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B Vitamins in Epileptics

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Vitamin B_6 is the only vitamin of the B group for which a relationship to epileptic seizures has been found until now. In newborns, pyridoxine-dependent seizures are described which can be treated successfully only by the intake of pyridoxine and not of the common anticonvulsants. A disturbance in binding between glutamic acid decarboxylase-apoenzyme and pyridoxal phosphate, with the consequence of a reduction of the inhibitory neurotransmitter γ-aminobutyric acid, is discussed as the underlying mechanism [13]. On the other hand, vitamin B_6 is the only vitamin of the B group which has been found to be reduced by the intake of anticonvulsants [3, 5, 11]. In a representative sample of treated epileptics we investigated the vitamin status and compared the values with those of a control collective of the same age group. A total of 620 patients of the Heidelberg outpatient clinic for epileptics, aged 20–40 years, were examined. All patients were treated for at least 1 year with anticonvulsants. Over 50% of them took more than one type of anticonvulsant drugs. Because of this predominance of combination therapies the total amount and the mean daily dose of anticonvulsants administered were calculated for each patient using equivalent units, based on the medium therapeutic dose of each anticonvulsant.[1] The Heidelberg study subjects served as control collective, in which

[1] 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.

Table I. Means, standard deviations, medians and 5th and 95th percentiles of B vitamins in epileptics and controls (M = males, F = females)

	n		Mean ± SD		Median		5th–95th percentile	
	epileptics	controls	epileptics	controls	epileptics	controls	epileptics	controls
α_{ETK}								
M	342	636	1.11 ± 0.07	1.12 ± 0.07	1.09	1.11	1.01–1.24	1.01–1.25
F	245	744	1.12 ± 0.08	1.13 ± 0.09	1.11	1.12	1.01–1.28	1.02–1.28
α_{EGR}								
M	350	640	1.15 ± 0.15	1.11 ± 0.11	1.12	1.10	0.98–1.40	0.96–1.31
F	249	749	1.18 ± 0.17	1.01 ± 0.07	1.15	0.99	0.97–1.42	0.92–1.14
α_{EGOT}								
M	350	636	1.82 ± 0.24	1.76 ± 0.20	1.81	1.75	1.43–2.21	1.45–2.10
F	249	748	1.81 ± 0.24	1.81 ± 0.25	1.81	1.80	1.41–2.21	1.44–2.25
PLP, µg/l								
M	347	640	4.05 ± 6.76	5.67 ± 6.06	3.20	5.00	1.80–6.86	3.10–9.29
F	246	746	4.52 ± 10.35	3.35 ± 2.96	3.00	2.90	1.73–6.10	1.50–6.16
B_{12}, pmol/l								
M	344	727	368.7 ± 163.3	571.7 ± 332.4	350	490	150–707.5	210–1,180
F	240	712	377.6 ± 209.4	400.3 ± 263.8	350	353	100.5–740	179.6–704.4
Biotin, ng/l								
M	334	70	236.6 ± 84.72	344.5 ± 106.7	225	327	107.5–374	230–505.6
F	243	73	223 ± 76.12	335.6 ± 74.56	213	335	100–336.4	207.1–459

more than 1,500 healthy persons, aged 20–40 years, were examined [1]. In this presentation we report our findings regarding the status of vitamins B_1, B_2, B_6, B_{12} and biotin in epileptics.

Vitamin B_6

The vitamin B_6 status was assessed in two ways: first by using the erythrocyte-glutamate oxaloacetate transaminase (EGOT) test, and second by assaying the level of pyridoxal-5-phosphate (PLP) in erythrocytes determined by the rate of decarboxylation of carbonyl-^{14}C-labelled tyrosine [14]. The activation coefficient α_{EGOT} was clearly higher and, even more pronounced, the PLP levels were lower in epileptic males than in controls (table I). Both these findings indicate an inferior vitamin B_6 status in male

