Transmembrane Potentials of Cultured Mouse Dentinal Pulp Cells.* (26870)

D. C. KROEGER, F. GONZALES,† AND W. KRIVOY
University of Texas Dental Branch and Baylor University College of Medicine, Houston, Texas

Odontoblasts are found in the pulp of teeth as a cellular layer adjacent to the dentin. The fine protoplasmic processes, Tome's Fibers, of these cells extend through the dentinal tubules to the dentino-enamel junction (D.E.J.) in the crown or the dentinocemental junction (D.C.J.) in the root. Some workers have suggested that sensory function is mediated by the odontoblast(1,2); however, the prevailing opinion is that these cells are agents of dentin formation exclusively and that sensory function is mediated by nerve endings extending through the dentinal tubules to the D.E.J. or the D.C.J.(3,4).

Because of the lack of definitive information on this and other pulpal cell types, we have begun a study of the electrophysiological properties of these cells. Ultramicroelectrode technics for determining the transmembranal potentials of cells have yielded very instructive results on the changes in cellular activity and function of the cell membrane. This is a report of the measurement of membrane potentials of mouse dentinal papilla cells grown in tissue culture.

Methods. Pulpal cells obtained aseptically from newborn mouse molar tooth germs were cultured in plasma clots on narrow cover slips in Leighton tubes with a liquid nutrient consisting of Puck's N16(5) containing 30% calf serum. After 4 to 10 days of cultivation the cover slips were transferred to a microscope stage-chamber through which fresh culture medium could be perfused.

Ultramicroelectrodes, machine-pulled from 1 mm soft glass capillary tubing, were filled with 3 M KCl by boiling under reduced pressure and mounted on an inclined micromanipulator to allow access to the cells in the perfusion chamber. Impalement of single cells was observed under a binocular phase-contrast microscope. The electrodes were connected to a single ended D.C. amplifier, Grass P6 with a M.E.P. 6 probe, input impedance of 1000 megohms with input grid current adjusted to 10^{-12} amps, and oscilloscope recordings obtained with a Polaroid camera. The slides were fixed and stained for further morphological studies. Only electrodes with resistances of 10 to 40 megohms were used.

Results. Transmembranal potentials were measured in approximately 200 single cells in isolation or in clumps of cells from 20 cultures. On the basis of visual observations and electrophysiological data these cells can be classified into 3 groups. Table I is a summary of the morphological cell types and their membrane potentials recorded at 20 to 25°C. We have called the polygonal cell an odontoblast. Fig. 1, 2, and 3 show typical examples of the above cell types.

The potential measurements were made on cells viewed through a phase contrast microscope. As the micropipette penetrated the cell membrane, there was an abrupt negative deflection of the oscilloscope beam to a value which generally remained quite steady for a number of minutes. Just before penetration, dimpling of the cell wall could occasionally be observed, and this was marked by a slight shift, 3 to 5 millivolt, of either polarity. This latter observation is perhaps due to the mechano-transducer actions of micropipettes described by Brown and Wiesel(6). No spontaneous activity, either visual or electrical, has been observed in these cultured cells.

In general, tissue culture cells grown *in vitro* lose many of their morphological characteristics especially at the glass-clot interface where they tend to flatten and spread out. The area presented by the spread-out polygonal cells is about 7.5×10^{-5} cm^2. Taking into consideration the top and the bottom of the cell and assuming negligible

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† U.S.P.H.S. Senior Research Fellow.

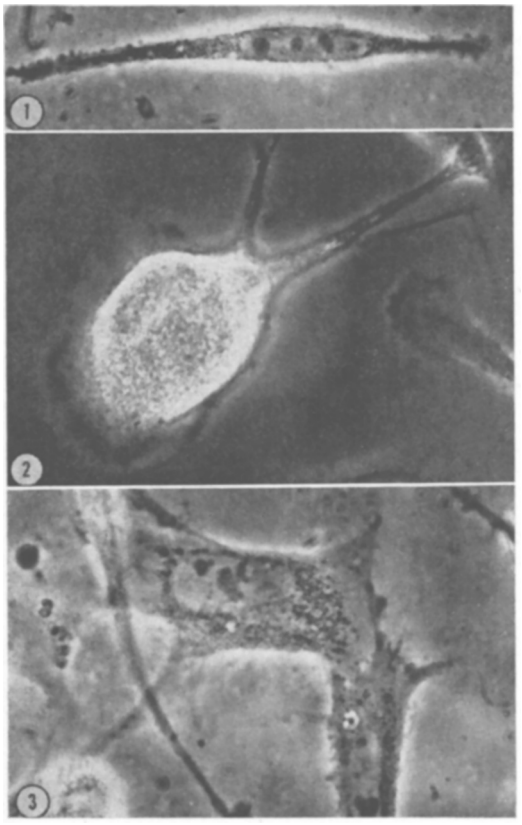
TABLE I. Cell Types and Membrane Potentials of Pulpal Cells Grown in Tissue Culture.

