Influence of Time on the Reversible Association between Large Molecules : the Collagen System

It is increasingly evident that reversible interactions between macromolecules underlie many important biological functions; the actomyosin system is a classical example. Time-related changes in association between large molecules, which give rise to altered solubility and tensile strength (often associated with increased order), are well known to polymer chemists. It is suggested here that biological systems which rely on controlled intermittent association between specific macromolecules may, by increasingly long periods of association, become fixed in an irreversible or very slowly reversible state, resulting in impaired or lost function.

This communication reports an example of time-related changes in solubility of fibrils formed in relatively homogeneous solutions of collagen. Cold neutral salt extracts of fresh skin from growing animals contain collagen in solution which may be precipitated as an opaque gel composed of cross-striated fibrils, characteristic of the native tissue, by warming to 37° C.[1]. Purified collagen isolated from such extracts behaves in like manner[2]. Dilute acetic-acid extracts of collagen dialysed against neutral buffers can be gelled in a similar way. The effect of certain electrolytes and non-electrolytes on the rate of heat gelation has been briefly described[3].

It was observed in the course of these studies that the opaque collagen gels would occasionally be re-dissolved on cooling in the refrigerator. Fessler[4] has independently reported that one fraction of purified collagen obtained from rabbit skin by neutral salt extraction would, after precipitation by warming, redissolve on cooling. The investigations reported here revealed that one factor determining whether or not a gel would dissolve on cooling was the time it remained at the higher temperature.

Clear viscous solutions of collagen were prepared from calf dermis by extraction with 0·5 *M* acetic acid and dialysis consecutively against phosphate buffer, *p*H 7·6, $\Gamma/2 = 0{\cdot}4$, and sodium chloride, $\Gamma/2 = 0{\cdot}45$. Other samples were similarly prepared at physiological ionic strength and *p*H. These solutions are 'homogeneous' electrophoretically and in the ultracentrifuge. Partial composition of the dissolved collagen was reported earlier[3].

When warmed to 37° C. the opacity of the rapidly formed gel increased with time along a sigmoid curve as observed in a 'Klett' photoelectric colorimeter (No. 540 filter). Opacity, in this system, was a direct function of precipitated protein sedimentable at 80,000*g* for 1 hr. This was demonstrated by analysis of the supernatant solution and weighing of the dried, salt-free precipitates. Series of samples of the two neutral solutions described above were allowed to stand in a water-bath at 37° C. for varying time-intervals ranging from 10 min. to 48 hr. after reaching maximum opacity. At the end of each period the tubes were transferred to an ice-bath and the fall in opacity was measured at 1-min. intervals in the colorimeter. After the rate of decrease in opacity had diminished nearly to zero the samples were centrifuged cold at 80,000*g* for 1 hr. and the collagen-content of the supernatant solutions was determined by hydroxyproline analysis, and the dry salt-free precipitates were weighed. Results of a typical experiment using a collagen solution, sodium chloride 0·14 ionic strength buffered to *p*H 7·4 with 0·008 *M* phosphate, are shown in Fig. 1. On cooling a gel for 10 min. after it had reached maximum opacity at 37° C., there was a fairly prompt fall in opacity, levelling off at about 40 per cent of the control value. After 48 hr. of incubation, less than 10 per cent reduction in opacity was observed on cooling. The same phenomenon in a greater degree occurred in solutions of 0·45 ionic strength. The loss in opacity on cooling after 10-min. incubation was 100 per cent and after 48 hr. of incubation was less than 10 per cent.

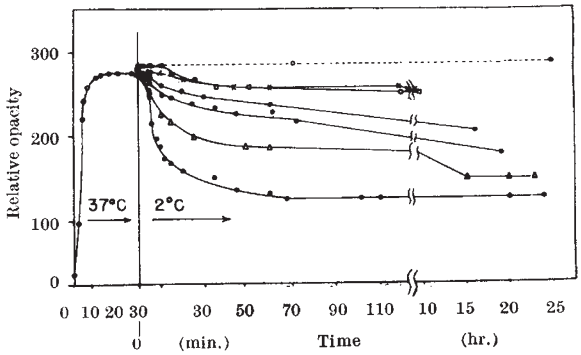

Fig. 1. Effect of cooling to 2° C. on the opacity of collagen gels incubated at 37° C. for different periods of time. Reading from the lowest curve upward: ●, 10 min.; △, 1 hr.; ◒, 3 hr.; ◓, 6 hr.; □, 24 hr.; ×, 48 hr.; ○ - - - - ○, 56 hr. (control, not cooled). Starting solution contained about 0·2 per cent calf-skin collagen dissolved in cold 0·14 *M* sodium chloride buffered with phosphate to *p*H 7·6

This phenomenon was also observed in cold saline extracts of fresh guinea pig skin which contained relatively high concentrations of a collagen fraction thought to be the precursor of the characteristic tissue fibrils.

Changes in solubility of the gels at acid *p*H as a function of time of previous incubation at neutral *p*H were also investigated. Between one and two weeks of incubation at 37° C. were required for the collagen gels to become almost completely insoluble in cold citrate buffer at *p*H 3·5.

It is improbable that the system reported here required interaction with non-collagenous components or enzymes. It is suggested that the increasing degree of irreversibility may result from the action of Brownian movement, allowing the associated molecules to find their most stable and sterically matched fit, thus permitting the establishment of more intermolecular cross-links.

The results of this study suggest the possibility that increasing time of association between biological high polymers may, at least in some instances, be contributing factors to ageing changes in structure and function in the organism.

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[1] Gross, J., Highberger, J. H., and Schmitt, F. O., *Proc. U.S. Nat. Acad. Sci.*, **41**, 1 (1955). Jackson, D. S., and Fessler, J. H., *Nature*, **176**, 169 (1955).
[2] Gross, J., *J. Exp. Med.*, **107**, 247 (1958).
[3] Gross, J., *Fed. Proc.*, **15**, 82 (1956).
[4] Fessler, J. H., *Fed. Proc.*, **16**, 37 (1957).