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TISSUE CULTURE MODELLING OF MICROWAVE INDUCED CATARACTS OF THE EYE LENS

Annual Summary Report

DR. JOHN R. TREVITHICK

AUGUST, 1979

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Intact rat lenses incubated in Medium 199 (M199) at 35.5° maintain their transparency for at least 9 days. When heated to various temperatures for 1 hour to model cataractogenesis by elevated temperatures, followed by 47 hours at 35.5°C, some lenses developed cataractous opacities, depending on the temperature of incubation. At 39°C slight globular degeneration of fiber cells appeared, localized in the equatorial region, with globules up to 20μ in diameter as has been reported for glucose-induced cataracts. At 41°C the

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globular degeneration was deeper at the equatorial region, extending towards the nucleus and extended to the anterior and posterior of the lens. At 47°C and 50°C some very large globules were formed (ca 200μ across) and the globular degeneration was more extensive. At higher temperatures (60°C) lenses did not become opaque. They had normal transparency and acuity, perhaps because they had been fixed, by a process similar to histological fixation. The morphological appearance of such lenses examined by scanning electron microscopy (SEM) was normal. D-α-tocopherol acetate (Vitamin E) 2.4 μM) when added to lenses before incubation at 41°C, prevented the globular degeneration observed at this temperature. These results indicate that the intact rat lens in M199 appears much more sensitive to elevation in temperature than does the lens in vivo. The fact that the process of cataractogenesis is prevented by Vitamin E indicates a possibility of further exploring the mechanism of heat-induced cataract; this effect may involve several possibilities: (a) a bulk effect on membranes; for instance on membrane fluidity or (b) as a scavenger of free radicals, or (c) some other effect on membranes mediated by its lipid solubility and anti-oxidant properties. The fact that cortical cataracts induced in this model system by both glucose and elevated temperatures may be prevented by Vitamin E (D-α-tocpherol) may indicate that some step common to both processes is involved in cortical cataractogenesis.

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Summary

The eventual aims of these experiments are to use intact rat lenses incubated in vitro in tissue culture medium to study the development of cataracts when the lenses are exposed to elevated temperatures and/or microwave irradiation by high energy-pulsed microwaves, to establish (a) cataractogenic conditions for irradiation and (b) the mechanism of cataractogenesis in such lenses.

Intact rat lenses incubated in Medium 199 (M199) at 35.5° maintain their transparency for at least 9 days. When heated to various temperatures for 1 hour to model cataractogenesis by elevated temperatures, followed by 47 hours at 35.5°C, some lenses developed cataractous opacities, depending on the temperature of incubation. At 39°C slight globular degeneration of fiber cells appeared, localized in the equatorial region, with globules up to 20μ in diameter as has been reported for glucose-induced cataracts. At 41°C the globular degeneration was deeper at the equatorial region, extending towards the nucleus and extended to the anterior and posterior of the lens. At 47°C and 50°C some very large globules were formed (ca 200μ across) and the globular degeneration was more extensive. At higher temperatures (60°C) lenses did not become opaque. They had normal transparency and acuity, perhaps because they had been fixed, by a process similar to histological fixation. The morphological appearance of such lenses examined by scanning electron microscopy (SEM) was normal. D-α-tocopherol acetate (Vitamin E, 2.4 μM) when added to lenses before incubation at 41°C, prevented the globular degeneration observed at this temperature. These results indicate that the intact rat lens in M199 appears much more sensitive to elevation in temperature than does the lens in vivo. The fact that the process of cataractogenesis is prevented by Vitamin E indicates a possibility of further exploring the mechanism of heat-induced cataract; this effect may involve several possibilities: (a) a bulk effect on membranes; for instance on membrane fluidity or (b) as a scavenger of free radicals, or (c) some other effect on membranes mediated by its lipid solubility and anti-oxidant properties. The fact that cortical cataracts induced in this model system by both glucose and elevated temperatures may be prevented by Vitamin E (D-α-toopherol) may indicate that some step common to both processes is involved in cortical cataractogenesis.

Foreword

A. List of Professional Personnel Employed on This Project

Principal Investigator	-	Dr. John R. Trevithick, Ph.D.
Research Associate	-	Dr. P. Jill Stewart-DeHaan, Ph.D.
Research Associate	-	Dr. William M. Ross, Ph.D.

B. Animal Care

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences - National Research Council, U.S.A.

