Primary retinal degeneration: evidence of normal phagocytosis in the retinal pigment epithelium. F. B. Reich-D'Almeida and D. J. Hockley.

In rats with primary retinal degeneration, lens extraction combined with total retinal detachment provided a model for injection of a tracer of colloidal carbon into the subretinal space. Electron microscopy and acid phosphatase cytochemistry were subsequently used to analyze the ingestion phases of the phagocytic process. It was found that the attachment, ingestion, and digestion phases of the phagocytic process were apparently preserved. From this evidence it is suggested that there is no lack of phagocytic power in the retinal pigment epithelium of affected rat strains.

In order to analyze the controversial involvement of the retinal pigment epithelium (RPE) in primary retinal degeneration we have recently studied the reactivity in situ of the RPE in normal and dystrophic rats. The experimental model was provided by a combined lens extraction and induction of total retinal detachment in the rat eye. Following injection of colloidal carbon into the subretinal space, a light and electron microscopic study of the RPE indicated that the two preliminary phases of phagocytosis, i.e., attachment and ingestion, were well preserved in both normal and dystrophic rats.

The objective of the present work was to try to analyze the third or lysosomal phase of the phagocytic process in the dystrophic rat. To this effect, acid phosphatase cytochemistry and electron microscopy were utilized in conjunction with the same model of experimental surgery. Materials and methods. Three dystrophic PETH (pink-eyed, tan-hooded) rats from the same litter were used in the experiment; they were 11 weeks of age. In the left eye of these animals surgical aphakia and total retinal detachment were induced and colloidal carbon was then injected into this artificial reaction chamber. Details of these techniques and criteria for the selection of ocular specimens have been described previously; all three eyes fulfilled the experimental criteria.

After enucleation, each ocular globe was immersed at room temperature in 3 per cent glutaraldehyde buffered to pH 7.4 with 0.1M sodium cacodylate. After 2 to 5 minutes a deep paraviewal incision was made through the ora serrata and the eyes were fixed for a further 20 minutes; they were then meridionally bisected and pieces of tissue were subsequently obtained in one of two ways: (1) by excising with a sharp razor blade small fragments of RPE and choroid located in the midperiphery and posterior pole or (2) by slicing ocular pieces from the same region with a microtome so as to provide sections approximately 100 μm in thickness. Both types of specimens were rinsed (three times) in cold 0.1M sodium cacodylate buffer containing 7.5 per cent sucrose (pH 7.4) and were left overnight in this buffer sucrose mixture at 4° C. At this stage some material was processed directly for normal ultrastructural studies.

For acid phosphatase cytochemistry, the fixed and washed specimens were incubated for 25 minutes at 37° C. in Comori's medium. After incubation the tissue was washed twice in 0.05M acetic acid between the washings. Incubation controls were performed by omitting sodium glycerophosphate substrate from the medium; otherwise they were treated in a similar manner.

All specimens were postfixed in 1 per cent osmium tetroxide buffered to pH 7.4 with 0.1M s-collidine; they were dehydrated in a graded ethanol series and embedded in Araldite. Thin sections were cut with a glass knife, stained in alcoholic uranyl acetate solution (1 per cent), and examined with a JEM 100C electron microscope.

For light microscopy one half of an eye was fixed in formal saline after initial fixation in glutaraldehyde. The specimen was embedded in paraffin and sections were stained with hematoxylin and eosin.

Results. The age of the animals ensured an advanced degree of retinal degeneration; light microscopy showed the detached retina to be virtually devoid of photoreceptor outer segments and there were numerous areas of glial reaction. The RPE appeared essentially intact although some changes were found which could be ascribed to the dystrophy or to the retinal detachment itself. Vesicles of phagocytosed carbon were visible in most RPE cells and electron micrographs showed these inclusions to be widely distributed in the cytoplasm (Fig. 1). The vesicles either contained the tracer alone or exhibited a mixed composition of carbon together with granular or membranous material. A well-defined, finely granular precipitate, interpreted as the reaction
Fig. 1. Low-power electron micrograph of the RPE in the dystrophic rat. Microvilli (MV) are present in the apical area facing the subretinal space where the tracer was available for phagocytosis. Numerous inclusions of colloidal carbon (C) are present in the cytoplasm. This specimen was not processed for acid phosphatase cytochemistry. (×13,250.) Inset a, Carbon vesicle at higher magnification which shows, for comparative purposes, the size and morphology of the tracer particles. (×54,000.)

Discussion. Phagocytosis can be divided into three phases: attachment, ingestion, and digestion.1 In dystrophic rats, a deficiency at the level of the first two phases would not appear to be compatible with the reactive behavior of the RPE as demonstrated in this and previous work.2 Carbon was consistently visible in the cytoplasm of the RPE cells, which would seem to indicate that the attachment and ingestion phases of phagocytosis were normal. A deficiency might still be postulated as belonging to the third or lysosomal phase. Nevertheless, in this case it would be difficult to understand why in previous work carried out in rats with retinal dystrophy, no intracytoplasmic phagosomes of photoreceptor origin have been found3 as evidence of preserved first and second stages. In the present investigation foreign particles of colloidal carbon have been observed inside a limiting membrane together with cellular components that by their morphology and presence of acid phosphatase reaction indicated a lysosomal nature. This composite structure fulfills the criterion for identification of a phagolysosome (digestive vacuole).4 A positive acid phosphatase reaction is found when the lysosomal enzymes are activated after stimulation of the RPE cell by a challenging factor. Under normal conditions this stimulus is provided by the photoreceptors; in our experiments the presence of colloidal carbon resulted equally in lysosomal activation and gave rise to a positive acid phosphatase reaction. Conversely, when the RPE cell is not fulfilling its normal phagocytic role, acid phosphatase may not be demonstrated in the lysosomal system.5 In this work, therefore, the presence of enzymatic hydrolytic activity within the vesicles of phagocyted carbon indicated that the third or digestive phase of the phagocytic cycle was probably taking place in the cytoplasm of the RPE cell.
Fig. 2. Electron micrograph illustrating the appearance of the carbon phagolysosomes in specimens incubated for acid phosphatase reaction. Carbon tracer (C) is present in conjunction with the reaction product (P) which appears as a well-localized fine precipitate. Inset b shows for comparison a lysosomal vacuole which is devoid of tracer and therefore demonstrates more clearly the reaction precipitate. (×54,000.) Insets c and d represent further examples of digestive vacuoles containing carbon and reaction precipitate. In some areas (arrows) the delimiting membrane is particularly evident. (Both ×50,000.)

