Prenatal Development after Diapause in the Marsupial *Macropus rufogriseus*

*M. T. Walker*AB and *R. Rose*A

A Zoology Department, University of Tasmania, Box 252C, G.P.O., Hobart, Tas. 7001.
B Present address: School of Anatomy, University of Queensland, St Lucia, Qld 4067.

Abstract

Prenatal development of *Macropus rufogriseus* was described in eight stages ranging from a quiescent blastocyst to a near-term foetus. Embryos and corpora lutea were induced to resume development after diapause by the removal of suckling pouch young (RPY). The diameter of unilaminar blastocysts ranged from approximately 260 μm at 0 days after RPY to 290 μm after 6 days. At 9 days after RPY (330 μm diameter) the zona pellucida and mucoid coat were apparently absent and endoderm had proliferated (early bilaminar). At 15 days, mesoderm had formed in the trilaminar blastocyst (4.8 mm diameter). By 18 days after RPY the shell membrane had ruptured and was 3-0 μm in thickness (7-0 μm at 0 days after RPY). At this time the pre-foetal embryo had acquired forelimb ridges, mesonephric kidneys, optic stalks and 25 paired somites; persistent features included an amniopore, posterior neuropore and an intact stomodeal membrane; the lung buds and allantois were rudimentary; cervical, lumbosacral and cranial flexures were present. By 20 days after RPY, digital anlagen were noted in the forelimb paddles of the pre-foetal embryo (32 paired somites); the gonadal ridges, glomeruli and optic cups had formed. At 25 days after RPY, the near-term foetus had forelimbs with claws on the separate digits, hindlimb paddles, pontine flexure and cardiac valves; the metanephric kidneys, pigmented and nervous retinas and villous intestine were present. A single birth was recorded at 26 days after RPY (delayed gestation length).

Introduction

Bennett's wallaby occurs in Tasmania and is a distinct subspecies of the red-necked wallaby *M. r. banksianus*, found on the eastern coast of mainland Australia. Both are polyoestrous and monovular (Sharman 1961). The Tasmanian subspecies appears most recently in the literature as *Macropus rufogriseus rufogriseus* (Johnston and Sharman 1979).

Marsupials of the family Macropodidae ovulate post partum; the resultant corpus luteum is inhibited by the suckling stimulus of the young and becomes quiescent. The fertilized egg develops to a unilaminar blastocyst, which then enters a period of embryonic diapause (Tyndale-Biscoe 1963; Sharman and Berger 1969). If the pouch young is lost, or removed, both the quiescent corpus luteum and the delayed blastocyst resume development (Clark 1967).

The marsupial egg at ovulation is surrounded by a thin transitory zona pellucida and subsequently acquires a mucoid coat and an outer shell membrane, which remains intact for up to two-thirds or more of pregnancy (Hughes 1974). The blastocyst expands during this phase and the three primary germ layers (endoderm, ectoderm, mesoderm) are established.
In most marsupials embryonic development proceeds beyond the somite stage and up to the formation of foetal rudiments before the shell membrane ruptures (Hughes 1974). This rupture corresponds with a rapid growth phase and organogenesis occupies the latter stages of marsupial gestation. Certain structures are precocious in development and correspond to adaptation for birth and early pouch life of the rudimentary neonate.

Little has been reported on the development of anatomical features in the embryos of marsupials. Certain relevant studies are those of McCrady (1938) on Didelphis virginiana and of Hill and Hill (1955) on Dasyurus viverrinus, and that on the embryology of Schoinobates volans by Bancroft (1973). The present study of M. rufogriseus covers aspects of prenatal development and illustrates the disproportionately long period in marsupial gestation occupied by the establishment of primary embryonic germ layers in relation to organogenesis.

Materials and Methods

Female Bennett’s wallabies were captured during April and May 1977 in north-eastern Tasmania. Nine blastocysts were induced to resume development by the removal of pouch young (RPY) from 13 wallabies. The captive wallabies were fed on water, kangaroo pellets, grass and shrubs in an enclosure (57 by 31 m) at the Department of Zoology, University of Tasmania. Before dissection, females were anaesthetized with a mixture of ether and air. The entire reproductive tract was excised and fixed in 10% buffered formalin. A small amount of fixative was perfused by syringe into the pregnant uterus. A minimum fixation period of 10 days elapsed before the tissue was sectioned. The duration of 'pregnancy' or 'delayed gestation' for each dissection is accurate to within 10 min. The pregnant uteri of 0, 3, 6, 9, and 15 days after RPY were serially sectioned (transversely) at 6 pm to determine the position and approximate dimension of the blastocyst. The pre-foetal embryos (18 and 20 days after RPY), and the foetus at 25 days after RPY, were photographed (Figs 1–3) and then serially sectioned in the sagittal plane at 6 pm. Sections were stained with Ehrlich’s haematoxylin and eosin, or Mallory’s triple stain. Anatomical measurements were made with a graduated eyepiece calibrated against a micrometer slide. These measurements were not corrected for post-fixation shrinkage artefacts.

