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METHODS FOR ISOLATION OF ODONTOBLASTS AND DETERMINATION OF INTRACELLULAR POTASSIUM*

by

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In most areas of modern histology chemical information of a particular group of cells under different conditions is desired in order to understand their functions. This is particularly true of cells in the dental pulp since cells in this region play an important role in the vitality of the whole tooth. Due to difficulties to reach cells in the dental pulp under living conditions, very little is known of their reactions to circulatory changes or alterations in the general metabolism. The technique developed by *Engström & Öhman* (1960) has offered a possibility of an easy approach to the pulpal tissues with a minimum of trauma.

On freshly extracted human teeth a groove is cut longitudinally round the tooth with a diamond saw using a modified Krebs-Ringer solution (see below) as cooling fluid. Then the tooth is split in two halves with a vice provided with two wedges made of stainless steel. The pulp is easily removed with a small forceps. Most of the odontoblasts remain on the surface of the pulp, and generally a minor portion of the odontoblasts are found at the end of the dentinal tubules on the mineralized parts. Under a dissecting microscope groups of odontoblasts can be isolated by hand with the aid of needles made of stainless steel and an "iris" knife, Fig. 2. As can be seen from Figs. 1 and 2 the Tomes fibers

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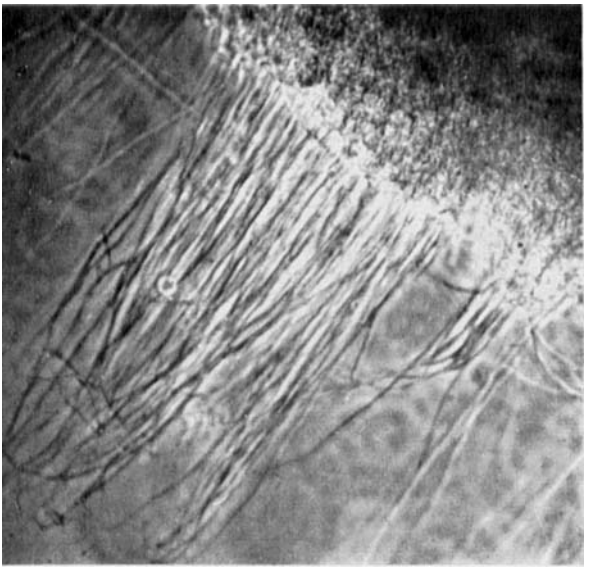

Fig. 1. Edge of an isolated dental pulp with odontoblastic processes seen under a phase contrast microscope (Orig. mag. × 512).

or parts of them quite often stay on the odontoblasts. It is possible to isolate groups of odontoblasts with approximately the same volume for quantitative elementary analysis. This might slightly increase the statistical variation in the results, but if the population is big enough significant differences are registered with great accuracy. For comparison of sampling problems the reader is referred to *Hamberger* (1963), who measured the respiratory activity of small groups of glial cells from the brain and to *Hamberger & Röckert* (1964) who made potassium determinations on glial cells. A trained person can dissect groups of cells having the same volumes with great accuracy within 15 minutes after the tooth extraction, particularly if a plastic sphere of known volume is constantly used during the dissection under the microscope.

As incubating medium is used a modified Krebs-Ringer solution of the following composition: NaBr 124 mM, KNO_3 5 mM, KH_2PO_4 1.24 mM, $MgSO_4$ 1.3 mM, $NaHCO_3$ 26 mM, Ca lactate 2.8 mM and glucose 10 mM. The solution is given this particular composition in order to cause a minimum of background x-ray fluor-