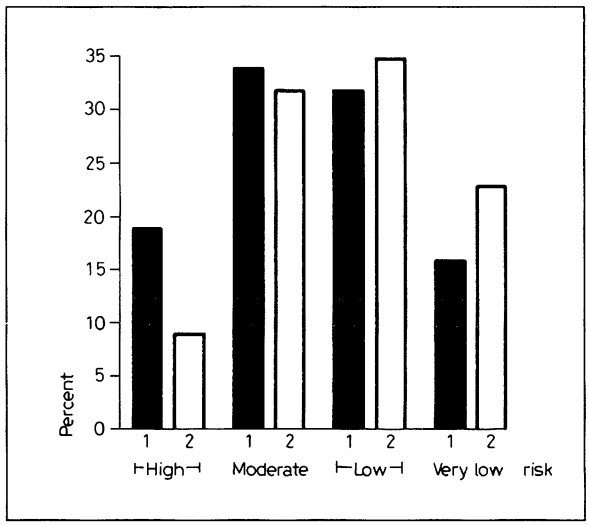

Fig. 1. Rates of high ($\alpha_{EGOT} > 2.00$), moderate (α_{EGOT} = 1.80–2.00), low (α_{EGOT} = 1.60–1.79) and very low ($\alpha_{EGOT} < 1.60$) risk of vitamin B_6 deficiency in male epileptics (1) and controls (2).

epileptics compared to controls. In females no difference in the mean α_{EGOT} values were noted between epileptics and controls (table I). Mean PLP values for the female epileptics are seemingly higher than in controls (table I), but there is a very high standard deviation in female epileptics caused by the extremely high PLP levels of about 100 µg/l in 7 females, probably due to the oral intake of vitamin B_6. When these 7 epileptics were not considered, the mean PLP level was 3.22 µg/l, not different to the females of the Heidelberg study. Of more interest than a comparison of means is the comparison of a risk of vitamin B_6 deficiency in both collectives. Regarding α_{EGOT}, a greater percentage of subjects at a high and moderate risk of vitamin B_6 deficiency was found in male epileptics than in controls (fig. 1). The risk of vitamin B_6 deficiency in male epileptics is much more pronounced when the PLP levels are considered (fig. 2). In females, no difference in the risk rates was noted for epileptics and controls. About 50% of the women in both groups had a high or moderate risk of vitamin B_6 deficiency (fig. 3, 4).

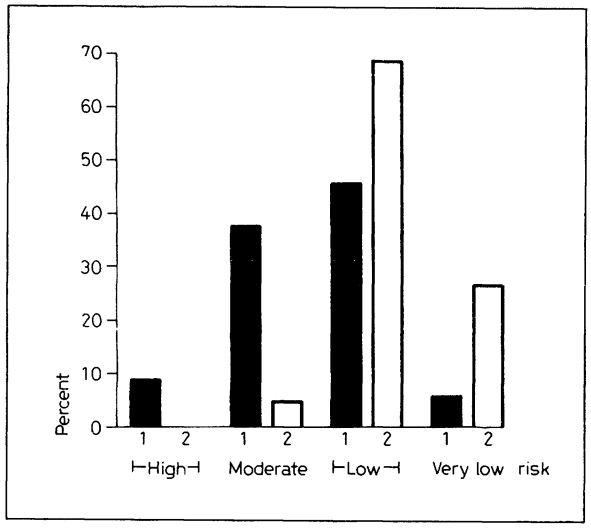

Fig. 2. Risk rates of deficiency of pyridoxal-5-phosphate (PLP) in erythrocytes (high: < 2.0 µg/l, moderate 2.0–3.0, low 3.1–6.0, very low > 6.0 µg/l) in male epileptics (1) and controls (2).

