Environmental Biology of Fishes **30**: 155–163, 1991.

Ultrastructure of the epidermis and digestive tract in *Sebastes* embryos, with special reference to the uptake of exogenous nutrients

Motohiro Shimizu[1], Muneharu Kusakari[2], Mary M. Yoklavich[3], George W. Boehlert[4] & Juro Yamada[1]
[1] *Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041, Japan*
[2] *Hokkaido Central Fisheries Experimental Station, Yoichi, Hokkaido 046, Japan*
[3] *College of Oceanography and Marine Science Center, Oregon State University, Newport, OR 97365, U.S.A.*
[4] *Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, NOAA, Honolulu, HA 96822, U.S.A. and Joint Institute of Marine and Atmospheric Research, University of Hawaii, Honolulu, HA 96822, U.S.A.*

Received 27.6.1989 Accepted 14.11.1989

Key words: Viviparity, Nutrition, Horseradish peroxidase, Ruthenium red, Pinocytosis, Rockfish, Pisces, Scorpaenidae

Synopsis

Ultrastructural features of the epidermis and rectum were studied in *Sebastes schlegeli* and *S. melanops* during the late stages of embryonic development, to confirm uptake of maternal substances. Ruthenium red (RR) and horseradish peroxidase (HRP) were used at fixation and in live embryos, respectively. Epidermal tissue of embryos after developmental stage 24 comprised two squamous cell layers. The outer, thinner cells and their intercellular spaces were easily infiltrated with RR, but the inner cells had no RR deposition. The HRP was not incorporated into the epidermis except in a few outer cells, which had well-developed microvillous projections of cytoplasm. Sacciform cells, chloride cells, and mucous cells distributed in the inner layer but protruding to the epidermal surface had no intracellular RR and HRP depositions. The rectal cells of embryos at about developmental stage 28 had many globular inclusions containing electron-dense substances. The rectal cells were found to take up and digest HRP actively. It is suggested that the embryonic epidermis is structurally loose and takes up low weight molecules, while rectal cells, after the opening of the mouth, actively ingest exogenous, high weight molecules.

Introduction

In larvae of many oviparous fishes, the hindgut has a unique feature for pinocytotic ingestion and intercellular digestion of high molecular weight nutrients (Iwai 1969, Tanaka 1969, Watanabe 1982). Embryos of many viviparous teleosts possess specific organs, such as the trophotaenia and the vasculated finfold, adapted for the uptake of nutrients from the maternal ovarian fluid (Turner 1947, Mendoza 1958, Wourms et al. 1988). Absorption of external nutrients through the embryonic epidermis has been reported in two viviparous species, *Goodea luitpoldii* and *Clinus superciliosus* (Mendoza 1958, Veith 1979, 1980).

The scorpaenid genus *Sebastes* has been categorized as ovoviviparous, because the internal embryos were considered to be lecithotrophic with no specific organs for taking up external nutrients. Recent studies on energetics, however, suggest that embryos of *S. schlegeli* and *S. melanops* utilize external materials as energy sources (Boehlert &

Yoklavich 1984, Boehlert et al. 1986). Those studies also present indirect histological evidence that the rectal cells of *Sebastes* embryos at late developing stages may function in the ingestion of exogenous substances, but offer no direct evidence of the absorption of nutrients by either rectal epithelial or epidermal cells.

Our paper presents direct ultrastructural evidence that the epidermis and the rectum in *Sebastes* embryos absorb external substances, using horseradish peroxidase (HRP) and ruthenium red (RR) as tracers.

Materials and methods

Intraovarian embryos and just hatched larvae of *Sebastes schlegeli* and *S. melanops* were used. Determinations of the developmental stages of the embryos followed Yamada & Kusakari (1991). Embryos before stage 32 were obtained by catheterization through the genital pore of maternal fish. For the experiments using HRP, live embryos of *S. melanops* were incubated for 1–4 h in physiological saline (230 mM NaCl, 8 mM KCl, 2.3 mM $CaCl_2$, 3.7 mM $MgCl_2$ and 2.4 mM $NaHCO_3$, pH 7.3) containing 0.5% HRP and then rinsed in saline for 5 min. The embryos were fixed in toto with Karnovsky's (1965) fixative and, after being washed with 0.1 M sodium cacodylate buffer (pH 7.4) were treated for 15 min with 0.05% diaminobenzidine tetrahydrochloride (DAB) in 0.1 M Tris-HCl buffer (pH 7.4) containing 0.01% H_2O_2 and 0.5% sucrose. After they were washed with the buffer, they were post-fixed with 1% osmium tetroxide, dehydrated through a graded series of ethanol and embedded in Epok 812. For the other experiments, embryos were fixed with Karnovsky's (1965) fixative containing RR (1 mg ml^{-1}), osmicated with osmic solution containing RR and embedded in Epok 812.

