



# FAUNA *of* AUSTRALIA



## 15. TACHYGLOSSIDAE

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Short-beaked Echidna–*Tachyglossus aculeatus* [CSIRO Wildlife & Ecology]

## DEFINITION AND GENERAL DESCRIPTION

Members of the Tachyglossidae – the echidnas – are Monotremata in which the snout has been modified to form an elongated beak-like structure. Teeth are absent and the tongue is long, vermiform and protrusible well beyond the end of the snout. Egg and young are carried in a pouch on the ventral surface. Both ovaries are functional. Some hairs on the sides and back are modified to form sharp spines and the tail is very short.

## DISCOVERY

Shaw (1792) published an account of a strange quadruped about ‘18 inches’ (450 mm) long with an elongated naked snout and long sharp spines on its back; the animal had been caught in New Holland ‘on a large red anthill’. He named it *Myrmecophaga aculeata*, thinking it might be related to the South American ‘ant bear’ (ant eater). The origin of this specimen is not known, but it was probably the specimen of a ‘porcupine’ caught near Port Jackson and sent on 18 December 1791 by Governor Arthur Phillip to Sir Joseph Banks. It went on H.M.S. *Gorgon* which arrived in England 7 July 1792 (Whitley 1975). The name of the animal has been changed from *Myrmecophaga aculeata* four times over the years: *Ornithorhynchus hystrix*, *Echidna hystrix*, *Echidna aculeata* and, finally, *Tachyglossus aculeatus* (Iredale & Troughton 1934), the Short-beaked Echidna.

The name ‘*Tachyglossus*’ means ‘quick tongue’ and refers to the rapidity with which this organ is used to catch ants and termites.

## MORPHOLOGY AND PHYSIOLOGY

### External Characteristics

The body of the Short-beaked Echidna is compressed dorso-ventrally, the ventral surface is flat or slightly concave, and the domed back is covered with hair and sharp spines of varying sizes and lengths (Figs 15.1 & 15.2). The belly is covered with hair except in the central concave area which is more or less devoid of hair, the pouch area. This occurs in both sexes and at its anterior end are located two laterally placed hairy patches about 5 mm in diameter, the milk areolae. In pregnant females the concave area becomes a true pouch by inward growth of its sides. The lips of the pouch become thick and tumescent (Fig. 15.15) through growth of the underlying mammary glands.

There is no outward sign of a neck, the head and body merging imperceptibly (Fig. 15.1). An ear hole is apparent on each side of the head and external pinnae usually are not discernible, though some individuals, particularly those from Tasmania, exhibit a small pinna dorsal to the ear hole. The maxillae, premaxillae and mandibles are prolonged to form the snout (Fig. 15.1) which is covered by soft skin sensitive to tactile stimuli. In an adult Echidna 450 mm long and weighing 4–5 kg, the snout is about 75 mm long. The horizontal ramus of the mandible is bound to the maxilla by a strong longitudinal marginal ligament which extends to within about 13 mm of the distal end of the snout (Murray 1981). The nostrils open dorsally at the distal end of the snout. The V-shaped mouth is located ventrally at the distal end of the snout. Captain William Bligh remarked of a specimen taken at Adventure Bay, February 1792 ‘it (the mouth) will not admit anything above the size of a pistol ball’. Murray (1981) has measured the gape and found it to be about 5 mm. The eyes are small, about 9 mm in diameter, situated at the base of the snout and are forwardly directed to some degree (Fig. 15.1).





**Figure 15.1** *Tachyglossus aculeatus multiaculeatus* the Kangaroo Island echidna. (From Griffiths 1972; with permission of the Australian Museum)

There is no scrotum and the testes are internal. There is only one orifice for the passage of faeces, urine and reproductive products, hence the name Monotremata for the Order. The limbs are short, stout and pentadactylous. The digits of the forefoot are furnished with spatulate claws (Fig. 15.1) used for digging in forest litter, rotten wood and ant and termite mounds to expose prey. The head of the femur is inserted horizontally into the acetabulum, projecting at right angles to the body. The tibia and fibula are twisted backwards so that the feet and claws are directed posteriorly. The claw on digit I is short, that on II is invariably long, and in some subspecies digit III is long too (Fig. 15.3). Those on digits IV and V are short. The long claws are used for grooming. The ankle of the juveniles of both sexes bears a hollow perforated spur which is shed by the female later in life, but which persists in the male. This spur is connected to a duct which leads to a gland buried in the muscles below the knee (Cabrera 1919). At the posterior end of the animal is located a naked stubby tail.

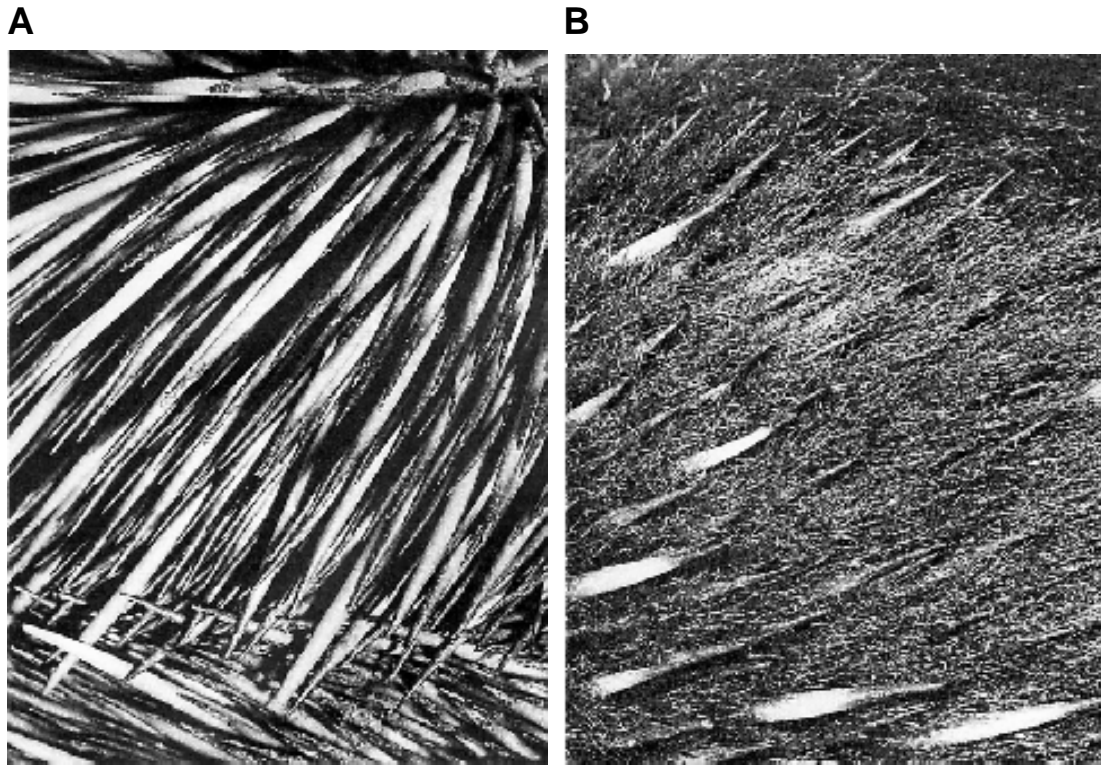
The five subspecies of the Short-beaked Echidna are externally recognisable by the degree of hairiness of the pelage and by the length of the claw on digit II of the hind leg (Griffiths 1978). Such criteria may seem trivial but all subspecies are readily distinguishable (Figs. 15.1 & 15.2). The marked differences in the pelage have implications for temperature regulation.

### Skeletal System

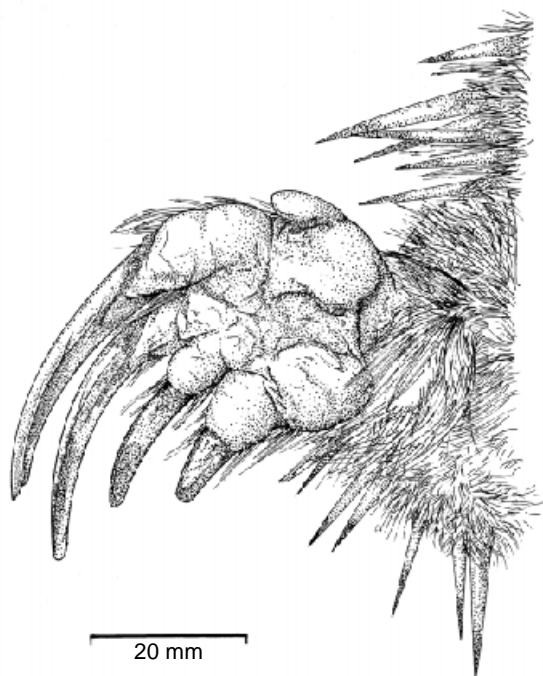
Members of the Monotremata exhibit a mosaic of reptilian and advanced mammalian characters in their physiology, reproduction and osteology.

In this and other sections, reference will be made to the occurrence of structures and processes in the Tachyglossidae that are also to be found in the living Reptilia (or Sauropsida), all of which are distant from the mammalian lineage. Such comparisons may seem dubious, but in the absence of extant mammal-like reptiles (Therapsida), it is a necessary compromise.

