The protective action of cysteine was apparent when its concentration was ten times greater than those of dehydroascorbic acid and alloxan. This is not surprising since only large amounts of exogenous cysteine can protect rats from the diabetogenic action of dehydroascorbic acid and alloxan[2]. The fact that the enzyme is protected by cysteine, but not by alanine, indicates that the sulphydryl group of cysteine is responsible for inactivating dehydroascorbic acid and alloxan.

I wish to thank Prof. F. C. Happold for laboratory facilities, and the University of Leeds for an Imperial Chemical Industries Fellowship.

S. K. Bhattacharya

Department of Biochemistry,
University of Leeds.

[1] Patterson, J. W., *J. Biol. Chem.*, **183**, 81 (1950).
[2] Lazarow, A., Council for International Organizations of Medical Sciences Symposia on "Experimental Diabetes and its Relation to Clinical Diseases", edit. by Delafresnaye, J. F., and Howard Smith, G., 49 (Blackwell Scientific Publications, Oxford, 1954).
[3] Bhattacharya, S. K., Robson, J. S., and Stewart, C. P., *Biochem. J.*, **62**, 12 (1956).
[4] Bailey, K., and Webb, E. C., *Biochem. J.*, **42**, 60 (1948).
[5] Sols, A., and Crane, R. K., *J. Biol. Chem.*, **206**, 925 (1954).
[6] Griffiths, M., *Arch. Biochem.*, **20**, 451 (1949).
[7] Crane, R. K., and Sols, A., in "Methods in Enzymology", edit. by Colowick, S. R., and Kaplan, N. O., **1**, 277 (Academic Press, New York, 1955).

Chromatographic Fractionation of Rat Tail Tendon Collagen

Physical studies[1] indicate that solubilized collagens give rise to two components when heated gently (13°–40° C. depending on the source of the collagen)[2] or when treated with other agents which break weak bonds. These two components have not been satisfactorily isolated[3] by the methods used to fractionate[4] 'gelatine', an end-product of more drastically altered collagen. We have succeeded in chromatographing warm dissolved rat tail tendon collagen into four components with a method which allows for the isolation of these components.

Tail tendons were obtained from Walter Reed Albino rats, weighing 300 ± 5 gm. The tendons were washed overnight at 4° C. in 0·2 *M* sodium chloride, rinsed in distilled water, immersed in measured volumes of acetic acid, and allowed to stand at room temperature for 24 hr. The mixtures were filtered through medium sintered glass funnels (pore size 10–15 micra) after high-speed centrifugation, yielding clear viscous solutions. The chromatography was done with jacketed columns kept at 40° C. by circulating water. The columns measured 0·9 cm. in diameter and were poured to a height of 7·0 cm. from a slurry made of carboxymethyl cellulose[5] and 0·2 *M* sodium hydroxide and 0·5 *M* sodium chloride. The gradient elution arrangement consisted of a separatory funnel reaching to the bottom of a side-arm flask which was stirred. The critical parameters of the gradient elution system were (1) concentrations of the lower solution— 0·05*M* sodium chloride, 0·05 *M* sodium dihydrogen phosphate, and of the upper solution—0·14 *M* sodium chloride, 0·05 *M* sodium dihydrogen phosphate, and (2) the volume of the lower flask up to its side-arm, 160 ml. 1·5 ml. of sample containing 45 mgm. per cent nitrogen were allowed to sink into the adsorbent which had been equilibrated with the lower solution, washed with distilled water and allowed to dry. A modification of the Folin method[6] was used to spot the effluent fractions which were collected at flow rates of 20 ml. per hour. A standard micro-Kjeldahl procedure was used to measure the total nitrogen in the identified peaks.

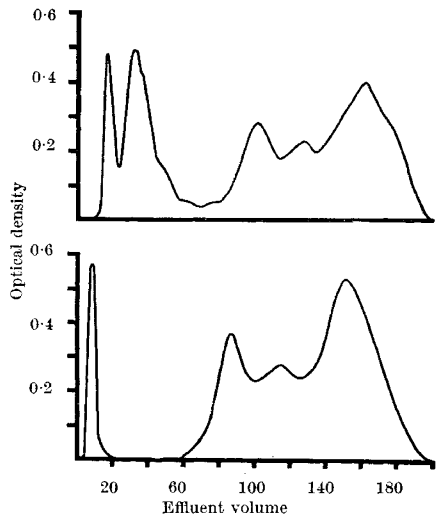

Fig. 1. Top, 0·6 *M* acetic acid ; bottom, 0·1 *M* acetic acid

All the chromatograms of solutions made with acetic acid ranging from 0·005 *M* to 1·0 *M* consist of at least 4 components (Fig. 1): three incompletely separated peaks found in the latter half of each chromatogram, called fractions 2, 3 and 4; and one or more peaks preceding these, collectively labelled fraction 1. Fraction 1 nitrogen makes up less than 1 per cent of the total at 0·05 *M* ; this percentage rises rapidly above 0·25 *M* so that fraction 1 contains 88 per cent of the total nitrogen at 1·0 *M*. This is associated with a proportional fall in fractions 2, 3 and 4. The hydroxyproline content of fraction 1 at 0·05 *M* is very low (0·7 per cent); it, too, rises sharply above 0·25 *M* almost attaining the levels of the unfractionated solutions of equivalent acid concentration. In fractions 2+3+4, the hydroxyproline to nitrogen ratio approximates that of the unfractionated solutions at all concentrations. The fractions 1, poor in hydroxyproline, obtained from acetic acid solutions below 0·25 *M* may represent the 'youngest' portions of the collagen structure; they appear to be loosely bound segments since they could be obtained from unwarmed fractions which was impossible for fractions 2, 3 and 4. Above 0·25 *M* the bulk of the collagen was altered dramatically at 40° C., giving rise to fractions 1 with normal amounts of hydroxyproline.

Alexander Kessler
Hyman Rosen
Stanley M. Levenson

Department of Surgical Metabolism and Physiology,
Division of Surgery,
Walter Reed Army Institute of Research,
Walter Reed Army Medical Center,
Washington 12, D.C.

[1] Matthews, M. B., Kulonen, E., and Dorfman, A., *Arch. Biochem. Biophys.*, **52**, 247 (1954). Orekhovich, V. N., and Shpikiter, V. O., *Doklady Akad. Nauk. S.S.S.R.*, **101**, 529 (1955). Boedtker, M., and Doty, P., *J. Amer. Chem. Soc.*, **78**, 4267 (1956). Tomlin, S. G., and Turner, K., *Biochem. Biophys. Acta*, **26**, 170 (1957).
[2] Doty, P., and Nishihara, T., in "Recent Advances in Gelatin and Glue Research", ed. G. Stainsby, p. 92 (Pergamon Press, London, 1958).
[3] Orekhovich, V. N., and Shpikiter, V. O., *ibid.*, p. 87.
[4] Pouradier, P., and Venet, A. M., *J. Chim. Phys.*, **47**, 11 (1950). Stainsby, G., *Disc. Farad. Soc.*, **18**, 288 (1954). Russel, G., *Nature*, **181**, 102 (1958).
[5] Peterson, E. A., and Sober, H. A., *J. Amer. Chem. Soc.*, **78**, 751 (1956).
[6] Lowry, O. H., Rosebrough, N. J., Farr, L. H., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).