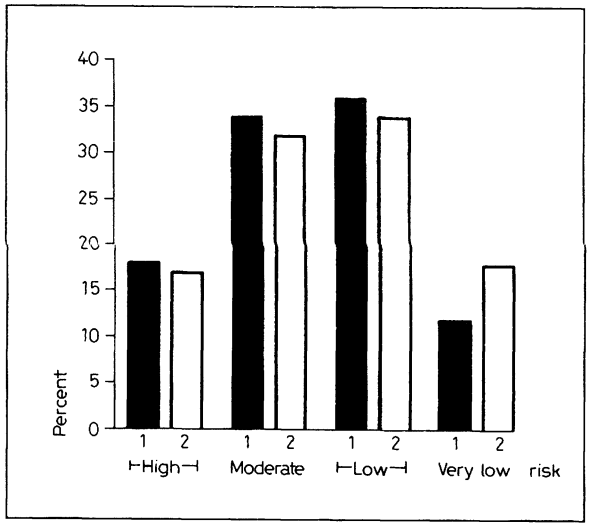

Fig. 3. Risk rates (α_{EGOT}) of vitamin B_6 deficiency in female epileptics (1) and controls (2) (see fig. 1).

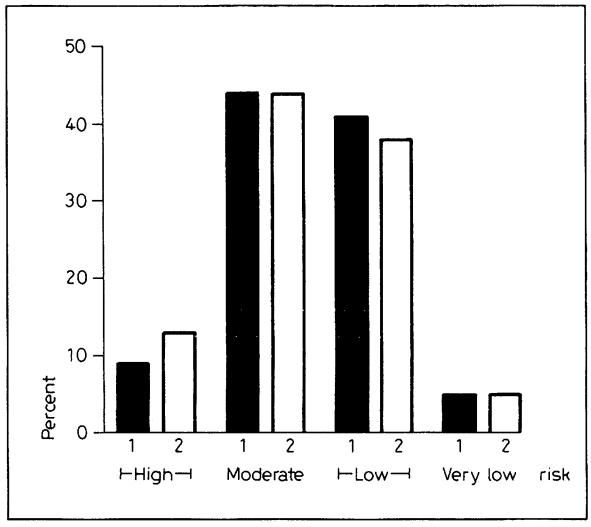

Fig. 4. Risk rates of PLP deficiency in female epileptics (1) and controls (2) (see fig. 2).

The reduced vitamin B_6 status in many females of the control group could be caused by the intake of hormonal contraceptives [7] which were taken by over 50% of these women, compared to less than 3% in the female epileptics. Therefore, it seems to be possible that in both female collectives the high rate of a risk of vitamin B_6 deficiency could be due to drug intake. If we assume that the reduced vitamin B_6 status in epileptics is caused by the intake of anticonvulsants, then correlations between vitamin levels and the anticonvulsants administered are of great interest. The female patients with an oral intake of pyridoxine at the time of examination were excluded from these calculations. In epileptic males – but not in females – the total amount as well as the mean daily dose and the duration of anticonvulsant therapy showed a negative correlation with the levels of PLP and unstimulated EGOT ($EGOT_0$), whereas no correlation was found for α_{EGOT} (table II). We also looked for correlations between the three parameters of the vitamin B_6 status and found that PLP correlated better with unstimulated EGOT than with the activation coefficient (table III). The question arises why PLP levels and $EGOT_0$ values correlated with the anticonvulsant parameters and why α_{EGOT} did not. A possible explanation would be that –

Table II. Spearman's rank correlation coefficients for B vitamins and data of anticonvulsant therapy

	ETK_0	α_{ETK}	α_{EGR}	$EGOT_0$	α_{EGOT}	PLP	Biotin	B_{12}
Mean daily dose of anticonvulsants (equivalent units per day)								
Males								
r	−0.1368	0.1163	−0.0089	−0.0832	0.0175	−0.1337	−0.1408	0.0197
p	0.0123	0.0336	0.8702	0.1276	0.7493	0.0145	0.0111	0.7217
n	334	334	341	337	337	334	325	331
Females								
r	−0.1236	0.1438	0.1762	−0.0079	−0.0591	−0.0167	−0.0753	−0.0728
p	0.0559	0.0259	0.0057	0.9026	0.3598	0.7968	0.2462	0.2685
n	240	240	245	242	242	239	239	233
Total amount of anticonvulsants (equivalent units)								
Males								
r	−0.0461	0.0822	−0.0118	−0.0959	0.00453	−0.1784	−0.1377	0.0475
p	0.4014	0.1340	0.8287	0.0789	0.9339	0.0011	0.0130	0.3888
n	334	334	341	337	337	334	325	331
Females								
r	−0.0876	0.0349	0.0865	−0.0200	−0.0652	−0.0292	−0.0674	−0.0133
p	0.1763	0.5906	0.1771	0.7566	0.3124	0.6539	0.2996	0.8405
n	240	240	245	242	242	239	239	233
Duration of therapy (years)								
Males								
r	0.0257	−0.0024	−0.0110	−0.1053	0.0082	−0.1929	−0.1139	0.0386
p	0.6403	0.9655	0.8402	0.0535	0.8807	0.0004	0.0401	0.4843
n	334	334	341	337	337	334	325	331
Females								
r	−0.0552	−0.0252	0.0278	−0.0235	−0.0571	−0.0278	−0.0286	0.0220
p	0.3943	0.6980	0.6649	0.7166	0.3769	0.6693	0.6602	0.7388
n	240	240	245	242	242	239	239	233

