

Placentation in Garter Snakes. III. Transmission EM of the Omphalallantoic Placenta of *Thamnophis radix* and *T. sirtalis*

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ABSTRACT The omphalallantoic placenta is a complex organ that is unique to viviparous squamates. Using transmission EM and light microscopy, we examined this placenta in garter snakes in order to understand its structural organization and functional capabilities. The omphalallantoic placenta is formed from the uterine lining and the bilaminar omphalopleure, the latter of which is associated with the isolated yolk mass and allantois. A thin shell membrane separates the fetal and maternal tissues throughout gestation. The uterine epithelium contains cuboidal cells with large droplets or granules and appears to be secretory. Epithelium of the omphalopleure is specialized for absorption and contains cells with prominent microvilli and others with large cytoplasmic droplets or granules. The brush-border cells are rich in mitochondria and Golgi bodies and interdigitate extensively with adjacent cells, forming elaborate intercellular canaliculi. Their morphology is consistent with their proposed role in sodium-coupled water movement. During development, the isolated yolk mass becomes depleted as yolk droplets are digested by cells of the omphalopleure and allantois. However, the allantois does not fuse to or vascularize the inner face of the omphalopleure. Consequently, the distance between fetal and maternal circulatory systems remains large (about 250–300 μm), precluding efficient gas exchange and hemotrophic transfer. The morphology of the omphalallantoic placenta strongly suggests that it functions in nutrient transfer through uterine secretion and fetal absorption. *J. Morphol.* 256:187–204, 2003.

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KEY WORDS: placenta; yolk sac; allantois; oviduct; fetal membranes; viviparity

The striking contrast between the omphalallantoic placenta and the chorioallantoic placenta of *Thamnophis* indicates that they are specialized for distinctly different functions—the former for histotrophic transfer, the latter for physiological exchange between maternal and fetal circulatory systems.

Among viviparous reptiles and mammals, several embryonic structures are available for physiological exchange with maternal tissues. Among them are the chorion, the chorioallantois, and various structures derived wholly or in part from the yolk sac (Yaron, 1985; Mossman, 1987; Stewart and Blackburn, 1988; Stewart, 1993, 1997). A persistent an-

thropocentric perspective probably accounts for the peculiar notion that the chorioallantoic placenta invariably is the sole placenta, or, at least, the “definitive” placenta. A corollary of this perspective is that effective placental exchange must be hemotrophic, i.e., that physiological transfer only occurs between uterine and fetal circulatory systems. Countering such views is the fact that vascular and avascular yolk sac placentas are widespread among mammals, and not just characteristic of marsupials but also rodents, bats, and insectivores, among others (Ramsey, 1975; Lockett, 1977; Mossman, 1987). Physiological and experimental studies have shown that yolk sac placentas can play important roles in the histotrophic transfer of nutrients (Noer and Mossman, 1947; Enders et al., 1976; Mossman, 1987).

Placentas formed out of the yolk sac appear to be universal among viviparous lizards and snakes. In fact, much anatomical work over the past 15 years has been devoted to clarifying the development, structural organization, and interspecific diversity of such placentas (e.g., Stewart, 1985, 1990, 1993; Stewart and Blackburn, 1988; Blackburn, 1993a; Blackburn and Callard, 1997; Stewart and Thompson, 1994, 1996, 1998, 2000; Flemming and Branch, 2001). While functions of these placental organs require further study, anatomical investigations have demonstrated that they can be sites of impressive cytological specializations that suggest roles in physiological exchange (Villagran-Santa Cruz, 1989; Stewart, 1990, 1993; Stewart and Thompson, 1996, 1998).

This article draws on transmission EM (TEM) to investigate the cytology and structural organization of the omphalallantoic placenta in *Thamnophis ra-*

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dix and *T. sirtalis*. The omphalallantoic placenta is an organ that is unique to viviparous squamates and which therefore has no equivalent among mammals. Its fetal component develops through apposition of the allantois to the inner face of the omphalopleure and isolated yolk mass (Stewart and Blackburn, 1988). As a consequence, although the omphalopleure is inherently avascular, in many or most squamates it becomes vascularized by allantoic vessels (Stewart, 1993). Thus, the omphalallantoic placenta is derived from components of the yolk sac as well as the allantois.

Previously published work on the omphalallantoic placenta in *Thamnophis* is largely confined to studies using light microscopy and scanning EM (SEM) (Hoffman, 1970; Blackburn et al., 2002); three micrographs derived from TEM have also been published (Hoffman, 1970). Because the omphalallantoic placenta is a complex structure with diverse components, a complete ultrastructural characterization of its components throughout development will require extensive study. This article focuses primarily on the maternal–fetal interface and certain other features relevant to physiological exchange across the placenta and fetal membrane function.

MATERIALS AND METHODS

Procedures are described in detail elsewhere (Blackburn and Lorenz, 2003). In brief, pregnant female *Thamnophis radix* and *T. sirtalis* from a commercial supplier in Wisconsin were housed in aquaria under conventional conditions (24°C; 14:10 L:D light cycle). From snakes sacrificed via Nembutal injection, reproductive tracts were harvested, dissected into pieces, and fixed in a modified Karnovsky's solution for 2 h. Following postfixation in osmium tetroxide and en bloc staining in aqueous uranyl acetate, tissue pieces were dehydrated and embedded in epoxy resin (Blackburn and Lorenz, 2003). The tissue was sectioned on glass knives and stained with Azur II / methylene blue. Thin sections (80 nm) were cut on diamond knives, collected on copper mesh and formvar-coated slot grids, and stained with lead citrate and uranyl acetate. Sections were examined and photographed using an Olympus BH-2 compound microscope (thick sections) and a Zeiss EM900 transmission EM (thin sections). Fetuses were staged developmentally by means of Zehr's (1962) classification system. Reported magnifications are of the actual photomicrographs, as calculated from the final photographic prints.

RESULTS

General Observations

The snake embryos in our study included Zehr stages 30–31 (mid-gestation) and 36–37 (late gestation). During the stages examined the omphalallantoic membrane lies in the ventral or abembryonic (anti-mesometrial) hemisphere of the egg, whereas the chorioallantois occupies the dorsal, mesometrial hemisphere. The omphalallantoic membrane consists of the omphalopleure and isolated yolk mass, with the allantois lying adjacent to its internal aspect. Figure 1, based in part on descriptions in the literature (see Stewart and Blackburn, 1988), is

largely consistent with our observations. As shown, the allantois has penetrated the yolk cleft, which is continuous with the exocoelom. Inside the yolk cleft, the inner and outer membranes of the allantois lie in contact, obliterating the allantoic lumen. We find that the allantois does not fuse to the inner surface of the isolated yolk mass and is only attached to it around the periphery. Thus, it does not vascularize the omphalopleure. The isolated yolk mass is easy to visualize in the osmium-fixed tissue, due to its black color. At Zehr stage 30, it is not as thick as implied in the diagram (Fig. 2). By the latest developmental stages (stage 37), it is largely depleted in many regions, with the result that the omphalopleure contains sparse patches of yolk.

The omphalallantoic placenta consists of uterine tissue in association with the omphalallantoic membrane. Likewise, the allantoic placenta consists of the uterus in apposition to the chorioallantois. From dissection and microscopic examination, we find that the omphalopleure and uterine lining are not attached and remain separated by the vestigial shell membrane until the end of gestation.

Mid-Gestation

Light microscopy. The omphalallantoic membrane complex is formed from the omphalopleure, the isolated yolk mass, and the allantois (Fig. 2). The omphalopleure itself is avascular and the isolated yolk mass separates it from the closest blood supply, that of the allantois. The omphalopleure is developmentally bilaminar, consisting of an epithelium (ectoderm) and yolk endoderm, associated with the isolated yolk mass. The epithelium consists of palely staining, cuboidal cells. Immediately beneath the epithelium lies the isolated yolk mass. Its yolk droplets are spherical and ovoid structures that vary in size from minuscule granules to large platelets of about 80–95 μm in diameter. On sections stained with Azure II / methylene blue, the large platelets appear yellowish, whereas the small granules stain intensely blue. Pale-staining cells of the yolk endoderm lie interspersed between the yolk droplets. The allantois is not attached to the inner surface of the isolated yolk mass, but is separated from it by the yolk cleft. The allantois in this region is very sparsely vascularized. Small, isolated yolk platelets are attached to its outer surface (i.e., the surface facing the omphalopleure) (Fig. 2). Some of the platelets appear to be embedded in the allantoic membrane itself.

A thin, but prominent, darkly staining shell membrane separates the omphalopleure from the inner lining of the uterus (Fig. 2). In section, the shell membrane takes an undulating path and, in some areas, folds back on itself. The uterine epithelium lies adjacent to the shell membrane. The uterine epithelial cells are very palely staining and vacuolated, with lightly staining nuclei. In some areas the

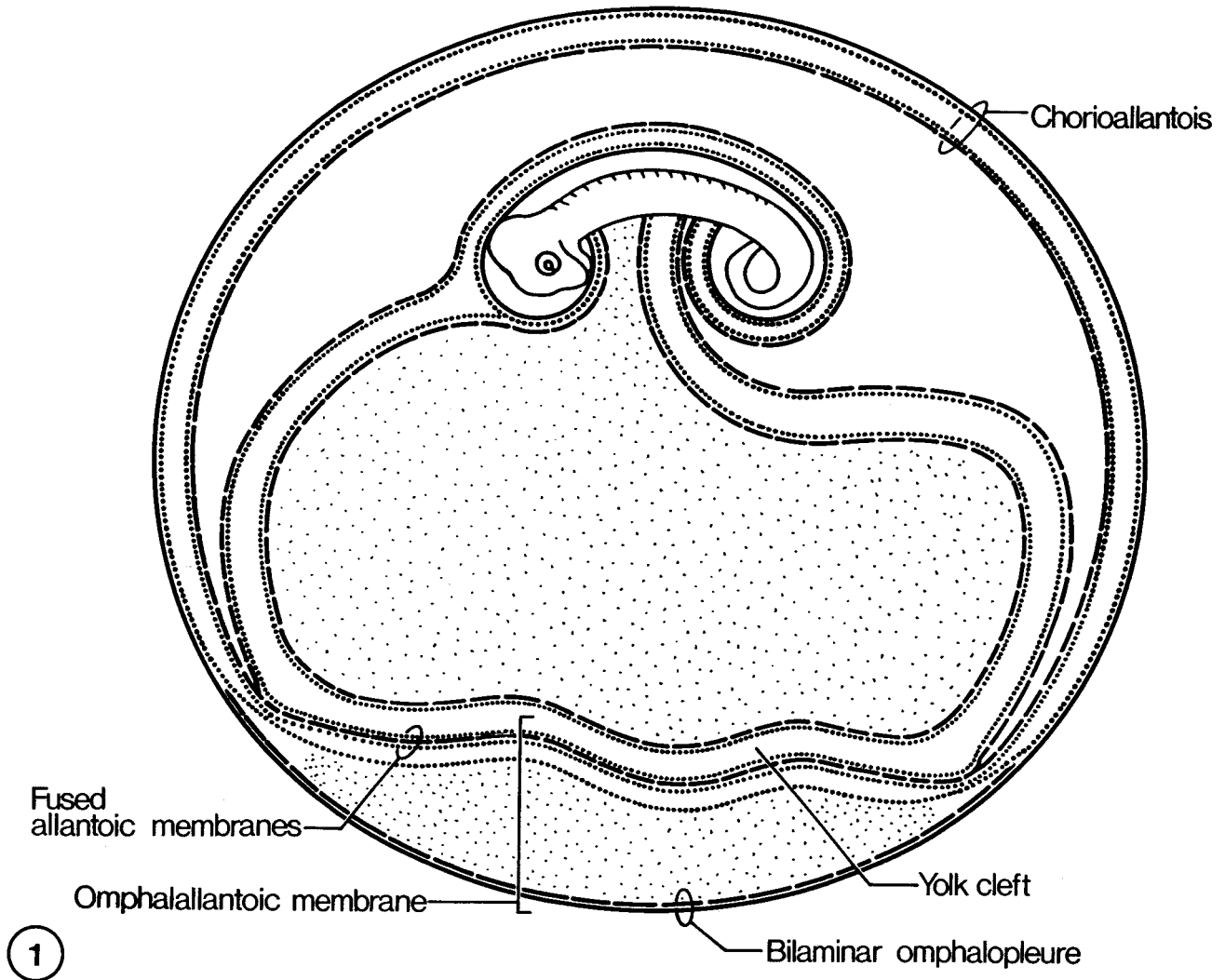


Fig. 1. Diagram of the fetal membranes in *Thamnophis sirtalis*. Two membranes surround the egg—the chorioallantois and omphalallantoic membrane. The latter is formed through invasion of the allantois into the yolk cleft (Stewart and Blackburn, 1988). The membranes are the fetal components of the chorioallantoic placenta (allantoplacenta) and omphalallantoic placenta. During development the isolated yolk mass progressively diminishes in thickness.

