ELECTRON MICROSCOPIC STUDY ON THE LENS OF RIBOFLAVIN-DEFICIENT ALBINO RAT

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Summary Although cataract was recognized as a typical symptom of ariboflavinosis soon after the discovery of riboflavin, some reports appeared thereafter which denied its occurrence. In the present study electron microscopic examination of the lens of rat fed on synthetic riboflavin-deficient diet revealed swelling degeneration of mitochondria and vacuolation of cytoplasm of the epithelial cells of the lens. These changes are similar to those observed in the initial period of cataract caused by other agents.

Some of the earlier studies on riboflavin deficiency in animals recognized cataract as one of the typical symptoms of ariboflavinosis. SALMON et al. (1) reported “opaque” eyeball in young rats suffering from ariboflavinosis and ascribed this opacity to that of the lens or the glass body. Subsequently, DAY et al. (2) and O'BRIEN (3) described the occurrence of cataract in riboflavin-deficient young rats. DAY and LANGSTON (4) reported 100% occurrence of cataract in riboflavin-deficient young rats. YUDKIN (5) found that cataract can be provoked in riboflavin-deficient young rats but not in adult rats. In addition, BOURNE and PYKE (6) observed only 31% occurrence of cataract in rats of 70 g body weight fed on riboflavin-deficient diet.

On the other hand, GYÖRGY (7), BESSEY and WOLBACH (8) and SUDA (9) reported that cataract cannot be detected in riboflavin-deficient rats, even though several other symptoms of the eye such as neovascularization can be observed. In this connection, the observation of BAUM et al. (10) should be pointed out. These workers reported that cataract cannot be observed in rats fed on diet completely deficient in riboflavin, but was observed frequently in animals fed on a diet which contained minute amounts of riboflavin. SHAW and PHILLIPS (11) reported the occurrence of cataract in rats fed on riboflavin-deficient diet which contained only carbohydrate or fat as basal nutrient. GERSHOFF et al. (12) ob-
served cataract in cats receiving low carbohydrate and high fat diet containing riboflavin below their minimal requirement.

The results cited above suggest that the grade of deficiency as well as the components of the nutrients in the diet could influence the occurrence of cataract in ariboflavinosis. In light of these observations, a detailed examination of the morphological changes in the lens of rats fed on well defined diets becomes important. In the present study, therefore, ariboflavinosis was produced by using a synthetic diet, and histological observations were made by using an electron microscope.

MATERIALS AND METHODS

Twenty-day-old male albino rats of Wistar strain (body weight about 50 g) were used. The animals were housed in individual cages in a room maintained at 25°C under constant humidity and were divided into 2 groups, control group and riboflavin-deficient group. Thirty animals were used for the control group and 50 for the riboflavin-deficient group. The basal diet was essentially the same as that described by FORKER and MORGAN (13). Riboflavin-free casein used as protein source was prepared as follows. Casein was dissolved in 1% NaOH, allowed to stand at room temperature for 1 hr, and sedimented by adjusting the solution pH to 4.6 with 20% acetic acid. The precipitates were washed 3 times with distilled water, once with 60% ethanol, and once more with 100% ethanol, and dried in the dark. The preparation thus obtained contained no riboflavin when determined by lumiflavin fluorescence method of YAGI (14). The daily supplement of B vitamins to each rat was, thiamine 5 μg, pyridoxine 5 μg, folic acid 5 μg, Ca-pantothenate 25 μg, p-aminobenzoic acid 25 μg, myoinositol 625 μg and choline chloride 1.25 mg. In addition, the controls received 10 μg riboflavin daily. A mixture of fat-soluble vitamins consisting of 10 I.U. of A, 1 I.U. of D and 0.1 mg of α-tocopherol was administered daily. These vitamins are given per os compulsorily.

The observation of the cornea and lens was made with slit-lamp.

After 4 weeks on the experimental diet, the animals under anesthesia with ketamine (2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone) were killed by decapitation and the eyeballs and the liver were rapidly removed. The total riboflavin content of the liver was determined by lumiflavin fluorescence method of YAGI (14).

For light microscopic observation, the cornea was taken, fixed in 10% formaldehyde, embedded in paraffin and sectioned by means of a microtome. The section was stained with hematoxylin-eosin. The specimens for electron microscopy were made as follows. The excised eyeball was pre-fixed in 2.5% glutaraldehyde-phosphate buffer (pH 7.4). After cutting the eyeball at equatorial zone in the medium, the lens was removed and its cortex was divided into three parts;
anterior zone, equatorial zone and posterior zone. These parts were double-fixed in 2.5% glutaraldehyde-phosphate buffer (pH 7.4) and in 1% osmic acid-phosphate buffer (pH 7.4) and embedded in Epon 812 after serial dehydration with ethanol. Ultra thin sections were obtained by means of a Poter-Blum type microtome with a glass-knife and stained with uranyl acetate and Pb. All specimens were examined in a Hitachi HU-11 electron microscope operated at 50 kV.

RESULTS

The growth rate of the rats is shown in Fig. 1. The rats fed on riboflavin-deficient diet failed to grow.

