the OH at the ethyl group, due to hydrogen bonding with the oxygen of the *peri*-OH group. Addition of a drop of D_2O caused the disappearance of the signals at 3.72 and 12.88 and the signal at 5.30 showed a normal quartet. The difference between the 2 OH groups was clearly demonstrated by measuring the NMR-spectrum of the original $CDCl_3$ solution after standing for 45 days. The signal at 3.72 was not observed, whereas the signal at 12.88 was still present (no exchange, due to strong hydrogen bonding of the *peri*-OH with the quinone carbonyl group). Thus the structure of C had been established as 2,7-dimethoxy-5-hydroxy-6-(1-hydroxyethyl)-1,4-naphthoquinone. Using the same gas chromatographic conditions as described earlier[3] compound C could be easily detected (retention time A = 4.40; B = 3.80; C = 4.75 min). In this way the presence of C was also demonstrated in the strains CBS 136.77 and M 48[3].

Efforts to synthesize C by hydrolysis of A in alkaline methanol failed. Only very small amounts of C were formed. The main product was a naphthoquinone derivative with a molecular weight of 292. IR, UV-vis. and PMR[4] studies indicated that the compound was 2,7-dimethoxy-5-hydroxy-6-(1-methoxyethyl)-1,4-naphthoquinone.

Since C showed no optical activity, the question was considered, whether the compound had been obtained as an artefact of the purification process. In our opinion this was not the case. Extraction procedures were carried out rapidly throughout this study. The presence of C in extracts of CBS 137.77 could already be demonstrated by TLC after an extraction period of only 2 min, whereas C was not found in extracts of cultures of CBS 131.78 and CBS 145.78, both good producers of A and B[3], even after a storage period of 1 week in the refrigerator. Therefore, we concluded that C is a natural compound.

Otomo et al.[5] recently reported the isolation of B and C (also optically inactive) from *Guignardia laricina* (Sawada) Yamamoto & Ito, a fungus belonging to the Botryosphaeriaceae[6] and considered to be the cause of shoot blight of larches. Its anamorph is classified as *Macrophoma* spec. by the authors[7] (*Phyllosticta* ss van der Aa[8]). The Botryosphaeriaceae include never conidial fungi belonging to *Hendersonula* and related genera, and *Scytalidium*. Campbell and Mulder[9] have described several strains belonging to the *Hendersonula toruloidea* complex as *Scytalidium hyalinum*. Although *H. toruloidea* is not strictly congeneric with the type species of the genus, *H. australis* (Sutton[10]), it can be concluded that neither *H. toruloidea* nor *Scytalidium* is related to *G. laricina* although they produce the same naphthoquinone pigments.

Physico-chemical data. Compound C: m.p. 202–204 °C; optically inactive; mol.wt 278.0795 (calculated for $C_{14}H_{14}O_6$ 278.0790). MS: 278 (M^+), 263 (100%), M* 209.98: transition $263^+ \rightarrow 235^+ + 28$ (CO); λ_{max} (MeOH): 222.5 (log ε 4.38), 232sh (4.19), 258 (4.18), 262 (4.18), 310 (3.98), 426 nm (3.62); ν_{max} (KBr): 1688, 1640, 1605 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.55 ($-CH_3$, d, J = 6.75 Hz), 3.72 (−OH, d, J = 11.95 Hz), 3.92 ($-OCH_3$, s), 4.00 ($-OCH_3$, s), 5.30 (>CH−, dq, J = 11.95, 6.75 Hz), 6.02 (>C=CH−, s), 7.27 (aromatic H, s), 12.88 δ_{TMS} (−OH, s).

