The Effect of Vitamin A Deficiency on the Fine Structure of the Retina[1]

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Introduction

FOR MANY YEARS it has been known that severe vitamin A deficiency causes histological degeneration in the retina (Tansley, 1933; Johnson, 1939), and it has been suggested that this structural damage might be responsible for the long lasting or permanent effects of night blindness that persist after the deficiency has been relieved (Johnson, 1943; Wald, 1955). However, experiments to examine this point have been limited by the fact that vitamin A has a function in general body metabolism in addition to its specific function in the retina. When animals are maintained on a diet completely free of vitamin A, they lose weight and die soon after the onset of retinal degeneration (Johnson, 1943; Dowling and Wald, 1958).

Recently, we have found that vitamin A acid, when fed to rats on a vitamin A-free diet, keeps them healthy and growing normally, but does not support the visual cycle (Dowling and Wald, 1960). Thus, rats maintained on a vitamin A-free diet, supplemented with vitamin A acid grow normally, but gradually become extremely night-blind.

The result of one such experiment is shown in Fig. 1. After exhaustion of its stored vitamin A, the rat given no supplement lost weight rapidly and died. The rat receiving vitamin A acid grew normally and appeared healthy throughout the experiment. Electroretinograms of this animal after 5 months are shown at the right of the figure compared with those of a normal rat. The deficient rat is highly night-blind: his visual threshold is 3.25 log units (about 1800 times) above normal. Independent measurements have shown that this rise of threshold corresponds to the loss of 96 to 98% of the rhodopsin content of the eye.

In such animals, therefore, we can study the effects of a dietary night blindness uncomplicated by other somatic symptoms.

In this paper we shall describe the effects of this deficiency and its

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relief on the structure of the retina. Detailed correlation of these histological changes with the simultaneous changes in biochemical composition and physiological activity has been made elsewhere (Dowling and Wald, 1960), and will be mentioned only briefly here.