Results

Blastocysts

The diapausing blastocyst (0 days after RPY) had a shell membrane approximately 7-0 μm thick, a mucoid coat 60 μm and a zona pellucida 4·5 μm (Fig. 4). The blastocyst diameter measured 260 μm. Below the zona pellucida, the protoderm comprised a single layer of attenuated cells with vacuolar cytoplasm. The nuclei of the protoderm cells were oval or spherical, usually with one prominent nucleolus and occasional clumps of heterochromatin. The unilaminar blastocyst at 3 days after RPY was 270 μm in diameter. The shell membrane (strongly eosinophilic) was 5·0 μm in thickness and surrounded a prominent basophilic mucoid coat. At 6 days after RPY the diameter was 290 μm, the shell membrane was 5·0 μm and the zona pellucida 2·5 μm in thickness. Between these two membranes the mucoid coat was abundant, but shrinkage (artefact) prevented an accurate estimate of the thickness. In situ fixation of the unilaminar blastocysts precluded an accurate measurement of the protoderm cells.

The early bilaminar blastocyst at 9 days after RPY measured 330 μm in diameter with an intact shell membrane (4·5 μm thick). The mucoid coat and zona pellucida were not identified with certainty, but a thin disrupted basophilic zone (presumptive remnant of these two acellular membranes) lining the inside of the shell membrane...
Fig. 1. Embryo of *Macropus rufogriseus* at 18 days after RPY. Scale line, 4 mm.
Fig. 2. Embryo of *Macropus rufogriseus* at 20 days after RPY. Scale line, 3 mm.
Fig. 3. Foetus of *Macropus rufogriseus* at 25 days after RPY. Scale line, 3 mm.

*a*, allantois; *am*, amnion; *b*, bilaminar yolk sac; *e*, embryo; *s*, shell membrane (ruptured); *st*, sinus terminalis; *u*, uterus; *v*, vascular yolk sac.
was noted. An entire layer of protoderm cells was closely apposed to the inside of the shell membrane (Fig. 5). These cells were linked by pseudopodial extensions of cytoplasm, and the flattened nuclei (13 by 6 \( \mu \)m) were strongly basophilic. The blastocoel contained acellular material and presumptive early endoderm cells. These cells were roughly spherical (18 \( \mu \)m diameter) with weakly basophilic oval nuclei (13 by 10 \( \mu \)m), and occupied a portion of the blastocoel immediately beneath
a limited area of protoderm. Mitoses were noted in the endoderm cells and several had become apposed to the protoderm cells, lodged beneath the thin area of cytoplasm at the lateral junctions of the protodermal cells.

At 15 days after RPY the embryo was an early trilaminar blastocyst, 4.8 mm diameter, with an intact shell membrane 6.0 μm thick. An entire layer of ectoderm was apposed to the inner surface of the shell membrane, and many of these cells were mitotic. The ectodermal cells were rectangular (17 by 12 μm), with mildly eosinophilic cytoplasm, and orientated with the long axis against the shell membrane. The majority of nuclei were oval (10 μm in length) with strongly basophilic reticular chromatin. A continuous layer of flattened endodermal cells was loosely apposed to the inner surface of the ectoderm. The cells were connected by long pseudopodial processes of strongly eosinophilic cytoplasm. The endodermal nucleus was oval, mildly basophilic and elongate (12 by 5 μm). The germinal disc was located in a restricted zone of the vesicle wall. In this region, cells of the superficial ectodermal layer (medullary plate) were cuboidal and strongly basophilic. The deepest layer comprised the notochordal plate (endoderm), and the middle layer of flattened cells was the presumptive early mesoderm. The endoderm and mesoderm cells were very similar at the light microscope level of magnification. There was no zona pellucida or mucoid coat.

Pre-foetal and Foetal Anatomy

At 18 days after RPY the pre-foetal embryo had acquired cervical, lumbosacral and cranial flexures (Figs 1, 8). This caused the head to dip into the proamnion. Slight ectodermal swellings at the level of the ventricle represented the rudimentary forelimb ridges. There were no external signs of the hindlimbs. The otic placodes had closed to form the otic vesicles. On the ventral surface the gut was open and in contact with the yolk-sac cavity. The amniopore persisted in the mid-dorsal region and a posterior neuropore was noted. The greatest length of the embryo measured 4.2 mm.

