

♀	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
S	18	21	21	10	13	14	16	11	16	24	21	21	18	21	25	11	19	16	24	15
mk	13	15	14	7	10	6	10	8	10	14	14	14	13	14	17	8	11	12	21	8
ok	5	6	7	3	3	8	6	3	6	10	7	7	5	7	8	3	8	4	3	7
mk/S	0.72	0.71	0.67	0.70	0.77	0.43	0.63	0.73	0.63	0.58	0.67	0.67	0.72	0.67	0.68	0.73	0.58	0.75	0.88	0.53
Zyklus	Met	Met	Met	Di	Oestr	Met	Met	Met	Di	Di	Met	Met	Di	Met	Di	Di	Oestr	Di	Pro	Oestr

♂	1	2	3	4	5	6	7	8	9	10	11	12
S	17	20	33	22	35	31	24	25	25	31	21	21
mk	10	12	22	14	25	22	14	15	17	19	16	20
ok	7	8	11	8	10	9	10	10	8	12	5	1
mk/S	0.59	0.60	0.67	0.64	0.71	0.71	0.58	0.60	0.68	0.61	0.76	0.95

S, Anzahl der Corpuscula mit getroffenem Harnpol im Schnitt; mk, Corpuscula mit kubischem Epithel im parietalen Blatt; ok, Corpuscula ohne kubischem Epithel im parietalen Blatt; Zyklus, Stadium nach der Vaginalcytologie (nach Allan-Doisy).

Blattes aus diesem Material. Diese Zellen zeigten alle die lichtmikroskopischen Charakteristika des proximalen Hauptstückepithels. Fast nie wurde der bei den Embryonen scharf abgesetzte Halsteil gesehen. Die Mündung in das proximale Hauptstück ist vielmehr scheidetrichterförmig ausgezogen (Figur 2). Es wurden auch die Nieren der weiblichen Tiere im Metöstrus und Diöstrus miteinander verglichen. Dabei zeigte sich, dass eine Kapselbeteiligung von kubischem Epithel in 66% im Metöstrus und in 68% im Diöstrus bestand (Tabelle).

Diskussion. Aufgrund der Befunde an Embryonen ist das Auftreten von kubischem Epithel im parietalen Blatt der Bowman'schen Kapsel schon primär als morphologisches Charakteristikum der Mäuseniere aufzufassen. Wir können uns damit nicht der Meinung von Crabtree³ anschließen. Bemerkenswert erscheint uns das verschiedenartige Verhalten des Harnpols bei den Embryonen und den erwachsenen Tieren. Das scheidetrichterartige Aussehen bei den adulten Mäusen führt unserer Meinung nach dazu, dass mehr Hauptstückepithelien in das parietale Blatt einbezogen

werden. Da bei beiden Geschlechtern gleichviele Corpuscula mit Beteiligung von Hauptstückepithelien gefunden werden, ist der Einfluss von Sexualhormonen^{3,4} auf diesen Differenzierungsprozess auszuschließen. Auch das gleichartige Verhalten der Corpuscula der Nieren von Weibchen im Metöstrus und solchen im Diöstrus gibt keinen Anlass, hier zyklusabhängige Unterschiede zu vermuten⁵.

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Endothelial nerve fibres in the cornea of the frog *Rana ridibunda*¹

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Summary. The ultrastructural study of the frog cornea revealed the existence of intraendothelial nerve fibres. These nerve fibres are unmyelinated elements but surrounded by cytoplasmic lamellae of Schwann cells.

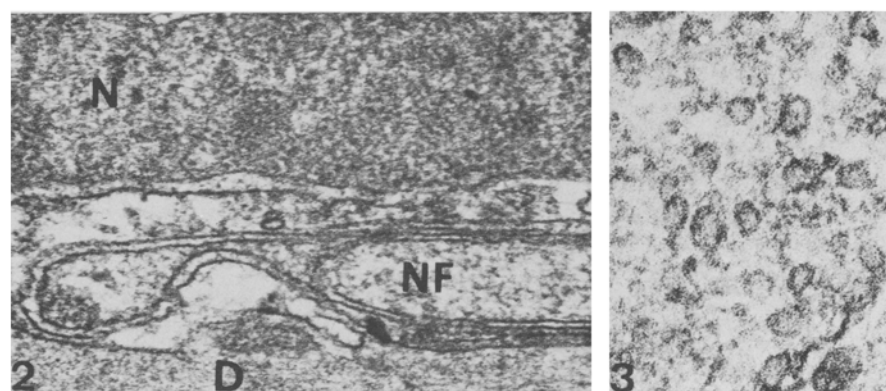
The innervation of the cornea in fishes, amphibians, reptiles, birds and mammals has been studied by many authors²⁻⁶. Most of the corneal nerves are of sensory nature, although sympathetic fibres have also been recognized. The nerves permeate the cornea as trunks of 60-80 myelinated fibres at its junction with the sclera. At variable distances from their entrance, they usually lose their myelin sheath and divide into an anterior and a posterior group. The anterior fibres pass through the corneal tissue and form a plexus under Bowman's membrane; Some branches permeate Bowman's membrane to form the subepithelial plexus, giving rise to the intraepithelial fibres. The question as to whether there are nerves in the endothelial layer of the cornea or not has been discussed in several papers^{3,8}. Most authors have found that the vertebrate corneal endothelium does not contain any nerve fibres. The sole positive assertion is that of Wolter⁹, who presented a description and photomicrographic evidence of nerves in

