Reports

Essential fatty acid deficiency and photoreceptor membrane renewal—a reappraisal. ROBERT E. ANDERSON.

The earlier reports from this laboratory that the renewal of rat photoreceptor membranes was dependent on essential fatty acids have not been confirmed in more recent studies.

The lipids of rod outer segments (ROS) contain high levels of long-chain polyunsaturated fatty acids.1 These fatty acids, which cannot be synthesized de novo by animals, are formed by the elongation and desaturation of essential dietary precursors. Since rat ROS are renewed every 9 to 10 days,2 Futterman et al.3 and our laboratory4 attempted to take advantage of the renewal process to reduce the high levels of polyunsaturates in the ROS of rats. To our surprise, only slight decreases in the polyunsaturated fatty acid content of rat ROS4 or whole retinas3 were found in animals deprived of essential fatty acids (EFA) for 10 or 41 weeks, respectively. In searching for an explanation for the mechanism of conservation of these fatty acids, we tested the possibility that polyunsaturated fatty acids are necessary for renewal of photoreceptor membranes by new disc formation. Our initial results from autoradiographic,5 phagosome density,6 and biochemical6 studies indicated that this was indeed the case; the turnover of ROS in EFA-deficient rats appeared to be very slow compared to controls. However, in more recent studies, we have been unable to confirm these earlier observations. A typical experiment is described below.

Three-week-old weanling albino rats were raised under cyclic fluorescent lighting (12L:12D) on an EFA-deficient diet alone or containing the following fatty acid supplements: (1) 0.9% 18:1o9 (a nonessential fatty acid which served as an isocaloric control) or (2) 0.45% 18:2o6 plus 0.45% 18:3o3. After 12 weeks on their respective diets, each animal was injected with 60 μCi of tritiated leucine per gram body weight; the eyes were removed at various times and fixed for autoradiography,5 or ROS were prepared6 for the determination of the specific activity of rhodopsin by polyacrylamide gel disc electrophoresis.7 Analysis of the lipids of plasma and adipose tissue from rats fed the three diets indicated that the two groups denied EFA were chemically deficient in EFA.8

Fig. 1, A to C, are autoradiograms of the retinas of rats 7 days after injection of the radioisotope. Fig. 1, A, is from an EFA-deficient rat fed no supplement, Fig. 1, B, is from an animal supplemented with 18:1o9, and Fig. 1, C, is from an animal supplemented with the essential fatty acids 18:2o6 and 18:3o3. In each case there is a band of silver grains near the apical tips of the ROS indicating renewal of membranes by new disc formation. The rate of renewal is the same in each group.

Disc electrophoresis of isolated ROS showed most of the radioactivity to be in rhodopsin. Plots of the specific activity of rhodopsin vs. time were compatible with a 9 to 10-day turnover of rhodopsin in the membranes.

Experiments intended to duplicate as closely as possible the conditions of our earlier studies produced results identical to those reported above. Varying such parameters as light intensity (60 vs. 6 foot candles), source of animals (Texas Inbred vs. Holtzman), or time of injection after the onset of light (2 vs. 10 hr) had no effect on the labeling pattern.

The reasons for the difference between these and our earlier data are not obvious. Although remote, one possibility is that the diets fed in our earlier studies lacked some essential nutrient other than EFA. Whatever the explanation, however, it is clear from our present data that EFA deficiency of 12 weeks' duration does not alter renewal of rat ROS by changing the rate of new membrane formation.

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REFERENCES

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Fig. 1. Autoradiograms of retinas of rats 7 days following injection of tritiated leucine. The animals were raised from 3 to 15 weeks of age on the following diets: (A) EFA-deficient, (B) EFA-deficient plus 0.9% 18:1a9, a nonessential fatty acid, and (C) EFA-deficient plus 0.45% 18:2a6 and 0.45% 18:3a3, both EFA. The bands of silver grains near the apical tips of the ROS are indicative of membrane renewal by new disc formation. (×975.)

The present study describes a model of multifocal septic choroiditis with serous retinal detachment after intracarotid injection of Staphylococcus aureus or Streptococcus faecalis. The fundus lesions occurred mainly in the tapetal area and, on ophtalmoscopic examination, were more extensive after S. aureus than after S. faecalis injection. On histopathologic examination there were microabscesses in the inner choroid and subretinal space, disrupting the outer retina but sparing the inner retina.

There are only a few reports in the literature on septic choroiditis. In several cases of human septicemia Friedenwald and Bones1 and Dienst and Gartner2 found foci of choroiditis without overlying retinal changes on pathologic or clinical examination. Foci of choroiditis with minimal serous detachment of the retina have been observed after injection of Histoplasma capsulatum into the carotid artery of rabbits or intravenously in dogs.3 4 This study describes a model of septic choroiditis with secondary serous detachment of the retina after intracarotid injection of bacteria in dogs.

Material and methods. Nineteen adult dogs, weighing 10 to 20 kg, were used in this study. The dogs were anesthetized with intravenous sterile sodium pentobarbital, 30 mg/kg body weight. The carotid artery was isolated through a vertical incision in the neck, and a 25-gauge needle was inserted. The needle was connected to a polyethylene tube. A bacterial or a control suspension was then injected slowly over 10 to 15 min.

The bacterial suspension was prepared as follows. The same strain of Streptococcus faecalis or American Type Culture Collection strain 25923 coagulase-positive Staphylococcus aureus was cultured in either 30 ml of the injected dog’s own plasma or 30 ml of trypticase soy broth at 37°C for either 5 or 16 hr. The resulting bacterial suspensions were diluted to 120 ml with sterile normal saline just prior to injection. The undiluted suspensions contained either 10^7-10^8 bacteria/ml (lower concentration, obtained from the 5 hr culture) or 10^9-10^10 bacteria/ml (higher concentration, obtained from the 16 hr culture). We used the urine colony count technique to determine the bacterial count. There was no difference in the bacterial count with trypticase soy broth or dog plasma used as the culture medium.

All 19 dogs were injected with bacterial suspensions, 14 with S. faecalis and five with S. aureus. Eleven of the 14 dogs injected with S. faecalis received the higher concentration (five cultured in tryptase soy broth, six cultured in the injected dog’s own plasma); three received the lower concentration (all three cultured in trypticase soy broth). Among the five dogs injected with S. aureus, four received the higher and one the lower concentration (all five cultured in trypticase soy broth).

Control suspensions consisted of 30 ml of either sterile trypticase soy broth or sterile plasma from the injected dog. A sterilized preparation of S. aureus was not used as controls because of the unknown effect of toxins from dead bacteria. Control suspensions were incubated at 37°C for the same duration as the bacterial suspensions and diluted to 120 ml with sterile saline just prior to injection. Control suspensions were injected into the carotid artery opposite to the side of bacterial injection. Five of the 19 dogs received control injections 3 to 7 days prior to the bacterial injection (sterile trypticase soy broth in three dogs, sterile autogenous plasma in two dogs). Eight of the 19 dogs received control injections at the same time as the bacterial injection (sterile trypticase soy broth in four dogs, sterile autogen-