uterine lining is thrown up into folds or papillae (Fig. 2). Small blood vessels are sparsely distributed beneath the uterine epithelium. A thin muscularis externa forms the external surface of the uterine tissue.

TEM. Uterine tissue in the anti-mesometrial (ventral) hemisphere of the egg exhibits the standard components of the squamate uterus: an epithelium, lamina propria, and muscularis externa. The epithelial cells are cuboidal, with large basal nuclei and small, irregular microvilli (Fig. 3). Large vacuoles fill the cytoplasm in our specimens, representing granules or droplets whose contents were extracted during processing. Very small vesicles occur at the apex of the epithelial cells, some of which open into the uterine lumen. The vesicles contain an electron-lucent secretion and their contents therefore appear different than the cytoplasmic vacuoles.

The uterine epithelial cells are attached laterally at their apices by tight junctions. The cells rest on a basal lamina, beneath which lie the lamina propria and uterine blood vessels.

Material of the shell membrane separates the uterine and extraembryonic tissue at the abembryonic pole. It consists of fibers of various sizes, intermixed with granular material and debris of unknown composition (Fig. 4). In the abembryonic hemisphere, the shell membrane is approximately 2–4.5 μm thick.

On the fetal side of the placenta, the surface of the omphalopleure is lined by cuboidal cells of two distinct types, one with microvilli, the other with large cytoplasmic droplets or granules (Figs. 5–7). Brush-border cells have irregular microvilli that extend into the uterine lumen towards the shell membrane. Their cytoplasm is rich in mitochondria, Golgi bod-

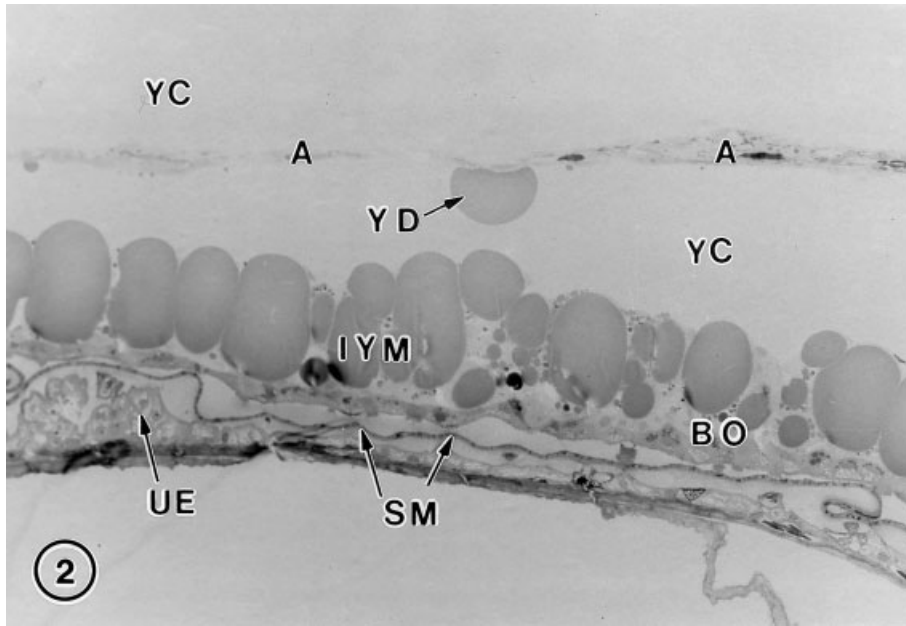


Fig. 2. Omphalallantoic placenta, *Thamnophis radix* (embryonic stage 30). The shell membrane (SM) delineates the maternal–fetal interface. The uterine epithelium (UE) is thrown up into papillae and ridges in some regions. On the fetal side of the placenta, the bilaminar omphalopleure (BO) overlies the yolk droplets of the isolated yolk mass (IYM). The allantois (A) occupies the yolk cleft (YC), but is not attached to the isolated yolk mass. A yolk droplet (YD) adheres to the outer surface of the allantois. Azur II / methylene blue. $\times 170$.

ies, and small rounded vesicles (Fig. 8). The nuclei are palely staining, ovoid structures without evident nucleoli, and occupy a basal position in the cell. The brush-border cells commonly lie surrounded by the granular cells (Figs. 5, 7). The granular cells are easily distinguished by the fact that their cytoplasm is full of large granules or droplets, which may be lipid inclusions (see Hoffman, 1970). Their cytoplasm contains modest numbers of mitochondria and numerous tiny vesicles. The granular cells have sparse, irregular microvilli that are much shorter than those of the brush-border cells. The epithelial cells are attached to one another at their apical borders by junctional complexes. Lateral borders of these cells interdigitate (Fig. 7) and adjacent cells are linked by desmosomes.

Beneath the epithelium of the omphalopleure lie the endodermal cells, interspersed with droplets of the isolated yolk mass (Fig. 2). The endodermal cells contain yolk droplets that vary widely in size (Fig. 9). These droplets appear as palely staining, round or ovoid structures that are not membrane-bound. The endodermal cells exhibit an abundance of elongate mitochondria, some of which are clustered around the lipid droplets, as well as Golgi bodies and numerous small vesicles. The cells exhibit oddly shaped, polymorphic nuclei.

As noted above, the inner and outer membranes of the allantois are fused together within the yolk cleft; thus, no allantoic lumen is present (Fig. 1). The resulting structure is shown in Figure 10. The outer surface of this double membrane faces the omphalopleure across the yolk cleft, whereas the inner surface faces the large body of yolk (vitellus). The outer surface of the membrane is lined by a thin layer of cells that extend small, irregular microvilli into the yolk cleft (Figs. 10, 11). These outer cells have a

granular cytoplasm with rough endoplasmic reticulum (ER), mitochondria, and small apical vesicles (Fig. 11). Adjacent cells are linked by tight junctions. Basally, the cells show unusual, irregular interdigitations with the underlying cells (Figs. 10, 11). Cells of the latter variety contain abundant ribosomal ER, as well as lipid droplets and mitochondria. Given the structure of the allantoic membrane (Blackburn and Lorenz, 2003), they presumably are endodermal in origin. At least two additional layers of cells contribute to the fused allantoic membrane (Fig. 10). These presumably represent mesodermal and endodermal cells of the inner, visceral leaf of the allantois, prior to fusion of the membranes.

Light microscopy reveals yolk droplets from the isolated yolk mass adhering to the outer surface of the allantoic membrane, where that membrane faces the isolated yolk mass across the yolk cleft (Fig. 2). Under TEM, extensions of the outer epithelial cells are seen attaching to the droplets (Fig. 11). Some yolk droplets appear to be in the process of being engulfed.

Late Gestation

Light microscopy. In terms of overall organization, the omphalallantoic membrane in late development is much like that of Zehr stage 30. However, the isolated yolk mass is considerably depleted, at least in some areas, and is represented by a few, isolated yolk platelets (Figs. 12, 13). The small, densely staining yolk granules such as were seen though light microscopy at Zehr stage 30 were not observed. Depletion of the isolated yolk mass has exposed the inner surface of the omphalopleure to the yolk cleft. The few remaining yolk droplets protrude into the cleft. The allantois is not fused or

apposed to the omphalopleure, but remains separated from it by the yolk cleft (Fig. 13). As a result, the omphalopleure is not vascularized. Occasional, small yolk droplets can be found attached to the outer (omphalopleuric) surface of the allantois, or embedded within it.

A prominent, darkly staining shell membrane separates fetal and maternal tissues at the placental interface (Figs. 12, 13). The shell membrane is folded and ridged, giving a scalloped, undulating appearance in section. It consists of a thin, darkly staining component (adjacent to the omphalopleure) and a thick, lighter-staining component (facing the uterus).

A simple cuboidal epithelium lines both the omphalopleure and the uterus. Cell detail is difficult to distinguish through light microscopy. The external surface of the omphalopleure exhibits ridges or folds that range from shallow to deep (Fig. 13). Some (but not all) of these ridges correspond to ridges in the shell membrane. The uterine tissue also exhibits shallow ridges or papillae in some regions (Fig. 13), although in other areas it appears flattened (Fig. 12). Compared to the earlier developmental stage, the uterus appears to be somewhat more vascular. However, capillaries are much less abundant than in the uterine portion of the adjacent allantoic placenta (Figs. 12, 13).

TEM. The uterine tissue in late pregnancy appears like that of mid-gestation. The epithelial cells are cuboidal elements with large cytoplasmic droplets, extracted in our specimens (Fig. 14). The apical cytoplasm exhibits small vesicles containing an electron-lucent material. The cells exhibit occasional, small, and irregular microvilli. They rest on a prominent basal lamina, beneath which lies the lamina propria and muscularis externa.

During advanced developmental stages the surface of the omphalopleure continues to be lined by two types of epithelial cells—the granular cells and brush-border cells (Fig. 15). As described earlier, the granular cells have large cytoplasmic droplets, possibly lipid in nature. The brush-border cells exhibit long slender microvilli, which extend apically as individual cell extensions that are not bound together by glycocalyx (Figs. 16, 17). Our observations suggest that the microvilli in late gestation may be longer than seen in mid-gestation; however, this inference needs to be corroborated. Cytoplasm of the brush-border cells exhibits abundant mitochondria and Golgi bodies. Laterally, the cells interdigitate extensively, with the extracellular space between cells forming elaborate intercellular channels (Fig. 17). Apically, they are bound to the surrounding cells by junctional complexes.

The shell membrane persists at the placental interface during late gestation. It consists of fibers interspersed with granular material (Fig. 15) and measures at least 2 μm in thickness. No regions were seen where the shell membrane had deteriorated,

such as characterizes the chorioallantoic placenta in late gestation (Blackburn and Lorenz, 2003).

