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Permeability of Normal and Cataractous Rabbit Lenses to Glutathione* (32727)

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The presence of high concentrations of reduced glutathione (GSH) in the mammalian lens has been demonstrated by several workers. Incorporation studies with glycine ^{14}C have suggested that GSH is synthesized in the lens and that its half-life is 2–8 days (1, 2). Recently Reddy *et al.* (3) have shown that the average turnover rate for glutamic acid and glycine in GSH is 1.8% per hour and have indicated that 21.6 μmoles of glutathione are formed per 100 gm of lens per hour, based on the incorporation studies. Although the lens actively synthesizes GSH, the further fate of the tripeptide has not been defined. However, in experimental cataract produced by cataractogenic agents and in senile cataract, the content of reduced glutathione is drastically diminished (1, 4, 5).

In the course of investigating possible mechanisms of senile cataract formation, a new *in vitro* method for producing cataracts has been developed. This consists of incubating lens in a culture medium (6) with added tyrosinase and tyrosine. The dopaquinone, formed as a result of the action of tyrosinase on tyrosine, probably enters the lens and oxidizes the reduced nicotinamide adenine dinucleotide phosphate (NADPH), the sulfydryl group of GSH, and proteins. The lens cultured in such a medium becomes opalescent and exhibits the same enzymic alterations and diminution in GSH content, which are found *in vivo* in the senile or experimental cataract.

Methods and Materials. Rabbits weighing 1.5–2.0 kg were sacrificed by injecting air into the ear vein. The whole eye was excised and the lens with intact capsule was removed by a posterior approach. The lenses were rinsed in the medium and blotted with moistened filter paper. One lens was placed into the culture tube containing 10 ml of culture medium (6), 300 enzyme units of mushroom tyrosinase (Sigma Chemical Company), and 1.0 mg of tyrosine; the other lens of the same rabbit was placed in the control tube which contained only culture medium. Prior to the addition of the lens, oxygen was bubbled into each tube for 5 min. The culture tubes were incubated for 22 hours under sterile conditions at 37°C.

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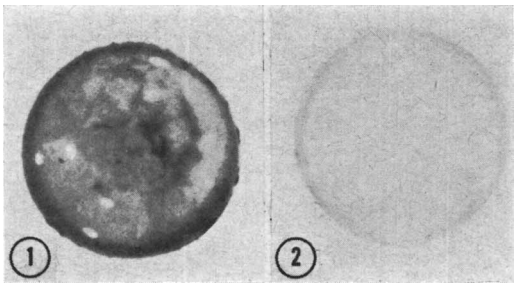

FIG. 1. Photograph of rabbit lens incubated in culture medium for 22 hours. (1) Cataractous lens (incubated with added tyrosine and tyrosinase in the culture medium). (2) Control lens (incubated in culture medium): about × 4.

Aliquots of the medium and 10% lens homogenate in buffered phosphate saline, pH 7.4 (1 part 0.15 *M* potassium phosphate buffer to 9 parts of 0.15 *M* NaCl) were taken for GSH and oxidized glutathione (GSSG) estimations. The GSH levels were measured using the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method (7). The GSSG levels were determined enzymatically using NADPH and glutathione reductase after alkylation of GSH with *N*-ethyl maleimide (8).

Results and Discussion. The lens incubated with tyrosine and tyrosinase became cloudy (Fig. 1) and the GSH content diminished to about 60% that of the control lens (Table I). No GSH was found in the culture medium either in experimental or control systems. Only small quantities of GSSG were detectable in both normal and cataractous lenses, but substantial amounts of GSSG were found in the medium surrounding the cataractous lens, accounting for about 50 to 60% of the GSH lost from the lens. The remainder of the GSH was not accounted for and may have been converted to mixed disulfides with lens proteins. Small amounts of GSSG were also found in the medium in which the control lens had been incubated but the average quantity of GSSG in the culture medium surrounding the cataractous lens was about 3.5 times higher than that in the medium surrounding the control lens. To ascertain that only GSSG leaks into the culture medium rather than GSH which is converted subsequently to GSSG, further studies were carried out. The *p*-hydroxy mercuribenzoate (PCMB) was added to the incubation medium. Under the experimental conditions PCMB was found to couple GSH and thus prevent its oxidation to GSSG. The same amount of GSSG was found in the medium with and without added PCMB, confirming that only GSSG, not GSH, leaks from the lens.