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Introduction

It has been known for many years that cortical cataracts of the eye lens may be induced by chronic exposure of the lens to elevated temperatures, as in glassblowing (Vogt, 1930, Goldman, 1933 and Langley *et al*, 1960). A number of papers on cataracts induced in whole animals by microwaves has appeared. Kramar *et al* (1975) have concluded that increased temperature in the lens is a necessary factor in cataractogenesis. Carpenter *et al* (1977) further indicates that *in vivo*, elevated temperature and microwaves may be required simultaneously to cause a cataract.

Recently it appears (Kramar, private communication) that an additional factor which should be considered in heat-and microwave-induced cataract formation may be the rate of temperature increase in the lens. Thus a rapid increase might be more likely to result in cataractogenesis.

Because of the difficulty of performing experiments *in vivo* to evaluate the role of elevated temperature *in vivo*, we decided, in performing studies of temperature, to utilize the *in vitro* system for incubation of rat lenses, in medium 199 (M199), which we developed for study of cataracts induced by various chemicals and drugs (Mousa *et al*, 1979).

Materials and Methods

Intact lenses from adult Wistar rats were removed after decapitation of the animals, checked by examination with a dissecting microscope (Zeiss) and incubated for 48 hr. in Medium 199 (M199) with 15% fetal calf serum in plastic tubes (Mousa *et al*, 1979). For the first hour the temperature of incubation was adjusted as necessary by incubation of the tubes in a thermostatted water bath. Following the first hour of incubation, the tubes were placed in a water-jacketed tissue culture incubator (Hotpack) at 35.5°C. Vitamin E was added to the medium (as D-α-tocopherol acetate) to a concentration of 2.4 μM; 5 ml M199 containing 15% fetal calf serum was removed from 100 ml of medium prior to filtering through a sterilizing filter (Nalgene) and the Vitamin E suspended by gently warming and stirring; just as the last 5 ml of this medium was passing through the filter, the warm medium containing suspended Vitamin E was added. The filter was checked visually to ascertain that no lipid droplets remained, so that all the Vitamin E had passed into the 100 ml of medium after the filter sucked dry. Determination of the Vitamin E content by fluorescence by extraction from the medium indicated the expected Vitamin E concentration.

Incubation with M199 containing glucose (0.0556) was performed as a positive control for cataract formation (Creighton and Trevithick, 1979).

Following incubation, lenses were fixed in Karnovsky's fixative (Graham and Karnovsky, 1966) at 4°C for 24 hr then placed in 0.1M sodium cacodylate buffer, pH 7.4 for 48 hr, also at 4°C. The lenses were quartered, dehydrated in alcohol and critical-point-dried from CO_2, then mounted on SEM stubs using silver daube paint and examined in a Hitachi Scanning Electron Microscope (Model HHS-2R).

For estimation of opacity, the lenses were placed in the inverted lid of a Petri dish in a raing stand, supported approximately 14.5 cm above the bulb of a high intensity lamp. Each focussed the light as a bright spot in the middle of its round shadow. Each lens was visualized on a sheet of translucent bond paper placed 6.5 mm above the dish bottom (ca.3.5 mm above the top of the lens) on top of the open dish. These lens images were photographed for identical times by our photography service, to show decrease in total light passing through the lens and the increased diffuseness or decreased focussing of the light.

Results

Shadowgrams (Fig. 1) of the fixed lenses which had been incubated at temperatures of 39°C indicated a very slight decrease in acuity (resolution of the light spot compared to a normal lens control as indicated by a slightly larger diameter of the spot. At 41°C, 47°C, 50°C and 55°C, the lens became much more opaque and no light was focussed into the central spot. For the lens incubated at 60°C, surprisingly, the lens appeared clear and its shadowgram was similar to that of a normal control, as was that of a lens placed in M199 containing Vitamin E, prior to exposure to 41°C for 1 hr.

In order to investigate these changes in detail, the lenses were examined by scanning electron microscopy. The control lens appeared normal (Fig. 1) with regular spacing of lens fiber cells interdigitating at the corners and, having ball and socket joints on their flat surfaces. At 39°C (Fig. 1) significant globular degeneration occurred at the lens equator, with the result that both a subcapsular layer of degeneration was observed in which globules could be seen and the cell surfaces appeared flaky (Fig. 1). In addition to the immediate subcapsular layer an area of relatively coarse globular degeneration extended at the equator deeper towards the nucleus. After exposure to 41°C, more extensive globular degeneration occurred which was similar to morphological appearance to that seen at elevated glucose levels; as after 39°C, a subcapsular layer extended both anteriorly and posteriorly. In lenses after exposure to 39°C or 41°C epithelial cells (at the equator and subcapsularly) could not be distinguished clearly from the fiber cells since all were involved in the area of globular degeneration.

