Photosensitization of the lens by 8-methoxypsoralen. Sidney Lerman,* ** Marcus Jocoy,* and Raymond F. Borkman,**

During the past decade, ambient ultraviolet radiation has been implicated in the age-related increase in fluorescence and pigmentation of the human lens nucleus. 8-Methoxypsoralen (8-MOP) (currently in vogue for the treatment of psoriasis) is a well-known photosensitizing agent. This drug interacts with specific macromolecules such as DNA and certain proteins, thereby enhancing their photosensitizing action.* It should be noted that since the lens is completely encapsulated and never sheds any of its cells, the possibility exists that 8-MOP might enter the lens, become bound to macromolecules in lenses exposed to ambient or UV light as described below. The purpose of this study was to evaluate the potential photosensitizing action of 8-MOP in rat lenses and to determine whether this compound becomes bound to macromolecules in lenses exposed to ambient light or UV radiation in vivo or in lenses exposed to monochromatic UV radiation in lens-incubation systems in vitro.

Materials and methods. In order to utilize phosphorescence spectroscopy to detect 8-MOP in the lens, it is important that these lenses not contain significant amounts of fluorescent material emitting in the 440 nm. region. We have noted that lenses with such fluorescent material give an ill-defined phosphorescence emission spectrum which lies in approximately the same region as that of 8-MOP and which interferes with detection of the phosphorescence. This problem can be overcome by utilizing rats which have been kept in the dark after weaning (4 weeks of age). Such animals do not develop the 440 nm. fluorescence, whereas littermates kept in ambient light develop this fluorescence region as they age (Fig. 1).

We used our own laboratory-bred strain of Sprague-Dawley rats which were put in the dark after 4 weeks of age. Some animals were maintained in these dark conditions throughout the experimental period. Other animals were exposed to ambient or UV light as described below. The
rats were given an intraperitoneal injection (0.1 ml.) of 4 to 8 mg./kg. 8-MOP in dimethylsulfoxide (DMSO) or a recrystallized suspension (0.25 ml.) of 4 to 8 mg./kg. 8-MOP in physiological saline. The control rats (also kept in the dark) were given DMSO or saline alone. The animals were sacrificed at varying periods of time after injection, and the lenses were immediately removed, gently rinsed with physiological saline, and analyzed by fluorescence and phosphorescence spectroscopy. Typical spectra and equipment descriptions for both fluorescence and phosphorescence spectroscopy have been previously reported.9 10 All fluorescence intensities at 440 nm. were measured relative to the intensity of intrinsic tryptophan fluorescence (290 nm. excitation; 332 nm. emission maximum) and expressed as a ratio:  

$$I_r = \frac{440 \text{ nm.}}{332 \text{ nm.}}$$  

This procedure provides an internal calibration standard.  

As previously mentioned, some animals were exposed to light following treatment with 8-MOP. Rats exposed to UV radiation were injected as above and placed unrestrained in 15 by 13 by 6½ inch plastic cages. Two UV lamps were mounted in each cage, each about one third of the way from the outside rim and 2 inches from the floor of the cage. The long-wave UV light utilized in these experiments was produced by black-light fluorescence lamps (No. F15T8BLB; General Electric). The spectral output of the lamps is in the range of 300 to 400 nm., with a peak output at approximately 365 nm. The animals were exposed to UV under these conditions for up to 48 hr. The animals exposed to ambient light were maintained in the same type of cage but without UV lamps, again for periods up to 48 hr. Control rats were kept under the same conditions but were injected with DMSO or saline alone. The animals exposed to ambient or UV light were sacrificed, and their lenses were removed and analyzed as described above for the dark-maintained animals.  

Results and discussion. Fluorescence spectra of whole rat lenses derived from animals maintained in the dark, given 1 dose of 8-MOP, and sacrificed 2½ to 5 hr. later, showed no 440 nm. fluorescence when excited at 360 nm. Phosphorescence spectra of these lenses showed the characteristic tryptophan emission spectrum when excited at 290 nm. and an emission characteristic of 8-MOP when excited at 360 nm. (Fig. 2). The control lenses from rats which received DMSO or saline alone showed no 360/440 nm. fluorescence emission and no detectable phosphorescence emission when excited at 360 nm. In order to determine whether 8-MOP had penetrated beyond the lens periphery, the capsules were removed from several lenses after phosphorescence spectroscopy was performed, and the measurement was repeated. The intensity of 8-MOP phosphorescence in these samples was approximately 75 percent of that observed in the intact lens, indicating that most of the 8-MOP was present in the nuclear and inner cortical regions of the lens.  