The epidermal and rectal tissues were cut into ultrathin sections, which were then stained with uranyl acetate and lead citrate and examined with a Hitachi H-6000 transmission electron microscope (TEM). Some of the specimens treated with HRP were examined by light microscopy. For scanning electron microscopy (SEM), some fixed embryos were dehydrated through ethanol and isoamyl acetate and dried by the critical point method using liquid CO_2. After being coated with a thin layer of evaporated platinum-palladium alloy, they were examined with a JEOL JSM-25 scanning electron microscope.

Results

Epidermis

Sebastes embryos during the late developmental stages had thin, delicate skin composed of epidermal and dermal tissues over the main body surface and only epidermal tissue over the finfold. The epidermis comprised of two squamous cell layers: an outer layer (0.5–3 μm thick) with thin cells and an inner layer with thick cells and other types of cells such as sacciform, chloride and mucous cells. The apico-lateral membranes of the outer epidermal cells were connected with tight junctions and desmosomes. Embryos treated with RR had particles of RR deposited in the outer layer of the epidermis (Fig. 1a), particularly at the intercellular spaces and interdigitating infoldings. Infoldings dotted with RR particles were commonly seen in the lateral and basal membranes of the outer layer cells (Fig. 1b). Small vesicles dotted with RR were distributed in the apical zone (Fig. 1b, c), which was characterized by an abundance of cytoplasmic filaments. Some vesicles were in direct contact with the apical membrane. The RR particles were deposited also in the intracellular membranous systems, cisternae of the endoplasmic reticulum (ER) and mitochondria (Fig. 1c). Numerous RR-dotted cisternae of ER were distributed in the middle zone of the cell, where expanded mitochondria also were present. The RR particles were found in the perinuclear space as well (Fig. 1d).

Squamous cells of the inner epidermal layer, as compared with the outer layer cells, had well-developed rough-ER and abundant free ribosomes in the relatively compact cytoplasm (Fig. 1d). The apical membranes of these cells interdigitated with the basal membranes of the outer cells. No RR

