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Cataract Formation following Limited Amino Acid Intake during Gestation and Lactation[1] (41902)

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Abstract. Female rats were fed defined diets limiting in one or more of certain amino acids and with or without vitamin E throughout gestation and lactation. Deficits of tryptophan, phenylalanine/tyrosine, or methionine/cystine reduced the body weight of progeny to about 50% or less of normal but only low tryptophan was cataractogenic. When total dietary amino acids were 12.4%, a low (65 mg%) level of tryptophan resulted in 34% incidence of cataract if vitamin E was simultaneously withheld. Elevation of total amino acids to 24.8% while maintaining tryptophan at 65 mg% caused 70 or 90% incidence of nuclear lens opacities in the presence or absence, respectively, of vitamin E. Maternal dietary amino acid imbalance was also associated with a 50% decrease in lens insoluble (membrane) proteins in the progeny independent of dietary vitamin E or the occurrence of opacities.

It has been reported that consumption throughout gestation and lactation of a diet simultaneously low in tryptophan and vitamin E resulted in the appearance of central nuclear opacities in about one-third of rat progeny (1, 2). The same level of tryptophan as the sole limiting nutrient was without effect on lens transparency. Restriction of vitamin E alone caused a 6% incidence of cataract. So far as we have been able to determine, the impact of deficits of amino acids other than tryptophan upon lens development during this period of life has not been investigated.

Simple tryptophan deficiency causes cataract in postweaning rats (3–8), guinea pigs (9), and trout (10). Hall *et al.* (11) determined that single deficiencies of either phenylalanine or histidine as well as tryptophan caused cataract in weanling rats within 3 weeks. Dietary omission of threonine, leucine, isoleucine, valine, lysine, methionine, or arginine for the same period of time resulted in changes in lens morphology such as haziness or separation of the superficial fibers but obvious opacific regions were absent. Methionine sulfoximine injections in rats induced cataracts which could be prevented by dietary supplements of methionine or cystine (12). A 90% incidence of cataract accompanied by less than one-fourth of normal growth rate was observed in fingerling lake trout fed a commercial soy protein isolate. Both problems were corrected by supplementation with methionine (13). A recent study has shown that the cataractogenic effect of a commercial milk replacement diet in wolf pups was prevented by addition of arginine (14). Lenses remained clear when young pigs (15) or rats (11, 16, 17) were maintained on very low (<5%) levels of total protein for as much as 146 days in the pigs or 600 days in the rats.

In this paper, we report further studies of the effect on lens development of limited intake of amino acids during gestation and lactation. We have confirmed the cataractogenic effect of coincident deficits of tryptophan and vitamin E, and have shown that comparable restrictions of vitamin E and phenylalanine/tyrosine, methionine/cystine, or lysine did not provoke a similar response. Tryptophan deficiency alone caused a 70% incidence of cataract when the total amino acid level of the diet was raised from 12.4 to 24.8% in the presence of vitamin E. Deletion of vitamin E caused a further increase of cataract frequency to 90%.

Materials and Methods. The complete basal diet is shown in detail in Table I. Crystalline L-amino acids were utilized as the sole nitrogen source permitting facile manipulation of the level of individual amino acids. Total amino acids were held constant by replacement of withheld indispensible amino acids with equal

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TABLE I. COMPOSITION OF BASAL DIET

Ingredient	Amount (g/100 g)
Glucose	58.00
Starch	8.06
Amino acid mixture[a]	12.44
Cellulose[b]	5.00
Salt mixture[c]	4.00
Vitamin mixture[d]	2.50
Corn oil (tocopherol stripped)[e]	10.00

[a] As g per 100 g diet: L-His 0.54; L-Lys-HCl 1.55; L-Leu 0.80; L-Ilu 0.50; L-Phe 0.60; L-Met 0.30; L-Cys-Cys 0.30; L-Tyr 0.30; L-Thr 0.50; L-Val 0.70; L-Arg 0.75; L-Glu 0.90; L-Asp 0.90; L-Pro 0.90; L-Ala 1.10; L-Ser 0.90; Gly 0.90. Obtained from Ajinomoto, New York, N.Y.

[b] Alphacel, ICN Pharmaceuticals, Inc., Cleveland, Ohio.

[c] Mixture No. 4164, ICN Pharmaceuticals, Inc., to which sodium selenite was added to give a dietary concentration of 25 μg Se per 100 g diet.

[d] Vitamin diet fortification mixture with α-tocopherol omitted, ICN Pharmaceuticals, Inc. Provides per 100 g diet: (in IU), retinyl acetate, 1980; cholecalciferol, 220; (in mg), inositol, 11; choline chloride, 165; menadione, 5; *p*-aminobenzoic acid, 11; niacin, 9.9; riboflavin, 2.2; pyridoxine-HCl, 2.2; thiamine-HCl, 2.2; calcium pantothenate, 6.6; (in μg) biotin, 44; folic acid, 198; and B-12,3.

