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Structural and Histochemical Aspects of the Pericellular Environment in Cartilage

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Phil. Trans. R. Soc. Lond. B 1975 **271**, 243-245
doi: 10.1098/rstb.1975.0048

Phil. Trans. R. Soc. Lond. B. **271**, 243–245 (1975) [243]
Printed in Great Britain

Structural and histochemical aspects of the pericellular environment in cartilage

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[Plate 1]

Mature cartilage contains pericellular regions of matrix of fine texture, consisting of filamentous material and granules containing proteoglycan. Intercellular matrix contains collagen fibres with structural elements resembling those of the pericellular regions in the spaces between the fibres. Membrane bound bodies may be present at the margin of the pericellular region. Histochemically, chondroitin sulphate is found in the pericellular region in all zones but keratan sulphate is similarly stainable only in the deep zones of ageing cartilage.

The constituents of the pericellular environment in general, and the particular significance of the evolutionary selection of polyanions have been described by Dr Scott. By contrast I would like to present very briefly some ultrastructural and histochemical characteristics of the pericellular region in one tissue, cartilage.

Ultrastructure

Chondrocytes are surrounded by a matrix which is essentially a proteoglycan gel permeated by collagen fibres. As seen in epiphyseal cartilage of the sheep foetus (Stockwell 1971), the matrix has a homogeneous texture, containing randomly disposed collagenous filaments about 15 nm in diameter. Processes of the chondrocytes penetrate into the matrix for a short distance and single cilia about 2 μm long may be found. Plaques of moderately electron-dense amorphous material about 50 nm thick are found abutting on the external surface of the plasma membrane; possibly these may be secretory material or perhaps a form of glycocalyx. The epiphyses are traversed by vascular canals: although cartilage is commonly considered to be avascular, it is noteworthy that at least some structurally normal chondrocytes can be found less than 5 μm from vascular endothelium, with no intervening shield of fibrous tissue. Thus in certain sites, blood vessels can encroach on the pericellular zone.

In adult cartilage, the texture of the matrix is no longer homogeneous. Thus, in the middle zone of articular cartilage the extracellular tissue is subdivided (figure 1) into pericellular regions of fine texture separated by intercellular coarsely fibrous matrix (Meachim & Stockwell 1973). The pericellular matrix contains slender filamentous material (with no measurable periodicity) arranged in a cobweb-like fashion (Meachim & Roy 1967). The intercellular matrix contains collagen fibres 30–80 nm in diameter, said to form basket-like enclosures around the cells (Weiss, Rosenberg & Helfet 1968). The spaces between collagen fibres in the intercellular matrix contain a delicate meshwork of fine filaments, resembling those in the pericellular material (Meachim 1972). In bovine nasal cartilage, Anderson & Sajdera (1971) describe angular or ovoid matrix granules 40 nm in diameter in both pericellular

and intercellular matrix; these represent proteoglycan seen after fixation and dehydration of the tissue. It is of considerable interest that it is the granules in the pericellular region which are most resistant to prolonged extraction with guanidinium chloride.

The pericellular matrix forms a sheath of varying thickness (1–2 μm) around the cell, tending to be thicker at the poles of the ovoid chondrocyte. It is narrower in young adult (figure 2) than in aged cartilage (figure 1), where there may be a much sharper boundary between the fine and coarse matrix. This corresponds to the lacunar rim seen with the light microscope. In the young adult, much of the scalloped margin of the chondrocyte may be in contact with the coarse intercellular matrix but, in aged cartilage, cell processes tend to be alined tangentially to the cell (figure 1), although some may radiate out through the pericellular zone to reach its border.

In older cartilage in particular, and in fibrillated tissue, electron dense particles are found at the periphery of the pericellular region (figure 3). Few particles are seen in the pericellular matrix itself, although cell processes pass through it. Many of the particles are membrane bound, and are up to 200 nm in diameter. It has been suggested that they are derived from chondrocyte processes which have become separated from the cell (Ghadially, Meachim & Collins 1965). Degradative enzyme activity has been associated with these bodies both in normal and fibrillated articular cartilage (Chrisman, Semonsky & Bensch 1967), perhaps affording a structural mechanism for turnover of matrix remote from the cell.