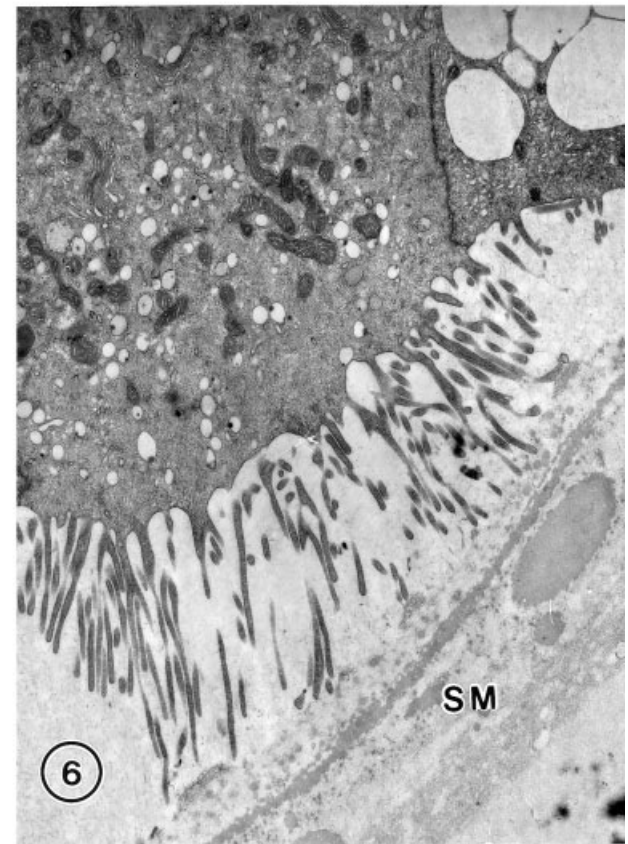
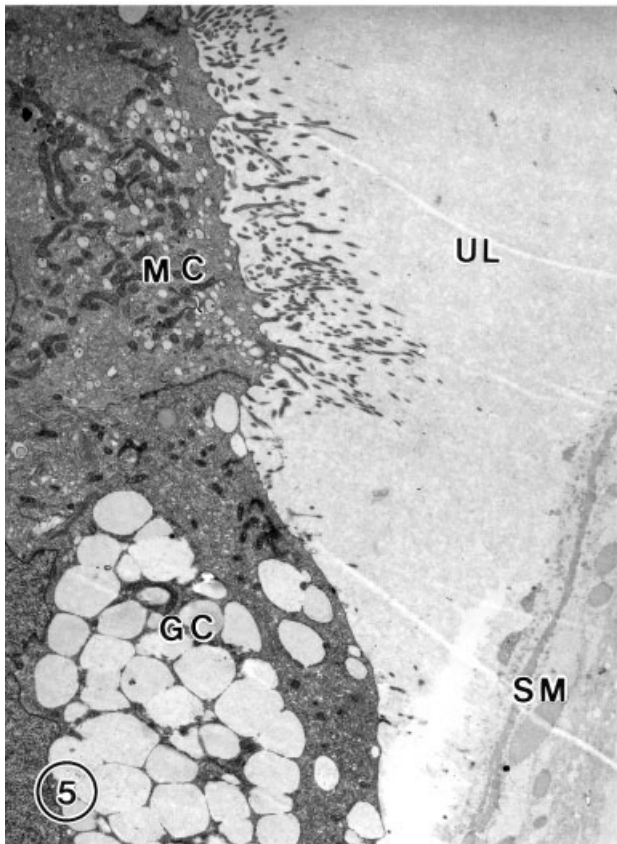
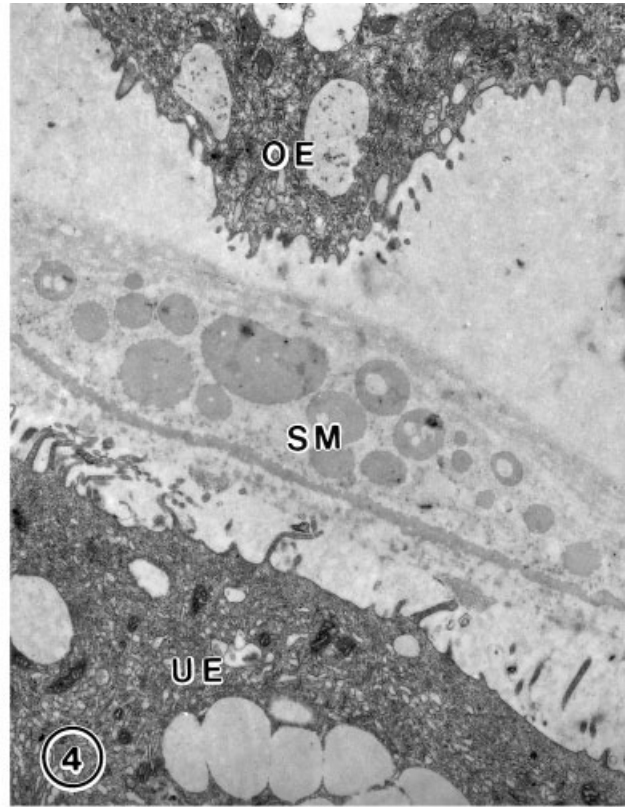
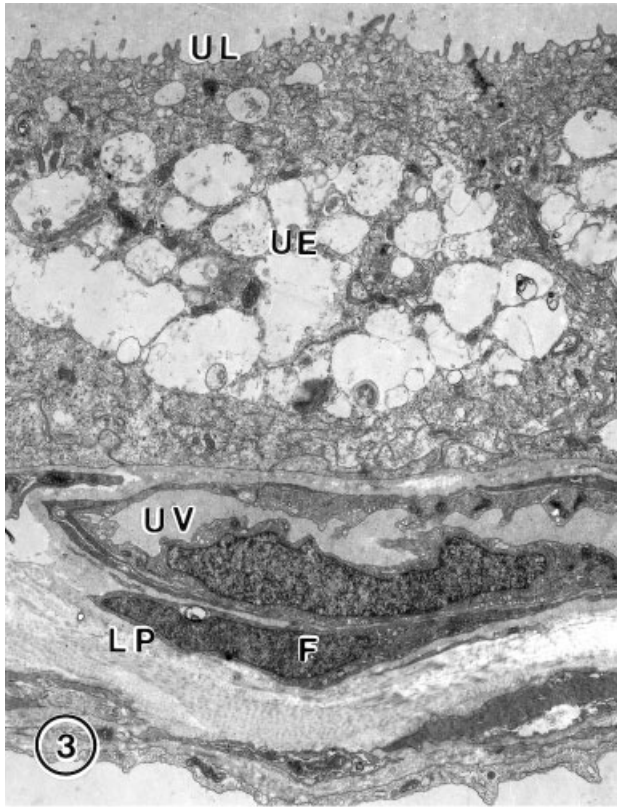
With the isolated yolk mass having been nearly depleted (Fig. 12), yolk primarily is found in intracellular locations. Endodermal cells of the omphalopleure contain yolk droplets and granules of various sizes. Their cytoplasm abounds with mitochondria, which often are aggregated around a droplet (Fig. 18). Also apparent are Golgi bodies and scattered ribosomes. Yolk droplets of various sizes also occur within the allantoic membrane (Figs. 19, 20). The surface endodermal cells of the allantois appear to surround yolk droplets with elongate cell extensions to take them into the cytoplasm (Fig. 19).

DISCUSSION

Ultrastructural examination reveals that the omphalallantoic placenta of garter snakes is anatomically very different from the chorioallantoic placenta, with specializations that reflect distinct functional attributes. Specifically, this placenta appears to be an organ specialized for secretion and absorption rather than for hemotrophic transfer. Our observations provide a basis for future experimental work on this placenta, as well as for descriptive studies focusing on details of developmental changes and functional morphology.

Fetal Membrane Development

Some familiarity with squamate patterns of embryonic development is necessary for an understanding of the structure of the omphalallantoic membrane in *Thamnophis*. Recent, illustrated accounts offer accessible descriptions of the development of the extraembryonic membranes (Stewart and Blackburn, 1988; Stewart, 1993, 1997). Figure 1, constructed to represent the situation in *Thamnophis sirtalis*, based largely on work by Hoffman (1970), shows the topographic arrangement of fetal membranes by mid-development. A major point to bear in mind is that in squamates (oviparous and viviparous alike), yolk sac development differs from that of all other vertebrates. During initial stages of development, ectoderm, endoderm, and mesoderm spread over the vitellus, forming a (trilaminar) choriovitelline membrane that lies close to the equatorial plane of the egg. In a pattern unique to squamates, mesoderm then invades into the vitellus and splits to form a space (the yolk cleft) that separates off an abembryonic segment of yolk material. This yolk material, the “isolated yolk mass,” lies in the abembryonic hemisphere of the egg. The cellular omphalopleure associated with it is formed from ectoderm and endoderm; thus, lacking mesoderm, the structure is avascular. However, in snakes and many lizards the allantois invades the yolk cleft and comes to line the inner face of the isolated yolk mass,



Figures 3-6

forming an “omphalallantoic membrane” (Fig. 1). In this way, the omphalopleure may become vascularized secondarily by allantoic vessels. The snake *Virginia striatula* appears to have modified the general pattern, through development of a secondary yolk cleft within the isolated yolk mass (Stewart, 1990). In some lizards, the allantois does not invade the yolk cleft and the omphalopleure remains entirely avascular (Stewart, 1985; Blackburn and Callard, 1997; Stewart and Thompson, 2000). Other departures from the pattern described above have been documented in particular lizard species (Boyd, 1942; Stewart and Florian, 2000; Stewart and Heulin, 2000).

General Observations

The snake fetuses used in this study ranged from Zehr stages 30–37. Previous studies of *Thamnophis sirtalis* and *T. ordinoides* have shown that the omphalallantoic placenta has been formed by stage 27 (Hoffman, 1970). Consequently, our specimens bracket most of the period during which the omphalallantoic placenta is present, missing only its very earliest and latest developmental stages.

At the developmental stages examined, our observations are largely consistent with previous descriptions of species of *Thamnophis* (Hoffman, 1970; Blackburn et al., 2002). As indicated in Figure 1, by mid-gestation the chorioallantois occupies the entire dorsal (embryonic) hemisphere of the egg and the omphalallantoic membrane occupies the abembryonic pole. Thus, two fetal membranes are available for placental formation in *Thamnophis* during the last half of gestation. The allantoic placenta is formed through apposition of the chorioallantois to the inner lining of the uterus. The omphalallantoic placenta is formed by combination of the omphalallantoic membrane and the uterine tissue (Figs. 2, 12, 13).

Although Figure 1 depicts the general arrangement of the fetal membranes, our samples indicate that by Zehr stage 30 the isolated yolk mass is not as thick as the figure implies. At that stage the isolated yolk mass largely appears to be represented by a single layer of yolk droplets (see Fig. 2). Given that

the isolated yolk mass is formed by Zehr stage 27 (Hoffman, 1970), perhaps it begins to diminish in thickness soon after its initial formation. Certainly by late development (e.g., stage 35–36), the isolated yolk mass is greatly depleted in some areas (Blackburn et al., 2002) (Fig. 12). Nevertheless, the yolk cleft continues to separate the allantoic membrane from the inner lining of the omphalopleure. The allantois does not fuse with the inner surface of the isolated yolk mass, but is only attached around the periphery of the latter. As a result, allantoic vessels do not invade the isolated yolk mass or vascularize the omphalopleure, both of which do occur in the only oviparous snake that has been examined (Blackburn et al., 2000).

