7239 C

Studies on the Crystalline Lens. Fate of Glutathione in Parathyroid Cataract.

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It is well established that in senile cataract two of the principal biochemical changes are (1) a great increase of calcium and (2) an apparent total loss of glutathione.[1] Evans and Kern[2] demonstrated that in experimentally produced parathyroid cataract (by extirpation of the parathyroid glands), the calcium content of these cataractous lenses increased to approximately 25 times the amount present in normal dog lens.

It is probable that glutathione plays a definite rôle in the internal oxidation-reduction and enzymic reactions of the lens (initially suggested by Goldschmidt[3]). We were therefore naturally interested to learn the fate of the glutathione of the crystalline lens after removal of the parathyroids. Preliminary study indicated that the normal dog lens contains approximately 0.385% reduced glutathione, and that any figure considerably below this might be considered as indicating a loss of glutathione from the lens. The opportunity to study the fate of glutathione in parathyroid cataract was afforded by the early development of bilateral lenticular opacities in 5 young dogs (2 mos. old) that had been thyroparathyroidectomized in connection with another study. A complete description of these lenticular changes has been made elsewhere.[4]

At the time the dogs were sacrificed because of severe distemper, they had been without parathyroids for 57-152 days. The earliest signs of lenticular degeneration were evident at about the 25th day. The dogs were kept on a sour milk-bread-liver diet; tetany was not observed.[5] The dogs were sacrificed by the intravenous injection of small amounts of sodium cyanide. Immediately after death the eyes were excised, the crystalline lenses removed and weighed on a watch glass.

Glutathione determination: Reduced glutathione was determined by the Okuda Method as modified by Hess[6] for blood. The 2 lenses

[1] See discussion in Duke-Elder's Textbook of Ophthalmology, London, Henry Kimpton, 1932, p. 481.

[2] Evans, E. I., and Kern, R., *Am. J. Ophth.*, 1931, **14**, 1029.

[3] Goldschmidt, M., *Arch. f. Ophth.*, 1924, **113**, 160.

[4] Evans, E. I., *Am. J. Ophth.*, in press.

[5] Evans, E. I., *Am. J. Physiol.*, 1933, **105**, 32.

were ground with 10 cc. 10% sulphosalicylic acid and filtered by suction through hard paper. The protein residue was reground twice with 5 cc. portions of the same acid and the cake washed with two 2 cc. portions of the acid. The combined filtrates were made up to 30 cc. and an aliquot taken for immediate titration with M/1200 KIO_3 under conditions as specified by Okuda.[7] For determination of oxidized glutathione, an aliquot was reduced with zinc and HCl. In 2 cases an aliquot was hydrolyzed with 20% HCl for 6 hours and the liberated cystine determined by the Sullivan Method.[8] Also, in 3 cases, a Sullivan test for free cystine or cysteine was made directly on an aliquot of the sulphosalicylic acid extract. These data and other unpublished data (in collaboration with Drs. Sullivan and Hess) on glutathione in the crystalline lens indicate that the Okuda Method, when used on the sulphosalicylic acid extracts of the crystalline lens, titrates only glutathione of the reducing substances present.

TABLE I.
Glutathione in the cataractous lenses.

Dog No.	Days alive after operation	Free cysteine or cystine	Reduced Glutathione %	Oxidized Glutathione %	Total Glutathione %
1	57	.....	0.115	.....	0.115
2	82	.....	0.132	0.021	0.153
3	142	None	0.110	0.009	0.119
4	138	"	0.184	0.014	0.198
5	152	"	0.121	0.015	0.137
6-15*	.....	"	0.385	0.007	0.392
16-23†	.....	"	0.391	0.002	0.393

* 10 normal adult dogs.
† 8 normal young dogs.

Summary. The data shown in Table I indicate that before any appreciable calcium deposition takes place in the developing "parathyroid cataract" there is a serious loss of glutathione from the lens, amounting in the cases described here, from 53 to 72% of the amount present normally. Also, the negative Sullivan reaction for the lens filtrates indicates that free cystine is not the end-product of the destroyed glutathione.

If glutathione is as necessary for certain enzymic reactions and internal autoxidations as it is held by many to be, it is very probable that its removal or destruction must seriously impair the metabolism of the lens. Just how glutathione is destroyed after removal of the

[6] Hess, W. C., *J. Wash. Acad. Sci.*, 1929, **19**, 419.

[7] Okuda, Y., *J. Dept. Agri., Kyushu Imp. Univ.*, 1929, **2**, 133.

[8] Sullivan, M. X., and Hess, W. C., *U. S. P. H. S., Public Health Reports*, Suppl. No. 86, 1930.

parathyroids is not clear but it may not be impertinent to suggest that it might be removed with glutathione from the other tissues, to detoxify certain injurious metabolic products that may arise after parathyroidectomy. Each of the 3 amino acids, glycine, glutamic acid, and cysteine are important detoxifying agents.[9]

These data on glutathione in parathyroid cataract suggest further the possible rôle of parathyroid deficiency (even in the absence of any tetany) in the pathogenesis of senile cataract.

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A Simple Method for Production of Vitamin D Milk of Known and Controllable Potency.

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Since the introduction of irradiated ergosterol and irradiated foods for the prevention and treatment of rickets, several methods have been employed in order to impart anti-rachitic properties to milk. Among these may be mentioned: (1) the irradiation of milk in both the powdered and liquid forms; (2) irradiation of the cow; and, (3) feeding to the cow substances containing large amounts of vitamin D, as for example cod liver oil or irradiated yeast. The irradiation of the cow as a means of imparting vitamin D to the milk is obviously most impractical from the commercial point of view, as is also the feeding of large amounts of cod liver oil. The irradiation of the milk itself has the practical disadvantage of being expensive since it involves the installation, maintenance and operation of expensive apparatus in the farm or dairy where it is employed. The direct irradiation of the milk and the indirect method of feeding irradiated yeast to the cow both have the disadvantage that they are not absolutely reliable methods of holding the vitamin D content at fixed concentration, so that repeated testing of the milk at regular intervals becomes necessary in order to be sure that the vitamin D content of the milk has not fallen below the standard.

In view of these difficulties it occurred to us that the method for adding fats to milk, which has been employed successfully by Holt, Tidwell and their associates, in our laboratories, for the past 4 years, might also be more practical for fortifying milk with vita-

[9] Brand, E., and Harris, M. M., *Science*, 1933, **77**, 589.