ies. The addition of the hydroxamate chelator to *Scenedesmus* suppressed growth. The colonies broke into single cells, and the cells quickly became chlorotic. There are two reasons for believing that the activity of this fraction is due to the hydroxamate and not a toxin that may have been in the same fraction. The uptake of iron per cell in *Scenedesmus* was only 20 percent of the control, and the addition of excess iron overcame the toxicity of this fraction. The addition of excess iron does not stimulate growth if the hydroxamates are not added to the culture. *Scenedesmus* does produce small peptides that can solubilize iron, but the *Scenedesmus* iron uptake system cannot compete with the hydroxamate system that is found in at least some blue-green algae and probably many planktonic bacteria.

It thus appears that the availability of iron may be an important factor in determining the stability and composition of aquatic ecosystems. The availability of iron is both a function of the biological demand for iron and the chelators excreted by microbes into lake water.

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## Neural Properties of Cultured Human Endocrine Tumor Cells of Proposed Neural Crest Origin

Abstract. *Cells from human endocrine tumors of proposed neural crest origin—five pheochromocytomas, two medullary carcinomas of the thyroid, and two bronchial carcinoids—were grown in monolayer culture. Cells from all nine tumors, including epithelial forms of medullary carcinoma of the thyroid and bronchial carcinoid cells, and epithelial and neuron-like pheochromocytoma cells demonstrated all-or-nothing, short-duration action potentials.*

Embryological studies employing ablation, grafting, and tissue culture techniques have demonstrated that the neural crest is the origin of sensory and sympathetic ganglia, melanocytes, and adrenal chromaffin cells (*1*). Migrating neural crest cells are able to decarboxylate and store precursors of aromatic amines which fluoresce after exposure to formaldehyde vapor, and studies using this fluorescence as an endogenous marker suggest that the neural crest also gives rise to widely dispersed endocrine cells including enterochromaffin and related cells of the gut, argyrophil cells of the bronchi, islets of Langerhans, and parafollicular cells of the thyroid. These endocrine cells have been collectively termed the amine precursor uptake and decarboxylase (APUD) system (*2*).

This report demonstrates that cells from three types of human endocrine tumors of the proposed APUD system are capable of generating all-or-nothing, short-duration action potentials, consistent with their neural crest origins. In the case of pheochromocytomas, the cultured cells can also morphologically resemble neurons.

Cells from five adrenal pheochromocytomas, two medullary carcinomas of the thyroid (MCT's), and two bronchial carcinoids (BC's) were grown in monolayer culture (*3–5*) sometimes in medium supplemented with nerve growth factor (NGF) (*6*). These tumors are derived, respectively, from the APUD cells of the adrenal medulla, thyroid, and bronchi (*2*). Each tumor was diagnosed from multiple histologic sections by at least two of the pathologists in two teaching hospitals, using accepted morphologic criteria. Tumor cells from each of the pheochromocytomas grew as polygonal epithelial cells, 15 to 20 $\mu$m in diameter, which usually formed clusters, and which to varying degrees produced thin, branching, argyrophilic processes (> 100 $\mu$m)