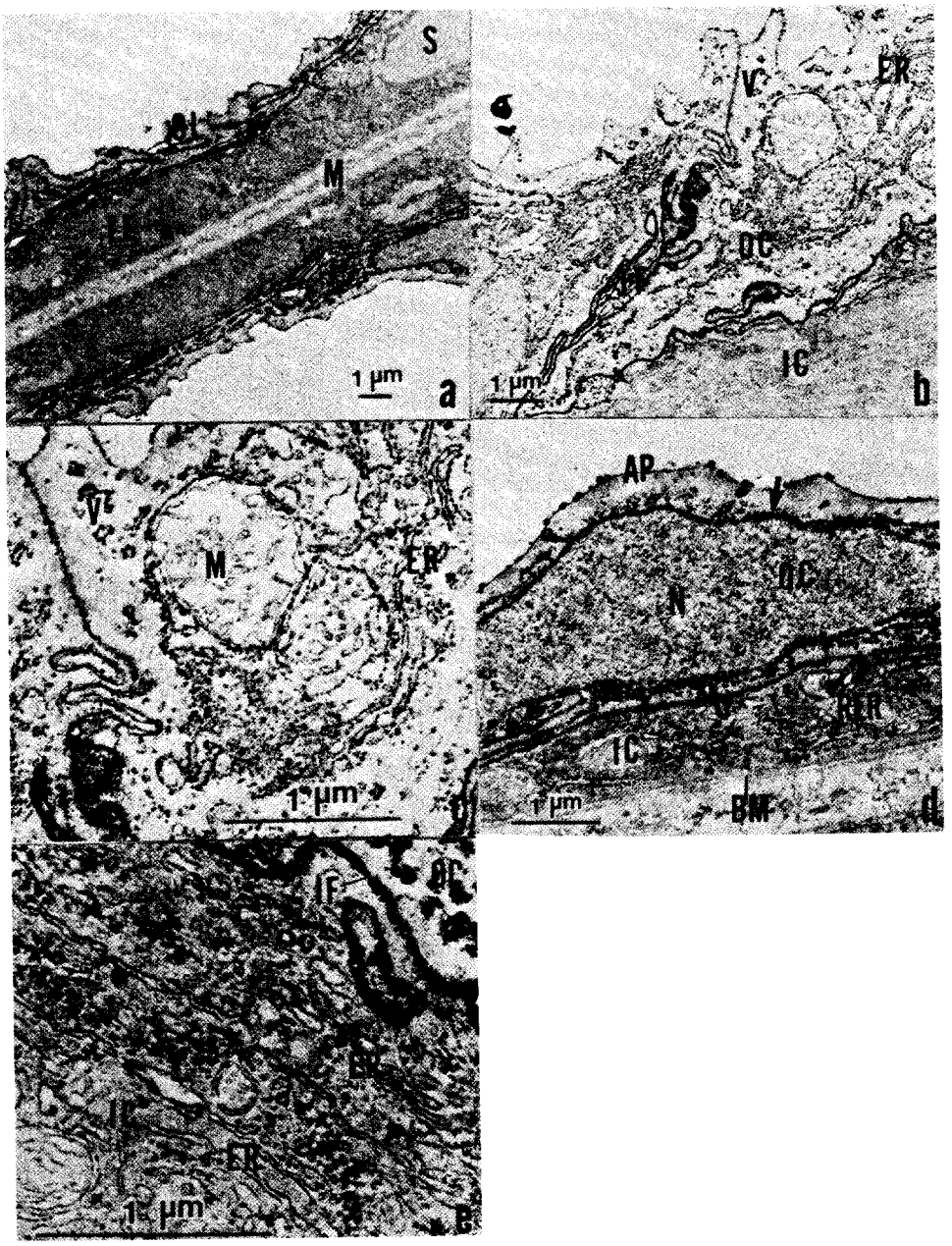

Fig. 1. Transmission electron micrographs of embryonic epidermal tissues, stained with ruthenium red, from *Sebastes melanops* at developmental stage 24: a – Transverse section of the finfold. IL = inner layer, OL = outer layer, M = muscle layer and S = sacciform cell. b – Interdigitations of the outer cells (OC) forming infoldings (IF) at the lateral and basal membranes. Arrows indicate vesicles attaching apical surface. V = vesicles, ER = endoplasmic reticulum and IC = inner cell. c – Membrane systems of endoplasmic reticulum (ER) and mitochondria (M).V = vesicles. d – Perinuclear space (arrows) in the outer cell (OC). AP = apical surface, BM = basement membrane, IC = inner cell, N = nucleus and RER = rough endoplasmic reticulum (ER). e – Inner cell (IC) interdigitating with an outer cell (OC). ER = endoplasmic reticulum, IF = infolding.

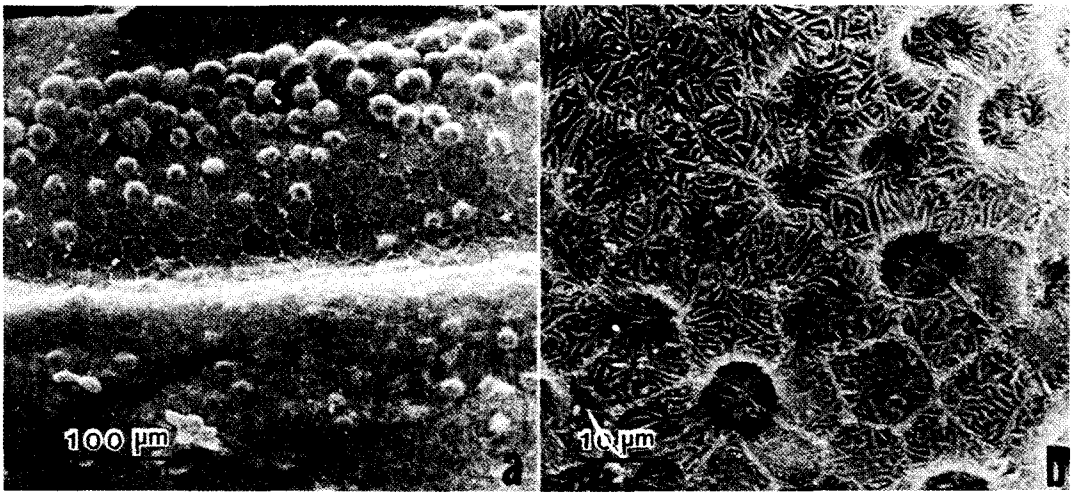

Fig. 2. Scanning electron micrographs of *Sebastes melanops* embryos: a – Swollen sacciform cells in the dorsal finfold (stage 28). b – Numerous depressions formed by shrunken sacciform cells in the jaw region (stage 28).