The skull of the Echidna (Fig. 15.4) is apparently almost purely mammalian, but certain precursors of bones found during development in the chondrocranium occur in form during the development of sauropsids. Viewed from the rear to the front, the floor of the skull consists of a basioccipital and a fused basisphenoid-presphenoid complex which includes paired ossifications of *pilae antoticae* along the sides of the *sella turcica*. *Pilae antoticae* are paired strips of cartilage arising from the basal plate of the chondrocranium. In the Sauropsida these ossify to form the pleurosphenoid bones. Within the Mammalia, *pilae antoticae* occur only in the Monotremata.



**Figure 15.2** Pelage characteristics of different subspecies of echidna. **A**, *Tachyglossus aculeatus setosus* x 0.62 (From Griffiths 1978; with permission of Academic Press); **B**, *Tachyglossus aculeatus acanthion* x 0.49 (After Griffiths 1978; with permission of Academic Press)



**Figure 15.3**  
*Tachyglossus aculeatus*  
right hind limb. (After Griffiths 1978; with permission of Academic Press; © ABRS) [T. Cochran]

Rostral to the basisphenoid-presphenoid complex, the floor consists of a median vomer. The anterior wall of the braincase is formed by the transverse mesethmoid, nasal capsules and *lamina infracribrosa* which dorsally bears a cribriform plate that extends forwards horizontally. This plate is pierced by many small pores for the passage of olfactory nerve fibres from the olfactory epithelium to the olfactory bulbs of the brain. The elongated snout is made up of extensions of the maxillae and premaxillae (Fig. 15.4). The roof of the mouth is formed, as it is in all mammals, by the union of mesially directed flat extensions from the maxillae and palatine bones to a ventrally directed ridge of the vomer. Paired nasopharyngeal passages are thus formed along with the very extended false palate. The nasopharyngeal passages open anteriorly to the exterior at the nostrils and posteriorly into the buccal cavity at the internal choanae (Fig. 15.4). At the posterior ends of the palatines and lateral to them are paired pterygoids which are overlain by robust ectopterygoids (found also in the skulls of Sauropsida). There are no jugals. The thin malar arch of each side is formed by zygomatic processes of the squamosal and maxilla.

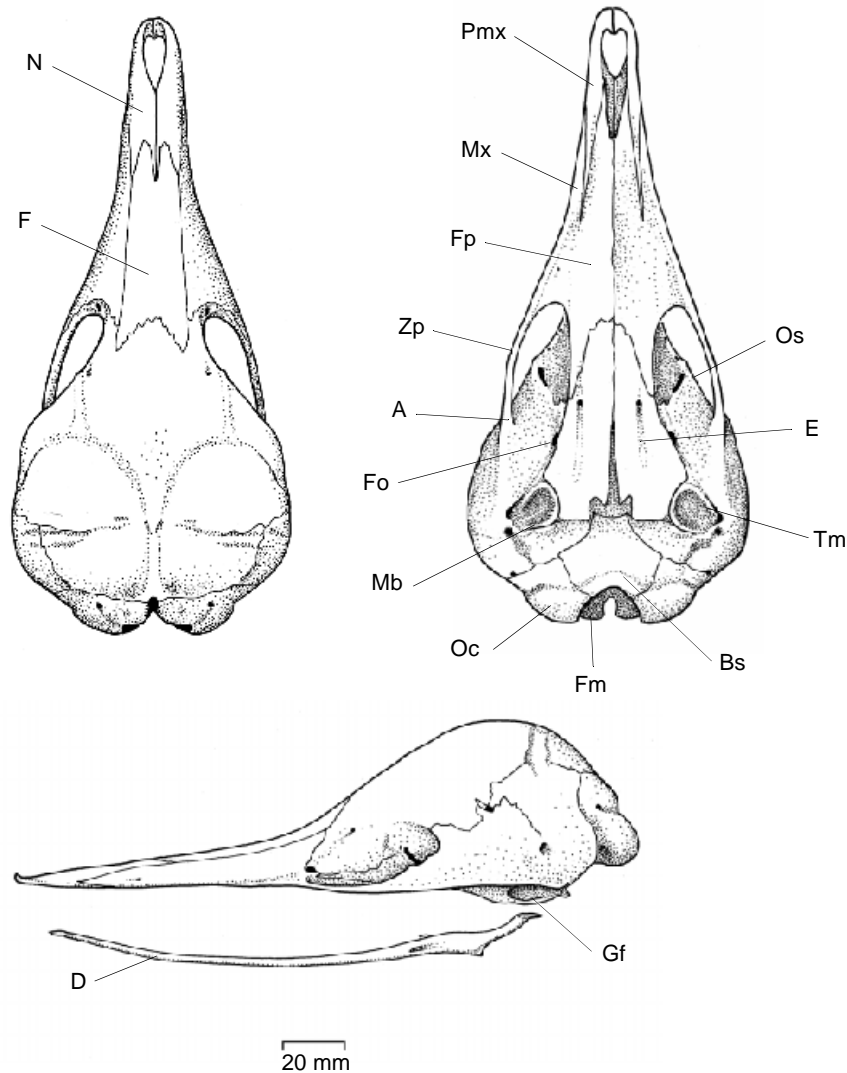
As in all mammals, the lower jaw consists of a single bone, the dentary, which in tachyglossids is elongated, thin and curved. Anteriorly, the left and right rami are joined by a ligament at the mandibular symphysis (Murray 1981). The postero-ventral aspect of each ramus exhibits a small angle (Griffiths 1968) and the condyles articulate with glenoid fossae in the squamosals – the hallmark par excellence of a mammal. The suspension of the reptile lower jaw, (including the Therapsida) differs and involves a number of bones among which are the dentary, articular, quadrate and angular. The articular meets with the quadrate to form the jaw suspension. In mammals, the articular, quadrate and angular are modified to form the ear bones: the malleus, incus and tympanic, respectively. The dentary articulates with the squamosal to form the jaw suspension (Gaupp 1908). This remarkable transformation of what were jaw bones in Therapsida into mammalian ear ossicles, is well attested by the fossil record and by ontogeny of mammals (Crompton & Parker 1978; Kermack, Mussett & Rigney 1981). In adult tachyglossids, the tympanics remain open so that the tympanic membrane is visible as does the handle of the hammer (manubrium of the malleus). The malleus articulates with the incus which in turn articulates with the stapes. In monotremes, the stapes is columelliform as it is in many marsupials and in the eutherian *Manis javanica* (Doran 1878).

The tympanic cavities are housed in the postero-ventral surface of the periotics which lie between the squamosals and the basioccipital. The fenestra ovalis, filled in by a membrane, is located dorsally in the domed tympanic cavity. The expanded base of the columelliform stapes is attached to this membrane.

The posterior portion of the skull comprises paired exoccipitals and an unpaired supraoccipital surrounding the foramen magnum. Each exoccipital bears a condyle (Fig. 15.4) which articulates with the first member of the post-cranial skeleton, the atlas vertebra. The roof of the skull is formed by paired parietals, frontals and nasals and the sides of the posterior end of the skull are formed by orbitosphenoids, the squamosals and the periotics.

The lateral wall of the brain case appears to be one large slab of membrane bone which, until recently, was considered to be an anterior extension of the periotic. In young echidnas, however, the side wall consists of a transparent membrane (*membrana spheno-obturatoria*) in which multiple independent ossifications appear and which is invaded on each side by a latero-dorsal projection of the palatine and a *lamina ascendens* (alisphenoid) of the *ala temporalis* (Griffiths 1978). The independent ossifications fuse to one another, to the squamosals and, later, to the periotic. There is no anterior flange to the periotic.

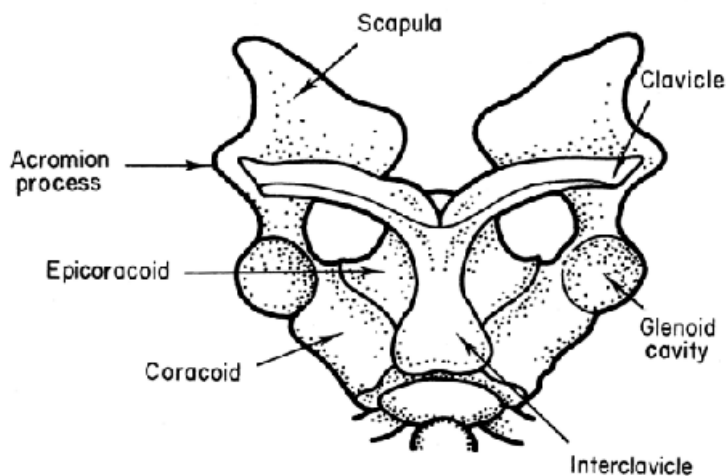




**Figure 15.4** Ventral, dorsal and lateral views of skull of the Short-beaked Echidna. A = alisphenoid; Bs = basisphenoid; D = dentary; E = ectopterygoid; F = frontal; Fm = foramen magnum; Fo = foramen ovale; Fp = false palate; Gf = glenoid fossa; Mb = manubrium of malleus (handle of hammer); Mx = maxilla; N = nasal; Oc = occipital condyle; Os = ossification in the sphenoparietal membrane; Pmx = premaxilla; Tm = tympanic membrane; Zp = zygomatic process of maxilla. (© ABRS) [S. Weidland]

These processes of ossification of the side wall of the brain case can be seen in progress in Fig. 15.4. Presley (1981) has shown that similar processes of ossification take place in the side wall of the brain cases of the Platypus (*Ornithorhynchus aculeatus*), the marsupial Virginia Opossum (*Didelphis virginiana*), and the placental Hedgehog (*Erinaceus europaeus*). The apparent structure of the side wall had hitherto served as a criterion for separation of fossil mammals into Monotremata and Theria (Hopson 1970), but as Presley (1981) remarks of the region: ‘In view of the variable pattern found in living forms and the difficulties of establishing detailed osteological homologies in fossils, it must surely be dangerous to erect two groups, however informally, based on the adult pattern of this region. Development shows that there is no monotone pattern and therefore fossils may not be categorised as sharing it’. Kemp (1983) also argues in depth that the monotremes are related to ‘modern therians’.