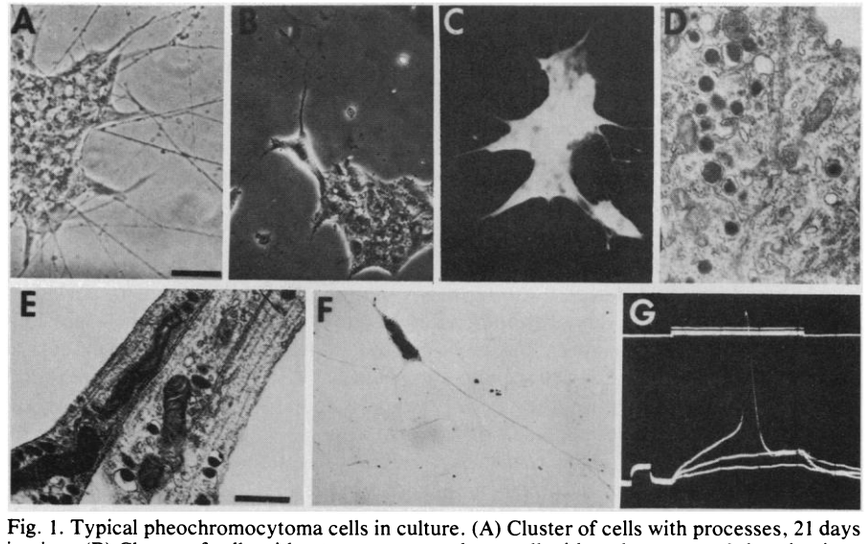


Fig. 1. Typical pheochromocytoma cells in culture. (A) Cluster of cells with processes, 21 days in vitro. (B) Cluster of cells without processes, and one cell with early process, 9 days in vitro. (A) and (B) are phase contrast pictures of live cells; scale bar, 50 $\mu$m. (C) Formaldehyde-induced fluorescence of cell bodies and processes. (D and E) Electron micrographs of flat-embedded cell bodies and proximal processes, showing large secretory granules typical of pheochromocytes 7 days in vitro; scale bar, 1 $\mu$m. Cells were fixed in 3 percent glutaraldehyde, *p*H 7.3, and postfixed in $OsO_4$. Processes often coursed side by side, forming fascicles. (F) Cluster of cells with branching fascicle of processes stained by the Holmes silver stain (*20*) with fibroblasts in background. (G) Typical intracellular recording from pheochromocytoma cells, showing three superimposed sweeps. (Upper trace) Current injected into cells. (Lower trace) Electrical potential responses: two subthreshold depolarizations and one suprathreshold depolarization triggering an action potential. Calibration pulse, 10 mv and 5 msec. Such action potentials could be elicited from all of the cell types illustrated.

similar to those of cultured neurons (Fig. 1). Cultured MCT and BC cells were epithelial, 12 to 15 $\mu$m in diameter, with dark cytoplasm and light, ovoid nuclei (Fig. 2). In pheochromocytoma cultures, NGF markedly increased the number of cells with neuron-like processes, and also appeared to improve tumor cell plating efficiency (*7*). Tumor cells with processes were occasionally observed in the absence of added NGF in cultures from two of the five pheochromocytomas, in contrast to the results of a previous study of one human pheochromocytoma in cell culture (*8*). Nerve growth factor had no effect on the morphology of MCT and BC cells, and no tumor cells with processes could be identified with certainty in MCT and BC cultures.

The identity of cultured tumor cells was established by electron microscopy in conjunction with a variety of other techniques. Cultured tumor cells of each type contained secretory granules (Fig. 1, D and E; Fig. 2, B and F) similar to those described by other authors for the respective tumors in vivo (*9*) or in vitro (*8*). Cultured pheochromocytoma cells with and without processes showed intense green fluorescence consistent with catecholamine content after exposure to hot formaldehyde vapor (*10*) (Fig. 1C). Varying numbers of cultured pheochromocytoma cells showed a chromaffin reaction by the method of Hillarp and Hökfelt (*11*). Cultured BC cells showed yellow-green formaldehyde-induced fluorescence consistent with indole-alkylamine content (Fig. 2E). Calcitonin was demonstrated in cultured MCT cells by the immunoperoxidase technique and amine storage by formaldehyde-induced fluorescence, as reported elsewhere (*12*).

Electrophysiological studies were performed on cells between 2 hours and 56 days in vitro (*13*). Pheochromocytoma cells (2 hours to 43 days in vitro) were fragile when penetrated with microelectrodes; initial resting potentials were low, usually less than −35 mv, and were not usually maintained. Most likely these values were underestimates because of cellular damage caused by impalement. Depolarizing potentials that reached threshold produced all-or-nothing, short-duration, frequently overshooting action potentials in epithelial cells as well as in cells with processes from all five tumors (Fig. 1G). Larger depolarizing potentials could produce repetitive firing. Electrical excitability was demonstrated in nearly 100 percent of consecutively penetrated cells with measurable resting potentials in two pheochromocytoma cultures so studied, and was seen in mechanically dissociated cells within 2 hours after plating. Many of the cells required background hyperpolarization to membrane potentials above −40 mv for activation of the spike mechanism.

