# EFFECT OF GANGLION NODOSUM EXTRACT ON GROWTH OF BASAL CELLS OF THE LINGUAL EPITHELIUM *IN VITRO*

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Nerve fibers stimulate proliferation of cutaneous epithelial cells and control regeneration of the limbs in amphibians [1, 8]. The trophic influence of nerves on epithelium is manifested most clearly on the taste buds of the tongue, which degenerate after denervation and blockade of axon transport [2]. Division of the lingual nerve causes complete cessation of DNA synthesis in cells of frog taste buds [7]. However, in Guth's opinion [5], proliferation of epithelium is independent of nervous influences, which merely stimulate differentiation of taste buds.

The aim of this investigation was to determine the competence of lingual epithelium in relation to the action of neurogenic chemical factors and the character of the response of target cells to long-term nonimpulse nervous influences.

## EXPERIMENTAL METHOD

The lingual epithelium of rats aged 1-3 months was dissected to isolate the basal cells by the method in [6]. The isolated basal cells were obtained from fragments of epithelium by pipeting. The cells were cultured on collagen gel [14] in DMEM medium (Gibso, USA) with the addition of insulin (5 μg/ml) in plastic multiwells (76015104, from Flow Laboratories, England) in an atmosphere of 5% $CO_2$ and 95% air at 37°C. In another series of experiments the basal cells were cultured in the same medium, but with 2.5% or 10% calf embryonic serum (Gibco), or with the addition of extract from rat ganglion nodosum (80 and 160 μg/ml as protein) at the time of transplantation. The concentration of extract corresponded to the concentration used in investigations to study neurotrophic control of other target cells [13]. The results of counting the cells in the cultures were subjected to statistical analysis by Student's test.

## EXPERIMENTAL RESULTS

In medium with 10% serum most of the cells 2 h after transplantation were adherent to the substrate, and later the cells acquired the ability to proliferate. The cells subsequently aggregated with the formation of colonies, by analogy with the behavior of typical keratinocytes *in vitro* [4].

On the first days after transplantation the number of cells in medium without serum was unchanged (Fig. 1). A similar picture was observed in cultures with 2.5% serum (Fig. 1). Addition of extract (80 μg/ml) from the ganglion nodosum to the culture medium caused an increase in the number of cells ($P < 0.05$) 24 h after transplantation, followed by a decrease (Fig. 2). Twice the concentration of extract (160 μg/ml) led to marked proliferation of the cells ($P < 0.05$), which was observed 1-2 days after transplantation. Evidently in this case the extract contained a growth factor which stimulates cell proliferation in a population which has not yet reached saturation density. The results suggest that sensory neurons of the ganglion nodosum are the source of a chemical factor with mitogenic properties toward the epithelial cells of the tongue. This observation is in agreement with other data obtained recently, indicating that neurons can synthesize and secrete substances with mitogenic activity. For instance, spinal neurons of chick embryos secrete a transferrin-like protein [10] which stimulates myogenesis. As regards transferrin itself, its role as a growth factor controlling proliferation

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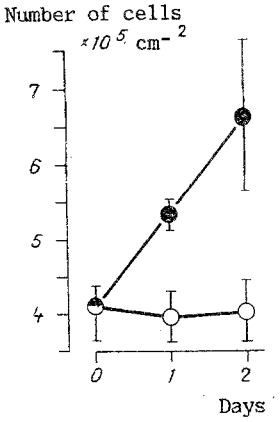


Fig. 1

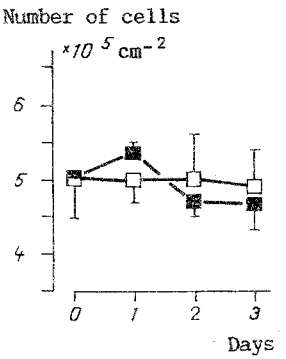


Fig. 2

Fig. 1. Growth of basal lingual epithelial cells in medium without serum and with extract (160 μg/ml) from ganglion nodosum and in medium without serum.

Fig. 2. Growth of basal lingual epithelial cells in medium without serum but containing extract (80 μg/ml) of ganglion nodosum and in medium with 2.5% serum.

of different cell types is considered to be proven [3, 9]. Another example of a neurogenic factor stimulating proliferation of epithial cells is vasopressin [11].

Growth of the basal cells of the lingual epithelium may also have another interpretation: sensory neurons are the source of a chemical factor which "permits" the action of a mitogen already present in the particular cell system. This possibility has been established in the case of human fibroblasts, the binding of transferrin with which increases considerably after interaction of the cells with epidermal growth factor [12].

The hypothesis of the mitogenic or permissive property of neurogenic factors can be applied to the analysis of mechanisms of neurotrophic control of the taste epithelium of the tongue, which is in direct contact with nerve fibers. The possibility of differentiation of taste buds in the competent epithelium of the vallate papillae of the tongue, demonstrated by Zalewski [15] in experiments on cocultivation with rat ganglion nodosum in the anterior chamber of the eye, justifies the further study of the effect of extract from this ganglion on cultures of basal cells of the lingual epithelium in order to confirm or refute the hypothesis of the role of neurogenic chemical factors in the realization of neurotrophic control.

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