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Effects of dietary deficiency of selective amino acids on the function of the cornea and lens in rats

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Summary. Effects of dietary deficiencies of tryptophan and methionin on the transparency of cornea and lens were investigated in young rats (Brown-Norway, BN; Sprague-Dawley, SD) over 3 months. Transparency of the cornea and lens were evaluated in weekly intervals using a photo-slitlamp microscope. After sacrifice and lens fresh weight determination the lenses were prepared for histopathology. Methionin deficiency had no effect on the parameters investigated. Tryptophan deficiency caused severe loss of body weight in both strains, with additional loss of hair in SD rats. These developed corneal neovascularisations and cataracts. BN rats showed an enhanced zone of discontinuity in the lens. Diet intermission arrested the pathological processes in the eye which restarted when feeding the diet again. This observation is supported by lens fresh weight data. DNA staining evidenced that tryptophan deficiency arrested lens fiber maturation in both strains but stimulated corneal neovascularisation only in SD rats.

Keywords: Tryptophan/methionin deficiency – Corneal/lens transparency – Cataract – Albino / pigmented rats – Fiber cell maturation

Introduction

The transparency of cornea and lens is an essential prerequisite for the complex visual function of the vertebrate eye. Both tissues are completely avascular, thus needing a constant supply of nutrients from the tearfilm and the aqueous humour respectively. The eye lens is the only organ of the vertebrate body which grows throughout life. Therefore a constant supply of nutrients, among them also amino acids (AA), from the aqueous humour is needed to maintain a proper lens protein synthesis which is the basis for its transparency. Blindness due to corneal opacification and cataract are still among the major health problems in "Third World" countries but surgical intervention to restore vision has failed to keep pace with this problem, so that there is an increasing backlog of blind patients needing surgery (Minassian and Mehra, 1990; Thylefors, 1992). To help reduce this problem, a better understanding of nutritional deficiencies and their role in the multifactorial process of cataract development and in corneal opacification is urgently needed.

Interventional studies in rats have demonstrated that deprivation of the entire set of amino acids needed for the synthetic machinery of the lens causes corneal neovascularization but does not induce cataract formation before this condition becomes life threatening (Hall et al., 1946). Deficiency of selective essential amino acids, in contrast, has been reported to lead to a very rapid formation of corneal neovascularisation and cataract in rats (Ohrloff et al., 1978). Among the most indispensible amino acids are L-tryptophan, L-phenylalanin, and L-histidine (Hall et al., 1948). The selective elimination of tryptophan from the diet has been found to cause the formation of nuclear cataracts in about 3 weeks (Bunce et al., 1978; Ohrloff et al., 1978). Tryptophan is an essential constituant of the lens crystallins and due to its aromatic ring it is important for the absorption characteristics of the lens in near UV-radiation. Methionin is one of the main sources of sulfhydryl groups for the lens which are an essential part of its defense system against oxidative damage.

Most of the experiments on nutritional deficiencies reported so far, have been performed in albino rats,

which are not the best model because of the absence of pigmentation in the eye. This leads to a significantly higher amount of light which hits the sensitive retinal rods of these nocturnal animals, potentially causing retinal damage which through liberation of lipid peroxides into the vitreous could also affect the posterior surface of the lens (Breadsell et al., 1994). In addition to the lack of pigmentation, the enzymes involved in the melanin synthesis are missing in albino animals. Also the effect of (undesired) storage of compounds (e.g. drugs) due to their melanin affinity is absent (Hockwin et al., 1992). Several author have reported evidence that the albino eyes react differently to several external influences or noxious factors than the pigmented eye (Eiben und Wegener, 1995). Therefore the effects of tryptophan and methionin deficiency on the maintenance of transparency of the cornea and lens was studied in female albino (Sprague-Dawley, SD) and female pigmented (Brown-Norway, BN) rats.