The pectoral girdle (Fig. 15.5) in Short-beaked Echidnas would be at home in a therapsid reptile since both exhibit two clavicles, two scapulae with acromion processes at the anterior borders, two coracoids, two epicoracoids (procoracoids) and a median T-shaped interclavicle. The scapula is expanded dorsally and has a shallow infraspinous fossa. It forms a unit on one side with the ventral coracoid and procoracoid. This unit is fixed dorsally to the rib cage and ventrally to the interclavicle which is united posteriorly to the sternum. The transverse portion of the interclavicle is clamped rigidly to the scapulae by the clavicles. The glenoid cavity is a laterally oriented wedge-shaped depression at the union of scapula and coracoid. The pelvic girdle consists of the usual three elements: dorsal ilia, ventro-posterior ischia and ventro-anterior pubes. The latter two are united ventrally at a symphysis, but are separated dorsally by an obturator foramen. The symphysis of the three bones at the acetabulum is incomplete and the latter exhibits a foramen. The anterior margin of each pubis bears an epipubic bone which projects forwards and is movably articulated as in the marsupial pelvis. The anterior portions of the ilia are elongated, narrow and extend forward and dorsally to unite with the two sacral vertebrae.



**Figure 15.5** Ventral view of pectoral girdle of the Short-beaked Echidna (After Gregory 1947; with permission of American Museum of Natural History)

Like all mammals, monotremes have seven cervical vertebrae, the first two of which are modified to form the atlas and axis. The atlas has the form of a ring. Its anterior ventral surface is concave and articulates with the laterally placed occipital condyles of the skull. The axis bears an odontoid peg which projects forwards into the centre of the atlas ring. The atlanto-occipital joint permits flexion and extension, while the atlanto-axial joint allows rotation (Jenkins 1969). The cervical vertebrae exhibit the cervical ribs found in reptiles, but are not present in other mammals. The vertebral formula of *Tachyglossus* is: seven cervical, 16 thoracic or dorsal, three lumbar, three sacral and 12 caudal.

### Locomotion

The glenoid cavity is wide for the reception of the broad head of the horizontally positioned humerus, having a 90° abduction to the parasagittal plane of the body. The radius and ulna articulate with a condyle of the humerus, exhibiting a quasi-spiral configuration. Jenkins (1970) has shown by a cineradiographic analysis that the principal locomotory movement of the humerus is rotation, not antero-posterior protraction. The plantar contact of the manus with the ground is beneath the glenoid, as is the case in generalised Eutheria and Metatheria. The principal locomotory movements of the femur are rotation about the proximal-



distal axis (from the head of the femur through the patellar groove) and elevation and depression of the femur; total rotation is estimated to be about 45°. The long axis of the femur also sweeps posteriorly during propulsion starting about 35° to the sagittal plane and ending at 50° or more. At this termination the foot is directed posteriorly in a final thrusting movement. From this study and another on locomotion in non-cursorial mammals, Jenkins (1971) concluded that there is no single mode of posture or locomotion among terrestrial mammals and that statements made to the effect that monotreme limbs are reptilian in posture 'are imprecise, and with regard to the Echidna, at least, are inaccurate'.

The energetics of this locomotion were determined by Edmeades & Baudinette (1975) by measuring O<sub>2</sub> consumption in echidnas trained to walk on a treadmill at a variety of speeds. Steady state O<sub>2</sub> consumption increased linearly with running speed. Total O<sub>2</sub> consumption per kg body weight while travelling a given distance ('cost of transport') was found to be less than that in eutherians.

Musculature. Four matters of interest concerning the musculature of tachyglossids are discussed: the panniculus carnosus, and the musculature of the face, jaw and tongue. The latter is discussed under feeding.

The rudiments of the organs of facial expression appear in tachyglossids. These are muscles derived from the sphincter colli, and although present in reptiles and birds, they are restricted to the neck region. In tachyglossids, the sphincter colli invades the face region and is differentiated into the platysma at the side of the head surrounding the ear hole, the frontalis platysmae around the eye and the buccinator muscles beneath the lower jaw. In the absence of this musculature, as is the case in reptiles and birds, the face is a 'rigid mask devoid of expression' (Hüber 1930).

Under the skin, the body is covered with an enormous muscle, the panniculus carnosus. It is thick over the back and sides of the animal but thin in the centre of the ventral surface where the pouch forms in the pregnant female. By contraction of various parts of the panniculus carnosus, an Echidna can achieve changes in shape, including rolling themselves into a ball thereby protecting the belly and presenting a defensive array of sharp spines.

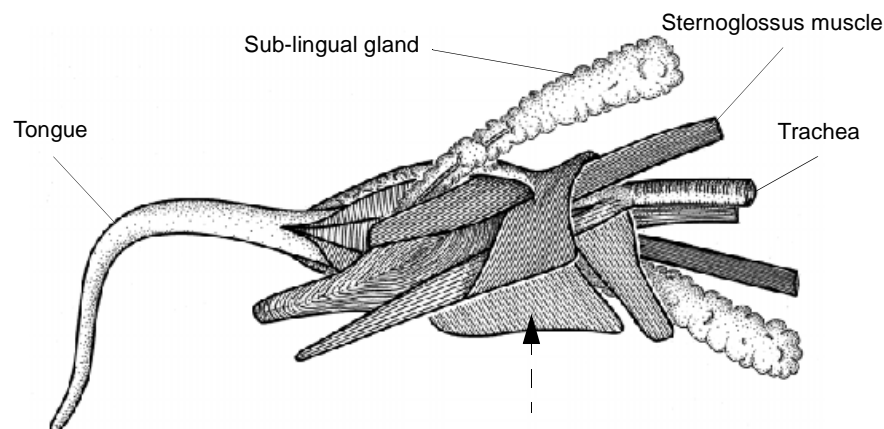
The jaw musculature of mammals has been discussed by Parrington (1974). The muscle responsible for the depression of the mandible in the monotremes is the detrahens, whereas opening of the jaw in Metatheria and Eutheria is accomplished by the digastric. This comprises an anterior and a posterior belly, joined by a ligament. The detrahens is a derivative of the masseter which has migrated posterior to the jaw hinge, thus becoming a depressor muscle. Both the detrahens and the anterior belly of the digastric are innervated by a branch of the trigeminal nerve (cranial nerve V), while the posterior belly is innervated by the facial nerve (VII). This difference has been advanced as one argument in support of the notion of polyphyly in the Class Mammalia. The detrahens mandibulae would appear to be practically functionless in *Tachyglossus*, since orthal hinge movements are prevented by the ligaments which attach the horizontal ramus of the dentary throughout most of its length to the maxilla and premaxilla (Murray 1981). Gentle squeezing of the skin covering the angles of the dentary causes the mandibles to rotate mesially. Since the latter are curved, this distortion results in lowering of the ends of the mandibles at their loose junction, thus opening the mouth about 5 mm.

Murray (1981) adduces evidence that this is brought about by contraction of a muscle that takes its origin at the posterior temporalis muscle and inserts on the medial side of the angle. Contraction of musculature inserted on the lateral aspect of the angle would close the mouth.

## Feeding and Digestive System

The tongue is the sole means of catching and ingesting prey. If use of this organ is impaired in any way the animal starves. The tongue is long, vermiform and sticky and can be protruded up to 180 mm from the end of the snout. The stickiness is due to lubrication with a mucus composed largely of glycoprotein (Lew, Heidelberger & Griffiths 1975) secreted by paired sub-lingual glands. The viscous mucus is passed via a duct from the anterior end of each gland to the floor of the buccal cavity. Each duct subdivides into a number of smaller ones and from these the mucus passes to the tongue. When the tongue is protruded, any ants or termites coming into contact with it are drawn back into the buccal cavity. When the tongue starts to protrude again, the insects are scraped off by rows of backwardly directed keratinous 'teeth' located along the roof of the cavity.

