tration, 2–20 μg/ml), 6-methyl-4-nitroquinoline-1-oxide, 6-methyl-4-hydroxyaminoquinoline-1-oxide, 7-chloro-4-hydroxyaminoquinoline-1-oxide·HCl, 4-hydroxyaminoquinoline-1-oxide·HCl, and 2-methyl-4-nitroquinoline-1-oxide; low activity (inducing concentration 20–100 μg/ml), 7-chloro-4-nitroquinoline-1-oxide; inactive (inducing concentration, > 100 μg/ml), 3-methyl-4-nitroquinoline-1-oxide, 4-nitroquinoline, 4-hydroxyaminopyridine-1-oxide·HCl, 4-nitropyridine-1-oxide, 3-nitroquinoline-1-oxide, 6-nitro-4-hydroxyaminoquinoline-1-oxide·HCl, and 5-nitroquinoline-1-oxide.

These results indicate a clear positive association between phage induction and carcinogenicity. All 6 non-carcinogens were also non-lysogenic and of the 10 carcinogens, all were lysogenic with the exception of 6-nitro-4-hydroxyaminoquinoline-1-oxide. It is of additional interest that of the 15 compounds for which mutagenicity data is available[15], this property is closely associated with lysogeny with 2 exceptions (Nos. 1 and 14)[16].

Zusammenfassung. In einer Serie von 16 Nitroquinolinen und verwandten Substanzen wurde eine gute Übereinstimmung zwischen Phageninduktion in *Escherichia coli* C-600 und Karzinogenese nachgewiesen.

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[15] S. S. EPSTEIN and J. A. ST. PIERRE, unpublished data.

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Excessive Proliferation of Epithelium of the Rabbit Cornea[1]

Epithelial growth into the damaged cornea is not a common phenomenon, although it is recognized that proliferation and movement of epithelial cells is responsible for closure of any gap in the corneal surface[2]. The usual limit of ingrowth appears to be several layers of cells, as in the case of mucopurulent conjunctivitis in man reported by SIHOTA[3], or in the epithelial thickening after injury of the rabbit cornea reported by BERENS et al.[4]. In the course of studies of the production of collagenous tissue in the rabbit cornea by injection of the polysaccharide carrageenan[5], uncontrolled epithelialization was observed in 13 out of 90 eyes, in both experimental and control rabbits. In several of these the excessive proliferation of epithelial cells obliterated the corneal stroma. We have failed to find corneal epithelialization of this magnitude described in the literature.

Methods. Details of the materials used and of the animal experiments have already been described in a previous publication[6]. Injections of 0.05 ml 1% λ carrageenan (Marine Colloids, Inc.) in 0.9% NaCl, or of 0.9% NaCl alone, were made into the centre of the cornea, using a 0.25-ml syringe and 30 gauge needle. No further treatment was applied to the cornea until the animals were killed for histological analysis at intervals up to 2 months postinjection.

Results and discussion. Microscopic examination of sections from the area into which material had been injected revealed a marked proliferation of epithelial cells in 5 out of 20 control (saline injected) corneas and in 8 out of 58 carrageenan injected corneas. The cells which participated in the epithelialization process stained like normal epithelial cells, viz. blue with hematoxylin and eosin, dark purple with Masson's trichrome, yellow with the von Gieson method, green with dialyzed iron, brick red in the periodic acid Schiff reaction and orthochromatically with toluidine blue. The corneas which exhibited this phenomenon were taken from corneas sacrificed 3–49 days after the original injection. Although 15 of 58 carrageenan injected corneas became ulcerated, the epithelialization process was not associated with this complication, as epithelialization occurred in none of these.

The epithelialization penetrated the stroma at various places and was not uniform at its various levels of penetration. The increasing mass of epithelial cells encroached upon the stroma to varying extents, as shown in Figures 1 and 2, sections from the same cornea. The proliferation was usually 30–40 cells thick, but in 2 cases it was approximately 75 cells thick and completely obliterated the stroma. One of these cases is illustrated in Figure 3. In a few cases, corneal endothelial cells also divided and invaded the stroma.