Relationship of the Allantois and Omphalopleure

For several years researchers who work on squamates have carefully distinguished between species that develop an omphalallantoic membrane and those in which the allantois does not enter the yolk cleft (e.g., Stewart, 1985, 1993; Stewart and Blackburn, 1988; Blackburn and Callard, 1997; Stewart and Thompson, 2000). In viviparous species exhibiting the first situation, the resulting placenta is termed “omphalallantoic.” In species showing the latter arrangement, however, the omphalopleure is avascular and the term “omphaloplacenta” is applied. The situation observed in garter snakes does not entirely conform to either arrangement. Although the allantois is present within the yolk cleft, it remains separated from the omphalopleure by a tissue space (Fig. 1). As a result, the allantois (which itself is sparsely vascularized) does not vascularize the omphalopleure. As commonly used, the term “omphalallantoic membrane” arguably does not precisely describe the condition seen in *Thamnophis* at the developmental stages examined. We have no wish to confuse an already complicated situation with additional terminology. However, we would caution that casual application of the term “omphalallantoic membrane” risks combining species in which the omphalopleure is vascularized by the allantois with those in which the allantois enters the yolk cleft but does not contact or vascularize the omphalopleure.

One further complication is that, in the absence of a complete developmental series, we cannot be certain that *Thamnophis* never develops an arrangement between the allantois and omphalopleure that might subsequently be obscured, through formation of a secondary yolk cleft. In the thamnophine snake *Virginia striatula*, a secondary cleft forms within the isolated yolk mass, separating a portion of yolk that adheres to the outer face of the allantois within the yolk cleft (Stewart, 1990). While we observed no evidence of a secondary yolk cleft, its development offers one potential explanation for adherence of

Fig. 3. Uterine component of the omphalallantoic placenta, *Thamnophis radix* (embryonic stage 30). Cells of the uterine epithelium (UE) are large and heavily vacuolated. F, fibroblast; LP, lamina propria; UL, uterine lumen; UV, uterine vessel. $\times 5600$.

Fig. 4. Omphalallantoic placental interface, *Thamnophis radix* (embryonic stage 30). OE, epithelium of omphalopleure; SM, shell membrane; UE, uterine epithelium. $\times 11,000$.

Fig. 5. Omphalopleuric epithelium, *Thamnophis radix* (embryonic stage 30). GC, granulated cell; MC, microvilliated cell; SM, shell membrane; UL, uterine lumen. $\times 4400$.

Fig. 6. Microvilliated epithelial cell of the omphalopleure, *Thamnophis radix* (embryonic stage 30). SM, shell membrane. $\times 7300$.

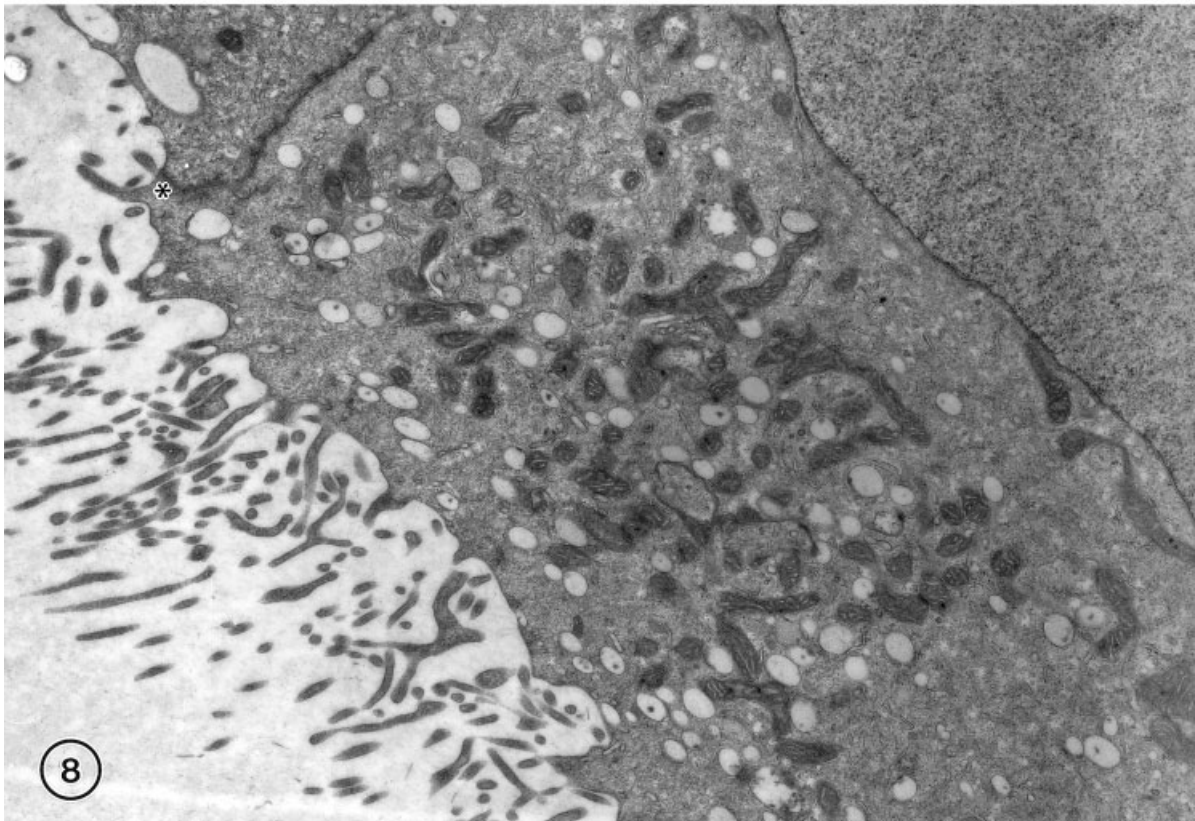
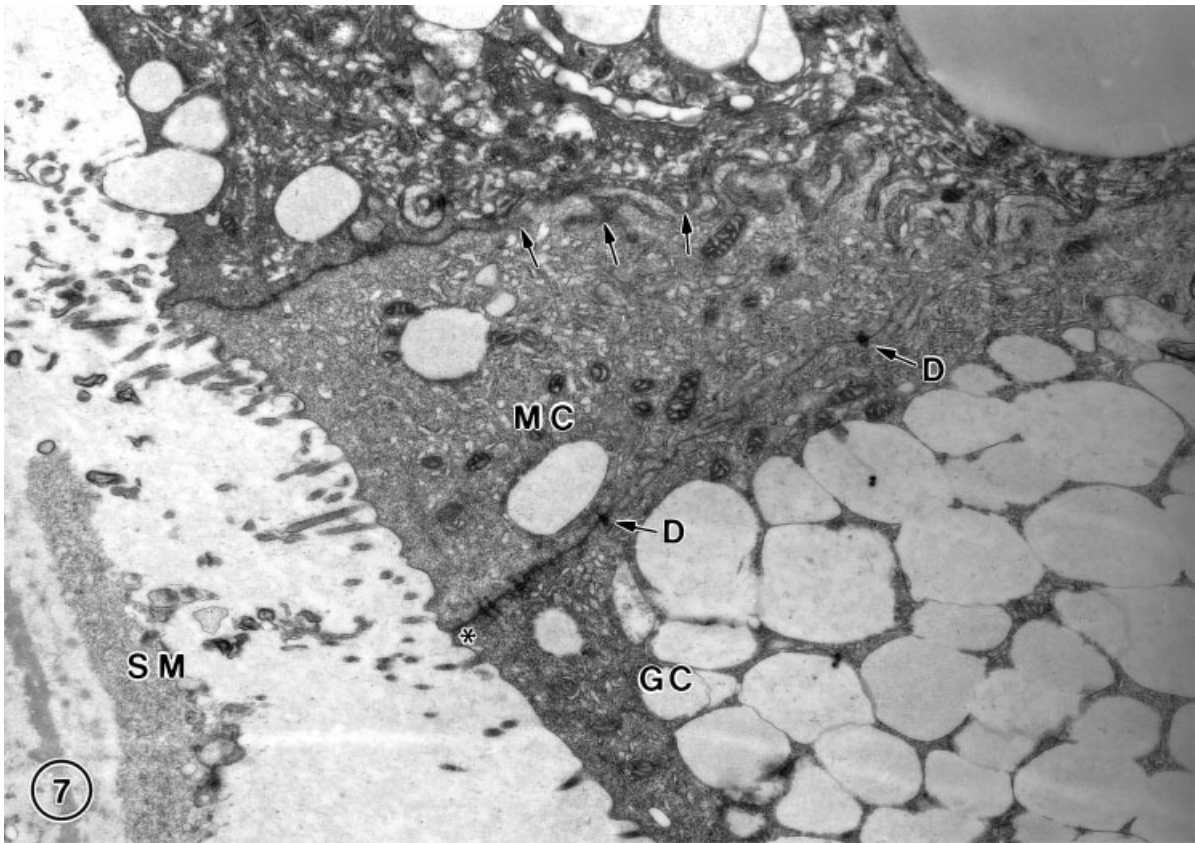


Fig. 7. Omphalopleuric epithelium, *Thamnophis radix* (embryonic stage 30). Adjacent cells are linked by desmosomes (D), and at their apices, tight junctions (asterisk). GC, granulated cell; MC, microvilliated cell; SM, shell membrane. $\times 11,000$.

Fig. 8. Microvilliated cell, *Thamnophis radix* (embryonic stage 30). Note the abundant mitochondria. A tight junction is indicated by the asterisk. $\times 11,000$.

