Contract No. DOE EV06021

COMPREHENSIVE PROGRESS REPORT

The long range goal of our research program has always been to understand the underlying mechanisms which make light both the adequate and the damaging stimulus for the retina. The phenomenon of retinal light damage has been known for about fifteen years (Noell et al., 1966) and its occurrence has been documented in a variety of animal species including monkeys (Adams et al., 1972; Ts'o, 1973); pigeons (Marshall et al., 1972); rabbits (Lawwill, 1973) and rats (Noell, 1966; Kuwabara and Gorn, 1968). Although this has been by no means a neglected area of investigation, the phenomenologic studies thus far reported have only served to show that a myriad of variables effect the production of light damage. These variables include such things as age, prior light history, body temperature, vitamin A status, intensity, wavelength and duration of light. When we embarked on our investigations of light damage it appeared obvious that if one wished to quantify this phenomenon, careful parametric studies must be undertaken. To date we have studied the intensity-duration function and the age function in detail. We have, in addition, started studies of the wavelength variable. The results of these investigations will be reported under the appropriate sub-headings below.

A. Parametric Studies

1. Intensity-duration function.

Historically, it has been well accepted that intensity and duration are to some extent interchangeable in producing light

DISCLAIMER

damage. This has been referred to as the Bunsen-Roscoe law. This law states that light intensity and duration are reciprocally related in producing light-mediated reactions. If one considers the photochemical meaning of the Bunsen-Roscoe law, i.e. that the intensity parameter can never be limited by a decrease in the absorbing product, it is obvious that one should not expect the reciprocal relationship to hold for light damage. In that case the absorber, namely rhodopsin, is being reduced in concentration; i.e., bleached. For this reason we have looked not simply at intensity-duration but at the relationship of the product of intensity-rhodopsin bleaching-and duration. These studies have been done in both albino (Sprague-Dawley) and pigmented (Long-Evans) rats. Measurements of the time required to produce a criterion photoreceptor loss (assayed histologically) as a function of the steady-state rhodopsin levels (assayed by rhodopsin extraction) have shown that a linear relationship holds for intensities between 20-160 lux. Another way of stating this is that the higher the steady-state rhodopsin bleach induced by a given intensity, the shorter the time needed to produce a criterion cell loss. This relationship is shown in Figure 1. It was also found that low intensities; e.g. 5 lux, caused no measurable bleaching and no measurable damage even at 16 days exposure. Ten lux intensity was found to bleach about 10% of the rhodopsin and criterion cell loss was attained in 12 days. The 10 lux exposure did not fit the linear relationship found in the range: 20 to 160 lux.

Several interesting and possibly far-reaching additional findings have come out of this parametric study of intensity and

duration. Since these investigations were done using both albino and pigmented rats, we are in the position of being able to comment on the role of eye pigmentation in protection against light damage. It was found that in pigmented rats, the same range of light intensities used for exposure of albino rats produced no measurable steady state bleaching and no detectable light damage. This was also true for light intensities an order of magnitude higher, a finding supported in a recent report by LaVail (LaVail, 1980). It was reasoned that the opaque iris of the pigmented rats was lowering the effective intensity by as much as 2 log units. This was shown to be the case when atropine sulfate was utilized to dilate the pupil. Pigmented rats with dilated pupils were found to be as susceptible to damage as albino animals whose pupils were not dilated with atropine. (The albino iris is not pigmented, does not effectively occlude the pupil and, hence, does not need to be dilated before it freely admits light.)

Another unexpected finding of these studies has been non-homogeneous susceptibility of the rat retina to light damage. In both albino and pigmented rats the superior hemisphere of the retina is more sensitive to damage. More than this: a smaller region between 500 and 2000 microns superior to the optic nerve head, is exquisitely sensitive to damage. This distribution is shown in Figure 2 which plots outer nuclear layer (photoreceptor nuclei) thickness vs position on the retina for both albino and pigmented rats exposed to 40 lux illumination for four days. This non-uniform distribution of damage is not an artifact. For example, even when gradients of intensity were purposely created so that the intensity of light falling on the inferior retina was 100 times greater than that on the superior, the

damage was still more severe in the superior area. In addition, anaesthetized rats whose eyelids were sutured open were rotated under a light-beam so as to uniformly irradiate all parts of the retina. The same distribution obtained. The reason for the higher susceptibility to light damage of the superior retina and, especially, the small area of superior retina has not been explained. Several possibilities exist but none has been really tested. The first possibility involves the optical properties of the rat eye. Even though our light cubicles are essentially a "ganzfeld" illuminator, light striking the cornea enters the eye through the pupil and is focused by the lens. The placement of eyes in the head, the degree of binocular overlap, and the refractive properties of the eye all combine to produce what is commonly called the fixation axis or area retinae (Mueller, 1861). In man as well as in many vertebrates with frontal eyes this axis is centrally located, but in animals with non-frontal eyes such areas are not central but situated in the temporal retina so as to form the visual pole of the forward fixation axis. The specialization of the area retinae arises from a locally increased ganglion cell density, increased retinal thickness or photoreceptor density, and the absence of overlying major blood vessels. When one looks at the ganglion cell density profile of the rat retina (Hughes, 1977) it appears that there is a close correspondence of a defined "area retinae" and the area of maximal light damage sensitivity. The area of greatest ganglion cell density occurs in superior retina and is primarily temporal. Thus the high light-damage sensitivity of this region may simply result from the fact that it is an area of higher receptor density.

Another feature of the highly "light damaged" sensitive area of the rat retina which was noted during this investigation involves the distribution of melanosomes across the retinal pigment epithelium (Howell, Rapp and Williams, 1981). The distribution is shown to be non-uniform: more melanosomes exist in the periphery than elsewhere and, importantly, there are almost no melanosomes in a restricted area of the midperiphery of the superior hemisphere; i.e., the retinal region showing high light-damage sensitivity. Because the sensitive region is the same in both pigmented and albinos, the paucity of melanin in this region is not the cause of its greater sensitivity to light-damage. Dark-rearing experiments also show that light is not required for the non-uniform distribution of melanin. The suggestion is made that the area represents a vestigial tapetum, a structure associated with amelanotic retinal pigment epithelium. This suggestion could again support the "area retinae" view of the cause of damage distribution but it could, in addition, suggest that as in true tapetal areas there exists an avascularity of the choroid which might lead to a reduced metabolic uptake in this area of the retina. This view gains some support from the findings of Noell et al. that systemically administered iodoacetate caused the greatest loss of receptor cells in the inferior retina. Carter-Dawson (1979) has also shown that inferior retina is also more susceptible to Vitamin A deficiency. Both of these findings support more extensive choroidal circulation in the inferior retina.

While this distribution of damage is extremely interesting and could conceivably provide insight into the underlying mechanism(s) of light damage, the studies suggested by this finding are currently

beyond the scope of our existing program. The experiments required to test the proposed suggestions would require elaborate fundus cameras, metabolic chambers, etc. not now available to us. We are, therefore, not pursuing this aspect at the present time.