Only a few mitotic figures were observed in normal basal cells of the epithelium, and none at all were seen in the proliferating epithelial cells. To ascertain whether they had been missed because of a diurnal rhythm in mitotic activity with a maximum at night, reported in other species[6,7], 6 additional rabbits were injected and killed 11 and 14 days later at 23.00, 03.00 and 07.00 with entirely negative results.

The significance of the usual restriction of the number of layers of epithelial cells in the normal cornea is really not understood. The observations of SIHOTA[3] and BERENS et al.[4] have already been mentioned. Even in rabbit corneas cultured in vitro, this restriction appears to be operative[8]. On the other hand, KNOWLES[9] found hyperplasia and downgrowth of epithelial cells 18–30 cell layers thick in rabbit corneas which had ulcerated after insertion

[1] This research was supported in part by grants from the National Research Council (Canada) and the National Cancer Institute of Canada.

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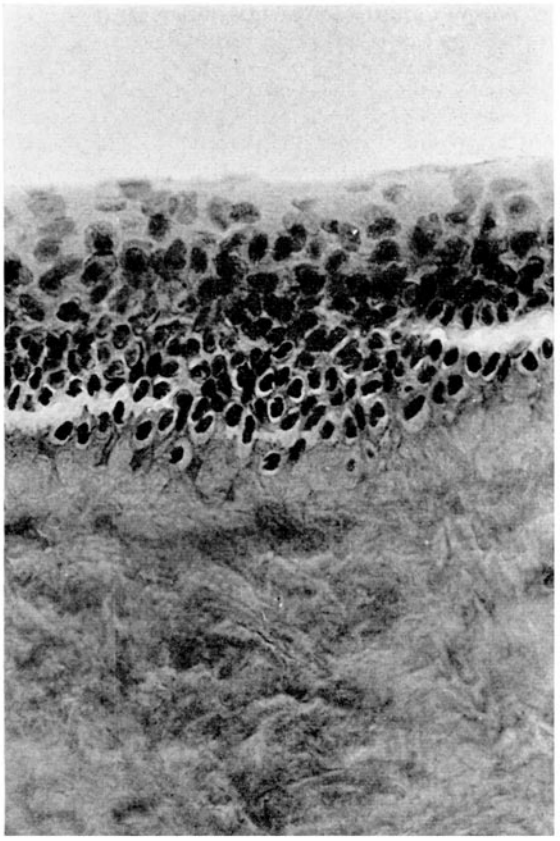

Fig. 1. Proliferation of epithelial cells; saline injected cornea, 15 days after injection. Von Gieson stain.

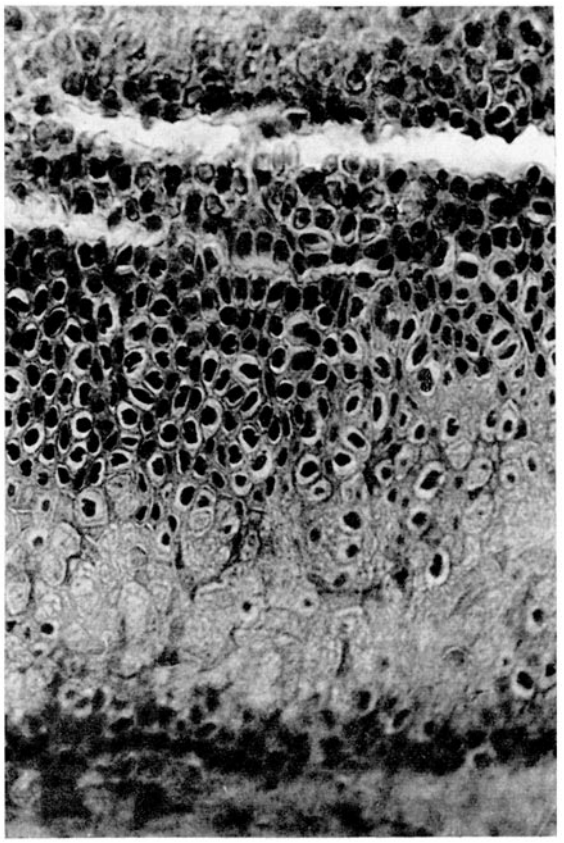

Fig. 2. Proliferation of epithelial cells; saline injected cornea, 15 days after injection. Von Gieson stain.

of plastic membranes, in which case both the epithelial basement membrane and Bowman's membrane had been injured. In the present study, where no restriction to downgrowth was provided, and where presumably the membranes had also been penetrated, the proliferation was more extensive, and occasionally continued to the endothelial surface. It is not clear how these last 2 situations differ from the former more usual circumstance, unless it is in damage to the membrane barriers.