Retraction is brought about by contraction of the sternoglossi, two internal longitudinal muscles, (Fig. 15.6), that extend from near the tip through the fixed caudal end of the tongue to the sternum, where they take their origin at the xiphoid process (Duvernoy 1830; Fewkes 1877; Doran 1973). Protrusion results partly by contraction of circular muscles (Fig. 15.7) and partly by contraction of two genioglossal muscles attached to the caudal end of the tongue and to the mandibles (Doran 1973). Since the base is fixed, contraction of the circular muscles deforms the tongue so that it is forced forwards. This is facilitated by the presence of a vascular stiffening mechanism discovered by Doran & Baggett (1970). It lies anterior to the section shown in Fig. 15.7 and consists of a centrally placed artery, a nerve ventral to the artery and vascular spaces located dorsally and ventrally. These spaces are supplied with blood from the central artery via a series of bulbous extensions, and small channels. The tongue is stiffened by engorgement of the vascular spaces with blood from the central artery. In this condition it is strong enough to penetrate termite-ridden wood. This stiffening of the tongue has no apparent effect on protrusion and retraction, which occur at a rate of about 100 actions per minute (Doran & Baggett 1970). Baggett (Griffiths 1978) has shown that the sinusoids of the erectile tissue unite in the mid-line anterior to a transverse sinus, forming a large space lined with endothelium and containing arterial blood. Striated fibres from the circular muscles are inserted into the wall of the sinus. Contraction of the circular muscles compresses and distorts the large sinus raising the pressure of the blood contained therein, and displacing blood into the dorsal and ventral sinusoids, leading to rapid protrusion of the tongue.



**Figure 15.6** Dissection of the musculature of the tongue of the Short-beaked Echidna (After Duvernoy 1830; © ABRS) [S. Collin]

In addition to rapid protrusion and retraction, the end of the tongue is capable of movement left, right, up, down and of recurving on itself (CSIRO 1969). This ability helps to apprehend ants and termites scurrying in many directions at once. It is achieved by the contraction of radially arranged musculature and three pairs of longitudinal muscles, all of which take their origin in the dermis of the anterior end of the tongue (Griffiths 1968, 1978).

As well as apprehending the prey, the tongue masticates it. The insects are passed to the posterior end of the buccal cavity where they are comminuted by the grinding action of a set of spines, located on the dorsal surface of the base of the tongue. The cuticular teeth, as Home (1802b) termed the latter, are directed backwards and project about 2.5 mm above the surface of the pad; they are covered with keratin (Doran & Baggett 1972). Located above the pad, along the palate, are seven transverse rows of keratinous teeth also directed backwards.

When prey arrive at the posterior end of the buccal cavity, the pad is pressed up against the palate and is worked backwards and forwards by the action of musculature described by Fewkes (1877) and Doran & Baggett (1970). Since both sets of teeth are directed backwards they can slide past one another and their oscillations grind the insect prey into fragments. An illustration of a sagittal section of a head in Doran & Baggett (1972) showing the lingual teeth forwardly directed is incorrect. The trituration process is quite audible and the sound, a scratching noise, has been recorded along with slow-motion photography of the tongue in action (CSIRO 1969).

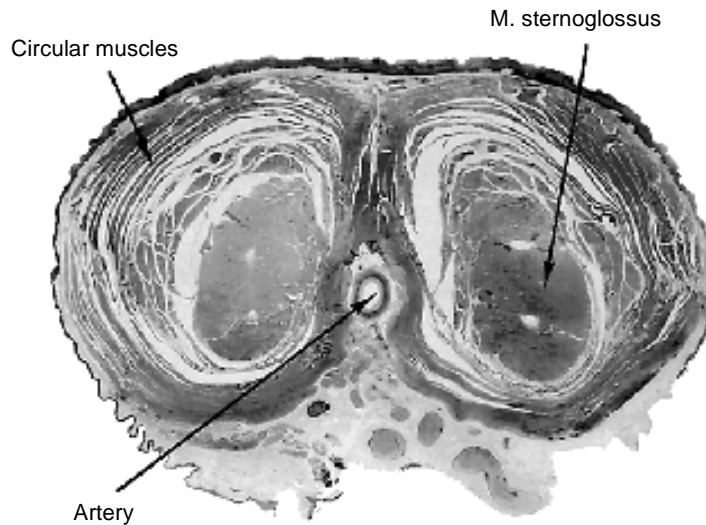
The epithelium bearing the palatal spines is applied to a concave surface of bone, made up of the posterior ends of the palatines and of the robust ectopterygoids (Fig. 15.4). This offers the necessary stout support for the grinding action of lingual pad against the palatal spines.

The trituated insects are passed through the oesophagus to a distensible stomach lined by stratified squamous epithelium (Griffiths 1965a; Krause & Leeson 1974; Oppel 1896).

Apart from a set of Brunner's glands (Krause 1970) at the posterior end, the stomach contains no other glands, and hence no peptic digestion occurs. The pH of the stomach 2–4 hours after ingestion of a meal of 200 g of termites (*Nasutitermes exitiosus*) does not fall below 6.2. Except for the possibility of self-digestion of the trituated insects in the stomach, all digestion of proteins, lipids and carbohydrates takes place in the small and large intestines. These exhibit the usual villi and crypts of Lieberkühn found in the intestines of other vertebrates. The villi are clothed distally with a simple columnar epithelium, centrally with goblet cells secreting mucigens. The deeper parts or crypts exhibit Paneth cells loaded with secretion granules (Griffiths 1965a; Krause 1971). At the anterior end of the small intestine a combined pancreatic and bile duct passes digestive secretions of the pancreas and liver into the lumen of the intestine. The succus entericus exhibits lipase, amylase and trypsin-like activities. Sodium taurocholate is the emulsifying salt in the bile of *Tachyglossus* (Bridgwater, Haslewood & Tammar 1962).

The amylase activity of the *succus entericus* would presumably contribute to digestion of glycogen, but as far as digestion of carbohydrates of shorter chain length is concerned, Kerry (1969) has shown that the intestinal mucosa of echidnas has maltase, isomaltase and trehalase activity but no sucrase and practically no lactase activity, a finding confirmed by Stewart *et al.* (1983). The presence of trehalase is interesting since trehalose (a disaccharide of two molecules of glucose) is the main sugar found in insects. The latter authors also found quantities of the enzymes fucosidase and N-acetyl-D-glucosaminidase. This is in contrast with the enzymes present in the intestinal mucosae of suckling echidnas, which have appreciable lactase, maltase, isomaltase, neuraminidase and practically no sucrase activities, but relatively enormous





**Figure 15.7** Cross section of proximal region of the tongue of the Short-beaked Echidna x 5.6 (From Griffiths 1968; with permission of Pergamon Press)

amounts of trehalase, fucosidase and N-acetyl-D-glucosaminidase. This doubtless reflects the need to digest the two main oligosaccharides found in the milk: sialyllactose (4-0-acetyl-N-acetylneuraminyllactose) and fucosyllactose (Messer & Kerry 1973; Messer 1974; Kamerling *et al.* 1982). Stewart *et al.* (1983) adduced evidence that digestion of these oligosaccharides does not take place at the border of the villi (as happens in the Eutheria), but intracellularly by the release of the digestive enzymes from lysosomes. The concept of lysosomal digestion entails a mechanism for entry of the milk oligosaccharides into the mucosal cells. Such a route has been described by Krause (1972) as an extensive network of tubules in the apical cytoplasm of the cells of the intestinal mucosa in suckling echidnas. These tubules are in communication with invaginations of the apical cell membrane and they lie close to small spherical vacuoles which are probably lysosomes.

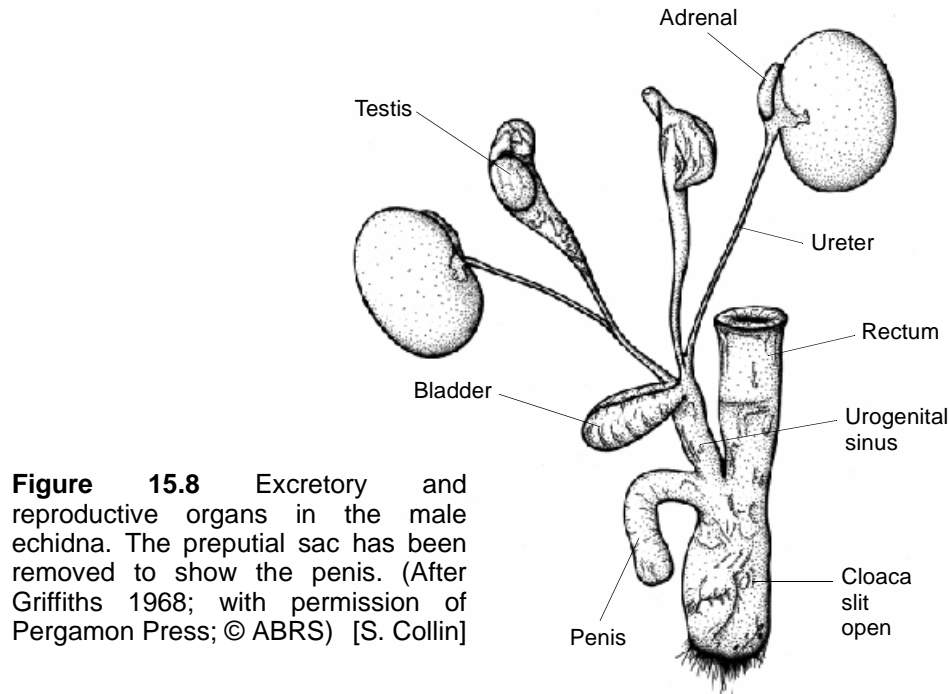
As an apparent compensation for the lack of peptide digestion, the adult has a very long intestine (some 3.5 m), which imposes a slow rate of passage. Clearance of a meal of termites takes upwards of 2 days (Griffiths 1965a) providing the pancreatic and intestinal digestive enzymes with ample time to act effectively on their substrates.

