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Enzymic and Electron-Microscopic Analysis of Extracellular Matrix Vesicles Associated with Calcification in Cartilage

By S. Y. Ali, H. C. Anderson and S. W. Sajdera. (*Institute of Orthopaedics, University of London, Stanmore, Middx. HA7 4LP, U.K., and Department of Pathology, State University of New York, Downtown Medical Centre, Brooklyn, N.Y. 11203, U.S.A.*)

The biochemical sequence of events that initiates calcification in epiphysial cartilage, and subsequently transforms it into cancellous bone, has not yet been fully elucidated. Electron microscopy has shown that the longitudinal septa of epiphysial cartilage contain extracellular matrix vesicles that are membrane-bounded and are about 0.1 μm in diameter (Anderson, 1967; Bonucci, 1967). The very first crystals of apatite that are seen in epiphysial cartilage are found in these vesicles, which thus play an important role in calcification (Anderson, 1969; Bonucci, 1970; Bernard & Pease, 1969). Ultrastructural histochemistry has shown that alkaline phosphatase and adenosine triphosphatase are present in matrix vesicles (Matsuzawa & Anderson, 1970).

We have attempted the isolation and characterization of matrix vesicles by using a collagenase digestion method that facilitates the isolation of intact chondrocytes and lysosomes from cartilage (Ali, 1970). Bovine foetal or rabbit epiphysial cartilage was disintegrated by incubation with collagenase for 18h at 37°C in 0.25M-sucrose, buffered at pH 7.4. After a cell count, the cartilage digest was partitioned into seven fractions by differential centrifugation (up to 300000*g*) to obtain the extracellular particles.

Electron microscopy and DNA analysis revealed that over 80% of the chondrocytes remained intact and sedimented in fractions I and II. Matrix vesicles, resembling those found in intact tissue and often containing apatite crystals, sedimented in fraction V. Alkaline phosphatase, adenosine triphosphatase, pyrophosphatase and adenosine monophosphatase activities were mostly localized in extracellular fractions and the highest specific activity was also present in fraction V. These phosphatases therefore appear to be associated with the matrix vesicles (Ali, Sajdera & Anderson, 1970). In contrast, acid phosphatase was mostly found in the cellular fractions, indicating that the matrix vesicles are distinct from lysosomes. β-Glucuronidase activity was too low for a satisfactory localization; glucose 6-phosphatase and an esterase were present in both the cellular and the matrix-vesicle fractions. This appears to be the first instance of isolation of extracellular membrane-bounded particles from any tissue.

The extracellular matrix vesicles could induce calcification (*a*) by increasing the local concentration of P_i, (*b*) by the hydrolysis of ATP for active transport of calcium or phosphate into the lumen of the vesicles, and (*c*) by the hydrolysis of PP_i, which is an inhibitor of the mineralization process.

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The Subcellular Distribution of Colchicine-Binding Protein(s) ('Microtubule Protein') in Rat Brain

By J. R. Lagnado, C. Lyons and G. Wickremasinghe. (*Department of Biochemistry, Bedford College, Regent's Park, London N.W. 1, U.K.*)

The ability of colchicine and other antimitotic alkaloids (e.g. vinblastine) to disrupt cytoplasmic microtubules in brain and other tissues appears to parallel the abundance, in these tissues, of a soluble protein that binds colchicine (Adelman, Borisy, Shelanski, Weisenberg & Taylor, 1968; Wilson & Friedkin, 1968). Much indirect evidence (for review see Schmitt & Samson, 1968) suggests that the colchicine-binding protein present in soluble extracts of brain and other tissues represents the main subunit of microtubules and that the interruption of several physiological processes in neural tissue by antimitotic alkaloids such as colchicine may be due to their interaction with microtubule protein.

More recently it was observed that at least 50% of the colchicine-binding activity in brain homogenates is associated with the crude particulate fraction obtained after high-speed centrifugation (see, e.g., Dahl, Redburn & Samson, 1970), although no intact microtubular structures could be detected in