A second problem is the mechanism by which the increase in cell number occurred. Although the corneal epithelium itself has great powers of regeneration, and although mitosis occurs in normal basal epithelial cells[2], very few mitotic figures were seen in the present study. In experiments of RIGG and RIGG[2], closure of a corneal wound by migrating epithelial cells occurred in as short a time as 6 h after injury. Immediately after wounding, mitosis appeared to be depressed, hence this migration occurred without concomitant cell division. Subsequently, however, mitotic activity was increased. This secondary increase was never seen in the present experiments; the rare mitotic figure which was observed could hardly account for the extensive wound healing process.

BINDER and BINDER[10] suggested that a mitotic division of endothelial cells is the normal mechanism. They also

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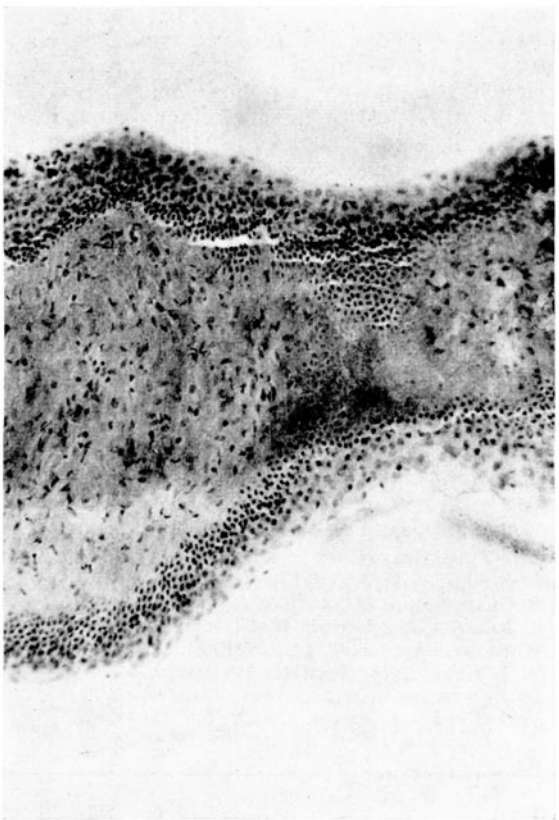

Fig. 3. Proliferation of epithelial cells, epithelial layer about 75 cells deep, obliterating the corneal stroma; carrageenan injected cornea, 15 days after injection. Von Gieson stain.

observed mitotic divisions which they postulated were due to injury. However, in the occasional instance of endothelial cell proliferation observed here, mitosis was not prominent, either.

Although the mechanisms involved in cellular proliferation in these experiments could not be ascertained, it is clear from the incidence of epithelialization in control as well as carrageenan injected corneas that the phenomenon was related to the production of a wound in the epithelial surface, and perhaps in the basement membrane and/or Bowman's membrane, rather than to the development of a collagenous carrageenan granuloma in the underlying stroma. Presumably this epithelialization represents an exaggeration of part of the healing process of the wound caused by the act of injection into the cornea.

Zusammenfassung. Die Schädigung der Basalmembran durch eine Stichverletzung hat eine Proliferation und Einwanderung von Corneaepithelien zur Folge. Eigenartigerweise sind bei diesem Prozess keine Mitosen nachweisbar, was darauf hinzuweisen scheint, dass Amitosen experimentell verfolgt werden können.

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McMaster University, Hamilton (Ontario, Canada), 1 July 1968.

Axo-Axonic Synapses in the Main Sensory Trigeminal Nucleus

Axo-axonic synapses have been found between the outer surfaces of axonal boutons in several locations in the central nervous system, including the spinal cord[1,2], the cuneate nucleus[3], spinal trigeminal nucleus[4] and the lateral geniculate body[5,6]. The purpose of this communication is to report the presence of axo-axonic synapses on unusual interdigitations of some axonal boutons in the main sensory trigeminal nucleus at the level of the motor trigeminal nucleus.

