

Do Pregnant Lizards Resorb or Abort Inviabile Eggs and Embryos? Morphological Evidence From an Australian skink, *Pseudemoia pagenstecheri*

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ABSTRACT Although pregnant viviparous squamates are sometimes claimed to be able to resorb inviable eggs and embryos from the uterus, definitive evidence for such resorption is not available. After placing pregnant female *Pseudemoia pagenstecheri* into conditions under which embryonic development is terminated, we periodically harvested the gravid oviducts and examined them histologically. Females contained abnormal and degenerating eggs and embryos that had died in various stages of development. Dead embryos had undergone extensive cytolysis, dissolution, and aseptic necrosis and vitelline masses showed signs of deterioration and passage down the oviduct. The uterine mucosa lay in direct contact with the vitelline material, with no intact shell membrane intervening between them. Yolk was sometimes displaced into the exocoelom and allantoic cavity due to rupture of the extraembryonic membranes. Histological examination revealed no evidence of the uptake of yolk by the uterine epithelium or its accumulation in the subepithelial connective tissue. In many specimens, the uterine epithelium showed minuscule, apical granules. The position, appearance, and staining properties of the granules suggests them to be secretory, a manifestation of placentotrophy. Our observations indicate that *P. pagenstecheri* females retain dead eggs and embryos for several weeks or longer, yet do not resorb them during that period. This lizard is the second placentotrophic skink species in which resorption has been suspected, but in which abortive eggs appear to be retained or extruded instead of being resorbed by the oviducts. Researchers should not assume that squamates can digest and resorb oviductal eggs without definitive morphological evidence. *J. Morphol.* 256:219–234, 2003.

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In viviparous amniotes, the prolonged association of a pregnant female with her developing embryos theoretically offers potential for the modulation of reproduction. Over the course of gestation, the female may provide extravitelline nutrients to the embryo, a pattern known as placentotrophy. Among amniotes, the capacity for substantial placentotrophy has evolved in therian mammals and in at least

three or four clades of squamate reptiles (Blackburn, 1998a, 2000; Stewart and Thompson, 1998,2000; Thompson et al., 1999; Flemming and Branch, 2001). Viviparous females also may have the potential to terminate reproduction, either through spontaneous abortion or resorption of developing eggs and embryos. Both mechanisms occur among therian mammals (Brambell, 1948; Henderson, 1954; Morton et al., 1982; Westlin et al., 1995), but their relative occurrence among viviparous squamates remains controversial.

For over a century, biologists have inferred that pregnant lizards and snakes can terminate reproduction by resorbing eggs and embryos from their oviducts (e.g., Mingazzini, 1892; Domini, 1928; Kasurirangan, 1951; Bustard, 1966; Hoffman, 1970; Yaron, 1972). The inferences are based chiefly on three lines of evidence: 1) the presence of degenerating and infertile eggs in maternal oviducts; 2) the apparent disappearance of embryos from the reproductive tract of captive, pregnant females, without evidence of parturition; and 3) histological observations suggestive of uterine resorption. The first two lines of evidence are very weak, because abortive eggs can be retained indefinitely or can be extruded from the reproductive tract, where they may go unnoticed or be ingested by the mother. Although anatomical observations potentially offer the most definitive evidence of resorption, a recent review

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concluded that the histological evidence was tenuous and inconclusive (Blackburn, 1998b). This review called for careful study of species in which resorption was suspected. A histological study of one such species, the placentotrophic lizard *Chalcides chalcides* (Blackburn et al., 1998), found no evidence of resorption of abortive eggs and embryos. This study concluded that the material from abortive eggs underwent necrosis and was extruded down the oviducts, rather than being taken up by the uterine epithelium. Further study is needed to determine whether the situation in *C. chalcides* characterizes other viviparous squamates.

The present study seeks anatomical evidence bearing upon uterine resorption vs. extrusion of eggs and embryos in the viviparous Australian lizard *Pseudemoia pagenstecheri* (Scincidae). This lizard is ideal for such a study because embryonic development can be terminated when pregnant females are held in captivity under certain thermal conditions. Furthermore, females are highly placentotrophic and ovulate ova that are among the smallest produced by reptiles (Thompson and Stewart, 1994; Thompson et al., 1999, 2000; Stewart and Thompson, 2000). Eggs of this size presumably would be much less difficult to resorb than the large eggs that are more typical of squamates (Blackburn et al., 1998). In addition, pregnant females of this species can modulate the degree of reproductive investment into their embryos according to nutrient availability (Shine and Downes, 1999). Moreover, structure of the uterus and placental membranes in *P. pagenstecheri* and its congeners are well understood (Harrison and Weekes, 1925; Weekes, 1930; Stewart and Thompson, 1994, 1996, 1998). An understanding of uterine structure is important, because the uterine oviduct is the organ presumed to accomplish the putative resorption of eggs and embryos in squamates.

This study extends our understanding of the potential capacity for uterine egg resorption to a new placentotrophic lineage of squamates, the second out of three such lineages that are known to exist. To our knowledge, this article represents only the second histological study bearing directly on reptilian egg resorption in the past 60 years.

MATERIALS AND METHODS

Pregnant female *Pseudemoia pagenstecheri* were collected by J. Stewart and A. Krockenberger on October 13, 1990, at Grundy Trig (elevation 1,460 m), in the Riamukka State Forest, near Nowendoc, New South Wales (Thompson and Stewart, 1994). Females were returned to the University of Sydney and housed in plastic aquaria with screen tops. Photoperiod, provided by 60W light bulbs placed at one end of the bank of cages, was 14:10 photophase:scotophase. Between October 18 and 20, the right oviducts were surgically removed and used for chemical composition analysis and histology (Thompson and Stewart, 1994; Thompson et al., 1999). Subsequent observations on both unoperated and operated females revealed that embryonic development was not proceeding normally. At periodic intervals between

November 19 and December 6, the lizards were sacrificed and the left oviducts were immediately harvested and fixed for microscopic examination.

The oviducts were fixed in a saturated picric acid–dioxane–formol solution (Griffiths and Carter, 1958) and stored in dioxane. Following transport to Trinity College, the tissue was dehydrated using cellosolve, parlodion-cellosolve, and benzene, following the procedure of Griffiths and Carter (1958). Tissue was infiltrated with Paraplast Plus (Fischer Scientific, Pittsburgh, PA) in a vacuum oven at 60°C, embedded in paraffin, and sectioned at 7 μ on rotary microtomes with steel knives. Although most uteri were sectioned transversely, some were sectioned longitudinally. Serial sections were mounted on albumen-coated slides and the slides were stained using an Autostainer XL (Leica Corp., Deerfield, IL). We used three histological stain protocols: iron hematoxylin and eosin, Milligan's trichrome (acid fuchsin, fast green, and orange G), and periodic acid-Schiff (PAS) reagent/hematoxylin. All stain recipes were adapted from Humason (1962).

Slides were examined using an Olympus BH-2 compound microscope equipped with S Plan Apochromatic objective lenses with correction collars. The tissue was photographed on Kodak T-Max 100 professional film using a 35mm camera. Magnifications reported in the figure legends are of the actual photomicrographs.

RESULTS

Gross Observations

The oviducts of *Pseudemoia pagenstecheri* are thin-walled, elongated tubes that attach to a mesentery along the dorsal aspect. Most of the oviducts in our study contained two eggs apiece (range, 1–3 eggs). Each uterus enclosed its eggs tightly; thus, expanded "incubation chambers" which contained the eggs were interspersed with unoccupied, constricted regions. Gross observation revealed that some of the incubation chambers (and the eggs that they contained) were misshapen and elongated in the direction of the cranial–caudal axis of the oviduct. In the oviducts used in this study, intact eggs were about 3 mm in diameter.

Egg Microstructure

Histological examination revealed substantial embryonic development in some eggs, whereas others showed little sign of development. Yolk was a prominent feature of all eggs, but differed in appearance among the specimens. In some eggs the yolk contained eosinophilic and strongly basophilic droplets or granules of various sizes, as well as spaces left by droplets that had been extracted during processing (Fig. 1). Compared to the rounded symmetrical granules of typical squamate eggs, the granules often appeared misshapen and shriveled. In other eggs the yolk was quite abnormal; it formed an eosinophilic mass in which basophilic droplets were rare and with layers of largely agranular material that varied in staining properties (Fig. 2). Unusually large, eosinophilic masses were found in the eggs, possibly representing coalesced droplets. The most normal-looking yolk appeared in eggs that had developed into embryogenesis before death (see below).

Fig. 1. *Pseudemoia pagenstecheri*. Inviably or unfertilized egg in the uterus (U). The yolk contains regions with misshapen granules and yolk droplets (D), as well as large homogenous areas in which droplets are sparse and scattered. Rounded, clear spaces presumably represent yolk particles extracted during processing. Hematoxylin and eosin. Bar = 100 μm .