Histochemistry

Histochemical methods employing Alcian Blue and the critical electrolyte concentration principle (Scott & Dorling 1965) can give information about the type of acid glycosaminoglycan present near the cells. In young adult articular cartilage (Stockwell & Scott 1965), the histochemical method demonstrates stainable chondroitin sulphate distributed fairly evenly throughout the matrix. However, at least in the deeper zones of the tissue, there is a more intense stain around the cells, in the so-called territorial matrix (a region including both the pericellular and the adjacent portion of the intercellular matrix described in electron micrographs). Stain attributed to keratan sulphate (using Alcian Blue at high salt concentration), the other major glycosaminoglycan of cartilage, is much less intense and is located at some distance from the cell in the interterritorial matrix (intercellular matrix remote from the pericellular regions). Since both glycosaminoglycans form part of the same proteoglycan molecule, the difference in histochemical localization may reflect the anatomical distribution of proteoglycans containing differing proportions of chondroitin sulphate and keratan sulphate. The histochemical localization may also be associated with differences in turnover of proteoglycan in the various regions of the matrix.

The type of glycosaminoglycan in the pericellular region need not remain constant. In older tissue, the histochemical method demonstrates a change in localization of stain due to keratan sulphate (Stockwell 1970). In the deep zone adjacent to the calcified layer of articular cartilage (as in the central zone of costal cartilage), keratan sulphate appears to be most intensely stained in the territorial matrix around the cell, with very little stain density in the interterritorial matrix. Chondroitin sulphate remains stainable in the territorial site. Presumably this change in keratan sulphate localization may be associated with changes in the type of proteoglycan molecule found in the pericellular environment.

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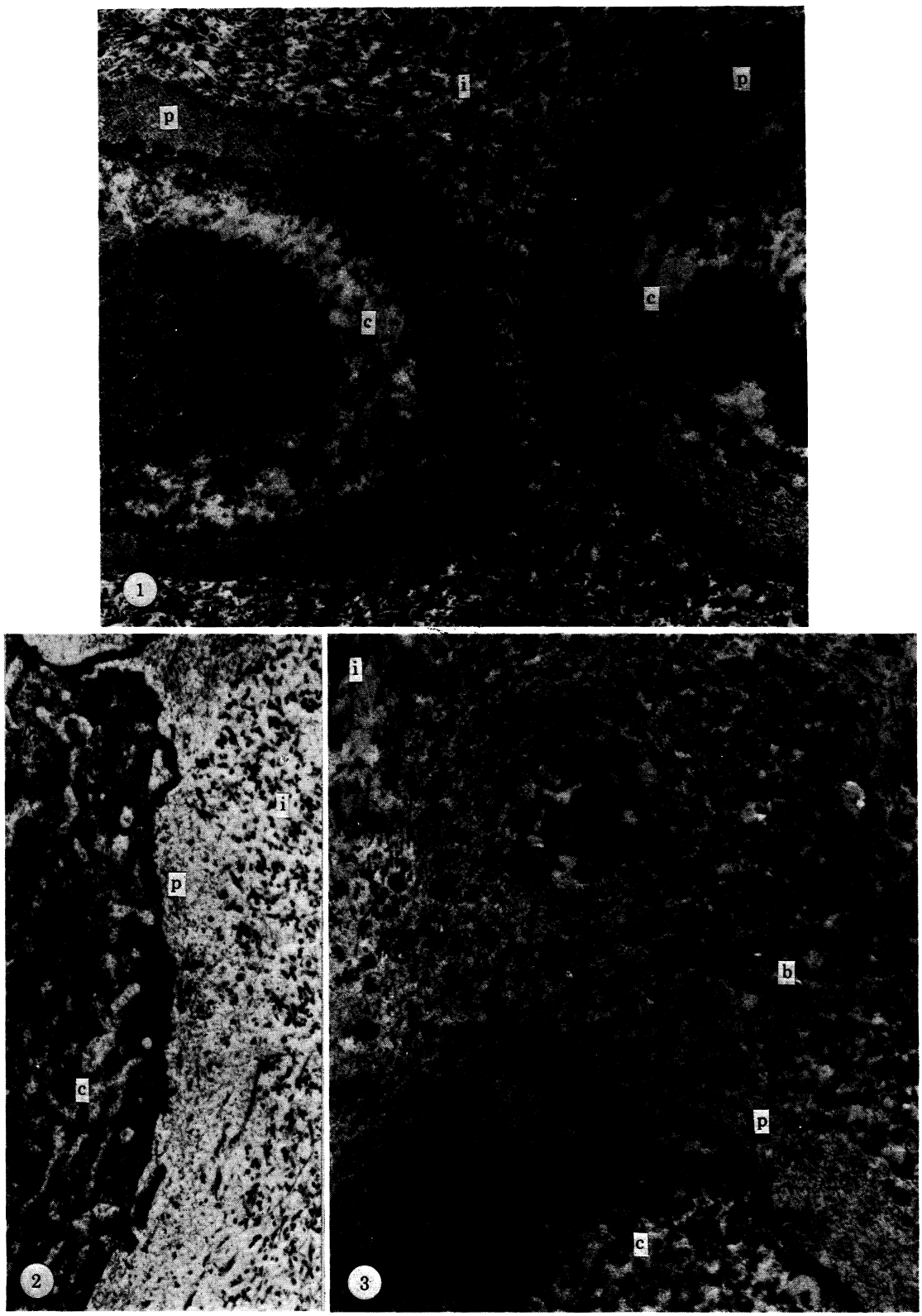