Methods

Young female Sprague-Dawley and Brown-Norway rats with an initial body weight of 80–90 g (SD) and 70–80 g (BN) were obtained from Charles-River Wiga (Sulzfeld, Germany). Standard lab chow for rats was obtained from Altromin® (Lage, Germany) and diets either highly deficient in tryptophan or in methionin were prepared by the same manufacturer. Regular and amino acid deficient diets were caloricly similar. The animals were housed in type 4 Macrolon® cages in groups of 6 in non-airconditioned rooms with an artificial 12 hours day/night rythm. Regular as well as deficient diets and water were fed ad libitum. Body weight and food consumption were controlled in weekly intervals. Alterations in the transparent media of the anterior eye segment, cornea and lens as well as the potential appearance of a Tyndall effect in the aqueous humor were monitored and documented with a photo-slitlamp microcsope. With the exception of group 4, diets were fed in both strains for 3 months. The treatment schedule is shown in figure 1. Groups 7 (SD) and 8 (BN) were fed the tryptophan deficient diet for 2 months, followed by a 1 month period on regular diet and another month on tryptophan deficient diet afterwards. Following the final ophthalmological examination, the animals were sacrificed by inhalation of CO_2, their lenses excised surgically and the lens wet weight determined on a Mettler microbalance. Thereafter lenses were fixed in 4% paraformaldehyde, dehydrated in ethanol and embedded in Technovit®. Histochemical staining for DNA was performed with Hoechst 33258. Handling of the animals and all experimental procedures were in compliance with the German Tierschutzgesetz.

Results

The essential difference between the 2 deficiency treatments was that methionin deficiency did not affect any of the parameters investigated in this study. All animals on this diet, BN (gr. 6) and SD (gr. 5), continued to gain body weight normally (Fig. 2), their corneae and lenses remained perfectly transparent and the lens fresh weight was normal (Fig. 3). These groups behaved like the control groups (grs. 1 + 2).

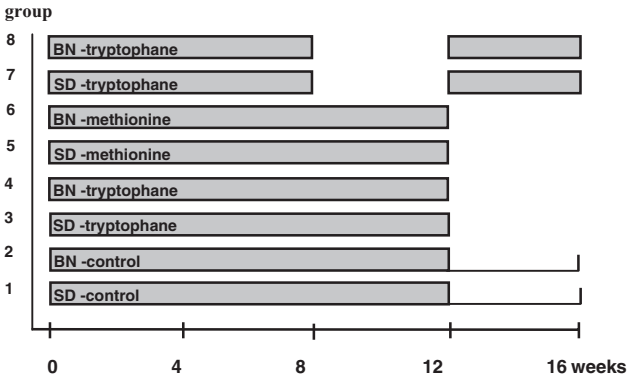

Fig. 1. Treatment schedule for all groups of the 16 weeks study

Tryptophan deficiency, in contrast, seriously affected all parameters investigated. Food consumption of SD rats normally is about 50% higher than of BN rats. In both strains, food consumption under tryptophan deficient diet dropped to about 1/3 of the regular intake. BN and SD rats stopped to grow and gradually lost body weight (Fig. 2). After about 2 months, the deficiency became life-threatening for the SD but not for the BN rats. In addition, SD rats almost completely lost their fur. Inspite of the severe starvation effects, the attentive behaviour and mobility of the rats of both strains were unaffected.

The eyes in both strains also reacted very differently to the deficiency: SD rats showed severe corneal clowding and stromal neovascularization from the limbal area. In some cases also leakage of the newly formed vessels with bleeding into the corneal stroma could be observed (Fig. 4). The corneas of the BN rats in contrast remained unaffected with only minute capillaries protruding into the peripheral stroma after 3 months. Lenticular changes were also markedly different between SD and BN rats. SD rats developed whitish striated opacities on the posterior lens surface (Fig. 5) after about 4 weeks which spread into the lens cortex and also affected the anterior suture system (Fig. 6). Intermission of the tryptophan deficient diet very rapidly lead to an increase of body weight, reappearance of the fur and an arrest of ophthalmopathological reactions. Corneal vascularizations regressed and the stroma cleared. Cataractous changes in the lens cortex condensed and it became obvious