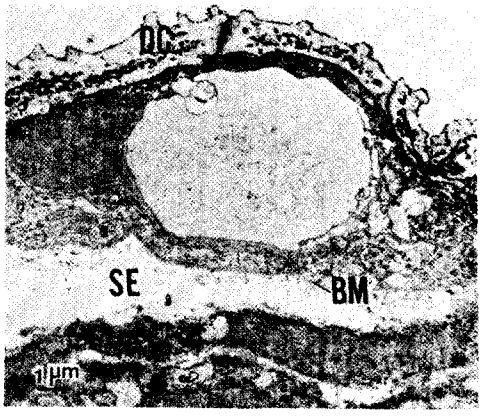

Fig. 3. Transmission electron micrograph of a swollen sacciform cell in a *Sebastes melanops* embryo at stage 24, stained with ruthenium red. BM = basement membrane, OC = outer cell and SE = subepidermal space.

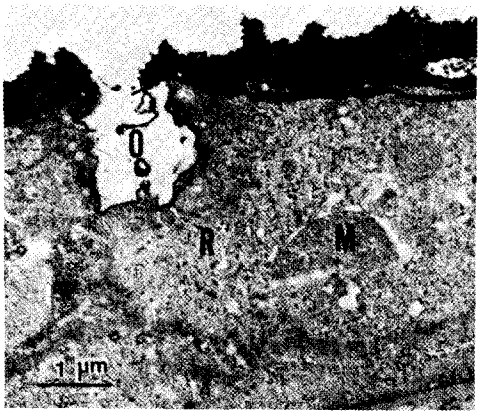

Fig. 4. Transmission electron micrograph of a chloride cell in a *Sebastes schlegeli* embryo at stage 32, stained with ruthenium red. M = mitochondria, R = reticular network, O = apical opening and OC = outer cell.

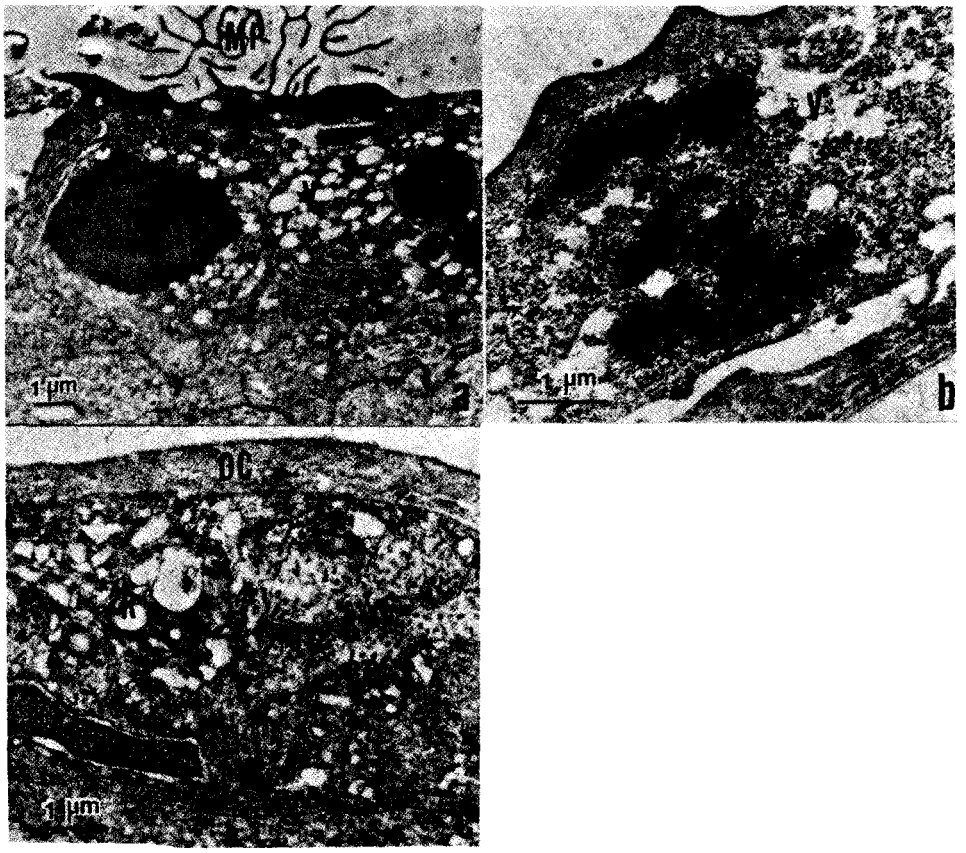

Fig. 5. Transmission electron micrographs of a *Sebastes melanops* embryo, treated with horseradish peroxidase (HRP): a – An outer epidermal cell with branched microvillous projections (MP) (stage 24). HG = large HRP globules, V = vesicles. b – An outer epidermal cell (stage 24). H = electron-dense HRP, V = vesicles. c – A chloride cell (CC) (stage 28) with electron-dense cytoplasm and expanded reticular network (R). *OC = outer cell.*

particles were present in the cytoplasm of the inner squamous cells (Fig. 1e). Numerous sacciform cells were anchored in the inner layer over the entire body surface and were particularly conspicuous in the dorsal and ventral finfolds.

