# Prevention of Cataract in the Progeny of Rats Fed a Maternal Diet Based on Vegetable Proteins[1] (36620)

GEORGE E. BUNCE, PRISCILLA CAASI,[2] BLANCHE HALL, AND NELSON CHAVES
*Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University; and the Instituto de Nutriçao, Universidade de Recife, Recife, Pernambuco, Brazil*

Chaves *et al.* (1) reported in 1964 the occurrence of unilateral and bilateral cataract and microphthalmia in about 30% of the progeny of female rats fed throughout pregnancy and lactation on a 10% protein diet in which cowpeas (*Vigna sinensis*) and cashew nuts served as the sole source of protein. No abnormalities were observed in a control group when the vegetable proteins were replaced by casein. Elevation of the protein level of the vegetable protein diet to 21% without changing the ratio of cowpeas to cashews (3:1) also prevented the ocular lesions.

We have sought to identify the cause of this congenital defect by supplementing the cataractogenic diet with purified nutrients. The results to date of these studies are reported below.

*Materials and Methods.* The ingredients of the standard cataractogenic diet are given in Table I. Cowpeas (black-eyed peas) were purchased in 100-lb lots, soaked for 12 hr in a volume (ml) of ion-free water equal to the weight in grams of the beans, autoclaved at 120° and 15 psi, dried at 80° in a forced draft oven for 20–30 hr and ground into a flour with a Wiley mill using a 1-mm mesh stainless steel sieve. Roasted dehulled cashew nuts were also ground in a Wiley mill but no sieving was employed and they were not subjected to a prior soaking or autoclaving.

The nutrient composition of the standard cataractogenic diet is compared to the NRC recommended intakes for gestation (2) in Table II. After examination of these values, a supplemented ration was devised in which a new mineral mix and a new vitamin mix were prepared to replace those of the standard ration so as to provide a quantity of these nutrients which met or exceeded the recommended levels. Although several amino acids were present in less than recommended levels, only tryptophan and methionine were added to the supplemented diet since these were the most limiting amino acids and those most directly related to lens health according to the available literature (3).

For all experiments, random bred Wistar strain rats were obtained (Microbiological Associates) at 90 days of age. They were fed the appropriate experimental diets for 2 weeks prior to mating, for 2 weeks of cohabi-

TABLE I. Cataractogenic Diet Composition.

| Ingredient | % of diet |
|---|---|
| Cowpeas (*Vigna sinensis*) flour | 30.8 |
| Cashew nut flour | 11.5 |
| Lard | 4.4 |
| Cornstarch | 50.6 |
| Mineral mix[a] | 2.7 |
| Vitamin mix[b] | 1.0 ml/100 g diet |

[a] Osborne-Mendel salt mix as modified by Hawk and Oser. ''Practical Physiological Chemistry'' (P. B. Hawk, B. L. Oser and W. H. Summerson, eds.), Chap. 13, p. 1375. Blakiston, New York.

[b] One hundred milliliters of aqueous solution contained (mg): thiamine • HCl, 19; riboflavin, 42; niacin, 16; calcium pantothenate, 216; pyridoxine • HCl, 25; biotin, 0.15; folic acid, 0.2; vitamin $B_{12}$, 1; inositol, 50; and choline chloride, 13 g.

[1] Supported in part by Public Health Service Grant NB07963. Portions of this work are taken from a thesis presented by P. C. in partial fulfillment of the requirements of the PhD in Biochemistry and Nutrition from the Virginia Polytechnic Institute and State University.

[2] Present address: Research Associate, Biochemistry Research Division, Department of Medicine, Sinai Hospital of Baltimore, Inc., Baltimore, MD 21215.

tation with male rats of the same strain and age, and throughout the subsequent gestation and lactation periods. The absence or presence of cataract in the progeny at weaning could easily be determined by examination of the eyes under a strong light.

