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.

S. U. KIM[13] and I. MUNKACSI

Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon (Canada), 10 December 1971.

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[13] New address: Division of Neuropathology, Department of Pathology, University of Pennsylvania, Philadelphia, Pa. 19104, USA.

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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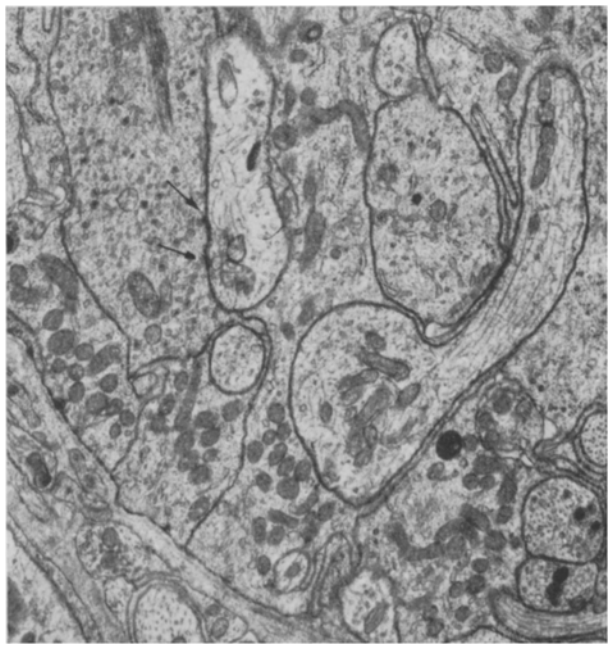

Fig. 1. Basal zone of the taste bud with many nerve fibers differently oriented. In the high and left part of the figure, a nerve fiber contacts a neurosecretory cell: 2 'active sites' are observed (arrows). Note the abundance of mitochondria in the basal region of clear cells. ×6750.

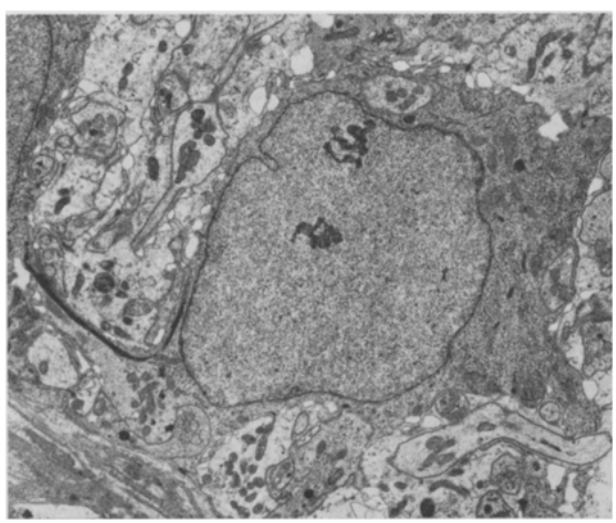

Fig. 2. Differentiating basal cell. Note the abundance of free ribosomes and in the left part of the figure the bundle of tonofilaments. ×6750.

differentiated cells, i.e. the basal cells and the clear cells. Mature cells, except for some neurosecretory cells, are rarely observed. The basal cells or IV type cells[4] are destined, together with peripheral cells, to transform into mature cells. Clear cells, i.e. cells characterized by the extreme clarity of the cytoplasmic matrix, have already been described[5] as 'scattered cells': they present filamentous bundles quite irregularly oriented, more or less elongated cisternae of the rough endoplasmic reticulum, small mitochondria and some dense inclusion bodies: such bodies which sometimes have the aspect of cytosegregosomes, are typically increased in number after denervation[5]. Other dense bodies with frequent lamellar

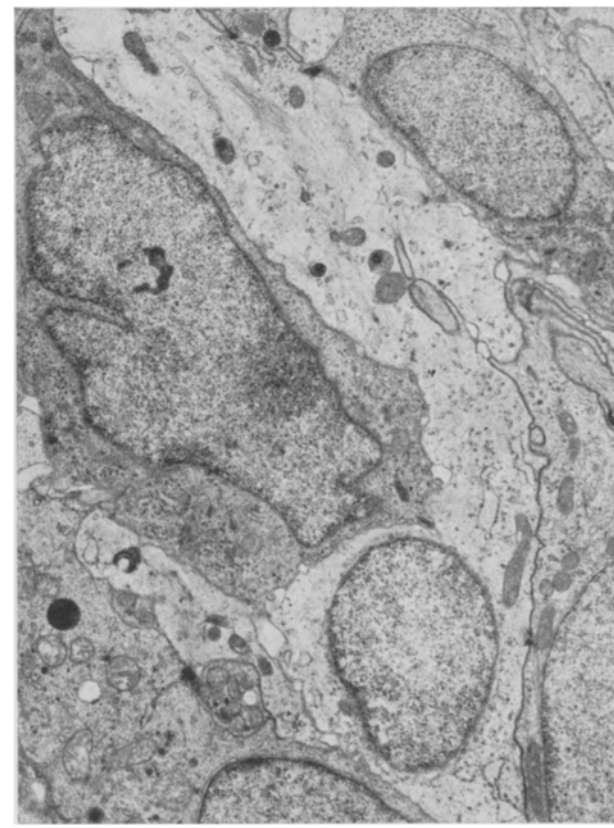

Fig. 3. Longitudinal section of a clear cell. It is possible to observe short cisternae of the rough endoplasmic reticulum, scattered filamentous structures and nucleus in the basal zone of the cell. At the left, we observe a basal cell. ×6750.

structure are quite similar to those found in mature I type cells: these cells further present elongated cisternae of rough endoplasmic reticulum so that it seems possible that clear cell represents a little-differentiated cell which subsequently evolves towards the mature I type cell. As far as the specialized synapse-like contacts between neurosecretory cells and nerve fibers are concerned, it must be emphasized that they are relatively frequent showing the possibility that afferent impulses may be transduced also at this level.

Conclusively, the complex organization of the basal region of the taste bud shows a clear cut prominence of little-differentiated cells, useful for the replacement of those lost during the turnover typical of the taste bud[6].

Riassunto. Nella presente nota è discritto l'aspetto ultrastrutturale della regione basale del calice gustativo ove si osservano in prevalenza cellule «chiare» e cellule basali, entrambe caratterizzate da scarsa differenziazione e presumibilmente destinate al rimpiazzo delle cellule degenerate.

C. OLIVIERI-SANGIACOMO

Centro per la Chimica dei Recettori del C.N.R., Istituto di Anatomia Umana Normale dell'Università Cattolica, Via Pineta Sacchetti 644, I–00168 Roma (Italy), 6 December 1971.

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