Embryonic adaptations and nutrition in the viviparous teleost *Clinus dorsalis* (Perciformes: Clinidae)

D.A. Cornish and W.J. Veith

Department of Zoology, University of Stellenbosch, Stellenbosch

Embryos of *Clinus dorsalis* absorb nutrients from the embryotrophe, secreted by the follicular epithelium. Autoradiographic studies revealed that the principal areas of nutrient absorption are the embryonic gut and epidermis. A histological and electron microscopic study of embryonic structure revealed an extensively hypertrophied gut with numerous fingerlike villi projecting into the gut lumen. A brushborder of microvilli is, furthermore, characteristic of the columnar gut epithelium. Epidermal surface area is increased apically on individual epidermal cells, particularly on the ventral pericardial surface. Microridges further increase epidermal surface area. Epidermal surface area is reduced and a mucous layer is secreted prior to parturition.

S. Afr. J. Zool. 1986, 21: 79–84

Embrio's van *Clinus dorsalis* absorbeer voedsel vanuit die embriotroof wat deur die follikulêre epiteel gesekreteer word. Outoradiografiese studies het getoon dat die belangrikste gebiede vir voedselabsorpsie die embrioniese spysverteringskanaal en epidermis is. 'n Histologiese en elektronmikroskopiese ontleding van hierdie embrioniese strukture het getoon dat die spysverteringskanaal gehipertrofeer is en dat talle vingeragtige villi in die S.V.K.-lumen voorkom. Die silinderepiteel van die S.V.K. is ook voorsien met 'n goed ontwikkkelde borselrant bestaande uit mikrovilli. Die epidermale oppervlakte van die embrio's word vergroot deur bulte wat gevorm word deur die apikale oppervlakte van individuele epidermale selle veral op die ventrale perikardiale oppervlakte van die embrio's. Mikroriwwe vergroot die epidermale oppervlakte verder. Voor geboorte word die epidermale oppervlakte verminder en 'n slymlaag word gesekreteer.

S.-Afr. Tydskr. Dierk. 1986, 21: 79–84

D.A. Cornish
Department of Physiology, University of the North, Sovenga, 0727

W.J. Veith*
Department of Zoology, University of Stellenbosch, Stellenbosch, 7600 Republic of South Africa

*To whom correspondence should be addressed

Received 10 June 1985; accepted 27 August 1985

The species of the genus *Clinus* are all live bearing with embryos developing intra-follicularly and in *C. superciliosus*, one of the largest clinids, Veith (1979a, 1979b, 1980) found extensive embryonic adaptations for the uptake of nutrients secreted by the follicular epithelium. Specialized embryonic absorptive structures have been described in other viviparous teleosts. The trophotaeniae in Goodeidae, Zoarcidae and ophidioid fishes (Turner 1940a; Bretschneider & De Wit 1947; Mendoza 1956, 1958, 1972; Wourms & Cohen 1975), the extended pericardial and yolk sac areas in Poeciliidae and Anablepidae (Turner 1938, 1940b; Scrimshaw 1944) and in most cases a hypertrophied embryonic gut plays a role in nutrient absorption (Igarashi 1962; Veith 1980).

Some of these specialized absorptive structures have been shown to possess microvilli which increase the surface area available for absorption of nutrients. The ophidioid fishes *Oligopus longhursti* (Wourms & Cohen 1975) and *Microbrotula randalli* (Lombardi & Wourms 1978) are studded with microvilli. Electron microscopic studies on absorptive structures of embiotocids (Dobbs 1974), poeciliids (Dépêche 1973), goodids (Mendoza 1972) and clinids (Veith 1980) all revealed concentrations of microvilli. The morphology of absorptive structures of viviparous species has thus been extensively studied, but very little physiological work on the relative importance of absorptive sites has been undertaken. Dépêche (1976) homogenized whole embryos of *Poecilia reticulata* to demonstrate uptake of labelled L-leucine and Veith (1980) reported uptake of [6-^{3}H] thymidine by embryonic structures of *C. superciliosus*.

In the present study the site of nutrient absorption as well as the relative importance of embryonic absorptive tissues present in *C. dorsalis* was investigated autoradiographically.

