Beta-Components in Collagen

Recently, Bornstein, Martin, and Piez (*1*), using a strong denaturing agent, *5M* guanidine, extracted a gelatin containing a large proportion of β-components from skin collagen. In their report, they suggest an alternate interpretation of work published from our laboratory as follows:

> Bakerman and Hersh [(*2*)] have also found a collagen fraction that contained largely β-components when examined in the ultracentrifuge, but their conclusion that the collagen molecule must therefore contain a whole number of β-components does not necessarily follow if intermolecularly cross-linked components were extracted. For example, two collagen molecules could give rise to three β-components as a result of one intermolecular and two intramolecular cross-links.

In the text of our *Nature* article, it was made clear, as follows, that intermolecular cross-linked components were not extracted from human skin collagen by our procedures:

> The undenatured collagen containing β-chains had the same physical parameters which have been recorded previously [(*3*)] of citric acid soluble collagen prepared by extraction with citrate at *p*H 4.3 with no prior neutral salt extraction: $S_{20,w}$ 3.1; intrinsic viscosity, 13.5; optical rotation, —414°; and temperature of denaturation, 36°C. From the sedimentation and viscosity data, the molecular weight of the collagens obtained by the different extraction methods would be expected to be the same, that is, about 320,000.

The parameters given above are not compatible with the intermolecular cross-linked model as suggested by Bornstein *et al.* The ultracentrifuge patterns of mixed aliquots from different undenatured extractions of all our salt, acetate, and citrate extractable collagens show only one peak; this indicates that the molecular parameters of the soluble collagens from different aliquots are identical by sedimentation criteria.

Bornstein *et al.* write:

> Acid-extracted collagens from the same species usually contain 50 to 60 percent but never more than 67 percent β-components.

This again is in direct contrast with the findings in this laboratory. If our extraction procedure (*2*) is followed, that is, with seven different solvents used four or five times each, then extractable undenatured collagens from human skin having greater than 67 percent β-components are obtained. This requires 25 to 35 consecutive extractions. We have tried unsuccessfully to extract undenatured molecules containing high beta by reducing the number of extractions and thus reducing our effort and have concluded that the multi-extraction technique is necessary. In the extraction procedure outlined by Bornstein *et al.* two different solvents are used, one of them five times. In our own experience, two solvents and six consecutive extractions will not yield undenatured soluble collagen molecules having greater than 67 percent β-component.

Our interpretation of the findings remains unaltered, that is, the molecular weight of the undenatured soluble collagen monomer must be a whole-number multiple of the molecular weight of the β-chain.

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References

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2. S. Bakerman and R. T. Hersh, *Nature* **201**, 190 (1964).
3. ———, Abstract, Cincinnati Meeting Am. Chem. Soc., Jan. 1963.

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The model for collagen proposed by Bakerman depends critically on the demonstration that the *p*H 1.5 collagen fraction he obtained after exhaustive extraction with neutral salt and *p*H 4.3 citrate consists solely or largely of collagen monomers. If intermolecularly cross-linked aggregates were present, the high proportion of β-components could arise by a combination of intra- and intermolecular cross-links, as suggested by us for collagen obtained by *5M* guanidine extraction. In our statement that denatured acid-extracted collagens never contain more than 67 percent β-components the method of acid extraction referred to was that described in the report. It is likely that further extraction with the same solvent would have yielded a small amount of collagen with some covalently bonded molecular aggregates resulting in a higher percentage of β-components after denaturation.

The criteria of intrinsic viscosity and sedimentation velocity employed by Bakerman to characterize his fractions are usually sensitive to the presence of aggregates, but this is not the case for collagen. Because the collagen molecule is highly asymmetric and forms overlapping side-to-side aggregates with little increase or even decrease in asymmetry, the intrinsic viscosity and sedimentation coefficient will not in general reflect the presence of aggregates. Examination by sedimentation velocity is further complicated by the strong dependence of sedimentation on concentration. A hypersharp boundary is formed which prevents a critical evaluation of heterogeneity. We therefore suggest that the last extracts in Bakerman's procedure consist largely of high-molecular-weight aggregates and that this accounts for his results.

Although it may be necessary in the future to modify some aspects of our present concept of the structure of the collagen molecule, Bakerman's data do not seem to warrant this. Moreover, his model is at variance with an increasing body of data from our laboratory and others regarding molecular weight determination of the α- and β-components and the stoichiometric relation of these components to the collagen molecule as judged by amino acid data and chromatography on carboxymethyl cellulose.

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