Cytochemical Demonstration of Catecholamines and Acetylcholinesterase in Cultures of Chick Sympathetic Ganglia

In sympathetic ganglia, noradrenalin (NA) can be demonstrated in the neuronal somas as well as in the nerve fibers by fluorescence microscopy[1]. Recently several authors have demonstrated that NA and acetylcholinesterase (AChE) occur in the same cells and fibers of sympathetic ganglia[2,3], while others have reported that a positive reaction for NA or for AChE is mutually exclusive in sympathetic neurons[4,5]. In the present communication, a study was made of the cytochemical localization of NA and AChE in chick sympathetic ganglia cultured in vitro to contribute some informations to this controversy.

Materials and methods. Sympathetic chains taken from 14–16-day chick embryo were explanted on the collagen coated coverslips and incorporated in Maximow's slides. The cultures were fed twice a week with feeding medium consisting of equal parts of horse serum, medium 199, Hanks' balanced salt solution, 9-day chick embryo extract, and supplementary glucose in a concentration of 600 mg/100 ml medium. Cultures were incubated at 36°C for 7–48 days.

Cultures were freeze-dried, exposed to formaldehyde vapor for 1 h at 80°C and processed to demonstrate NA fluorescence as described by FALCK et al.[6]. The preparations were examined with a Zeiss fluorescence microscope equipped with HBO 200 mercury lamp, BG-12 3 mm excitation filter, Zeiss 50 barrier filter and dark field condenser.

The method used for demonstrating AChE activity was that of KARNOVSKY and ROOTS[7]. The cultures were fixed in icecold formol-calcium for 5 min, rinsed in distilled water, and placed in incubating medium for 1–2 h at 36°C. A specific inhibitor for pseudocholinesterase, iso-OMPA (tetraisopropyl pyrophosphoramide), was included in the medium in the concentration of $10^{-5}M$. Some cultures were fixed in formol-ammonium bromide and silver stained by the modification of Bodian's protargol method[8].

Results. The normal development of chick sympathetic ganglia in vitro has been described by previous authors[9]. It takes 7–10 days to recognize individual symapthetic neurons (12–15 μm in diameter) until the explant has thinned sufficiently. Numerous outgrowing axons are also recognizable radiating from the explant in this stage. Relatively well-differentiated neurons with increased size (20–25 μm in diameter) are observed by 4 weeks in vitro and onward (Figure 1A).

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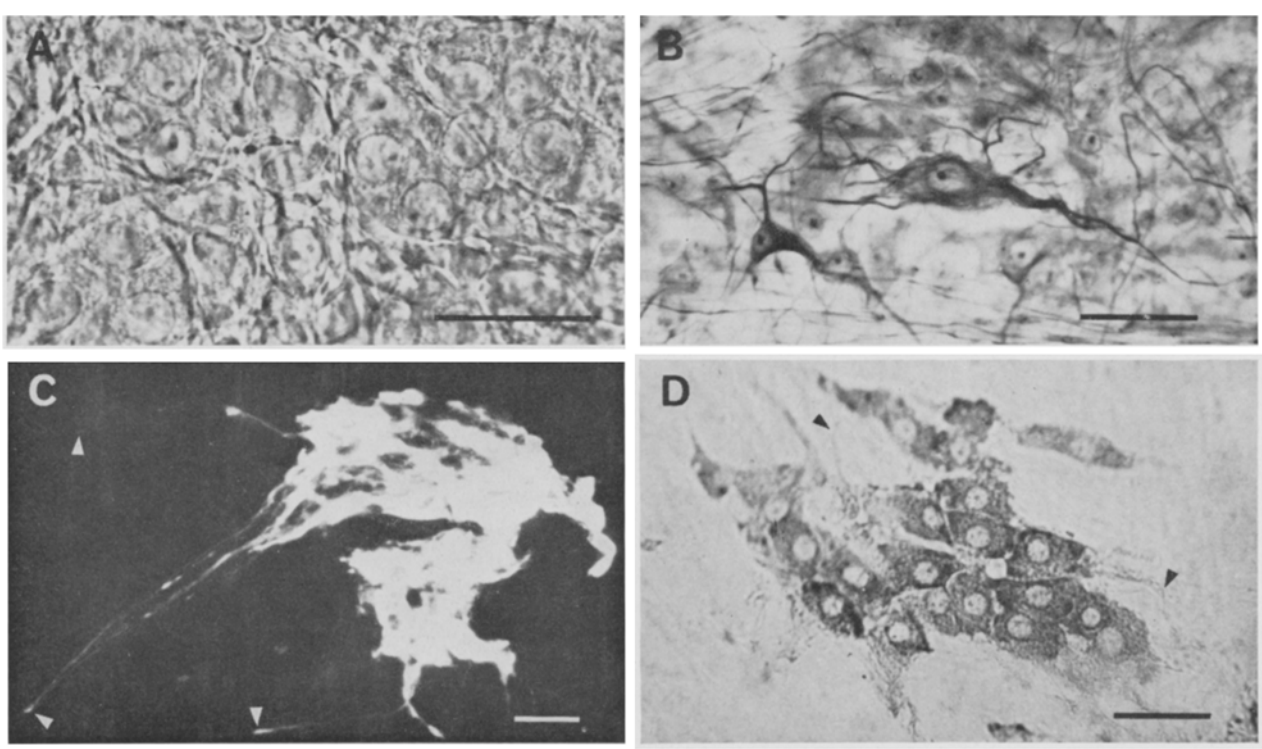

Fig. 1 A) Living chick sympathetic neurons, cultured 28 days in vitro. B) Multipolar sympathetic neurons in 32-day-old culture stained by Bodian silver method. C) Noradrenaline fluorescence of sympathetic neurons and outgrowing fibers (arrows). 28 days in vitro. D) Intense reaction of acetylcholinersterase in cytoplasm of sympathetic neurons. Moderate enzyme activity is also seen in outgrowing fibers (arrows). Neurons in the periphery are necessarily out of focus. 32 days in vitro. Bars represent 50 μm.