Table III. Correlation coefficients r between α_{EGOT}, unstimulated EGOT activity ($EGOT_0$) and pyridoxal-5-phosphate (PLP) in the epileptics

	α_{EGOT}	PLP
PLP	−0.5040	
$EGOT_0$	−0.6419	0.7001

as postulated for alcoholics [2] – a long-standing pyridoxine deficiency leads to a decrease of apoenzyme levels. In such cases a low α_{EGOT} value would be found on stimulation with PLP, and these seemingly normal values would erroneously indicate an adequate vitamin B_6 status. Assuming that such reduced apoenzyme levels occur in our epileptics, the occurrence of seeminlgy normal α_{EGOT} values would explain why only the PLP concentrations and $EGOT_0$ values correlated with drug parameters. We cannot explain why in the females, who also show high risk rates, no correlations have been found between vitamin B_6 parameters and anticonvulsant therapy.

Vitamin B_1

The thiamin status was assessed using the activation coefficient α_{ETK}, calculated from the coenzyme stimulation of the transketolase activity in erythrocytes [14]. α_{ETK} values in epileptics of both sexes were similar to those in controls (table I). Nevertheless, anticonvulsants seem to have an influence on the vitamin B_1 status: in male and female epileptics a positive correlation between α_{ETK} and a negative one between unstimulated ETK activity and the mean daily dose of anticonvulsants was found (table II). These findings would then indicate that long-term medication with anticonvulsants could increase the risk of vitamin B_1 deficiency. About 25% of both epileptics and controls had a high or moderate risk of thiamin deficiency (fig. 5, 6).

Vitamin B_2

When examining the vitamin B_2 status using the erythrocyte glutathione reductase activation test [14], unexpected results were obtained: in male epileptics a slight, in female epileptics a distinct deterioration of the riboflavin status was found, compared with controls (table I). This is confirmed when risk groups are considered (fig. 7, 8): about 40% of the female epileptics showed a high or moderate risk of vitamin B_2 deficiency, whereas practically none of the females of the control group did. Epileptic males showed no correlation between the activation coefficient α_{EGR} and anticonvulsants, but in females a positive correlation between the daily dose of anticonvulsants and α_{EGR} was found (table II).

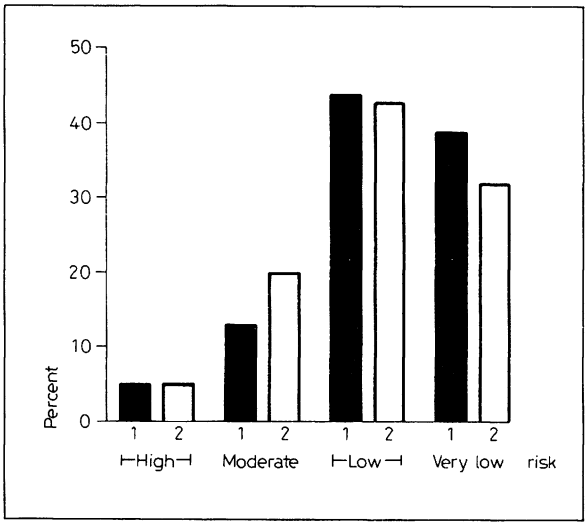

Fig. 5. Rates of high ($\alpha_{ETK} > 1.25$), moderate ($\alpha_{ETK} = 1.16–1.25$), low ($\alpha_{ETK} = 1.08–1.15$) and very low ($\alpha_{ETK} < 1.08$) risk of thiamine deficiency in male epileptics (1) and controls (2).