Materials and Methods

Female specimens were collected at the Strand, a locality in False Bay (34°07'S/18°49,5'E). They were caught by hand or with a hand net, and transported live to the laboratory where they were kept in 140-ℓ seawater tanks fitted with undergravel filters, a Hykro protein skimmer and a Turbinette filter.

Embryonic nutrient uptake

Nutrient uptake by the embryos was examined autoradiographically using the technique described by Veith (1980).

Fourteen gravid fish were injected subcutaneously with 1 μCi/g (1 Ci = 34,4 GBq) L-[4,5-^{3}H] leucine supplied by the Radiochemical Centre, Amersham. Injections were administered with a Terumo microsyringe above the lateral

line half-way along the trunk and the specimens were then killed as per the schedule outlined in Table 1. After removal of the reproductive tracts, the embryos of one of the two specimens, killed at each time interval, were removed from their follicular sacs and fixed in Bouin's Fixative. The reproductive tract of the other specimen was fixed intact in the same fixative. The material was then subjected to standard histological procedure using an embedding medium of 3 g Phenanthrene to 100 g Paraplast Plus. Longitudinal sections of 8 μm of embryos ranging from 7,5–17 mm standard length were made, whilst the intact gonad was serially sectioned at 8 μm.

Table 1 Record of sacrifice time after injection of 1 μCi/g L-[4,5-^{3}H] leucine

Specimen	Mass of fish (g)	Sacrifice time (Hours after injection)
1 and 2	0,978 and 0,994	2,5
3 and 4	1,002 and 0,989	6
5 and 6	0,921 and 0,901	12
7 and 8	1,013 and 0,994	24
9 and 10	1,024 and 0,993	30
11 and 12	1,001 and 0,975	48
13 and 14	0,982 and 0,941	60

Coating of sections with Kodak NTB_3 nuclear tracking emulsion was carried out in the darkroom using techniques described by Gude (1968) and Veith (1980). The emulsion was allowed to dry for 2 h after which the slides were placed in light-tight boxes containing silica gel as a drying agent. The boxes were sealed with masking tape, placed in black photographic bags and stored in a refrigerator at 4°C for a period of ten days before being developed. All sections were subsequently stained with azorcarmine.

The uptake of L-[4,5-^{3}H] leucine was quantified by using an Ernst Leitz Mikrometer grid of 25 mm^2 and counting the exposure dots produced on the autoradiograph over embryonic gut, epidermal and trunk muscle tissue. For this purpose the mean of ten counts for each tissue type minus a background count, determined on the sample, was used as a measure of radioactive uptake in both whole ovaries and individual embryos.

Histology of the embryos

For the purpose of an *in situ* histological examination of the embryonic structure, whole ovaries were removed from six gravid females, fixed in Bouin's, embedded in a mixture of Phenanthrene and Paraplast Plus and serially sectioned at 8 μm. In addition, embryos were removed from a further six ovaries and allowed to uncoil. The embryos were arbitrarily divided into five size classes ranging from 2 mm to 22 mm standard length, with 3-mm intervals between groups. Six embryos of each size class were then fixed and embedded as described above and longitudinally sectioned at 7 μm.

Scanning electron microscopy of the embryos

Embryos were removed from six ovaries and grouped into size classes as described above. They were fixed in Bouin's Fixative, dehydrated in alcohol and subsequently taken through a series of Amyl Acetate solutions ranging from 10% to absolute Amyl Acetate and critically point dried in a Balzers Union CPD 010 critical point dryer, using the carbon dioxide fluid-replacement method.

Dried specimens were transferred to specimen holders, coated with Gold-Paladium in a Hammer sputter coater and examined with a Jeol JSM-35 scanning electron microscope.

Results and Discussion

As is the case in *C. superciliosus* (Veith 1979a), gestation in *C. dorsalis* is intrafollicular and embryos in various stages of development occur simultaneously in the same ovary. It is interesting to note that the spawning size of *C. dorsalis* embryos (18,0–21,9 mm) is similar to that reported for *C. superciliosus* although the latter species is much larger, attaining a body mass of up to 120 g.

Embryonic nutrient transfer

Embryotrophe secreted by the follicular epithelium of *C. dorsalis* is particularly rich in lipids and amino acids suggesting major nutritional roles for these two components (Cornish 1983). In the present investigation it was found that the major sites of L-[4,5-^{3}H] leucine uptake by the embryos, were the epidermis and the gut. The amino acid is, however, first concentrated in the follicular epithelium itself (Figures 1a, 1b). Certain areas of the follicular epithelium are sometimes hypertrophied to form a thick region presumably active in the secretion of nutrients.