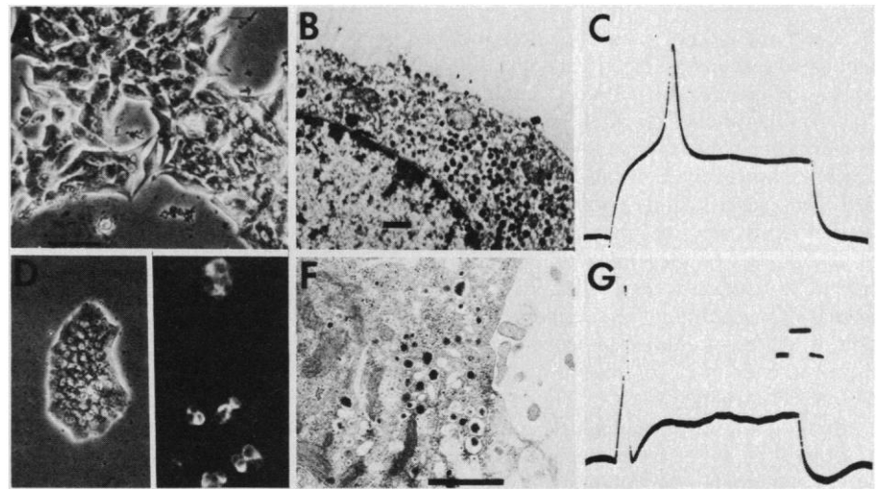


Fig. 2. Typical MCT cells (A to C) and BC cells (D to G) in culture. (A) and (D) are phase contrast pictures of live cells; scale bar, 50 $\mu$m. (B) and (F) are electron micrographs, showing secretory granules; scale bar, 1 $\mu$m. (E) Formaldehyde-induced fluorescence of cultured BC cells. Fluorescence faded rapidly on exposure to ultraviolet light or after immersion in 1 percent sodium borohydride in 70 percent isopropanol. (C) and (G) are intracellular recordings with all-or-nothing action potentials. Calibration pulse, 10 mv and 5 msec.

Electrically excitable cells were equally numerous in cultures with and without NGF. Action potentials in pheochromocytoma cells were reversibly inhibited by tetrodotoxin ($10^{-6}$ g/ml), and were readily demonstrable in calcium-free medium, indicating that they are predominantly or exclusively Na potentials. No synaptic potentials, either spontaneous or evoked, were noted when pairs of cells from one pheochromocytoma were impaled simultaneously and sequentially stimulated. Occasionally, cells that were close to one another in a cluster showed mild electrical coupling.

Penetration of MCT cells (2 to 56 days in vitro) and BC cells (2 to 22 days in vitro) by microelectrodes was difficult because of the cells' small size, and resting membrane potentials were thought to be unreliable. After activation by hyperpolarizing the cells to membrane potentials greater than −40 mv, depolarizing pulses produced short-duration, all-or-nothing action potentials (Fig. 2). Tetrodotoxin ($10^{-6}$ g/ml) reversibly inhibited BC cell action potentials in cells of one tumor so studied.

Although many cells from all nine tumor cultures did not produce action potentials, these cells generally had poor resting potentials and low input resistances, indicating that they were probably injured. We cannot, however, rule out the possibility that some of the cells might be electrically inexcitable as a function of genetic variation, as are some neuroblastoma cells (*14*). It is most unlikely that neuronal cells could have "contaminated" our tumor cultures since neurons were not seen in histological sections from areas of the original tumors used for cultures and spiking cells were numerous in cultures from all three tumor types.