By 20 days after RPY the pre-foetal embryo was facing anteriorly in the posterior part of the pregnant uterus, with forelimb paddles, hindlimb buds and an enlarged cephalic region (Fig. 2). The amnion was entire and neural groove entirely closed. There was a prominent cervical flexure and the tail had formed. A sacculate allantois (1.2 mm diameter) was located ventral to the tail. The greatest length of this embryo was 6.90 mm.

In the near-term foetus at 25 days after RPY the forelimbs were well developed and disproportionately large, with claws on the separate digits of the manus (Figs 3, 10). In contrast, the hindlimbs resembled paddles with digital anlagen. Two large omphalomesenteric veins merged just before entry into the umbilical hernia. The latter measured 1.3 by 1.8 mm and contained folds of small intestine, mesentery and the omphalomesenteric vein and artery. Retinal pigment, an enlarged mesencephalon, gaping mouth and non-protrusive tongue were observed. The allantois was much enlarged (13.5 by 8.0 mm) and deflated. The pontine flexure had lifted the head off the chest. The greatest length of the foetus (effectively crown-rump at this stage) was 15.30 mm.

A neonate was intercepted before reaching the pouch. It was born 30 min before 26 days after RPY. The nostrils were gaping, the forelimbs were well developed
M. T. Walker and R. Rose

and the body was covered by an epitrichium. The crown–rump length was 16.90 mm.

**Organogenesis**

(i) **Cardio-vascular system**

At 18 days after RPY the bulbus arteriosus, ventricle, atrium and sinus venosus were lateral dilations of the heart tube (Fig. 8). The endocardium was a sparse array of mesenchymal cells and the epicardium a more compact layer. Valves had not formed and the anlagen were mesothelial thickenings of the endocardium. The omphalomesenteric vein returned blood to the atrium from the trilaminar yolk-sac via a ventral vascular plexus and the sinus venosus. Aortic arches I–V, and a presumptive sixth, were noted in the mandibular arch, the hyoid, the third, fourth and fifth pharyngeal arches respectively (Fig. 7). The dorsal aorta was single, except in the medial zone just posterior to the fourth pharyngeal arch. Here it was paired, corresponding to the entry of the aortic arches. The atrium was directly posterior to the ventricle, and received blood on both dorsolateral margins from the anterior cardinal veins (paired) and the inferior vena cava. The pericardial cavity had not formed. Intersomitic vessels branched off the dorsal aorta, which passed dorsal to the paired mesonephric kidneys. The dorsal aorta connected with the ventral vascular plexus at both ends of the coelom, and provided blood to the vascular yolk-sac via the omphalomesenteric artery. The ventricle was lying to the left side of the median line.

By 20 days after RPY the heart had enlarged and the ventricular endocardium was forming trabeculae. The epicardium of both the atrium and ventricle was two or three cells thick. Endocardial cushions formed the atrioventricular valve. The atrium had altered position in relation to the ventricle and was anteriodorsal. In contrast to the condition in eutherian mammals, choriovitelline placentation was evident and the enlarged omphalomesenteric vein, which entered the embryo mid-ventrally, passed via the mesentery into the liver. Blood was conveyed thence to the definitive right atrium (Fig. 12). Major vessels were distended with blood. The pericardial cavity was formed and the atria had not separated.

By 25 days after RPY the sino-atrial valves were functional (Fig. 10) and the atrium had small trabeculae. The vascular system was in contact with all zones of the body, and capillaries supplied endocrine and other organs. The right ventricle was in direct contact with the left ventricle by a medial interventricular foramen. Two consecutive valves in the truncus arteriosus controlled blood flow into the aortic arch (just to the left of and merging with the pulmonary trunk). Basal semilunar valves were noted in the pulmonary trunk, and a single atrium was present.

![Fig. 6. Photomicrograph of the eye, in the sagittal plane, from the foetus at 25 days after RPY. Scale line, 0.1 mm.](image1)

![Fig. 7. Photomicrograph of the aortic arches, in the sagittal plane, from the pre-foetal embryo at 18 days after RPY. Scale line, 0.2 mm.](image2)

1-6, aortic arches in cephalocaudal sequence; e, ectoderm; h, hyoid arch; lv, lens vesicle (fibre core, outer epithelium); m, mandibular arch; n, nervous retina; ov, primitive optic vesicle; ph, pharynx; p, pigmented retina; s, stomodeum; v, ventricle; vb, vitreous body.
Fig. 8. Photomicrograph and labelled drawing of the pre-foetal embryo at 18 days after hatching, in sagittal section. Scale line, 0.5 mm.
(ii) **Alimentary and respiratory systems**

