PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 444-448 (1976)

Tissue Culture of Rat Lenses[1] (39565)

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Sugar cataracts associated with diabetes and galactosemia have been studied extensively in animals (1-4). Numerous short-term experiments have also been conducted *in vitro*. A system which will permit long-term culture of lenses *in vitro* through the entire cataractogenic period, including the appearance of nuclear cataracts, is desired so that studies and correlations can be made under conditions that can be controlled and manipulated. In animals, the development of sugar cataracts is commonly studied in rats that are on a high galactose diet. A period of approximately 3 weeks is required. This paper describes, for the first time, a system that can be used to culture rat lenses for periods of 3 weeks and longer and permits galactose cataracts with morphological changes comparable to those found in rats to be produced and studied.

Methods. Apparatus. The apparatus is depicted in Fig. 1. The lens is supported on a perforated dialysis membrane (B), which is prepared by cutting it into single thickness squares about 3 × 3 cm, washing it for 1 hr in three to five changes of distilled water, making a depression to hold the lens in a stable position by stretching the wet membrane over the bottom of a 10 × 75-mm test tube and attaching it to the glass ring (C), securing it with a stainless steel wire, and perforating it with an 18-gauge stainless steel needle. A notch is cut in the membrane in line with the gaps in the glass ring and steel wire (E) and it is placed in a 15-ml beaker that has had the upper portion ground off so that it serves as the lower portion of a petri dish. The notches in the glass ring and perforations in the membrane permit components of the media to diffuse more freely and the notch in the membrane permits air bubbles to escape from the under surface of the membrane. The notches and slit in the glass ring are made with a glass cutting wheel and the cover is made from the bottom of a 20-ml beaker. A drop of distilled water is added to the assembled unit (F) so that the membrane will remain moist while it is autoclaved for 20 min. Subsequently incubation medium is added and removed with pipets and suction using customary sterile procedures. This culture setup permits the lenses to be observed and measured with a low-power dissecting microscope and ocular micrometer.

Medium. The basic culture medium used for long-term incubation is a modified T.C. 199 (Gibco and Hanks' salts). It is fortified with additional buffer, magnesium, calcium, glutamine, fetal calf serum, and antibodies so that the final concentration (millimoles) of salts is 1.4 $CaCl_2$, 1.0 $MgSO_4$, 0.4 KH_2PO_4, 0.5 Na_2HPO_4, 10.4 $NaHCO_3$, 5.0 KCl, and approximately 112 NaCl. The medium contains 5.2 m*M* glucose, 13 m*M* glutamine, 100 units/ml of penicillin-G-potassium, 1 μg/ml of streptomycin sulfate, 2.5% fetal calf serum and the organic components of T.C. 199. Galactose and fructose may be added in the amount of 500 mg% for experimental and control media, respectively, if a cataractogenic medium is required. The concentration of NaCl may be varied to obtain the desired osmolarity, and the CO_2/air mixture of the incubator may be adjusted to the desired pH. The medium was sterilized by filtration through a 0.22-μm Millipore filter.

Incubation. Lenses are incubated in National CO_2/air-humidified incubators that are maintained in a special tissue culture room with a filtered positive-pressure air system and ultraviolet irradiation when the room is not in use. Surfaces are periodically washed with 1% Wescodyne solution to decrease contamination and the atmosphere within the incubators is continuously monitored with culture plates to be sure that the

[1] This research was supported in part by Research Grant No. EY-00902 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

