

concluded that the fundamental mechanical response of muscle has a different time course than that of tension development of the whole muscle. It is hoped that further research on sarcomere behavior by means of the muscle diffraction patterns will help elucidate some of these problems of the mechanism of contraction.

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Ascorbic Acid Oxidase in Determining Vitamin C in Lens and Aqueous Humor.

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The vitamin C content of the eye has been the subject of a great deal of speculation by investigators. In the normal lens and aqueous, there is an appreciable quantity of the vitamin. In the cataractous lens, however, this quantity is markedly decreased.^{1, 2}

At first, biological methods were used for determination of vitamin C. Later, it was estimated by titration with Tillman's reagent, 2, 6-dichlorophenolindophenol. It was accepted that reduction of the dye at pH 2 was entirely due to vitamin C. However, so far as the lens is concerned, this has been challenged by Fujita, Iwatake, and Miyata,³ who, by use of Folin's tungstate reagent, have found that only 79% of the reduction of indophenol depends upon vitamin C. Tauber, Kleiner, and Mishkind⁴ recently found in the Hubbard squash an oxidase enzyme which is specific for vitamin C. Use of this, then, should furnish direct evidence as to whether the substance in the lens and aqueous, which reduces the dye, is totally vitamin C.

We first undertook to substantiate the work of Tauber, *et al.* The results of our experiments on the action of the oxidase at various pH values are given in Table I.

As seen from our data, the optimum pH range of the enzyme is 5.5-6.0,—which confirms the work of the previous investigators.

¹ Mueller, H. K., and Buschke, W., *Arch. f. Augenheilkund*, 1934, **108**, 592.

² Bellows, J., *Arch. of Ophth.*, in press.

³ Fujita, A., Iwatake, D., and Miyata, T., *Biochem. Z.*, 1935, **277**, 296.

⁴ Tauber, H., Kleiner, I. S., and Mishkind, D., *J. Biol. Chem.*, 1935, **110**, 211.

TABLE I.
Effect of Reaction of Medium upon Activity of Ascorbic Acid Oxidase (Squash).

pH	Amount of Vitamin C added mg.	Amount of Vitamin C after Incubation at 37° for 30 min.	
		With Buffer alone mg.	With Buffer and enzyme mg.
4.7	.083	.034	.008
5.0	.083	.023	.005
5.3	.083	.020	.004
5.5	.083	.016	.003
5.8	.083	.011	.000
6.0	.083	.007	.000

To determine vitamin C in the lens, it was extracted twice with 5% metaphosphoric acid to precipitate the proteins, centrifuged, washed, and made to a total volume of 50 cc. A one cc. aliquot of this was titrated with the dye. Another one cc. aliquot was incubated for 30 minutes at pH 5.5 with one cc. of the enzyme extract. The solution was then brought to pH 2 with sulphuric acid and titrated. Control portions were incubated without the enzyme. Similar experiments were done upon the aqueous. The data are given in Table II.

TABLE II.
Determination of Ascorbic Acid Content of Lens and Aqueous Humor of Cattle.
1 cc. dye = 0.043 mg. Vitamin C.
Wt. lens = 4.14 gm.

	cc. of Dye	Amount of Vitamin C (Cattle Lens) mg./gm.
1 cc. lens extract	0.645	0.335
1 cc. lens extract with buffer alone (incubation)	0.620	
1 cc. lens extract with buffer and enzyme (incubation)	0.000	
		Amount of Vitamin C (Aqueous Humor Cattle) mg./cc.
1 cc. aqueous	4.650	0.199
1 cc. aqueous with enzyme and buffer (incubation)	0.000	

The results clearly indicate that at pH 2 the total reducing action of the lens and aqueous on Tillman's reagent is due to vitamin C. The fact that there was very little oxidation of vitamin C in the buffered solution without enzyme was to be expected, for, as was shown in a previous report by one of us,² the lens contains an anti-oxidative mechanism. This mechanism, however, does not, evidently, protect the vitamin against oxidation by the enzyme.

These experiments are offered as direct evidence that reduction of Tillman's reagent at pH 2 by lens and aqueous is entirely due to their vitamin C content.