The embryo at 18 days after RPY had an unruptured stomodeal membrane. The floor of the pharynx was thicker than the roof. Pharyngeal arches protruded into this region of the foregut and conveyed the aortic arches across the cavity to the dorsal aorta. At the level of the atrioventricular constriction of the heart, the pharynx divided into a ventral trachea and the oesophagus. The latter was continuous with the yolk-sac through a narrow medial tube. More anteriorly, the pharynx was shallow but laterally expanded. The anterior projection (Seessel’s pocket) of the pharynx formed the ectodermal invagination of Rathke’s pouch. Two primary bronchi diverged from the short trachea and passed into each of the paired lung buds. These lay in the laterally expanded coelom, and were joined dorsally and ventrally to the body wall (Fig. 8). No pleural cavity had formed and the coelom extended on the dorsal and ventral side of the mesonephric kidneys. Hepatic cells were noted near the sinus venosus and a ventral diverticulum of the hindgut formed the rudimentary allantois.

At 20 days after RPY the stomodeal membrane had ruptured and a long, shallow pharynx passed through the pharyngeal arches. The endodermal lung buds comprised three main bronchi and were surrounded by a pleural cavity (Fig. 12). The stomach and intestine had formed but did not contain any villi. The intestine (endoderm) was surrounded by mesenchymal thickenings comprising the definitive mesentery. Haemopoiesis was evident in the liver, with nucleated foetal erythrocytes and a few presumptive megakaryocytes. The hindgut had folded to resemble the definitive colon.

By 25 days after RPY the pharynx divided just anterior to the nasopharyngeal and thyroid ducts into an elongated trachea (with precartilaginous rings of mesenchyme) and the oesophagus. The latter terminated in a large villous stomach on the left side of the foetus. Several loops of small intestine extended into the umbilical hernia and were surrounded by mesenchymal cells and capillaries (Fig. 10). Secretory cells in the villous endodermal lining had apparently not differentiated. The endodermal layer was four cells thick with convex apical surfaces. The gall bladder lay on the ventroposterior margin of the liver. A bile duct, which contained secretory material (cellular), passed from the gall bladder and encircled the duodenum. Pancreatic tissue and ducts were formed. The spleen was connected to the stomach by the gastro-splenic ligament and to the duodenum by the dorsal mesogastrium. An omental bursa was formed. The lungs each comprised a single lobe with many endodermal bronchioles (approximately 60 μm diameter, and lumen 45 μm). These were surrounded by a mass of mesenchyme cells with scattered capillaries. Several capillaries contained blood cells but the majority were devoid of them. The apical surfaces of the endodermal bronchiolar cells were convex. The liver was large, with three lobes composed of parenchymal cells with chromophobic cytoplasm and basophilic nuclei. Sinusoids were surrounded by a thin mesothelial lining. Megakaryocytes with double nuclei were scattered throughout the liver. A tooth bud was observed below the tongue.

(iii) **Urogenital system**

The paired kidneys, at 18 days after RPY, contained a few degenerating pronephric tubules and an elongate, single layer of mesonephric tubules on both
sides of the median ventral line. A mesonephric duct lay dorsal to these tubules, which were curved into a U-shape.

A gonadal ridge had appeared by 20 days after RPY as a thickening of the dorsal peritoneum on the ventromedial surface of each mesonephros. Presumptive germ cells were present in the gonad. The mesonephros was an elongate mass of mesonephric tubules and a single ventral layer of glomeruli (Fig. 12).

In the near-term foetus (25 days after RPY), each functional mesonephros was enlarged and in contact with the liver, adrenal gland (0.75 by 0.42 mm), gonad (0.50 by 0.16 mm), and the roof of the coelom. Bowman's capsule in the glomerulus measured approximately 75 μm in diameter. Cells of the distal mesonephric tubules were small, with an estimated height of 6 μm. They were cuboidal with basophilic nuclei, one nucleolus, and acidophilic cytoplasm. The proximal tubules had large cuboidal or low-columnar cells with an average height
of 17 μm and chromophobic nuclei. The mass of tubules was enclosed by two or three layers of mesoderm. Below the gonads and in the same sagittal plane, the paired rudimentary metanephric kidneys had appeared; each measured 0.18 by 0.35 mm (0.8 by 2.0 mm for mesonephros). The narrow ureter passed from

the metanephros posteriorly to the urogenital sinus. The mesonephric duct, in contrast, had a swollen lumen and opened near the genital eminence into the allantois. Germ cells were noted in the gonads and were spherical with round nuclei. The gender could not be determined.

Fig. 9b. Labelled drawing of the same section as in Fig. 9a.
(iv) Nervous system

Eighteen days after RPY the neuropore was open dorsally from the medial zone. The brain comprised three chambers, a forebrain, midbrain and hindbrain. A lateral diverticulum from either side of the prosencephalon (forebrain) formed the optic stalks. Formation of the lens vesicles had not commenced. The paired otic placodes were closed to form vesicles, each with a mesiodorsal endolymphatic duct.