FIGURE 1. Human articular cartilage, 56 years. Note the well-defined margin to the pericellular zone. (Osmium fixation, uranyl acetate, magn. 12000.) c, chondrocyte; p, pericellular matrix; i, intercellular matrix; b, electron-dense membranous bodies.

FIGURE 2. Human articular cartilage, 21 years. In the young adult, the pericellular zone is narrower and merges imperceptibly with the intercellular matrix. (Osmium fixation, uranyl acetate, magn. 16000.)

FIGURE 3. Human articular cartilage, 56 years. Extracellular membranous bodies are seen at the periphery of the pericellular zone. (Osmium fixation, uranyl acetate, magn. 16000.)

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References (Stockwell)

Anderson, H. C. & Sajdera, S. W. 1971 The fine structure of bovine nasal cartilage – extraction as a technique to study proteoglycans and collagen in cartilage matrix. *J. Cell Biol.* **49**, 650–663.

Chrisman, O. D., Semonsky, C. & Bensch, K. G. 1967 Cathepsins in articular cartilage. In *The healing of osseous tissue* (ed. R. A. Robinson), pp. 169–192. Washington D.C.: Nat. Acad. Sci. Nat. Res. Council.

Ghadially, F. N., Meachim, G. & Collins, D. H. 1965 Extracellular lipid in the matrix of human articular cartilage. *Ann. rheum. Dis.* **24**, 136–146.

Meachim, G. 1972 Meshwork patterns in the ground substance of articular cartilage and nucleus pulposus. *J. Anat.* **111**, 219–227.

Meachim, G. & Roy, S. 1967 Intracytoplasmic filaments in the cells of adult human articular cartilage. *Ann. rheum. Dis.* **26**, 50–58.

Meachim, G. & Stockwell, R. A. 1973 The matrix. In *Adult articular cartilage* (ed. M. A. R. Freeman), pp. 1–50. London: Pitman Medical.

Scott, J. E. & Dorling, J. 1965 Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian Blue in salt solutions. *Histochemie* **5**, 221–233.

Stockwell, R. A. 1970 Changes in the acid glycosaminoglycan content of the matrix of ageing human articular cartilage. *Ann. rheum. Dis.* **29**, 509–515.

Stockwell, R. A. 1971 The ultrastructure of cartilage canals and the surrounding cartilage in the sheep fetus. *J. Anat.* **109**, 397–410.