*Results.* The results of the diet supplementation studies are summarized in Table III. Only D,L-α-tocopherol (40 mg %) or a combined supplement of 0.5 g % methionine, 0.5 g % tryptophan and 10 mg % niacin were as effective in prevention of the ocular lesion as the entire supplement. The efficacy of tryptophan alone has not yet been tested although neither 0.5 g % methionine nor 1 mg % niacin were active. It should be noted that cataracts were never observed in the mated does nor in weanling rats maintained on the standard diet for 10 weeks. Twelve young rats with cataracts were placed on a standard lab chow diet immediately after weaning. Reexamination of these animals after 3 weeks revealed no change in appearance but after 6 weeks only three had opacities of the original intensity; the other nine had either clear or cloudy lenses.

TABLE II. Nutrient Content[a] of Cataractogenic and Supplemented Diet.

| Nutrient | Cataractogenic diet | Fully supplemented diet | N.R.C. recommended level for gestation |
|---|---|---|---|
| Vitamins | | units/100 g diet | |
| Vitamin A (IU) | 145 | 2000 | 1200 |
| Vitamin D (IU) | 50 | 500 | Not estab. |
| Vitamin E, as mg α-tocopherol | 0.1 | 40 | 3 |
| Vitamin K, as mg menadione | 0 | 0.5 | Not estab. |
| Thiamine (mg) | 0.19 | 2.0 | 0.4 |
| Riboflavin (mg) | 0.42 | 2.0 | 0.4 |
| Pyridoxine (mg) | 0.25 | 1.0 | 0.06 |
| Niacin (mg) | 1.21 | 11.2 | 1.5[b] |
| Calcium pantothenate (mg) | 2.16 | 4 | 0.8 |
| Choline chloride (mg) | 130 | 200 | <100 |
| Vitamin $B_{12}$ (μg) | 10.0 | 5 | 0.5 |
| Biotin (mg) | 0.002 | 0.05 | Not estab. |
| Folic acid (mg) | 0.002 | 0.4 | Not estab. |
| Inositol (mg) | 0.5 | 50 | Not estab. |
| Minerals | | | |
| Calcium (mg) | 333 | 600 | 600 |
| Phosphorus (mg) | 361 | 500 | 500 |
| Sodium (mg) | 82 | 500 | 500 |
| Potassium (mg) | 442 | 237 | 140 |
| Chlorine (mg) | 286 | 185 | 25 |
| Magnesium (mg) | 121 | 50 | 50 |
| Manganese (mg) | 0.1 | 6.5 | 5 |
| Iron (mg) | 11 | 20 | 2.5[b] |
| Copper (mg) | 4 | 8 | 0.5[b] |
| Zinc (mg) | 2.4 | 10 | 1.2[b] |
| Iodine (mg) | 8.30 | 2 | 0.015 |
| Selenium (μg) | 0 | 20 | 0.4 |
| Cobalt (μg) | 0 | 30 | Not estab. |
| Amino acids | | | |
| Tryptophan (g) | 0.1 | 0.6 | 0.2 |
| Methionine (g) | 0.1 | 0.6 | 0.6 |
| H-Cystine (g) | Trace | Trace | |
| Lysine (g) | 0.8 | 0.8 | 1.24 |
| Histidine (g) | 0.3 | 0.3 | 0.54 |
| Phenylalanine (g) | 0.6 | 0.6 | 0.9 |
| Threonine (g) | 0.3 | 0.3 | 0.5 |
| Valine (g) | 0.6 | 0.6 | 0.7 |
| Leucine (g) | 0.8 | 0.8 | 0.8 |
| Isoleucine (g) | 0.5 | 0.5 | 0.5 |
| Arginine (g) | 0.9 | 0.9 | 0.75 |

[a] Amino acid values, except tryptophan obtained by analysis on a Beckman-Spinco 120 B amino acid analyzer after acid hydrolysis in 6 N HCl under $N_2$ for 24 hr. Tryptophan analyzed on protein samples after alkaline hydrolysis (15). Vitamin E and niacin analyzed by Wisconsin Alumni Research Foundation, Madison, WI. All other values based on quantities added during preparation.

[b] Recommended allowance for growth; no value set for gestation.