Cell type	No. of cells	Membrane potentials E_m = millivolts
1. Spindle-shaped cells, fibroblast-like	52	7.0 ± .6*
2. Round cells with or without a visible protoplasmic extension	37	19.3 ± 1.5
3. Polygonal cells with a long protoplasmic process	90	34.6 ± 1.4

* Stand. error of mean.

thickness one arrives at about 15×10^{-5} cm^2 as an estimate of total area of the cell.

Membrane resistances were determined on approximately 10 of the polygonal cells (odontoblasts) by the technic described by Sperelakis *et al.*(7) which consists of determining the increase in deflection height of a square-wave pulse when the electrode is intracellular. Membrane resistances of the range of 3 to 4 megohms were observed. No attempt was made to estimate possible errors in the determination *e.g.*, an artificial increase in resistance due to pressure on the electrode, etc.



Phase micrographs of living cells in a 14-day-old culture of molar tooth germ dental papilla from a one-day-old mouse.

FIG. 1. Spindle-shaped fibroblast-like cell. ×213.
FIG. 2. A round cell with protoplasmic processes. ×213.
FIG. 3. Polygonal cells with protoplasmic processes. ×213.

Discussion. It is not possible from these studies to determine the physiological role of these pulpal cells. The moderately high membrane potentials recorded for the polygonal cells, odontoblasts, indicate that they might be classified with such cells as smooth muscle(8) and glandular epithelium(9). The anatomic position of the odontoblast, the presence of cholinesterase in its membrane (1) and a moderately elevated membrane potential could support the idea of sensory mediation. It is equally possible, however, that these data support the much older view that the odontoblast acts in a secretory manner for governing the deposition of calcium salts in the dentin. Nylen and Scott(10) found extensive concentrations of rough surfaced endoplasmic reticulum in rat's odontoblasts. This suggests a secretory function but does not rule out a sensory function for these cells.

In confirmation of an observation by Crill *et al.*(11), it was noted that the membrane potentials of grouped cells were higher than those of any isolated cell. Whether this is the result of different electrical resistive values of a cell membrane in isolation compared with cell membranes in intimate contact is not known. Other properties of cells, for example cytochemical features, are known to differ in isolated cells as compared to their counterparts in clusters(12). Just what mechanism is involved remains to be determined. It is possible that the round cell is an odontoblast in the terminal stages. The rounding or "balling up" of a cell is generally taken as a sign of necrosis. These cells were not present in all cultures and when seen were usually found as solitary cells.

Preliminary studies on the *in vivo* pulps of young dogs and rats have indicated the presence of at least 2 of the cell types found in tissue culture; many scattered cells throughout the pulp having membrane potentials of 5 to 10 millivolts and a few cells located along the dentinal border with membrane potentials of 25 to 45 millivolts. Work is continuing along these lines.

Summary. It has been possible to grow in tissue culture viable cells from tooth germs of young mice. Three cell types have been determined based on morphological and electrophysiological characteristics. The mouse odontoblast grown in tissue culture possesses a membrane potential which is considerably greater than that of the fibroblast and is in the range reported for smooth muscle and glandular cells. At present it is not possible to state if the physiological role of the odontoblast is sensory, glandular, or both.

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Iodine-Induced Submandibular Sialadenitis in the Hamster. (26871)

RICHARD H. FOLLIS, JR.
Veterans Administration Central Laboratory for Anatomical Pathology and Research, Armed Forces Institute of Pathology, Washington, D.C.

During the course of some studies on the pathogenesis of iodine-induced thyroiditis in the hamster(1), other tissues, particularly those which are known to concentrate this element, have been examined microscopically. Damage to the submandibular (submaxillary) glands has been found uniformly; in contrast, the parotid and sublingual glands, as well as the gastric mucosa, appear to be normal. This communication presents a description of the changes in affected glands together with comparative data on radioiodine uptake, which may help to explain the difference in reaction between the submandibular glands and other structures.

Materials and methods. Histological observations have been carried out on the salivary glands of approximately 100 normal male hamsters, each weighing about 125 g, which had received various quantities of iodide as the potassium or sodium salts for periods lasting several hours to 10 days. The iodide was administered intraperitoneally in distilled water. Animals were sacrificed at intervals from one hour after a single injection up to after 10 days of multiple doses, usually once daily. Submandibular, sublingual and parotid glands as well as glandular portions of the stomach, were studied after fixation with neutral formalin and staining with H and E, PAS, and alcian blue technics. Iodide dosages ranged from 1.0 to 200 mg, since it was found that smaller amounts were ineffective.