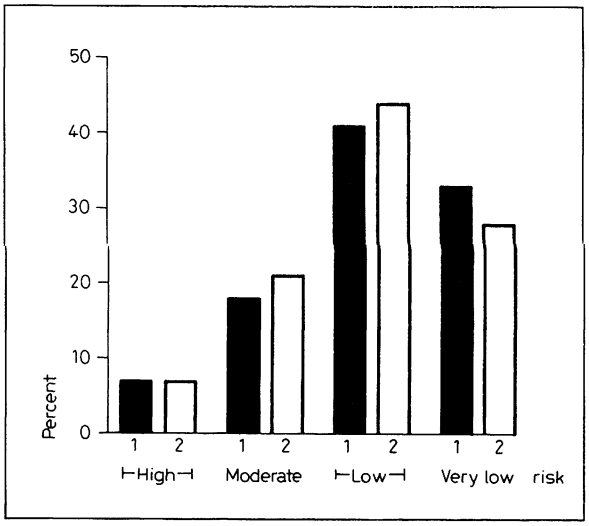

Fig. 6. Risk rates of thiamin deficiency in female epileptics (1) and controls (2) (see fig. 5).

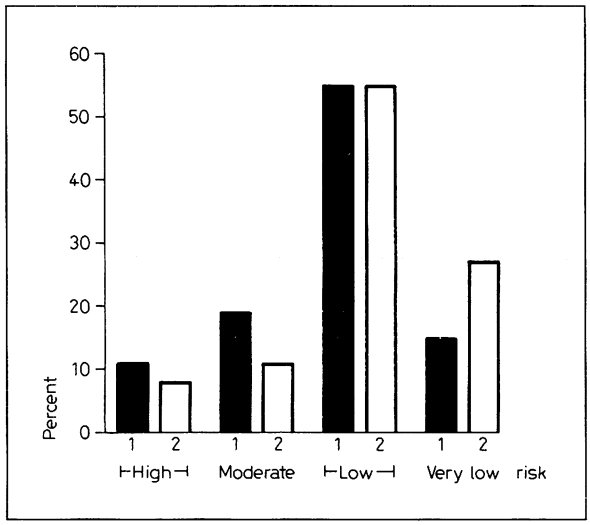

Fig. 7. Rates of high ($\alpha_{EGR} > 1.28$), moderate ($\alpha_{EGR} = 1.20–1.28$), low ($\alpha_{EGR} = 1.03–1.19$) and very low ($\alpha_{EGR} < 1.03$) risk of riboflavin deficiency in male epileptics (1) and controls (2).

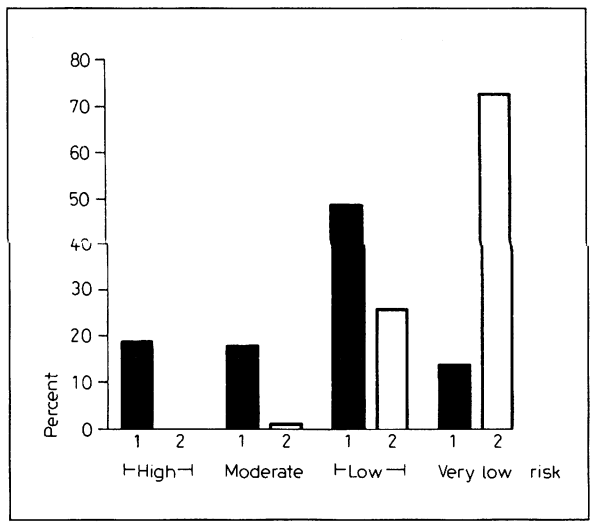

Fig. 8. Risk rates of riboflavin deficiency in female epileptics (1) and controls (2) (see fig. 7).