The results of the in vivo experiments in which rats were injected with 8-MOP and then exposed to ambient light or UV radiation are shown in Fig. 3 and Table I. The lenses from rats exposed to ambient light or UV radiation showed a significant loss of the characteristic 8-MOP phosphorescence emission as compared with lenses from rats.
kept in the dark. Instead of the sharp 8-MOP phosphorescence peaks present in the "dark" lenses (Fig. 2), lenses treated with 8-MOP and then exposed to UV or ambient light showed a broad, structureless phosphorescence emission (Fig. 3). A low-level phosphorescence is present in lenses from control rats subjected to UV radiation. The latter phosphorescence appears to be related to the development of the 440 nm. fluorescence-emission region in these lenses. These results indicate that 8-MOP undergoes photochemical changes in the lens, but the nature of these changes remains to be elucidated. One possibility is that the 8-MOP present in the lens in free form (as indicated by the phosphorescence spectra in Fig. 2) might have become bound to macromolecules (probably proteins) within the lens as a result of exposure to ambient or UV light, thereby altering the shape and the intensity of the phosphorescence emission.

The intensity of phosphorescence of lenses excited at 360 nm. from animals injected with 8-MOP and exposed to ambient light or UV radiation does not increase significantly with time of exposure; on the other hand fluorescence spectra of these lenses indicate an increase in 440 nm. fluorescence intensity with time of exposure (Table I). Control lenses given only DMSO or saline injections showed much lower levels of 440 nm. fluorescence. It is noteworthy that exposure to ambient light results in a significant increase of 440 nm. fluorescence in lenses from rats subjected to a single dose of 8-MOP. The increase in 440 nm. fluorescence was smaller by a factor of 2 in lenses from animals exposed to ambient light than in animals exposed to UV radiation. Furthermore, if the rats are given two doses (one every 24 hr.) of 8-MOP, the 440 nm. fluorescence shows an even more marked increase (Table I).

For the in vitro studies, lenses were extracted 24 hr. after the rats were given one dose of 8-MOP, and these lenses were then exposed to monochromatic UV light for 4 hr.10 The results (Table II) indicate that irradiation wavelengths between 300 and 320 nm. are the most effective in increasing the 440 nm. fluorescence. These results suggest that 8-MOP is capable of functioning as a photosensitizer in the lens and that it enhances UV-induced lenticular changes, i.e., the generation of 440 nm. fluorescent material.

Preliminary results in our laboratory indicate that when rats are maintained for at least 24 hr. in the dark after receiving an injection of 8-MOP, little if any phosphorescence excited at 360 nm. can be detected. This suggests that most of the 8-MOP, which appears to enter the lens in a free form, eventually diffuses out of the lens or is modified, provided that the animal is kept completely in the dark for at least 24 hr. However,

**Table I. Fluorescence intensities of rat lenses after in vivo exposure to ambient light or UV radiation following 1 or 2 intraperitoneal doses of 8-MOP or DMSO**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Time (hr.)</th>
<th>Source</th>
<th>( \text{fluorescence (332 nm.)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-MOP (1 dose)</td>
<td>24</td>
<td>Ambient light</td>
<td>0.0190</td>
</tr>
<tr>
<td>8-MOP (1 dose)</td>
<td>24</td>
<td>UV</td>
<td>0.0226</td>
</tr>
<tr>
<td>DMSO (1 dose)</td>
<td>24</td>
<td>Ambient light</td>
<td>0.0000</td>
</tr>
<tr>
<td>DMSO (1 dose)</td>
<td>24</td>
<td>UV</td>
<td>0.0067</td>
</tr>
<tr>
<td>8-MOP (1 dose)</td>
<td>48</td>
<td>Ambient light</td>
<td>0.0210</td>
</tr>
<tr>
<td>8-MOP (1 dose)</td>
<td>48</td>
<td>UV</td>
<td>0.0718</td>
</tr>
<tr>
<td>DMSO (1 dose)</td>
<td>48</td>
<td>Ambient light</td>
<td>0.0000</td>
</tr>
<tr>
<td>DMSO (1 dose)</td>
<td>48</td>
<td>UV</td>
<td>0.0150</td>
</tr>
<tr>
<td>8-MOP (2 doses)</td>
<td>48</td>
<td>Ambient light</td>
<td>0.0465</td>
</tr>
<tr>
<td>8-MOP (2 doses)</td>
<td>48</td>
<td>UV</td>
<td>0.0957</td>
</tr>
<tr>
<td>DMSO (2 doses)</td>
<td>48</td>
<td>Ambient light</td>
<td>0.0000</td>
</tr>
<tr>
<td>DMSO (2 doses)</td>
<td>48</td>
<td>UV</td>
<td>0.0080</td>
</tr>
</tbody>
</table>