The observed absence of photoreceptor phagosomes in the RPE of the dystrophic rat is possibly due to a structural change of the outer segments which might prevent their uptake because of the likely discriminatory phagocytic behavior of the epithelial cell. This behavior is exhibited by several types of phagocytic cells and has been postulated for the RPE of lower vertebrates. Recent work on the reactivity of the RPE of the rat toward carbon and photoreceptor material is also in agreement with this interpretation in that it emphasizes a parallel reaction in both normal and dystrophic animals. Our own in vivo and in vitro studies have also clearly demonstrated phagocytosis of an outer segment suspension and various other tracers by the RPE of dystrophic extraction. The above observations are therefore in favor of the existence of a capacity for discrimination in the pigment epithelial layer.

In conclusion, the evidence of this work appears to demonstrate that there is no lack of phagocytic power towards colloidal carbon in the RPE of rats with primary retinal degeneration, a finding which possibly transfers the responsibility for this disease to the adjacent photoreceptor layer.

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Key words: acid phosphatase, lysosomes, phagocytosis, primary retinal degeneration, retinal dystrophy in the rat, pathogenesis, retinal pigment epithelium.

REFERENCES

Ocular damage induced by near-ultraviolet laser radiation. JOSEPH A. ZUCLICH AND JOHN S. CONNOLLY.

A quantitative study was conducted of ocular damage thresholds in the rhesus monkey eye from krypton, argon, and nitrogen laser radiation. Corneal and lenticular thresholds are reported for various laser beam parameters. Corneal damage was found to occur following incident energy doses of ~60 to 70 Joules per square centimeter (J/cm²) for pulsewidths ranging from 250 v-sec to 120 sec. The results are consistent with a photochemical damage mechanism. With certain exposure parameters, cataracts were induced with lower energy doses than required to cause corneal damage. The lenticular thresholds, however, appear to be consistent with a thermal rather than a photochemical mechanism. Corneal and lenticular hazards of near-ultraviolet (near-UV) lasers are discussed in terms of existing safety standards for laser radiation.

In the past several years, numerous ultraviolet lasers possessing a wide variety of beam parameters have become commercially available. These include continuous wave (cw) lasers such as argon- and krypton-ion lasers, short-pulsewidth, high-power units such as nitrogen gas lasers, and various solid-state and tunable dye lasers. Existing safety standards have not anticipated the wide ranges of beam parameters available with these lasers. In the near-UV (315 to 400 nm.) range, there is a paucity of experimental threshold data even for noncoherent sources and the safety standards appear to be based on the assumptions that only corneal damage would occur and it would be thermal in nature.

Recently, several papers have appeared concerning ocular damage induced by near-UV radiation. Ebbers and Sears reported on corneal and lenticular damage induced in the rhesus eye by the 325 nm. output of a helium-cadmium laser. Also, Zigman and Vaughan have reported pathologies in the lenses and retinas of mice subjected to repeated exposures of noncoherent, near-UV radiation. From these studies, it is clear that in addition to corneal hazards there are potential lenticular and retinal hazards from near-UV radiation. Little evidence is available regarding the mechanism for damage at each site, and thus little experimental support exists for any safety standard which attempts to set maximum permissible exposure (MPE) levels for wide ranges of beam parameters.

We have undertaken this study in an attempt to identify the most sensitive ocular components to near-UV radiation under a variety of exposure conditions. This report is part of a continuing study of the primary sites and mechanisms of near-UV-induced ocular damage, designed to provide an experimental basis for more comprehensive safety standards for the use of near-UV lasers.

Experimental. The subjects used in these experiments were rhesus monkeys (Macaca mulatta). The animals were maintained and the experiments conducted in accordance with procedures outlined in the "Guide for Laboratory Animal Facilities and Care" of the National Academy of Sciences—National Research Council. The pupils were dilated with a topical application of atropine administered up to 24 hours prior to exposure. The animals were preanesthetized with an intramuscular injection of ketamine hydrochloride and anesthetized with an intravenous injection of sodium pentobarbital. During exposure and observation the eyelids were held open by a wire speculum. Corneal drying was prevented by periodic irrigation with normal saline. Observations of the corneas and lenses of the subjects were made with a Nikon "Zoom-Photo" slit-lamp microscope.

Ultraviolet laser sources used were as follows: Spectra Physics 164-01 krypton-ion laser with cw output simultaneously at 350.7 and 356.4 nm. (~ 3:1 intensity ratio); Spectra Physics 170 argon-ion laser with cw output simultaneously at 351.1 and 363.4 nm. (~ 1:1 intensity ratio); Molelectron UV-1000 nitrogen laser emitting at