### Excretion

Tachyglossid kidneys are like those in other mammals (Fig. 15.8), bearing no resemblance to the lobulated renal organs found in Sauropsida. Further, there is no renal portal system as in the sauropsids, but like other mammals a renal artery conveys oxygenated blood to the kidney with drainage via a renal vein.

For the most part, renal anatomy is typically mammalian (Zarnik 1910), though echidnas do exhibit one reptilian characteristic. This is the presence of a curious kind of nephron: the 'Zwerg-kanälchen' of Zarnik (1910) or dwarf nephron, also found in the kidneys of many reptiles. The ureters discharge into a urogenital sinus, thence to a bladder which depends from the ventral surface of the sinus. In females, contraction of the bladder forces urine into the sinus, from where it exits at its posterior end into a cloaca (Fig. 15.8). The male system is identical, since the penis is for insemination only.

Like all other mammals, monotremes are ureotelic, with urea as the end-product of protein catabolism (Neumeister 1898; Mitchell 1931). Urea nitrogen accounts for 82–90% of total urinary nitrogen. The end-product of purine metabolism is uric acid, some of which probably is further oxidised to allantoin by liver uricase, as in most eutherians (Griffiths 1965a).



**Figure 15.8** Excretory and reproductive organs in the male echidna. The preputial sac has been removed to show the penis. (After Griffiths 1968; with permission of Pergamon Press; © ABRs) [S. Collin]

The kidney can produce a hypertonic urine. This might be expected from Zarnik's description of its tubular-loop structure, which in Eutheria permits urine concentration by counter current multiplication. In echidnas deprived of food and water, urine volume is small and urine concentrations may be as high as 2300 mOsm/kg water, compared with a plasma concentration of only 281 mOsm/kg water (Bentley & Schmidt-Nielsen 1967). This represents a urine plasma ratio of approximately eight. From calculations of evaporative water loss, and from data of Griffiths (1965a) for individuals eating the termite *Nasutitermes exitiosus*, Bentley & Schmidt-Nielsen (1967) were able to strike an approximate water balance for echidnas living on termites alone at 25°C in dry air without free water intake. They conclude that animals could maintain a positive water balance on such a diet in an arid climate. However, direct determination of the performance of individuals eating a fixed ration of termites (*N. exitiosus*) without drinking water showed, as far as maintenance of body weight and nitrogen balance were concerned, that they can do quite well at temperatures ranging from 21–28°C in moist air of absolute humidity (AH) 9.0–15.6 g H<sub>2</sub>O/m<sup>3</sup> (Griffiths 1978). In relatively dry air, AH 3.3 g H<sub>2</sub>O/m<sup>3</sup> at 28°C an individual that had performed well at AH 15.6 and eaten the same amount of termites, lost weight and secreted larger amounts of nitrogen. When offered drinking water the animals drank it at average rates ranging from 40–80 ml per day. The subspecies used in these experiments was *T. a. aculeatus*; it is quite possible that the arid-land *T. a. acanthion* would offer a better performance at 28°C and AH 3.3 g H<sub>2</sub>O/m<sup>3</sup>.

### Circulatory System

Monotremes have two completely separate circulations and the systemic aorta is derived from the left fourth arch, as in the other mammals (Hochstetter 1896).

The left ventricular valve is tricuspid, though its movement is not controlled by *chordae tendinae* as in other mammals, but by extensions of papillary muscles from the ventricular wall which are inserted into the membranous flaps of the valve. The right atrioventricular valve is also controlled by papillary muscles (Dowd 1969b). The two coronary arteries 'have a typical mammalian course on the surface of the heart'. The venous drainage of the heart walls is achieved by the great cardiac vein which, as in mammals and birds, opens directly into the right atrium. In the Monotremata, however, venous blood is also carried to the right atrium by a coronary vein which also opens directly into the atrium (Dowd 1969b). Coronary veins do not occur in other mammals, nor in birds.

The fibres of the sinus and atrioventricular nodes appear as discrete entities since they are paler in colour and have rounder nuclei than those of the contractile fibres. Both nodes are richly innervated with some of the axons spiralling around the nodal fibres; boutons terminaux are prominent features among the fibres (Dowd 1969b).

The cardiac output is 59 ml/kg/min, pulse rate 115 beats per minute, arterial blood pressure systolic/diastolic 123/96 mm Hg and stroke volume 1.4 ml (Parer & Metcalfe 1967c). Vasomotor responses to changes in ambient temperature are discussed below.

The erythrocytes in *Tachyglossus* are non-nucleated biconcave discs, as they are in all mammals except camels. The mean haemoglobin level is 17.4 g/100 ml (Bollinger & Backhouse 1960), a value confirmed by Parer & Metcalfe (1967b).

A mysterious feature in red blood cells in *Tachyglossus* and *Ornithorhynchus* is their minuscule levels of adenosine triphosphate (ATP), the primary source of energy for utilisation of glucose in adult mammalian red cells (Kim *et al.* 1984). Monotreme erythrocytes can nevertheless metabolise glucose to lactate at a rate comparable to those of other mammals. Kim *et al.* (1984) remarked: 'it will be interesting to characterise the monotreme glycolytic enzymes which apparently can carry out hexose phosphorylation with a trace amount of ATP'.

Bollinger & Backhouse (1960) found that the granular leucocytes are polymorphonuclear as in other mammals. Of the white blood cells, neutrophils account for 12%, monocytes 1% and lymphocytes 87% of the total; basophils and eosinophils are practically absent. These two kinds of white cells, however, are present in Platypus blood (Whittington & Grant 1983). The ultrastructural study carried out on Platypus blood cells (Canfield & Whittington 1983) has yet to be extended to Echidnas.

Tachyglossid serum exhibits seven clearly defined fractions as shown by cellulose acetate electrophoresis:  $\alpha_1$ -,  $\alpha_2$ -,  $\alpha_3$ -,  $\beta_1$ -,  $\beta_2$ -, and  $\gamma$ -globulins and albumin (Jordan & Morgan 1969). The mobilities of these fractions were found to be similar to those in human serum. The  $\beta_1$  globulin (transferrin) binds iron and is essential for transport to a variety of cells, such as reticulocytes (developing red corpuscles) and bone marrow cells. Its amino-acid composition and sequence are the subjects of a study into the structural evolution of the serum and milk whey proteins in various mammals (Griffiths *et al.* 1985).

Atwell, Marchalonis & Ealey (1973) have further characterised the  $\gamma$  globulins or immunoglobulins observed by Morgan & Jordan from the serum of a Short-beaked Echidna immunised with *Salmonella adelaide* flagellae. Atwell *et al.* (1973) isolated IgM and IgG, the two kinds of immunoglobulin found in the sera of mammals, including man. In terms of electrophoretic mobility, size and polypeptide structure, they resemble the immunoglobulins of man in particular. IgG predominates, and there is striking similarity between the  $\gamma$  heavy chain of human IgG and that of echidnas. The predominance of IgG immunoglobulin in



the serum of *T. aculeatus* resembles more closely the mammalian pattern than that of reptiles, including the Tuatara, *Sphenodon punctatus*, (Marchalonis, Ealey & Diener 1969).

### Respiration

Mammalian lungs, including those of the Monotremata, differ from those of birds and reptiles in many ways, but principally in the great development of a branching tree-like system of intrapulmonary bronchi. The bronchi divide and subdivide forming bronchioli which lead to alveolar ducts ending blindly in alveoli. The bronchial tree is usually asymmetrical and this is the case with echidnas where two lobes of lung are present on the right side and only one on the left (Home 1802b). Narath (1896) described the bronchial tree in *Tachyglossus* in detail.

Monotremes exhibit that characteristic mammalian feature, a diaphragm, which is very broad, muscular towards the periphery, but tendinous at the centre immediately under the heart (Home 1802a, 1802b).

Parameters of respiration have been determined by Bentley, Herreid & Schmidt-Nielsen (1967) and by Parer & Hodson (1974). The former found end tidal (alveolar) CO<sub>2</sub> concentration was 5.3% while the concentration of O<sub>2</sub> was 14.5%; similar concentrations of CO<sub>2</sub> and O<sub>2</sub> in end-tidal air are found in man (5.95% CO<sub>2</sub>, 14.0% O<sub>2</sub>) seals (6.3%, 11.6%) and dogs (5.68%, 13.6%). Mean tidal volume was found to be 26.9 ml per breath and mean minute volume 137 ml/kg body weight. Parer & Hodson (1974) recorded an average minute volume of only 76 ml/kg. Bentley *et al.* (1967) found, however, that minute volume could be altered. The changes related to changes in respiratory rate: a decrease in respiratory rate is accompanied by a decrease in minute volume.