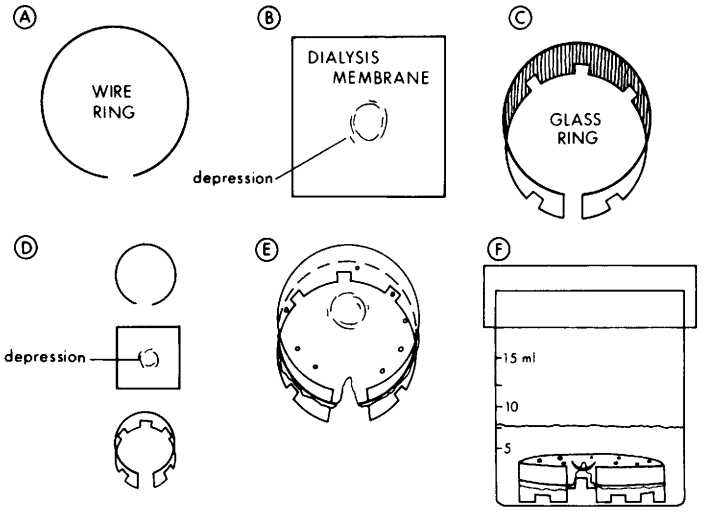

FIG. 1. Diagram of the culture flask with the membrane bed that supports a lens during incubation.

possibility of contamination is minimal. Incubation conditions are considered to be standard with a medium volume of 5 ml, an osmolarity of 295 mOsm, a pH of 7.3, and a temperature of 37°. For long-term cultures, the medium is changed at 48-hr intervals.

Lenses. Lenses are obtained from young male Sprague–Dawley strain rats generally weighing 100 ± 10 g. Animals are sacrificed by decapitation, the whole eye is removed and put in a beaker containing 1% Wescodyne in a 0.9% saline for 1 min. The eye is then placed on a sterile towel and taken into the tissue culture room where the lens is removed under sterile conditions under a LabCon hood. The adhering muscles are resected with a pair of straight scissors and toothed forceps. A cruciform incision is started with a scapel in the posterior surface and extended with the air of fine curved scissors and forceps. Care is taken to avoid cutting through the vitreous body. The eye is then transferred to a depression slide containing sterile media of the sort that is to be used in the experiment. The remaining dissection is done within the medium. The corners of opposite flaps are held with forceps, pulled apart, and inverted so that the tips of the forceps come together over the cornea. The lens and vitreous body become free with the lens resting on the vitreous with its anterior surface exposed. The ciliary body can be removed with very fine forceps and iris scissors. If the ciliary body is to be retained, it is readily separated from the vitreous body with scissors. The lens is washed several times with sterile medium and transferred with the aid of a platinum scoop and wire loop to a culture dish. Similarly, following incubation, lenses are transferred with a loop to a piece of filter paper, rolled to remove excess media, weighed, and analyzed as desired.

Chemical. Sorbitol is determined as previously described (2).

Results. The results of incubating 27-mg lenses are shown in Fig. 2. The lenses were photographed with transmitted light so that opacities show as dark spots and transparent areas are white. Since the lenses of animals are usually observed with reflected light showing white opacities, the lenses are also shown after photographic reversal (2-B). The numbers indicate the days of incubation in standard medium (N) or galactose medium (G).

In a standard medium (N), lenses are transparent and normal in size at 23 days.

In a galactose medium (G), lenses go through the stages of cataract development that have been described for intact rats with nuclear cataract formation in 21 days (2, 4, 5, 6). In simplest terms, four stages may be recognized.

The first or early vacuolar stage involves changes in the equatorial region as seen in the photographs for Days 1–11. These changes are reversible *in vivo* (7).