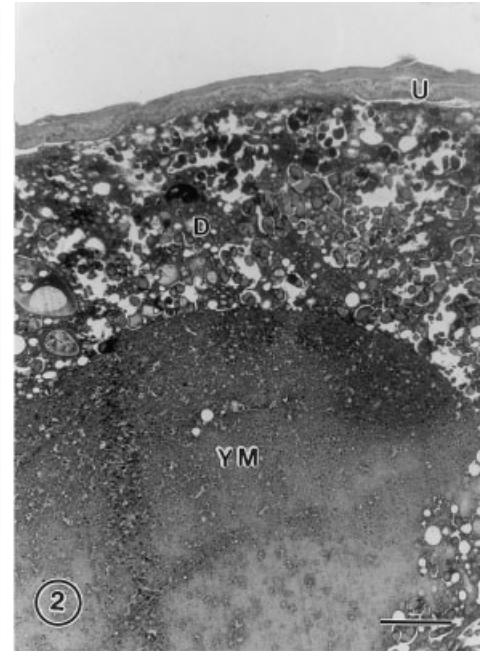
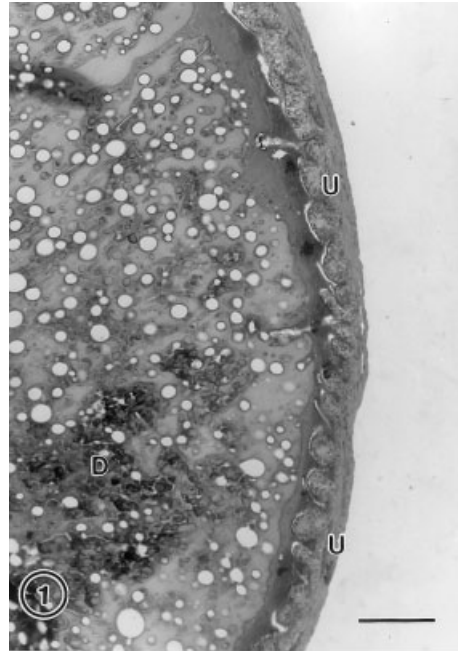


Fig. 2. *Pseudemoia pagenstecheri*. Inviably oviductal egg. An unusual accumulation of eosinophilic yolk material (YM) occupies the center of the vitellus, surrounded by yolk droplets (D). U, uterus. Hematoxylin and eosin. Bar = 50 μm .

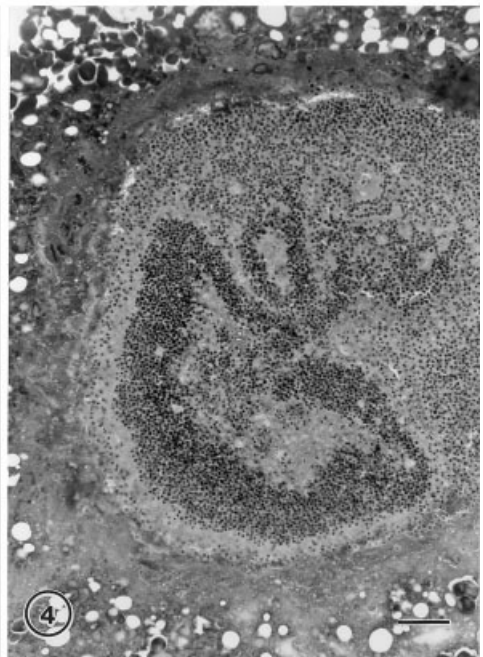
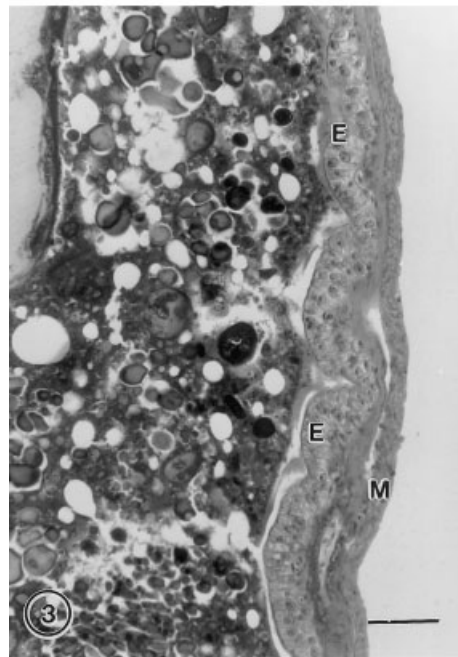


Fig. 3. *Pseudemoia pagenstecheri*. Yolk material conforms to the general shape of the uterine lining. No shell membrane is visible around the yolk mass. E, epithelium of the uterus, M, muscle. Hematoxylin and eosin. Bar = 50 μm .

Fig. 4. *Pseudemoia pagenstecheri*. Inviably egg, containing masses of dead embryonic cells with pyknotic nuclei. Specific embryonic origins of such cells were often difficult to determine. Hematoxylin and eosin. Bar = 50 μm .

No intact shell membrane or vitelline membrane entirely surrounded any of the eggs. As a result, yolk material lay in immediate contact with the uterine epithelium in each. In histological sections, the yolk substance bore the shape of the ridges and troughs of the uterine lining, as if it had flowed to fill the available space (Figs. 1, 3). Occasional pieces of what may have been shell membrane were located in both the mesometrial and antimesometrial hemispheres of the incubation chambers, where they commonly had sunken into the vitellus. In a few of the eggs, a very thin acellular eosinophilic layer (about 2–3 μm in thickness) lay external to the yolk

in some restricted regions; possibly it represents the vitelline membrane (sensu Blackburn and Callard, 1997) or, alternatively, a remnant of the shell membrane.

Embryonic Development

Most of the eggs appeared to have died at an early ontogenetic stage. In these eggs, evidence of development was confined to patches of cellular material that lay embedded within the body of the yolk. Commonly, this material consisted of clumps of very small cells with dense, pyknotic nuclei (Fig. 4). The

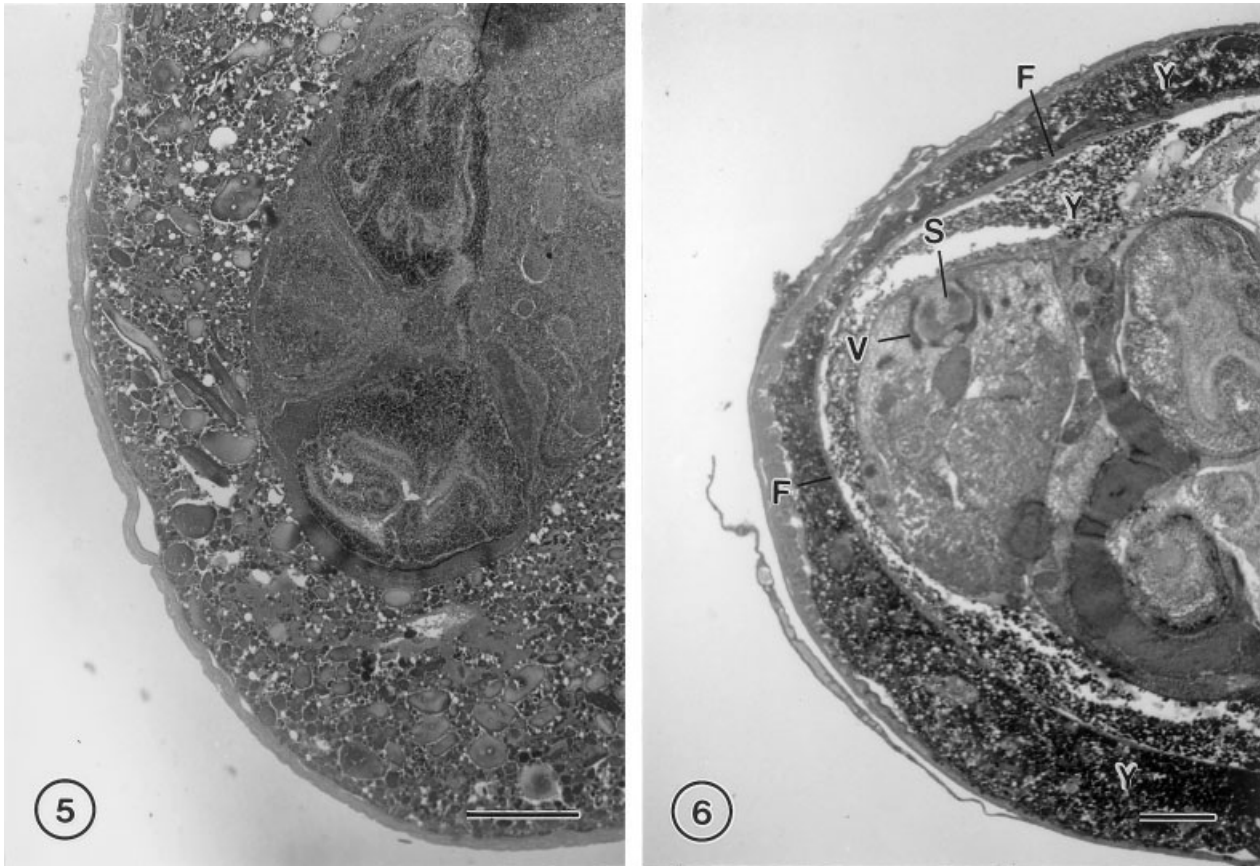


Fig. 5. *Pseudemoia pagenstecheri*. Degenerating dead embryo, embedded deep in the yolk. Embryonic tissue has undergone dissolution. Hematoxylin and eosin. Bar = 195 μ m.