[e] ICN Pharmaceuticals, Inc., and General Biochemicals, Chagrin Falls, Ohio.

weight of L-alanine. The mineral mix (No. 4164), as originally formulated, lacked selenium but sodium selenite was added to all diets to insure a level of 25 μg selenite/100 g diet. All of the lipid was supplied by tocopherol-stripped corn oil so that vitamin-E content could be adjusted by its deletion from the otherwise complete vitamin mix.

Eight- to 10-week-old female Sprague-Dawley rats weighing between 175 and 225 g were fed for 1 week a diet containing the complete amino acid mixture but lacking vitamin E. This was to assure that the plasma levels of vitamin E would be low throughout the entire 6 weeks of gestation and lactation in those animals which would be in the low vitamin-E groups. Earlier studies have shown that plasma levels of vitamin E fall rapidly during the first week of depletion but remain relatively constant for several weeks beyond (18). After the adjustment period, male and female rats were housed in a one-to-one pairing in stainless-steel wire mesh cages. Upon detection of copulation plugs, female rats were assigned to either the low tryptophan–low vitamin-E diet, a fully supplemented control diet, or a diet modified by manipulation of the content of one of the other essential amino acids.

The female rats were housed in opaque polypropylene "shoebox" cages from conception to weaning. The cages were provided with shredded softwood bedding and enclosed with a bonnet-type cage filter. The animal quarters were temperature (25–28°C)- and humidity (65%)-controlled with circulating filtered air and a 12-hr light/dark cycle.

Evaluation of the progeny for cataract was conducted under artificial light without a magnification aid. Animals were then decapitated and the lenses removed for analysis. Measurements included lens wet weight/dry weight ratio, total soluble and insoluble lens proteins (7), total glutathione (19), and Na and K by atomic absorption spectrophotometry following wet ashing. Representative soluble fractions were also fractionated by gel filtration chromatography to determine proportional distribution of the major crystallins (7).

Results. Data on animal size at weaning and cataract frequency are summarized in Table II. Control populations in our laboratory have never displayed congenital cataract. Weight at weaning (Day 23 postpartum) for rat pups from dams fed the fully supplemented diet averaged 39 ± 1 g (mean ± SEM). Simultaneous restriction of L-tryptophan to 65 mg/100 g of diet and omission of vitamin E reduced the mean weight at weaning to 24 ± 3 g and resulted in the appearance of unilateral or bilateral (1:3 ratio) cataract in 63 of 184 progeny (34%). Approximately one-half (9 of 20) of the litters were free of evident lenticular damage. Elevation of the vitamin-E content of this diet to 40 mg/100 g diet did not affect weight at weaning but prevented the appearance of cataract in the test group (71 rats from 10 dams). Increasing the total amino acid content of the diet from 12.4 to 24.8% while leaving tryptophan at 65 mg/100 g diet aggravated the cataractogenic effect whether vitamin E was absent (90% of 98 rats) or present (70% of 107 rats). When phenylalanine (450 mg/100 g diet) and tyrosine (none present) were limiting instead of tryptophan, size at weaning was restricted to 18 ± 3 g but only one animal in 48 (2%) showed a cataract (not

TABLE II. CATARACT FREQUENCY

Maternal diet	Total litters (*N*)	Litters with opacities (*N*)	Total pups (*N*)	Pups with opacities (*N*)	Percentage incidence (*N*)	Mean weight at postpartum Day 23[e] (g)
L-Trp 65 mg/100 g diet Vitamin E absent Total amino acids 12.4%	20	11	184	63	34[b]	24 ± 3
L-Trp 65 mg/100 g diet Vitamin E 40 mg/100 g diet Total amino acids 12.4%	10	0	71	0	0[a]	22 ± 2
L-Trp 65 mg/100 g diet Vitamin E absent Total amino acids 24.8%	11	10	98	88	90[c]	20 ± 1
L-Trp 65 mg/100 g diet Vitamin E 40 mg/100 g diet Total amino acids 24.8%	11	9	107	75	70[d]	20 ± 1
L-Phe 450 mg/100 g diet L-Tyr absent Vitamin E absent Total amino acids 12.4%	7	1	48	1	2[a]	18 ± 3
L-Met 300 mg/100 g diet L-Cys–Cys absent Vitamin E 40 mg/100 mg diet Total amino acids 12.4%	8	0	90	0	0[a]	15 ± 1
L-Lys 450 mg/100 g diet Vitamin E absent	5	0	55	0	0[a]	38 ± 3

[a–d] All values with letters different from one another are highly significantly different ($P < 0.005$) according to chi-square test.

[e] Mean ± SEM.

significant). Limitation of sulfur amino acids to 50% of the minimal requirement (20) reduced weight at weaning to 15 ± 1 g. Although lenses frequently appeared hazy, distinct opacities were not detected in any of 90 pups. Reduction of lysine to 50% of the stated minimal requirement did not reduce size at weaning (38 ± 3 g) and no cataracts were seen.