(Fig. 8). Anlagen of the paired semilunar ganglia were noted anterior to the otic vesicles.

By 20 days after RPY the spinal ganglia were formed, the neuropores had closed and the mantle layer of the spinal cord was distinguished from the ependymal layer. Cervical ganglia were enlarged and rami from the semilunar ganglia (cranial
nerve V) had penetrated the mandibular and maxillary arches. The paired optic vesicles were invaginated to form cups. The lens vesicles had pinched off from the head ectoderm and proximal mesenchymal cells appeared invasive.

At 25 days after RPY the nervous system of the foetus was extensively developed. The paired geniculate ganglia (apposed and anterior to the acoustic) were located just anterior to the precartilaginous otic capsules in which the cochlear ducts were formed. The semilunar ganglion was connected to the floor of the myelencephalon
by the trigeminal nerve (Fig. 10). The ophthalmic (sensory), mandibular and maxillary branches of this ganglion innervated the eye, lower jaw and upper jaw respectively. Spinal ganglia had increased in complexity. The brain cavities were large and the walls poorly developed. The lateral telocoels projected anteriorly and the posterior choroid plexus extended into the myelocoel. The stomach was innervated by large splanchnic nerves and the tongue by the hypoglossal nerves (12th cranial). Paired optic recesses were identified in the floor of the diencephalon. A notochordal remnant was located in the centre of the vertebral column. The eyes had both pigmented and nervous retinas (Fig. 6). The vitreous body and inner neural layer of the lens vesicles were formed. By 25 days after RPY the olfactory apparatus was well developed.

(v) Muscular and skeletal systems

The embryo at 18 days after RPY had 26 paired somites lying on either side of the spinal cord. The somites were bathed on the ventral portion by both the dorsal aorta and postcardinal plexus, which send capillaries into the intersomitic fissures.

At 20 days after RPY 32 paired somites were counted. These had enlarged since 18 days after RPY and were still bathed directly by the dorsal aorta. Precartilaginous condensations of mesenchyme were associated with the somites. These areas surrounded the notochord. As with the embryo at 18 days after RPY, there were extensive areas of mesenchyme.

By 25 days after RPY the voluntary musculature was well developed, particularly in such regions as the tongue, shoulders and forelimbs. Histologically, the nuclei of voluntary muscles were aligned in a single axial row. The striated fibrillae were confined to the periphery. Both internal and external intercostal muscles were developed between the ribs (Fig. 10). Chondrification was widespread in the cartilage of the axial skeleton and ossification was in the initial stages in the mandible, maxilla and claws of the forelimbs.

(vi) Endocrine system

These areas were identified mainly in the foetus at 25 days after RPY. The pituitary anlage at 18 days after RPY was an anterior pharyngeal invagination of mesenchyme and ectoderm. This made contact with the floor of the midbrain and formed Rathke’s pouch. The ectodermal portion had thickened by 20 days after RPY. In the near-term foetus, Rathke’s pouch was connected to the pharyngeal roof by a thin line of cells which was almost severed by the sphenoid cartilage (Fig. 11). The residual lumen of Rathke’s pouch separated the pars intermedia from the anterior lobe of the hypophysis (pars distalis). The pars neuralis was

---

Fig. 11. Photomicrograph of the pituitary gland, in the sagittal plane, from the foetus at 25 days after RPY. Scale line, 0.3 mm. b, basilar artery; d, diencephalon; i, infundibulum; m, myelencephalon; p, pharynx; pd, pars distalis; pi, pars intermedia; ps, pituitary stalk (remnant); s, sphenoid cartilage.

Fig. 12. Photomicrograph of the cardio-vascular system, in the sagittal plane, from the pre-foetal embryo at 20 days after RPY. Scale line, 0.5 mm. a, atrium; am, amnion; b, lung bud; g, glomerulus; i, intestine; l, liver; m, mandibular arch; me, mesonephros; os, omphalomesenteric artery; ov, omphalomesenteric vein; fns, sinus venosus; v, ventricle.
Prenatal Development in *Macropus rufogriseus*
forming as an infundibulum from the posterior floor of the diencephalon. The pituitary was vascularized, and comprised compact basophilic ectodermal cells.

Conical papillae were scattered regularly over the tongue surface and projected approximately 10 μm.