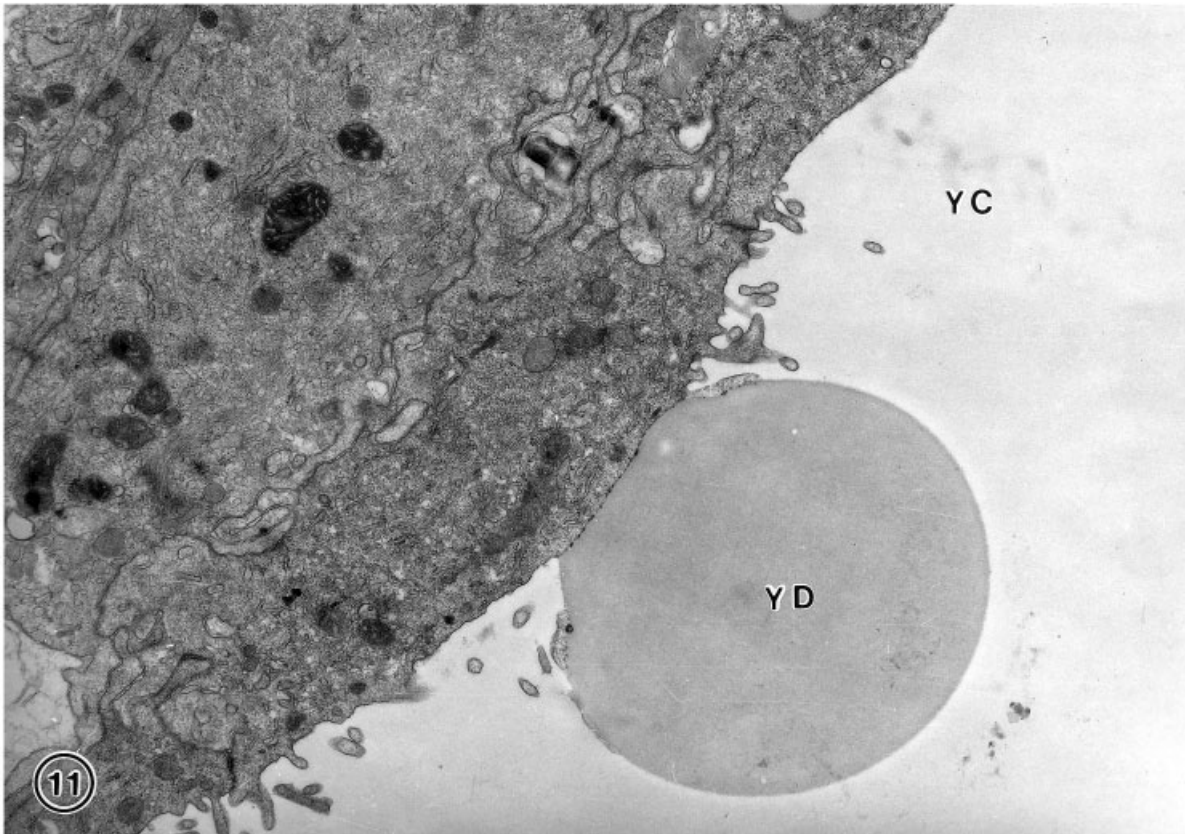
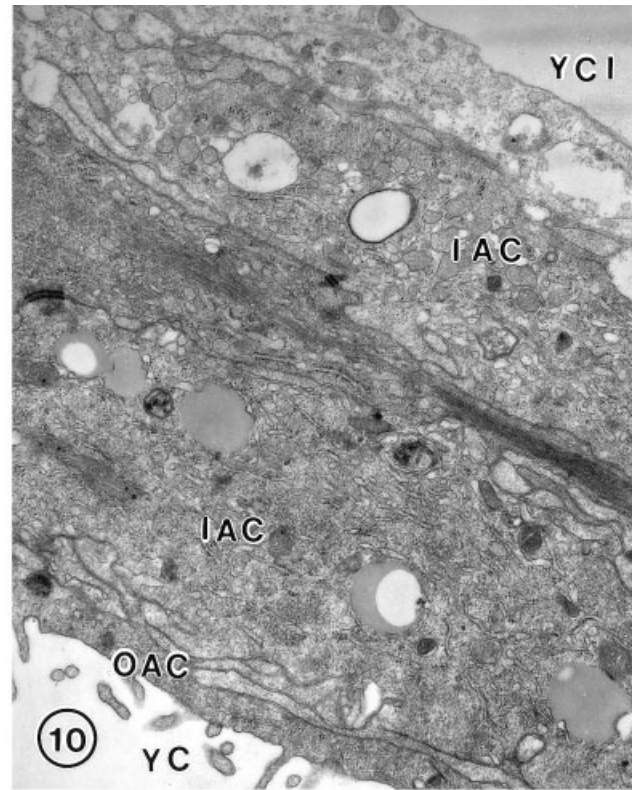
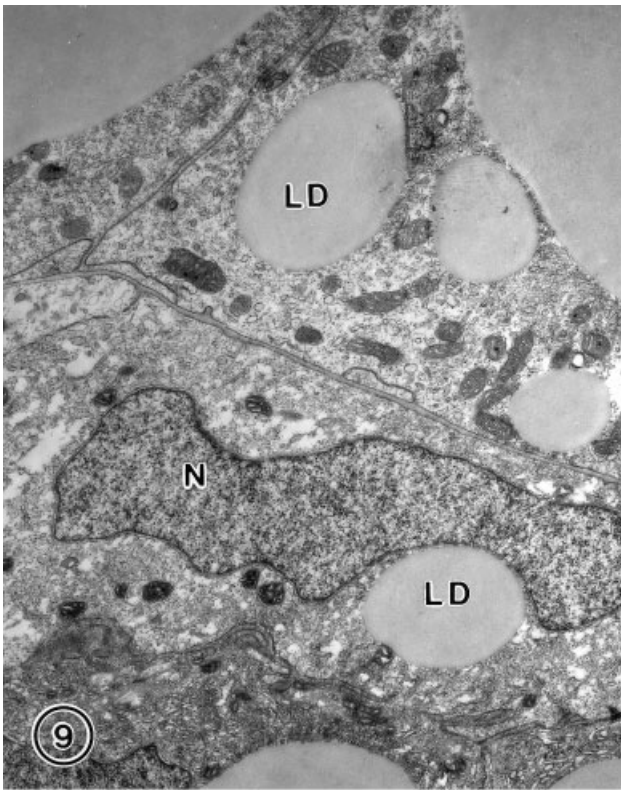


Fig. 9. Endodermal cells of the omphalopleure, *Thamnophis radix* (embryonic stage 30). LD, lipid droplets; N, nucleus. $\times 7800$.
 Fig. 10. Allantoic component of the omphalallantoic placenta, *Thamnophis radix* (embryonic stage 30). Within the yolk cleft, the outer and inner portions of the allantoic vesicle are fused into a single membrane. A cell of the outer allantoic membrane (OAC) extends microvilli into the outer portion of the yolk cleft (YC) in the direction of the isolated yolk mass and omphalopleure. IAC, inner allantoic cells (presumably of endodermal origin); YCI, inner portion of the yolk cleft, between the allantois and the vitellus. $\times 13,000$.
 Fig. 11. Allantoic membrane, *Thamnophis radix* (embryonic stage 30). A yolk droplet (YD) adheres to the outer allantoic cell. YC, yolk cleft. $\times 7800$.

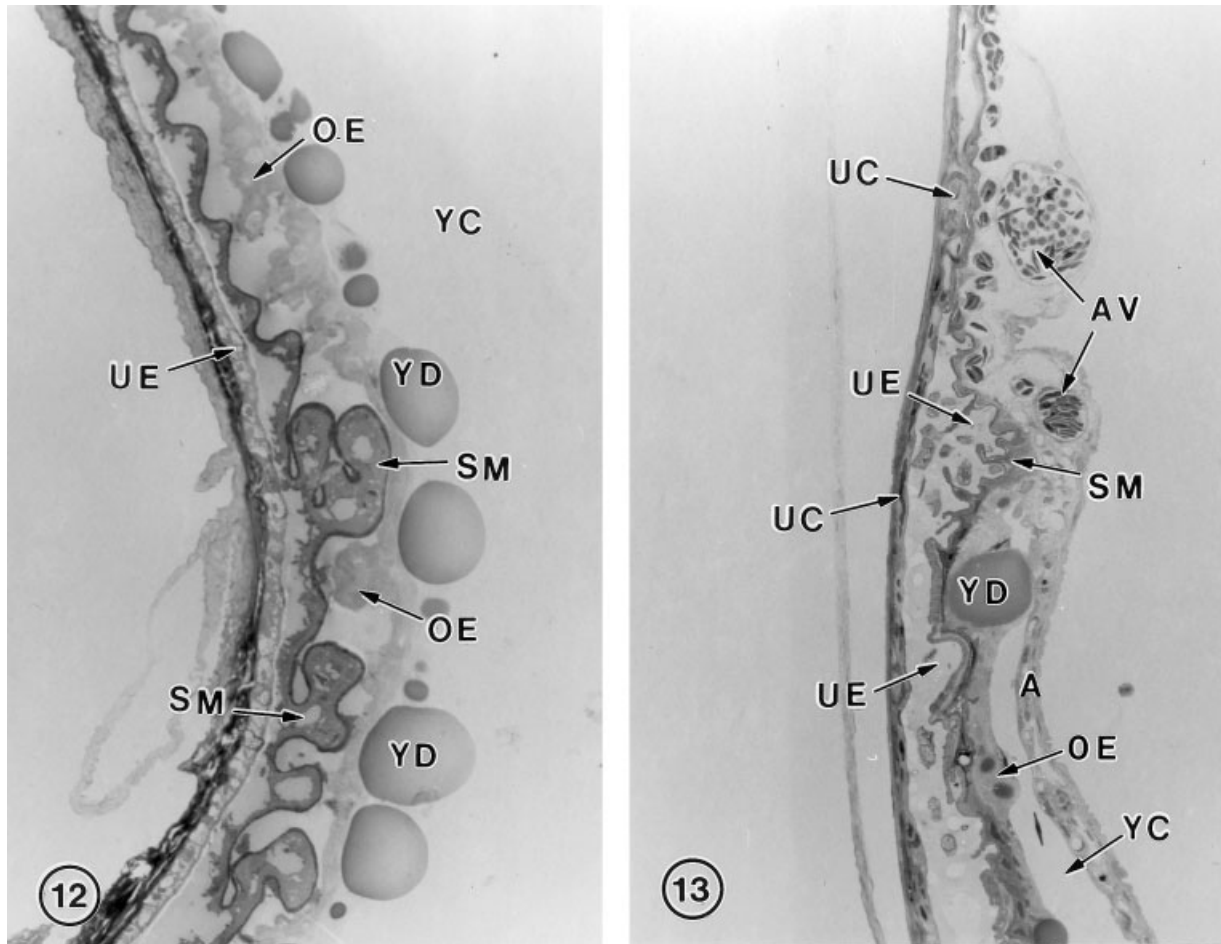


Fig. 12. Omphalallantoic placenta, *Thamnophis sirtalis* (embryonic stage 36). The isolated yolk mass is represented by scattered yolk droplets (YD). The shell membrane (SM) takes an undulating course at the placental interface. The omphalopleuric epithelium (OE) forms ridges. In the region shown, the uterine epithelium (UE) is flattened (see Fig. 13). The allantois (not shown) occupies the yolk cleft (YC). Azur II / methylene blue. $\times 130$.

Fig. 13. Placental membranes, *Thamnophis sirtalis* (embryonic stage 36). In the allantoic placenta (top half of the micrograph), the uterine capillaries (UC) and allantoic vessels (AV) are abundant, and the maternal-fetal interface is smooth. In the omphalallantoic placenta (bottom half of the micrograph), capillaries are sparse, and the uterine epithelium (UE) exhibits ridges and papillae that extend towards the shell membrane (SM). The isolated yolk mass is depleted, and represented here by a single yolk droplet (YD). The yolk cleft (YC) separates the allantois (A) from the omphalopleuric epithelium (OE). Azur II / methylene blue. $\times 260$.

small yolk droplets to the outer face of the allantois (see below). More detailed study of a complete developmental series would be helpful in clarifying the issues.

In any case, despite the lack of contact between the allantois and omphalopleure, the tissues arguably contribute to a functional placenta. In species in which the allantois does not enter the yolk cleft, fetal membranes at the abembryonic pole are avascular but nevertheless contribute to the definitive omphaloplacenta (Stewart and Blackburn, 1988; Stewart, 1993). In such species, a yolk cleft separates the omphalopleure from the closest vascular supply, the vitelline circulation. Fetal membranes do not require a vascular supply to be classified as placental; marsupials as well as certain eutherians exhibit yolk sac placentas with an avascular fetal component (Mossman, 1987).