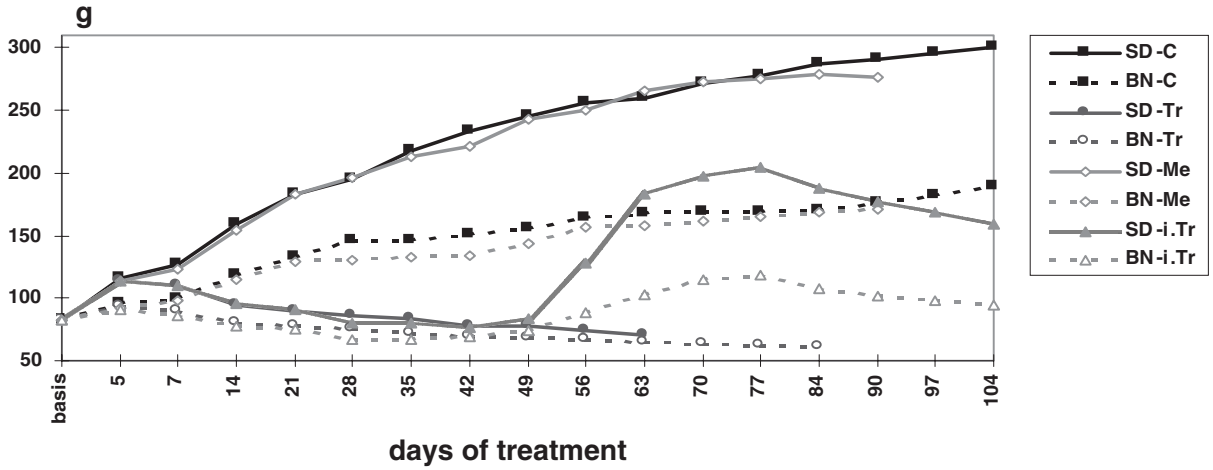

Fig. 2. Mean body weight (*g*) of all groups over the diet treatment period

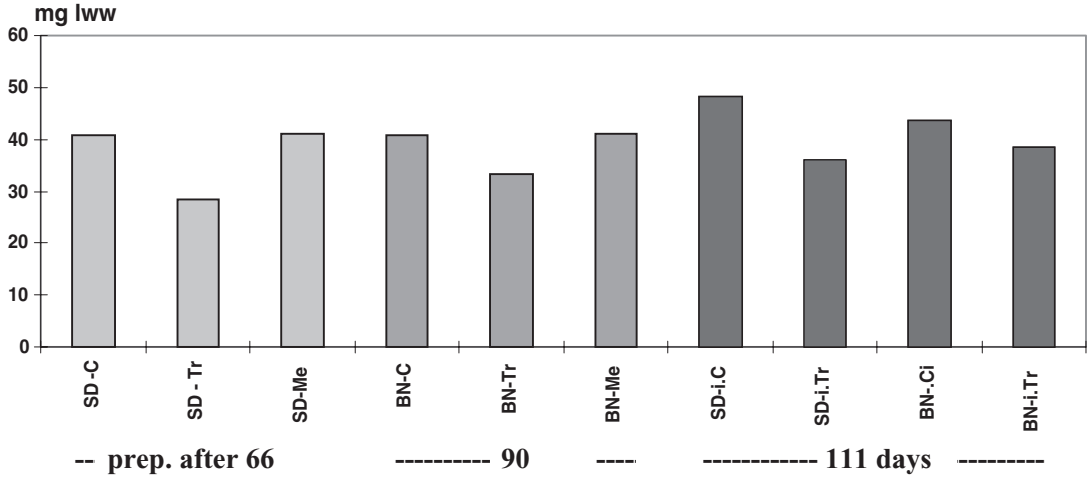

Fig. 3. Lens fresh weight (*mg*) determined immediately after excision of the lens

that transparent newly formed fiber cells covered the cataractous layers of the cortex (Fig. 7). Reinstallation of the diet treatment after 1 month again triggered the effects described above.

