Merkel cells of cat's vibrissae following denervation or application of colchicine to the nerve

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Summary. A decrease in the number of, and ultrastructural changes in, Merkel cells in cat vibrissae was observed after infraorbital nerve transection and colchicine application.

The nerve's trophic effect on peripheral tissues is usually explained by the influence of unidentified substances which are synthesized in the neuron's perikaria, transported along the axons, and released from terminals[1,2]. This hypothesis has been confirmed in experiments with axoplasmic transport blockade with colchicine or vinblastine[3]. The influence of the sensory neurons on the maintenance of specialized cells in the peripheral sensory receptors has been similarly explained and confirmed by many experiments with denervation of receptor organs and, above all, with taste buds[4]. The data on this problem[5,6] suggest something about the nerve fibers' inductive influence on the differentiation of receptor cells. The axoplasmic transport has been thought to be directly related to the neurotrophic effect on target cells of tissue mechanoreceptors[7,8].

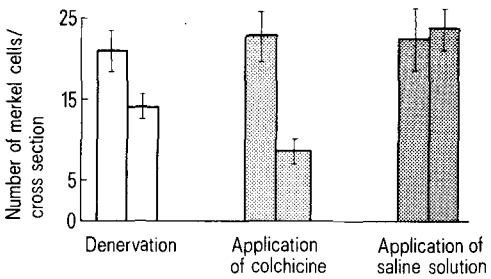

Figure 1. A number of Merkel cells per section of middle region of cat's vibrissae 30 days after denervation, application of colchicine and application of saline solution to the nerve. In each pair of bars: right, a number of Merkel cells on ipsilateral side; left, on the contralateral side. Statistically significant differences from controls are denoted by mean ± SEM.

The experiment presented here was undertaken to determine whether such is the case for the Merkel cell-neurite complex of cat vibrissae.

13 adult cats were used in the experiment. The animals were divided into 3 groups. The cat were anesthetized with ether and the right infraorbital nerves of the 1st group (5 cats) were transected at the level of the infraorbital foramen. The same nerves of the 2nd group (5 cats) were treated with colchicine solution (30.5 mM, Merck) for 10 min. Colchicine was applied topically to the infraorbital nerve by means of a cotton pledget soaked with the drug. In the control group (the 3rd groups, 3 cats) the right infraorbital nerves were treated with saline solution in the same manner in which colchicine was applied. On the 30th, 45th and 60th post operation days, the cats were re-anesthetized with ether. Skin from the upper lip bearing vibrissae was excised and fixed in glutaraldehyde fixative. For all experiments (nerve transection, treatment of the nerves with colchicine or saline solution) vibrissae from the contralateral (left) side were used as controls. The material was subsequently postfixed with OsO_4. Tissue blocks were dehydrated and embedded in Epon 812. Semithin sections were cut at 1 μm and stained with alkaline fuxin and methylene blue. In addition, material was thin-sectioned, stained with lead and uranyl, and examined with an electron microscope. The number of Merkel cells and keratinocytes was counted within the basal-cell layer in cross-sections of vibrissae. Only sections in the middle region of the ring sinus were used. The data were grouped for statistical analysis according to the experimental procedure. Significance of differences was tested by Student's t-test.

A decrease in the number of Merkel cells was observed after the nerve transection (fig. 1). This effect was most evident on the 45th day after denervation. The short-term

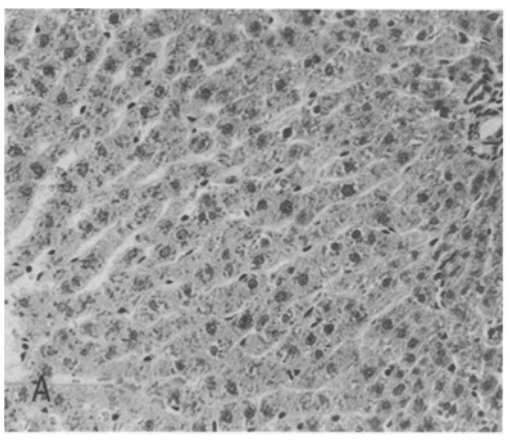

Figure 2. A Merkel cell-neurite complex from intact vibrissa. A, Nerve terminal; M, Merkel cell; g, specific granules; GM, glassy membrane; Merkel cell forms desmosome with keratinocyte (× 10,000).