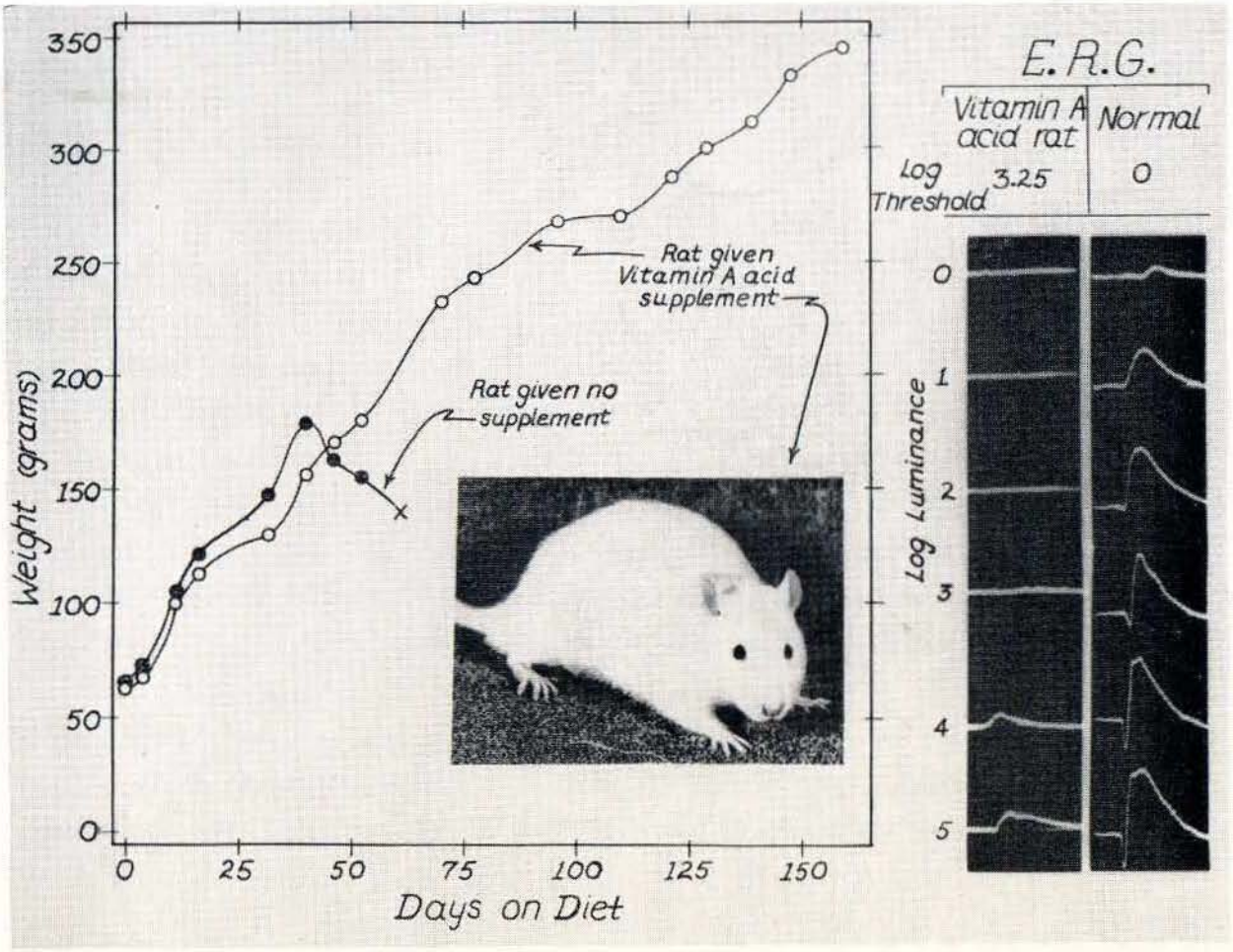

FIG. 1. Biological activity of vitamin A acid.

Litter mates were placed on vitamin A-deficient diet. The animal receiving no supplement grew until vitamin A stores were exhausted, then lost weight rapidly, and died on the 57th day of the experiment. The animal given vitamin A acid grew throughout the experiment (5 months) and remained in good condition. The picture of this animal was taken at the end of the experiment, as were the electroretinograms shown at the right, compared with those of a normal animal. They show that this rat is highly night-blind: it has a visual threshold 3.25 $\log_{10}$ units above normal, corresponding to loss of 96 to 98% of the rhodopsin from the eye (from Dowling and Wald, 1960).

Experimental

REARING OF THE RATS

Groups of albino, weanling rats were raised on Standard U.S.P.

similarly on vitamin A-free diets, supplemented with vitamin A alcohol. The animals were sacrificed at times ranging from 2 to 10 months after having been placed on the diet.

In certain cases, deficient rats were fed large doses of vitamin A to permit us to examine the course of recovery from the deficiency.

Preparation for Light and Electron Microscopy

Without regard to conditions of light or dark adaptation, animals were anesthetized with Nembutal and the eyes enucleated. The cornea and the lens were removed and the whole back of the eye fixed for 1 hr in a 2% solution of osmium tetroxide, buffered to pH 7.8 with Veronal acetate, and containing 45 mg per milliliter sucrose and 0.002 *M* calcium chloride. The specimens were dehydrated in graded acetone-water mixture and embedded in Araldite epoxy resin (Glauert and Glauert, 1958). The whole back of the eye was embedded in an attempt to retain the normal relationship of the retina to the pigment epithelium. However, some separation usually did occur, and only in occasional areas was the normal approximation preserved.

Thin sections were cut with a Porter-Blum microtome and stained with saturated uranyl acetate in 50% ethanol. They were examined in an RCA EMU-3D electron microscope operated at 100 kv.

For light microscopy, thick (2–4 μ) sections were cut from the same specimens with the same microtome. The sections were mounted on slides with Mayer's albumen, and stained for 6 to 24 hr with 2% Giemsa blood stain. They were then washed briefly with ethanol, allowed to dry in the air, and mounted in paraffin oil.

Observations

Retinal Degeneration

The changes observable with the light microscope during the course of degeneration are shown in Fig. 2. The retina of a control animal that had been kept 10 months on a vitamin A-free diet supplemented with vitamin A appears entirely normal (Fig. 2a).

In rats on the diet supplemented with vitamin A acid, the first signs of degeneration are noted after about 2 months, when the outer segments of the visual cells begin to stain less intensely than the normal and present a somewhat fragile and broken appearance (Fig. 2b). The inner segments and the nuclei appear normal, along with the other

teriorated considerably: only fragments of the outer segments remain. and the nuclei and inner segments are greatly reduced in number. The inner segments that remain are shorter and thicker than normal. However, the other retinal cells—bipolar and ganglion cells—and the pigment epithelium—appear normal.