The lenses of BN rats in contrast remained transparent until about 2 months, when a demarkation of the outer zone of discontinuity became visible. This remained the only change in optical quality which could be observed in the pigmented rats over 3 months diet treatment.

Histochemical staining for DNA in the nuclei of the epithelial and superficial fiber cells showed a regularly formed nuclear bow area in the lenses of the control groups and the methionin deficient animals, characterized by disapperance of stainable nuclear DNA in the deeper fiber cells (Fig. 8a). In the bow area of the lenses from tryptophan deficient animals, however, nuclear DNA remained stainable deep into the lens cortex (Fig. 8b). Diet intermission restarted the regular fiber cell denucleation process, evidenced by a clear interval between the outer part of the lens bow and the deeper lens cortex with fragmented nuclei.

Discussion

The data from the 2 rat strains (SD + BN) presented here evidence marked differences in reaction to a severe nutritional deficiency of tryptophan. Among the more general effects e.g. on mean body weight development, both strains, albino and pigmented, showed similar reactions, but the rapid loss of hair in SD rats pointed to a strain specific lower tolerance

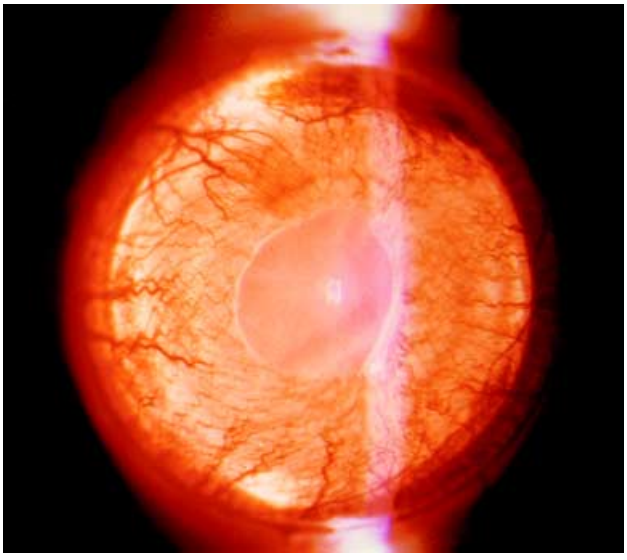

Fig. 4. Slitlamp micrograph of a SD rat eye demonstrating extended neovascularization of the cornea. In addition leakage of blood into the corneal stroma can be observed

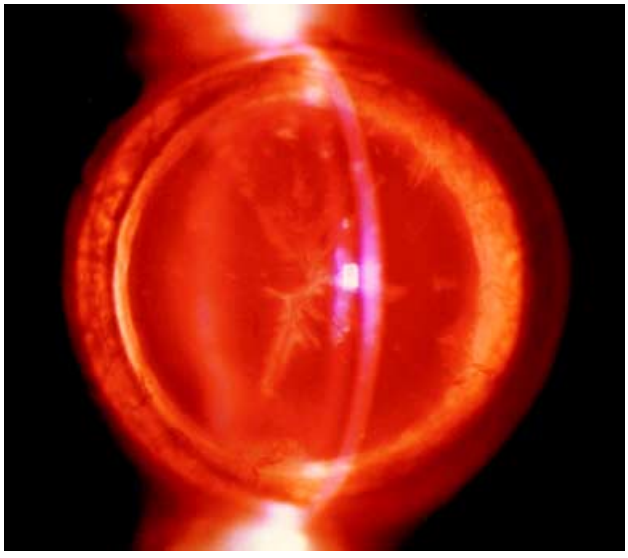

Fig. 6. Slitlamp micrograph of a SD rat eye with spoke-like cataracts also affecting the anterior suture system