2. Age function.

In 1972, Ballowitz and Dammrich reported that the retinas of newborn rats were less damaged than those of adult animals after exposure to rather high intensity continuous illumination. The increasing susceptibility to light damage was confirmed by O'Steen et al. (1974) and Kuwabara and Funchashi (1976). We are currently completing a study of light damage (80 lux for 2 days) as a function of age in the albino rat. We have looked at 80 lux light-damage in animals from 4 to 16 weeks of age and, in general, find that younger animals, up to about 8 weeks of age are not as susceptible to damage. However, at no age investigated are animals resistant to the damage of continuous 80 lux illumination. The design of our age experiments has been somewhat different than our earlier parametric investigation of the intensity-duration function. In the current study we have evaluated cell loss (ONL thickness) at four different times after the light period; immediately after, 4, 6 and 8 days in the dark. The outer nuclear layer in the rat is made up of cell bodies of the photoreceptors. As photoreceptors die the ONL becomes reduced in thickness (O'Steen et al., 1972). In addition, we have analyzed ONL thickness by integration across the entire retina in the region of the optic nerve head (i.e. central retina). This analysis has revealed that damage assessed as cell loss in the ONL continues in the dark for at least six

days following the damaging exposure. Although this study is not complete, preliminary evidence suggests that the "sensitive area" develops between the fourth and fifth week of age and that, aside from the area of high sensitivity, cell loss in the inferior and superior retina is of similar magnitude. In addition, the rate of cell loss is different when comparing the sensitive area and the remainder of the retina. When assessment of cell loss is made immediately after the light exposure most of the damage is located in the sensitive area with little or no cell loss seen in the remainder of the retina. However, after four days in the dark after light exposure there is a generalized cell loss across the retina. The question posed by such a finding is whether the faster cell death seen in the sensitive area represents more rapid damage or more rapid removal of damaged cells. Perhaps assay at the photoreceptor outer segment level would help to answer these questions. Little is known as to the cues used in the retina for phagocytic, macrophage activity so it is difficult to predict how "damaged" a cell must be before it is considered debris for disposal. At the present time we are assessing the time between the end of light exposure and four days in the dark. Upon completion of this study we should be able to evaluate the rate of cell death following light exposure in both the sensitive area and the remainder of the retina.

One further experiment which has been done to look at cell loss after light exposure involves 15-week old albino rats subjected to 80 lux illumination for two days. At the end of the 48 hour light-period, animals were either placed in darkness or

returned to 12 hr cycle light. This is similar in design to our age experiment except we additionally evaluated cell loss in the cyclic environment. There was no difference in cell death between animals maintained in darkness after the light period and those maintained in 12 hr cyclic light after the light period. Both showed a 25% decrease in ONL thickness when compared to unilluminated animals (i.e., normal 12-12 cycle) and an 11% decrease in ONL thickness when compared to animals evaluated immediately following the light period. Thus the 12 hours of light, which was of very low intensity (10 lux), experienced by the cyclic animals, had no effect on the extent of damage seen four days after exposure.

B. Mechanistic Studies

1. Action spectrum.

Thus far only one type of experiment has been completed to evaluate the action spectrum for cellular loss in light damage. In this experiment the decrease in ONL thickness was again used as an assay. The experiment involves short duration-high intensity damage. Animals were anaesthetized with nembutal, one eye sutured opened, the other patched as a control eye. The open eye was subjected to six hours of xenon illumination through narrow band interference filters. The illumination at each wavelength was equated by means of neutral density filters. The variance of illumination was 7.5% and the mean intensity was 1.97×10^9 photons/sec/cm^2 at the cornea. The relative integrated cell loss vs wavelength is shown in Figure 3. The dashed line is the rhodopsin spectrum. The fit is quite good although it deviates somewhat at

longer wavelength. The possibility of cone damage in the sensitive area may account for the increased long wavelength sensitivity. Most importantly, this action spectrum is not maximal in the blue. The significance of this finding will be discussed in our proposal.

2. Photodynamic action.

Much of the progress in this area has been made with in vitro investigation of photodynamic action. Rod outer segments (ROS) from frogs, cattle and rats have been subjected to oxygen and light and the behavior of rhodopsin and the presence of malonaldehyde and its fluorescent cross-linking products assayed. Malonaldehyde was found to increase initially but within 24 hours the levels begin to fall. As malonaldehyde falls fluorescent products are built up and the spectra observed are shown in Figure 4. When rhodopsin from these ROS is bleached it shows a change in the bleaching kinetics. Figure 5 shows the time-resolved difference spectra of fresh normal digitonin-extracted rhodopsin and that of the digitonin extract of peroxidized membranes.

The presence of fluorescent products similar to those of Figure 4 have so far not been demonstrated after in vivo exposure of the retina to constant light. We have been able to show increased fluorescent products in rats maintained in constant 40 lux illumination for 24 hours and a greater increase when illumination is extended to 48 hours. In these experiments animals are removed from the light box, killed by CO_2 inhalation and the retinas removed. The retinae of three animals are combined and subjected to Bligh-Dyer extraction. The resulting chloroform and methanol-water layers are assayed for fluorescence. Three animals from the colony are

used as controls. The difficulty with interpretation of these experiments arises from the fact the fluorescence produced in vivo, does not match that seen in our in vitro studies or that reported by many investigators of peroxidative production of lipofuscin. Obviously, more work is needed in this area.

3. Dystrophic animals.

Because of the possible role of light damage in the etiology and progress of retinal dystrophies, we have also done some work on two animal models, namely the RCS rat and the pcd mouse. This work was not described in our original proposal but was undertaken with the hope that some of our findings in light damage would have relevance for these diseases. The two animal models are quite different in their course and the role of light in the progress of the disease has only been shown for the RCS rat. The dystrophy seen in the RCS rat involves a build-up of a rhodopsin-rich material between the photoreceptor cell layer and the pigment epithelium This debris, as it is called, forms mainly from the breakdown or shedding of ROS. The defect in the RCS retina appears to be a lack of phagocytosis by the pigment epithelium (Herron et al., 1969; Bok and Hall, 1971; Mullen and LaVail, 1976). The pigment epithelium of the RCS rat will phagocytize carbon particles presented to it (Custer and Bok, 1975; Reich D'Almeida and Hockley, 1975), but does not perform its normal role of phagocytizing shed ROS. The possibility that there exists a lack of recognition on the part of the pigment epithelium for the ROS material due to some abnormality of the ROS membranes, prompted us to investigate the bleaching characteristics of RCS rat rhodopsin (Chaitin and

Williams, 1977). We found no difference in the bleaching rate, thermal stability or rate of metarhodopsin II production of rhodopsin isolated from normal and dystrophic rats. In a series of elegant studies just completed by Michael Chaitin in the laboratory of Dr. Michael Hall at the Jules Stein Institute it has now been shown that the defect of RCS pigment epithelium is not in the recognition of shed photoreceptor material, but in the ingestion phase of the phagocytic activity. By the use of indirect immunofluorescence techniques it has been shown that the pigment epithelium cell of the RCS rat readily binds ROS but the bound ROS material is not ingested. This is shown in Figures 6 and 7. Michael Chaitin was a graduate student trained under our contract. However, since his problem required tissue culture of pigment epithelium Dr. Michael Hall kindly agreed to allow the work to be done in his laboratory. Dr. Chaitin has now completed the requirements for his Ph.D. and has gone on to post-doctoral work in the laboratory of Dr. David Papermaster at Yale University.