After 10 months (Fig. 2d), the visual cells have almost completely disappeared. Only one irregular row of visual cell nuclei remains, and no inner or outer segments can be distinguished. The rest of the retina

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FIG. 2. Retinal histology of rats raised on vitamin A-free diets and supplemented with vitamin A acid.

FIG. 2a. The retina from a control animal that had been raised for 10 months on vitamin A-free diet with vitamin A (alcohol) supplementation. The structure is entirely normal.

FIG. 2b. The retina of an animal raised for 2 months on vitamin A-free diet supplemented with vitamin A acid. The primary change has occurred in the outer segments which are disoriented and stain less intensely. The rest of the visual cell appears normal, as do the other layers of the retina.

FIG. 2c. After 6 months, the outer segments have almost entirely disappeared. Only fragments and occasional large spherical structures remain. The inner segments and visual cell nuclei are reduced to about half the normal number, and the inner segments have become squat and rounded. The rest of the retina and the pigment epithelium appear normal.

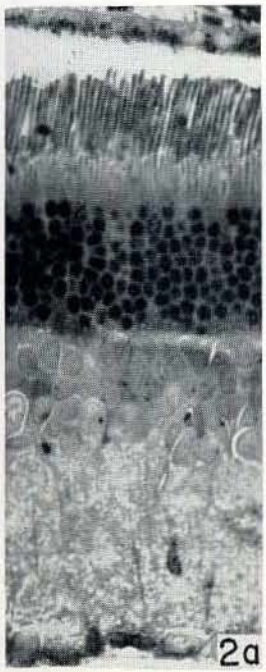
FIG. 2d. Retina from an animal maintained for 10 months on the diet with vitamin A acid supplementation. The visual cells have disappeared, except for one irregular row of visual cell nuclei. The rod-bipolar synapse layer is thinner than normal. Other parts of the retina appear normal.

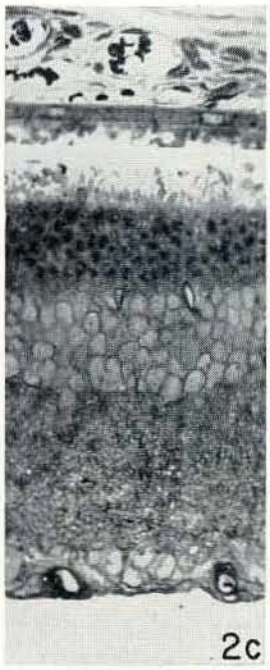
FIG. 3. An electron micrograph of a longitudinal section of a normal rat rod from the retina shown in Fig. 2a. As in other animals, the outer segment consists of stacks of transverse disks, enclosed within the cell membrane. The disks are about 150 Å in over-all thickness and 1.5 μ in diameter, and are separated from one another by a space of about 100 Å. The short tubules associated with the incision appear here, in transverse section, as small circles similar in diameter to the thickness of the disk. Magnification: × 16,500.

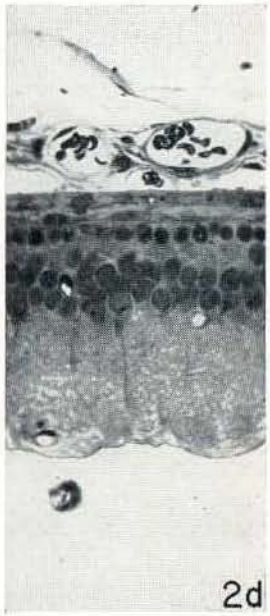
FIG. 4. Cross section of the proximal end of a normal rod outer segment showing the circular shape of the disks, and the single incision. The incisions of adjacent disks are lined up so that together they form a small channel running the length of the outer segment. Numerous, short tubular projections extend from the disk into the incision. Occasional tubular projections can be seen also at the periphery of the disk, extending into the space between the disk and the cell membrane. A cluster of singlet outer fibers from the connecting cilium appears just within the cell membrane opposite the incision. In the upper left of the figure is a cross section of a connecting cilium belonging to another cell. Magnification: × 13,000.