Fig. 6. *Pseudemoia pagenstecheri*. Degenerating embryo that died in an advanced stage of development. The spinal cord (S) and portions of vertebrae (V) are visible, as are other skeletal elements and components of various organs. Embryonic cells are dead and tissues have undergone degeneration and displacement. Displaced yolk (Y) occupies the uterine lumen and lies internal to the fetal membranes (F). Hematoxylin and eosin. Bar = 300 μ m.

cells apparently had died and degenerated well before harvesting of the tissue. Although the cells seldom bore features that would allow them to be identified as to type or origin, some appeared to be hemopoietic elements. Isolated large cells with the morphology of yolk endoderm were also evident. These cells also appeared abnormal and dead; their cytoplasm was pale-staining and heavily vacuolated and some seemed to be ruptured.

In uterine sites that contained more-developed embryos, those embryos also seemed to have died well before the oviducts were harvested. The embryos had undergone degeneration and necrosis and cytolysis and pyknotic nuclei were common (Fig. 5). Like the above-mentioned cells, the embryonic material was sunken into the vitellus and was represented as masses of cells with small pyknotic nuclei and scattered cellular debris. No evidence of infective microorganisms was observed.

Those eggs that were the most advanced developmentally had proceeded into middle and later stages of embryogenesis. Although dissolution made pre-

cise staging impossible, the most advanced of the degenerative embryos had an identifiable notocord, spinal cord, and abdominal organs, as well as a chorioallantois (Fig. 6; also, see below). Cartilaginous portions of the braincase, vertebral centra and arches, ribs, and epididial forelimb elements had formed and ossification had begun. Cell nuclei were pyknotic throughout, but were especially evident in cells of the spinal cord and abdominal organs. The spinal cord commonly had lost its structural integrity; tissues lying dorsally had invaded the gray matter, obliterating the central canal (Fig. 6). The yolk appeared relatively normal, with an abundance of rounded, basophilic, and eosinophilic droplets. The chorionic epithelial cells ranged from thin and squamous to enlarged and cuboidal in the placental region. They had strongly basophilic nuclei indicative of inactive or dead cells. The apical region of the enlarged chorionic cells stained with PAS, possibly revealing the existence of microvilli or a glycocalyx. Sparse granules were present in their cytoplasm; like granules in the uterine epithelium

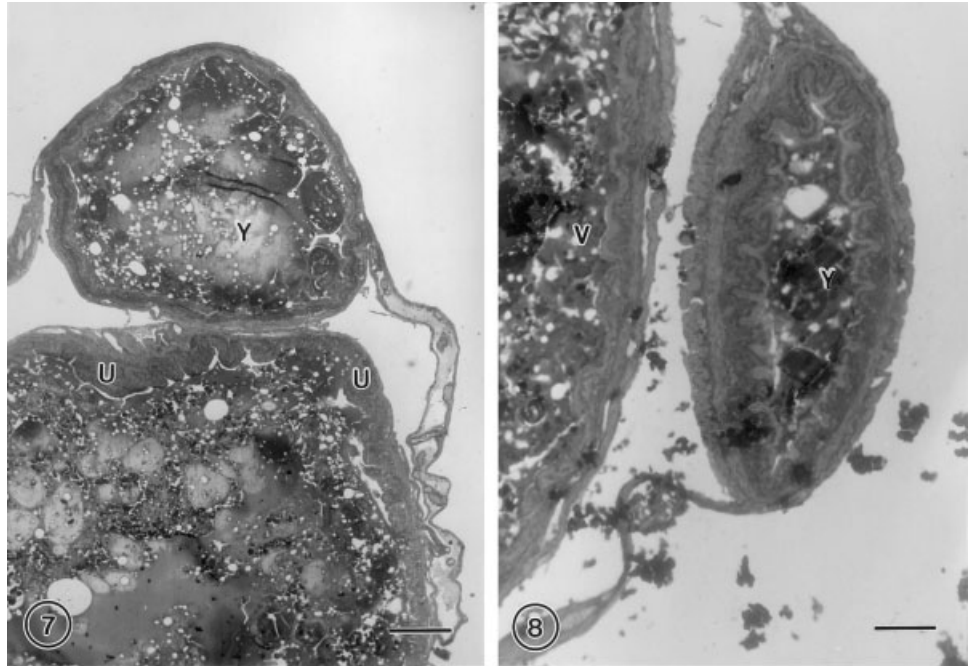


Fig. 7. *Pseudemoia pagenstecheri*. The constricted region of the oviduct contains yolk (Y). The main mass of yolk is located in the adjacent incubation chamber of the uterus (U). Hematoxylin and eosin. Bar = 200 μ m.

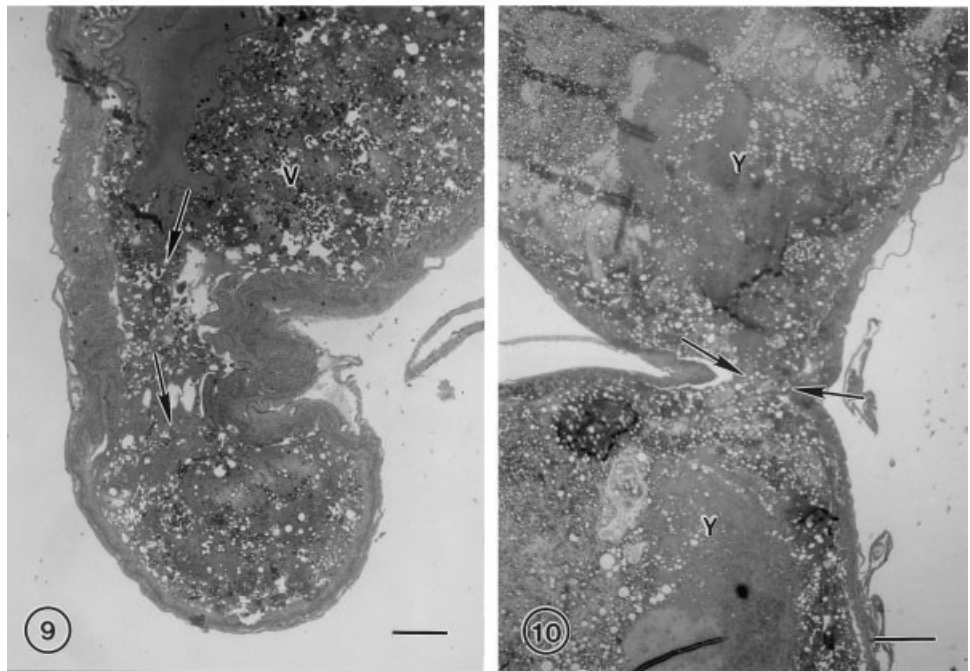


Fig. 8. *Pseudemoia pagenstecheri*. Displaced yolk. The constricted region of the oviduct, contains yolk (Y) derived from the adjacent vitellus (V) or another egg. Hematoxylin and eosin. Bar = 100 μ m.

Fig. 9. *Pseudemoia pagenstecheri*. Displaced yolk. Yolk from the vitellus (V) in the incubation chamber has passed down the oviduct into the constricted region (arrows). Hematoxylin and eosin. Bar = 200 μ m.

Fig. 10. *Pseudemoia pagenstecheri*. Yolk from the eggs in adjacent incubation chambers has intermixed (arrows). Hematoxylin and eosin. Bar = 200 μ m.

(described below), they stained with PAS and with iron hematoxylin.

Although all of the oviducts in this study contained inviable eggs and embryos, a few also contained living fetuses. For example, a female sacrificed on 6 December contained one necrotic, fairly well-developed embryo, plus two living fetuses in a very late stage of development (Dufaure and Hubert, 1961; Stage 39). Another female (sacrificed on 4 December) contained two dead eggs (terminated very early in development) plus a viable, lightly pigmented embryo.

Topographic Displacements

Many of the eggs were misshapen and conformed to the shape of the oviduct. Commonly, yolk was displaced out of the incubation chambers. Consequently, substantial quantities of yolky material could be found in the constricted regions lying between incubation chambers (Figs. 7, 8). Longitudinal sections of the oviduct sometimes revealed yolk from the incubation chamber passing into the adjacent constricted regions (Fig. 9). In addition, yolk of adjacent eggs had sometimes intermixed through its