The other animal model which has been investigated is the pcd mouse (Mullen, Eicher and Sidman, 1975). Pcd is the gene symbol for a new autosomal recessive mutation that results in loss of virtually all Purkinje cells of the cerebellum between the ages of 3 and 5 weeks. This neurological mutant shows, in addition, a slow progressive photoreceptor degeneration (Mullen and LaVail, 1975). The retinae of pcd/pcd mice seen relatively normal at 18 days but by 25 days the photoreceptor nuclei are showing some pyknosis and ROS are somewhat disorganized. Photoreceptor loss continues over the next year with outer segments becoming shorter and more variable in length. There are regional

differences in the rate of photoreceptor degeneration similar to those reported by LaVail and Battelle (1975) in the RCS rat. Thus far, our work with this mutant has involved evaluation of the rhodopsin content of the retina as a function of age (Figure 8). In collaboration with Dr. Anne Fulton, Children's Hospital, Boston the relationship between rhodopsin content, sensitivity in background adaptation and state of retinal degeneration has been investigated. A manuscript (preprint enclosed in the Appendix) has been submitted to Investigative Ophthalmology and Visual Science. Our findings with regard to the electrical activity of the pcd/pcd retina have led us to start an evaluation of ouabain-binding as a function of age. We wish to know if the Na^+ pump involved in the production and maintenance of receptor dark current is normal during the course of this degeneration. The results of these experiments are very preliminary and not enough ages have been investigated to allow any statement to be made.

OVERVIEW

In our initial proposal we spelled out the need for careful parametric studies of retinal light damage. To this end we have looked at two important variables, namely, intensity-duration and age. In addition, we have contributed somewhat to the understanding of the variable of eye-pigmentation, although some questions remain in this area. Our studies have also demonstrated the regional differences of the rat retina to light damage. This finding gains increased importance when considered with the body of literature of regional differences of photoreceptor degeneration

in dystropic conditions. Thus these parametric studies have given us information which suggests new mechanistic studies.

The most disappointing aspect of these investigations has been our inability to tie down a role for photodynamic action in light damage. The results of our in vitro, studies suggest that peroxidative damage is prevalent in the retina and more specifically in the rod outer segment. To date, however, we have failed to demonstrate this clearly in the in vivo, situation. This area of our research will be continued with increased effort since we feel that our experience with both light damage and pigment chemistry make this area one in which we are uniquely qualified. While investigations of the underlying causes of regional differences in retinal light damage should be pursued it is our belief that to undertake these at the present time will only dilute our effort. Instead, we intend to focus on the problem of photodynamic action as a causal agent in light damage. To this end some new approaches are described in the accompanying proposal.

Only one student has been trained under this contract. He is Dr. Michael Chaitin who has completed his degree program and the Ph.D. will be awarded in June.

Present state of knowledge and its significance

To date very few inroads have been made in understanding the phenomenon of light damage. At the present time the body of information available has stimulated concern in several areas about the quality and quantity of light to which the retina is exposed. During the last 18 months this concern has led to (1) the formation of a working group of the National Research Council

Vision Committee; (2) a session on ophthalmic exposure in a conference on applied vision sponsored by the National Academy of Sciences and (3) a special conference sponsored by the National Eye Institute and organized by Dr. Harry Sperling of the Sensory Science Center, University of Texas Health Science Center in Houston. In addition in April of 1979 The Psychobiology Research Center of Florida State University sponsored a symposium on the Effects of Constant Light on Visual Processes. A third symposium on Light Damage to the Retina nad Retinal Pigment Epithelium was held in June, 1978, under the sponsorship of the Society for Photobiology.

At the recent NEI sponsored conference on intense light hazards in ophthalmic diagnosis and treatment held in Houston (October, 1979) it was apparent that considerable concern exists in both the research and clinical community with regard to light exposures of patients during opthalmic evaluation and/or surgery. Particular concern was expressed about the short wavelength (blue) exposure of both normal and diseased eyes. However, no real recommendations have been forthcoming, only warnings. The reluctance to defind thresholds for exposures of patients stems from the lack of understanding of all the variables controlling light damage as well as the known variation in the clinical picture of retinal disease. Sliney and Wolborsht (1981) have suggested that the retinal hazard criteria presently in operation (ACGIH, 1978) lack a completeness in their standards which require very careful application if total safety is to be insured. The general feeling among workers in this area is that an appreciation of light-toxicity is lagging far behind phenomenalogical observation. Until more

quantitative evaluation of intensity-duration functions and the role of eye pigmentation in light damage are better understood, specific guidelines or standards will probably not be available. In the meantime, warnings of the type issued by Calkin and Hochheimer (1980) are of utmost importance to the protection of patients. Obviously, more investigations with animals and clincal population are indicated.

Bibliography of work associated with this project

1. Rapp, L. M. and T. P. Williams, A Parametric Study of Retinal Light Damage in Albino and Pigmented Rats in THE EFFECTS OF CONSTANT LIGHT ON VISUAL PROCESSES, T. P. Williams and B. N. Baker, eds., Plenum Press, New York, p. 135-159 (1980).

2. Rapp, L. M. and T. P. Williams, The Role of Ocular Pigmentation in Protecting Against Retinal Light Damage, Vis. Res. 20, 1127 (1980).

3. Howell, W. L., L. M. Rapp and T. P. Williams, Distribution of Melanosomes Across the Retinal Pigment Epithelium of a Hooded Rat: Implications for light-damage. Accepted by Investigative Ophthal. (1981).

4. Fulton, Anne B., Karen A. Manning, Barbara N. Baker, Susan E. Schukar and Clark J. Bailey, Background adaptation in Purkinje Cell Degeneration (pcd/pcd) Mice. Invest. Ophthal. and Vis. Sci., in press.

Other Support

(1) Active Support.

a. National Science Foundation, BNS 78-05842, Rhodopsin Bleaching: Role of the Microenvironment, Theodore P. Williams, 25% T, $56,148 annual direct cost, 6/15/78 to 11/30/81. No overlap with project.

b. Department of Energy, EVO 6021, Damaging Effects of Visible Light, Theodore P. Williams, 10% T, $33,320 annual direct cost, 6/1/80 to 5/31/81.

c. National Eye Institute, I R01EY03501, ^{13}C Retinals as Probes for the Rhodopsin Spectrum, Theodore P. Williams, 5% T, $72,442 annual direct cost, 9/1/80 to 8/1/83. THE PI IS ONLY THE COORDINATOR AND HIS LAB DERIVES ALMOST NO SUPPORT FROM THIS PROJECT. This project does not overlap with proposed project.

(2) Pending Support.

a. National Eye Institute, Damaging Effects of Visible Light, Theodore P. Williams-PI, 15% T, requested $78,740, 2/1/81 to 1/31/86. This project does not overlap with proposed project. Due to federal cut-backs, we will not know about support until September, 1981.

b. National Science Foundation, Damaging Effects of Visible Light, Theodore P. Williams-PI, 15% T, requested $94,271, 2/1/81 to 1/31/84. This project does not overlap with proposed project. NSF is holding on support until NIH makes a decision.

c. National Eye Institute, Studies of Single Visual Photoreceptors, Theodore P. Williams-PI, 5% T, annual direct cost requested-$74,640, 7/01/81 to 6/30/86. This project does not overlap with the proposed project.

d. National Science Foundation, Studies of Single Visual Photoreceptors, Theodore P. Williams-PI, 5% T, annual direct cost requested-$99,078, 7/01/81 to 6/30/86. This project does not overlap with this proposed project.