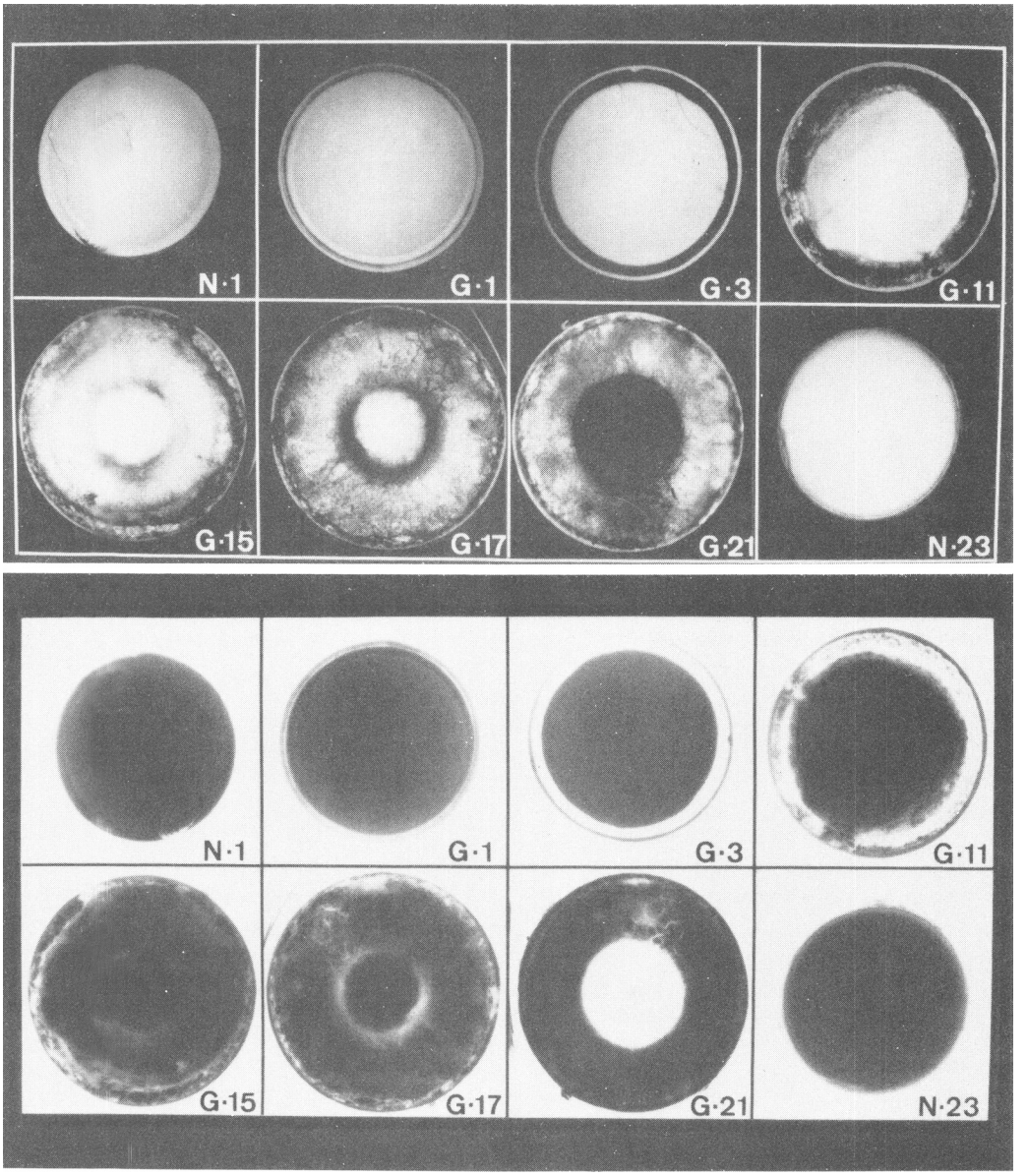

FIG. 2. (A) Direct photographs of lenses with transmitted light. The numbers indicate the days of incubation for lenses in standard medium (N) or galactose medium (G). (B) Photographic reversal to give the appearance of reflected light.

The second or late vacuolar stage is characterized by an extension of vacuolization to more central portions of the lens as seen for Days 15 and 17. At this stage the process is irreversible *in vivo* (7).

The third or nuclear cataract stage matures over a period of 24–36 hr and is easily recognized as a white nuclear opacity in reflected light and as a dark central area in transmitted light as in the photograph for Day 21. This relatively distinct and sudden change serves as an important end point in cataractogenic studies. This change is accompanied *in vivo* by a destruction of the lens fiber membranes (2).

