The Subcellular Fractionation of Embryonic Chick Tendon and Cartilage Cells: a Re-examination

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(Received 8 February 1979)

A re-examination of the subcellular fractions obtained from matrix-free chick tendon and cartilage cells has been made since the discovery that three out of four of the micrographs of chick tendon microsomal fractions published in an earlier paper from this laboratory were not authentic. The present studies demonstrate that by using the procedures previously reported it is possible to isolate microsomal and submicrosomal fractions from tendon and cartilage cells which exhibit typical morphology when examined by electron microscopy. These observations are consistent with our original biochemical characterization of subcellular fractions, which we know to be valid. Other publications from this laboratory in which these fractionation procedures have been applied to studies of collagen biosynthesis are in no way compromised, and indeed, most of our data have been confirmed by several other laboratories.

The removal of the extracellular matrix by digestion with bacterial collagenase and trypsin has facilitated the subcellular fractionation of both tendon and cartilage cells from 17-day chick embryos (Harwood *et al.*, 1973, 1974a, 1975a; Bhalla, 1974) and permitted an investigation of the site and timing of some of the post-translational events occurring during collagen biosynthesis. The application of this approach depended on the successful fractionation of the cells and in our initial studies the subcellular fractions obtained were characterized by chemical analysis and the location of specific marker enzymes (Harwood *et al.*, 1973, 1974a, 1975a,b). The data obtained indicated that the fractions isolated by differential centrifugation and also by discontinuous sucrose-density-gradient centrifugation were of a purity comparable with those obtained with non-connective tissues. Further confirmation of this conclusion was demonstrated in electron micrographs of the microsomal fractions obtained from leg tendon fibroblasts (Harwood *et al.*, 1974a).

The preparation of these micrographs was the responsibility of a colleague who left us in 1976 to

take up another university post, from which he has now resigned. We believed the micrographs to be authentic but it has been brought to our attention that, with the exception of that of the tendon cell smooth microsomal fraction, these micrographs were of rat liver fractions obtained by other workers and used without their knowledge. We have examined the original micrographs and although they clearly demonstrated the successful fractionation of chick tendon cells, they were technically unsuitable for publication.

In view of this situation, we have re-examined subcellular fractions prepared from tendon and cartilage cells. The isolation of cells and their fractionation to yield total microsomes, rough microsomes and smooth microsomes, were carried out exactly as described previously (Harwood *et al.*, 1974a). Electron microscopy of the fractions from tendon cells revealed microsomal pellets rich in ribosomes present in membrane-free clusters and in association with membranous vesicles (Plate 1a). Also present were smooth membranous vesicles but there was a marked absence of mitochondrial or lysosomal

EXPLANATION OF PLATE I

Electron micrographs of microsomal fractions from tendon cells

Total microsomes (a) were isolated by differential centrifugation procedures after homogenization of chick tendon cells (approx. 10^9 cells) in 0.25 M-sucrose in 0.05 M-Tris/HCl buffer, pH 7.5, containing 0.025 M-KCl and 0.5 mM-MgCl₂, as described by Harwood *et al.* (1974a). Rough microsomes (b) and smooth microsomes (c) were obtained by discontinuous sucrose-density-gradient centrifugation of tendon cells homogenized in 0.25 M-sucrose in water (Harwood *et al.*, 1974a). Pellets were fixed in 2.5% (v/v) buffered glutaraldehyde solution, post-fixed with 1% (v/v) OsO₄ and dehydrated in a graded ethanol series and propylene oxide. Examination of the microsomal pellet in a Philips EM301 electron microscope after staining with uranyl acetate and lead acetate revealed the presence of smooth membranous vesicles (SV) as well as ribosomes, which occurred as membrane-free clusters (R) and in association with membranous vesicles (RV). The rough microsomal fraction comprised ribosome-studded vesicles (RV) and free ribosomes (R) whereas ribosomes were absent from the smooth microsomal fraction, which consisted of smooth membranous vesicles (SV). (Magnification $\times 50000$).

contaminants. Analysis of the rough and smooth microsomal fractions revealed the characteristic morphology of ribosome-studded vesicles and smooth vesicles in the respective fractions (Plate 1b and 1c). Very similar electron micrographs were obtained from the corresponding fractions isolated from embryonic chick cartilage cells (not shown). These results are totally consistent with the biochemical data originally reported (Harwood *et al.*, 1974a), which we know to be valid.

In all the studies on procollagen biosynthesis conducted in this laboratory, in which the subcellular fractionation of tendon and cartilage cells has been employed (Harwood *et al.*, 1973, 1974a,b, 1975a,b, 1976, 1977), the data obtained support or have been supported by the work of others (Dehm & Prockop, 1972, 1973; Diegelmann *et al.*, 1973; Olsen *et al.*, 1973, 1975; Uitto & Prockop, 1973, 1974; Cutroneo *et al.*, 1974; Fessler & Fessler, 1974; Schofield *et al.*, 1974; Olsen & Prockop, 1974; Brownell & Veis, 1975; Kerwar, 1974; Nist *et al.*, 1975; Guzman *et al.*, 1976; Oikarinen *et al.*, 1976; Peterkofsky & Assad, 1976). Any doubt concerning the validity of the subcellular fractionation has, we believe, been removed by the re-examination described in this paper.

We express our thanks to Dr. C. Jones, Dept. of Pathology, University of Manchester, for assistance in the preparation of the electron micrographs. The financial support of the Arthritis and Rheumatism Council is gratefully acknowledged.

Note by the Chairman of the Editorial Board

The *Biochemical Journal* has received written permission from the author's colleague for the publication of this paper.

J. T. DINGLE

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