At higher temperatures, 47°C (Fig. 1) the lenses exhibited giant globules, an unusual feature not previously noticed for glucose-or sugar-

induced cataracts. These giant globules were up to 20 times as big as globules usually found in other cataracts, and may be a distinctive feature of cataracts caused by heating since they have not been observed in our experiments with other potential cataractogenic agents.

At 60°C, the morphological appearance of the lenses viewed by scanning electron microscopy was normal, with no globular degeneration. The lens may have been "fixed" by the heating, to result in this effect.

When the lens was incubated in M199 containing Vitamin E (D-α-tocopherol acetate, 2.4 μM) prior to and during heating, only minimal changes in morphology occurred (Fig. 2) when examined by SEM. By comparison to normal, and control lenses only a slight alterations in ball and socket joints and interdigitation at cell corners occurred.

Conclusions

These results extend to elevated temperatures our previous observations of induction of cataracts following the incubation of rat lenses with intact capsules in M199 modified by added metabolities, drugs and chemicals (Creighton, Trevithick, Mousa, Sanford and Percy, 1978, Mousa et al, 1979, Creighton and Trevithick, 1979). In particular, three important observations were made:

(1) The observation, that a minimum temperature of 39°C is required to induce a cataractous opacity with associated cortical globular degeneration in an intact rat lens, is significant. In vivo, the lowest temperature at which lenses have been reported to form cataracts is 43°C. The lens in vitro thus seems to be much more sensitive to changes in temperature than it is in vivo. Nevertheless, the changes in cellular morphology in the cataractous regions appear to be rather similar to those we previously described in vivo in human PCSC, although they are distributed slightly differently. This indicates that the rat lens in vitro may be a suitable system, because of its greater sensitivity to temperature, for determining under controlled conditions dose-response and time-temperature relationships, which might be useful in indicating the relationships which might be obtained in vivo.

(2) The observation that, when added to M199, prior to and during incubation of the lens in M199, Vitamin E (D-α-tocopherol acetate) appeared to prevent cataractous morphological changes in rat lenses, may indicate a useful approach to the prevention of heat cataracts in vivo which should be explored further. The mechanism by which Vitamin E is acting is unknown, although at least two prominent possibilities should be considered:

(a) an effect as a scavenger of free radicals which are produced ubiquitously in tissues in the presence of oxygen and

(b) a bulk effect on the fluidity of membrane lipids, due to its lipid solubility. Other possibilities may involve some other effect on membranes mediated by its lipid solubility and anti-oxidant properties.

The surprising similarity of action of Vitamin E in preventing cataracts in vitro induced by either heat, or glucose as we previously reported (Creighton and Trevithick, 1979) may indicate some common process occurring in cataracts in intact lenses in vitro caused by either process.

(3) The observation that at elevated temperature of 47 and 50°C giant globules were formed is a unique finding. These globules could possibly originate as a result of fusion of several cells as is suggested by the picture in Fig. 2e, at 47°C. Although their exact significance is not clear, it seems possible that the large globules result from an exaggeration of the process by which small globules are formed, which may involve a breakdown of several functions which maintain the cell surface integrity. We have previously suggested that such a process might involve breakdown of cell subsurface actin microfilaments and herniation outwards of the cell cytoplasm into globules. In an exaggerated form such a process could lead to cell fusion as membrane integrity is lost. Alternatively, it seems possible that each globule might represent the contents of one fiber cell which are extruded into one globule. Another alternative is that the globules are actually vacuolar in nature. This is currently being investigated by histology and transmission electron microscopy.

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Fig. 1. Scanning electron microscopy and shadowgrams of intact rat lenses incubated for 1 hour at different temperatures, followed by 47 hr at 35.5°C before fixation.
Temperatures are shown on the figures.
Magnifications of all scanning electron micrographs are identical, 156X. Magnifications of shadowgrams are all 2.3X. The shadowgrams are as follows: (1) clear plastic ball; (2) opaque ball bearing; (3) fresh intact rat lens, fixed immediately; (4) intact rat lens incubated in M199 for 48 hr at 35.5°. (5) - (12) were incubated at the temperature indicated for 1 hr then for 47 hr at 35.5°C: (5) 37°C, (6) 39°C, (7) 41°C, (8) 43°C, (9) 45°C, (10) 47°C, (11) 50°C, (12) 60°C.