Vitamin B_{12}

No difference in the mean vitamin B_{12} levels, which were determined in serum by a radioimmunoassay, were noted between female and male epileptics, whereas in the Heidelberg study distinctly lower mean vitamin B_{12} concentrations were found in females than in males (table I). When comparing the two collectives, no difference in mean vitamin B_{12} levels was detected in females, but the male epileptics had much lower levels than the controls. This tendency is confirmed in the risk rates: 15% of the male and 1% of the female epileptics had a high or moderate risk of vitamin B_{12} deficiency compared with 6 and 12%, respectively, in the controls (fig. 9, 10). The relatively frequent occurrence of deficient levels in the female controls could be due to the intake of oral contraceptives for which a negative correlation with vitamin B_{12} levels had been detected in the Heidelberg study [1]. The reason for the low levels in our epileptics is not clear, because no correlations with anticonvulsant intake were found either in males or in females. In 1966, *Reynolds* et al. [12] reported a small reduction of B_{12} levels in epileptics; other studies, which were done after the description of megaloblastic anemias as side effect of anticonvulsant therapy, failed to show an influence of anticonvulsants on the vitamin B_{12} status [4, 8].

Biotin

The last vitamin discussed here, possibly with the most striking results, is biotin. This vitamin has not been investigated in the Heidelberg study; therefore, a collective of blood donors served as controls. Their plasma was analyzed in the same laboratory using the same method, namely a microbiological assay with *Lactobacillus plantarum* as test organism [6]. The plasma biotin levels were clearly reduced in epileptics, both in males and in females (table I), and very high rates for a high and moderate risk of biotin deficiency were found in our epileptics (fig. 11, 12). In male epileptics there was a negative correlation with anticonvulsant medication (table II, fig. 13).

As our further investigations have shown, this reduced biotin status has biochemical consequences: in epileptics with low biotin levels abnormal organic acids (such as methylcrotonylglycine, methyl citric acid or propionylglycine) are excreted in the urine [10]. Interesting hypotheses concerning the possible mode of action of anticonvulsants can be postulated from these

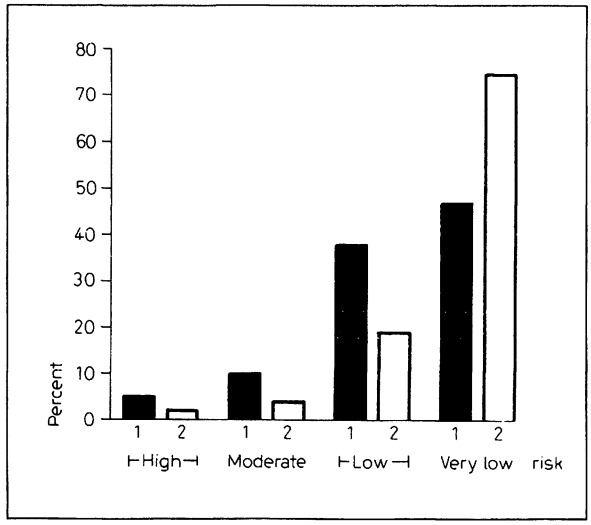

Fig. 9. Rates of high (< 150 µmol/l), moderate (150–220), low (221–350) and very low (> 350 µmol/l) risk of vitamin B_{12} deficiency in male epileptics (1) and controls (2).

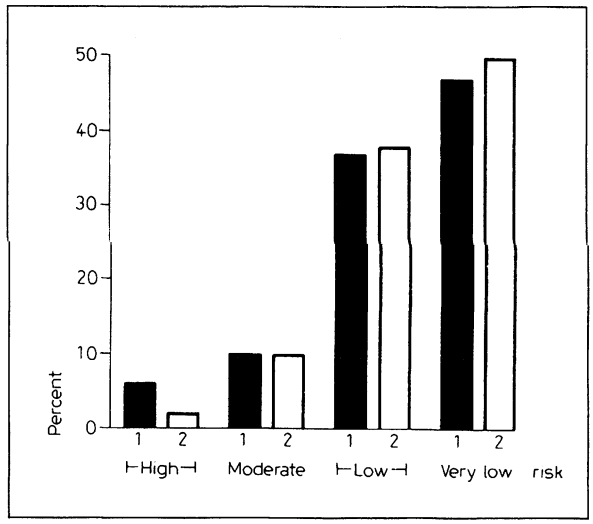

Fig. 10. Risk rates of vitamin B_{12} deficiency in female epileptics (1) and controls (2) (see fig. 9).