Gut

The embryonic gut plays the major role in the nutrition of the developing embryos. Veith (1979) found that the yolk sac provides nutrients in the earliest stages of the development of *C. superciliosus*. However, no yolk-containing yolk sac was found in *C. dorsalis* and embryos thus rely solely on embryotrophe for nutrients. Six hours after injecting the parent fish with tritiated leucine the embryonic gut showed moderate uptake of the amino acid. Uptake increased with time, being substantial 48 h after injection (Figures 1c, 1d).

Epidermis

In the smallest embryos (2,0–9,9 mm) the epidermis plays an important part in the uptake of nutrients, as the embryonic gut has not developed sufficiently. Epidermal uptake of leucine was noted 6 h after injection of the parents, becoming particularly noteworthy after 48 h (Figures 1c, 1d). A quantitative analysis of the leucine uptake by embryonic gut, epidermis and trunk muscles revealed that epidermal tissue absorbed more leucine per unit area than did gut tissue (Figure 2). Muscle tissue was taken into account as a reference, as there is no direct contact of the muscles with embryotrophe. It is evident that the quantity of leucine absorbed by gut and epidermal tissue increased with time, reaching a maximum after 48 h. After 60 h there is a decline in the exposures counted, being particularly dramatic in the case of gut epithelium. This fall-off can probably be ascribed to depletion of the quantity of leucine injected. The fact that gut absorption is lower per unit area than epidermal absorption is misleading, as the total surface area of the gut is probably much greater than that of the epidermis in view of the convolutions, villi and dense brushborder of microvilli present in the gut. Moreover, if only gut microvilli were taken into account, rather than total epithelial surface, gut absorption would probably be comparable to epidermal absorption (Figures 1c, 1d).