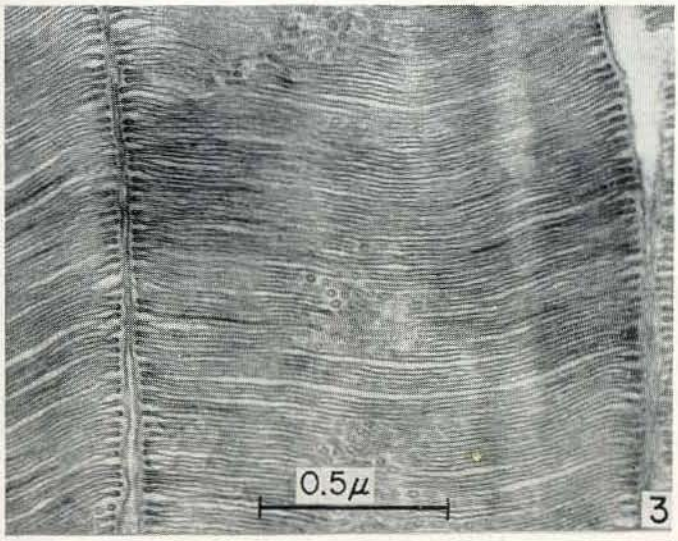
FIG. 5. A longitudinal section of a rod outer segment showing an early stage of

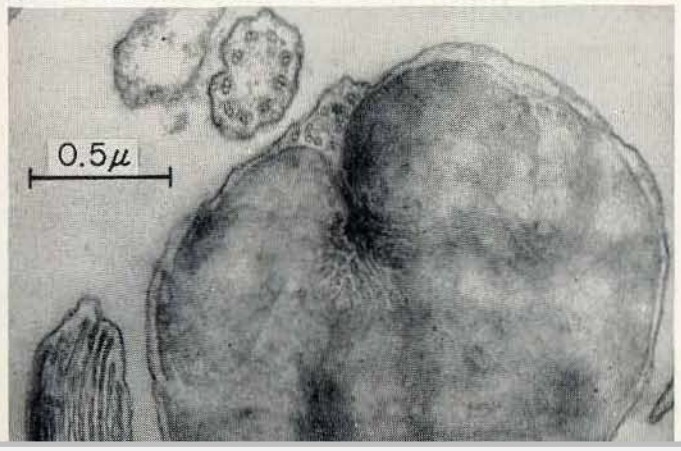
and the pigment epithelium still appear normal, except for the suggestion of some thinning of the layer of bipolar cells. The retina and the pigment epithelium adhere tightly to one another in this condition, and

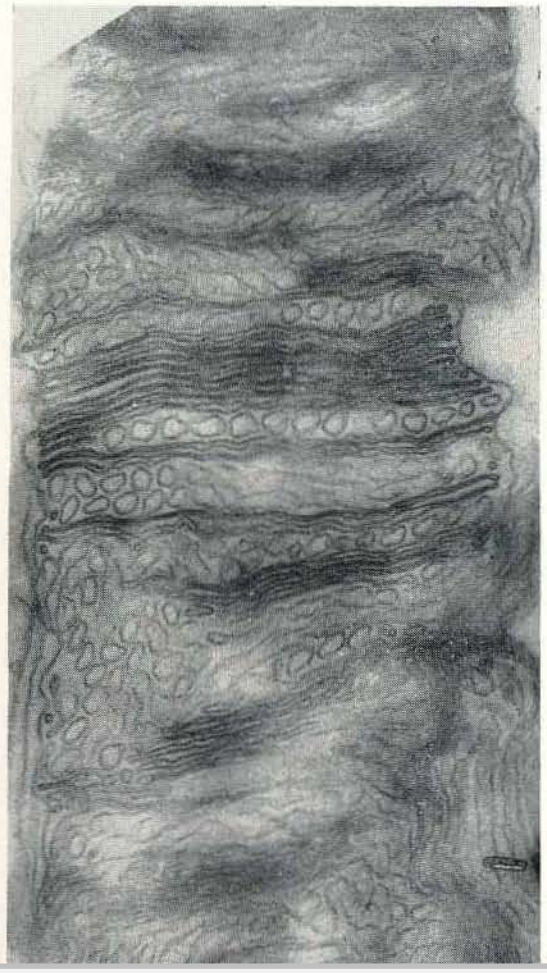












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