REFERENCES

1. Adams, D. O., Beatrice, E. S. and Bedell, R. B. (1972). Retina: Ultrastructural alterations produced by extremely low levels of coherent radiation. Science 177, 58-60.

2. Ballowitz, L. and Dämmrich, K. (1972). Retinaschäden bei Ratten nach einer Fototherapie. Z. Kinderhulk 113, 42-52.

3. Bok, D. and Hall, M. O. (1971). The role of the pigmented epithelium in the etiology of inherited retinal dystrophy in the rat. J. Cell Biol. 49, 664-682.

4. Carter-Dawson, L., Kuwabara, T., O'Brien, P. J. and Bieri, J. G. (1979). Structural and biochemical changes in vitamin A-deficient rat retinas. Invest. Ophthal. and Vis. Sci. 18, 437-446.

5. Chaitin, M. H. and Williams, T. P. (1977). Bleaching Characteristics of rhodopsin from normal and dystrophic rats. Exp. Eye Res. 24, 553-558.

6. Custer, N. V. and Bok, D. (1975). Pigment epithelium-photoreceptor interactions in the normal and dystrophic rat retina. Exp. Eye Res. 21, 153-166.

7. Herron, W. L., Riegel, R. W., Meyers, O. E. and Rubin, M. L. (1969). Retinal dystrophy in the rat-a pigment epithelial disease. Invest. Ophthalmol. 8, 595-604.

8. Howell, W. L., Rapp, L. M. and Williams, T. P. (1981). Distribution of melanosomes across the retinal pigment epithelium of a hooded rat: Implications for light-damage. Invest. Opthal. and Vis. Sci., in press.

9. Kuwabara, T. and Gorn, R. A. (1968). Retinal damage by visible light: an electron microscopic study. Archs. Ophthalmol. 79, 69-78.

10. Kuwabara, T. and Funahashi, M. (1976). Light damage in the developing rat retina. Arch. Ophthalmol. 94, 1369-1374.

11. LaVail, M. M. (1980). Eye Pigmentation and constant light damage in the rat retina. In The Effects of Constant Light on Visual Processes (Edited by T. P. Williams and B. N. Baker), Plenum Press, New York, pp. 357-387.

12. Lawwill, T. (1973). Effects of prolonged exposure of rabbit retina to low-intensity light. Invest. Ophthalmol. 12, 45-51.

13. Marshall, J., Mellerio, J. and Palmer, D. A. (1972). Damage to pigeon retinae by moderate illumination from fluorescent lamps. Exp. Eye Res. 14, 164-169.

14. Mullen, R. J. and LaVail, M. M. (1976). Inherited retinal dystrophy: Primary defect in pigment epithelium determined with experimental rat chimeras. Science 192, 799-801.

15. Noell, W. K., Walker, V. S., Kang, B. S. and Berman, S. (1966). Retinal damage by light in rats. Invest. Ophthalmol. 5, 450-473.

16. O'Steen, W. K., Anderson, K. V. and Shear, C. R. (1974). Photoreceptor degeneration in albino rats: dependency on age. Invest. Ophthalmol. 13, 334-341.

17. Reich D'Almeida, F. B. and Hockley, D. J. (1975). In situ reactivity of the retinal pigment epithelium. II. Phagocytosis in the dystrophic rat. Exp. Eye Res. 21, 347-357.

18. Ts'o, M. O. M. (1973). Photic maculopathy in rhesus monkey: A light and electron microscopic study. Invest. Ophthalmol. 12, 17-34.

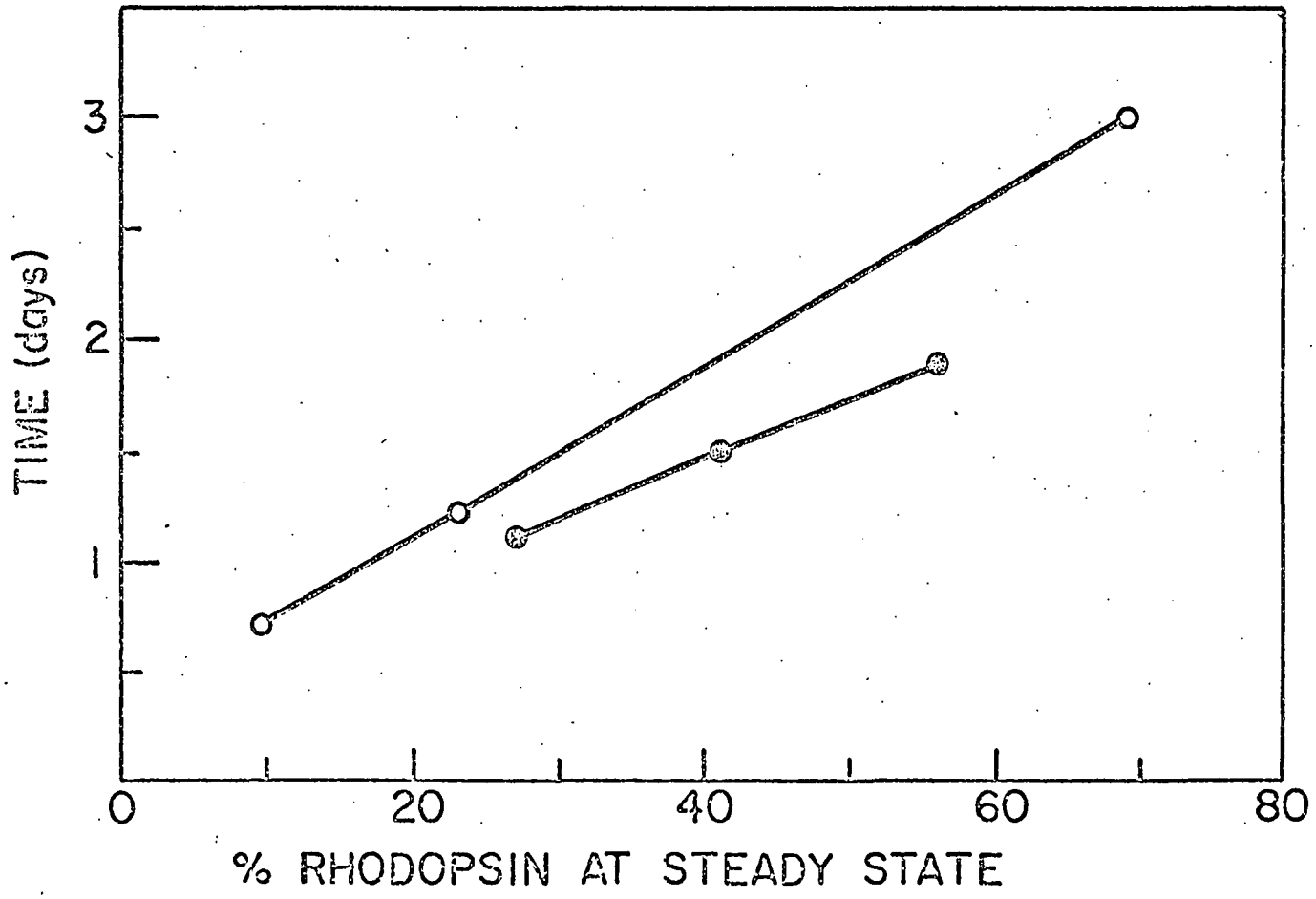


Figure 1. Time required to produce criterion damage as a function of the steady-state rhodopsin level in the retina. Open circles: albino rats; closed circles: pigmented rats.