A paired cervical thymus was located in the ventral cervical region, superficial to the hyoid muscle and just beneath the skin (Fig. 10). The two structures were almost apposed to the medial line and were divided laterally into two main lobes, one of which divided again at its extremity. The main medial lobe was 0.20 mm in diameter and composed of compact basophilic endodermal cells. There was a dense peripheral supply of blood capillaries. The thoracic thymus (paired) was an unlobed, spherical mass of cells. Each was located in the superior mediastinum on the ventral aspect of the trachea and just anterior to the pericardial cavity. It was lying in a small cavity (possible artefact) in close proximity and ventral to the right anterior cardinal vein. The thoracic thymus measured 0.12 mm in diameter.

The thyroid gland was composed of compact basophilic endodermal cells, interspersed by channels of mesenchyme and capillaries. It measured 0.14 mm in diameter and was connected to the pharynx by a thyrroglossal duct which emerged just posterior to the epiglottis. The thyroid was located ventral to the trachea and dorsoposteriorly from the hyoid cartilage.

As mentioned previously, the adrenal gland was in contact with the functional mesonephros in the near-term foetus. Submaxillary, submandibular and sublingual glands were noted. The latter had a duct passing below the tongue.

Discussion

Three unilaminar blastocysts (the earliest one quiescent) are described for *M. rufogriseus* at 0, 3 and 6 days after rpy. The early development of marsupials differs from that in most eutherian mammals in that there is no morula and no inner cell mass (Wimsatt 1975). Cell division does not normally occur in the quiescent blastocyst of macropodid marsupials (Tyndale-Biscoe 1963; Clark 1966), although it is common in the delayed blastocysts of eutherians (Enders 1963).

The macropodid embryo, during embryonic diapause, is a unilaminar blastocyst, with a vesicle of 80–90 cells (Clark 1966; Smith and Sharman 1969). The size of these blastocysts is very similar amongst macropodid marsupials. The mean diameter of 21 unilaminar dormant blastocysts of *Setonix brachyurus*, the quokka, is $262 \pm 3 \ \mu m$ (Tyndale-Biscoe 1963) and the specimen of Bennett’s wallaby at diapause is 260 μm. Kirkpatrick (1965), reports that 10 dormant blastocysts from *Macropus major* range in diameter from 220 to 310 μm. Other recordings are for the rat kangaroo *Bettongia lesueur* of 286 and 294 μm (Tyndale-Biscoe 1968), and the red kangaroo *Megaleia rufa*, $330 \pm 26 \ \mu m$ (Clark 1966). In contrast to that in eutherians, the definitive embryonic area in the marsupial unilaminar blastocyst cannot be distinguished. Polarity is not achieved until presumptive endoderm cells become detached from the protoderm cells (Tyndale-Biscoe 1973).

There is much variation among marsupial species in the thickness of the three acellular egg membranes which surround the vitellus. The shell membrane of Bennett’s wallaby (present study) is thickest during diapause and measures 7.0 μm. Hughes (1974) notes seven unilaminar blastocysts of *Trichosurus vulpecula* with
Prenatal Development in *Macropus rufogriseus*

A mean shell thickness of 7.4 μm, and the maximum shell thickness in *Didelphis aurita*, *Dasyurus viverrinus* and *Phascolarctos cinereus* is 3.8 and 10 μm respectively. The shell membrane is a resistant, proteinaceous material, rich in disulphide bonds and with the histochemical properties of ovokeratin. Its deposition continues after the egg enters the uterus (Hughes 1974). Persistence of the shell membrane prevents close foeto-maternal contact over an extended portion of gestation. This may function to inhibit an allograft reaction (Tyndale-Biscoe 1973). The mucoid coat of Bennett’s wallaby measures a maximum thickness of 60 μm in the quiescent blastocyst. The thickness of the fully formed mucoid coat in other marsupial species is recorded as: *D. aurita*, 140 μm (Hill 1918); *T. vulpecula*, 45-90 μm (Hughes 1974); *D. viverrinus*, 15-22 μm (Hill 1910).

By 9 days after RPY the blastocyst of *M. rufogriseus* (330 μm diameter) is an early bilaminar stage with prospective endoderm cells being proliferated into the blastocoel. Several of these cells are apposed to the inner surface of the protoderm. Hill (1910) notes both these cell types in a 350-μm blastocyst of Bennett’s wallaby.

All primary embryonic germ layers are evident before rupture of the shell membrane. The enlarged vesicle at 15 days after RPY (4.80 mm diameter) is lined with an outer layer of ectodermal cells, closely apposed to the intact shell membrane, and an entire inner endoderm layer. The few isolated cells (presumptive mesoderm) between these two cell types are also found in a blastocyst (1.8 mm diameter) of *Isoodon macrourus* (Hollis and Lyne 1977). Bancroft (1973) describes cells of the medullary plate in *Schoinobates volans* as denser and more chromophilic than the remaining ectoderm. This corresponds with the same zone in Bennett’s wallaby. The increased thickness of the shell membrane in the early trilaminar blastocyst is noted, and tentatively explained on the basis of individual embryonic variation. Hollis and Lyne (1977) mention that the shell membranes of two fully bilaminar blastocysts in *Isoodon macrourus* varied by a factor of two (0.9 and 1.8 μm thickness).