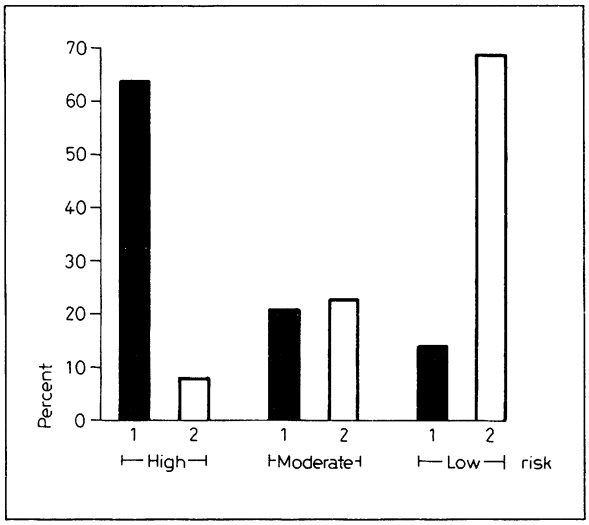

Fig. 11. Rates of high (< 250 ng/l), moderate (250–300) and low (> 300 ng/l) risk of biotin deficiency in male epileptics (1) and controls (2).

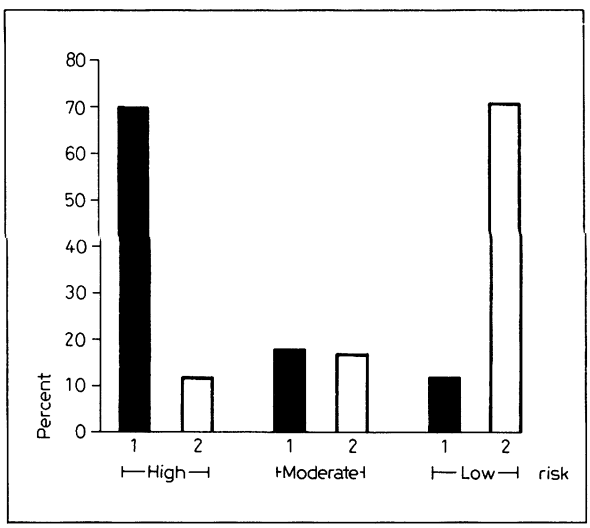

Fig. 12. Risk rates of biotin deficiency in female epileptics (1) and controls (2) (see fig. 11).

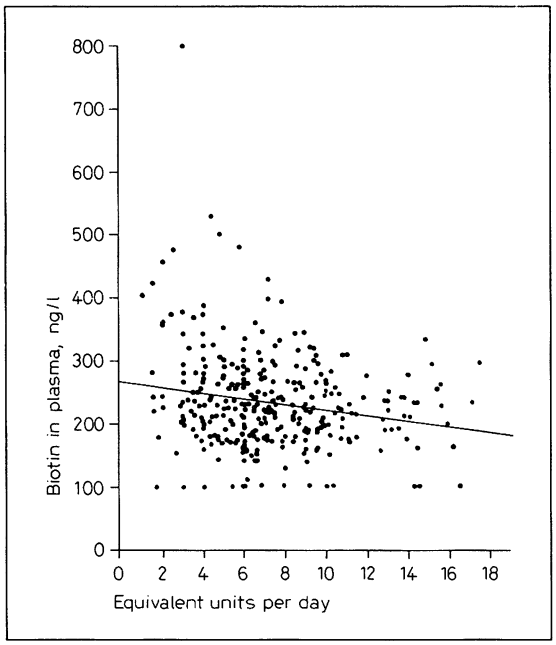

Fig. 13. Correlation between daily intake of anticonvulsants and biotin in male epileptics.

findings [9]: the biotin-dependent carboxylases are involved in biochemical reactions which influence inhibitory and excitatory neurotransmitters. Furthermore, the carboxylases are important in the metabolism of carbon dioxide, which directly influences the seizure threshold. Further investigations are planned to determine the effect of the biotin status on the clinical expression of epilepsy.