In SEM profiles of the body surface of embryos around stage 28, sacciform cells were often characterized by a swollen appearance (Fig. 2a), whereas others were evident as depressions (Fig. 2b). The swollen sacciform cells contained fine, granular, moderately electron-dense materials in a large vacuole, which occupied the majority of the cytoplasm (Fig. 3). No deposition of RR was observed in these cells. Chloride cells rarely were observed in the inner layer of embryonic epidermis, but were conspicuous in larvae. No RR deposition was evident in the chloride cells, although their apical openings were dotted with RR particles (Fig. 4).

In embryos of *S. melanops* treated with HRP in toto, the main body surface was stained strongly with brownish HRP. The finfold was pale brown. Transmission electron microscopy revealed that some outer epidermal cells contained inclusions of electron-dense HRP (Fig. 5a). Branched microvillous projections were visible at the apical surface of these cells. Other cells contained electron-dense

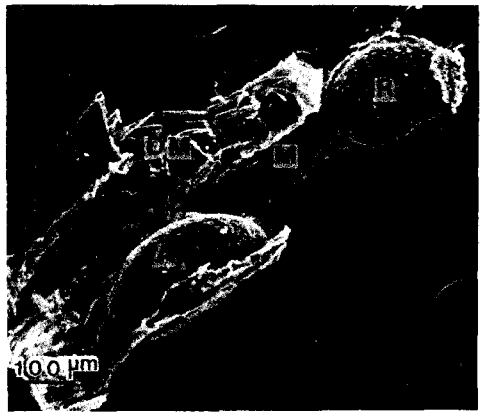

Fig. 6. Scanning electron micrograph of the embryonic digestive tract of *Sebastes schlegeli* at stage 28. A = anterior gut, DM = dorsal muscle, M = midgut and R = rectum.

HRP that appeared to be diffusing in the cytoplasm (Fig. 5b). However, most of the outer epidermal cells revealed no intracellular HRP. Chloride cells revealed no signs of HRP (Fig. 5c).

Digestive tract

In embryos of *S. schlegeli* during the late stages, epithelial cells of the rectum contained an amorphous granular substance, which has been described by Shimizu & Yamada (1980) and suggested to be evidence of the absorption of exogenous nutrients (Boehlert et al. 1986). In embryos at stage 28, the digestive tract had a hindgut or rectum that was distinguishable from the midgut by its enlarged appearance (Fig. 6). The anterior midgut was differentiated into several parts with varying diameters and a few constrictions. The mouth and the anus were already open. The rectum was separated from the midgut by a valve composed of an epithelial fold. The rectal epithelium consisted of high pseudostratified columnar cells, which had numerous microvilli forming a well-developed, striated border at the apical surface (Fig. 7a). Numerous pinocytotic invaginations were seen between the bases of adjacent microvilli. Amorphous or fine, granular electron-dense materials were present in the invaginations as well as in the rectal lumen. Some invaginations formed coated vesicles. In the apical cortex, many tubular structures containing electron-dense materials collectively constituted a canalicular system. Numerous vesicles of various sizes were confined to the subapical region. Large globules, apparently formed by fusion of the vesicles, were distributed in the supranuclear zone. Some globular inclusions were filled with electron-dense materials, while others were empty or partially filled. Inclusions that appeared to be degrading were present near the nucleus. Similar inclusions were found also in a large Golgi field (Fig. 7b).

When treated with HRP, the digestive tract, especially the rectum, was stained dark brown, indicating active uptake of HRP (Fig. 8a). Vesicles containing HRP were observed by TEM in the rectal cells (Fig. 8b). Small degrading HRP inclusions were observed near the lateral and basal membranes, but no Golgi apparatus was associated with these inclusions. Fine, electron-dense granules were conspicuous in the cytoplasm.