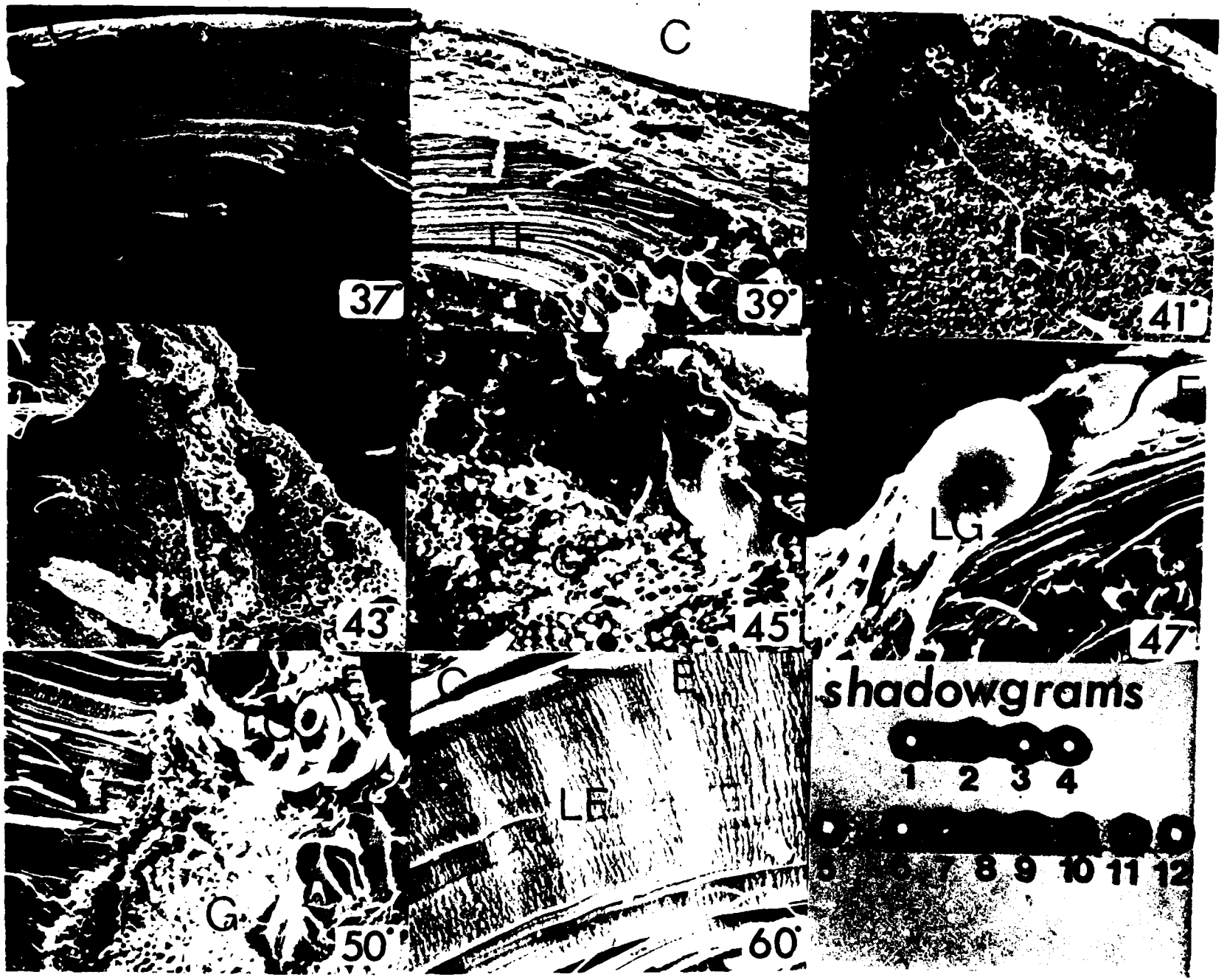
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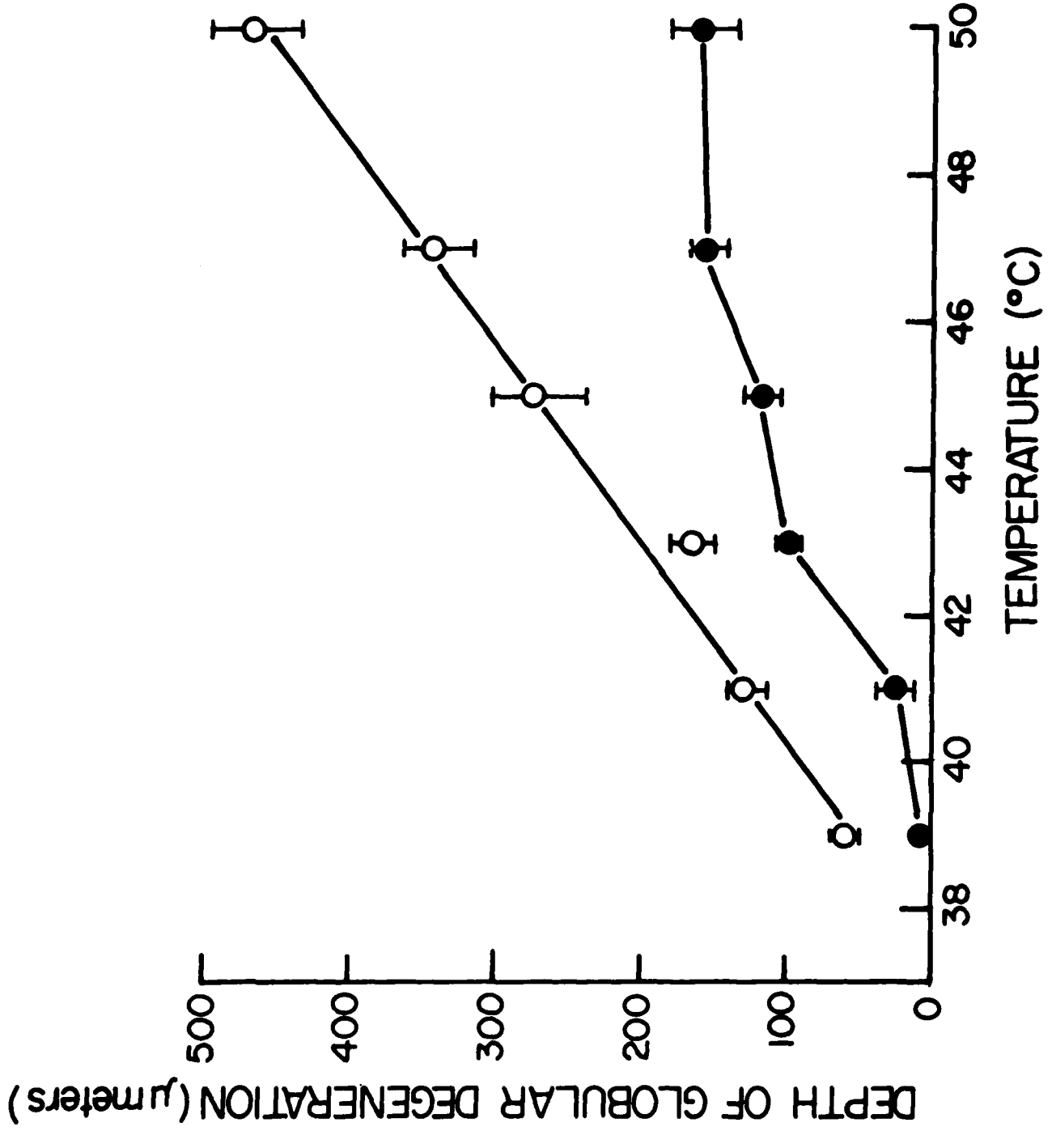
Fig. 2. Effect of vitamin E on temperature-induced globular degeneration of intact rat lenses incubated in M199 at 41°C for 1 hour. Lenses were placed in M199 at room temperature with or without vitamin E (D-α-tocopherol acetate, 2.4 μM) for a period of 10-15 minutes prior to exposure to the elevated temperature, and incubated after the exposure at 35.5°C for a period of 47 hr prior to fixation. After 24 hr the medium was replaced by fresh medium: (a) control lens, in M199 (156X), (b) treated lens incubated in M199 containing vitamin E acetate (D-α-tocopherol acetate, 2.4 μM) (156X).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