Synthesis of Collagen

Gerber and Altman have suggested[1] that the mechanism involved in the synthesis of fibrous proteins is different from the mechanism involved in the synthesis of globular proteins. In particular, they suggested that collagen is synthesized by the addition of polypeptides containing 70–140 amino-acids to pre-existing collagen fibres. Although the communication raised a number of interesting questions, it should be noted that the conclusions which were reached are not entirely consistent with several recent reports. They stated that the mechanism underlying the biosynthesis of fibrous proteins must be unique, since experiments on the isotopic labelling of collagen and silk fibroin indicated that specific amino-acids are labelled to varying degrees in different parts of these molecules. It is now apparent, however, that a similar situation exists in the synthesis of a number of proteins and polypeptides, including ovalbumin, ribonuclease and insulin[2]. Recently, Dintzis[3] demonstrated non-uniform labelling of specific amino-acids in the synthesis of the globular protein hæmoglobin.

Also in support of their hypothesis, Gerber and Altman observed that most fibrous proteins are considerably larger than 140 Å., the diameter of the ribosomes on which protein synthesis is assumed to occur. Since several non-fibrous proteins are also larger than 140 Å., the problem raised by the relatively small size of ribosomes has been recognized for some time. Recent studies suggest that ribosomes may be considerably larger than was originally supposed[4]. In any event, it seems premature to conclude that the problem which exists in explaining the synthesis of fibrous proteins by ribosomes is entirely different from the problem of explaining the synthesis of large globular proteins. Our own observations in chick embryos[5] indicate that collagen is synthesized by the same ribosomal system which has been postulated for the synthesis of other proteins. Lowther *et al.*[12] have recently reported similar results in work on carrageenin granulomas.

Several other suggestions which were made in the paper also appear to be unlikely. Because the number of C- and N-terminal amino-acids increase after the degradation of collagen to gelatin[6,7], it was suggested that intramolecular cleavage of peptide bonds occurs when collagen is fractionated into soluble forms and that the resulting solutions of 'soluble' collagen consist of peptides from near the termini of the collagen molecule. It was further suggested that these soluble peptides represent 'building blocks' of collagen. Although some peptide bonds are ruptured by relatively small energies[7], the mild conditions generally used to solubilize collagen cannot be expected to rupture peptide bonds. "Neutral salt soluble collagen"[8], for example, is prepared by extracting tissues with dilute, neutral salt solutions at 4° C. It is very unlikely that peptide bonds are broken by this procedure. In addition, it may be pointed out that the molecular weight of neutral salt soluble collagen has been estimated as being 340,000–500,000 (ref. 9), a size which is considerably larger than the peptides postulated by the authors.

It was also suggested that the turn-over time of the peptide 'building blocks' of collagen can be determined from the turn-over time of free hydroxyproline in urine and the turn-over time of collagen itself can be determined from the turn-over time of the peptide-bound hydroxyproline in urine. In previously published experiments, Gerber *et al.*[10] injected proline labelled with carbon-14 into rats and found a turn-over time of about 0·5 days for free urinary hydroxyproline-^{14}C and 15 days for bound urinary hydroxyproline-^{14}C. Using different analytical techniques, we were recently unable to confirm their results and instead we found comparable rates of decay for both the free and bound hydroxyproline-^{14}C in urine[11]. My own results indicated that both the free and bound hydroxyproline in rat urine originate from pools of body collagen which are both synthesized and degraded rapidly.

Although the synthesis of fibrous proteins probably involves unique mechanisms not encountered in the synthesis of globular proteins, there is at present little evidence to indicate what these unique mechanisms may be. For the moment the similarities between collagen synthesis and the synthesis of other proteins are as apparent as the differences.

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Use of a Thin Layer of Calcium Sulphate for the Chromatographic Separation of Corticoids

In experiments where we sought a suitable adsorbent for the separation of corticoids by partition chromatography, we found that in calcium sulphate columns a good separation is obtained of less-polar corticoids when chloroform is used for elution. Based on this fact, a detailed analytical procedure was worked out for the fractionation of some corticoids in urinary extracts. The desirable properties of calcium sulphate were applied successfully in a method using thin layers, which substantially speeded up and simplified chromatographic analysis of steroid mixtures and separated the polar 17-OH-corticoids.

Calcium sulphate was prepared by precipitation of an aqueous solution of calcium chloride with a stoichiometric amount of sulphuric acid under constant stirring at a temperature of 70°–80° C. The precipitate of calcium sulphate was rinsed on a Büchner funnel with distilled water until the water gave a neutral reaction. The obtained precipitate was ground and spread in a 2–3 cm. thick layer and allowed to dry for 48 hr. at a temperature of 115°–120° C. Thus prepared, calcium sulphate was suspended in water (20 gm. calcium sulphate per 100 ml. water) with vigorous shaking. The suspension was rapidly poured on a thoroughly degreased glass plate 4 × 40 cm. and allowed to dry in a horizontal position for 2 hr. at 80° C. A compact layer of calcium sulphate was