Embryonic adaptions for viviparity

As the embryonic gut and epidermis are the principal areas

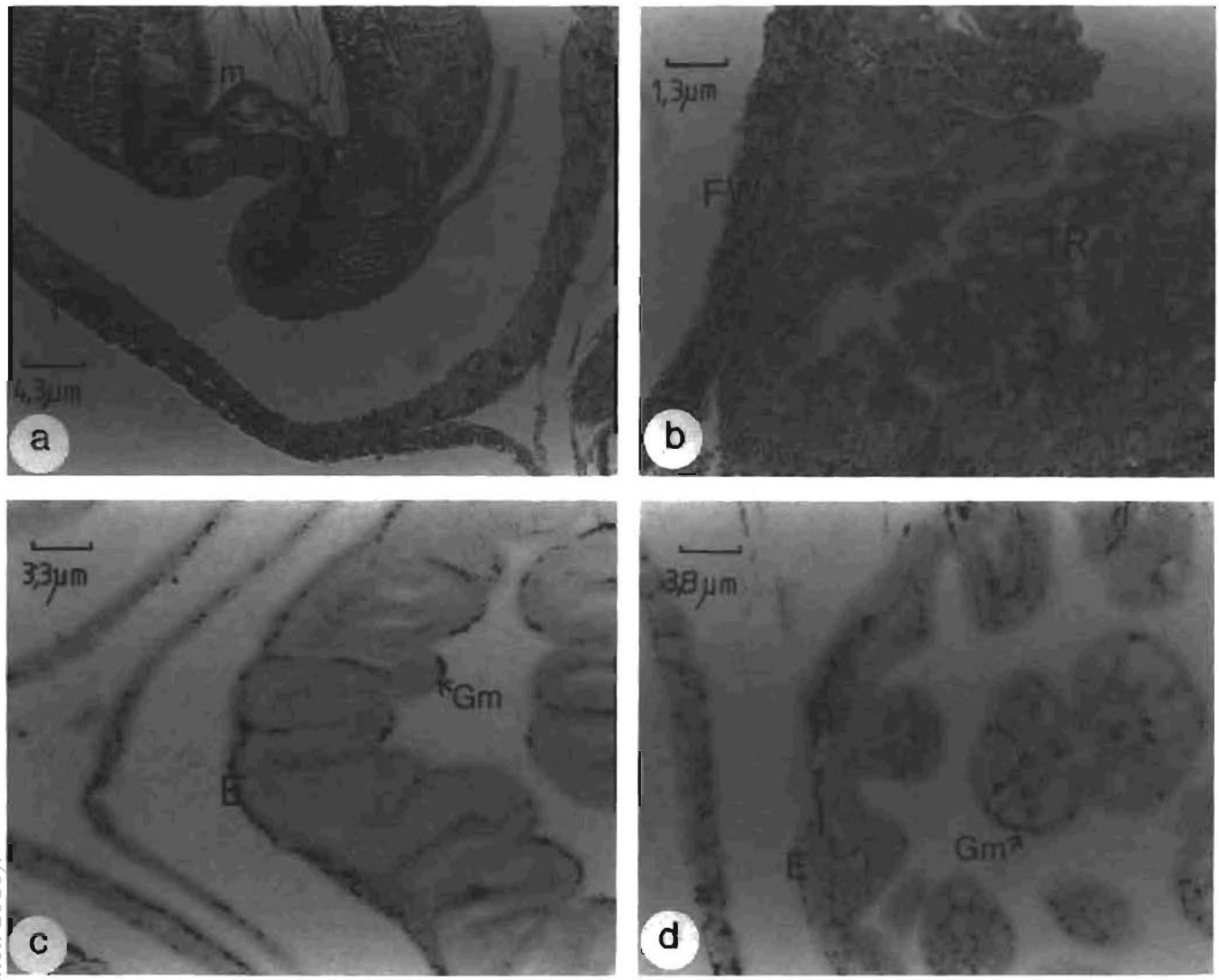

Figure 1 (a) Autoradiograph of a cross-section through the ovary of *C. dorsalis* showing moderate uptake of leucine by the follicle wall 6 h after the injection. FW — follicle wall; Em — developing embryo. (b) Autoradiograph of a cross-section through the ovary of *C. dorsalis* showing extensive uptake of leucine by the follicle wall 12 h after injection. FW — follicle wall; TR — thick region of follicular epithelium. (c) Autoradiograph of a cross-section through the ovary of *C. dorsalis* showing leucine uptake by the embryonic epidermis and anterior gut villi 48 h after injection. E — epidermis; Gm — gut microvilli brushborder. (d) Autoradiograph of a cross-section through the ovary of *C. dorsalis* showing leucine uptake by the epidermis and posterior gut villi 60 h after injection.

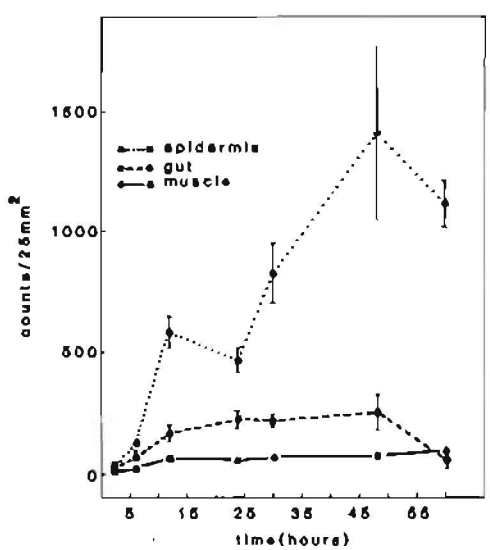

Figure 2 Distribution of tritiated leucine in the gut, muscles and epidermis of *C. dorsalis* embryos at various time intervals after injection of the parent.

for nutrient absorption, a histological and scanning electron microscopic study of these areas was undertaken.

Gut

The embryonic gut of *C. dorsalis* is a hypertrophied sac-like structure occupying virtually the whole of the body cavity. Initially the gut consists of a single sac but with increase in embryo size it differentiates into distinct anterior and posterior regions separated by a sphincter muscle (Figures 3a, b & c). Unlike the anterior gut region, the posterior region is characterized by numerous goblet cells in the columnar epithelium.

