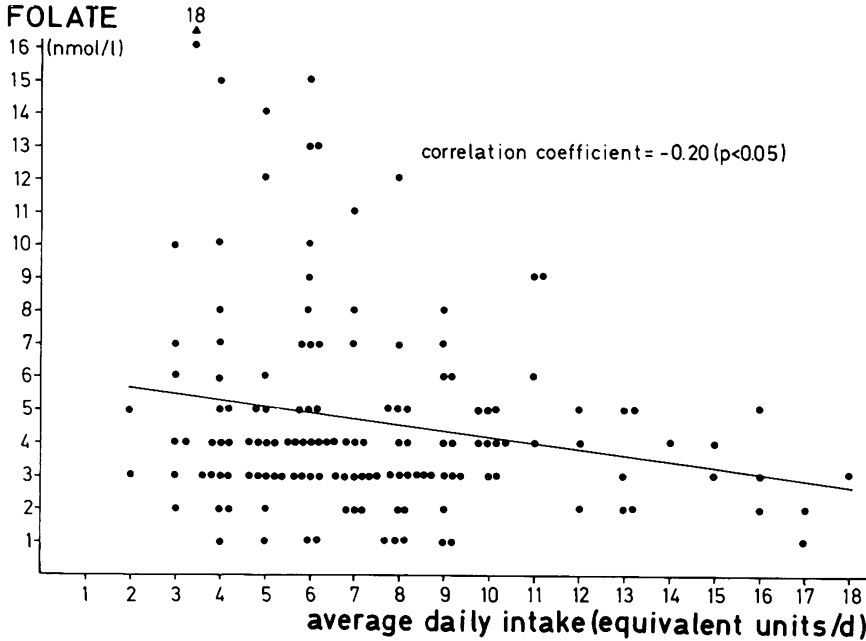


Fig. 7: Correlation between average daily intake of anticonvulsants (equivalent units/d) and plasma folate level.

Discussion

Slightly higher mean values were found for vitamin A, E, and C and for β -carotene in the female epileptics than in the females of the "Heidelberg Study" [1]. In male patients slightly lower mean values for vitamins A and E and higher ones for vitamin C were noted than in male controls. The mean control values used for comparison were taken from the "Jura Study" [26], as in the "Heidelberg Study" these vitamins had not been determined for males. No increased risk of vitamin A, E and C deficiency was found in male and female epileptic patients. RBP and the ratio vitamin A/RBP were within normal limits for most epileptics. Therefore, a chronic anticonvulsant therapy does not seem to influence the status of vitamins A, E, and C; these findings do not confirm the observations made by OKUNMEGA [28] and HIGASHI *et al.* [18] who reported reduced vitamin E levels in epileptic children treated with anticonvulsants.

The activation coefficient (α_{ETK}) for vitamin B₁ tended to be lower in male and female epileptic patients than in controls indicating a good vitamin B₁ status in epileptics; also the risk of a vitamin B₁ deficiency was higher in controls. A higher mean α_{EGR} and a substantially higher risk of a vitamin B₂ deficiency was noted in the female patients with epilepsy, whereas in males no such

differences were seen. The reason for this sex-specific distinction has not yet been elucidated; but should this be confirmed in a larger number of patients, investigations into a possible correlation with the hormonal condition of the female epileptics seem to be worthwhile.

Impairment of the vitamin B₆ status in patients treated with anticonvulsants has been reported [9, 30, 33]. Our investigations, using the EGOT activation test, noted a deterioration in the vitamin B₆ status for male patients only; both the mean values and the percentage with abnormal levels were higher than in controls. For females no such difference was found. This might be because female epileptics less often use oral contraceptives than do their healthy controls. These agents are known to lower the vitamin B₆ status [29]. Furthermore, some controversy exists about the usefulness of the EGOT activation test as an indicator of the vitamin B₆ status. Not only are various methods used to measure activities [36] but in some cases the results may be misleading. For instance, in alcoholic subjects known to have an inadequate intake of vitamin B₆ often a low α_{EGOT} , indicating no risk of a vitamin B₆ deficiency, is found [4]; a similar phenomenon might occur in anticonvulsant therapy. Therefore, a determination of the level of pyridoxal-5'-phosphate in erythrocytes as an additional parameter related to the vitamin B₆ status seems to be useful in further studies of the vitamin B₆ status in epileptics.