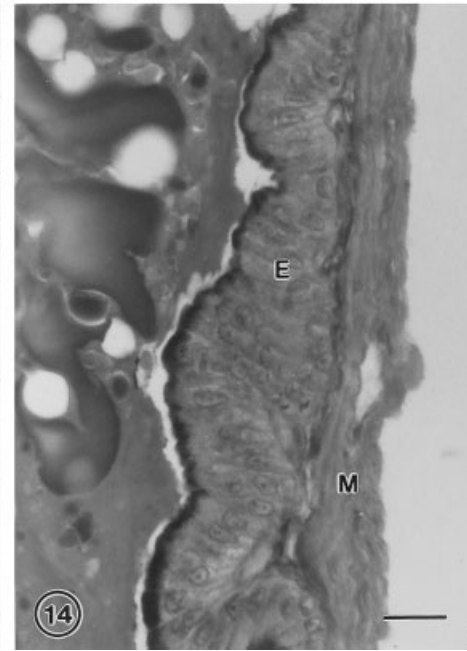
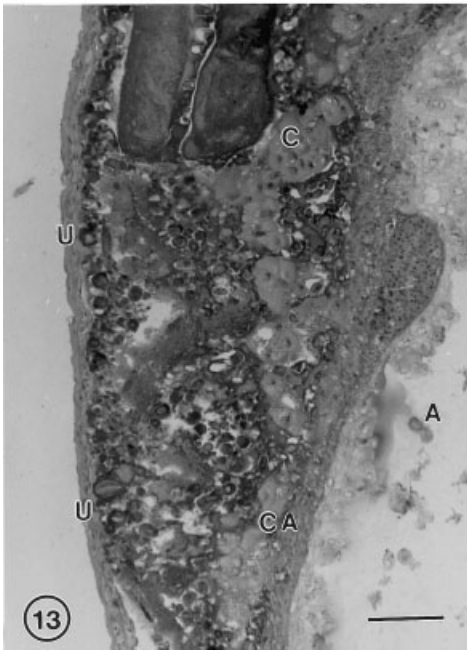
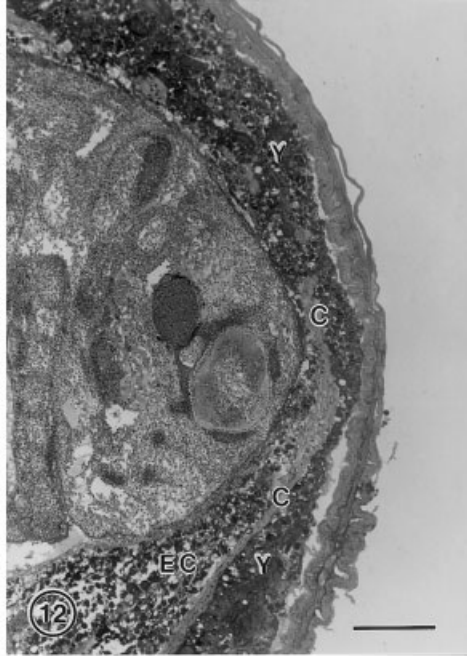
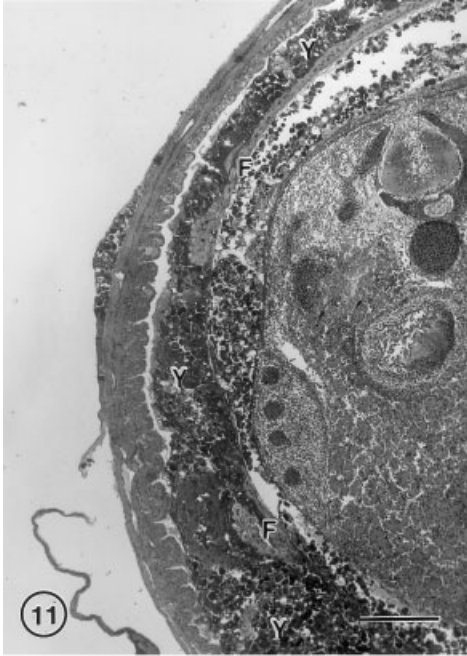


Fig. 11. *Pseudemoia pagenstecheri*. Displaced yolk (Y) occupies the lumen of the oviduct, between the uterine lining and the fetal membranes (F), as well as internal to the fetal membranes. Hematoxylin and eosin. Bar = 200 μm .

Fig. 12. *Pseudemoia pagenstecheri*. The chorion (C) has ruptured and displaced yolk (Y) in the uterine lumen has entered the exocoelom (EC) between the chorion and amnion. Hematoxylin and eosin. Bar = 200 μm .

Fig. 13. *Pseudemoia pagenstecheri*. Displaced yolk (Y) has penetrated between the chorioallantois (CA) and the uterine tissue (U). Cells (C) of the chorionic epithelium have been shed into the lumen, and lie surrounded by yolk material. A, allantoic cavity. Hematoxylin and eosin. Bar = 50 μm .

Fig. 14. *Pseudemoia pagenstecheri*. Uterus, containing inviable egg. Apical cytoplasm of cells of uterine epithelium (E) has basophilic staining properties. No multicellular glands are present. M, muscle. Hematoxylin and eosin. Bar = 25 μm .

movement between adjacent incubation chambers (Fig. 10). In some oviducts the eggs were distorted and had coalesced to such an extent that the original incubation chambers could not be distinguished. In such areas the fact that more than one egg was represented was evident from the volume of yolk.

In the most advanced of the abortive embryos, yolk could be found in the lumen of the oviduct, interposed between the fetal membranes and the uterine epithelium (Fig. 11). In such cases, thick masses of yolk separated the chorion as well as the chorioallantois from the uterine lining (Figs. 11, 12). In some embryos, the chorion was ruptured. As a

result, yolk had invaded the extraembryonic coelom, occupying the space between the chorion and amnion (Figs. 12, 13). In such cases, whether the displaced yolk originated from the embryo itself or from an adjacent embryo was not readily apparent.

Uterine Histology

The squamate uterus is thin-walled and consists of three layers: epithelium, lamina propria, and smooth muscle. Orientation on the histological sections was easily determined by the attachment of

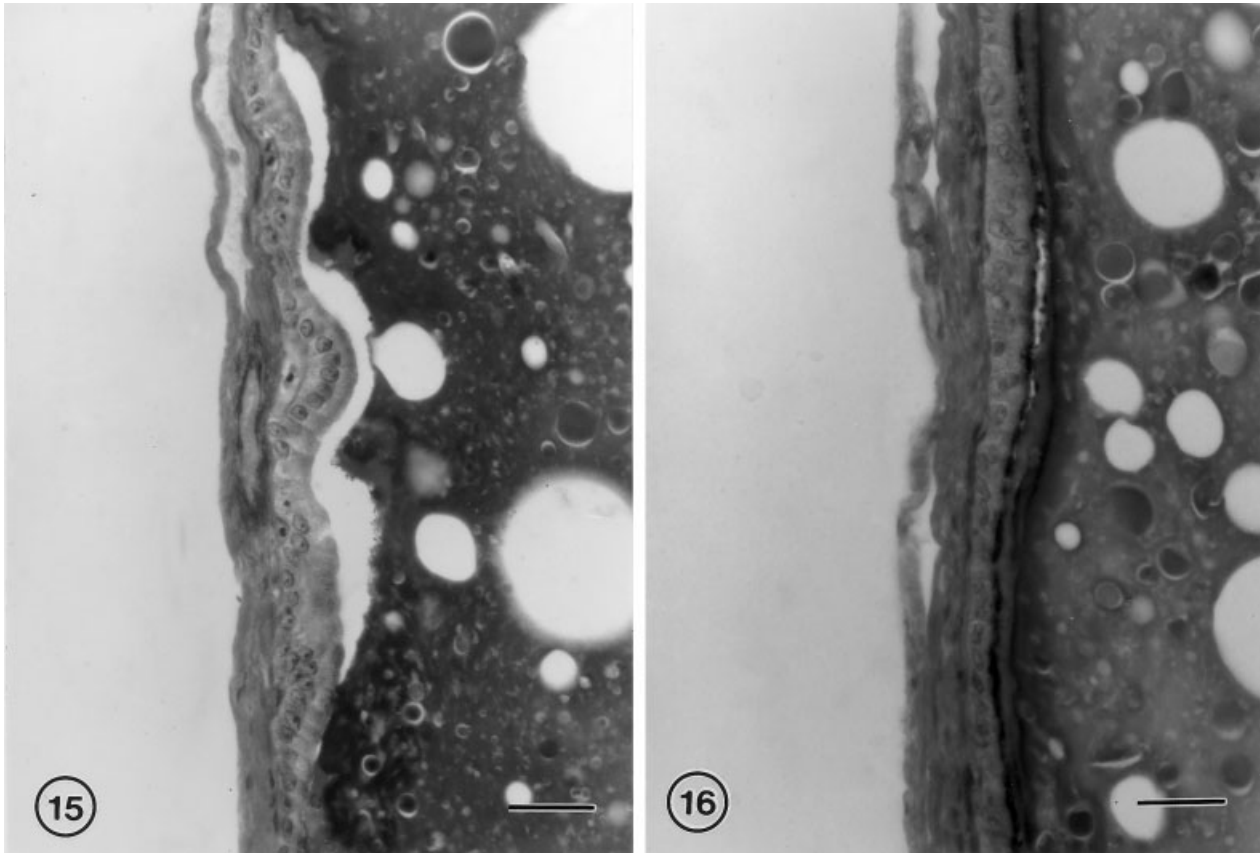


Fig. 15. *Pseudemoia pagenstecheri*. Mesometrial (embryonic) pole of the uterus. No trace of yolk particles can be observed in the epithelial cells. Hematoxylin and eosin. Bar = 25 μm .

Fig. 16. *Pseudemoia pagenstecheri*. Antimesometrial (abembryonic) pole of the uterus. Epithelial cells in this region also show no evidence of absorbed yolk. Hematoxylin and eosin. Bar = 25 μm .

the mesentery along the dorsal border of the uterus. The epithelial lining of the uterus formed shallow ridges, randomly distributed (Figs. 3, 14). In specimens containing eggs that proceeded through embryonic stages, the mucosa of the mesometrial pole formed folds that protruded into the lumen and the yolky material therein. These folds contained epithelium and underlying connective tissue, but not muscle. This region may represent the uterine portion of a placentome (see Weekes, 1935; Stewart and Thompson, 1998).

The uterine epithelium consisted of a monolayer of cells, typically cuboidal and columnar in character, but occasionally appearing pseudostratified (Figs. 3, 14). Columnar cells predominated in the embryonic (mesometrial) hemisphere of the egg, whereas cuboidal cells were common at the abembryonic pole (Figs. 15, 16). Squamous cells were only seen in uterine sites containing advanced embryos, and there, only in some locations. Epithelial cell nuclei were rounded or oval and usually located towards the base or longitudinal center of the cells; each contained a prominent nucleolus. The cyto-

plasm appeared relatively homogenous and stained lightly with basic dyes.