Stockwell, R. A. & Scott, J. E. 1965 Observations on the acid glycosaminoglycan (mucopolysaccharide) content of the matrix of ageing cartilage. *Ann. rheum. Dis.* **24**, 341–350.

Weiss, C., Rosenberg, L. & Helfet, A. J. 1968 An ultrastructural study of normal young adult human articular cartilage. *J. Bone Jt Surg.* **50**A, 663–674.

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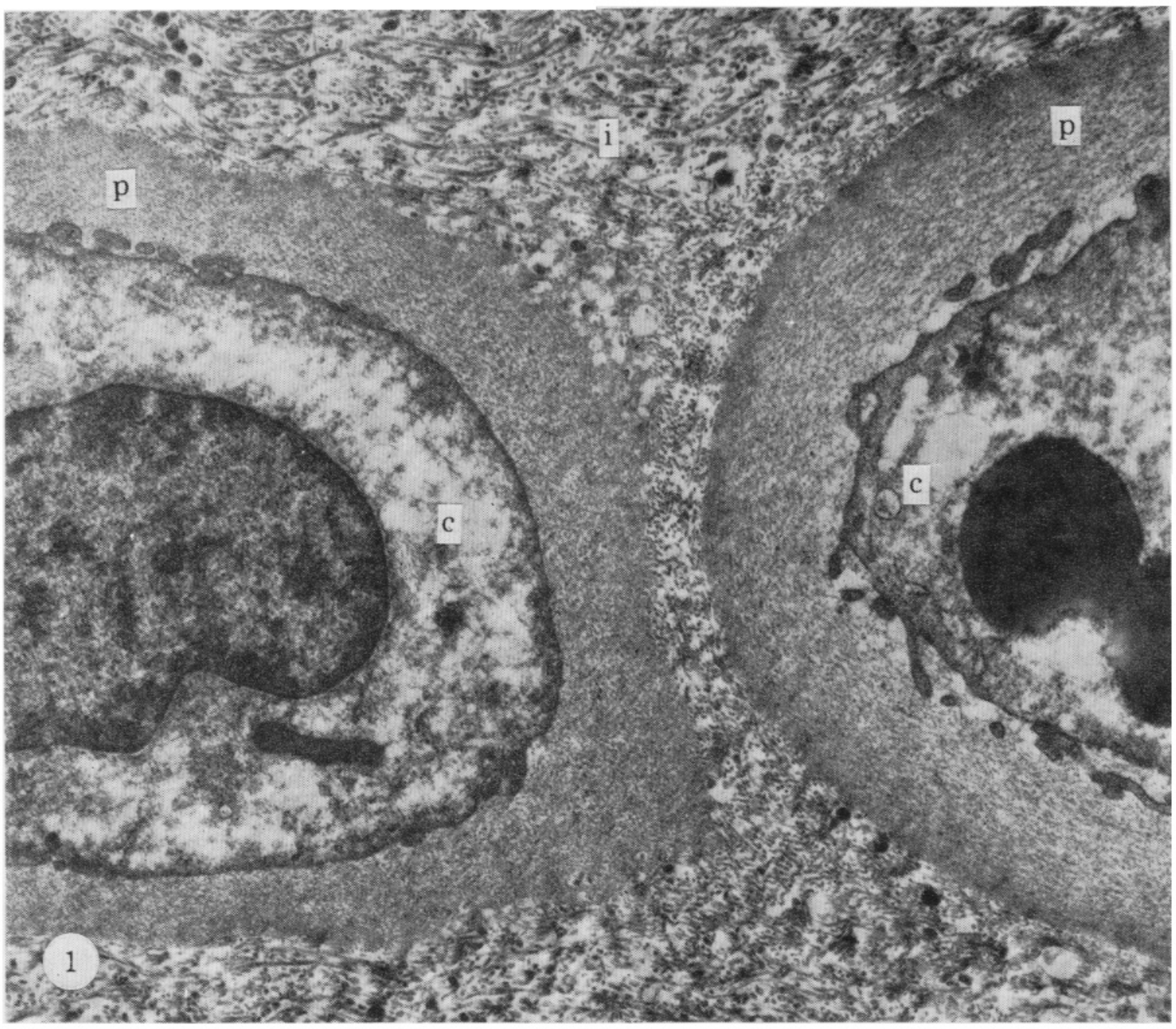