Marsupials follow the amniote pattern of development. The middle germ layers differentiate into splanchnic mesoderm, which is later found in a proximal vascular zone of the extraembryonic membranes, and somatic mesoderm. The pre-foetal embryo at 18 days after RPY corresponds to McCrady stage 27 or 28 of the American opossum *Didelphis virginiana* (first third and middle of the 10th day) according to the anatomical description (McCrady 1938). The prenatal development of *M. rufogriseus* differs temporally from that described for other marsupials, particularly in terms of gestation length and maternal weight. Eye pigment is lacking in neonates of the marsupials *Marmosa* sp. and *Dasyurus* sp., whereas the pigmented retina is present in Bennett’s wallaby at 25 days after RPY (Fig. 6) and in *D. virginiana* at birth (McCrady 1938). The *M. rufogriseus* neonate is also more advanced in terms of lung and forelimb development. Certain features of the 3-4-mm *Sch. volans* embryo with 18 somites (Bancroft 1973) are similar to the 26-somite, 4-2-mm Bennett’s wallaby. The tail amnion is partly differentiated and neural folds are fused along most of the length of the embryo, but anterior and posterior neuropores persist. An embryo of *Sch. volans* with 25 somites and greatest length of 4.5 mm is similar to the 26-somite stage (4.2-mm embryo) of Bennett’s wallaby in the pharyngeal pouches, lung and allantois rudiments. However, in *Sch. volans* at this stage the liver anlage is present and the amniopore is closed. This is in
advance of Bennett's wallaby. A 5.9-mm embryo of *Sch. volans* corresponds to the 6.9-mm *M. rufogriseus* at 20 days after RPY in the extent of gonad, stomach, intestine and liver development (Bancroft 1973). In a *Se. brachyurus* embryo of greatest length 4.0 mm, the neural groove is still open along its entire length (Sharman 1955). The allantois of *M. rufogriseus* at 18 days after RPY extends from the posterior hindgut as a ventral diverticulum. This resembles, as does the incomplete fusion of the amnion folds, a *Se. brachyurus* embryo of 5 mm greatest length (Sharman 1955). Sharman (1961) did not recognize an allantoic primordium in a 4.8-mm *Se. brachyurus* embryo (9 days before birth).

The 6.9-mm Bennett's wallaby pre-foetal embryo at 20 days after RPY is intermediate between McCrady stages 31 and 32 (first and second halves of the 11th day of gestation). Corresponding features include the forelimb clubs which are flattened into paddles, a dorsoventral increase in body dimension, and size of the allantois. The hindlimb buds persist in the *M. rufogriseus* embryo. The embryo of *Sch. volans* corresponding to McCrady stage 31 is 6.0 mm (Bancroft 1973) with hindlimbs at the club stage. The Bennett's wallaby foetus at 25 days after RPY (15.3 mm crown–rump) resembles McCrady stage 33 or 34 and a 10.2-mm embryo of *Sch. volans*.

In Fig. 3 it is apparent that the foetal mouth at 25 days after RPY is more gaping than that of the neonate. Shortly before birth in most marsupials a transitory seal develops by means of a filling substance. This moves from the corners of the mouth and narrows it to just large enough to accept the mother's nipple. This lip closure seals the mouth and limits jaw movement. During early pouch life the jaw transforms from an articular–quadrate form to the mammalian dentary–squamosal jaw (Lillegraven 1975). A keratinized external covering on the neonate functions to prevent it desiccating while crawling from the external genital opening to the pouch (Hill and Hill 1955). The single observation of delayed gestation length in the Tasmanian subspecies of *M. rufogriseus* (26 days after RPY) is shorter than the range of 27.5–29.5 days given by Calaby and Poole (1971). Further observations are required to clarify this discrepancy.

Although certain features of marsupial organogenesis resemble that of eutherian mammals, there are deviations from this pattern. Organs may develop precociously to cope with the extrauterine existence in a rudimentary form. The well developed pancreas, gall bladder and villous stomach of the marsupial neonate enable it to utilize a new source of food at birth. Within the foetal liver of *M. rufogriseus* (present study) and *Di. virginiana* (McCrady 1938), the omphalomesenteric vein is not completely destroyed or replaced by sinusoids as in placental mammals, but maintains an open channel through to the sinus venosus. The umbilical vein is relatively undeveloped, in accordance with the choriovitelline placenta of macropodid marsupials. The non-placental allantois contrasts with eutherian placentation, in which the highly vascular allantois enlarges and invades the maternal tissues to establish a placenta.