Discussion

As in our study, numerous inclusions of electron-dense substances and their degradation products have been observed in the rectal cells of *S. schlegeli* embryos at stage 28 (Boehlert et al. 1986). *Sebastes melanops* embryos treated with HRP have a conspicuous accumulation of tracer in the digestive tract, especially in the rectum. The embryos at stage 28 are characterized by the opening of the mouth and the anus (Yamada & Kusakari 1991). Therefore, the observations indicate that orally ingested materials are actively absorbed by the rectal cells in the same pinocytotic process described in HRP-injected larvae of *Cottus nozawae* by Watanabe (1984). The ingesta in viviparous *Sebastes* embryos with a functional mouth probably are derived from the ovarian fluid. Substances absorbable in the rectal cells are probably high molecular weight protein complexes, such as glycoproteins and lipoproteins, because contents of the inclusions varied in texture.

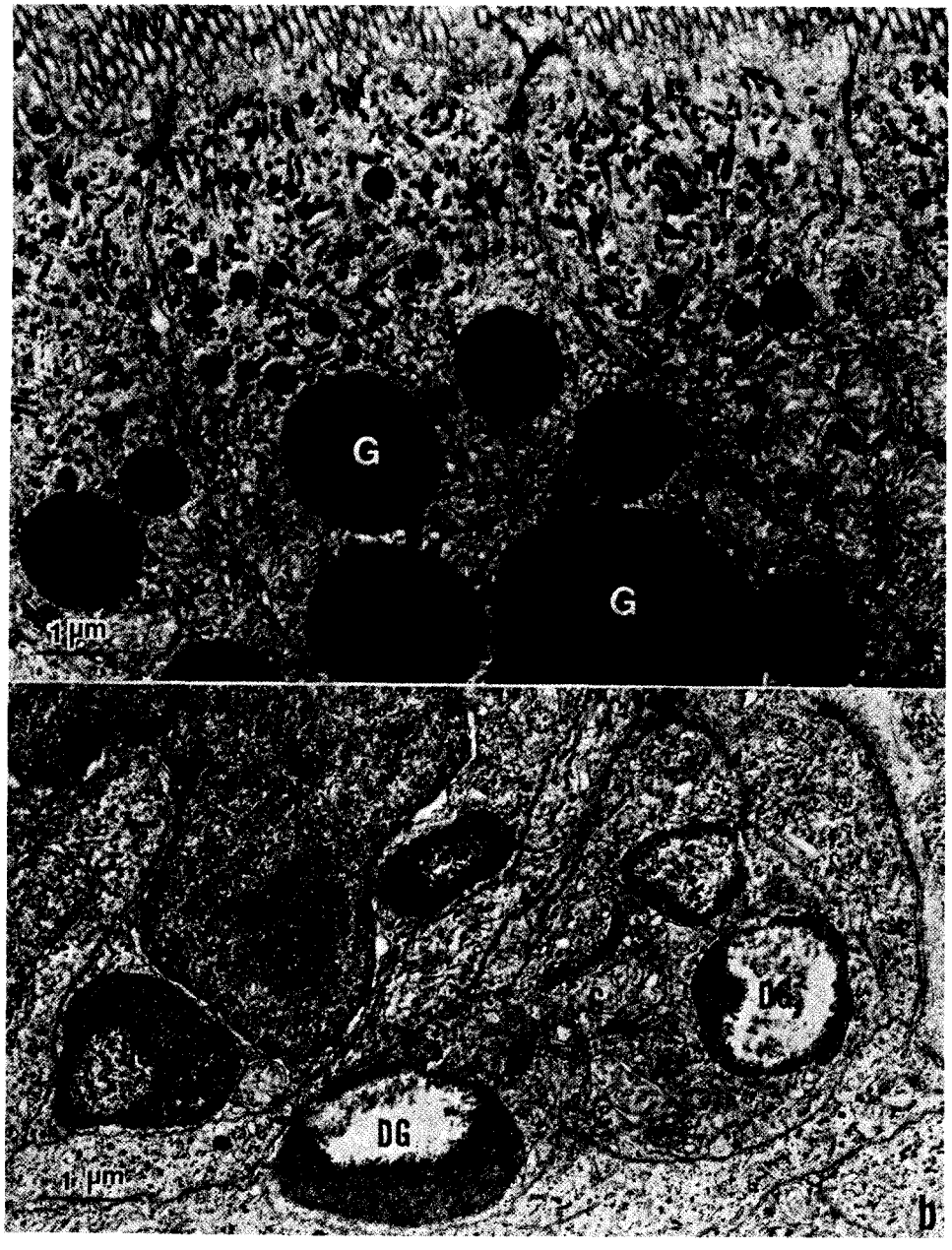

Fig. 7. Transmission electron micrographs of a *Sebastes schlegeli* embryo at stage 28, stained with ruthenium red: a – Embryonic rectal cells actively taking up external materials. G = electron-dense globules in the supranuclear region, MV = microvillus, T = tubular structures, Arrow = pinocytotic invagination. b – A rectal cell with degrading globules (DG) in a large Golgi field (G). N = nucleus.