In embryos smaller than 12 mm there is a subepidermal vascular sinus between the outer surface of the gut and the epidermis (Figure 3a). This sinus initially projects into the gut villi but in embryos larger than 5 mm a capillary loop is formed in each villus (Figure 3d). The gut of *C. dorsalis* embryos has numerous finger-like villi projecting into the gut lumen (Figures 3a & b) and in this regard resembles the gut of *Micrometrus minimus* (Dobbs 1974). Moreover, the gut epithelium has a distinct brushborder of microvilli

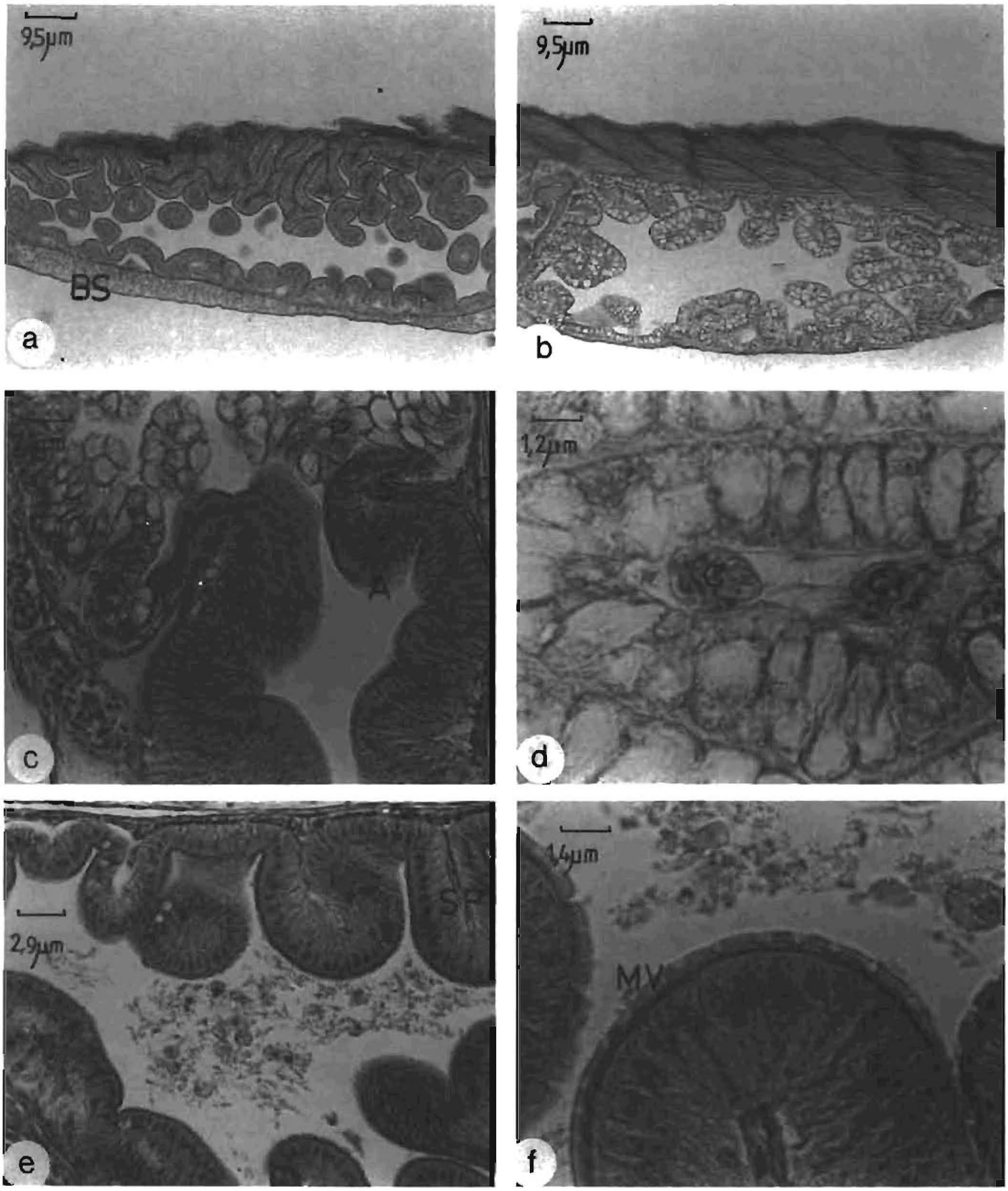

Figure 3 (a) Longitudinal section through a 10,2-mm embryo showing the anterior gut region and the sub-epidermal blood sinus. BS — blood sinus. (b) Longitudinal section through a 10,2-mm embryo showing the posterior gut region. Note the numerous goblet cells and absence of the subepidermal blood sinus. (c) Longitudinal section through a 9,4-mm embryo showing the junction between the anterior (A) and posterior (P) gut regions. S — sphincter muscle; C — capillary. (d) Cross-section through a finger-like posterior gut villus of a 9,4-mm embryo, showing a section through the capillary loop. C — capillary. (e) Longitudinal section through the anterior gut region of a 14,1-mm embryo showing the villi with subepidermal vascular projection (SP). (f) Section through an anterior gut region of a 14,1-mm embryo, showing the microvilli brushborder. MV — microvilli.

(Figures 3e & f) which further increases the surface area for the absorption of nutrients.

The embryonic gut of *C. dorsalis* thus differs markedly from that of *C. superciliosus* in that it is larger, occupies most of the abdominal cavity even in small embryos and undergoes earlier differentiation into distinct gut regions.

Epidermis

The role of the epidermis in absorption of nutrients is reflected in the surface topography of the embryos. Small