1 The authors are indebted to Mr C. Versluys, Analytical Laboratory, State University of Utrecht, for measuring the mass-spectra.
2 Thanks are due to Mr A. George, Laboratory for Organic Chemistry, State University of Utrecht, who measured and interpreted the PMR-spectrum.
3 van Eijk, G.W., and Roeymans, H.J., Experientia *34* (1978) 1257.
4 We thank Dr H. Sato, Hokkaido University, Sapporo for PMR-data of this major product.
5 Otomo, N., Sato, H., and Sakamura, S., Agric. Biol. Chem. *43* (1982) 861.
6 von Arx, J.A., and Müller, E., Stud. Mycol. *9* (1975) 1.
7 Yamamoto, W., Sci. Rep. Hyogo Univ. Agric. Ser. Agric. Biol. *5* (1961) 1.
8 van der Aa, H.A., Stud. Mycol. *5* (1973) 1.
9 Campbell, C.K., and Mulder, J.L., Sabouraudia *15* (1971) 161.
10 Sutton, B.C., The Coelomycetes, p. 347. Commonwealth Mycological Institute, Kew 1980.

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Disruption of the goblet cell intercellular junction following histamine infusion of the rabbit ileum[1,2]

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Summary. Histamine infused into the mesenteric artery of the rabbit ileum ruptured tight junctional complexes linking goblet cells with neighboring absorptive epithelial or other goblet cells. Loss of tight junctional integrity induced by histamine could contribute to extra-intestinal endotoxemia reported after a variety of injuries.

Histamine and other vasoactive substances, increased in mammalian tissues following trauma, could enhance intestinal permeability[6]. For example, infusion of vasoactive substances into rabbits permitted endotoxin from gram negative enteric microorganisms to move transmurally into the peritoneal cavity[7]. A mechanism for increased permeability of the intestine to endotoxin has been suggested. Following challenge with endotoxin[8], radiation trauma[9,10], or full dermal thickness wounds (Porvaznik, unpublished), the tight junctional barriers (zonulae occludens) between goblet-epithelial cells in the ileum became disrupted. In irradiated animals this disruption was associated with the presence of endotoxin in extra-intestinal tissues[11].

If disruption of junctional barrier provides a pathway for the escape of endogenous endotoxin present in the intestine, a substance such as histamine, which has been associated with escape of endotoxin from the intestine, may also mediate the disruption of these junctional barriers. We tested the hypothesis that histamine could be responsible for disruption of tight junctions by infusing normal rabbits with histamine and subsequently determining the integrity of their junctional barriers.

New Zealand White male rabbits weighing 1.5–2.0 kg were anesthetized by delivery of methoxyflurane (Metofane, Pittman-Moore, Inc., Treton, NJ 08619) and oxygen via a Frazier-Sweatman gas anesthesia apparatus. The descend-

ing aorta was isolated via a laparotomy incision, and a polyethylene catheter (ID 0.58 mm × OD 0.96 mm) was introduced approximately 1 cm posterior to the left renal artery. The catheter was premeasured and marked to facilitate placement of its free end approximately 0.5 cm anterior to the superior mesenteric artery.

Histamine free base (Sigma Chemical Co., St. Louis, MO 63178) was administered at 1 mg/kg b.wt, dissolved in 30 ml of 0.9% sterile saline, and delivered intra-arterially at a rate of 10 ml/h. Individual animals were exposed for either 1, 2, or 3 h of histamine infusion. Control animals were exposed to the same procedures but with the infusion of 0.9% sterile saline.

After infusion of histamine, segments of the ileum were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2). Specimens were processed further for freeze fracture studies as described elsewhere[8].

The tight junctional complexes in the ileal epithelium from rabbits infused with sterile saline (controls) appeared normal whether observed on membrane fracture faces of goblet cells (fig. 1) or absorptive epithelial cells (fig. 2). The tight junctional fibrils formed a loosely inter-connected