While echidnas, forced to inspire high concentrations of CO<sub>2</sub>, increased their respiratory minute volume, the change was brought about by change in tidal volume, not by increase in respiratory rate. The tidal and minute volume levels observed are similar to those of eutherians, as is the extraction of O<sub>2</sub> from inspired air, although O<sub>2</sub> consumption at thermoneutrality is only 0.22 ml/g/h, about half that of eutherians of comparable size (Schmidt-Nielsen, Dawson & Crawford 1966). Such a comparison is dependent on the eutherian selected; sloths, for example, averaging the same weight as adult Echidnas (Nagy & Montgomery 1980), have a very low O<sub>2</sub> consumption (see below). Augee (1976) and Dawson, Grant & Fanning (1979) confirm that tachyglossids at thermoneutrality have a low O<sub>2</sub> consumption reporting levels of 0.15 ml/g/h and 0.132 ml/g/h, respectively.

Echidnas are tolerant of very high levels of CO<sub>2</sub> in inspired air, and voluntarily remain in situations where CO<sub>2</sub> concentrations range as high as 6.9% (Bentley *et al.* 1967). At least one other mammal, the rabbit *Oryctolagus cuniculus*, voluntarily withstands equally high CO<sub>2</sub> concentrations: the air in nesting chambers containing litters exhibits concentrations of 7–14% (Hayward 1966).

Augee *et al.* (1971) studied the respiratory and cardiac responses of echidnas buried in earth and breathing asphyxial air created by their own respiration. Following burial with about 200 mm of earth over the dorsal surface, bradycardia or slowing of the heart occurred; little change in respiration rate and a sustained rise in CO<sub>2</sub> concentration (hypercapnia) in the air around the snout were observed. When levels of 10–12% CO<sub>2</sub> were reached, the surface of the soil was seen to heave and crack open. After a few seconds this activity stopped, but the ‘bellows’ action was repeated at regular intervals leading to

maintenance of about 10% CO<sub>2</sub> in the inspired air for the duration of the experiments (4 hours). The Short-beaked Echidna is well adapted physiologically for burrowing. Apart from CO<sub>2</sub> insensitivity, the blood has a high oxygen affinity and a well-defined Bohr effect of 0.49 (Parer & Metcalfe 1967b). This ensures that the circulating blood of buried animals has a degree of O<sub>2</sub> saturation assisting it to cope with hypoxic inspired air. Measurement of plasma in individuals buried in soil reveals that pCO<sub>2</sub> increases to quite high values with a concurrent drop in plasma pH (Hochachka, Baldwin & Griffiths 1984). During hyperventilation or 'bellows' episodes, bradycardia was replaced by tachycardia for a short period. No major adaptations of enzyme content, enzyme organisation, myoglobin content or tissue and blood buffering capacity were found to be required to deal with the two accumulations other than the tachycardia and coupled hyperventilation. These were found to be adequate to maintain metabolic homeostasis and to sustain tachyglossids submerged in soil for hours at a time.

### Thermoregulation

The body temperature and temperature regulation of monotremes has interested physiologists since the end of the 19th Century. In view of their many reptilian characters, it was hoped, notably by Martin (1902), that their temperature regulation physiology might throw light on the evolution of thermoregulation. Of this notion Schmidt-Nielsen *et al.* (1966) wrote 'the Echidna in some respects is an excellent temperature regulator, in other respects less capable, but probably no more so than 'higher' mammals'. At ambient temperature ( $T_A$ ) ranging from 0–24°C, mean deep body temperature ( $T_B$ ) is 30.7°C with a maximum diurnal variation of 4.1°C at 24°  $T_A$ . At 0°C,  $T_A$ ,  $T_B$  remains within the usual range of 29–32°C. After exposure to 5°C for 12 h there was no drop in  $T_B$ . The response to low  $T_A$  is a several-fold increase in O<sub>2</sub> consumption (that is, an increase in metabolic rate) and shivering, which prevent a fall in  $T_B$ . The thermoneutral zone, the ambient temperature range over which no change in O<sub>2</sub> consumption occurs, is 20–30°C. At  $T_A$  lower than 20°C, the metabolic heat produced to maintain  $T_B$  must therefore be conserved by decreasing heat flow to the environment.

The latter is measured as calories cm<sup>2</sup>/h per °C. At 20°C, conductance in the Short-beaked Echidna is 0.15 cal cm<sup>2</sup>/h per °C, suggesting that vasoconstriction is already at a maximum at 20°C. Above 20°C there is a gradual increase in conductance until at  $T_A$  34°C it is 0.7 cal cm<sup>2</sup>/h per °C. At this temperature Short-beaked Echidnas become hyperthermic,  $T_B$  rising to about 37°C. If  $T_B$  rises above 38°C, the animals usually die (Augee 1976). Echidnas do not sweat or pant (Augee 1976). As vasomotor responses to high  $T_A$  are at their maximum at  $T_A$  34°C, it is apparent that what little evaporative cooling it has is insufficient to prevent lethal apoplexy. At  $T_A$  34°C only one-third of the metabolic heat is dissipated by evaporation (Schmidt-Nielsen *et al.* 1966). Clearly, the Echidna is not a good thermoregulator at high ambient temperatures, so must avoid such extremes. It achieves this by retreating to the relatively equable conditions of caves and breakaways or by burrowing (see below). Animals from hot arid regions may have an advantage in combating high  $T_A$  not shared by those living in equable climates. Augee (1978a) has shown that the three subspecies *Tachyglossus acanthion*, *T. aculeatus* and *T. setosus*, (hairless, hairy and very hairy, respectively) have different conductance. After 16 days at  $T_A$  20°C the conductance in arid-zone *T. acanthion* was 0.336 cal cm<sup>2</sup>/h per °C, that in

*T. aculeatus* was 0.254 and that in *T. setosus* 0.195, the respective  $T_b$ s being 28.2, 29.6 and 30.1°C. The high conductance of *T. acanthion* would give it an advantage in shedding a heat load.

The converse was demonstrated by lowering  $T_A$  over the next 42 days to 5°C. The mean  $T_b$  in *T. acanthion* was now a very low 23°C, that in *T. aculeatus* 27°C and in *T. setosus* was 28.5°C. Very hairy individuals with low conductance can therefore maintain normal  $T_b$  at very low  $T_A$ . When  $T_A$  was raised gradually over the next 35 days to 30°C, all three subspecies acquired a conductance of 0.17 cal cm<sup>2</sup>/h per °C. Since there were no observable changes in pelage at the end of the experiment, Augee (1978a) postulated that sub-cutaneous fat storage could have affected insulation, or vasomotor adjustments could operate to decrease conductance in *T. aculeatus acanthion*. He further surmised 'Perhaps some of the differences in published  $T_b$  and metabolic levels in monotremes relate to unpublished differences in acclimation due to different conditions under which the animals were kept prior to the studies being made'.

Augee (1978b), in agreement with Schmidt-Nielsen *et al.* (1966), found that mean  $T_b$  of individuals kept at 25°C was a stable 32.2°C, fluctuating at most 2°C. In those kept continuously at  $T_A$  10°C, however,  $T_b$  exhibits regular diurnal fluctuations of as much as 10°C. For this reason, *T. aculeatus* is considered a heterotherm. The lower temperatures are observed in the early morning when the animals are inactive. The increase in  $T_b$  during the day coincides with an increase in activity and feeding. The main source of heat thus appears to be the activity of striated muscle (Augee 1978b). This notion is supported by the results of injecting "Flaxedil", which abolishes muscle tonus, into individuals introduced to a  $T_A$  of 5°C. The response is an immediate and profound fall to a  $T_b$  of about 22°C in 3.5 h, as occurs in other mammals injected with curare-like substances. The hypothermia induced by "Flaxedil" in echidnas can be reversed by returning the animal to room temperature.

Occasionally, the Echidna spontaneously abandons homeothermy in winter and sinks into a torpor characterised by a  $T_b$  only a fraction of a degree above  $T_A$  (Martin 1902; Griffiths 1965a). Torpor can be induced at any time of the year by withdrawing food and keeping the animals at 5°C (Augee & Ealey 1968). Small echidnas become torpid within 3–9 days of this treatment. Larger ones take longer, upwards of 32 days. Torpid specimens kept at  $T_A$  5°C when slightly disturbed can exhibit a rise of  $T_b$  from 6°C to normal values,  $T_A$  being maintained at 5–6°C throughout the 20 hour arousal period (Augee & Ealey 1968). The ability to arouse from torpor is lost after repeated long exposures to  $T_A$  5°C, but arousal can always be elicited by raising  $T_A$ . If  $T_A$  is raised suddenly from 5°C to 25°C, a  $T_b$  of 28°C can be attained in 11 hours. Echidnas living on Kangaroo Island occasionally exhibit torpor in winter. Recaptured regularly by means of radio-tracking, some individuals have been taken in a torpid condition (stiff, cold and barely able to move). One such individual of body weight 2.6 kg had  $T_b$  of 13.6°C at recapture. Transferred to warm conditions ( $T_A$  18–20°C), during an arousal period of 22 hours, it exhibited  $T_b$ s of 13.5, 16.0, 17.5, 24.0 and 26°C. At the latter temperature it was lively (Green, Newgrain & Griffiths unpublished data). Torpid animals on Kangaroo Island have been observed basking in the afternoon sun.