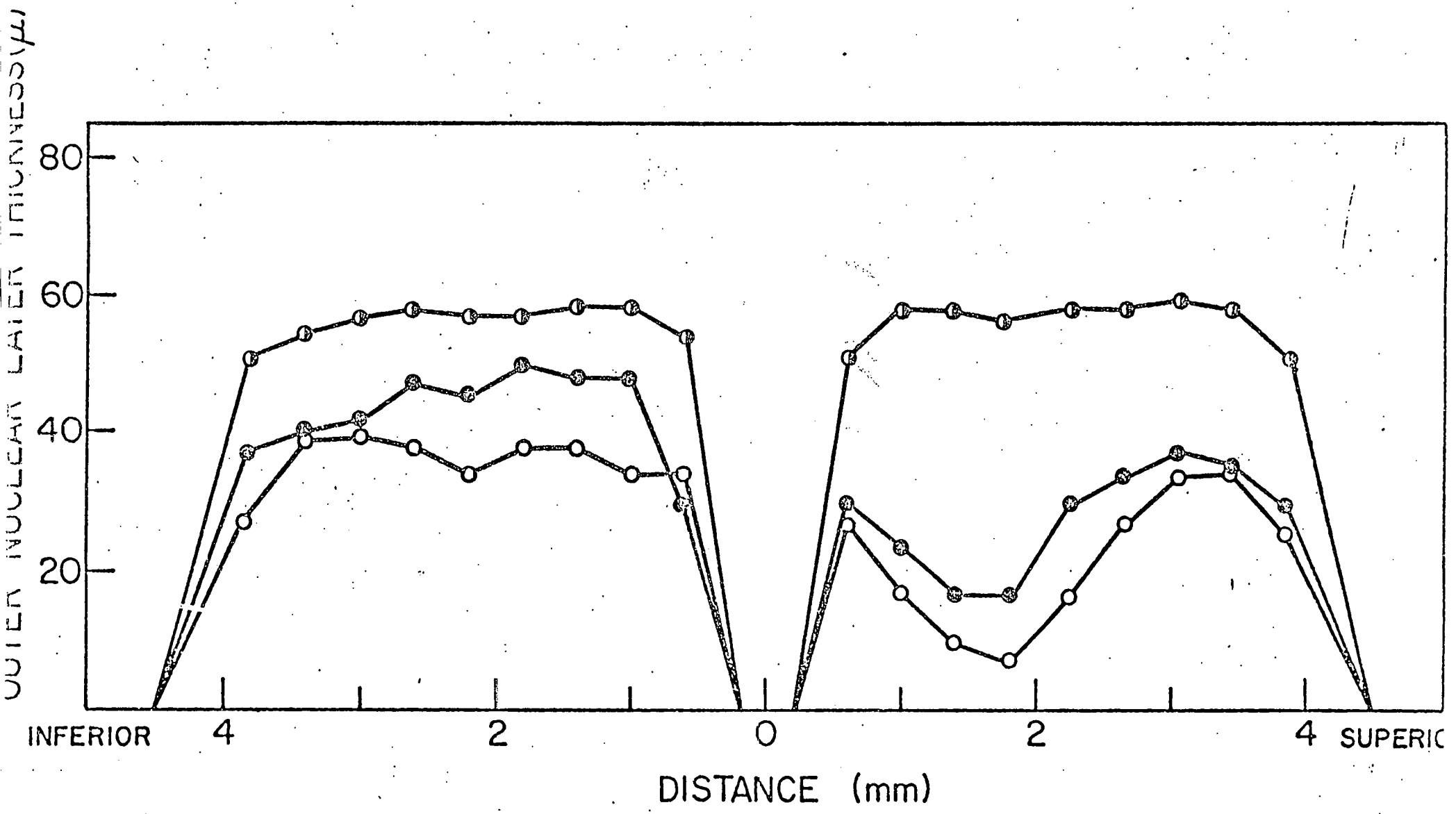


Figure 2. Distribution of light-damage across rat retinas. Outer nuclear layer thickness is the measure of damage. The optic nerve head is represented at "0" on the abscissa. Half-filled circles: Control--averages of a pigmented rat and albino ONL thickness. Filled circles: pigmented rat. Open circles: albino rat. Conditions of damage: four days at 40 lux.