The apical cytoplasm of uterine epithelial cells commonly stained intensely with basophilic dyes (Fig. 14). Close examination revealed an abundance of tiny granules, which stained strongly with hematoxylin (Fig. 17) as well as with PAS (Fig. 18). The granules failed to stain with Milligan's trichrome. These staining properties differed from yolk in the oviduct lumen, which stained lightly with PAS (if at all) and intensely with the trichrome stain. The cell granules were particularly concentrated at the apex of the cells; more rarely, they could be found elsewhere in the cytoplasm. These granules are interpreted here as secretory rather than absorptive in nature.

The cytoplasmic granules were present not only in epithelial cells facing the yolk and embryos, but also in cells lining deep uterine folds located in the mesometrial hemisphere (Fig. 19). In addition, granules were abundant in extensive folds at the mesometrial pole, in epithelial cells that did not directly border the lumen (Fig. 20). These folds may

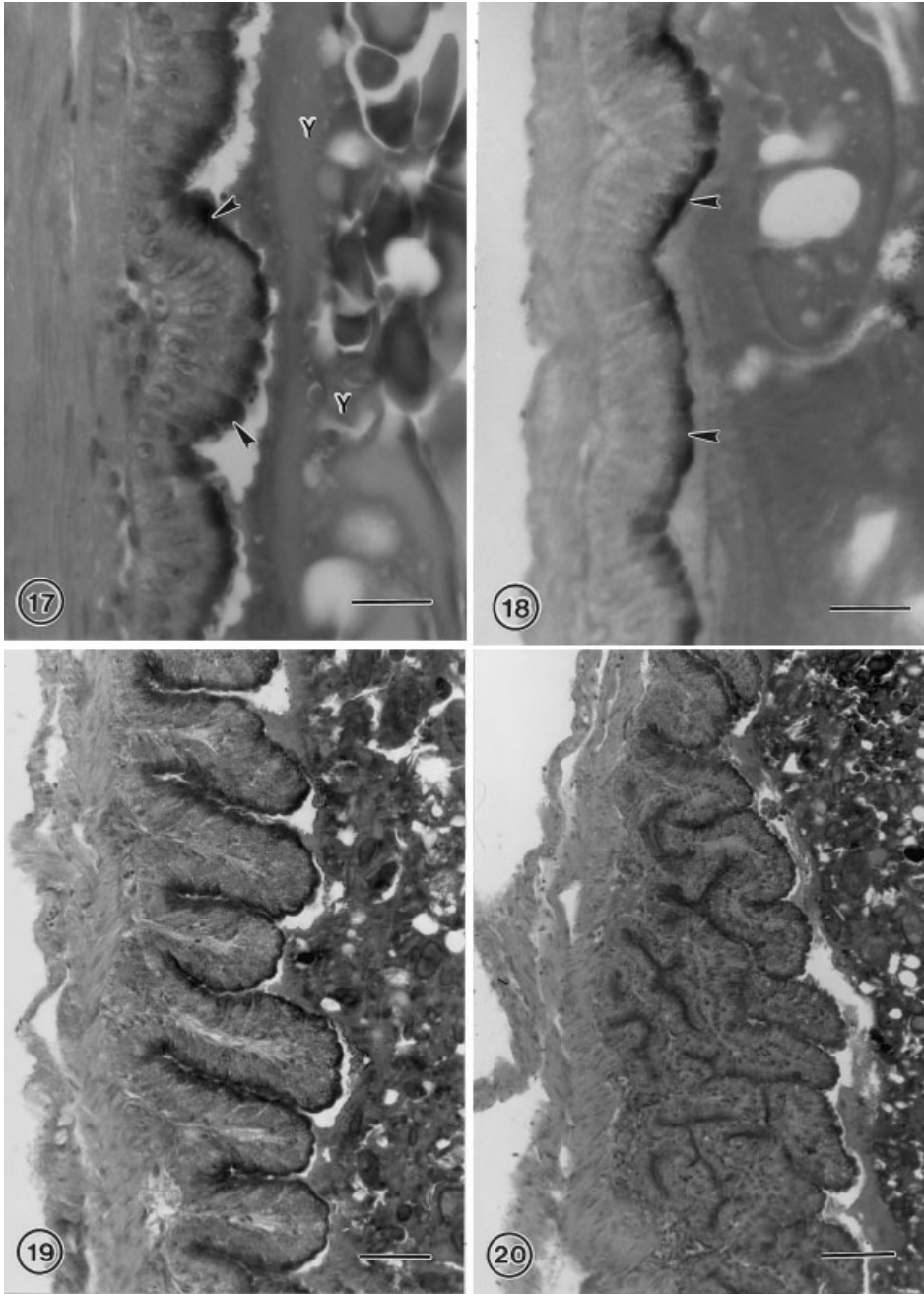


Fig. 17. *Pseudemoia pagenstecheri*. Apices of the uterine epithelial cells commonly stain intensely with hematoxylin (arrowheads), due to the abundance of basophilic cytoplasmic granules. Y, yolk. Hematoxylin and eosin. Bar = 20 μm .

Fig. 18. *Pseudemoia pagenstecheri*. The epithelial granules also stain intensely with the Periodic acid-Schiff procedure (arrowheads). This section is adjacent to that shown in Figure 17, and the image is focused. PAS, very light hematoxylin. Bar = 20 μm .

Fig. 19. *Pseudemoia pagenstecheri*. The basophilic granules occur in apices of epithelial cells that line the ridges of uterine folds. Hematoxylin and eosin. Bar = 50 μm .

Fig. 20. *Pseudemoia pagenstecheri*. The granules also are abundant in epithelial cells of elaborate folds at the mesometrial pole. These cells do not directly face yolk material. Hematoxylin and eosin. Bar = 50 μm .

be the maternal component of a placentome (see below). In some sections, identical granules were present in the uterine lumen in the immediate vicinity of cells that contained granules (Fig. 21). Here they typically lay interposed between the epithelial cells and the yolk material, sometimes adhering to the surface of the latter.

Located in the apical cytoplasm of the columnar epithelial cells of the uterus, parallel with the apex of the cell, lay an irregularly shaped structure that stained an intense pink with Milligan's trichrome stain (Fig. 22). It had the appearance of an organelle rather than an inclusion; it was located in the same

position in adjacent cells and was oriented longitudinally across each cell. In sections stained with hematoxylin and eosin, this cell structure was not differentially stained. In some cells it appeared entirely unstained and surrounded by basophilic cytoplasm, presenting the classic appearance of a "Golgi ghost" of other vertebrate tissues.

Although the eggs of *Pseudemoia pagenstecheri* are small, the uterus was so stretched in regions containing them that the boundary between the lamina propria and muscle was indistinct. The two layers could best be distinguished in trichrome-stained sections and both appeared unremarkable