Comparison of cataractous and clear lenses from progeny of the low tryptophan and control regimens with regard to wet-wt/dry-wt ratio, sodium, potassium, or glutathione revealed no significant differences. Lens glutathione was depressed (4.5 ± 0.28 vs 1.9 ± 0.35 μmole/g lens) only in the progeny from the maternal low-sulfur amino acid diet. The appearance of cataract in rats fed a low (50 mg/100 g diet) tryptophan ration beginning at weaning is accompanied by a significant decline in the β_H-crystallin fraction of soluble lens protein and an increase from 28–29 to 41% of lens protein which is insoluble (7). When the tryptophan deficit was imposed in the maternal diet, no change in the β_H-crystallin fraction was evident in the cataract-containing lenses. In general, total lens protein was elevated slightly and the percentage insoluble protein was depressed from 13–14 to 5.4–10.5% in the lenses from all progeny whose dams were fed an imbalanced amino acid ration regardless of whether or not the diet was cataractogenic (Table III).

Discussion. These data confirm and extend our earlier observations on the effects of maternal diets simultaneously low in tryptophan and vitamin E (1, 2). The incidence of cataract (34%) in the progeny when both nutrients were low was virtually identical to that reported earlier (33%) and, as before, a vitamin-E supplement of 40 mg/100 g diet prevented the lesion. It should be noted, however, that in the original study the opacific spot often oc-

TABLE III. LENS WEIGHT AND PROTEIN CONTENT OF PROGENY AT WEANING

Maternal diet	Lens wet wt (mg)	Lens insoluble protein (μg/mg lens)	Lens soluble protein[i] (μg/mg lens)	Percentage insoluble protein (%)	Percentage total protein (%)
L-Trp 65 mg/100 g diet Vitamin E absent Total amino acids 12.4%	17.4 ± 0.5[b]	24 ± 11[h]	418 ± 28	5.4	44.2
L-Trp 65 mg/100 g diet Vitamin E 40 mg/100 g diet Total amino acids 12.4%	17.9 ± 0.5[a]	23 ± 4[h]	368 ± 35	6.9	39.1
L-Trp 65 mg/100 g diet Vitamin E absent Total amino acids 24.8%	14.3 ± 0.3[c]	28 ± 7[h]	401 ± 25	6.5	42.9
L-Trp 65 mg/100 g diet Vitamin E 40 mg/100 g diet Total amino acids 24.8%	14.8 ± 0.4[d]	25 ± 9[h]	422 ± 32	5.6	44.7
L-Phe 450 mg/100 g diet L-Tyr absent Vitamin E absent Total amino acids 12.4%	13.5 ± 0.8[e]	41 ± 3[g]	349 ± 10	10.5	39.0
L-Met 300 mg/100 g diet L-Cys–cys absent Vitamin E 40 mg/100 mg diet Total amino acids 12.4%	13.0 ± 0.3[f]	26 ± 1[h]	387 ± 26	6.3	41.3
L-Lys 450 mg/100 g diet Vitamin E absent	18.0 ± 0.3[a]	53 ± 3[g]	316 ± 12	14.4	36.9
Complete amino acids at 12.4% Vitamin E 40 mg/%	19.1 ± 0.4[a]	50 ± 4[g]	331 ± 33[a]	13.1	38.1

[a-f] Mean ± SEM. All values with letters different from the control are statistically different (*b* vs *a*, $P < 0.05$; all others, $P < 0.01$) according to Dunnett's test.

[g,h] Mean ± SEM. All values with letters different from the control are significantly different ($P < 0.05$) according to Dunnett's test.

[i] Mean ± SEM. No values significantly different from the control at $P < 0.05$ according to Dunnett's Test.

cupied as much as 20% of the lens volume whereas in the present study there was a much higher frequency of "pinhead" opacities (<5% of total lens volume). The reason for this difference is not known. Increasing the pool of amino acids which compete with tryptophan for transport across the plasma membrane by doubling the total dietary amino acids, had only a slight effect on animal size at weaning but significantly ($P < 0.005$) increased cataract frequency in the absence or presence of vitamin E. Apparently the lack of vitamin E plays a permissive or contributory but not obligatory role in the emergence of the opacity. The failure of comparable deficits of phenylalanine/tyrosine or sulfur amino acids to generate a similar level of lens pathology reveals a unique importance of tryptophan. Because cataracts caused by a low tryptophan intake are limited to the fetal or embryonal nucleus, a region of the lens composed of the first fiber cells produced during differentiation, it is reasonable to suggest that tryptophan and vitamin E may play key roles in this process.

The 50% decline in percentage insoluble protein in the lenses of rats whose growth was severely restrained by a maternal dietary amino acid imbalance was independent of the presence or absence of opacities. At this early stage of life, the structural crystallins comprise the soluble fraction and membrane proteins constitute the bulk of the insoluble fraction (21). The observed decline may reflect the relative importance of amino acids such as tryptophan, phenylalanine, tyrosine, and cysteine as components of membrane proteins. The

failure of the reduced level of dietary lysine to impose a growth depression suggests a need to reevaluate the stated minimal requirement for this amino acid during pregnancy in the rat.

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