Vitamin B_6 Depletion in Women Using Oral Contraceptives as Determined by Erythrocyte Glutamic-Pyruvic Transaminase Activities[1] (35735)

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Observations of increased urinary excretion of xanthurenic acid after an oral load of L-tryptophan have led to the suggestion of vitamin B_6 deficiency in women using oral contraceptives (1–4). Price *et al.* (4) demonstrated that increased excretion of xanthurenic acid and other metabolites of tryptophan in women using steroid hormones (Enovid - E) for ovulation control could be overcome by pyridoxine supplementation. More recently, Baumblatt and Winston (5) have reported that pyridoxine supplementation alleviates five symptoms associated with depression in women using oral contraceptives.

Cheney *et al.* (6) and Raica and Sauberlich (7) have shown that erythrocyte transaminase activities may be readily detected in man. Subsequently, Cinnamon and Beaton (8) induced a dietary deficiency of vitamin B_6 in male subjects; they demonstrated a marked fall in erythrocyte glutamic-pyruvic transaminase (EGPT) activity which was restored to normal by addition of pyridoxal phosphate (PALPO) *in vitro* or by prolonged oral administration of pyridoxine. Comparing this assay with such tests as the tryptophan load and oxalate excretion, Cinnamon and Beaton concluded that stimulation *in vitro* of EGPT by PALPO addition constitutes a sensitive and valid criterion of vitamin B_6 status (8). The present investigation was undertaken to ascertain if evidence of vitamin B_6 depletion could be elicited using the EGPT-PALPO stimulation procedure in women using oral contraceptives.

Materials and Methods. Venous blood samples (5 ml) were drawn and heparinized from 24 healthy, nonpregnant, female subjects of whom 13 were using an oral contraceptive and 11 had never used oral contraceptives. A brief medical history was taken for each subject to ensure health and the absence of any current medication which could conceivably affect the assay results. Although there was considerable intragroup variation, the mean age of the two groups of subjects was comparable. A 24-hr recall of food intake was recorded and in each case the subject indicated this to be approximately her usual intake; it should be noted that three subjects were dieting for weight loss with intakes of about 800 cal per day. Records and blood samples were coded by the physician and the presence or absence of oral contraceptive use was not revealed until after all blood samples were analysed. Approximate intakes of protein and calories were estimated from standard tables of food composition since protein intake has been reported to have an inverse effect on vitamin B_6 status in animals (9) and man (10, 11).

Immediately upon obtaining blood samples, erythrocytes were separated by centrifugation, washed with physiological saline, hemolysed in 2 vol of glass-distilled water, and frozen. For EGPT assay, samples were thawed and centrifuged to remove cellular debris. To assess PALPO stimulation *in vitro*, hemolyzates were preincubated with 100 μg pyridoxal-5-phosphate in buffer at 37° for exactly 60 min prior to assay. The procedures for preparation of hemolyzate, for EGPT assay, and for PALPO stimulation have been described previously (6, 8).

Statistical significance of difference between means was determined by application of Student's t test. Only values of $p < .05$ or less were considered to be statistically significant.

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TABLE I. Erythrocyte Glutamic-Pyruvic Transaminase Activities of Women Using Oral Contraceptives.

Group	Subject no.	Age (years)	Oral contraceptive	Intake/days[a]		Erythrocyte glutamic-pyruvic transaminase		
				Protein (g)	Calories	Basal[b]	+ PALPO[b]	% Stimulation
I. Control	1	46	—	66	2180	174	239	37
	2	51	—	94	1760	69	92	32
	3	19	—	102	1600	284	292	3
	4	20	—	—	—	150	207	38
	5	28	—	76	1620	200	243	22
	6	22	—	48	1140	160	171	7
	7[c]	19	—	40	800	143	193	35
	8	25	—	71	1320	64	79	24
	9	23	—	137	4700	342	415	21
	10	24	—	116	2900	243	275	13
	11	20	—	—	—	117	135	15
	Mean	27.0		73.1	2002.2	176.9	212.8	22.4
	SEM	3.32		17.65	543.98	25.89	29.10	3.64
II. Oral contraceptive users	1	37	Norinyl (2.2 yr)	66	1080	335	115	228
	2[c]	37	Norinyl (5.5 yr)	39	895	58	164	184
	3	27	Ovulen (2 yr)	64	2130	77	166	116
	4	21	Ovral (1.2 yr)	51	1530	61	86	40
	5	23	Ovral (1.3 yr)	31	1450	72	101	40
	6	25	Norinyl (1 yr)	68	2320	51	88	72
	7[c]	34	C-Quens (5 yr)	35	770	35	123	251
	8	24	Ovral (4 months)	89	2380	48	156	226
	9	23	Ovral (2.5 yr)	45	1510	86	219	156
	10	22	Ovral (2 yr)	88	2060	70	99	41
	11	32	Enovid/Ovulen (8 yr)	75	2245	172	265	54
	12	20	Ovulen (4 months)	50	2325	239	405	69
	13	23	Norinyl (1.7 yr)	92	2040	172	261	52
	Mean	26.8		61.0	1748.8	90.4	172.9	117.6
	SEM	1.68		5.82	159.29	17.29	25.80	22.40