Ultrastructure of the Omphalallantoic Placenta

The omphalallantoic placenta is a complex structure, consisting of a diversity of components. Two features will be discussed here: the morphology of elements at the maternal-fetal interface and the relationship of the isolated yolk mass to surrounding tissues. These features have particular bearing on functional attributes of the placental membranes.

Maternal-fetal interface. In the abembryonic hemisphere the maternal tissue exhibits the standard uterine components of epithelium, lamina propria, and muscularis externa (Figs. 3, 14). The structure of the uterine epithelium is noteworthy. The epithelial cells are cuboidal during the latter half of gestation. In addition, the epithelium develops papillae or ridges that protrude towards the omphalo-

pleure (Fig. 2). SEM reveals that apices of the cells bulge luminally, but they show no specialized surface features (Blackburn et al., 2002). The uterine epithelium is markedly different from that lying opposite the chorioallantois, which consists of low cuboidal to squamous cells. The latter are extremely attenuated over the uterine capillaries, forming a very thin barrier to gas diffusion (Blackburn and Lorenz, 2003). In contrast, in the omphalallantoic placenta the uterine epithelium remains relatively thick and is not significantly reduced superficial to the capillaries. In fact, a quantitative study of *Thamnophis radix* found that height of the uterine epithelial cells increases markedly during gestation (Stearns, 1986).

The uterine epithelial cells in the omphalallantoic placenta show evidence of synthetic and secretory activity. Small vesicles with granular contents occupy the apical cytoplasm of the cells (Figs. 4, 14). They are membrane-bound and their contents are similar in electron density to material in the uterine lumen. In addition, cytoplasm of the uterine epithelial cells is highly vacuolated in our samples, representing loss of organic components during tissue preparation (Fig. 14). Hoffman (1970) found that these vacuoles contained material similar in appearance to material in the uterine lumen, from which he inferred that they function in secretion. Thus, both the vesicles and vacuoles may reflect secretory activity. Although small vesicles can also be found in uterine epithelium in the dorsal hemisphere of the uterus (Blackburn and Lorenz, 2003), they are more abundant in the region of the omphalallantoic placenta.

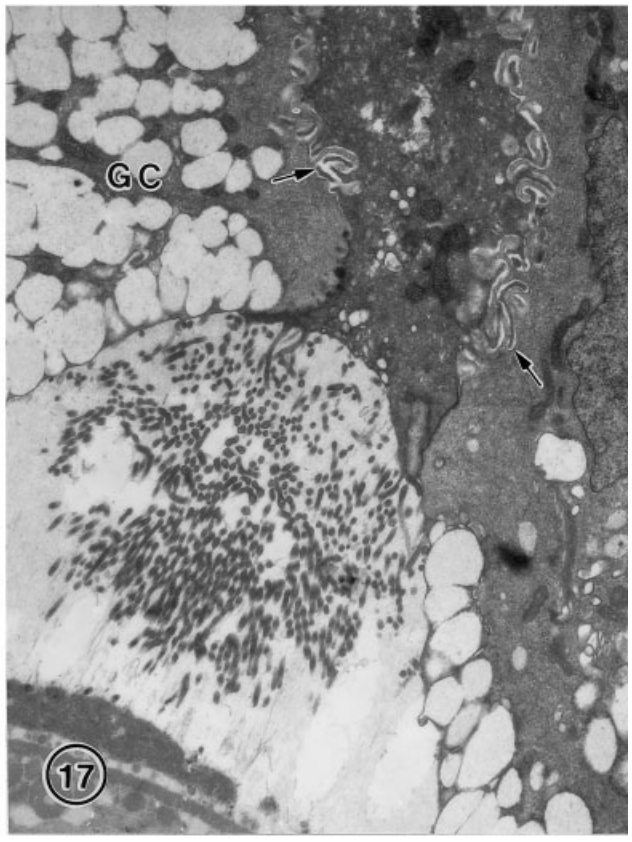
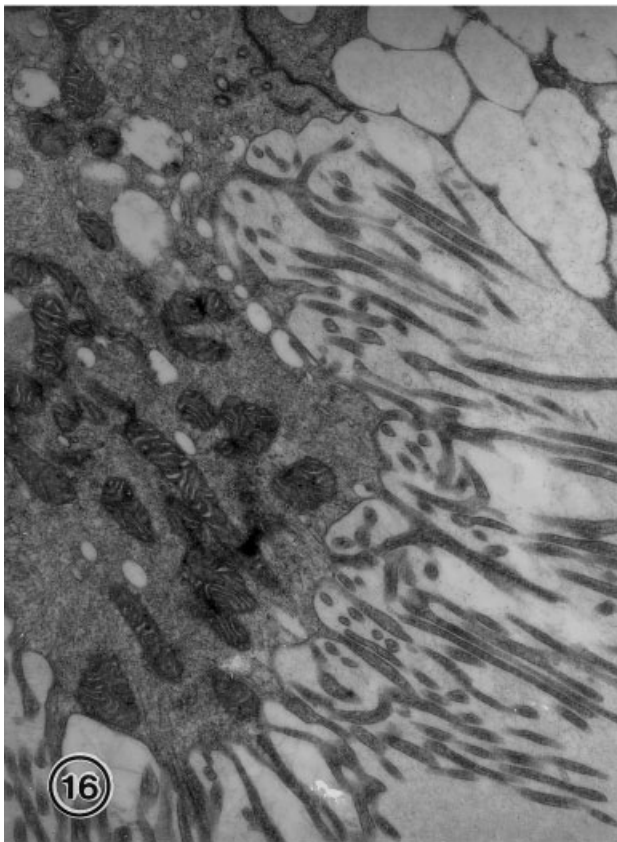
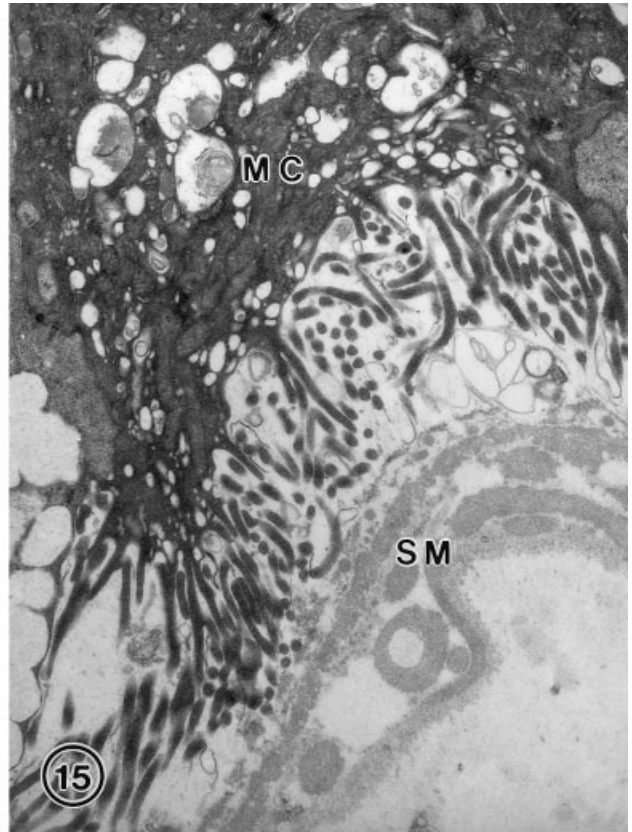
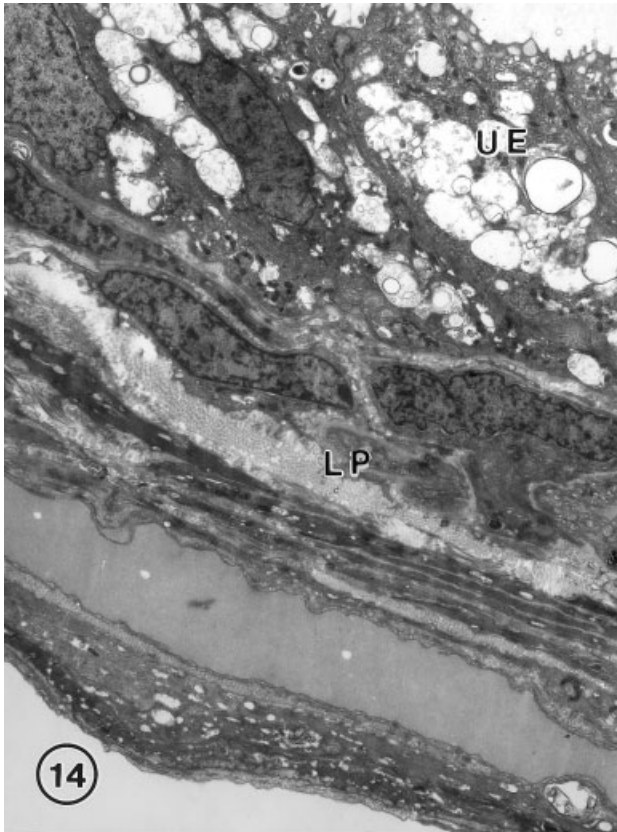
The epithelium lining the surface of the omphalopleure develops ridges that protrude towards the shell membrane (Fig. 12). Cytologically, the epithelium of the omphalopleure is highly specialized and includes two distinct types of cells. Following Hoffman (1970), these are termed "brush-border cells" and "granular cells" (Figs. 7, 8). SEM of large expanses of the omphalopleure in late gestation has revealed that the brush-border cells are surrounded laterally on all sides by granular cells (Blackburn et al., 2002). Under TEM, the microvilli of the brush-border cells appear unusual, compared to those of other vertebrate tissues. Not only are they quite elongated, but they extend outward as separate structures not bound together by a glycocalyx (Fig. 15), giving the impression of an unruly tangle of elements. The brush-border cells show cytoplasmic evidence of metabolic and absorptive activity; they are replete with mitochondria and Golgi bodies and contain an abundance of small cytoplasmic vesicles that contain particulate matter (Fig. 8). The granular cells are very different in appearance. Their microvilli are minuscule, sparse, and irregular, as previously seen through SEM (Blackburn et al., 2002). Their cytoplasm contains sizeable inclusions or droplets. A previous study inferred that the inclu-

sions were lipid in composition (Hoffman, 1970), which may help account for their loss during tissue processing in our samples. The granular cells also contain small inclusions in the apical cytoplasm. Histochemical analysis has shown that they have a staining profile of sulfated acid mucosubstances (Hoffman, 1970).

Brush-border cells of the omphalopleure interdigitate extensively with the neighboring granular cells (Fig. 17), particularly in late gestation. Adjacent cells are attached apically by junctional complexes, but are separated laterally by a thin extracellular space into which lateral protrusions of the cells extend. SEM indicates that lateral borders of the cells are extensively sculpted, forming elaborate interconnecting channels between the cells (Blackburn et al., 2002).

Material of the shell membrane separates tissue of the omphalopleure and uterus at the abembryonic pole (Figs. 4, 15). TEM reveals that it consists of thick fibers and particulate matter, interspersed with debris of uncertain composition. In our samples the membrane did not appear to have thinned or undergone deterioration between mid- and late development. In contrast, in the allantoic placental region the shell membrane becomes thin and fragmented late by gestation, so that in some regions uterine and chorionic tissues become directly apposed (Blackburn and Lorenz, 2003). However, in the omphalallantoic region the shell membrane remains as a thin but continuous barrier between fetal and maternal tissues (Figs. 4, 12).

