In this communication, the authors report morphological changes of neutrophil granulocytes after a combined treatment of L-DOPA and prednisolone.

Materials and methods. Wistar male rats weighing 250 g were used. 4 mg of L-DOPA and 4 mg of prednisolone were concomitantly injected into experimental animals. At 1 h after the administration, the uptake of L-DOPA by granulocytes was ascertained by a reflected fluorescent microscope with the authors' method[1,2]. Granulocytes from untreated rats served as controls. For performing the electron microscopic study on a morphology of granulocytes, bloods were collected by heart puncture, heparinized and centrifuged at 1500 rpm. Buffy coats were fixed in 3% glutaraldehyde fixative adjusted to pH 7.4 with 0.1 M sodium cacodylate and postfixed in 1% osmium tetroxide buffered with the same solution. Halves of the specimens were used for a transmission electron microscope (TEM); that is, specimens were embedded in Epon 812, and ultrathin sections were stained with uranyl acetate and lead acetate. Parallel samples were prepared for a scanning electron microscope (SEM) by the critical point method[6]. For examination, a JEM-100B transmission- and a SSM-2 scanning electron microscope were used.

Results and discussion. By TEM, control granulocytes presented a relatively small number of cytoplasmic projections and vacuoles, and endoplasmic reticula were thin and scarcely visible (figure 1a). On the other hand, as shown in figure 1b, most of granulocytes obtained from the experimental group possessed a lot of complicated cytoplasmic projections on their free surface. Further, intracellular vacuoles of various size increased, and endoplasmic reticula became expanded. No difference could be seen in nuclear shape and contents between control and experimental groups. Through this experiment, erythrocytes, eosinophil and basophil granulocytes took normal shapes. In figures 2a and 2b, eosinophil and basophil granulocytes from the same specimens were depicted. Figure 3 shows scanning electron micrographs of granulocytes from control (figures 3a and 3b) and experimental groups (figures 3c and 3d). Granulocytes looked to be somewhat shrunken by a preparation procedure of specimens for SEM. Their surfaces were uneven and sometimes platelets adhered to them.

Comparing figures 3a and 3b with figures 3c and 3d, the following evidence was obtained. Granulocytes in experimental group had elongated cytoplasmic projections originating from whole surface, and the projections appeared twisted and irregular in direction. Their length ranged between 200 nm and 650 nm, and the profiles of granulocytes took the form of a starfish. On the other hand, the surfaces of granulocytes in control specimens were relatively smooth and had a small number of projections. They were short, measuring about 200 nm in length. These findings were comparable with those in cross sections of transmission electron micrographs. So that, L-DOPA could induce a morphological change only for neutrophil granulocytes. From these findings, the authors postulate that this is evidence that neutrophil granulocytes are specifically sensitive and reactive to some biogenic amines - especially for L-DOPA, and such morphological changes of granulocytes are closely associated with an enhancement of adenylate cyclase activity in neutrophil granulocytes after combined treatment of L-DOPA and prednisolone.

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Fine structure of modified photoreceptor cells in the pineal of the goby, *Clevelandia ios* (Pisces: Gobiidae)

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Summary. Photoreceptor cells with a system of long, thin cytoplasmic processes (50–80 nm thick), which may represent a modification of the outer segment saccules, are described in the pineal of the goby, *Clevelandia ios.*

Previous electron microscopic studies have demonstrated the presence of well developed photoreceptor cells in the pineals of fishes[3-5] and a photoreceptive function has been confirmed by electrophysiological experiments[6-8]. During a recent study on the pineals of several species of fishes, photoreceptor cells with an ultrastructure different from those described in previous studies were observed in the pineal of the goby, *Clevelandia ios.*

Materials and methods. 7 specimens ranging in size between 18 and 27 mm standard length were examined. Because of their small size it was necessary to decapitate the fish and dissect the pineal together with parts of the dorsal cranium in a petri dish containing glutaraldehyde fixative (2.5% glutaraldehyde in 0.16 M monosodium phosphate buffered to pH 7.4 with NaOH). Following dissection the tissue was immersed in fresh fixative for a period of 1 h. After postfixation with 1% osmium tetroxide in phosphate buffer the tissue was dehydrated and embedded in Araldite 502 plastic resin. Thin sections were stained with both uranyl acetate and lead citrate and photographed with an RCA EMU-3F electron microscope operated at 50 kV.