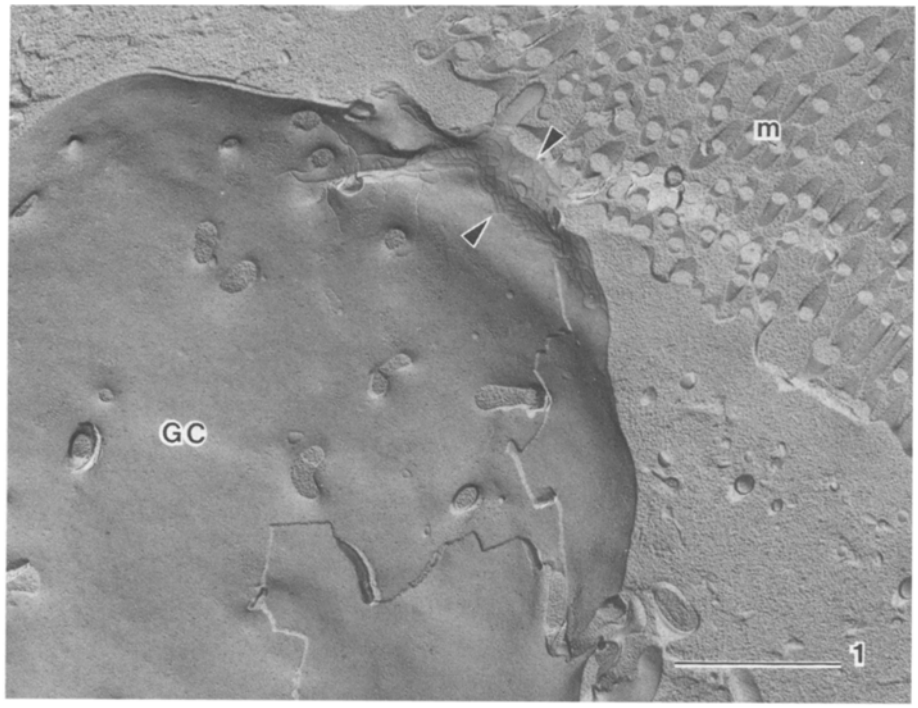


Figure 1. Zonula occludens (between arrow heads) from 2-h control preparation. This is a goblet cell membrane fracture face (GC). Cross-fractured apical microvilli (m). Scale bar = 1.0 μm.