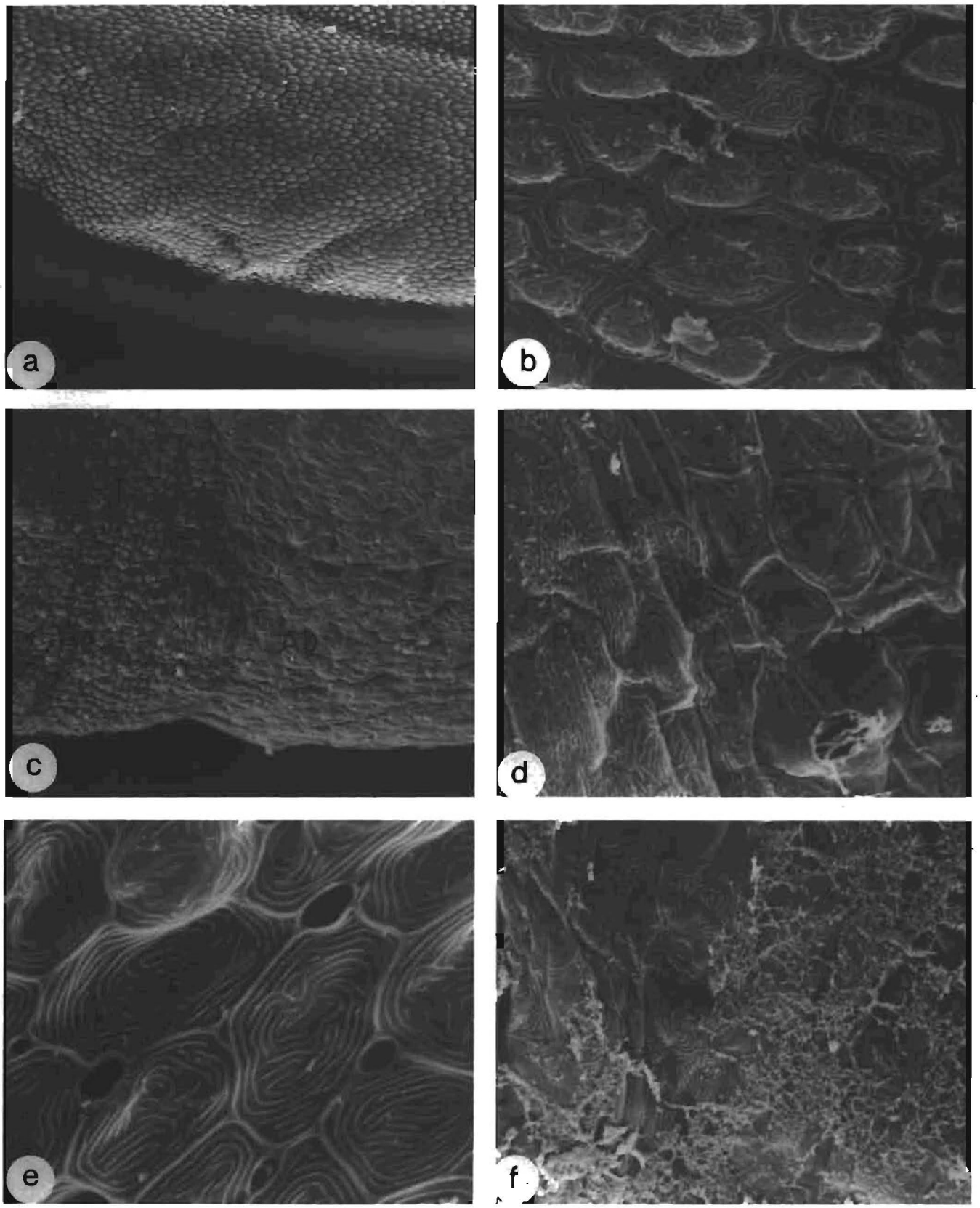

Figure 4 (a) Scanning electron micrograph of the pericardial region of a 3,5-mm embryo showing the epidermal surface projections (× 192,4). (b) Scanning electron micrograph of the epidermal surface projections on the pericardial region of a 3,5-mm embryo (× 1933). (c) Scanning electron micrograph of a 12,8-mm embryo contrasting the surface appearance of the pericardial (Pe) and the abdominal (Ab) regions (× 241,6). (d) Scanning electron micrographs of a 14,3-mm embryo contrasting the microridge pattern and abundance on the pericardial (Pe) and abdominal (Ab) regions (× 1611). (e) Scanning electron micrograph of the microridge pattern on the epidermal surface of a 20,3-mm embryo stripped of its cuticular coating (× 2416,5). (f) Scanning electron micrograph of the abdominal surface of a 20,4-mm embryo showing part of the cuticular coating overlying the epidermis (× 805,5).

embryos (2,0–5,9 mm) exhibit epidermal surface projections which are particularly apparent on the ventral surface of the pericardial region (Figure 4a). The surface appearance of *C. dorsalis* embryos differs markedly from that described for

C. superciliosus. In the case of *C. superciliosus* the entire epidermis, excluding the head region and fins, is folded to produce large epidermal ridges which increase the surface area (Veith 1980). In *C. dorsalis* each surface projection is produced by the apical surface of a single cell and these cells possess numerous microridges (Figure 4b).

Cellular surface projections in *C. dorsalis* are virtually confined to the pericardial region of the embryo and are not nearly as numerous on the abdominal region (Figure 4c). Furthermore, the concentration of microridges in the pericardial region is also greater than in the abdominal region (Figure 4d). The total epidermal surface area available for the absorption of nutrients, in the case of *C. dorsalis*, is thus considerably smaller than in the case of *C. superciliosus* but is probably compensated for by the extensive gut hypertrophy specialization which was found in this species.