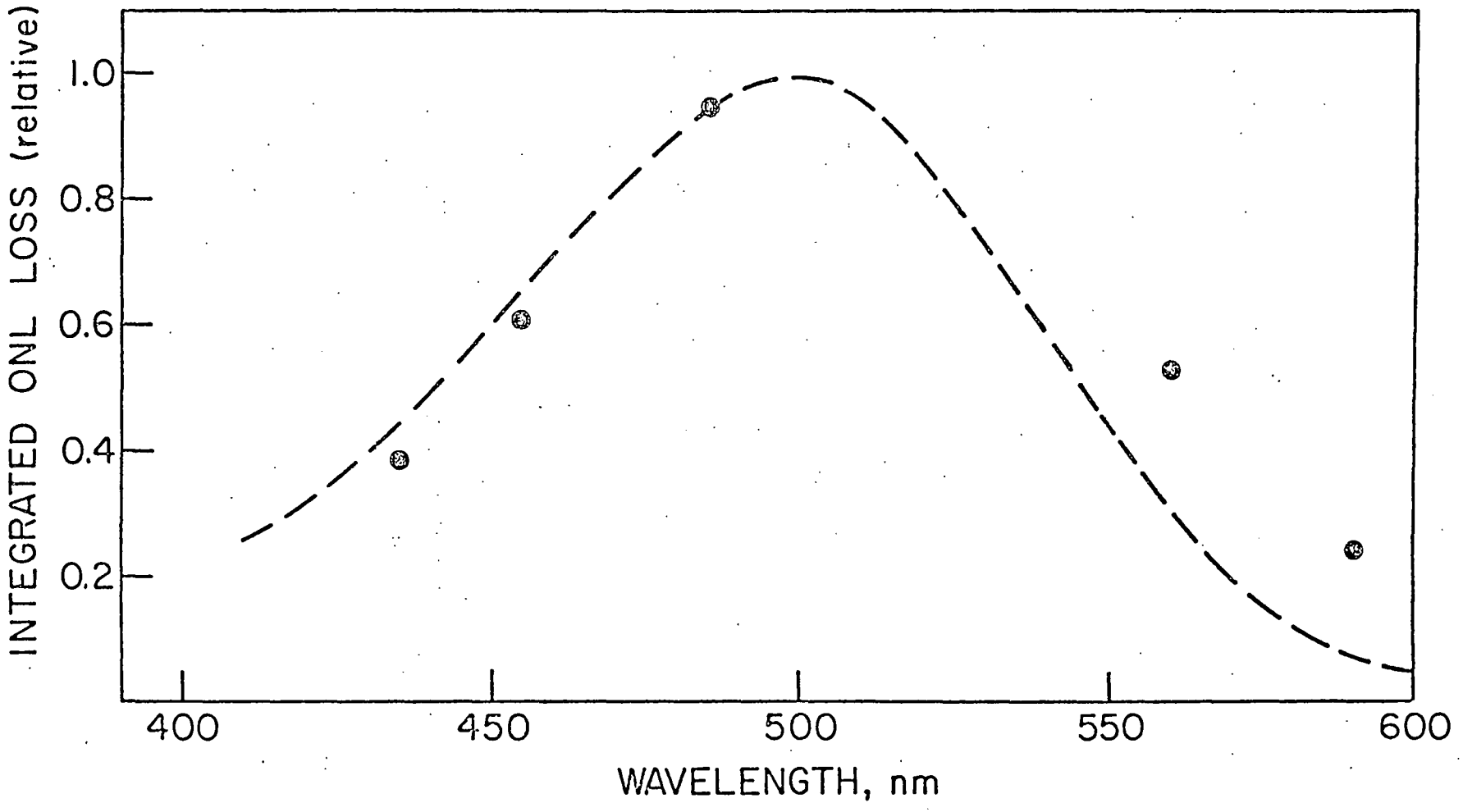

INTEGRATED ONL LOSS (relative)
1.0
0.8
0.6
0.4
0.2
400
450
500
550
600
WAVELENGTH, nm

Figure 3

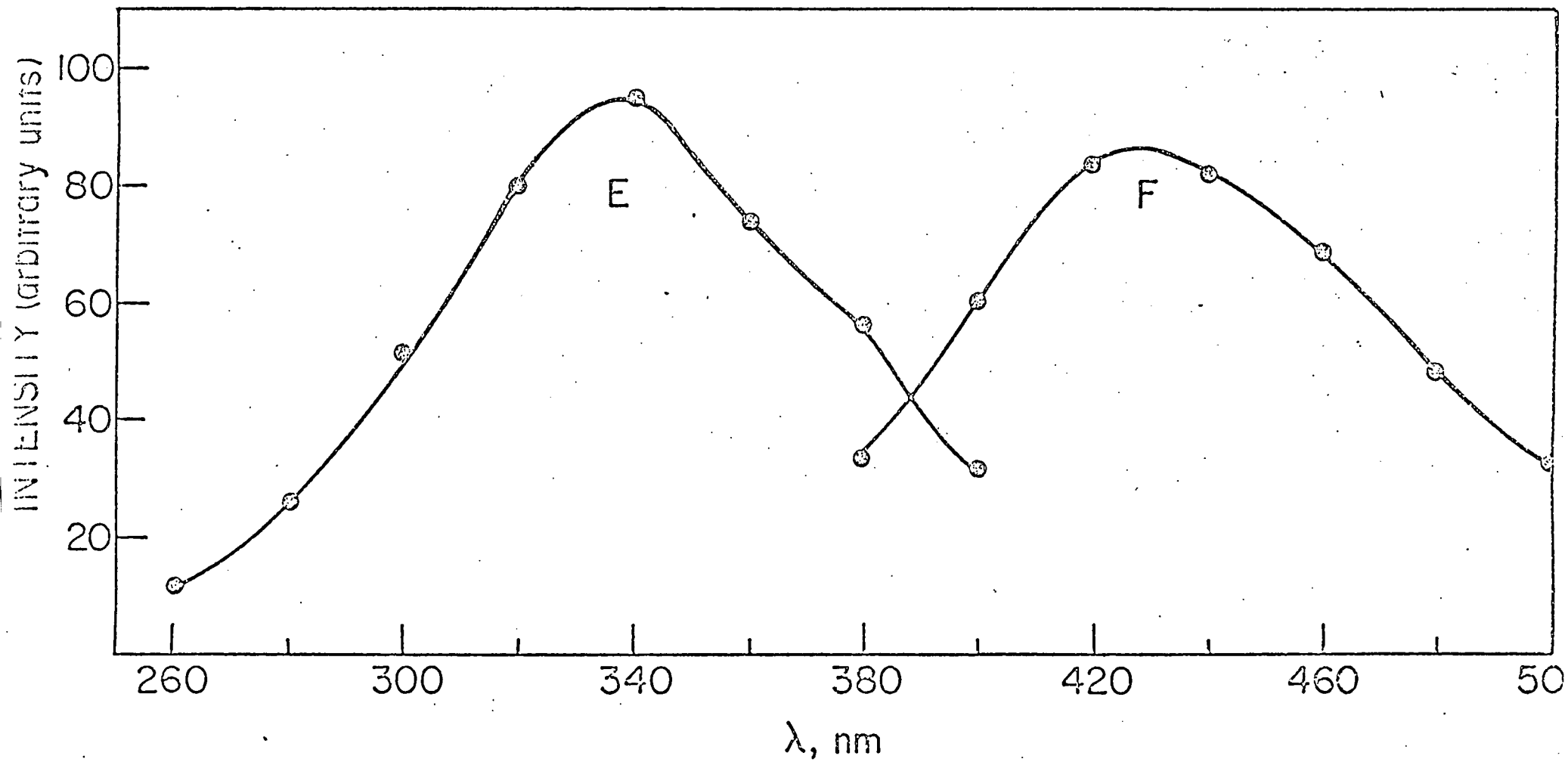
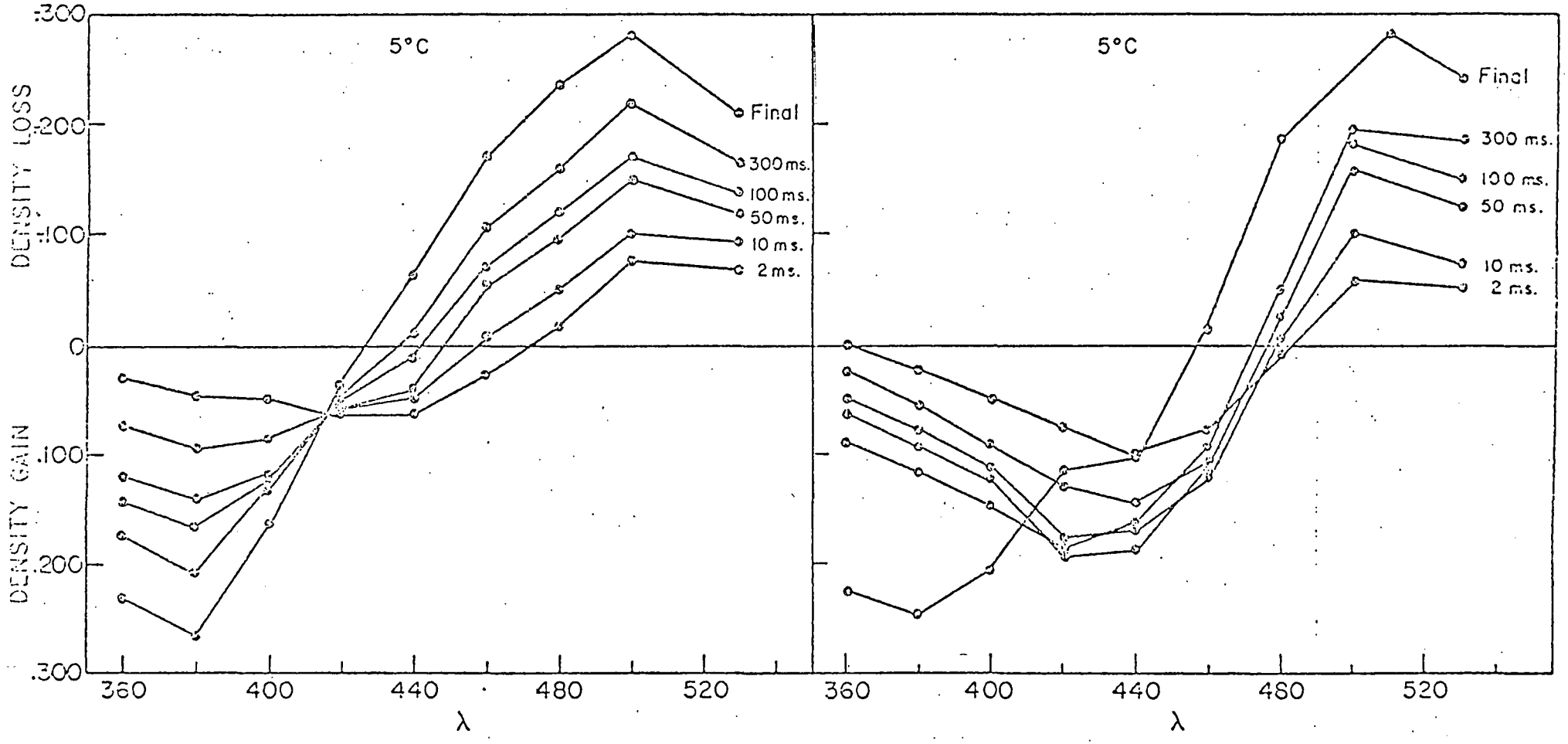