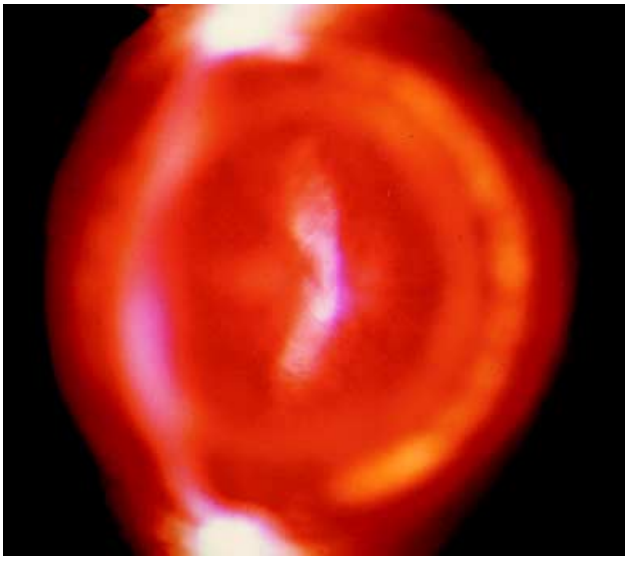

Fig. 5. Slitlamp micrograph of a tryptophan-deficiency induced subcapsular cataract appearing as striations on the posterior lens capsule

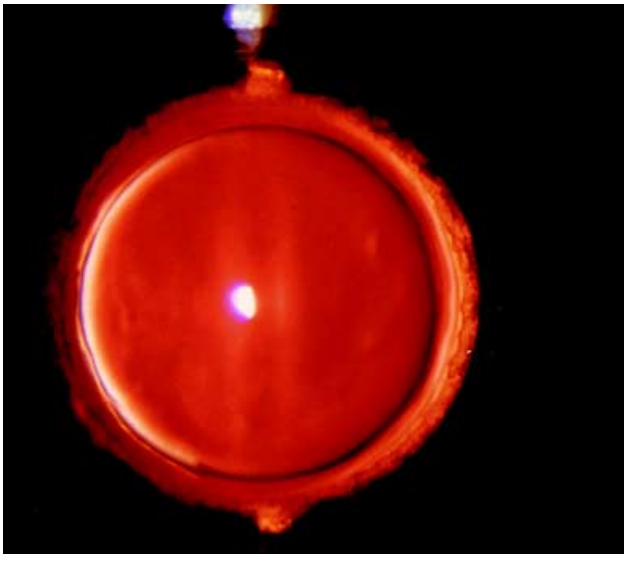

Fig. 7. Slitlamp micrograph of a SD rat eye showing a clear zone of newly formed fiber cells overlying a zonular cataract in the lens cortex

towards this dietary condition. Inspite of the fact that both, the normal and the deficient diet were caloricly similar, animals on diet from both strains showed severe starvation effects in their body weight. The reactions in the ocular tissues, cornea and lens, again evidence marked strain differences. Transparency of the cornea and lens in BN rats remained almost unaffected, in contrast SD rats developed complex pathological reactions involving cornea, iris and lens. In our study these reactions developed on a time scale of 4–6 weeks, which differs from the data published by Ohrloff anf coworkers (1978). They reported 21 days for complete corneal vascularization and opacity, which could be potentially due to traces of tryptophan in some protein constitutants in our diet. There is an obvious difference in reaction between cornea and lens to tryptophan deficiency. Neovascular events in the corneal stroma with accidental leakage of new blood vessels point to an inflammatory reaction cascade triggered by the deficiency. Occasional adhesions