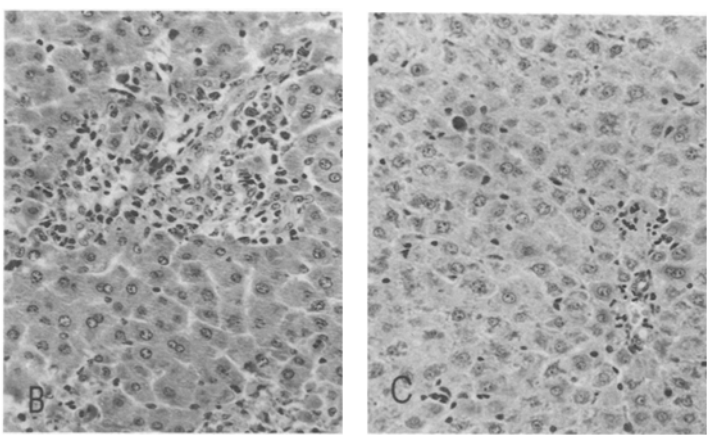

Figure 3. A Merkel cell-neurite complex from cat's vibrissa 30 day after application of colchicine to infraorbital nerve. A, Nerve terminal; V, vacuole (× 10,000).

application of colchicine to the nerve leads to a decrease in the number of Merkel cells too (fig. 1). Thus 30 days after colchicine application to the nerve, the number of Merkel cells was 55% of control ($p < 0.05$). The structure of vibrissae on the side where saline solution was applied to the nerve was compared with the structure of vibrissae on the contralateral side. There were no statistically significant differences in the number of Merkel cells in these 2 groups of vibrissae. The ultrastructural examination of the remaining Merkel cells revealed the following alterations 30, 45 and 60 days after the nerve transection: a greater density of the cytoplasm and nucleus, an increment in number and size of the vacuols and greater heterogenity in the density of specific granules. These ultrastructural changes in Merkel cells were accompanied by Wallerian degeneration of nerve terminals in the Merkel cell-neurite complexes. At 45 and 60 days after nerve transection numerous nerve fibers appear again in the connective tissue of vibrissae beneath glassy membrane. 30, 45 and 60 days following colchicine nerve treatment, ultrastructural examination of ipsilateral vibrissae revealed signs of morphological modifications in the remaining Merkel cells (fig. 3). Their ultrastructure was similar to that observed after the nerve transection. However, the contacts between Merkel cells and nerve terminals were always observed after colchicine nerve treatment. Some Merkel cell-neurite complexes showed normal morphology. On the other hand no structural alterations were detected in the contralateral vibrissae in the cases either of nerve transection and colchicine nerve treatment, nor were alterations detected in the ipsilateral vibrissae of cats whose nerves were treated with saline solution alone. No changes in the number and structure of keratinocytes could be observed in vibrissae as a result of the experiments.

It has been reported that denervation of tactile corpuscles in cats was followed by the loss of Merkel cells[9,10]. Authors believe that the effect does not appear to be the result of the generalized skin alterations. It seems to be most likely that sensory innervation of the epithelium is requisite for maintaining Merkel cell integrity. This supposition was confirmed by the observations on tactile corpuscles of cat skin which indicated a close connection between Merkel cells' regeneration and the reappearance of sensory nerve fibers in the epithelium[9,11]. Consequently, the decrease in number of Merkel cells after nerve transection is caused by the interruption of the trophic influence of nerves on the epithelium. The nature of nerve influence on the target (inducing and/or supporting differentiation) in the case of Merkel cells is still unknown. It is highly probable that blockade of axonal transport with colchicine prevents the secretion of chemical factors which can be released from the nerve terminals and provide control of Merkel cells integrity. The nature of these factors remains to be demonstrated.

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The protective effect of polyriboinosinic acid-polyribocytidylic acid against the occurrence of galactosamine-induced liver cell injury in rat[1]

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Summary. A marked increase of serum transaminase activities, histological changes of livers similar to those seen in viral hepatitis in man, and inhibition of hepatic protein synthesis were observed in rats following a single injection of D-galactosamine-HCl. These galactosamine-induced phenomena were prevented by the pretreatment of polyriboinosinic acid-polyribocytidylic acid 24 h before the galactosamine administration.

The administration of D-galactosamine (GalN) to rats has been known to induce alterations in livers which closely resemble human viral hepatitis[2-4]. It is interesting to note that GalN-induced liver cell injury is different in its histological features from other experimental hepatitis caused by various hepatotoxic chemicals. Such observations suggest that a similar mechanism may be involved in producing liver cell lesions in GalN-induced hepatitis and human viral hepatitis. Interferon or interferon-inducing agents have been used for therapy or protection from viral infection. Since it has been recently reported that interferon has a therapeutic effect against human viral hepatitis[5,6], we are interested in the effect of these agents on GalN hepatitis.

In the present report we will describe the protective effect of polyriboinosinic acid-polyribocytidylic acid (poly IC), which has been known to induce interferon in vivo and in vitro[7], against hepatocyte damage caused by GalN.

Materials and methods. Wistar strain male rats weighing 180–200 g were used and fed laboratory chow and water ad libitum. GalN-HCl and poly IC were purchased from Sigma Chemical. [U-^{14}C]-amino acid mixture (10 mCi/mmole) was obtained form Radiochemical Centre, Amersham.

All reagents used for injections were dissolved in physiological saline solution, neutralized immediately before use and given by a single i.p. injection. Control animals received an