INTENSITY (arbitrary units)
100
80
60
40
20
E
F
260
300
340
380
420
460
λ, nm

Figure 4


5°C
Final
300ms.
100ms.
50ms.
10 ms.
2ms.
DENSITY LOSS
DENSITY GAIN
.300
.200
.100
0
.100
.200
.300
360
400
440
480
520
λ
5°C
Final
300 ms.
100 ms.
50 ms.
10 ms.
2 ms.
360
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480
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λ

Figure 5

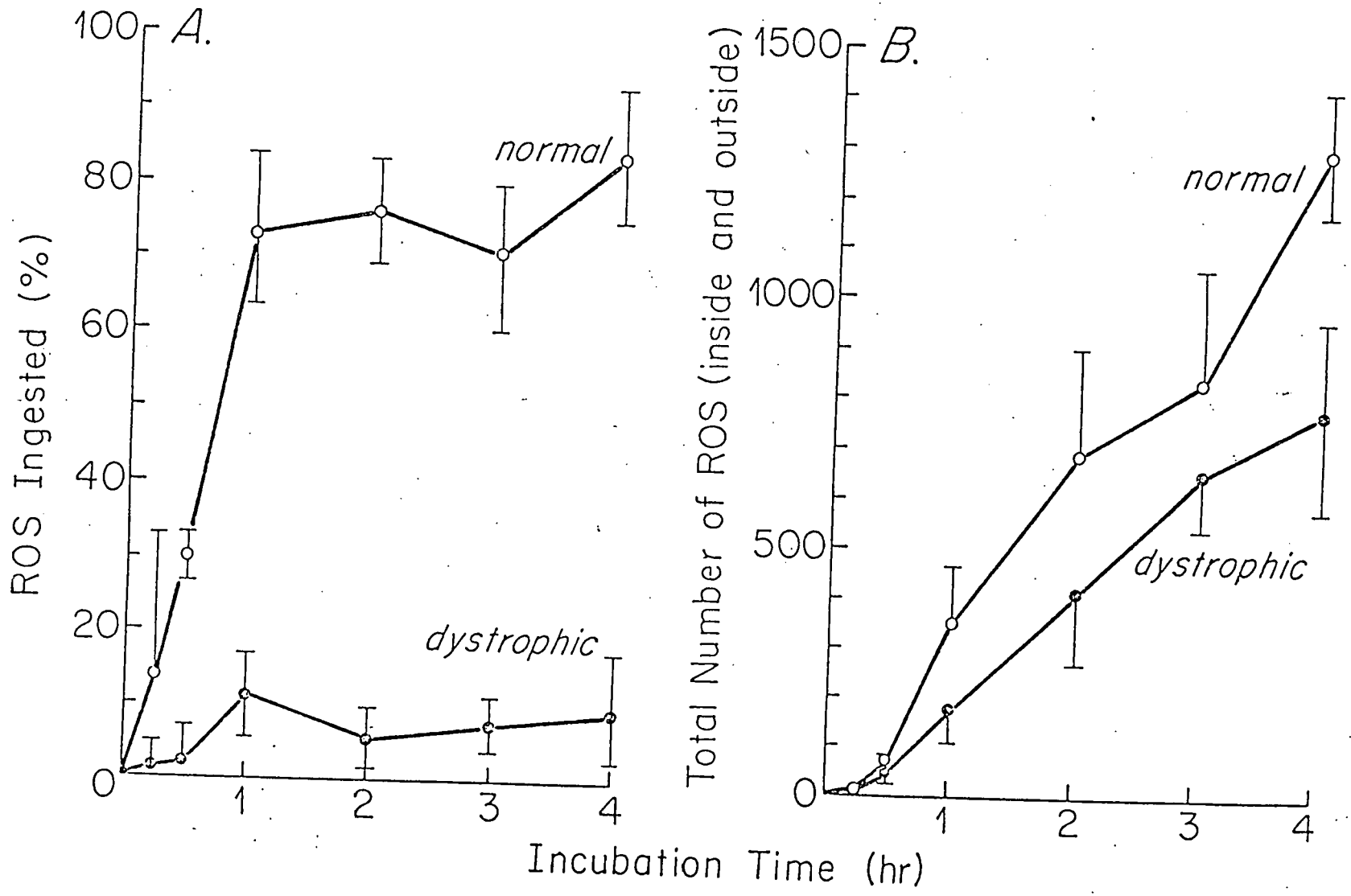
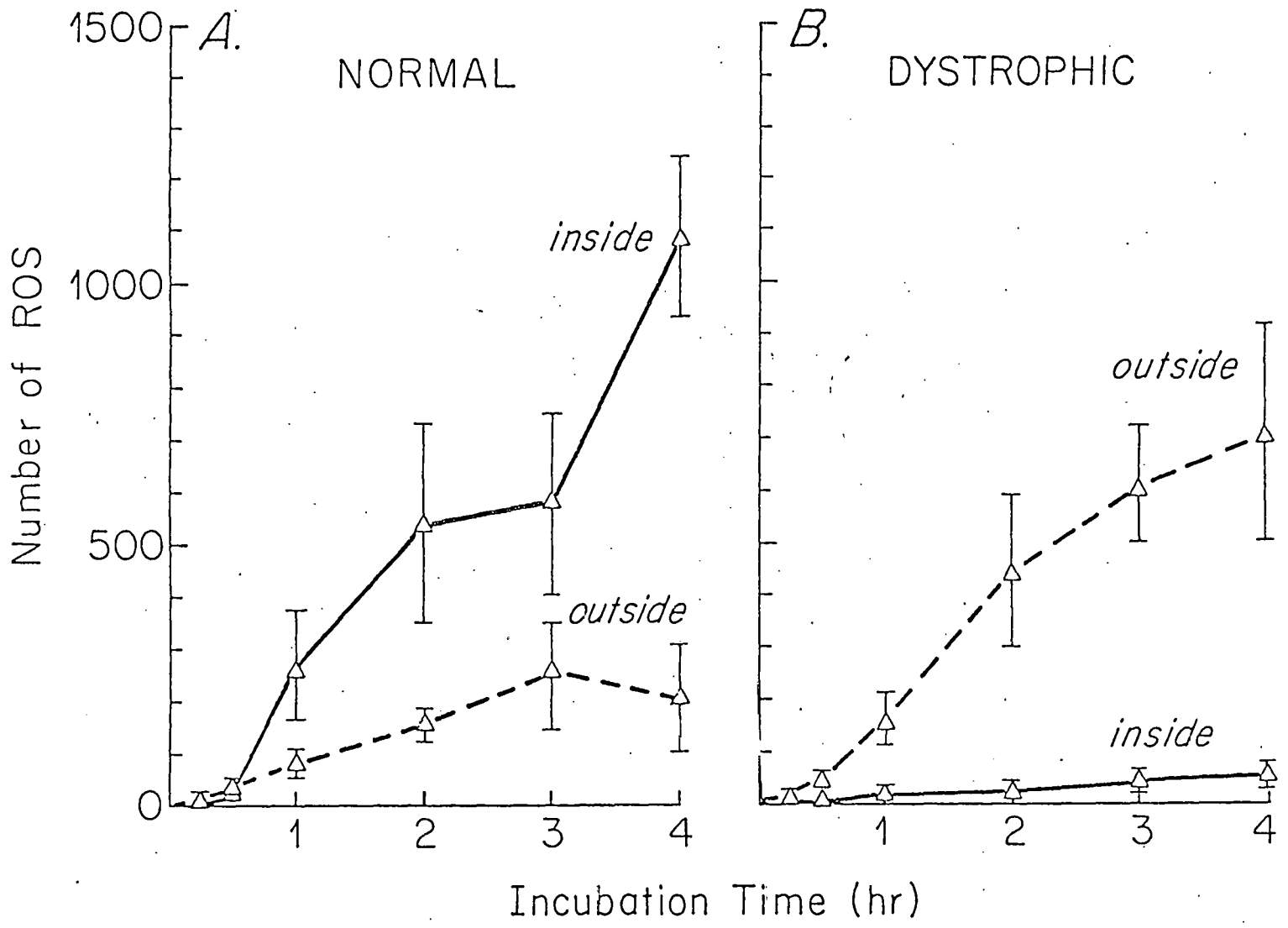