The GSH has been shown to keep the lens proteins in the reduced, soluble form (9). Progressive lowering of lens GSH content in the development of senile and experimental cataract is associated with the formation of high molecular weight mixed disulfides which include insoluble protein polymers leading to lenticular opacity (9).

These observations demonstrate that the rabbit lens is permeable to oxidized glutathione. At least a portion of GSH lost in the course of initial experimental cataract formation has been shown to leave the lens. It is suggested that a similar mechanism may be operative *in vivo*, and may account not only for the GSH lost in cataract formation, but also for normal lens GSH turnover.

Summary. Rabbit lenses were made cataractous by incubating in culture medium with added tyrosine and tyrosinase. The content of GSH was drastically lowered in the cataractous lens. The GSH which was lost from the lens could be partially recovered as GSSG in the medium. This finding may explain the

TABLE I. The GSH and GSSG Content of Normal and Cataractous Rabbit Lens and Culture Medium.[a]

	Additions to medium	GSH/lens	GSSG/lens	GSSG/10 ml of medium
Control (normal)	None	3008 ± 265	16.6 ± 3.5	82 ± 11.0
Experimental (cataractous)	Tyrosine + tyrosinase	1760 ± 273	55.6 ±15.5	289 ± 37.8

[a] All GSH and GSSG values are given as mμmoles and represent the mean ± SE of values obtained in studies on 13 rabbits.

fate of lens GSH, both normally and in cataract formation.

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Western Equine Encephalitis and Eastern Equine Encephalitis Virus Antigens Derived from Sucrose–Acetone Treated Chicken Embryos (32728)

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Subsequent to the initial report by Mohler (1) describing the use of chicken embryos for the production of Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE) virus complement-fixation (CF) antigens, several modifications have been suggested for rendering the antigens more useful in serological tests (2–4). DeBoer and Cox (3) determined that extraction of these antigens with certain organic solvents minimized the frequency of false positive reactions with human sera. Although useful CF antigens have been made from unextracted tissues infected with WEE and EEE viruses (4), extraction processes have been preferentially employed for obtaining high titered hemagglutinating (HA) antigens (5,6).

Murine tissue has been regarded as the material of choice for HA antigens made with EEE and WEE viruses even though the use of mice presents certain problems of maintenance, yield of antigen per animal, and attendant economic factors. In an effort to minimize some of the disadvantages encountered in production of mouse brain antigens, the use of sucrose–acetone extracted chicken embryo homogenate was investigated as a source of WEE and EEE antigens.

Materials and Methods. Viruses. The strain of EEE virus used for production of antigens was isolated from mosquitos collected in New Jersey in 1960 and passaged three times in mice (EEE/NJ, M_1, SM_2).[1] It was supplied by Dr. Roy Chamberlain of the National Communicable Disease Center, Atlanta, Georgia. The Fleming strain of WEE was used in its eighth egg passage and third mouse passage subsequent to its receipt in this laboratory (WEE/Fleming, XE_8, SM_3).

Antigen preparation. Ten batches of antigen for each of the two types of virus were prepared with virus from common seed pools. Eleven-day-old embryonated chicken eggs were inoculated intra-allantoically with 0.2 ml of virus diluted to 10^{-2} in phosphate buffered saline (PBS), pH 7.2, containing 200 μ of penicillin and 200 μg of streptomycin per ml. At least 120 eggs were inoculated for each batch. Embryos dying after 18 hours of incubation at 37°C were removed along with the allantoic fluid for processing. A portion of the embryos was mixed with an equal volume of allantoic fluid and homogenized. After remaining at 4°C overnight, the homogenate

[1] The letters M, SM, and E, and the numerals designated in subscripts indicate passage history in the adult mouse, suckling mouse, and egg, respectively. The letter X indicates that the history of WEE virus prior to its acquisiton at the National Communicable Disease Center is unknown.