Sympathetic neurons maintained in culture are rarely stained by silver technique and the chance of having well-impregnated neurons is 1 in 100 preparations. When the neurons are successfully stained, they have fine, argyrophilic neurofibrils running through the neuronal perikarya and dendrites (Figure 1B). By 4 weeks in vitro, the neurons acquire multipolar morphology and the size of most neurons falls in the range of 20–25 μm in diameter. Occasionally giant neurons in size of 40–50 μm are observed (Figure 1B).

Under the fluorescence microscope, strong NA fluorescence reaction is observed in neuronal perikarya and fiber bundles (Figure 1C). Fluorescence reaction in non-neuronal elements including Schwann cells is negative, though non-specific fluorescence is occasionally observed in round-shaped non-neuronal elements in outgrowth zone, probably macrophages or mast cells. The fluorescence in nerve fibers is of a beaded nature, showing small fluorescent varicosities along the course of the fibers (Figure 1C).

When the cultures are processed for AChE enzyme histochemistry, most of the sympathetic neurons reveal intense AChE activity. The reaction of AChE is observed in perikarya of sympathetic neurons and moderate to weak enzyme activity is also seen in the dendrites and outgrowing fibers (Figure 1D). The enzyme activity is always negative in non-neuronal elements including Schwann cells.

Our attempts to demonstrate NA fluorescence and AChE reaction consecutively in a same culture met with considerable success. For that purpose the following procedures were undertaken: cultures were dried in the air current, exposed to formaldehyde vapor for 1 h at 40°C to produce NA fluorescence, and examined under the fluorescence microscope. After brief rinsing in distilled water, the prepatarions were incubated in the AChE medium (vide supra) for 2–3 h at 36°C. Photomicrographs were taken of the same cultures after each histochemical procedure. We have repeatedly seen the presence of NA fluorescence and AChE reaction in the same or in closely associated fibers.

Discussion. Although the presence of NA fluorescence in cultured sympathetic neurons has been demonstrated in rat and mouse[10] and in chick[11], no information is available on AChE activity in cultured sympathetic neurons. It is the first time, to our knowledge, that AChE activity is demonstrated histochemically in cultured sympathetic neurons.

It is generally accepted that the nerve cell population of the sympathetic ganglia is mostly noradrenergic and a small population of cholinergic cells are also present. There does not seem to be an obvious explanation as to why most of sympathetic neurons cultured in vitro exhibit both NA fluorescence and AChE activity.

From our results, it appears that the sympathetic neurons, grown and maintained in vitro, are capable of synthesizing cathecholamines and at the same time are well equipped with AChE, the enzyme metabolizes the cholinergic transmitter. Our results agree with previous studies which suggest the presence of AChE in adrenergic neurons or fibers. This fact supports the hypothesis of BURN and RAND[12] which involves ACh in adrenergic transmission.

Zusammenfassung. Es wurde die Katecholamin-Fluoreszenz und Acetylcholin-esterase-Reaktion an Nervenzellen des sympathetischen Grenzstrangs an Hühnerembryonen in Gewebekultur demonstriert.

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Ultrastructural Aspects of the Basal Zone in the Taste Bud

The study of the apical and middle region has been the main subject of most papers concerning the taste bud. On the contrary, this report deals with the ultrastructural investigation of the basal region of taste buds.

Materials and methods. Foliate papillae of adult rabbits were fixed in glutaraldehyde, postfixed in osmium, dehydrated in ethanol and embedded in Epon. Fixation in glutaraldehyde was performed by immersion or by perfusion.

Results and discussion. The contour of the basal region, formed from the basal contour of the taste bud cells, is gently curved and is lined by a basement membrane approximately 500 Å thick (Figure 1). The cells more frequently found in this region are the basal cells and the 'clear cells'. The basal cells are roundish or elongated in shape, and show abundance of free ribosomes and tonofilaments, whit a relative scarcity of other cytoplasmic organelles (Figure 2). The 'clear' cells, interspersed among other cells, are elongated in shape and contain short scattered cisternae of the rough endoplasmic reticulum, fine filamentous bundles, mainly longitudinally oriented, and some dense bodies. The cytoplasmic matrix is quite clear (Figure 3). Sometimes they possess dense bodies with the aspect of cytosegregosomes[1]. The low portion of these cells often shows a characteristic clumping of mitochondria. (Figure 1). It is frequently possible to observe nerve fibers just entering the taste bud which are rich in microtubules and differently oriented (Figure 1). Some nerve cells come into contact with cells which have been described as neurosecretory or gustatory[2,3], and considered as neurosensory in function. In these points it is quite possible to observe synapse-like 'active sites' with several synaptic-like vesicles crowded along the plasma membrane on the cytoplasmic side (Figure 1). Sometimes one or more polimorphonuclear leucocytes are observed underlying the basal region of the taste bud.

The basal region of the taste bud assumes a remarkable importance because of the relationship with subgemmal connective tissue and with the nerve fibers entering the taste bud. Such region shows the presence of little

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