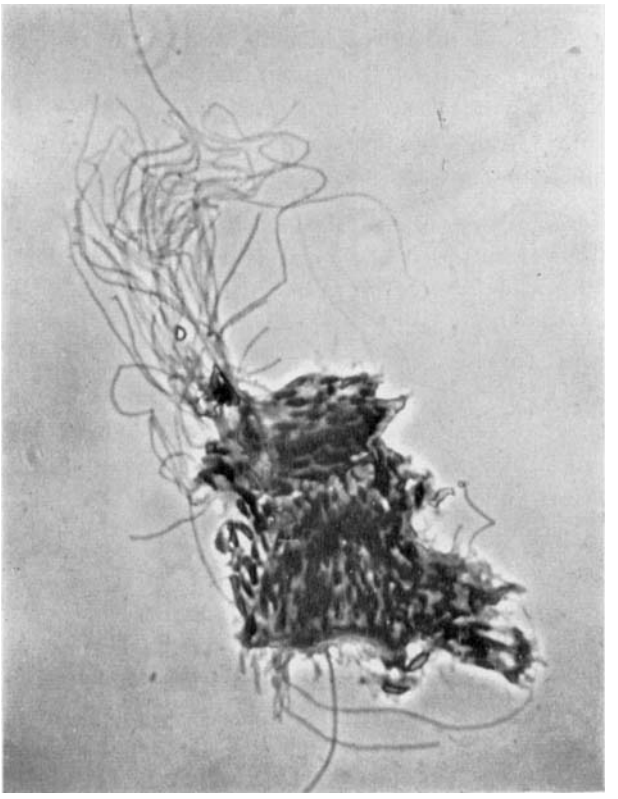

Fig. 2. Group of isolated odontoblasts with some of their processes seen under the phase contrast microscope (Orig. mag. × 512).

escence in the wavelength region 1.7—3.8 Å, which is the wavelength region within which the method is operating for these analyses.

Generally, leakage of substances from the cytoplasm when processes are cut off is not great provided the cell membrane is not ruptured over an extensive area. For enzymes this has been demonstrated by *Hydén* (1959) on isolated nerve cells where all the dendrites and the neurite were cut off. The enzymatic activity remained unchanged for more than one hour. For potassium there is an initial rapid loss from nerve cells after isolation but K^+ levels are rapidly restored when the isolated cells are incubated at 37° C in physiological saline (*Cummins & McIlwain,*

1961). It should be noted, however, that K^+ levels in nerve cells gradually decrease during an hour's incubation at 37° C and at 20° C. The glial cells on the other hand show a corresponding increase but only at 37° C (*Hamberger & Röckert,* 1964). Because of these findings the dissections of odontoblasts were carried out both at 20° C and at 37° C.

The inference that odontoblasts behave similarly to nerve cells on isolation from surrounding tissue is supported by electron micrographs, *Schroff et al.* (1954, 1956), showing that Tomes fibers are morphologically distinct from the cytoplasm of the odontoblasts themselves and suggest that the damage of the odontoblast by isolation is of the same minute order as that of the nerve cell. Besides that, the potassium content of a small group of odontoblasts is approximately of the same order as that of a group of glial cells or one nerve cell analysed with the same method.

The intracellular potassium is determined by means of x-ray fluorescence micro-analysis according to the method described by *Long & Röckert* (1963). The specimen is placed on a mylar foil on top of two electron microscope molybdenum apertures with 100 μ and 200 μ diameters, respectively. These are placed on a Cosslett-Nixon x-ray microscope run at 20 KV and 50 μA producing a high energy primary x-ray beam from a focal spot of about 0.5 μ. When hitting the specimen, secondary x-rays (x-ray fluorescence) are radiated spherically and a sector is picked up in a proportional counter (run at 1700 V). The counter is connected to a pulse height analyser (256 channels). This system can discriminate different wavelengths, thus showing on an oscilloscope a curve with different peaks for each element present in the specimen. The height of each peak is determined by the amount present. The characteristic curves are also registered by an electrical typewriter and an x-y recorder. By using standards with known amounts of potassium, quantitative measurements can be made. The method allows analyses of potassium in amounts down to 10^{-11} g. For further details the reader is referred to *Long & Röckert* (1963).

SUMMARY

A method is described by which small groups of odontoblasts can be isolated manually under a dissection microscope. Determination of intracellular potassium was performed with x-ray fluorescence micro-analysis.

RÉSUMÉ

MÉTHODE POUR L'ISOLATION DES ODONTOBLASTES ET RECHERCHE DU POTASSIUM INTRA-CELLULAIRE

L'auteur décrit une méthode permettant d'isoler manuellement de petits groupes d'odontoblastes sous un microscope de dissection. La recherche du potassium intra-cellulaire fut faite par micro-analyse par fluorescence aux rayons X.

ZUSAMMENFASSUNG

EINE METHODE ZUR VEREINSAMUNG VON ODONTOBLASTEN UND KALIUMBESTIMMUNG DERSELBEN

Eine Methode wird beschrieben, wodurch kleine Gruppen von Odontoblasten manuell unter einem Sezierungsmikroskop isoliert werden können. Die Bestimmung von intrazellularem Kalium wird durch Röntgenfluoreszenzmikroanalyse ausgeführt.

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