Observations and discussion. Light microscopically, the structure of the pineal in *Clevelandia ios* generally agreed with observations on the pineal of another goby, *Acanthogobius flavimanus*[9]. The photoreceptor cells lined the highly folded epithelium with their inner and outer segments projecting into a narrow lumen. The outer segments of most photoreceptor cells consisted of a series of membranous saccules, which measured between 20–40 nm in thickness, similar to those described in other fish species[3-5,10]. In many cells the saccules were highly irregular and often vesiculated.

Modified photoreceptor cells also were observed which lacked the typical membranous saccules of the outer segments. These cells were identified as photoreceptor cells by the presence of a sensory cilium (connecting piece) with a

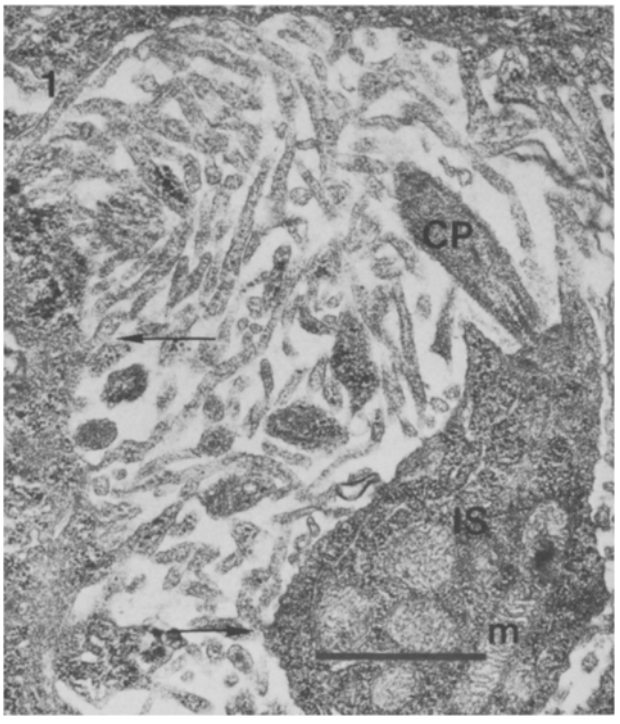
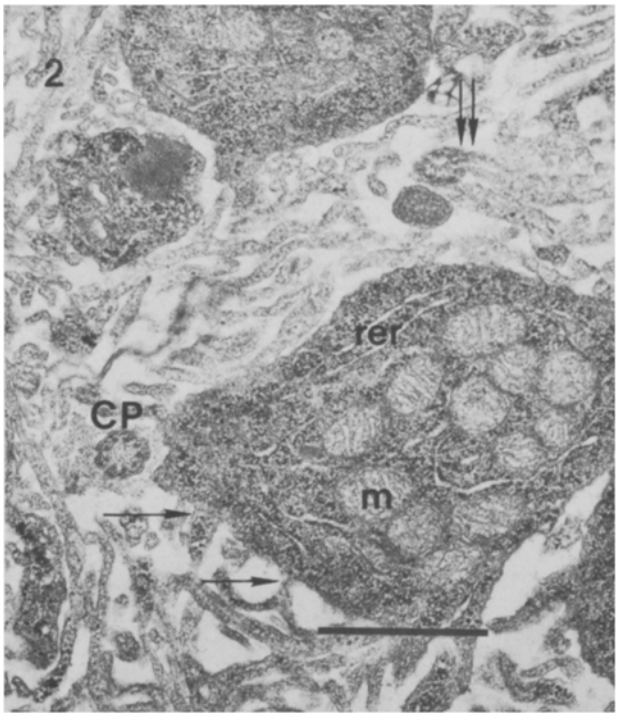
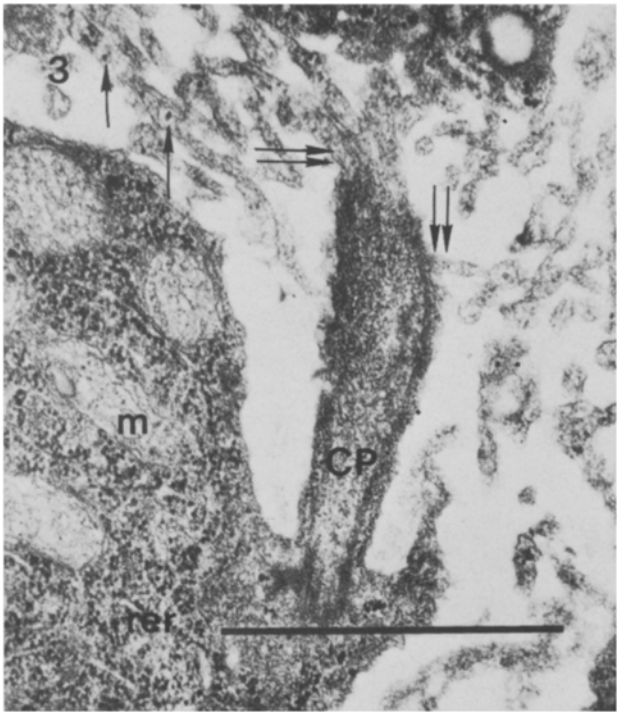