Although a complete histopathological examination was not performed, there was no gross evidence of abnormalities in any other parts of the body. Weight gain of the weanling rats fed the cataractogenic diet was substantially less than normal but a significant

TABLE III. Effect of Dietary Modification on Incidence of Cataract.

| Diet | No. of litters | No. progeny examined | Progeny with cataracts No. | Progeny with cataracts % |
|---|---|---|---|---|
| Standard cataractogenic | 13 | 70 | 17 | 24 |
| + entire supplement | 5 | 38 | 0 | 0 |
| + mineral supplement | 5 | 31 | 10 | 32 |
| + vitamins A, D, K | 4 | 28 | 4 | 14 |
| + water-soluble vitamins except niacin | 12 | 64 | 5 | 8 |
| + 1.0 mg % niacin | 5 | 29 | 6 | 21 |
| + 0.5 g % methionine | 7 | 45 | 11 | 24 |
| + 40 mg % D,L-α-tocopherol | 14 | 87 | 0 | 0 |
| + 0.5 g % methionine, 0.5 g % tryptophan, 10. mg % niacin | 22 | 123 | 0 | 0 |

stimulation of growth was observed only with the combined supplement of niacin, tryptophan and methionine. There were no differences in fertility, the ability to complete pregnancy and produce live young, or the number of live young as a function of the dietary regimens.

*Discussion and Summary.* Each of the four nutrients found to be associated with a protection against congenital cataract formation in this study has been implicated previously as being necessary for normal lens health and development. Ferguson, Atkinson and Couch (4), and Ferguson, Rigdon and Couch (5) described a cloudiness in the central portion of the lens in embryos from eggs laid by turkey hens fed an all vegetable protein diet without added vitamin E. The diet consisted of soybean oil meal, ground yellow corn and ground milo with supplements of minerals and selected vitamins. Associated with the lens disorder was a high embryonic mortality. Supplementation with α-tocopheryl acetate (20 mg/lb) prevented the eye anomalies and restored normal hatchability rates. In mammals, a deficiency of vitamin E has been associated with resorption of the young. We have been able to find only two reports (6, 7) where eye abnormalities were reported as a consequence of a low intake of vitamin E by rats during gestation and in neither case were cataracts specifically noted.

The interaction of tryptophan and niacin in producing congenital cataract was studied by Pike (8). Her basal diet consisted of acid-hydrolyzed casein, 14.7%; sucrose, 15%; cornstarch, 42%; Brewer's yeast, 4.3%; salts, 2%; agar, 2%; Crisco, 15%; and cod liver oil, 5%, and was supplemented with 0.15 g of cystine and 10 mg of nicotinic acid/100 g diet. Quantities of tryptophan varying from 0.01 to 0.2% were added to the basal diet to determine the requirement for reproduction. Of 11 young selected at random from female rats fed diets containing 0.01–0.025% tryptophan, 7 had cataract. No cataracts were seen in 10 young when the female rats received a 0.2% tryptophan diet. Omission of the nicotinic acid supplement from the latter diet resulted in the observation of 3 cataractous offspring from among 11 examined. One may conclude that tryptophan is probably directly involved in biochemical alterations which yield congenital cataract. The direct involvement of nicotinic acid may be more difficult to establish in that when nicotinic acid is the limiting dietary nutrient, the cataract may actually arise from the depletion of available tryptophan for nicotinic acid synthesis. A low intake of tryptophan has been observed to induce cataract in post weaning rats (9, 10), pigs (11), and guinea pigs (12).

Studies on the influence of methionine deficiency seem to be even more sparse, despite the fact that the importance of sulfur compounds in lens structure makes it a logical candidate for investigation. McLaren (3) presented limited data suggesting that a deficiency of protein in general and sulfur amino acids in particular are of importance in the intrauterine development of the eye. The limitations necessary to produce the observed changes, however, also markedly diminished the fertility of the female rat.