the corneal endothelium of the rabbit. No histological or ultrastructural reports, indicating the existence of nerves and nerve-endings in the corneal endothelium can be found in the recent literature¹⁰⁻¹³. The present paper is concerned with the occurrence of nerve fibres in the corneal endothelium of the frog *Rana ridibunda*. This was observed in the course of experiments described elsewhere on cryopreservation of the cornea¹⁴.
Materials and methods. 10 regular specimens of the frog *R. ridibunda* were used. The animals were immobilized by pithing the spinal cord as well as the brain. The corneas were surgically removed and cryopreserved in liquid nitrogen at -196 °C according to the method of Kaufman et al.¹⁵ for 30 days. After temperature recovery and rehydration, these were fixed for 3 h in glutaraldehyde-formaldehyde buffered with 0.2 M collidine at 4 °C, fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were double-stained with

Fig. 1. Endothelial cell of a cryo-preserved frog cornea. NF, nerve fibre surrounded by mesaxon-like structures. N, nucleus. $\times 20,000$.



Fig. 2. Groups of nerve fibres (NF) in the endothelial cells of the frog cornea. N, nucleus; M, mitochondria; D, Descemet. $\times 25,000$. Fig. 3. Nerve terminal in the endothelium of a frog cornea. $\times 110,000$.



uranyl acetate-methanol and lead citrate, and observed in a Philips 301 electron-microscope.

Results and discussion. The ultrastructural analysis of the frog cornea revealed the occurrence of non-myelinated nerve fibres within the endothelial cell layer.

These nerve fibres are covered by one or more cytoplasmic lamellae resembling Schwann cell mesaxons. The fibres appear either isolated (figure 1) or in small groups of 2 or 3 elements (figure 2). Most are located beneath the basal plasmalemma in the infranuclear region of the endothelial cells. It was possible to find occasional nerve terminals, but neither synaptic contacts nor typical synaptic specializations were observed (figure 3). The corneal epithelium in the same specimen showed nerve fibres similar to those described by other authors.

Both light and electron microscopic studies on corneal innervation showed the occurrence of nerve fibres in the anterior stroma, Bowman's membrane and epithelium. Schwann cells accompany the fibres all their way to Bowman's membrane, but do not permeate the epithelium.

The description of nerve fibres in the rabbit corneal endothelium by Wolter⁹ is the sole evidence for the existence of innervation in the endothelial layer. Wolter carried out his observations using the Del Rio-Hortega staining of nerve fibres for light microscopy. The nature of these fibres was not established, but it was suggested that they may belong to the autonomic system; they were supposed to come directly from the endothelial periphery and not from the nerve plexus of the stroma¹⁶. However, it has been suggested⁸ that these fibres may run the outer surface of Descemet's membrane and may not be truly intraendothelial fibres.

Present results provide full evidence of the existence of intraendothelial nerve fibres in the frog cornea. Attention was drawn to the existence of mesaxon-like structures surrounding the endothelial fibres. These are typical spe-

cializations of Schwann cells, but it may be that endothelial corneal cells themselves may develop a similar system of lamellae. This is the case for the Deiter's cells lamellae surrounding the acoustic fibres in the organ of Corti of mammals¹⁷.

In the frog cornea, the active chloride transport occurs in the epithelium which accounts for 90% of the total potential difference through the isolated cornea. This characteristic is typical of the amphibian cornea, to which we have to add the existence of nerve fibres in the endothelial layer. Keeping in mind the relatively small functional significance assigned to the amphibian corneal endothelium as compared with that of mammals, the physiological meaning of the endothelial nerve fibres remains uncertain.

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