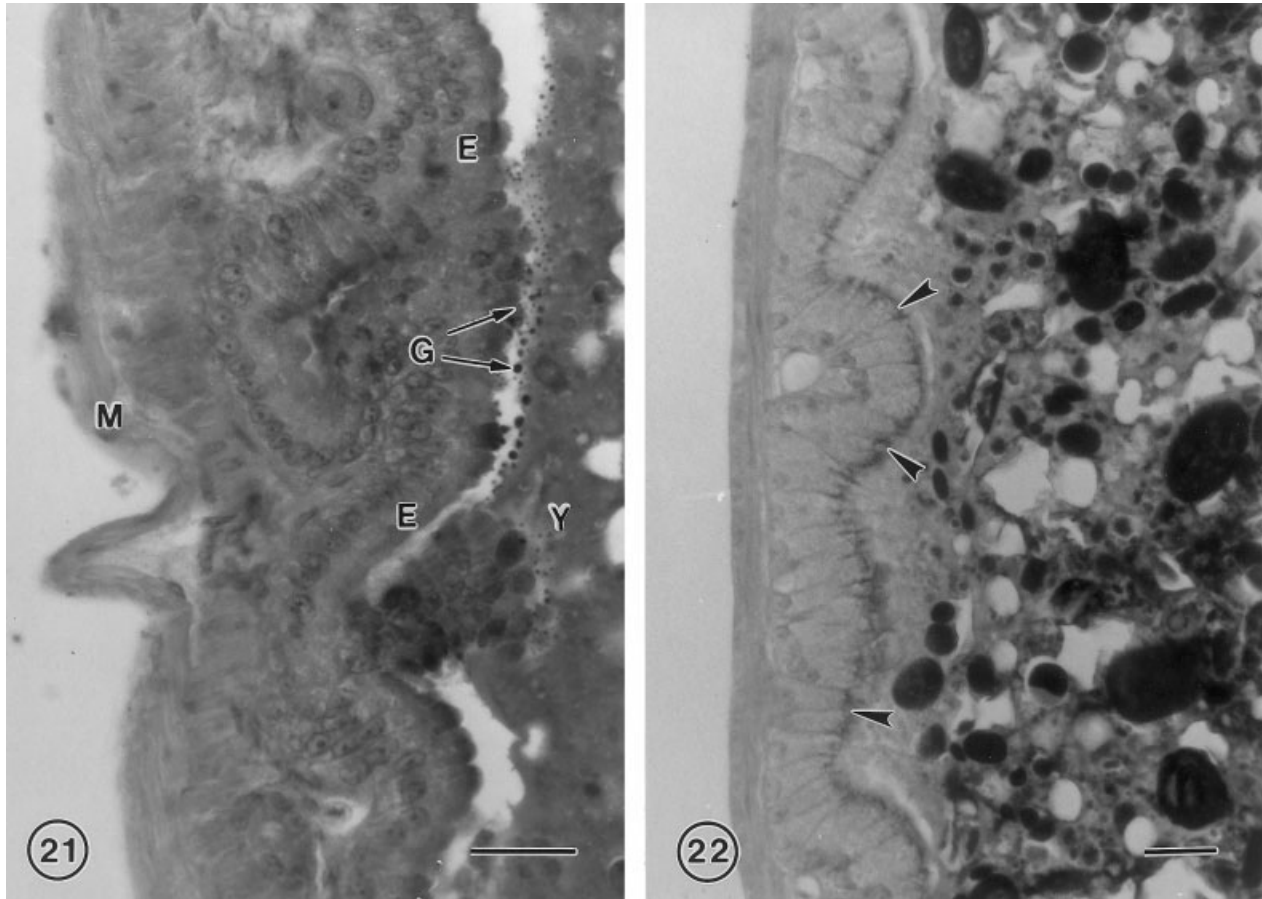


Fig. 21. *Pseudemoia pagenstecheri*. Extracellular granules lie between the uterine epithelial cells (E) and the yolk (Y), and presumably have been secreted by the cells. M, uterine muscle. Hematoxylin and eosin. Bar = 250 μ m.

Fig. 22. *Pseudemoia pagenstecheri*. Milligan's trichrome procedure reveals a red-staining organelle that is oriented transversely across each of the epithelial cells (arrowheads), below the cell apices. It may represent a Golgi apparatus. Bar = 20 μ m.

(Figs. 3, 14, 19). The lamina propria consisted of collagenous fibers oriented transversely beneath the epithelium. Occasional small blood vessels were apparent, particularly in the mesometrial hemisphere. No granules or phagocytosed particles were observed, such as have been described in an unrelated lizard said to resorb its eggs (Jacobi, 1936), nor were macrophages or mast cells observed. Signs of infection such as accumulating leukocytes or lymphocytes were also not observed in the lamina propria of any of the specimens.

DISCUSSION

Timing of Reproduction and Its Termination

Pseudemoia pagenstecheri females in New South Wales reproduce seasonally and produce one litter per year. Ovulation occurs in the spring (October) and gestation lasts approximately 2 months. Follow-

ing parturition, females mate and store the sperm over the winter, until ovulation the following spring (Greer, 1989).

Females in our study were collected in mid-October and subsequent examination indicated that all of them had ovulated. Although we do not know (or expect) that all of the eggs were fertilized, most females contained eggs that showed signs of development. The eggs evidently had ceased developing well before they were harvested for this study. From their appearance, we infer that development usually had halted at a very early stage and that deterioration had ensued. Yolk material commonly appeared as large masses of homogeneous material. Distinct basophilic granules that typify squamate yolk were relatively scarce, and those granules that were present were often misshapen and distorted (Figs. 2, 3). This appearance is similar to that of the yolk of early, abortive eggs of the viviparous lizard *Chalcides chalcides* (Blackburn et al., 1998). In both spe-

cies these granules probably had deteriorated during the period that had elapsed since embryonic death. In *Pseudemoia pagenstecheri*, the granules probably had coalesced into the large eosinophilic masses of material found in some specimens of (Fig. 2).

The extraembryonic germ layers can envelop small squamate eggs relatively early in development (Blackburn and Callard, 1997). However, evidence of such envelopment was lacking in most of the abortive eggs in our study, even those that appeared relatively intact. Typically, extraembryonic cell layers were not located on the yolk surface, but small, dead cells with pyknotic nuclei, as well as scattered nuclear material, were located in the body of the vitellus itself (Fig. 4). The nondescript appearance of the dead cells commonly obscured their origins and identity. However, hemopoietic elements suggestive of mesoderm sometimes appeared to be present, as well as small cells that could have been epithelial. In addition, isolated yolk endodermal cells lying deep in the yolk occasionally could be distinguished by their relatively large size. Compared to those of viable embryos (e.g., Blackburn, 1993a; Stewart, 1993; Stewart and Thompson, 1996; Blackburn and Callard, 1997), the endodermal cells were abnormal and appeared to be dead; they were heavily vacuolated, with pale-staining cytoplasm that showed no sign of phagocytosed yolk granules.

Like those eggs terminated early in development, eggs with evidence of moderately well-developed embryos exhibited cell death and disruption that must have preceded the harvesting of the tissues by a substantial period (Figs. 5, 6). Pyknotic nuclei were ubiquitous and the embryonic tissues were necrotic, showing massive cytolysis, liquification, and displacement. As a result, cells and tissues often could not be identified as to type. These features are characteristic of advanced stages of necrosis in mammalian tissues (Thomson, 1978; Sheldon, 1988). In the most advanced embryos, death and disruption had occurred after formation of the chorion and chorioallantois. In these advanced embryos, identifiable tissues and organs lacked structural integrity and cell material from adjacent structures were intermixed. In addition, fetal membranes had been ruptured, spilling yolk into the oviductal lumen and the exocoelom (Figs. 12, 13).

In sum, histological observations indicate that development had ceased in most of the eggs at an early stage, probably close to the time the females were brought into captivity. Some of the abortive eggs had proceeded through the limb bud stage and beyond, but those also commonly had died and degenerated well before harvesting of the oviducts. Based on the state of egg development, we infer that the lizards in this study typically contained eggs whose development was terminated as many as 5–7 weeks earlier. However, abortive eggs and embryos sometimes cohabited in oviducts with normal embryos; in one

female the latter were near term. Thus, embryos were not necessarily terminated synchronously in a given female.

Uterine Microstructure

For the squamate oviduct to accomplish resorption, the epithelium presumably would have to digest and phagocytose the uterine contents and pass the products of digestion to the subepithelial connective tissue, where they can be removed by phagocytic cells and the blood vascular system. Consequently, one could expect histological evidence of both extracellular and intracellular digestion, phagocytosis, and accumulation of the products of digestion in the epithelium or underlying lamina propria. In fact, in previous studies egg resorption has been inferred from degeneration of uterine eggs and embryos, as well as the accumulation of putative yolk material in the subepithelial connective tissue (Mingazzini, 1892; Domini, 1928; Jacobi, 1936). However, embryo degeneration certainly does not imply enzymatic digestion by the uterus, since such degeneration may be a result of autolysis. As the only empirical study showing histological evidence of uterine resorption of eggs, Jacobi's (1936) monograph on *Anguis fragilis* deserves consideration. Although this monograph includes no illustrative photomicrograph, it does show a drawing of an oviductal site containing an abortive egg (his fig. 8), with a small number of putative yolk particles in the uterine connective tissue. Based on their size and appearance, these structures have been reinterpreted as granules of mast cells (Blackburn, 1998b). Intact and degranulating mast cells are common components in lizard oviducts (Uribe et al., 1988; Blackburn, 1998a) and have been found in pregnant females with abortive eggs (Blackburn et al., 1998). Jacobi's (1936) study also shows other features that are hard to reconcile with what we know about oviduct morphology. For example, it illustrates uterine "glands" as comprising a continuous monolayer of cells lying parallel to the luminal surface, an arrangement that is entirely different from what has been described in other squamates, in which the glands are multicellular and tubuloalveolar (e.g., Fox, 1977; Perkins and Palmer, 1996; Blackburn, 1998a).

In our study, the uterine sites containing abortive eggs presented no unusual microscopic features that can easily be attributed to egg resorption (Figs. 1, 3, 14). On the contrary, in overall histological appearance the uterine tissues were similar to those described in other lizards (Blackburn, 1993b, 1998a), including congeneric Australian skinks (Stewart and Thompson, 1994, 1996, 1998). Likewise, they are consistent with past descriptions (Harrison and Weekes, 1925) of normal oviducts that are thought to have been made on *Pseudemoia pagenstecheri* (see Thompson and Stewart, 1994). An epithelium of columnar and cuboidal cells (such as the abortive egg

sites exhibited) is characteristic of the saurian oviduct in early pregnancy, as well as nonpregnancy (Blackburn and Callard, 1997; Blackburn, 1998a). No indication of epithelial phagocytosis of yolk particles or embryonic tissue was observed in our study. Likewise, the subepithelial connective tissue showed no evidence of yolk particles or accumulations of macrophages or other phagocytic elements (Figs. 14, 17).

One noteworthy specialization of the uterine tissues of various *Pseudemoia* is the placentome (Weekes, 1930; Stewart and Thompson, 1996, 1998, 2000). This region is characterized by uterine folds lined by enlarged epithelial cells, apposed to an enlarged chorionic epithelium; details vary interspecifically. Placentomes are thought to function in maternal–fetal nutrient transfer in placentotrophic skinks (Blackburn, 1993b, 1998a; Stewart and Thompson, 2000; Blackburn and Vitt, 2002). In our study, the mesometrial folds in uteri containing advanced abortive embryos appear to be the maternal component of a placentome. However, in the absence of apposed chorionic tissue the extent to which its morphology was normal is not certain. This feature of the uterus is of interest in the present context because its epithelial cells bear an abundance of granules that appear to be secretory and which may be a means of nutrient transfer. The uterus of *Pseudemoia pagenstecheri* supplies substantial quantities of nutrients to the offspring; in fact, this species is one of the most highly placentotrophic reptiles known (Thompson and Stewart, 1994; Thompson et al., 1999).