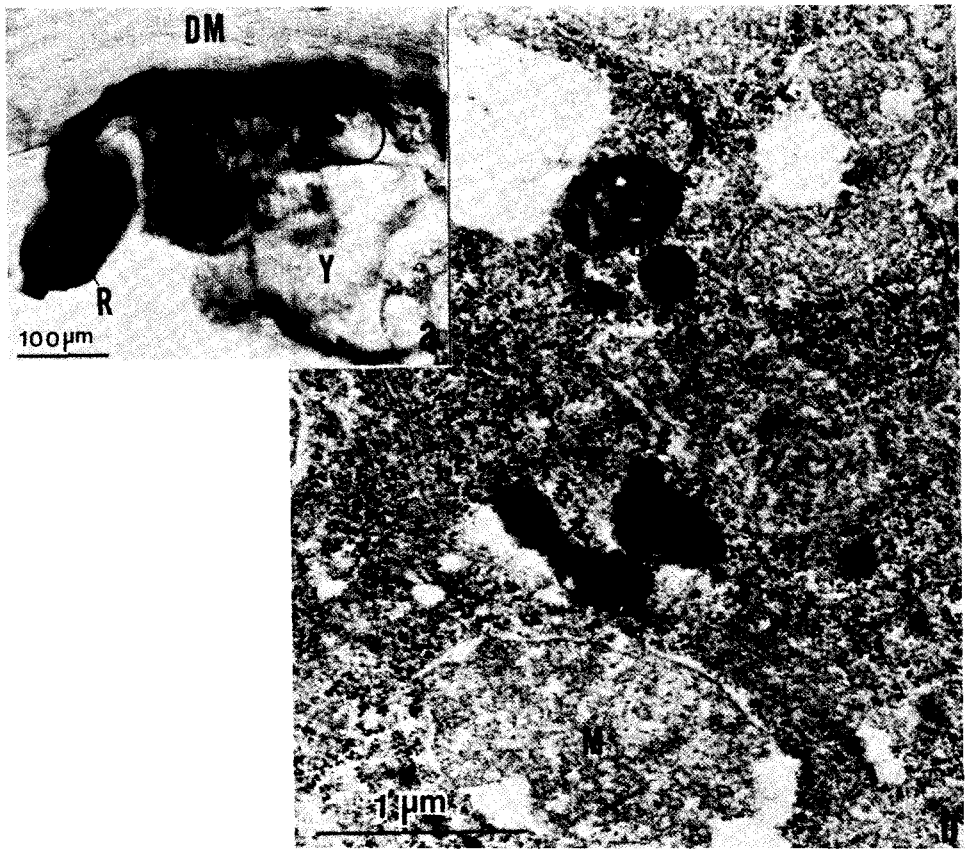

Fig. 8. Sebastes melanops embryos at stage 28, treated with horseradish peroxidase (HRP): a – Light micrograph of the embryonic digestive tract with abundant HRP, DM = dorsal muscle, M = midgut, R = rectum and Y = yolk sac. b – Transmission electron micrograph of a rectal cell containing globules of HRP (HG) among mitochondria (M).

The epidermis of *Sebastes* embryos at the late stages consists of two cell layers, as do the embryos of *Oncorhynchus keta* (Aso et al. 1977) and larvae of *Clupea harengus* (Jones et al. 1966) and *Hypomesus olidus* (Yamada 1968). The outer epidermal cells allow infiltration of RR, a tracer known to be impermeable to cell membranes and commonly used as a marker substance to show intercellular spaces (Brooks 1969). This suggests that at least the outer cell layer of the embryonic epidermis of *Sebastes* is permeable to some low weight molecules contained in the intraovarian environment. In addition, the outer cells commonly have well-developed tubular structures, which probably consist of cisternae of the ER. Similar structures have been described as the tubular-cisternae complex in the trophotaenial epithelium of the viviparous teleost *Characodon eiseni* (Mendoza 1972). Membranes of the tubular system of *Sebastes* embryos in our study were dotted with RR, suggesting that this membrane system opens outward at the apical surface.