![Graph showing growth curve of rats fed on riboflavin-deficient diet. I: Riboflavin-deficient rats (n=50); II: controls (n=30). The values of M are given.](image)

Fig. 1. Growth curve of rats fed on riboflavin-deficient diet. I: Riboflavin-deficient rats (n=50); II: controls (n=30). The values of M are given.

After 4 weeks of feeding on the experimental diets, the content of riboflavin in the liver was 27.0±6.7 and 13.9±4.4 μg/g wet tissue for the control and the riboflavin-deficient groups, respectively.

Rats fed on riboflavin-deficient diet, showed characteristic symptoms of arbofavinosis, such as rough hair or loss of hair (Photo. 1), inflammation of the eyelids and conjunctivas. Neovascularization in the cornea was also observed (Photo. 2), and it was confirmed by light microscopic observation that this vascularization occurred in the parenchyma of the cornea (Photo. 3). The opacity of the lens was not observed with naked eye, even by the slit-lamp examination. There were also no remarkable changes in light microscopic appearance.

The results of electron microscopy of the lens are shown in Photos. 4–7. Photograph 4 shows the electron micrograph of the epithelial cells of the lens near the equatorial zone of normal animal. There was uniform distribution of many mitochondria, endoplasmic reticulum and Gorgyi-body. In the case of
riboflavin-deficient animals swelling degeneration of mitochondria and vacuolation of cytoplasm were observed in the same area of the lens (Photo. 5). In the cells near the cortex, however, normal shape of mitochondria was still observed even in rats suffering from ariboflavinosis. It should be noted that the changes were observed in the superficial part of the lens. Photograph 6 shows the presence of vesicular substances which might be some degenerated cellular material. They appear to be displaced leading to the appearance of vacuoles. Photograph 7 shows the electron micrograph of the superficial layer of the posterior cortical cell, showing a rough appearance of the cytoplasm with many vesicles and vacuoles.

Thus, it appears that the swelling degeneration of mitochondria and vacuolation of cytoplasm of the epithelial cells of equatorial zone were notable among the early changes in the lens of rats suffering from ariboflavinosis.

DISCUSSION

The present investigation clearly demonstrates that the lens of rats fed on riboflavin-deficient synthetic diet showed only a slight histological change, even though these rats displayed typical symptoms of ariboflavinosis, and decrease in flavin concentration in the liver.

The slight, but definite histological changes revealed by electron microscopic observation, are similar to those of the initial changes observed in the lens of rabbits irradiated with X-ray (15,16), β-ray (17,18) or fast neutron (19). The fact described by Usui (18) that the vacuolation of the lens proceeds from the surface to inside of the epithelial cell of the lens, was also true in the case of riboflavin-deficient rats.

The initial changes typical of the cataract mentioned above as well as those caused by the administration of naphthalene (15,20,21), dithizone (15,22), alloxane (22) or streptozotocin (23) are equally characterized by both the swelling of mitochondria and vacuolation of the epithelial cells of the lens. Thus, the presently observed changes in the lens can be considered as the initial changes observed in the lens, when cataract is being provoked. Accordingly, it is obvious that ariboflavinosis caused under the present experimental conditions provoked only initial changes characteristic of cataract in the lens.

As mentioned in the introductory part of this paper, typical cataract was described in the earlier reports on riboflavin deficiency. Therefore, the present experimental condition is less favorable for provoking cataract than that described in the older reports. Some of the earlier observations might be ascribed to the incomplete deficiency of riboflavin in the diet and/or simultaneous deficiency to some extent of other vitamins in the diet. The report that feeding of a minute amount of riboflavin provoked cataract to a greater extent (10), supports this view.

All these results imply that metabolic disorder caused by incomplete deficiency of riboflavin (or probably combined deficiency in vitamin B complex) is
probably correlated with the manifestation of cataract. The report that riboflavin deficiency with unbalance of the basal diet provokes the cataract (11,12) coincides with this view.

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LEGENDS FOR PHOTOGRAPHS

Photo. 1. The feature around the eyelids of rat suffering from riboflavin deficiency.
Photo. 2. Neovascularization in the cornea of rat suffering from riboflavin deficiency.
Photo. 3. Light microscopic observation on the corneal vascularization of rat suffering from riboflavin deficiency.
Photo. 4. Electron micrograph of the epithelial cells of the lens of normal rat. The specimen was made from the lens near the equatorial zone. Bar shows 1 μ. AC: anterior capsule.
Photo. 5. Electron micrograph of the epithelial cells of the lens of riboflavin-deficient rat. The specimen was made from the lens near the equatorial zone. Bar shows 1 μ. AC: anterior capsule.
Photo. 6. Electron micrograph of the epithelial cells of the lens of riboflavin-deficient rat. The specimen was made from the lens near the equatorial zone. Bar shows 1 μ. AC: anterior capsule. Arrows show the vesicular substance.
Photo. 7. Electron micrograph of the superficial layer of the posterior cortical cells of the lens of riboflavin-deficient rat. Bar shows 1 μ. PC: posterior capsule.