The granules in question lie in the apical region of the epithelial cells, particularly those of the uterine folds (Figs. 17, 18, 19). Several observations suggest them to be secretory granules, rather than absorbed particles of yolk. First, the granules were of consistent size and appearance, unlike what one would expect of phagocytosed pieces of yolk and embryo undergoing intracellular digestion. Second, the granules commonly were concentrated in the apical portions of the epithelial cells, above a possible Golgi apparatus (Figs. 17, 18, 22). This location is what one would expect for secretory granules. Phagocytosed particles, in contrast, presumably would be distributed more extensively throughout the cytoplasm. In addition, evidence of accumulating granules or their breakdown products was not found in the lamina propria, to which products of catabolism presumably would be transferred. Third, cytoplasmic granules were especially abundant in epithelial cells lining uterine folds of the mesometrial region (Figs. 19, 20). Because of the elongated nature of these folds, these epithelial cells generally did not directly face yolk in the lumen and were not positioned to have phagocytosed yolk readily. Thus, the overall location of the granules is indicative of their secretory nature.

Fourth, granules occasionally lay in the uterine lumen, clustered in the immediate vicinity of the putative secretory cells, from which they apparently had been released (Fig. 21). If the granules were the product of widespread extracellular digestion, they should have been more consistently distributed in the uterine lumen. Fifth, similar granules were located (although sparsely) in the chorionic cells of some specimens, where they presumably had been absorbed. This location is consistent with their postulated role in nutrient transfer. Sixth, the granules had different staining properties from yolk. They stained strongly with hematoxylin (Figs. 17, 19) (including in specimens in which yolk was mainly eosinophilic) and were unstained by Milligan's trichrome. Unlike the yolk of such specimens, they were strongly PAS-positive (Fig. 18), suggesting a carbohydrate component. Taken together, these points make a strong case that the uterine granules are secretory in character. Interestingly, a carbohydrate moiety has also been documented in uterine secretions in the placentotrophic Brazilian skink, *Mabuya heathi* (Blackburn and Vitt, 2002).

Histological observations alone cannot eliminate the possibility that small amounts of yolk material are taken up by epithelial cells, particularly given past inferences that the abembryonic uterine epithelium has phagocytic properties (Weekes, 1930; Stewart, 1993; Blackburn, 1998a). It is also quite conceivable that water or organic molecules released during autolysis are absorbed across the uterine epithelium. However, if such resorption does occur, it had not depleted the abortive eggs noticeably in the several weeks since their cessation of development. The only eggs that appeared to have been depleted substantially in mass were ones in which the yolk material had passed out of the incubation chamber into the intervening segments. Furthermore, no differences in depletion of the egg material were noted between oviducts harvested in mid-November and those taken in December. Parturition would normally have been expected by mid-December; in fact, two healthy fetuses harvested on 6 December were at Dufaure and Hubert Stage 39, the stage before birth. Our observations suggest that even if any resorption was occurring, it would not have significantly depleted egg mass during the normal gestation period.

In summary, uterine histology revealed no definitive evidence for egg and embryo resorption. On the contrary, the most prominent feature of the oviductal eggs was their necrotic degeneration in situ. Moreover, the uterine tissue appeared strikingly normal. Rather than showing uptake of yolk, the uterine epithelium appeared to be secreting into the uterine lumen, as could be expected during normal pregnancy in this placentotrophic species. But if they are not resorbed, what is the fate of the inviable eggs and embryos?

Fate of Abortive Eggs

Resorption is one of several possible fates of abortive embryos and eggs (see Blackburn, 1998b, for a review). Observations on various squamates indicate that inviable eggs can 1) be retained in the oviducts for varying (and sometimes prolonged) periods of time; 2) be extruded en masse at the time of parturition, along with any normal fetuses or eggs; 3) pass down the oviduct and out of the cloaca as single, relatively intact masses; and 4) undergo dissolution and ooze out of the cloaca. Eggs can also be passed through the infundibulum into the peritoneal cavity, where they either degenerate or develop for a while ectopically. Of these possibilities, resorption is the only one for which no definitive evidence exists in squamates. The other outcomes have been reported in multiple species (Blackburn, 1998b).

In *Pseudemoia pagenstecheri*, our observations indicate that the oviductal eggs and embryos underwent substantial postmortem degeneration, necrosis, and homogenization. As indicated above, the deterioration of yolk and embryo probably is autolytic. Certainly no multicellular glands that might produce enzymatic breakdown are apparent in the uterus. The lack of evidence of invading leukocytes, lymphocytes, mast cells, macrophages, or infective microorganisms indicates that the necrosis was aseptic and did not extend to the uterine tissues. Advanced eggs also underwent mechanical disruption, through rupture of the chorion and movement of yolk into the exocoelom (Figs. 11, 12). Likewise, yolk had moved into the space between the uterine lining and the chorion and chorioallantois (Fig. 13). Given the importance of the chorioallantoic placenta to embryonic gas exchange, a squamate embryo could hardly have withstood such destruction.

While this study found no evidence of yolk digestion or uptake by the uterine epithelium, our observations did find that some yolk material had passed out of the incubation chambers and moved down the oviduct. Some degenerative eggs clearly had broken into pieces and their yolk had flowed or been extruded out of their incubation chambers (Fig. 9). As a result, yolk material had moved into constricted regions of the oviduct, where it lay isolated from the adjacent vitelline masses (Figs. 7, 8). In addition, the yolk of adjacent incubation chambers became intermixed (Fig. 10). The passage of yolk along the oviduct from adjacent eggs may account for the presence of yolk between fetal membranes (e.g., the chorioallantois) and the uterine lining. Alternatively, in such cases the yolk may have been extruded from the yolk sac of the egg itself.

How long the degenerating eggs and embryos would have been retained in the oviducts is unknown. Given that their development was terminated early in most females, probably soon after the time of capture, inviable and dead eggs may have been retained for as many as 7 weeks—most of the

expected length of gestation. Whether any yolk material was exuded from the cloaca, as occurs in some squamates (Blackburn, 1998b), is not known. Presumably, any eggs that persisted in the oviducts along with normally developing fetuses would have been extruded at parturition. This pattern also has been reported in other squamate species (Blackburn, 1998b). As another alternative, persistent abortive eggs may have been retained for more extended periods of time. Viviparous garter snakes (*Thamnophis sirtalis*) can retain abortive oviductal embryos and adnexae into the following breeding season (Hoffman, 1970). Likewise, oviparous snakes have been reported to retain inviable eggs in their oviducts for many months (Blanchard, 1925; Watanabe et al., 1989).

Thus, degenerative eggs in *Pseudemoia pagenstecheri* appear to be retained in the oviducts for some time, although portions thereof move down the oviducts prior to the normal time of parturition, possibly to be extruded from the cloaca. Evidence for this pattern exists for two other viviparous skinks, *Chalcides ocellatus* (DGB, pers. obs.) and *C. chalcides* (Blackburn, 1998a; Blackburn et al., 1998). In addition, extrusion of abortive eggs has been widely reported among other squamates (Rollinat, 1904; Domini, 1928; Neill and Boyles, 1957; Bustard, 1966; Fitch, 1970; Ronne, 1996). Reports in the literature may substantially underestimate the incidence of oviductal extrusion of eggs or embryonic material if these elements are ingested by the mother. Ingestion of abortive eggs or embryos has been reported in squamates (Osadnick, 1984; Seufer, 1985; Ronne, 1996) as well as mammals (Brambell et al., 1948). How widespread the phenomenon may be taxonomically is unknown.

Mechanism of Termination

Although the proximate cause of the cessation of development in *Pseudemoia pagenstecheri* is not clear, our observations offer insight into its nature. Four points should be noted. First, reproduction was interfered with in all of the females, since each contained dead embryos or eggs. As indicated above, development typically was disrupted very early, probably soon after the time of capture. Second, the cessation of development can be attributed indirectly to the stress or conditions of captivity, rather than to surgical procedures. Other captive females, which had not been operated on (to remove the contralateral oviduct), also contained eggs in which development had been terminated (JRS, pers. obs.). Furthermore, study of female *P. pagenstecheri* from this population (Thompson et al., 1999; Shine and Downes, 1999) indicates that normal pregnancy is maintained in captive females that are given optimal conditions for basking. A third point to be noted is that within a given female, the termination of development was, at least in some cases, a local

rather than a general phenomenon. A given female could contain eggs that had died at a range of stages of development. One even contained dead, undeveloped eggs adjacent to normal fetuses that were nearing the time of parturition. Fourth, termination of reproduction involved the disruption of cell layers, the breakage of extraembryonic membranes, and movement of yolk into exocoelomic cavities (Figs. 11, 12).

Based on the evidence, we can postulate two possible mechanisms for the cessation of development. The simplest explanation is that the eggs were simply unable to survive thermal conditions or other aspects of maternal captivity. Accordingly, following their death, eggs and embryos may have deteriorated through autolytic necrosis. While this explanation is quite plausible, it does not specifically account for the fact that some females contained eggs that had developed through the limb bud stage, and others to the point of parturition. Nor does it explain why a given female could contain eggs in which development was terminated early, lying adjacent in the oviduct to living embryos.