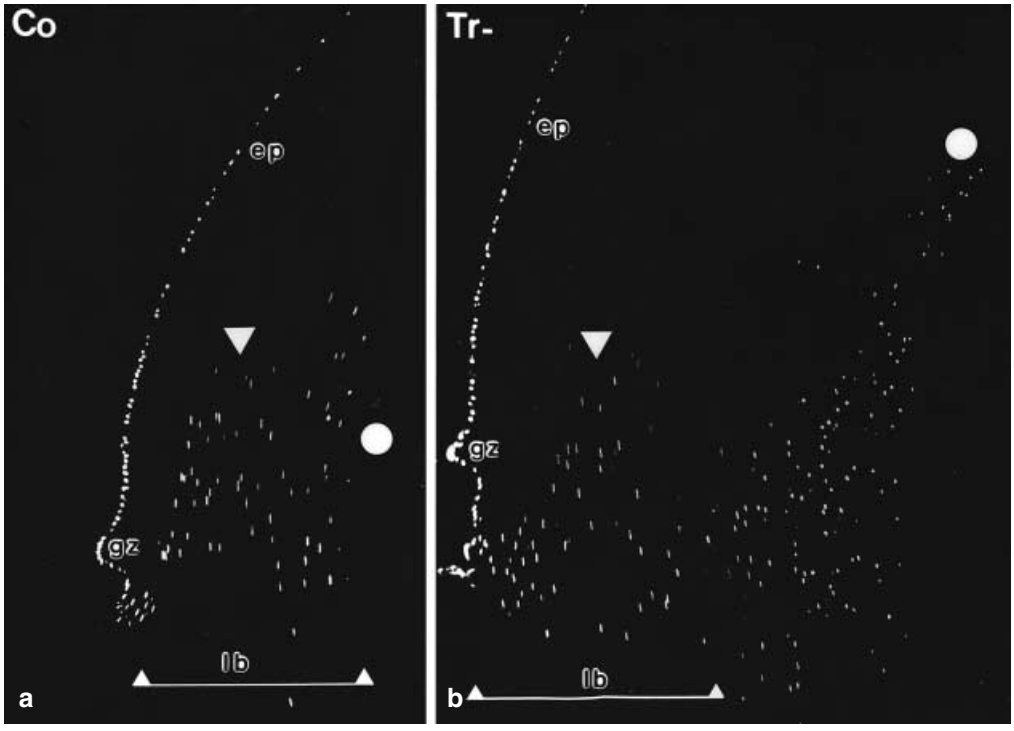

Fig. 8. **a** Histological section of the equatorial bow of a normal lens. The cell nuclei have been stained for DNA; **b** equatorial lens bow of a tryptophan deficient animal, DNA stained nuclei extend far into the lens cortex, the diameter of the bow area corresponds to that part of the lens cortex which has been formed during diet treatment. *Co*, control group; *Tr-*, tryptophan deficient animals; *ep*, epithelium; *gz*, germinative zone; *lb*, lens bow. White arrowhead = normal fiber cell nuclei. White circle = fragmented fiber cell nuclei

of the posterior sheet of the iris to the anterior lens capsule, which have been found only in albino eyes, support this assumption.

In the lens, however, no angiogenetic effects from the iris are found, but the deficiency interfers with fiber cell maturation, as evidenced by the persistent fiber cell nuclei, though with fragmented DNA, deep in the lens cortex. This observation is in line with the data published by MacAvoy and coworkers in 1979, who already described alterations in the germinative zone of the epithelium and the nuclear bow area. Our observations from the animals in groups 7 and 8 clearly demonstrate, however, that this arrest of fiber cell maturation is a temporary phenomenon. Maturation of lens fibers, even of those deeper in the cortex, continues as soon as tryptophan is available again. This observation is coroborates with the lens fresh weight data, where mean values of the lenses from animals on intermittant diet are in between the fresh weight of normal lenses and those on permanent diet. It remains to be elucidated, however, why maturation interference triggers cortical cataract formation only in albino eye lenses although maturation interference has been observed in SD + BN rats.

Biochemical investigations by Bunce et al. (1978) and Koch et al. (1982) showed effects of tryptophan deficieny on β-crystallins synthesis, but further investigations will be needed, to shed more light into the complex process of gene activation and suppression during fiber cell elongation and maturation which obviously is influenced by tryptophan deficiency. The data from this study in line with the literature data (Taylor, 1999) demonstrate the complexity of nutritional factors in their effects on the eye together with the influence of racial variation which also are involved in the human situation in "Third World" Countries (Minassian and Mehra, 1990).

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