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Action of Ascorbic Acid and Hydrogen Peroxide on Collagen and Thymonucleohistone.*†

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A degradation of mucins and polysaccharides by ascorbic acid or other enediols and hydrogen peroxide, which consisted of a loss of viscosity and a reduction in particle size of the substrate, was reported recently.¹ We have further observed that these same agents cause a similar irreversible reduction of viscosity of solutions of two other biologically significant substances, collagen and thymonucleohistone. The changes which occur in collagen, so-called "depolymerization," are of special interest in the light of Wolbach's work demonstrating the necessity of ascorbic acid for the formation of collagen fibers *in vivo*.²

Experimental.

Collagen. Collagen was prepared by extracting the tendons of rats' tails according to the method of Bessey.³ The resulting solution is water clear and extremely viscous. Upon dialysis, it forms a firm gel in the dialyzing sack. Collagen can be precipitated from such an extract as a filamentous precipitate by the addition of an equal volume of 2.3 M potassium phosphate, pH 6.3.

The degradation of collagen by ascorbic acid and hydrogen peroxide was illustrated by the following experiment. One ml of M/5 phosphate buffer containing 20 mg of ascorbic acid and 1 ml of 1% hydrogen peroxide were added to 50 ml of collagen extract. The control solution contained collagen, phosphate buffer and hydrogen peroxide. The pH of both solutions was 4.2. The temperature was kept at 6° to inhibit spontaneous degradation of collagen. The changes in relative viscosity which occurred are shown below.

| | Immediately after mixing | Sixteen hrs later |
|--|-----------------------------|----------------------|
| A Collagen + ascorbic acid + hydrogen peroxide | 8.1 | 2.1 |
| B Control | 8.1 | 7.0 |

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¹ Robertson, W. v. B., Ropes, M. W., and Bauer, W., *Biochem. J.*, 1941, **35**, 903.

² Wolbach, S. B., *Am. J. Path.*, 1933, **9**, 689.

³ Bessey, O. C., personal communication.

Solution B yielded a typical precipitate with 2.3 M phosphate buffer, whereas solution A gave none. The irreversibility of this degradation of collagen was shown by the fact that upon dialysis solution B formed a firm gel, whereas solution A exhibited further decrease in relative viscosity to 1.7.

Thymonucleohistone. Minced thymus gland was extracted twice with 10% sodium chloride at 4° for 2 days. Following centrifugation the viscous solution was precipitated by pouring into 3 volumes of 1% acetic acid. The resulting fibrous precipitate was redissolved in M/5 phosphate buffer, pH 6.2, containing 5% NaCl.

The addition of 2 ml of M/5 phosphate buffer containing 20 mg of ascorbic acid and 1 ml of 5% hydrogen peroxide to 25 ml of this solution caused a breakdown of the nucleohistone within one hour as indicated by the flocculent precipitate obtained in 1% acetic acid. The control solution, lacking ascorbic acid, yielded a fibrous, ropey precipitate even at the end of 24 hours.† The relative viscosity of the degraded nucleohistone after one hour was 1.28 as compared with 4.51 for the control solution.

Summary. Ascorbic acid and hydrogen peroxide cause an irreversible reduction of viscosity of solutions of collagen and thymonucleohistone.

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An Unsuccessful Search for Dopa in Protein Hydrolysates.

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Dopa (*l*(—)-3,4-dihydroxyphenylalanine) occurs in free form in the broad bean (*Vicia faba*)¹ in the Georgia velvet bean (*Stizolobium deeringianum*),² and in certain insects.³ It has not been

† The change in the precipitate was similar to that observed in the case of synovial fluid mucin, which was illustrated in a previous publication.⁴

¹ Robertson, W. v. B., Ropes, M. W., and Bauer, W., *J. Biol. Chem.*, 1940, **133**, 261.

² Torquati, T., *Arch. farmacol. sper.*, 1913, **15**, 213, 308; Guggenheim, M., *Z. physiol. Chem.*, 1913, **88**, 276.

³ Miller, E. R., *J. Biol. Chem.*, 1920, **44**, 481.

⁴ Schmalfuss, H., and Muller, A., *Biochem. Z.*, 1927, **183**, 362.