A.
B.
ROS Ingested (%)
Total Number of ROS (inside and outside)
normal
dystrophic
normal
dystrophic
Incubation Time (hr)
0
20
40
60
80
100
500
1000
1500
1
2
3
4

FIGURE 6


A.
NORMAL
B.
DYSTROPHIC
1500
1000
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Number of ROS
inside
outside
outside
inside
1
2
3
4
1
2
3
4
Incubation Time (hr)

FIGURE 7

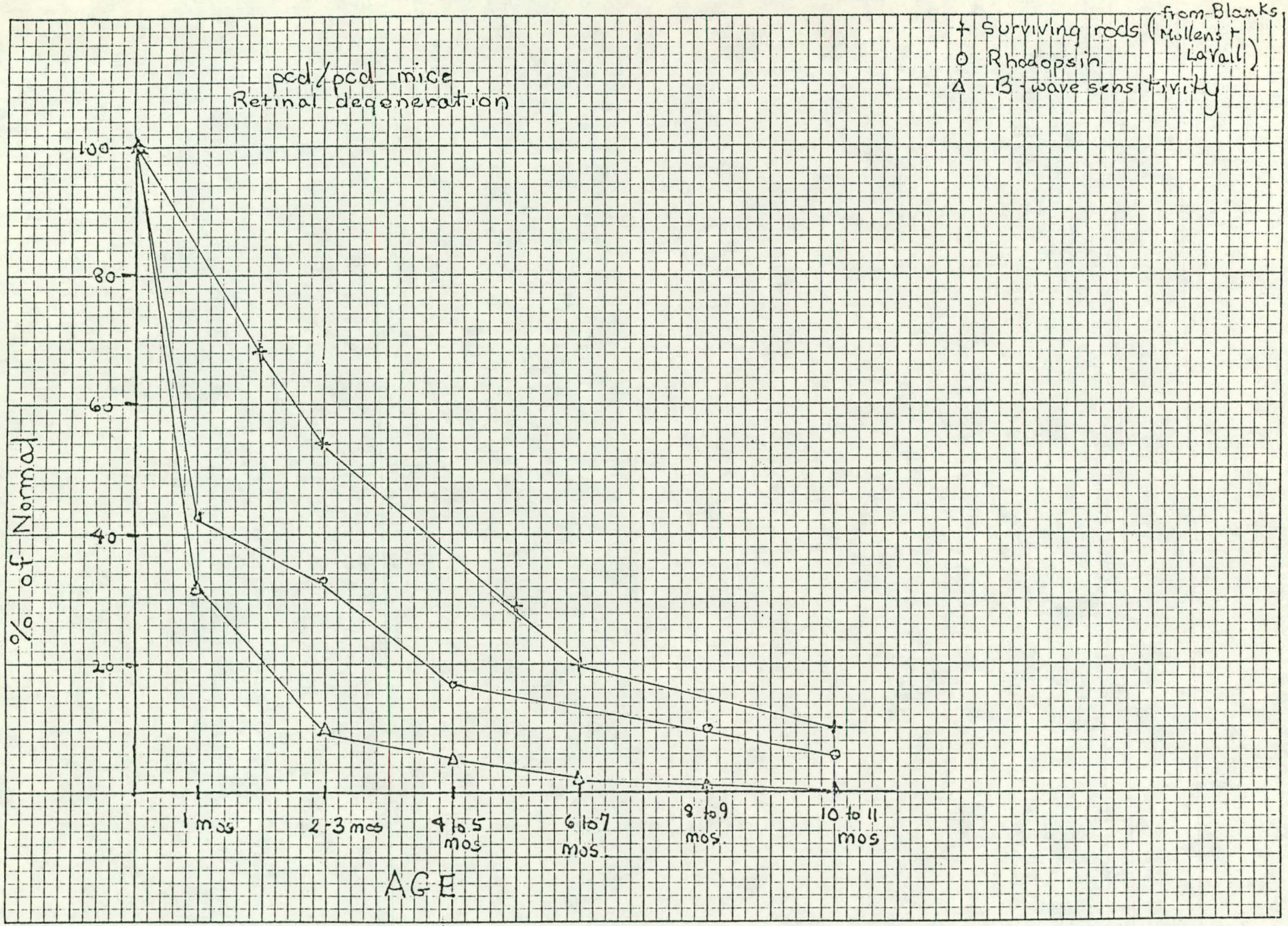

Figure 8