Fig. 1. A modified photoreceptor cell with a system of cytoplasmic projections surrounding the inner segment (IS) and connecting piece (CP). Some processes are seen originating from the inner segment and cells bordering the lumen (arrows). Bar indicates 1 µm.

Fig. 2. Modified photoreceptor cells showing a connecting piece (CP) cut in transverse section with its 9 + 0 arrangement of microtubules. Cytoplasmic processes can be seen originating from the inner segment (arrows) as well as from one connecting piece (double arrows). m, mitochondira; rer, rough endoplasmic reticulum. Bar indicates 1 µm.

Fig. 3. Higher magnification of a connecting piece (CP) showing continuity of the cytoplasmic processes with the connecting piece (double arrows). Occasional ribosomes are seen within the processes (arrows). m, mitochondria; rer, rough endoplasmic reticulum. Bar indicates 1 µm.





9 + 0 arrangement of microtubules extending from their inner segments (figures 1–3). Surrounding the inner segments of these cells were numerous long, thin cytoplasmic processes measuring between 50–80 nm in thickness which originated from cells bordering the lumen (figure 1), the inner segments (figures 1 and 2), as well as the connecting piece (figures 2 and 3). The cytoplasmic processes differed from the more typical membranous saccules of other photoreceptor cell outer segments in that they were approximately twice as thick, were disorganized, and contained some small particles which may be ribosomes (figure 3).

Cytoplasmic processes from the inner segments of photoreceptor cells have been described in previous studies of the fish pineal[5,11,12], but are usually few in number. In 1 species, the deep-sea fish, *Triphoturus mexicanus,* similar processes appeared to be continous with the outer segment saccules[10]. Since the cytoplasmic processes in *C. ios* originated from the connecting piece it is possible that they are a

modification of the photoreceptor-cell outer segment. Whether or not they represent a developmental stage of the outer segment saccules could not be determined since definite intermediate stages between the 2 were not observed. Although the outer segments of photoreceptor cells are known to be susceptible to fixation artifact[5,13], the presence of ribosomes in the processes of these modified photoreceptor cells makes it unlikely that they are distorted outer segment saccules. The disorganized appearance of the cytoplasmic processes raises the question of whether these cells function in photoreception or in some other respect. Their functional significance cannot be determined by the present study.