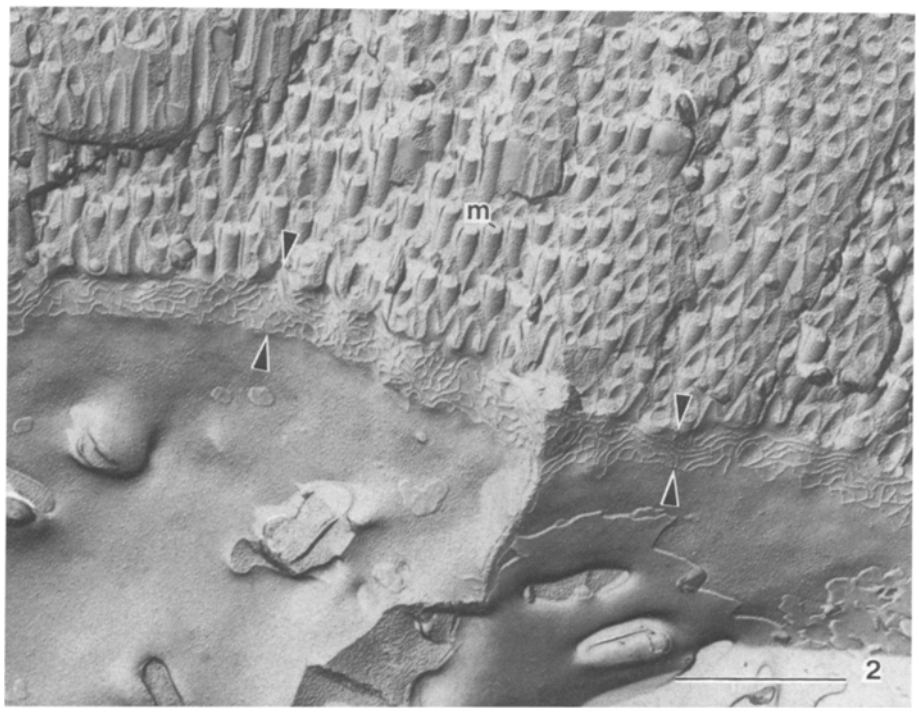
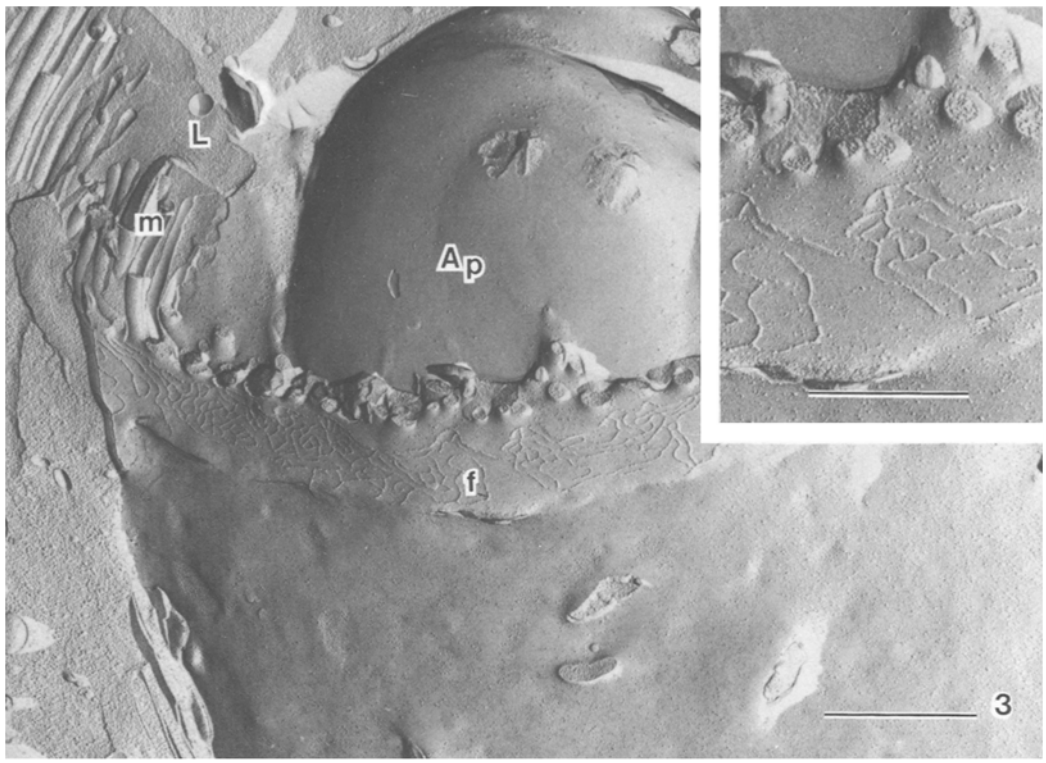


Figure 2. Zonula occludens (between arrow heads) from 2-h control preparation. This is an absorptive epithelial membrane fracture face. Cross-fractured apical microvilli (m). Scale bar = 1.0 μm.