The fourth or mature cataract stage is less

definite and consists of the gradual and complete opacification of the lens. It is observed in culture after 25 to 30 days.

Retention of the ciliary body decreases the trauma of dissection (8). This is noted in the incubation of lenses in standard medium. Lenses with intact ciliary bodies could be incubated 30% longer (28 days) without increasing in size. Loss of transparency followed the onset of swelling.

Lenses incubated in galactose medium had dulcitol levels of 1100 mg/100 g at 2 days with a resultant increase in size from 18 to 30 mm^3 at 9 days (Table II). These changes are in accord with observations *in vivo* (3).

Discussion. The method that is described provides a means of studying lenses for transparency and size over a period of time without the necessity of sacrificing lenses. This will serve as a base for investigating cataract formation and other parameters of lens viability.

The presence of the ciliary body lengthens the time that lenses can be cultured without loss of size or transparency. The ciliary body seems to limit swelling (Table II). The high proportion of burst lenses among those cultured in the galactose medium is thought to be due to this limitation. Lenses are known to undergo rapid colloidal swelling following the third stage of cataractogenesis (3). If the ciliary body and intact capsule resists such swelling then enough pressure might develop so that the capsule will burst rather than accommodate to the pressure of gradual expansion. As indicated in Table I, the time at which lenses burst coincides with the appearance of nuclear cataracts in other lenses. This lends credibility to this explanation and in addition indicates that lens bursting should be equated with the appearance of nuclear cataracts as an endpoint in the study of cataractogenesis in tissue culture.

Summary. A system is described which

TABLE II. VOLUME OF LENSES INCUBATED IN GALACTOSE MEDIA.

	Without ciliary body		With ciliary body	
Day	Number	Volume ± SE	Number	Volume ± SE
1	7	18.5 ± 0.7	7	17.9 ± 0.6
2	7	20.5 ± 1.2	7	19.5 ± 1.0
5	7	23.6 ± 1.4	7	22.6 ± 1.0
7	7	27.2 ± 1.4	7	25.0 ± 1.5
9	7	30.9 ± 1.6	4	29.6 ± 1.8
12	7	29.6 ± 1.5	3	22.6 ± 2.2
14	7	28.4 ± 1.0	2	28.4
16	7	27.2 ± 0.9	2	28.4
19	7	23.6 ± 1.4	1	24.8

TABLE I. DISTRIBUTION OF LENSES BY THE TIME REQUIRED FOR THE APPEARANCE OF NUCLEAR CATARACT IN GALACTOSE CULTURE MEDIUM.[a]

	Without ciliary body			With ciliary body		
Day of culture	Cataract	Burst	Total	Cataract	Burst	Total
6	1	0	1	0	0	0
7	1	0	1	0	0	0
8	2	0	2	0	0	0
9	2	0	2	0	1	1
10	1	0	1	2	4	6
11	1	1	2	1	1	2
12	5	0	5	1	1	2
13	8	1	9	2	0	2
14	1	0	1	2	2	4
15	1	0	1	4	5	9
16	1	0	1	2	2	4
17	0	0	0	1	0	1
18	1	0	1	1	0	1
Number of lenses	25	2	27	16	16	32
Median (day)	12	12	12	14.5	14	14
Mean (day)	11.8	12	11.8	14	12.9	13.5
SE	0.6	–	0.5	0.6	0.6	0.4

[a] Control lenses without (8) and with (15) ciliary bodies incubated in standard medium were all clear and intact at 21 days.

permits rat lenses to be cultured and studied for periods in excess of 3 weeks without loss of transparency or an increase in size. When galactose is added to the medium, the lenses undergo morphological changes comparable with those found *in vivo* with the eventual production of nuclear cataracts. Lenses cultured with the ciliary body attached are less likely to be injured during dissection and can be cultured as effectively as isolated lenses.

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Received April 28, 1976. P.S.E.B.M. 1976, Vol. 153.