Isolated yolk mass and allantois. As noted above, the isolated yolk mass becomes depleted during development (Figs. 2, 12, 13). Our observations indicate that yolk droplets become associated with the allantois within the yolk cleft (Fig. 2). At Zehr stage 30, yolk droplets can be seen adhering to the outer surface of the allantois, where they are held by extensions of the epithelial cells (Fig. 11). In our samples from late gestation, droplets have been engulfed by the allantoic epithelium (Figs. 19, 20). Given the overall pattern of fetal membrane development, we can suggest two possible explanations for how the allantois and yolk droplets become associated. One possibility is that individual yolk droplets are simply released from the isolated yolk mass and pass across the narrow yolk cleft to the nearby allantois, where they are engulfed by allantoic cells. This possibility would require that the yolk droplets traverse the thin cellular barrier presented by the intravitelline cells lining the yolk cleft. However, it would explain the fact that some droplets extend into the yolk cleft early in mid-development and, later, appear to be being engulfed by allantoic epithelium (cf. Figs. 11, 19). A second possibility is that the position of the yolk droplets reflects formation of a secondary yolk cleft, as described in the thamnophine snake *Virginia striatula* (Stewart, 1990). In this species, a band of yolk is separated off from the



Figures 14-17

inner side of the isolated yolk mass and attaches to the allantois. A secondary yolk cleft has not been described in any other species and to determine whether it forms in *Thamnophis* would require further histological study.

At least two cell populations contribute to the ontogenetic depletion of the isolated yolk mass—the endodermal cells of the bilaminar omphalopleure and the allantoic epithelium. Cells of the omphalopleuric endoderm lie in between the yolk droplets (Figs. 12, 13; see also Hoffman, 1970; Blackburn et al., 2002). These cells appear to digest and absorb the yolk, because smaller droplets lie inside the cells (Fig. 9). The intracellular yolk droplets are surrounded by mitochondria, which probably play a role in their digestion. Yolk droplets also appear to be phagocytosed and then digested by the epithelial cells of the allantois within the yolk cleft (Figs. 2, 11, 20).

Functional Considerations

Although yolk sac placentas have been studied histologically in various viviparous squamates, their role in maternal–fetal exchange has been a source of uncertainty. The views of many researchers appear to be similar to those expressed by Giacomini (1906): that physiologically, the yolk sac placenta is much less important than the allantoplacenta (e.g., Weekes, 1927; Bauchot, 1965). After all, the omphalopleure is inherently avascular and is commonly separated from the nearest embryonic blood vessels (those of the yolk sac or the allantois) by the isolated yolk mass and, at least in some species, the yolk cleft. In addition, pieces of shell membrane accumulate along with cellular debris at the abembryonic pole of the uterus, forming a barrier to physiological exchange between maternal and fetal circulatory systems. Consequently, according to one conceptual tradition, the yolk sac placenta is considered to be primitive, having been supplanted developmentally and evolutionarily by the allantoplacenta (Harrison and Weekes, 1925). However, several investigators have observed cellular specializations in squamates that could indicate secretion and absorption, such as uterine folds, enlarged epithelial cells at the

maternal–fetal interface, and omphalopleuric papillae (Kasturirangan 1951a,b; Parameswaran, 1962; Hoffman, 1970; Villagran-Santa Cruz, 1989; Stewart, 1990; Blackburn, 1993a; Stewart and Thompson, 1996). Furthermore, experimental studies and histochemical analyses of two snake species have provided evidence for a maternal–fetal transfer of material across the omphalallantoic placenta (Hoffman, 1970; Baxter, 1987).

Intervascular transfer. Our observations, coupled with those from previous studies, provide insight into the functional capabilities and limitations of the omphalallantoic placenta in *Thamnophis*. First, it should be noted that efficient transfer of gases or nutrients between maternal and fetal bloodstreams is highly unlikely as a possibility. In order to diffuse between maternal and fetal capillaries across the omphalallantoic placenta, an oxygen molecule would have to traverse the following layers in sequence: the maternal vascular endothelium, the cuboidal uterine epithelium, the uterine lumen, the shell membrane and associated cell debris, more uterine lumen, the bilayered epithelium of the omphalopleure, a layer of endoderm with yolk granules and platelets, the yolk cleft, and finally a bilayered allantois—only to reach the center of a membrane that is poorly vascularized. Only one of these intervening layers, the isolated yolk mass, is diminished substantially during the second half of development, and that change does not bring the allantois physically closer to the omphalopleuric epithelium. The allantois apparently does not vascularize or even adhere to the omphalopleure in *Thamnophis*; rather, it remains free within the yolk cleft (Figs. 2, 13). Furthermore, another one of these intervening layers increases in thickness during development. Quantitative study has shown that height of the uterine epithelial cells at the abembryonic pole increases significantly during pregnancy (Stearns, 1986). Thus, the diffusion barrier remains substantial in mid- to late gestation, when fetal needs for oxygen are at their greatest.

The omphalallantoic placenta contrasts markedly with the allantoplacenta (Fig. 13), in which fetal and maternal components are highly vascularized and closely apposed and separated by extremely attenuated epithelia and a thin remnant (if any) of the shell membrane (Blackburn and Lorenz, 2003). Whereas the intervacular diffusion distance across the allantoplacenta is as small as 2 μm (Blackburn and Lorenz, 2003), in the omphalallantoic placenta it is on the order of 250–300 μm . Thus, the distance between fetal and maternal bloodstreams across the omphalallantoic placenta is about 100–150 times the minimum distance across the allantoplacenta. The differences reflect the specialization of each of these placental types for different functions.

Uterine secretion. While the omphalallantoic placenta cannot realistically be viewed as a site of significant intervacular exchange, it does show

Fig. 14 Uterine lining at the omphalallantoic placenta, *Thamnophis sirtalis* (embryonic stage 36). LP, lamina propria; UE, uterine epithelium. $\times 4200$.

Fig. 15. Omphalopleuric epithelium, *Thamnophis sirtalis* (embryonic stage 36). MC, microvilliated cell; SM, shell membrane. $\times 12,000$.

Fig. 16. Microvilliated cell of the omphalopleure, *Thamnophis sirtalis* (embryonic stage 36), showing the elongate microvilli. $\times 12,000$.

Fig. 17. Cell junctions in the omphalopleuric epithelium, *Thamnophis sirtalis* (embryonic stage 36). Lateral borders of the microvilliated cells (arrows) are elaborately sculpted, and interdigitate with the surrounding granular cells (GC). $\times 7000$.

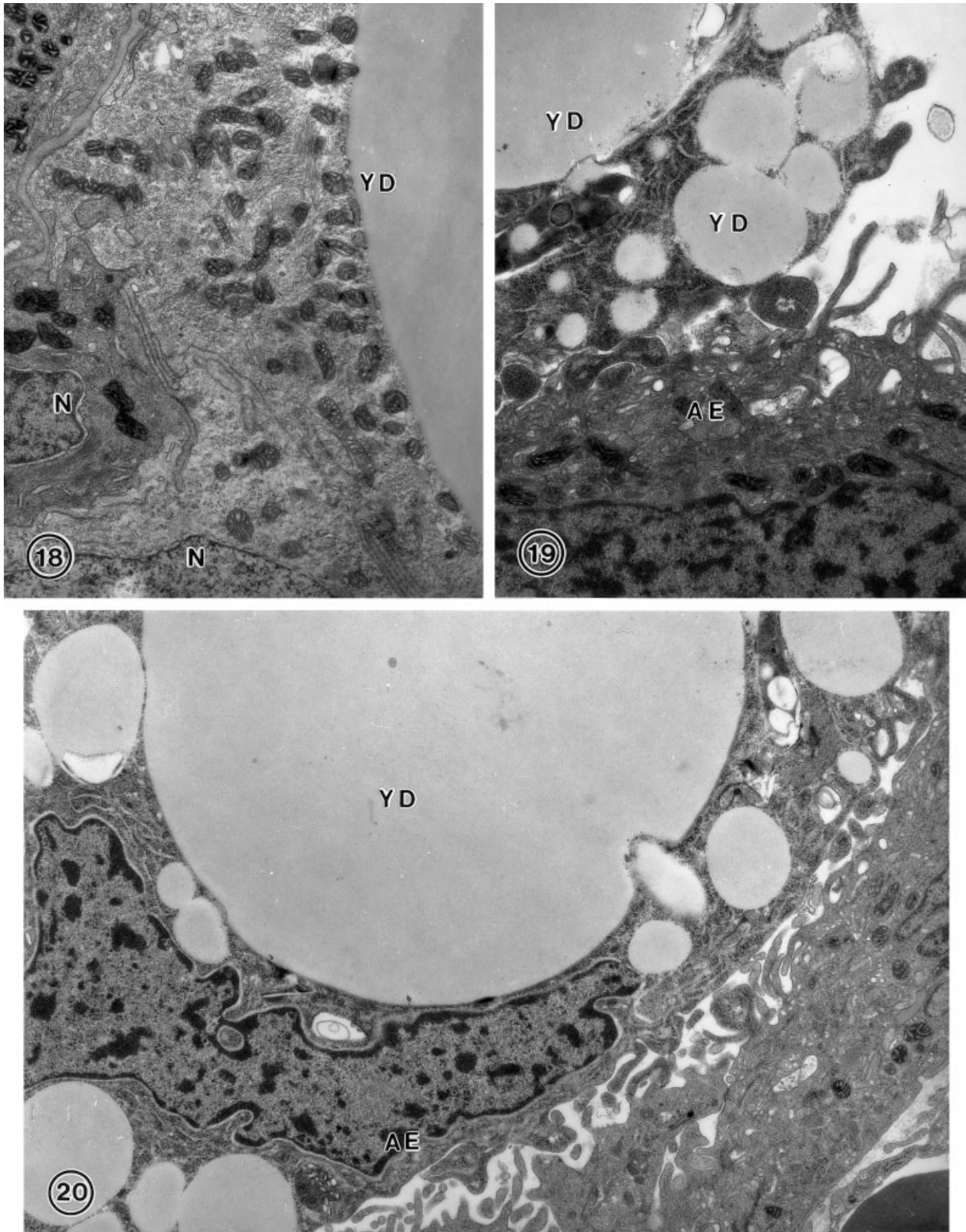


Fig. 18. Endodermal cells of the bilaminar omphalopleure, *Thamnophis sirtalis* (embryonic stage 36). N, nuclei of endodermal cells; YD, yolk droplet. $\times 12,000$.

Fig. 19. *Thamnophis sirtalis* (embryonic stage 36). Yolk droplets (YD) of various sizes have been engulfed by the allantoic epithelium (AE). $\times 12,000$.

Fig. 20. *Thamnophis sirtalis* (embryonic stage 36). Yolk droplets (YD) lie within cells of the allantoic epithelium (AE). $\times 11,000$.

strong evidence of secretion and absorption. Microscopic anatomy offers modest, indirect evidence that the uterine epithelium at the abembryonic pole produces organic secretions. The cytoplasm has large vesicles (Figs. 3, 14), which Hoffman (1970) found to contain an amorphous substance similar in appearance to material in the uterine lumen. In addition, the apical cytoplasm of the cells contains an abundance of membrane-bound secretory granules (Fig. 14). These granules have staining properties indicative of a mucoid secretory product, much like material in the uterine lumen (Hoffman, 1970). Experimental study has provided additional evidence for a uterine secretory function. When radiolabeled glycine was injected into pregnant females, it was transferred across the placental membranes, either as amino acid or as newly synthesized protein (Hoffman, 1970). The label was concentrated at the abembryonic pole of the uterus, suggesting that the omphalallantoic placenta was involved.