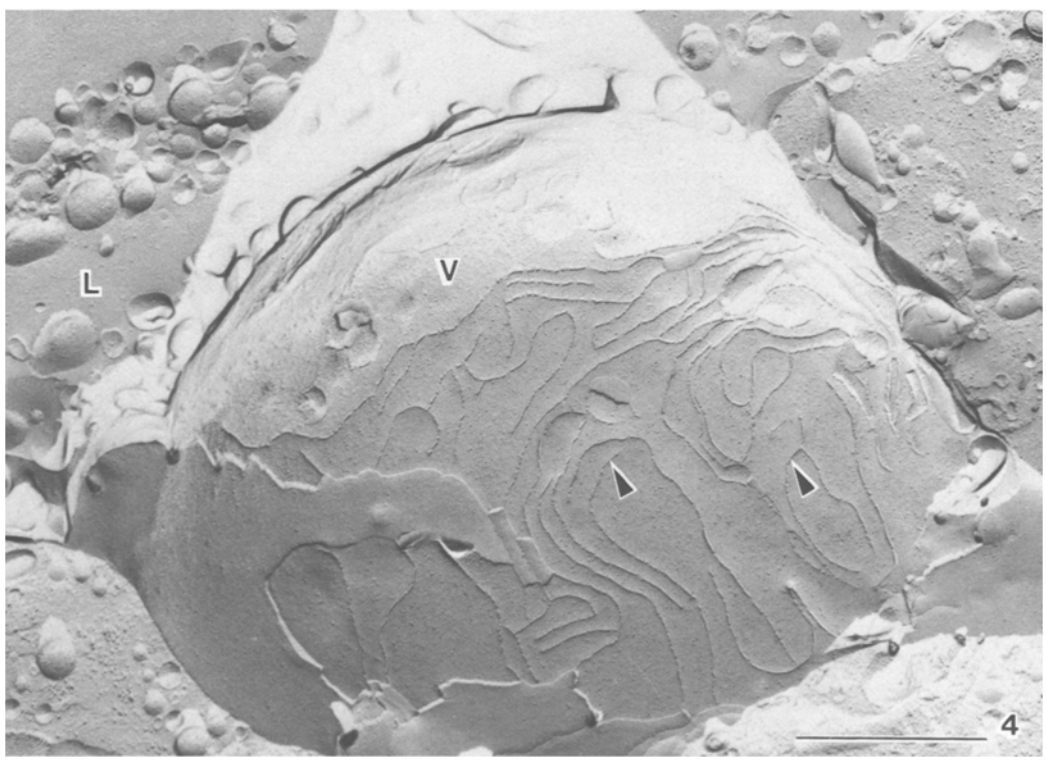


Figure 3. Zonula occludens 1 h after continuous histamine infusion from a goblet cell fracture face. Notice bulging apical surface (Ap) and loosely organized tight junctional fibrils (f). Intestinal lumen (L), apical microvilli (m). Scale bar = 1.0 µm. *Inset:* Higher magnification of a breach in permeability barrier shown in Fig. 3. Scale bar = 0.5 µm.

Figure 4. Large membrane vescle (V) expelled into intestinal lumen (L) 1 h after continuous histamine infusion. Notice numerous tight junctional fragments (arrow heads) associated with this membrane fracture face. Scale bar = 1.0 µm.