The data of the current study suggest that a multistep process is involved in the development of cataract. Tryptophan would seem to be the key nutrient of the group tryptophan–methionine–niacin. As noted earlier, it has been shown that an inadequate intake of this amino acid will result in cataract appearance in both weanling and newborn rats and neither methionine nor niacin supplements alone were effective in cataract prevention in our studies. The level of tryptophan in our standard diet was not low enough to yield cataract, however, unless there was also a relative insufficiency of tocopherol. It seems reasonable to suggest that the low vitamin E diets employed by Ferguson, Atkinson and Couch (4) and Ferguson, Rigdon and Couch (5) might also have been low in tryptophan considering the protein sources cited. Dische (13) has studied the pathogenesis of the three main types of congenital cataract; that is, sugar cataracts, X-ray cataracts, and nutritional deficiency (tryptophan restricted) cataracts and has concluded that there are two stages in the appearance of the opacity. The first stage involves the inhibition of protein synthesis and the consequent disruption of the normal interactions between fibers. Loss of transparency appears to occur in the second stage when insoluble proteins arise following the formation of disulfide links and other oxidative processes. If $\alpha$-tocopherol is acting to inhibit or retard a secondary oxidative step in the development of lens opacity, it might be worthwhile to explore the potential benefit from dietary supplements of this compound in the prevention or amelioration of senile cataract and support would be lent to the theory that the role of vitamin E is that of an intracellular antioxidant.

One must still speak of the question of the protective benefit of the control diet employing casein as the sole protein source in place of the vegetable proteins (1). According to the analytical data, both diets were equal in their content of methionine, tryptophan and niacin. Evaluations of the protein quality and digestibility of the two diets have failed to reveal any striking differences (14). It does appear, however, that the tocopherol content of the casein-based diet was inadvertently elevated when cottonseed oil was added (1.6%) to the casein diet to restore the lipid calories provided by the cashew nut flour in the vegetable protein diet. Based upon current estimates of the $\alpha$-tocopherol content of cottonseed oil, this could have raised the $\alpha$-tocopherol level of the diet to 1 to 2 mg/100 g, a value only slightly below the suggested NRC requirement for gestation of 3 mg/100 g.

Regardless of the interpretation of the data presented here, it does seem advisable to suggest that persons working in applied nutrition programs using vegetable protein blends be alert to the relative intakes of tocopherols, tryptophan, sulfur amino acids and niacin and to the potential for congenital lesions of the lens should these nutrients be provided in suboptimal quantities.

1. Chaves, N., Barreto, S. P., Leal, M. R. B. P., Lapa, A. G., Costa, L. P., Mayer, R., and Costa, S., Proc. Int. Congr. Tropical Med. Malaria, 7th **4**, 13 (1964).

2. National Academy of Sciences–National Research Council Publication 990, "Nutrient Requirements of Domestic Animals", No. 10, Washington, DC (1962).

3. McLaren, D. S., "Malnutrition and the Eye." Academic Press, New York (1963).

4. Ferguson, T. M., Atkinson, R. L., and Couch, J. R., Proc. Soc. Exp. Biol. Med. **86**, 868 (1954).

5. Ferguson, T. M., Rigdon, R. H., and Couch, J. R., AMA Arch. Ophthalmol. **55**, 346 (1956).

6. Callison, E. C., and Orent-Keiles, E., Proc. Soc. Exp. Biol. Med. **76**, 295 (1951).

7. Cheng, D. W., Chang, L. F., and Bairnson, T. A., Anat. Rec. **129**, 167 (1955).

8. Pike, R. L., J. Nutr. **44**, 191 (1951).

9. Curtis, P. B., Hauge, S. M., and Kraybill, H. R., J. Nutr. **5**, 502 (1932).

10. Totter, J. R., and Day, P. L., J. Nutr. **24**, 159 (1942).

11. Cartwright, G. E., Wintrobe, M. M., Buschke, W. H., Follis, R. H., Jr., Suksta, A., and Humphreys, S., J. Clin. Invest. **24**, 268 (1945).

12. Von Sallmann, L., Reid, M. E., Grimes, P. A., and Collins, E. M., AMA Arch. Ophthalmol. **62**,

662 (1959).

13. Dische, Z., "Biochemistry of the Eye, Symposium," p. 413. Tutzing Castle, Aug. (1966).

14. Chaves, N., Barros, M. M. R., Madruga, I., Lapa, M. A. G., Freitas, C. P., de Lima, J. A. L., and da Costa, L. P., Rev. Brasil. Med. **19**, N. 7 (1962).

15. Graham, C. E., Smith, E. P., Hier, S. W., and Klein, D., J. Biol. Chem. **168**, 711 (1947).

Received Mar. 24, 1972. P.S.E.B.M., 1972, Vol. 140.