Adult cats and rats were perfused through the heart with potassium dichromate-buffered osmium tetroxide. In some instances, the brainstem blocks were placed in 2% uranyl acetate before dehydration (Figure 1b). Maraglas[7] was used as the embedding medium. Thin sections were stained with lead citrate for electron microscopy.

Throughout the main sensory trigeminal nucleus, axonal boutons of different size have been found which relate to each other by small evaginations. In Figure 1a, a large bouton receives an evagination from a smaller bouton. The synaptic vesicles in the bouton receiving the evagination appear larger than those in the evaginating one. The reverse relationship is shown in Figure 1b; a bouton with small synaptic vesicles receives an evagination containing large synaptic vesicles.

One synapse is usually seen on small evaginations (Figure 1b); however, 2 may be seen on some larger ones (Figure 2a). Such axo-axonic synapses are rather short and characteristically occupy a small part of the apposed axonal plasma membranes. For this reason synapses are not seen in all sections through the axonal evaginations. It is our interpretation however, that at least 1 axo-axonic synapse is present on some part of the circumference of these evaginations. The axo-axonic synapses are characterized by a widening of the intercellular space and the presence of electron dense material within the synaptic cleft (Figures 1b and 2b). The boutons containing small synaptic vesicles usually show small clusters of synaptic vesicles near the axo-axonic synapse (Figures 1b and 2b).

In some instances 3 axonal processes have been seen related to each other by evaginations. In Figure 3 a large bouton surrounds a smaller axonal process which in turn surrounds a third axonal process (AX 3). Axon 3 may

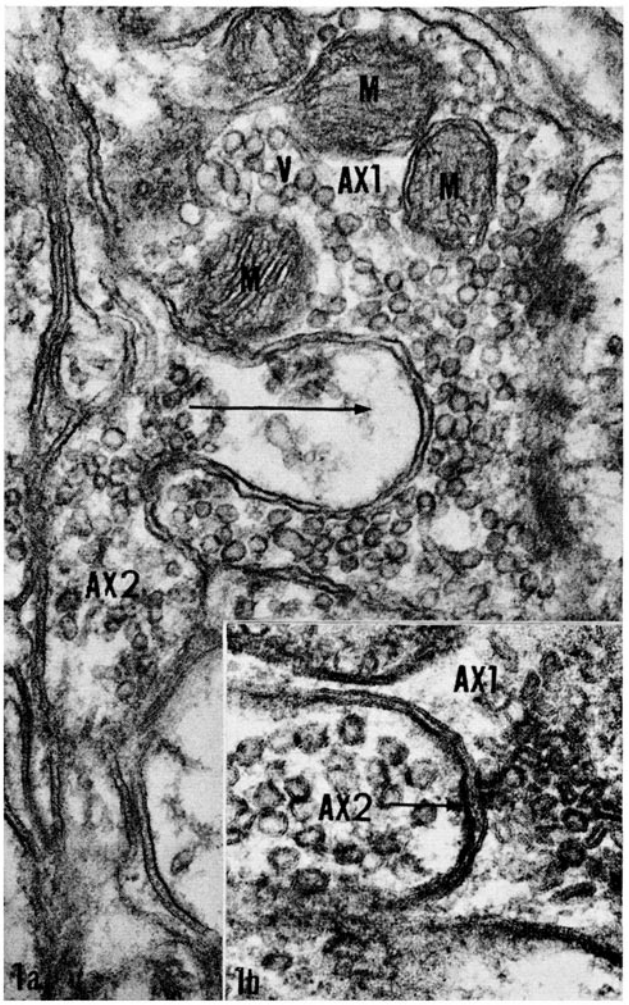

Fig. 1 (a) An axonal bouton en passant (AX 2) sends a projection (arrow) into a larger axonal bouton (AX 1). The synaptic vesicles (V) in AX 1 appear larger than those in AX 2. Mitochondria (M). × 60,000. (b) An axon containing small vesicles (AX 1) receives an axonal evagination containing large vesicles (AX 2). An axo-axonic synapse is seen at the arrow. At the synapse, note the widened intercellular space containing dense material. Synaptic vesicles in AX 1 are accumulated in the vicinity of the synapse. × 70,000.

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