Given the relatively thick uterine epithelium at the abembryonic pole, as well as anatomical and histochemical evidence of secretion, histotrophic secretion of the epithelium is more likely than simple transfer from uterine blood vessels. In view of the above findings, the chemical nature of the epithelial cell contents should be characterized further. In addition, experimental studies are required to demonstrate that uterine secretions are actually absorbed by the cells of the omphalopleure. However, several lines of evidence indicate that the epithelium of the omphalopleure does have absorptive capabilities.

Absorption by the omphalopleure. Brush-border cells of the omphalopleure appear to be highly specialized for uptake of material from the uterine lumen. The cells have prominent, elongated microvilli that extend into the uterine lumen (Figs. 8, 15). These cells appear to be highly active metabolically, with an abundance of mitochondria and Golgi bodies, as well as smooth ER and PAS-positive vesicles in the cytoplasm (see Hoffman, 1970). In addition, SEM and TEM show that the lateral cell membranes are extensively sculpted, forming intracellular channels that separate them from the surrounding cells (Fig. 17).

Based on their similarities to transport epithelia of other vertebrates, a recent study (Blackburn et al., 2002) proposed that the brush-border cells function in sodium-coupled water movement across the omphalopleure. According to a general conceptual model, ion pumps in lateral and basal membranes of epithelial cells transport sodium into the extracellular space, producing an osmotic gradient that draws water across the apical plasma membrane (Friedman, 1960; also see Komnick, 1984). Such functions may be reflected in the morphological specializations of the brush-border cells in *Thamnophis*. The elongate microvilli and lateral evaginations of the plasmalemma of these cells provide a greatly expanded surface area for movement of sodium and

water. In addition, junctional complexes at the apex of adjacent epithelial cells of the omphalopleure separate the extracellular and intracellular compartments, a prerequisite for the establishment of such a gradient. Indirect evidence for sodium-coupled water movement across placental membranes is provided by a study of *T. ordinoides* (Stewart et al., 1990). This study demonstrated that placental transport accounted for 57% of the sodium and 43% of the water content of the neonate. The amounts of sodium and water provision were statistically correlated in individual snakes, suggesting that their movement across the placenta was functionally related. This study did not determine which of the placental membranes was responsible for sodium and water movement. However, our anatomical observations and those of a previous study (Blackburn et al., 2002) indicate that the omphalallantoic placenta exhibits cellular specializations consistent with this functional role.

The omphalopleure is known to be capable of absorbing substances other than water and sodium in garter snakes. Various experiments on *Thamnophis sirtalis* have shown that the epithelium has absorptive and phagocytic properties (Hoffman, 1970). Upon removal of the shell membrane, embryos cultured in physiological saline take up the dye trypan blue, especially in the region of the omphalopleure. Methylene blue injected into pregnant females is transferred to the fetus and shows a particular accumulation in the omphalopleure. In addition, as noted above, the omphalallantoic placenta has been implicated in transfer of amino acid molecules and/or protein. Furthermore, the epithelial cells of the omphalopleure exhibit a histochemical profile that is associated with lipid absorption and high mitochondrial activity (Hoffman, 1970). Absorption of organic components may account for the membrane-bound granules located in the apical region of the epithelial cells and perhaps the large cytoplasmic inclusions of the granular cells (Figs. 7, 8). From their staining properties, Hoffman (1970) considered the apical granules to be mucopolysaccharides in nature and the large cytoplasmic inclusions to be lipid. The presence of lipid is not definitive evidence of uptake from the uterine lumen, since the cells conceivably could have accumulated products of digestion of the isolated yolk mass. Nevertheless, available evidence leaves little doubt that the omphalopleure has absorptive capabilities.

Characterization of the nature and quantity of the material absorbed by the omphalopleure will require physiological and biochemical studies. Based on available evidence, uterine secretions offer one potential source of material. The uterine lumen also accumulates shed epithelial cells and other material that might conceivably be absorbed, including material from the shell membrane. In some viviparous scincid lizards the omphalopleure develops papillae that extend into the debris at the abembryonic pole

and which are postulated to be involved in its uptake (ten Cate-Hoedemaker, 1933; Blackburn, 1993a). Early studies on viviparous squamates have postulated that epithelial cells of the omphalopleure phagocytose such debris, along with pieces of the shell membrane (Weekes, 1935). The epithelial extensions of the omphalopleure in *Thamnophis* (Fig. 12) may well perform similar functions.

Shell membrane. The shell membrane is a vestige of the eggshell and is deposited around the egg in most or all viviparous squamates (Blackburn, 1993b, 1998; Stewart, 1993). Typically, it thins or ruptures at the embryonic hemisphere of the egg in the region of the allantoplacenta. However, pieces of shell membrane accumulate at the abembryonic hemisphere of the egg and, in some species, they contribute to a thick mat of material separating the uterine and extraembryonic tissue (Weekes, 1935).

If the omphalallantoic placenta is a site of nutrient transfer in garter snakes, the persistence of a thick shell membrane in the abembryonic region (Figs. 2, 4, 12) is difficult to explain on adaptive grounds. One possibility is that the membrane may act as a dialyzing membrane, preventing large molecules from passing between maternal and fetal tissues. This possibility is supported by Hoffman's (1970) discovery that in cultured embryos the membrane prevents trypan blue as well as Evans blue-labeled albumen from passing into the omphalopleure. However, why a dialyzing membrane might be adaptive in the placenta of viviparous squamates is unclear. Perhaps the membrane is simply a vestige—of no particular functional utility, but not sufficiently disadvantageous as to prevent maternal–fetal transfer of small organic molecules, sodium, and water across the omphalallantoic placenta.

Evolution and Differentiation of Placental Function

One noteworthy feature of the omphalallantoic placenta in *Thamnophis* is the presence of marked specializations for physiological transfer in species that are relatively lecithotrophic. In garter snakes and other thamnophines that have been studied, females ovulate yolk-laden eggs that are larger in dry mass than the fetuses that develop from them (Stewart and Castillo, 1984; Stewart, 1989; Stewart et al., 1990; Sangha et al., 1996). Thus, the yolk provides most of the nutrients for development. Nevertheless, in each of the thamnophine species studied significant maternal–fetal transfer of inorganic nutrients occurs. In *T. ordinoides*, for example, although the embryos show a net loss of organic material during gestation, sodium and calcium are provided in significant quantities by placental means (Stewart et al., 1990). Likewise, as discussed above, placental transfer of both inorganic and organic nutrients occurs in *T. sirtalis* (Hoffman, 1970).

Traditionally, nutrient transfer in squamates has been viewed as the province of the allantoplacenta, leaving as uncertain the functions of placentas derived from the yolk sac (Weekes, 1935). This perspective receives support from the fact that highly placentalotrophic squamates exhibit allantoplacentas with elaborate specializations for nutrient transfer (e.g., Blackburn, 1993a; Stewart and Thompson, 1996; Flemming and Branch, 2001; Jerez and Ramírez-Pinilla, 2001; Blackburn and Vitt, 2002). In fact, broad correlations exist between allantoplacental types and the extent of placentalotrophy (Blackburn, 1993b, 1998; Stewart and Thompson, 2000; Thompson et al., 2000). However, as noted in several recent publications (e.g., Stewart, 1990, 1992, 1993; Stewart and Thompson, 1996, 2000), placentas derived from the yolk sac offer alternative sites for physiological transfer in squamates, including transfer that may be qualitatively different from that occurring across the allantoplacenta. Cytological specializations of such placentas are found in some highly placentalotrophic species (Blackburn, 1993a; Stewart and Thompson, 1996), but also have been documented in viviparous squamates that appear to be relatively lecithotrophic (Stewart, 1989, 1990; Villagran-Santa Cruz, 1989; Attaway, 2000).

Our observations support the idea that placentas formed from the chorioallantois and the yolk sac are specialized in distinctly different directions for qualitatively different functions. In the allantoplacenta, the emphasis is on exchange between maternal and fetal circulatory systems. Both the chorioallantois and uterus are highly vascularized and the interhemal diffusion distance is extremely small (on the order of 2 μm), due to progressive reduction of the intervening cell layers and the shell membrane. In contrast, the omphalallantoic placenta of *Thamnophis* exhibits evidence of secretion and absorption. Epithelium of the fetal omphalopleure is highly specialized, with two distinct populations of cells, one of which shows adaptations for absorption and the other of which stores organic molecules as cytoplasmic inclusions. Likewise, the uterine epithelium may be secretory.

The key to understanding placentas derived from the yolk sac in reptiles may be to view them as organs that are specialized in their own right—incapable of significant hemotrophic transfer, but having instead the capacity for secretion and absorption. Thus, dramatic morphological differences between the two types of placentas reflect qualitative differences in the potential for physiological exchange. In studies on eutherians, the anthropocentric focus on chorioallantoic placentas has led researchers to overlook the distinct functional attributes of yolk sac placentas—hence, the traditional (and parochial) characterization of marsupials as “aplacental.” In studies on squamates, to focus exclusively on the allantoplacenta is to neglect a placental arrangement that is much more complex

structurally, that changes dramatically during development, and that shows, in some species, strong evidence of maternal–fetal transfer of nutrients.

Future Placental Investigations

Many questions must yet be answered about omphaloplascentas and omphalallantoic placentas of squamates. Among them are questions about functional capacities, developmental modifications, interspecific diversity, and, ultimately, the roles that such placentas have played in the evolution of viviparity. Furthermore, while two placental types clearly differ in terms of their potential for gas exchange and hemotrophic transfer, we cannot assume that the allantoic placenta engages in no histotrophic transfer. Modest evidence for such transfer is provided elsewhere (Blackburn and Lorenz, 2003).

Regarding the functional and evolutionary issues, a particularly intriguing question is whether functional properties of omphaloplascentas and omphalallantoic placentas in viviparous squamates are exaptations that predate the evolution of the live-bearing reproductive mode (Stewart, 1989, 1992; Stewart and Thompson, 2000). The chorioallantois is one such exaptation. Having originated under conditions of oviparity back in the Paleozoic Era, it has been coopted for placental gas exchange in over 100 separate squamate lineages. Given evidence that the uterine epithelium has secretory functions in oviparous forms (Blackburn, 1998), the uterus similarly may have been coopted under viviparous conditions. Likewise, the extraembryonic membranes of oviparous squamates are likely to function in calcium uptake, a capacity that may have been retained and modified under conditions of viviparity (Stewart and Thompson, 2000). As one further example, the omphalopleure probably functions in water uptake by oviparous eggs, and may well play an analogous role in viviparous forms (Blackburn, 1993b).

Ultimately, explanations of the evolutionary morphology of the placental membranes in viviparous squamates will require considerably more information about oviparous forms than is now available. An understanding of the structure and function of extraembryonic membranes in both oviparous and viviparous squamates should therefore be of paramount concern to biologists interested in the evolution of viviparity. Future work should not only include consideration of the chorioallantois, but also membranes derived from the yolk sac, since the latter appear to play roles in physiological exchange that are both distinctive and important.

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