In pre-partum embryos (18,0–21,9 mm) it was found that the epidermal surface projections had disappeared, thus giving the embryo a smoother appearance and reduced epidermal surface area. The microridge pattern now also resembles that found in adult teleosts which secrete a cuticular mucopolysaccharide layer (Whitear 1970). Figure 4e shows the embryonic surface of a 20,3-mm embryo stripped of its cuticular coating. The cuticular coating is probably produced by the numerous goblet cells in the epidermis and it is noteworthy that it is deposited prior to parturition. In Figure 4f part of the coating is visible.

Rendering surface embryonic exchange surfaces non-functional prior to or just after parturition probably has adaptive value for teleosts and also occurs in other viviparous species. In the goodid fishes the trophotaeniae are resorbed just prior to or just after parturition (Turner 1933, 1937; Mendosa 1937), in embiotocids blood is shunted away from the fin-web capillary bed at birth (Webb & Brett 1972a, 1972b) and in *Clinus superciliosus* epidermal macroridges disappear prior to birth and according to Veith (1980) this is seen as an osmotic adaptation as teleosts are either hypo-osmotic or hyperosmotic to their environment depending on whether they inhabit a marine or a freshwater environment.

Acknowledgements

We are indebted to the Council for Scientific and Industrial Research for supporting this study financially.

References

BRETSCHNEIDER, L.E., & DE WIT, J.J.D. 1947. Monograph on the process of research in Holland during the war. No. 11, Amsterdam.

CORNISH, D.A. 1983. Reproductive biology and viviparity in the teleost *Clinus dorsalis*. (Perciformes: Clinidae). M.Sc. dissertation. Zoology Department, University of Stellenbosch.