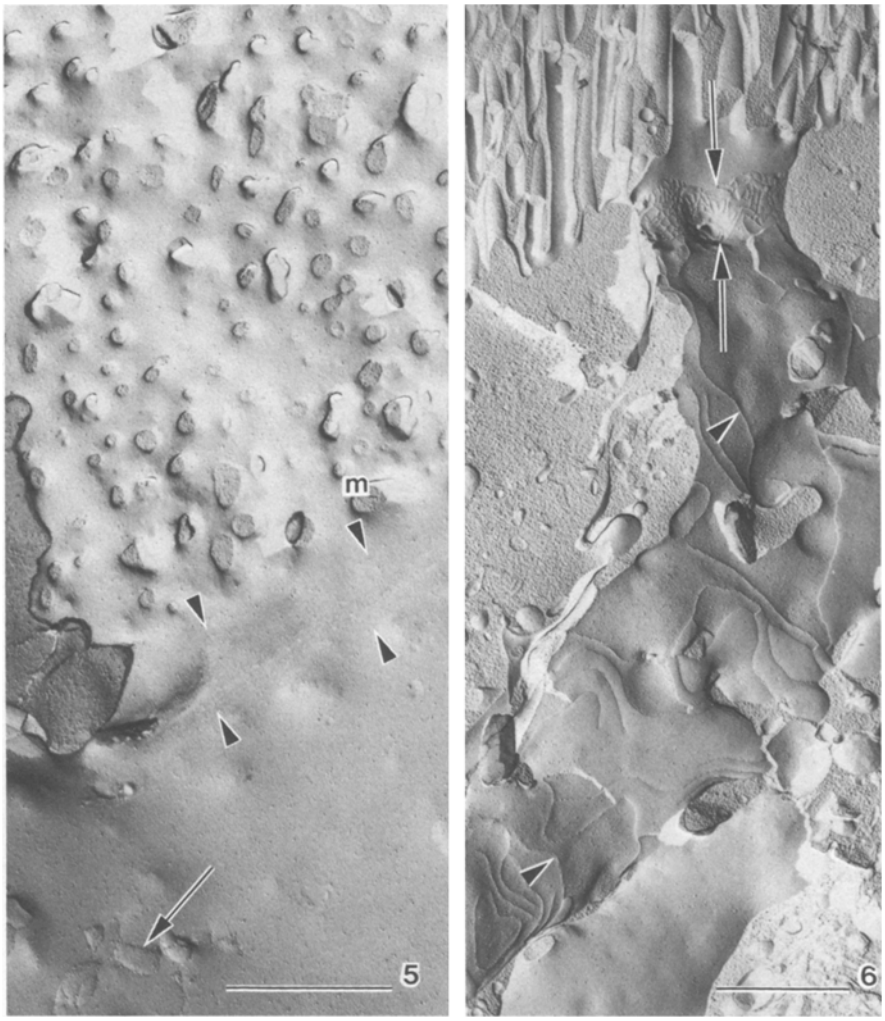


Figure 5. Membrane fracture face showing apical cross-fractured microvilli (m) and absence of zonula occludens (arrow heads) 1 h after continuous histamine infusion. Notice small tight junctional fragments (arrows). Presumably these are remnants of zonula occludens that normally occupied locus marked by arrowheads. Scale bar = 1.0 μm.

Figure 6. At 2-3 h after continuous histamine infusion, tight junctional fragments (arrow heads) on lateral membrane fracture faces can be observed basally as far as basal lamina. Zonula occludens (between arrows) appears normal at this time. Goblet cell fracture faces were infrequently observed. Scale bar = 1.0 μm.

zonula occludens 0.30 ± 0.04 μm (SD) wide in the apical to basal direction. A characteristic of this network was the irregularity in the shape of the enclosed regions formed by the intersection of the curvilinear fibrils.

The tight junctional complexes in the ileal epithelium from rabbits sampled at 1 h after infusion with histamine were normal in appearance on membrane fracture faces between absorptive epithelial cells. However, a different phenomenon was observed on membrane fracture faces of goblet cell membranes. During the 1st h after infusion of histamine, goblet cells appeared to bulge at their apical ends (fig. 3). The tight junctional fibrils were curvilinearly arrayed with few intersections and frequent interruptions, and each created a breach in the permeability barrier (fig. 3 and inset).

In the lumenal compartment, large spherical membranes were often observed bearing tight junctional fragments (fig. 4). These membranes appeared to be the result of an explosive event from the epithelium observed 1 h after histamine infusion. In some cases, membrane fracture face areas were completely devoid of tight junctional elements where such structures were normally positioned (fig. 5).

After 2-3 h of histamine infusion, interruptions in continuity of the tight junctional complexes seen after 1 h were not observed. However, extensive areas of the lateral membrane fracture faces contained tight junctional fragments extending below the zonula occludens to the basal lamina (fig. 6).

Disruption of some tight junctions observed in the ileum after histamine infusion may be a mechanism for increased intestinal permeability after trauma. Since histamine infusion does induce endotoxemia[7], our finding further substantiates the concept that losses of tight junctional integrity in traumatized animals may permit escape of endogenous endotoxin from the intestine.

As observed previously in endotoxin-challenged or traumatized animals[8,10] (Porvaznik, unpublished), disrupted permeability barriers in animals infused with histamine were also associated only with goblet cells. This finding may indicate that activity of these secretory cells is altered in a way which could contribute to mechanical damage of the tight junctional complexes. Apparent repair of tight junctions following longer infusion time may indicate production of substances that degrade histamine or that enhance repair processes[9].

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2 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals', prepared by the Institute of Laboratory Animal Resources, National Research Council.

