

Summary. A new medium was developed for tissue-culturing of the human squamous cell carcinoma of the skin. The morphology of the outgrowing tumour cells is described, particularly with regard to the projections of cell surface. There are 2 forms to be differentiated: one which is similar to the desmosomes of the normal epithelium of the skin, and one of intercellular connective

strands. The different functions of both forms are discussed with regard to the exchange of cell matter.

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Cholinergic Mechanism in the Release of Catecholamines from Intra-Ganglionic Inhibitory Terminals?

Inhibition of sympathetic ganglion cells is exerted by intraganglionic adrenergic nerve endings¹ arising either from axon collaterals or from special 'chromaffine' cells. Recently we have shown² that a number of adrenergic terminals is present in the feline ganglion cervicale superius, displaying a formaldehyde-induced fluorescence under the light microscope. These terminals contain electron microscopic 'dense-core vesicles' and establish axo-somatic contacts with ganglion cells.

Electron histochemical studies performed by means of the uranyl-thiocholine technique³ revealed that adrenergic axo-somatic nerve endings exhibit an acetylcholinesterase (AChE) activity. The reaction is confined to the intervesicular space within the terminal (Figure). No enzyme activity could be observed, however, at the junctional area between the terminals and the pseudodendritic protrusions of the ganglion cells, in striking contrast to axo-dendritic (excitatory) synapses, characterized by a heavy enzyme reaction of the synaptic area.

Supposing that the site of an enzyme activity points to the site of the breakdown of its physiological substrate, one arrives at the conclusion that acetylcholine, being the physiological transmitter substance in excitatory ganglionic synapses⁴, plays a role also in the liberation of

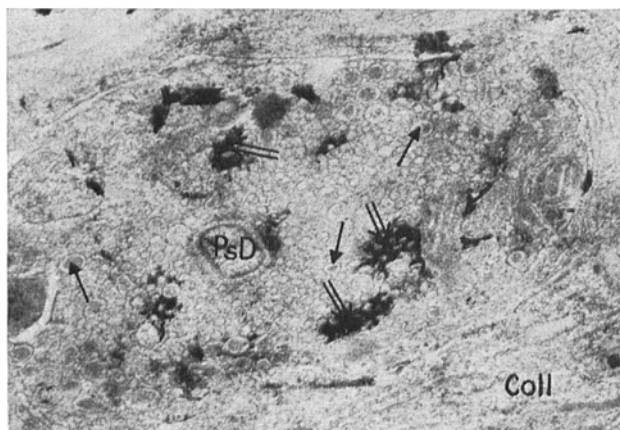
catecholamines from inhibitory (adrenergic) terminals. Catecholamines are known to exert a blockade of ganglionic transmission⁵ by means of increasing the membrane potential of the ganglion cells⁶. The electron histochemical patterns suggest that the liberation of catecholamines from their bound form within the adrenergic nerve endings is effected by a cholinergic mechanism.

An interaction between acetylcholine and norepinephrine at the level of the peripheral autonomic innervation apparatus has been proposed repeatedly⁷. Histochemical studies on the structure, development and regeneration of the peripheral autonomic innervation apparatus failed to support this theory⁸. A transmitter interaction of this kind appears to be possible, however, at the level of the inhibitory ganglionic synapses that might, at least partly, account for the startling physiological findings of BURN and his collaborators.

Zusammenfassung. Elektronenmikroskopisch werden einfache und osmiophile («dense core») synaptische Bläschen in den axo-somatischen (hemmenden) adrenergischen Nervenendigungen im Ganglion cervicale superius der Katze gefunden. Die Anwesenheit einer elektronenhistochemischen Acetylcholinesterase-Reaktion um die synaptischen Bläschen herum scheint für die funktionelle Bedeutung des Acetylcholins bei der Freisetzung von Catecholaminen aus solchen Nervenendigungen zu sprechen.

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Electron histochemical localization of acetylcholinesterase in an axo-somatic synapse in the ganglion cervicale superius of a cat. A large number of synaptic vesicles is present in the presynaptic axon; most of the vesicles are of the simple ('regular') type, whereas others belong to the dense-core type (arrows). The intervesicular space within the terminal axoplasm is, here and there, filled by the electron dense end product of the acetylcholinesterase reaction (double arrows). PsD, pseudodendritic protrusion of the postsynaptic ganglion cell. Coll, collagenous fibres. $\times 36,800$.

¹ B. HAMBERGER, K.-A. NORBERG and F. SJÖQUIST, *Int. J. Neuropharmac.* 2, 279 (1963).

² B. CSILLIK, G. KÁLMÁN and E. KNYIHÁR, *Experientia* 23, 477 (1967).

³ Fixation with an isotonic and isohydric 10% formaline solution through the carotid artery. Thick frozen sections are incubated at pH 6.2 for 10–60 min in the following solution: acetylthiocholine, 7 mg; acetate buffer, 0.1 M, 2.5 ml; copper glycinate, 0.1 M, 0.1 ml; uranyl acetate (concentrated solution), 0.1 ml. After incubation, sections are treated with an isotonic and isohydric yellow ammonium sulphide solution, rinsed, dehydrated, embedded in Durcupan, sectioned on an LKB Ultratome and photographed under a Tesla 413 B electron microscope.

⁴ W. FELDBERG and J. H. GADDUM, *J. Physiol., Lond.* 81, 305 (1934).

⁵ A. S. MARRAZZI, *Am. J. Physiol.* 127, 738 (1939). – R. M. ECCLES and B. LIBET, *J. Physiol., Lond.* 157, 484 (1961).

⁶ A. LUNDBERG, *Acta physiol. scand.* 26, 252 (1952).

⁷ J. H. BURN and M. J. RAND, *Nature* 184, 163 (1959). – J. H. BURN, *Pharmac. Rev.* 25, 377 (1966).

⁸ B. CSILLIK, *J. Neurochem.* 11, 351 (1964). – B. CSILLIK and G. B. KOELLE, *Acta histochem.* 22, 350 (1965); *Acta neuroveg.* 29, 177 (1966).