DÉPÊCHE, J. 1973. Infrastructure superficielle de la vésicule vitellaire et du sac péricardique de l'embryon de *Poecilia reticulata*. (Poisson Téléostéen). *Z. Zellforsch. Mikrosk. Anat.* 141: 235–253.

DÉPÊCHE, J. 1976. Acquisition et limites et l'autonomie trophique embryonnaire au cours du développement du poisson téléostéen vivipare *Poecilia reticulata*. *Bull. Biol. Fr. Belg.* 110(1): 45–97.

DOBBS, G.H. 1974. Scanning electron microscopy of intraovarian embryos of the viviparous teleost *Micrometrus minimus* (Gibbons), (Perciformes: Embiotocidae). *J. Fish. Biol.* 7: 209–214.

GUDE, W.D. 1968. Autoradiographic techniques. Localization of radioisotopes in biological material. Prentice-Hall Inc. Englewood Cliffs, New Jersey.

IGARASHI, T. 1962. Morphological changes of the embryos of a viviparous teleost, *Neoditrema ransonneti* during gestation. *Bull. Fac. Fish. Hokkaido Univ.* 13: 47–52.

LOMBARDI, H. & WOURMS, J.P. 1978. SEM of trophotaeniae, embryonic adaptations in an ophidioid, *Microbrotula randalli* and other viviparous fishes. *Am. Zool.* 18: 607.

MENDOZA, G. 1937. Structural and vascular changes accompanying the resorption of the proctodaeal processes after birth in the embryos of the Goodeidae, a family of viviparous fishes. *J. Morphol.* 61: 95–125.

MENDOZA, G. 1956. Adaptations during gestation in the viviparous cyprinodont teleost, *Hubbsina turneri*. *J. Morphol.* 99: 73–96.

MENDOZA, G. 1958. The fin fold of *Goodea luitpoldi*, a viviparous cyprinodont teleost. *J. Morphol.* 103: 534–560.

MENDOZA, G. 1972. The fine structure of an absorptive epithelium in a viviparous teleost. *J. Morphol.* 136: 109–115.

SCRIMSHAW, N.S. 1944. Embryonic growth in the viviparous poeciliid *Heterandria formosa*. *Biol. Bull. (Woods Hole, Mass.)* 87: 37–51.

TURNER, C.L. 1933. Viviparity superimposed upon ovoviviparity in the Goodeidae, a family of cyprinodont teleost fishes of the Mexican Plateau *J. Morphol.* 55: 207–234.

TURNER, C.L. 1937. The trophotaeniae of the Goodeidae, a family of viviparous cyprinodont fishes. *J. Morphol.* 61: 495–515.

TURNER, C.L. 1938. Adaptations for viviparity in embryos and ovary of *Anableps anableps*. *J. Morphol.* 62: 323–349.

TURNER, C.L. 1940a. Pericardial sac, trophotaeniae, and alimentary tract in embryos of goodeid fishes. *J. Morphol.* 67: 271–283.

TURNER, C.L. 1940b. Follicular pseudoplacenta and gut modifications in anablepid fishes. *J. Morphol.* 67: 91–101.

VEITH, W.J. 1979a. Reproduction in the live-bearing teleost *Clinus superciliosus*. *S. Afr. J. Zool.* 14(4): 208–211.

VEITH, W.J. 1979b. The chemical composition of the follicular fluid of the viviparous teleost *Clinus superciliosus*. *Comp. Biochem. Physiol.* 32A: 37–40.

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

WEBB, P.W. & BRETT, J.R. 1972a. Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. *J. Fish. Res. Bd. Can.* 29: 1525–1542.

WEBB, P.W. & BRETT, J.R. 1972b. Oxygen consumption of embryos and parents and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Racochilus vacca* and *Embiotoca lateralis*. *J. Fish. Res. Bd. Can.* 29: 1543–1553.

WHITEAR, M. 1952. The innervation of the skin of teleost fishes. *Q.J. Microscop. Sci.* 93: 289–305.

WHITEAR, M. 1970. The skin of bony fishes. *J. Zool. Lond.* 160: 437–454.

WOURMS, J.P. & COHEN, D.M. 1975. Trophotaeniae embryonic adaptations, in the viviparous ophidioid fish, *Oligopus longhursti*: a study of museum specimens. *J. Morphol.* 147: 385–402.