Augee (1978b) stated that 'modern therian mammals are homeothermic and endothermic' and advances the notion that the heterothermy of *Tachyglossus* is far greater than any observed in eutherians or metatherians of comparable size, reflecting an early stage in the evolution of endothermy/homeothermy. This



represents a recapitulation of the views of Martin (1902), and ignores the fact that the taxon Eutheria is a very heterogeneous collection of mammals exhibiting widely varying degrees of thermoregulatory capacity. Eutherians of the same size as echidnas that closely mimic their imperfect thermoregulation include the three-toed sloths *Bradypus tridactylus*, *B. griseus* and *B. variegatus* (Almeida & Fialho 1924; Britton 1941; Irving, Scholander & Grinnell 1942; Nagy & Montgomery 1980).

Dawson *et al.* (1979) found O<sub>2</sub> consumption in two specimens of the New Guinea Echidna *Zaglossus bruijnii* that had been living for years in captivity to be 0.081 ml/g per hour, but their body weights were 10.7 and 16.5 kg. The heaviest of eight *Zaglossus* weighed when captured was 8.0 kg (M. Griffiths unpublished data). It would appear that the captive *Zaglossus* of the earlier study were carrying much fat, respiration of which is very low (Schmidt-Nielsen 1977). The O<sub>2</sub> consumption rate of normal *Zaglossus* may thus prove to be much higher than that calculated by Dawson *et al.* (1979). Indeed, McNab (1984) reports that O<sub>2</sub> consumption of a specimen of *Z. bruijnii* weighing 10.3 kg was 0.118 ml/g/hour.

The response in *Bradypus* to high T<sub>A</sub> is similar to that in *Tachyglossus*. Britton (1941) found that at T<sub>A</sub> 35–40°C lethal apoplexy intervenes. Like in *Tachyglossus*, there are few sweat glands in *Bradypus* and these are of small size.

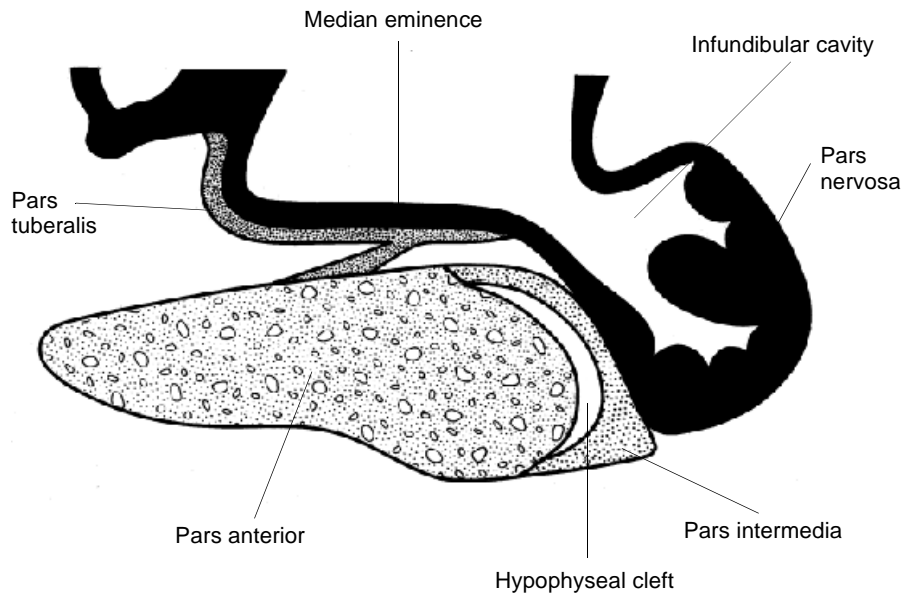
Data similar to the above have been recorded for Nine-banded Armadillos, *Dasypus novemcinctus* (body weight 2.8–4.0 kg), by Scholander, Irving & Grinnell (1943).

The Southern hairy-nosed Wombat, *Lasiorhinus latifrons*, (body weight 25 kg) is a cryptic burrower that lives in the same habitat as some echidnas and exhibits a response to mild heat stress as ineffective as that in *Tachyglossus* and *Bradypus*. At T<sub>A</sub> 35°C, *Lasiorhinus* lies on its back, breathes through its mouth and as T<sub>B</sub> rises to 38°C (normal T<sub>B</sub> is 35°C), it salivates, loses muscle tone and becomes limp. Like *Tachyglossus*, *Lasiorhinus* cannot sweat, is a good thermoregulator at low T<sub>A</sub> and can withstand hypothermia (Wells 1973). From all these data it is clear that some mammals of similar or even greater body weight to that of echidnas are just as heterothermic. Perhaps as Schmidt-Nielsen *et al.* (1966) remarked: ‘it may be more profitable to consider physiological findings as such without the requirement that they should fit into a preconceived evolutionary pattern’. The ability to enter torpor is connected with adrenal gland activity.

### Endocrine and Immune Systems

The pituitary gland is a pyriform organ dependent from the ventral surface of the infundibulum of the brain. Hanström & Wingstrand (1951) described the anatomical relationship of the various partes (Fig. 15.9). As in all vertebrates, the pituitary consists of two separate endocrine glands: the pars nervosa, a swollen postero-ventral extension of the infundibulum and the pars distalis, an elongated gland, pyriform in longitudinal section. The pars distalis consists of a pars tuberalis, pars anterior and a pars intermedia, the latter being plastered over the anterior face of the pars nervosa and separated from the pars anterior by the hypophyseal cleft. In longitudinal section, the anatomy very much resembles a reptilian pituitary. The presence of luteinising and adrenocorticotrophic hormones in the pars anterior has been established (Fink, Smith & Augee 1975).

As in all vertebrates, the pars nervosa consists largely of unmyelinated fibres in the endings of which the nonapeptide hormones formed in the hypothalamus are stored. From these endings the hormones are released into the blood stream. As



**Figure 15.9** Median longitudinal section of the pituitary gland of the Short-beaked Echidna (After Hanström & Wingstrand 1951; with permission of *Acta Universitatis Lundensis*; © ABRS) [S. Collin]



**Figure 15.10** Median longitudinal section of right adrenal gland of the Short-beaked Echidna 10.4 (From Griffiths 1968; with permission of Pergamon Press)

in the eutherian partes nervosae, these hormones are oxytocin and vasopressin (Acher, Chauvet & Chauvet 1973). Oxytocin is of particular interest since it is important for suckling (see below).

The adrenal gland is pear-shaped externally and lies in close contact with the antero-medial surface of the kidney; in an adult it is about 9 mm long and 4 mm wide. Internally, instead of a central medulla of chromaffin (adrenalin-secreting) tissue surrounded by the various zones of cortical tissue as in metatherian and eutherian adrenals, the chromaffin tissue is located in the posterior half of the gland (Fig. 15.10). The rest of the gland is called 'cortical' tissue simply because the steroid hormones (corticoids) are synthesised there as in the eutherian adrenal cortex. The 'cortical' tissue in the tachyglossid adrenal actually bears a startling resemblance to that seen in reptile adrenals (Griffiths 1978).

The principal corticoids secreted by the steroidogenic cells are cortisol, corticosterone and aldosterone (McDonald 1978). The rates of secretion of the glucocorticoids, corticosterone and cortisol are extremely low (Weiss & McDonald 1965; Sernia & McDonald 1977a; Weiss, Oddie & McLance 1979), as is the concentration of plasma corticosterone. These facts, coupled with observations on adrenalectomised echidnas kept under laboratory conditions (McDonald & Augee 1968), suggest that adrenocortical secretions are not very important in metabolic regulations. Nevertheless, adrenalectomised echidnas exposed to  $T_A$  4–10°C, even if provided with food, become torpid within 12 to 28 hours of this treatment. The adrenalectomised individual can be made to behave normally if injected with cortisol. Infusion of glucose into adrenalectomised animals also prevents the fall in  $T_B$  (Augee & McDonald 1973). This suggests that the action of the glucocorticoid in tachyglossids, as in eutherians, is to stimulate hepatic gluconeogenesis from protein and so to replenish carbohydrate energy reserves depleted by stress. Sernia & McDonald (1977a, 1977b) have further elucidated adrenocortical functions in *Tachyglossus*.

Injection of ACTH over a 4 day period increases cortisol and corticosterone levels, but does not effect significant changes in levels of other blood corticosteroids (Weiss *et al.* 1979). Adrenal homogenates from control animals convert progesterone to 11-deoxycorticosterone and 11-deoxycortisol, but after ACTH treatment the principal product of the homogenates is corticosterone. These effects of ACTH stimulation are similar to those found in eutherians.

The pancreas is discrete as in carnivores, not diffuse as in rodents and lagomorphs. The Islets of Langerhans exhibit the usual alpha, beta and indifferent cells and are distributed throughout the exocrine tissue. Storage of insulin has not been demonstrated in the *Tachyglossus* pancreas, but the quick return in blood sugar to normal levels in glucose tolerance tests (Griffiths 1965a) argues that a rapid secretion of stored insulin takes place in response to hyperglycaemia. Injection of exogenous insulin induces hypoglycaemia in *Tachyglossus* (Griffiths 1965a; Sernia & McDonald 1978). While insulin has potent actions on carbohydrate, protein and fat metabolism, the antagonistic effects of glucocorticoids on these actions are minimal (Sernia & McDonald 1978).