3 We thank Luther G. Milton, Jr, for his assistance as surgical technician, Joseph L. Parker for preparing the tissue for microscopy, and Donna Boyle for editorial assistance. We thank the Departments of Anatomy at the Uniformed Services University of Health Sciences and the University of Chicago Medical School for the use of their Balzers freeze-fracture apparatus.
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6 Cuevas, P., Ishiyama, M., Koizuni, S., Woodruff, P., Kaufman, A., and Fine, J., Surgery Gynec. Obstet. *138* (1974) 725.
7 Cuevas, P., and Fine, J., Gastroenterology *64* (1973) 285.
8 Walker, R.I., and Porvaznik, M.J., Infect. Immun. *21* (1978) 655.
9 Porvaznik, M., Radiat. Res. *78* (1979) 233.
10 Walker, R.I., and Porvaznik, M., Life Sci. *23* (1978) 2315.
11 Walker, R.I., in: Experimental hematology today 1979, p. 55. Eds S.J. Baum and G.D. Ledney. Springer, New York 1979.

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Scanning electron microscopy of the gills of *Trachurus mediterraneus*

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Summary. Scanning electron micrographs have shown a significant difference in surface sculpture of the epithelial cells of the secondary lamellae and those of the gill filaments. The filament epithelium is covered with many microridges which appear to be interrupted periodically by swellings of various sizes. The secondary lamellae have few microridges.

The gills of *Trachurus mediterraneus* are similar to those of other carangid fish in having a relatively large surface area[2]. This is achieved by the high frequency of the secondary lamellae (40/mm) although each of them is smaller than those of similar-sized species which are less active. The secondary lamellae are relatively thin (12 μm) and the space between them is a little wider.

In this way water is brought into close contact with the gas exchange surfaces. The shape of the secondary lamellae varies along the length of each filament, being more triangular at the tip and rectangular at the base of the filaments. These differences are clearly visible under the scanning electron microscope as are differences in their surface architecture. Pieces of gill filament were removed from fish and fixed in 2% glutaraldehyde in phosphate buffer (pH 7.35) and after critical point drying were observed in a Phillips scanning electron microscope.

The surface of the epithelial cells which cover the secondary lamellae are characterized by many microvilli of varying densities (fig. 1). Boundaries between individual epithe-

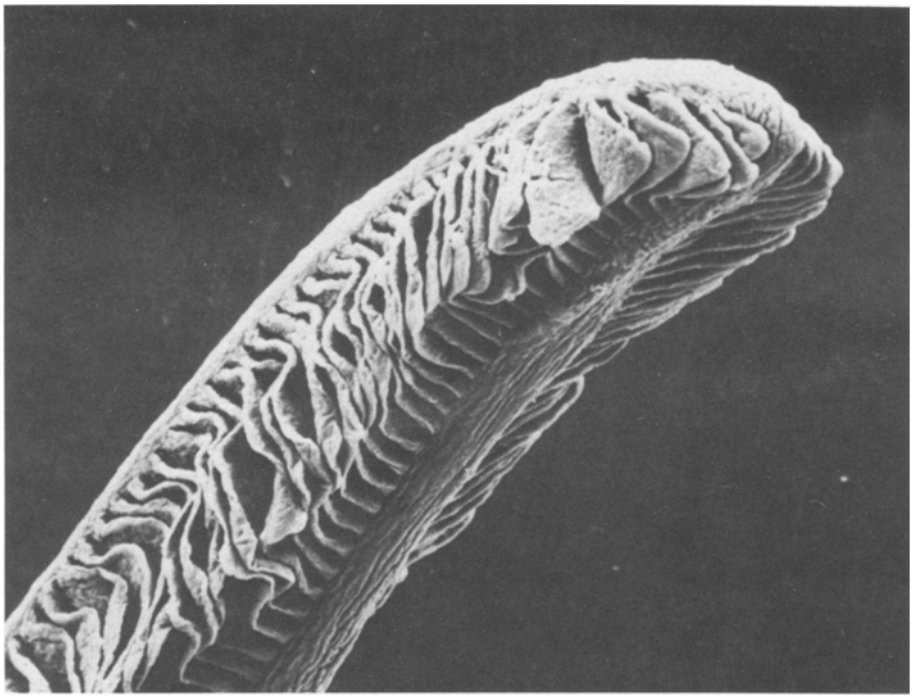

Figure 1. *T. mediterraneus* scanning electron micrograph of a single gill filament showing variations in lamellar shape in different regions of the filament (× 135).