Conclusion

All in all, we found a poorer status of the vitamins B_2, B_6, B_{12} and biotin in long-term treated epileptics compared with a control collective from the same area. These changes cannot be explained by alimentary factors alone, as the epileptics investigated were selected from an outpatient clinic and generally lead a normal life. The direct influence of antiepileptic

medication on the status seen for vitamins B_1, B_2, B_6 and biotin is a factor to be considered. Further investigations elucidating the mechanism of this influence of anticonvulsants on the vitamin B status are therefore of high interest. At this time we cannot definitely say whether a substitution with B vitamins should be recommended in long-term treated epileptics.

References

1 Arab, L.; Schellenberg, B.; Schlierf, G.: Nutrition and health. A survey of young men and women in Heidelberg. Ann. Nutr. Metab. *26:* suppl. 1, pp. 1–244 (1982).

2 Bonjour, J.P.: Vitamins and alcoholism. III. Vitamin B_6. Int. J. Vitam. Nutr. Res. *50:* 215–230 (1980).

3 Botez, M.I.; Joyal, C.; Maag, U.; Bachevalier, J.: Cerebrospinal fluid and blood thiamine concentrations in phenytoin-treated epileptics. Can. J. neurol. Sci. *9:* 37–39 (1982).

4 Carney, M.W.P.: Serum vitamin B_{12} values in 374 psychiatric patients. Behav. Neuropsychiatry *1:* 19–28 (1969).

5 Davis, R.E.; Reed, P.A.; Smith, B.K.: Serum pyridoxal, folate, and vitamin B_{12} levels in institutionalized epileptics. Epilepsia *16:* 463–468 (1975).

6 Frigg, M.; Brubacher, G.: Biotin deficiency in chicks fed a wheat based diet. Int. J. Vitam. Nutr. Res. *46:* 314–321 (1976).

7 Heilmann, E.: Orale Kontrazeptiva and Vitamine. Dt. med. Wschr. *104:* 144–146 (1979).

8 Klipstein, F.A.: Subnormal serum folate and macrocytosis associated with anticonvulsant drug therapy. Blood *23:* 68–86 (1964).

9 Krause, K.-H.: Biotin-Erniedrigung unter antiepileptischer Behandlung. Ein Beitrag zur möglichen Wirkungsweise von Antikonvulsiva (Hochschul-Verlag, Freiburg 1983).

10 Krause, K.-H.; Kochen, W.; Berlit, P.; Bonjour, J.P.: Excretion of organic acids associated with biotin deficiency in chronic anticonvulsant therapy. Int. J. Vitam. Nutr. Res. *54:* 217–222 (1984).

11 Reinken, L.: The influence of antiepileptic drugs on vitamin B_6 metabolism. Acta vitaminol. enzymol. *29:* 252–254 (1975).

12 Reynolds, E.H.; Chanarin, I.; Milner, G.; Matthews, D.M.: Anticonvulsant therapy, folic acid and vitamin B_{12} metabolism and mental symptoms. Epilepsia *7:* 261–270 (1966).

13 Tada, K.: Pyridoxine dependency with seizures; in Vinkens, Bruyns, Handbook of clinical Neurology, vol. 42, pp. 716–717 (North Holland, Amsterdam 1981).

14 Vuilleumier, J.P.; Keller, H.E.; Rettenmaier, R.; Hunziker, F.: Clinical chemical methods for the routine assessment of the vitamin status in human populations. II. The water-soluble vitamins B_1, B_2 and B_6. Int. J. Vitam. Nutr. Res. *53:* 359–370 (1983).

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