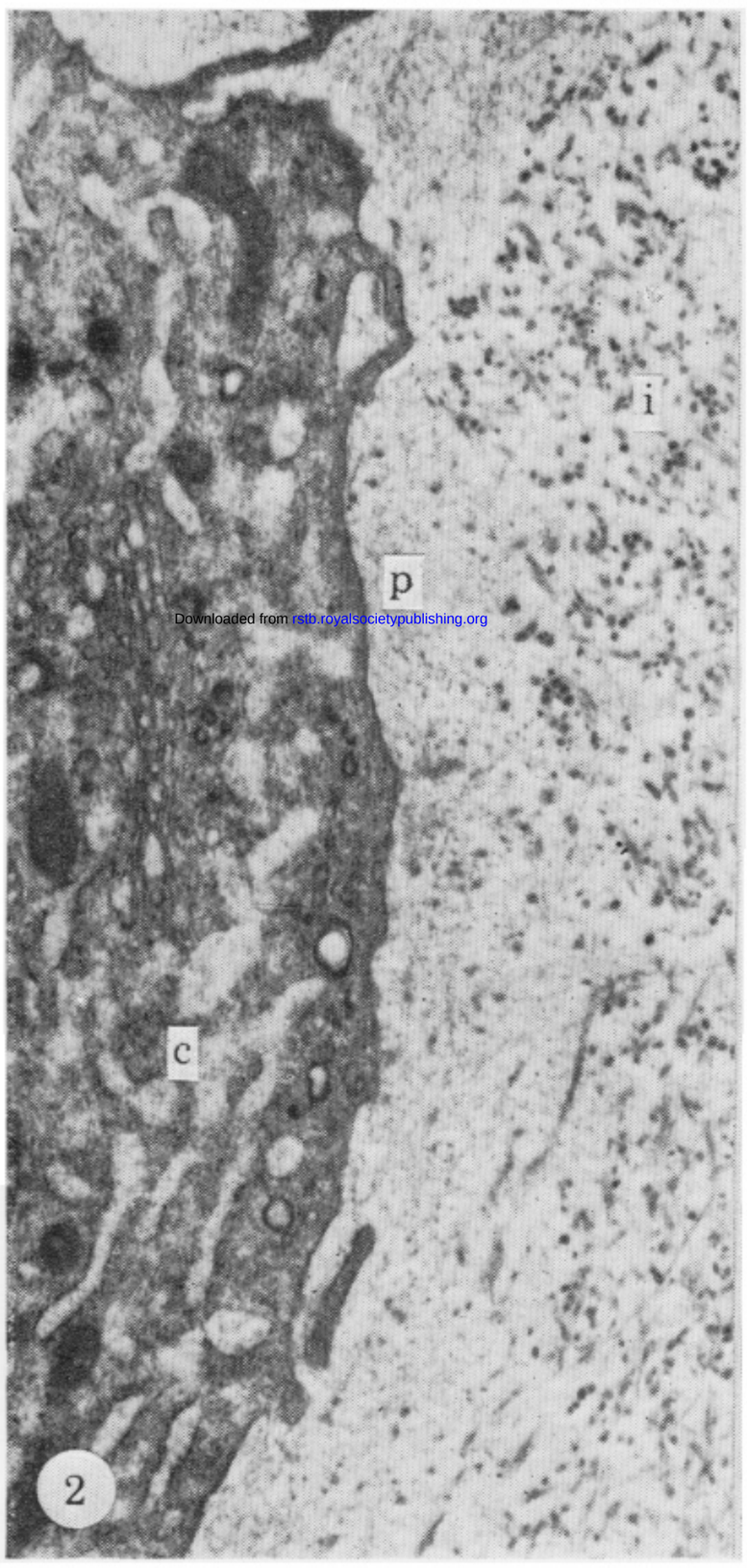

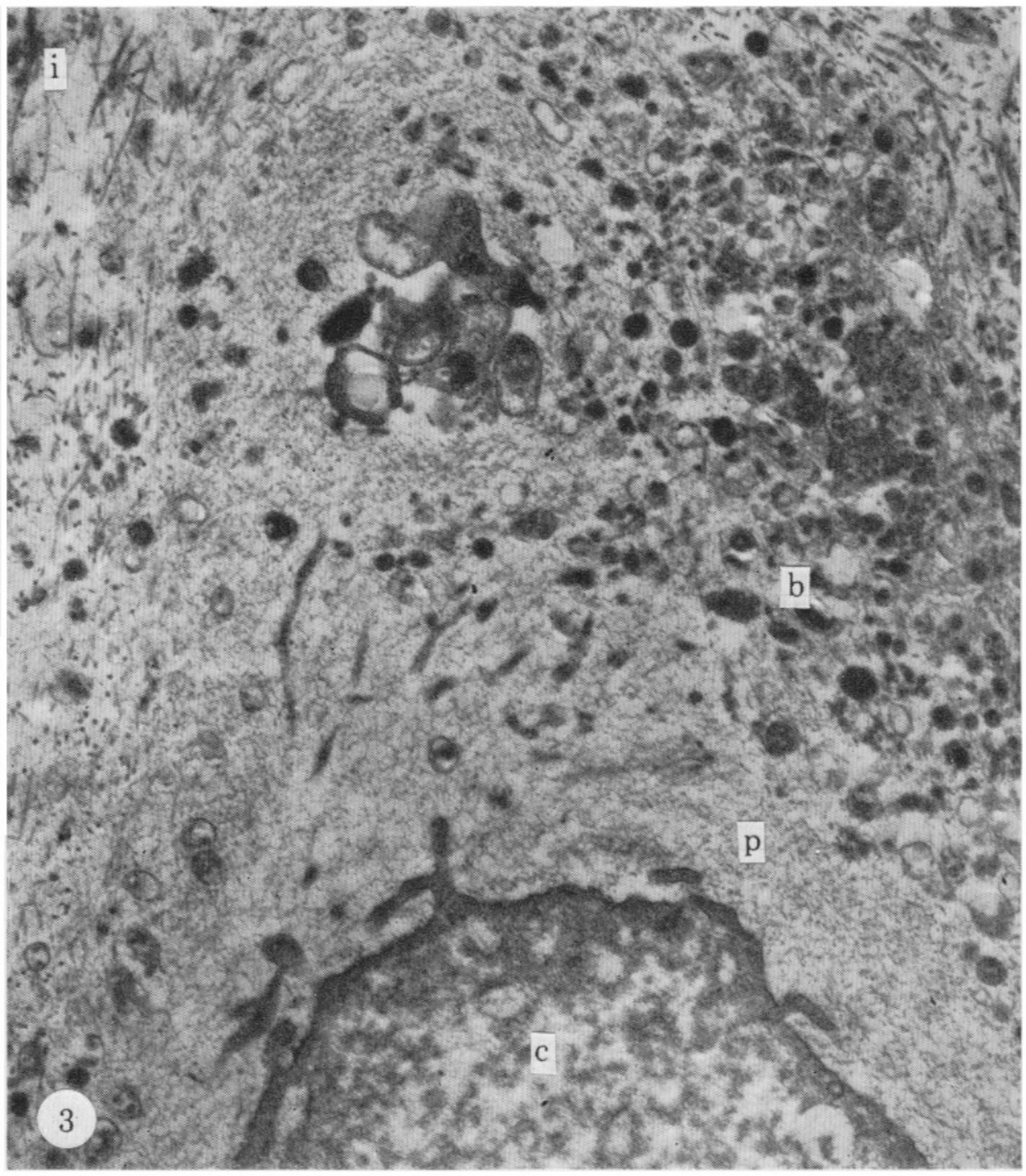

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