The marsupial urogenital system varies from the basic eutherian pattern. In the embryonic marsupial, the ureters (metanephric ducts) pass between the Wolfian (mesonephric) and Müllarian ducts, whereas in the embryonic eutherian mammal they pass to the outside of the future sex ducts (Sharman 1970). In *Di. virginiana* the gonadal rudiment appears during the late 10th or early 11th day of uterine development. Differentiation of the ovary begins at the 3rd day of pouch
life (Nelson and Swain 1942a, 1942b). Morgan (1943) notes that the gonads of *Di. virginiana* at birth are not sexually differentiated. In Bennett's wallaby the gonad appears as a thickening of the dorsal coelomic epithelium on the ventromedial surface of each mesonephros, as in the human embryo (Valdés-Dapena 1957). The mesonephros of the marsupial neonate is functionally active before and for some time after birth (Buchanan and Fraser 1918). Although the mesonephros of Bennett's wallaby never reaches the relative size of that in the 8-mm or 10-mm pig embryo (Barnett 1969), it has attained a single layer of glomeruli up to 6 days before birth (20 days after RPY). The mesonephric kidney of *Marmosa* sp. and *Dasyurus* sp. still lacks glomeruli at birth (Lillegraven 1975).

The functional anatomy of the foetal mesonephros may be correlated with the type of placenta. The rat has a rudimentary embryonic mesonephros, with relatively few nephrons, and an efficient haemochorial placenta, which assumes the entire burden of excretion. The pig has a large embryonic mesonephros, comprising several hundred long and convoluted nephrons, and an epitheliochorial placenta (Torrey 1967). The uterine mucosa and chorion of the pig do not grow together. The placenta has a low excretory capacity and this function is primarily dependent on the mesonephros. The human, Bennett's wallaby and many other mammals lie between these two extremes.

The embryonic nervous system in the marsupial reflects the transition to a latent development in the pouch. Otic invagination, which closes over at 9 days in the mouse (Rugh 1968) occurs just under 18 days after RPY in Bennett's wallaby. Cervical ganglia develop early, for the control of the forelimbs at birth, and the myelencephalon is precocious because nerves to the suckling mechanism, lungs and stomach must be used at an early age. However, in the near-term stage, the mouse brain is more developed (Rugh 1968) than it is in *M. rufogriseus*, in which the brain cavities are vacuous. The rodent neonate must attain independence more rapidly than the marsupial, in which immediate postnatal nervous action is essentially reflex. Although the shoulder and forelimb muscles are relatively advanced, for the climb to the pouch, the nuclei of voluntary muscles in the *M. rufogriseus* foetus at 25 days after RPY are located in axial rows, with the striated muscle fibres along the periphery. This also occurs in *Di. virginiana* (McCraday 1938) and is considered a transitional stage between the foetal form and adult form, in which the nuclei are peripheral.

The aortic arches are formed during organogenesis and appear in cephalo-caudal sequence. In contrast to the majority of eutherian mammals, the six aortic arches of *M. rufogriseus* appear simultaneously in the embryo at 18 days after RPY. The first aortic arch, which passes around the pharynx in the mandibular arch, has diminished, and the sixth aortic arch is rudimentary. In most mammals, the cephalic arches undergo regression before the sixth arch is formed (Patten 1964). This effect in Bennett's wallaby may be due to the short period of intrauterine existence, during which the body systems must achieve a functional state. In *M. rufogriseus, Marmosa* sp., *Dasyurus* sp. and *Di. virginiana*, the separation of the heart ventricles is incomplete in the near-term foetus. In the human heart the interventricular foramen closes when the embryo is between 15 and 17 mm in length (Patten 1964).

Thus, as in other marsupials, the establishment of primary embryonic germ layers in Bennett's wallaby occupies a disproportionately long period in gestation,
while foetal differentiation and organogenesis are largely confined to the terminal third of pregnancy. In contrast to that of eutherian mammals, the macropodid gestation period corresponds mainly to embryogenesis and the major growth phases are postnatal.

Acknowledgments

The authors wish to thank Dr R. L. Hughes and Dr W. Whitten for criticism of the manuscript, and the National Parks and Wildlife Service of Tasmania for assistance in the trapping of the wallabies.

References

Morgan, C. F. (1943). The normal development of the ovary of the opossum from birth to maturity and its reaction to sex hormones. J. Morphol. 72, 27–85.
Rugh, R. (1968). 'The Mouse: its Reproduction and Development.' (Burgess: Minneapolis.)


Manuscript received 11 March 1980; accepted 10 September 1980