[a] From 24-hr food intake (considered typical by subjects).
[b] Micrograms pyruvate formed/ml erythrocytes/hr.
[c] Dieting to lose weight.

Results. Mean basal EGPT activity was significantly lower ($p<.02$) in women using oral contraceptives; mean activities with PALPO stimulation were not different between the two groups. Mean PALPO stimulation (%) was significantly greater for women using oral contraceptives than for controls ($p<.001$) although there was considerable variation within each group. PALPO addition *in vitro* did not yield a statistically significant stimulation of EGPT activity in controls but did so ($p<.02$) in women using oral contraceptives (Table I). Mean protein and calorie intakes were somewhat lower for women using oral contraceptives than for controls.

Discussion. Results of this investigation support evidence of a vitamin B_6 depletion in women using oral contraceptives as suggested previously using the tryptophan load as the test procedure (1–4). Furthermore, the data (Table I) do not indicate any apparent relationship between the type of contraceptive and the EGPT values. It is doubtful that the lower intakes of protein and calories of women using oral contraceptives can be a contributing factor. Decreased protein intake should yield higher EGPT activities (9); the reverse was observed.

It appears that oral contraceptives decrease basal EGPT activity, a premise supported by the marked stimulatory effect of PALPO *in vitro*. This observation is comparable to that of Cinnamon and Beaton (8) who noted decreased basal EGPT values in vitamin B_6-deprived male subjects with a return to normal activities with PALPO stimulation *in vitro*. Although ranges of PALPO stimulations (%) were relatively large, being 3–38 and 40–251 for controls and contraceptive users, respectively, no overlapping was observed between groups. In contrast, the ranges of basal values (64–342 and 35–239) showed considerable overlapping. These observations support the conclusion that although basal EGPT activity *per se* is inconclusive, PALPO stimulation *in vitro* of EGPT activity is a sensitive index of vitamin B_6 nutriture (8, 12).

Cinnamon and Beaton (8) reported PALPO stimulations of 15–26% in three normal male subjects and recently, Woodring and Storvick (12) have noted stimulations of 0–15% in seven normal female subjects. The results for normal subjects reported in the present investigation are comparable.

Although the present study provides additional biochemical evidence for vitamin B_6 deprivation, it should be noted that no clinical or subjective symptoms of vitamin B_6 deficiency were recorded by the examining physician among contraceptive users. It will be of value to determine what effect, if any, pyridoxine supplementation has upon EGPT activity and PALPO stimulation in women using oral contraceptives. As noted earlier, pyridoxine supplementation alleviates the apparent abnormalities of tryptophan metabolism of oral contraceptive users (4). With respect to EGPT activity and PALPO stimulation, it has been shown that daily supplementation with pyridoxine results in increased EGPT activity (6, 12).

Summary. Erythrocyte glutamic-pyruvic transaminase (EGPT) activities were measured, with and without stimulation by pyridoxal phosphate (PALPO) *in vitro*, in 11 control females and in 13 females using oral contraceptives. In women taking oral contraceptives, mean basal EGPT activity was significantly lower ($p<.02$) and percentage of stimulation by PALPO was significantly greater ($p<.001$) than in controls. Mean values of EGPT activity with PALPO stimulation were not significantly different between the two groups. It would appear that biochemical evidence of vitamin B_6 depletion is elicited by the use of oral contraceptives. Further evidence is provided in support of the conclusion that PALPO stimulation of EGPT activity *in vitro* is a valid criterion of vitamin B_6 status.

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