Incubation of live embryos (stage 24) with HRP proved that some epidermal cells can take up exogenous, high weight molecules. These cells, characterized by well-developed microvilli, were not commonly found in all examined embryos. In em-

bryos of stages 21, 28 and 31, such microvilli-bearing epidermal cells have not been reported (Boehlert & Yoklavich 1984, Boehlert et al. 1986). Stages 24 and 25 are the midpoint in epidermal cell differentiation from a smooth surface (stage 21) to developing numerous microridges (stage 28), which are probably involved in the initial uptake of external nutrients. The lack of involvement of sacciform cells in material uptake suggests that the cells are not absorptive, and we cannot speculate on their function.

The morphological features observed in the present study indicate that the embryonic epidermis is structurally loose and absorbs low molecular weight substances, whereas the rectal cells are involved in active uptake of high molecular weight substances after the opening of the mouth. Further research with specified substrates will be necessary to define the source and identity of such substances.

Acknowledgements

This research is part of a project of the U.S.-Japan Cooperative Science Program. We acknowledge funding from the Japan Society for the Promotion of Science and the U.S. National Science Foundation.

References cited

Aso, N., H. Saito & T. Takagi. 1977. Microridge formation of epidermal cells of the developing salmon (*Oncorhynchus keta* Walbaum): scanning and transmission electron microscopic study. Zool. Mag. 86: 208–221. (In Japanese.)

Boehlert, G.W., M. Kusakari, M. Shimizu & J. Yamada. 1986. Energetics during embryonic development in kurosoi, *Sebastes schlegeli* Hilgendorf. J. Exp. Mar. Biol. Ecol. 101: 239–256.

Boehlert, G.W. & M.M. Yoklavich. 1984. Reproduction, embryonic energetics, and the maternal-fetal relationship in the viviparous genus *Sebastes* (Pisces: Scorpaenidae). Biol. Bull. 167: 354–370.

Brooks, R.E. 1969. Ruthenium red stainable surface layer of lung alveolar cells: electron microscopic interpretation. Stain Tech. 44: 173–177.

Iwai, T. 1969. Fine structure of gut epithelial cells of larvae and juvenile carp during absorption of fat and protein. Arch. Histol. Jap. 30: 183–199.

Jones, M.P., F.G.T. Holliday & A.G.E. Dunn. 1966. The ultrastructure of the epidermis of larvae of the herring (*Clupea harengus*) in relation to the rearing salinity. J. Mar. Biol. Ass. U.K. 46: 235–239.

Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137A.

Mendoza, G. 1958. The fin fold of *Goodea luitpoldii*, a viviparous cyprinodont teleost. J. Morph. 103: 539–560.

Mendoza, G. 1972. The fine structure of an absorptive epithelium in a viviparous teleost. J. Morph. 136: 109–130.

Shimizu, M. & J. Yamada. 1980. Ultrastructural aspects of yolk absorption in the vitelline syncytium of the embryonic rockfish, *Sebastes schlegeli*. Jap. J. Ichthyol. 27: 56–63.

Tanaka, M. 1969. Studies on the structure and function of the digestive system in teleost larvae-2. Characteristics of the digestive system in larvae at the stage of first feeding. Jap. J. Ichthyol. 16: 41–49. (In Japanese.)

Turner, C.L. 1947. Viviparity in teleost fishes. Sci. Mon. 65: 508–518.

Veith, W.J. 1979. The chemical composition of the follicular fluid of the viviparous teleost *Clinus superciliosus*.Comp. Biochem. Physiol. 63A: 37–40.

Veith, W.J. 1980. Viviparity and embryonic adaptations in the teleost *Clinus superciliosus*. Can. J. Zool. 58: 1–12.

Watanabe, Y. 1982. Intracellular digestion of horseradish peroxidase by the intestinal cells of teleost larvae and juveniles. Bull. Jap. Soc. Sci. Fish. 48: 37–42.

Watanabe, Y. 1984. An ultrastructural study of intracellular digestion of horseradish peroxidase by the rectal epithelium cells in larvae of a freshwater cottid fish *Cottus nozawae*. Bull. Jap. Soc. Sci. Fish. 50: 409–416.

Wourms, J.P., G.D. Bryon & J. Lombardi. 1988. The maternal-embryonic relationship in viviparous fishes. pp. 1–134. *In:* W.S. Hoar & D.J. Randall (ed.) Fish Physiology, Vol. 11B, Academic Press, New York.

Yamada, J. 1968. A study on the structure of surface cell layers in the epidermis of some teleosts. Annot. Zool. Jap. 41: 1–8.

Yamada, J. & M. Kusakari. 1991. Staging and the time course of embryonic development in kurosoi, *Sebastes schlegeli*. Env. Biol. Fish. 30: 103–110.