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Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus[1]

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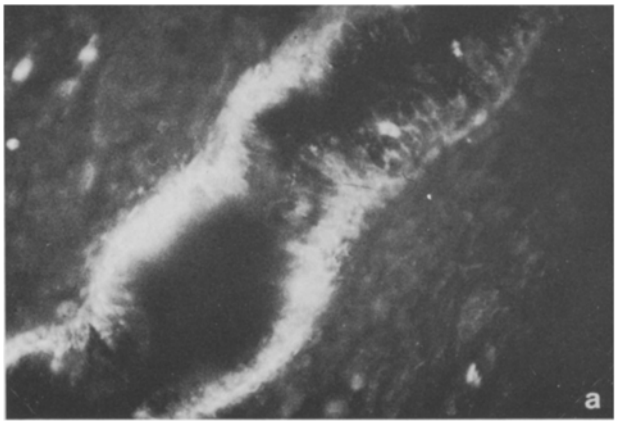
Summary. Localization of the acid-stable proteinase inhibitor of human cervical mucus within the epithelium of the upper cervix was possible by indirect immunofluorescence.

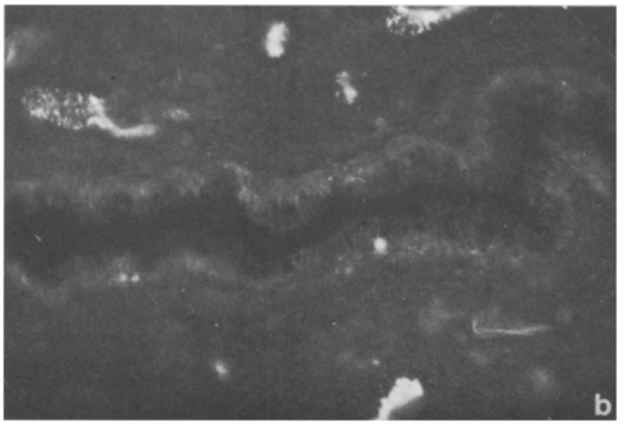
Human cervical mucus contains an acid-stable low molecular weight trypsin-chymotrypsin inhibitor[3] which has been recently characterized in more detail[4]. Inhibition characteristics, mol. wt, amino acid analysis and immunological properties indicate identity of this proteinase inhibitor with human seminal plasma inhibitor I (HUSI-I) produced by the seminal vesicles[5-8]. Remarkably, an acid-stable inhibitor with very similar characteristics is present in human nasal and bronchial secretions[9]. This class of inhibitors has a high affinity to neutral granulocytic proteinases (elastase, cathepsin G) present in most mucus secretions. The rapid and permanent inhibition of granulocytic proteinases by this antileukoprotease suggests a protective function of the cervical epithelium against proteinases liberated from disintegrating leukocytes[7].

In this communication, the side of secretion of the antileukoprotease in the uterine cervix is demonstrated by indirect immunofluorescent technique employing an anti-HUSI-I immunoglobulin obtained from rabbits immunized with highly purified HUSI-I.

Material and methods. The cervix of 5 women (32–45 years) at cycle day 8–10 undergoing hysterectomy for various reasons was collected immediately after surgery. Small tissue pieces of different parts of the cervix and the endometrium were preserved in isopentan/liquid nitrogen. The frozen material was transferred to a cryostate (SLEE, London). Slices of 8–10 µm thickness were cut, mounted on microscopic slides, air-dried and fixed with acetone for 10 min. The indirect immunofluorescent staining method according to Nairn[10] was performed, using either treatment



a Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus in the columnar epithelium of the upper cervix, using HUSI-I-directed immunoglobulins. Magnification 250:1.



b Control treated with HUSI-I-directed immunoglobulins incubated prior to the experiment with highly purified HUSI-I.