Another possibility is that the pregnant females took an active role in terminating egg development in response to the conditions of captivity. From the standpoint of life history theory, it should be adaptive for females to modulate reproductive investment according to environmental circumstances. Consideration of the functional morphology of the oviduct reveals a possible mechanism by which female squamates could terminate reproduction. The oviduct of squamates tightly surrounds the eggs and its musculature is able (perhaps along with the hypaxial abdominal musculature: Blackburn, 1998a) to expel eggs during oviposition and fetuses at parturition. Thus, compression by the oviductal musculature theoretically could disrupt eggs mechanically, effectively terminating reproduction. Indirect, histological evidence of this mechanism exists for *Chalcides chalcides*; observations of abortive eggs indicated that embryonic cell populations apparently continued to proliferate and spread after the fetal membranes had ruptured (Blackburn et al., 1998). Whether such a mechanism operates in *Pseudemoia pagenstecheri* remains to be determined. However, in the absence of a thick shell membrane the eggs would have little defense against oviductal contraction. If females can kill eggs through myometrial contraction, that would account for the rupture of extraembryonic membranes and intrusion of substantial quantities of yolk into the exocoelom (Figs. 6, 11, 12). It could also account for the extensive displacement of cell populations and tissues in the abortive embryos. The hypothesis that the oviduct played a role in killing eggs is also consistent with our observation that some yolks were separated into pieces and leaked and/or were squeezed out of the incubation chambers down the oviduct.

As suggested above, the ability to terminate pregnancy theoretically could allow females to modulate the timing and extent of reproductive investment in a given reproductive season. Further study is necessary to reveal whether its termination in *Pseudemoia pagenstecheri* occurs in response to nutritional shortages or physiological stress, as is true of the placental *Chalcides chalcides* (Domini, 1928; Blackburn et al., 1998). However, experimental evidence indicates that females can modulate nutrient investment according to food availability (Shine and Downes, 1999). We have good reason to believe that the particular conditions of captivity played a central role in our study, since females that are allowed to bask freely and that are housed with cycling temperatures carry their fetuses to term. Factors such as stress and suboptimal temperature regimes during pregnancy are known to lead to abnormal embryonic development in other squamates as well (e.g., Fox et al., 1961; Badir, 1968).

If female squamates can modulate reproduction after ovulation, such modulation may be other than a simple all-or-none phenomenon. Our observations on *Pseudemoia pagenstecheri* indicate that normal eggs can cohabit in an oviduct with others that had undergone mechanical disruption and deterioration. This situation also occurs in *Chalcides chalcides* (Domini, 1928; Blackburn et al., 1998). In addition, the literature contains many other reports of pregnant females that contain abortive embryos cohabiting in an oviduct with normal fetuses (Badir, 1968; Hoffman, 1970; Blackburn, 1998b). The possibility that female squamates may be able to control reproductive output following ovulation deserves to be investigated. Such control is unknown for reptiles, but is not unprecedented among other viviparous vertebrates. In a number of mammalian species, prenatal death mediated by the uterus is an adaptive response to such factors as inadequate maternal nutrition, physiological stress, genetic defects, and embryonic abnormalities (Hafez and Jainudeen, 1974; Short, 1979; Jacobs, 1982; Wilmut et al., 1986).

Uterine Morphology and Resorption: Squamates vs. Mammals

Common assumptions that squamates reabsorb uterine eggs are based largely on negative evidence, notably, the apparent disappearance of eggs from pregnant oviducts. However, as outlined above, reabsorption is one of several potential fates for non-viable eggs, and the only such fate for which definitive evidence is not readily available. Because reabsorption has obvious theoretical advantages (e.g., allowing females to recycle nutrients), the likelihood of such a pattern might seem quite plausible, at least to biologists who have not had occasion to consider uterine structure and function in detail.

Nevertheless, from a morphological standpoint, the resorption of entire eggs by the squamate uterus is not easy to imagine. Put simply, an oviduct is not an intestine. The small intestine of amniotes has multicellular glands (including the pancreas and liver) that can synthesize and secrete lipases, proteases, and emulsifying agents in great quantities—and which act on products of mechanical and chemical breakdown by the stomach and mouth. The intestine also exhibits folds and villi to increase internal surface area; enlarged epithelial cells with microvilli and glycocalyx-bound enzymes for absorption and digestion; and an elaborate system of blood vessels and lymphatics for carrying away products of digestion. Such features have not been observed in the oviduct of any squamate (Blackburn, 1998a). To note such points is not to deny the possibility of uptake of small quantities of yolk by uterine epithelial cells, nor epithelial uptake of fluid containing organic molecules originating from the egg. Further, given squamate diversity, one cannot disallow the possibility that elaborate specializations for large-scale digestion and absorption of inviable eggs have evolved in one or more of the many viviparous squamate clades. However, such specializations presumably would be evident morphologically, but have never been observed. Furthermore, given the tubular shape of the oviduct, it is difficult to imagine how inviable eggs could be digested extracellularly, in sites lying immediately adjacent to places where sibling eggs are undergoing normal development. In other words, known reproductive functions of the uterus would seem to be incompatible with a putative, simultaneous role in embryo digestion.

One reason why the idea of uterine resorption of eggs by squamates may seem plausible to some researchers is that such resorption occurs among eutherian mammals. However, viviparous mammals differ markedly from squamates with regard to structure of the female reproductive tract, relationship of the pregnant uterus to the developing embryo, and size and structure of the egg and early embryo. Eutherians exhibit a glandular, vascularized endometrium into which the blastocysts implant (Luckett, 1977; Mossman, 1987). In most species, implantation is invasive; the blastocyst penetrates through the uterine epithelium and becomes partly or completely surrounded by the vascularized stroma. Extraembryonic tissues are thereby in direct contact with the uterine stroma, the capillary endothelium, or the uterine blood itself (Ramsey, 1975; Mossman, 1987). As a consequence, embryonic tissues are directly exposed to maternal leukocytes as well as to macrophages and other phagocytic elements—structures that can function in resorption of the embryonic tissues (Fortuyn, 1929; Henderson, 1954; Gendron and Baines, 1989).

Squamates differ dramatically from such mammals, in that no invasive implantation occurs. The squamate egg invariably remains entirely confined

to the oviductal lumen, where an unbroken barrier of uterine epithelium and, usually, a remnant of the eggshell, separate the uterine connective tissue from the egg (Blackburn, 1993b, 1998a; Stewart, 1993). Because the egg never comes in contact with the uterine stroma, it is inaccessible to leukocytes, macrophages, and blood vessels that might otherwise be able to invade and help to remove cellular debris and extracellular components. Another major difference is that the squamate egg is massive compared to that of therians, even in the most placentotrophic squamates. Egg diameter in squamates vastly exceeds the width of the uterine stroma and, indeed, the entire uterine wall (for example, see Fig. 1). The connective tissue stroma of the pregnant uterus is far too thin to accommodate a macrolecithal egg, even if the barrier imposed by the uterine epithelium was able to be breached.

Conditions under which egg and embryo resorption occurs in mammals may offer further insight into the situation in squamates. Prenatal death and degenerative uterine fetuses have been widely reported among mammals, including in ungulates, lagomorphs, insectivores, rodents, primates, and carnivores (Marshall, 1922; Fortuyn, 1929; Hafez and Jainudeen, 1974). However, while ejection by the uterus of a dead fetus is common in eutherians, embryonic resorption generally occurs in early development, when the conceptus is small. For example, in domesticated ungulates, resorption occurs when embryos die in preimplantation and early blastocyst stages. Fetal death at advanced stages of development is associated with spontaneous abortion or fetal mummification (Hafez and Jainudeen, 1974). This situation is understandable, given the large size of the advanced fetus. In an experiment on rabbits involving surgical and hormonal induction of embryonic death, early stage embryos were resorbed, whereas later ones were aborted (Brambell et al., 1948). Embryos of intermediate stages were partially resorbed before uterine ejection of the remainder. These studies and others on rodents (see Morton et al., 1982) suggest that resorption by the uterus is a function of size of the embryo. The ability of eutherians to resorb early embryos, therefore, cannot readily be extrapolated to reptiles, whose macrolecithal eggs are large at ovulation and massive in comparison to the oviducts in which they are housed. It is notable that morphological evidence for uterine resorption is lacking even in *Chalcides chalcides* and *Pseudemoia pagenstecheri*, species whose ova are far smaller than those of typical squamates.

In sum, reproductive characteristics of squamates, in comparison to those of mammals, reveal no obvious means by which egg and embryonic material could be resorbed by the uterus in sizeable quantities. Most likely, this situation helps account for the fact that definitive evidence for oviductal egg resorption in squamates has remained so elusive, despite more than a century of speculation.

CONCLUSION

This study offers evidence that oviductal retention and probably extrusion, rather than uterine resorption, was the likely fate of abortive eggs and embryos in the *Pseudemoia pagenstecheri* in our study. Assumptions that pregnant females of this and other squamate species reabsorb inviable eggs lack definitive empirical support and are incompatible with known functional attributes of the squamate oviduct. Given the theoretical advantages of such a pattern, possibilities for its existence in other reptilian species, including snakes, cannot be ruled out. Nevertheless, in the absence of morphological evidence that the uterus of reptiles can digest and absorb egg material, claims that squamates resorb eggs and embryos from their oviducts remain speculative and uncorroborated.

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