Overshooting, short-duration (1 to 10 msec), all-or-nothing action potentials are generally considered properties of nerve or muscle. They have been seen in cell lines derived from one mouse neuroblastoma (*14*) and two primary human neuroblastomas in our laboratory, but they have not been reported in other types of normal or neoplastic tissue (*15*). Pheochromocytomas are members of a group of tumors which include neuroblastomas, ganglioneuroblastomas, and ganglioneuromas. In culture neuroblastomas will extend processes and can contain dense core granules in addition to synthetic enzymes for acetylcholine and catecholamines. The close relationship between pheochromocytoma and neuroblastoma makes the finding of "neuronal" properties in pheochromocytomas less surprising. However, the finding of action potentials in BC and MCT cells is more unexpected and raises the question of whether electrical excitability is involved in hormonal secretion by these cells' normal counterparts, as it is in hypothalamic neurosecretory cells.

Previously reported studies of the electrical properties of normal gerbil adrenal chromaffin cells indicated that these cells had low resting potentials (about −25 to

−30 mv) and did not produce action potentials in response to electrical depolarization or application of acetylcholine (*16*). Similar studies on adult gerbil and human adrenal medullary cells in our laboratory, however, indicate that these cells can have resting potentials of at least −55 mv, and that they are capable of generating overshooting action potentials (*17*). If action potentials do prove to mediate some forms of endocrine secretion, it will be of interest to see whether the phenomenon is confined to cells of neural crest origin.

Many specific physiological considerations such as the ionic mechanism of action potentials, the relation between excitation and secretion, and receptor sensitivity could not be adequately studied in our human tumor systems because of the small amounts of tissue available in surgical specimens. We have, therefore, extended our investigations to reported animal models of APUD tumors (*18*). We have studied rat pheochromocytoma and bovine MCT cells, and have observed that these cells, like their human counterparts, are electrically excitable, and that the pheochromocytoma cells grow processes in response to NGF (*19*). These animal tumors might therefore be useful models for expanded studies.

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4. Cells from two human MCT's were mechanically dissociated and grown on Falcon tissue culture dishes in McCoy's 5A medium with 20 percent FCS, sometimes supplemented with 5 mg of L-thyroxine or 5 BU's of NGF, or both, per milliliter.
5. Cells from two human BC's were mechanically dissociated and plated in McCoy's 5A medium with 20 percent FCS, sometimes supplemented with 5 BU of NGF per milliliter either directly on Falcon tissue culture dishes, or on monolayers of fibroblasts whose growth had been arrested with cytosine arabinoside.
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13. Cultures were placed in a phosphate-buffered physiological salt solution on an inverted phase-contrast microscope (Zeiss). Single cells were impaled under direct vision by use of glass microelectrodes filled with 3.4*M* potassium acetate or 3*M* KCl (20 to 80 megohms) and connected to a high-input impedence electrometer amplifier equipped for passing current through the microelectrode while recording (WPI Instruments) and to a differential amplifier for display on oscilloscope and penwriter. Experiments were done at both room temperature and 35°C.
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## Nuclear Magnetic Resonance Patterns of Intracellular Water as a Function of HeLa Cell Cycle

Abstract. *Nuclear magnetic resonance relaxation time ($T_1$) of the intracellular water protons and water content were measured in synchronized HeLa cells. The $T_1$ was maximum (1020 milliseconds) in mitotic and minimum (534 milliseconds) in S phase cells. The cyclic pattern of $T_1$ values correlated well with the chromosome condensation cycle. By treating cells with spermine, it was possible to alter $T_1$ without a significant change in the water content. The results of this study suggest that an additional variable, namely, the conformational state of macromolecules, should be included in any expression explaining the shortened relaxation times of water protons in biological systems.*

The importance of water in biological systems is obvious, since it constitutes 70 to 90 percent of the mass in most living systems. The structure and function of this simple molecule in biology, however, is not yet completely understood. One of the quantitative methods available to study the physical properties of water is nuclear magnetic resonance (NMR) spectroscopy. Primarily, NMR spectrometers are comprised of a magnet and a high-frequency radio transmitter which produce perpendicular magnetic fields. The hydrogen nuclei of water molecules will absorb energy when placed in a strong magnetic field at a specific resonance frequency. In pulsed NMR, the water hydrogen protons absorb energy during a brief (microseconds) pulse of radio-frequency ener-