Mean circulating vitamin B₁₂ levels were reduced in patients suffering from epilepsy more so in males than in females, but the percentage of patients with a deficient level was lower than in the control population. Also other studies usually found no reduction of vitamin B₁₂ levels in epileptics [9, 33, 38] and, therefore, a chronic treatment with anticonvulsants should not lead to a more frequent occurrence of vitamin B₁₂ deficiency symptoms in these patients than in the general population.

Major differences in the vitamin status of epileptics and controls were only found for folate, vitamin D and biotin. A reduction of folate levels on anticonvulsant therapy has been known for some time [24]. This reduction in folate levels is being discussed as a factor in the mode of action and as a cause for side-effects of anticonvulsants such as psychosis and haematological abnormalities [31, 32]. About 40% of the epileptics investigated by us were found to have deficient folate levels, a figure which fits well into the published frequencies of 29% to 91% [33]. Some investigations have found a correlation between folate levels and the doses of anticonvulsants administered [32, 34] whereas others have not [19]. Our results seem to indicate a certain dose-dependency (fig. 7), but the number of patients investigated, especially of those on monotherapy, are as yet too small to draw any definite conclusions.

An impaired vitamin D status is considered to be the cause of the anticonvulsant osteomalacia which has been described for the first time by KRAUSE [22]

and SCHMID [37] in 1967/68. Deficient 25-hydroxy-cholecalciferol levels have been reported to occur in 23–39% of the epileptics investigated [16, 21, 25, 35]. A frequency of 35% for the female and of 43% for the male patients (table II) seems to be quite high. However, most of our patients have been investigated during winter and spring, when 25-hydroxy-cholecalciferol levels are known to be low [39]. This seasonal variation might also be the reason why no correlation between plasma 25-hydroxy-cholecalciferol levels and the total amount or the mean daily dose of anticonvulsants administered has been found. Also the connection between 25-hydroxy-cholecalciferol levels and the skeletal mineralisation is not clear: in manifest osteopathy low 25-hydroxy-cholecalciferol levels are generally noted [12, 21, 25], but in a number of patients treated with anticonvulsants low levels are seen without concurrent radiological signs of osteopathy. A retrospective study has shown that slight skeletal changes as demonstrated by radiology occur frequently in the first few years of anticonvulsant therapy. These changes tend to revert to normal in the majority of patients in the next few years; only in a few patients, mostly those treated with high doses for a long period, does a severe osteopathy develop [25]. Similarly, 25-hydroxy-cholecalciferol levels might also decline during the first years of treatment with a subsequent normalization in most patients and a further decrease in only a few. This possibility will be investigated in a larger number of patients. Furthermore, bone density measurements which are more sensitive than the conventional radiology [8], should be used to assess the diagnostic value of 25-hydroxy-cholecalciferol levels.

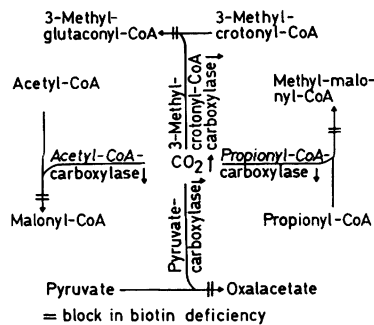


Fig. 8: Increased carbon dioxide as hypothetical contribution of reduced biotin levels to the mode of action of anticonvulsants.

The reduction of biotin levels in 80% of the epileptics has been discussed elsewhere [20]. This reduction might be a factor in the efficacy of anticonvulsants: a decrease in the activity of the biotin-dependent carboxylase could lead to an increase in carbon dioxide (figure 8), and via the decrease of the

pyruvate carboxylase activity to a reduction of aspartate in brain (figure 9). Both these possibilities have to be considered as antiepileptic mechanisms of action. The pharmacological effect of a biotin deficiency in epileptics deserves further attention.

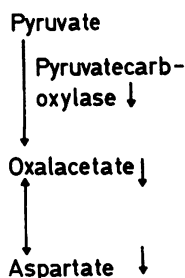


Fig. 9: Decreased aspartate as hypothetical contribution of reduced biotin levels to the mode of action of anticonvulsants.

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