**Table II. Fluorescence intensities of in vitro incubated lenses from rats given 1 dose of 8-MOP (intraperitoneally) followed by 4 hr. exposure to UV (E) or 4 hr. in dark (C)**

<table>
<thead>
<tr>
<th>Wavelength (( \lambda ))</th>
<th>( \text{fluorescence (332 nm.)} )</th>
<th>( \text{fluorescence (440 nm.)} ) of control lenses (no 8-MOP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>280</td>
<td>E = 0.0300</td>
<td>C = 0.0010</td>
</tr>
<tr>
<td>300</td>
<td>E = 0.0625</td>
<td>C = 0.0110</td>
</tr>
<tr>
<td>320</td>
<td>E = 0.0480</td>
<td>C = 0.0100</td>
</tr>
<tr>
<td>360</td>
<td>E = 0.0130</td>
<td>C = 0.0140</td>
</tr>
</tbody>
</table>

even ambient laboratory light appears to be capable of binding 8-MOP in the lens. Thus patients receiving 8-MOP therapy for psoriasis should not only have their eyes shielded during the time they are undergoing treatment but also should avoid exposure to any significant level of ambient light for at least 24 hr. in order to prevent retention of 8-MOP within the lens.

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**Key words:** 8-methoxypsoralen, lens fluorescence, lens phosphorescence, lens photosensitization, UV radiation.
REFERENCES


Vitreal syneresis in rhesus monkeys.*

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The eyes of 15 rhesus monkeys were evaluated. Various degrees of vitreal syneresis were observed in 28 of the 30 eyes. The observed vitreous structures varied from fine strands randomly spaced throughout the vitreous to thick, intertwining, fibrous networks with some clumping of the collagogenous condensate at the fiber junctions. Qualitatively, the degree of syneresis was slightly more extensive in the eight older mature males than in the seven younger animals. In all animals a clear view of the fundus could be obtained with the ophthalmoscope. The vitreous structures may be one cause of variability in ocular dose-response relationships for exposure to laser radiation. The effect on retinal exposure experiments of the finer vitreal structure is considered minimal.

Vitreal syneresis is documented in human1,2 and described in other mammalian species (horse, dog, chimpanzee).3,4 Vitreal syneresis is a pathological condition caused by a change in the normal colloidal gel. In this paper we will describe the characteristics of vitreal syneresis found in normal rhesus monkeys and will illustrate some aspects of these structures which may cause variability in ocular exposure experiments.

Materials and Methods.1 Fifteen rhesus monkeys (eight males, 5 to 7 years of age; four males and three females, 2 to 3 years old) were tranquilized with 50 mg of ketamine (intramuscularly) and anesthesized with pentobarbital (30 mg/kg, intravenously). Their pupils were dilated with a cycloplegic-mydratic combination. The anterior ocular structures and the retina were observed with the slit lamp and the ophthalmoscope.

A narrow beam of the light from a helium-neon (HeNe) laser was projected into the animal's vitreous, and the scatter of the light by the strands was observed with the slit-lamp biomicroscope. The eyes from one of these animals were enucleated. Samples of vitreous were placed on a microscope slide and examined.

Results. Vitreal syneresis was observed in 28 of the 30 rhesus monkey eyes examined with the slit lamp. The degree of observed syneresis varied from the formation of thin, white, opacified strands randomly distributed within the vitreal space to the formation of a thick, intertwined network of the collagenous condensate (Fig. 1, A), with some clumping of structure at various intervals (Fig. 1, B). The light from a 3 mm HeNe laser beam incident on the cornea (left arrow, Fig. 1, B) was diffused and scattered by the dense vitreal clump (right arrow, Fig. 1, B). The observed structures extended from the posterior lenticular pole to deep into the vitreous. In eyes where the syneresis was more extensive, the viscosity of the vitreous appeared to be comparable to that of water. The vitreal structure moved or floated within the vitreal space when the animal's head was gently tapped. In all cases, a clear view of the fundus was obtained with no evidence of vitreal structure (Fig. 1, C). In some animals a fine, milky network appeared to fill the vitreous.

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1In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care' as outlined by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.