The Echidna can survive bilateral adrenalectomy indefinitely in the laboratory without developing disturbance in electrolyte and carbohydrate metabolism. This suggests that the mineralocorticoid, aldosterone, plays only a minor part in the regulation of plasma electrolyte levels. Sernia & McDonald (1981) found very low levels of aldosterone in plasma and low secretion rates. Infusion of ACTH and of angiotensin II, however, brought about marked increases in these parameters of adrenal function. The ability of unstressed adrenalectomised tachyglossids to survive in the study of McDonald & Augee (1968) may have been due to a high sodium level in the diet.

The thymus, parathyroid glands and the thyroid are all intermingled with fat and are located dorsal to the sternum. The thyroid exhibits the usual collection of follicles lined by a single layer of secretory cells surrounding a central mass of colloid. Thyroids of animals that have been in torpor become hypertrophied so that they could be distinguished as discrete glands (Augee 1969). Serum thyroxine levels (16 nmol/L), are roughly the same as those in many marsupials (Hulbert & Augee 1982a). Since destruction of the thyroid gland by injection of iodine<sup>131</sup> leads to no significant decrease in metabolic rate or in  $T_B$ , the notion was tentatively advanced that the low metabolic rate of the Echidna is due to its relative hypothyroidism.

Lymphoid organs such as the spleen, thymus, appendix, tonsils, lymph nodules and Peyer's patches occur in anatomical situations in *Tachyglossus* similar to those in eutherians (Diener, Ealey & Legge 1967a).

Submucosal extensions of intestinal glands into lymphoid aggregations, lymphoepithelial glands, are present in the ileum and similar structures in the colon and cloaca (Schofield & Corkill 1969). These structures bear a strong resemblance to those found in the bursa of Fabricius in young birds.

The Short-beaked Echidna exhibits both primary and secondary (anamnestic) responses to injection of an antigen (*Salmonella flagellae*) by production of antibodies (Diener, Wistar & Ealey 1967b). Although an anamnestic secondary response can be elicited, its magnitude is erratic and always much smaller than that of eutherians. The latter authors suggest that the antibodies formed seemed to be analogous to IgG and IgM, which Atwell *et al.* (1973) demonstrated in the serum of specimens injected with the same antigen. Marchalonis, Atwell & Goding (1978) found that the IgG was made up of the two distinct isotypes also found in the sera of 'higher mammals'. The anamnestic response of *Tachyglossus* is little better than that observed in reptiles (Griffiths 1978 for discussion).

### Sense Organs and Nervous System

The brain has been described in great detail by Ziehen (1897, 1905) and by Abbie (1934). These works have been summarised by Griffiths (1968, 1978). Only some matters of phylogenetic and physiological interest are discussed here.

Monotreme brains exhibit many typically mammalian structures. The cerebellum is large and divided into a number of lobes whose surfaces are thrown into folds. The lateral aspects of the cerebellum are joined by the pons Varolii, a massive band of ventrally directed nerve fibres found only in brains of mammals. The roof of the midbrain is also distinctively mammalian in that there are four prominences, the corpora quadrigemina or superior and inferior *colliculi*, instead of the two optic lobes of the sauropsidan brain. The presence of huge trigeminal nerves is a feature of prototherian brains. The trigeminal is a mixed sensory and motor nerve, but the sensory component is by far the greater, conveying impulses from the plethora of tactile organs in the snout to the cortex via a 'way station' in the thalamus.

The forebrain bears large olfactory bulbs which, enigmatically, have no mitral cell monolayer (Switzer & Johnson 1977). The forebrain is divisible into a pyriform cortex, hippocampal cortex and neocortex. The latter, as in the marsupial brain, lacks the corpus callosum which interconnects the two halves of the eutherian neocortex.

The neocortex or neopallium, the hallmark of a mammalian brain, consists of a hypertrophy of the lateral and dorsal aspects of the cerebral hemispheres. This brings about a ventral displacement of the pyriform cortex and where it and the neocortex meet, a longitudinal fissure, the rhinal sulcus, occurs. The latter is a landmark for palaeontologists studying brain endocasts of fossil mammals. Such an endocast of the brain of the mammal-like reptile, *Probainognathus*, indicated that a neocortex had evolved at least once in the Middle Triassic (Quiroga 1980). A neocortex does not occur in the brains of the Sauropsida.

The neocortex of Echidnas is markedly gyrencephalic (convoluted; Fig. 15.11), whereas the brains of Platypus and many marsupials are smooth or lissencephalic. The huge gyrencephalic neocortex of Echidnas has been a source of wonderment to neuroanatomists. Elliott Smith (1902a) wrote:

'The most obtrusive feature of this brain is the relatively enormous development of the cerebral hemispheres which are much larger, both actually and relatively, than those of the platypus. In addition the extent of

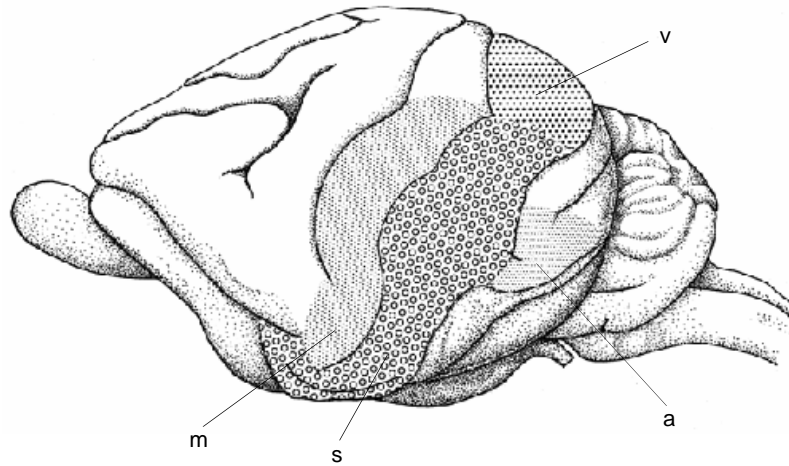


the cortex is very considerably increased by numerous deep sulci. The meaning of this large neopallium is quite incomprehensible. The factors which the study of other mammalian brains has shown to be the determinants of the extent of the cortex fail completely to explain how it is that a small animal of the lowliest status in the mammalian series comes to possess this large cortical apparatus’.

This mammal ‘of the lowliest status’ is, however, the equal of the Wistar rat in position-habit and habit-reversal learning (Saunders, Chen & Pridmore 1971a; Saunders *et al.* 1971b) and in its ability to learn to operate a simple piece of machinery (Buchmann & Rhodes 1978). The latter authors state:

‘Further studies of learning will undoubtedly disclose important facts about the intelligence of these remarkable animals and modify the quaint, explicitly or tacitly-held views that echidnas are little more than animated pin cushions or, at the best glorified reptiles’.

In all mammals the neocortex is organised for reception and analysis of sensory impulses and for dispatch of motor impulses. Lende (1964, 1969) maps auditory, visual, somatosensory and motor areas in the cortex of *Echidnas* (Fig. 15.11). The somatosensory, visual and auditory areas are clustered at the caudal pole of the cortex. Coincident and exactly coextensive with the somatic sensory area is the motor cortex, but rostral to this sensorimotor area is an area with motor function only. There is considerable representation of the snout and tongue in the sensorimotor area, but nowhere near as great as that of the muzzle in the *Platypus* cortex (Bohringer & Rowe 1977; see Chapter 16 this Volume).



**Figure 15.11** Somatosensory and motor (s); motor (m); visual (v) and auditory (a) areas of the cortex of the Short-beaked Echidna (After Lende 1969; with permission of the New York Academy of Sciences; © ABRS)  
[G. Scott]

Pathways of projection of sensory impulses to the cortex from various parts of the body and sense organs are reviewed by Griffiths (1968, 1978). The final way stations for relaying impulses to the cortex are in the thalamus. From here visual, auditory, tactile, temperature and proprioceptive impulses are projected onto the cortex. Two architectonic fields in the sensorimotor amalgam and the thalamic afferents to them have been identified. These fields are possibly homologous areas in eutherian cortices known as 3a and 3b (Ulinski 1984).

Only mammals have direct connection between the motor cortex and the lower reaches of the spinal cord. As might be expected, these corticospinal or pyramidal tracts occur in the brains of monotremes. They have been studied experimentally in *Tachyglossus* by Goldby (1939) and Draper (1971).

### Special Senses

The eye of *Tachyglossus* has been described by Gresser & Noback (1935), Walls (1942), O'Day (1952), Gates (1973), Stone (1983). As Gates (*op. cit.*) pointed out, there are inconsistencies in these descriptions.

The eyeball is roughly spherical, about 9.5 mm in diameter. The fibrous sclera exhibits a cartilaginous cup which is characteristic of the eyes of reptiles but not of other mammals, except the Platypus. The retina shows the layers typical of the retinas of all vertebrates: a thin layer of nerve fibres, a ganglion cell layer and a receptor layer. Stone (1983) has shown unequivocally that the receptors exhibit oil droplets and Locket (1985) examined well-fixed retinas by light and electron microscopy and found both rods and cones in a ratio of about 5:1.

The nerve fibres from the retina form the optic nerve and the number of fibres therein has been estimated at about 15 000. This is small compared with that in monkeys, for example, but it is double that estimated for the bat, *Eptesicus fuscus*, a mammal capable of making a single visual discrimination (Gates 1973). On this basis, the Echidna has a reasonable degree of visual function. Gates (1978) has shown that the Short-beaked Echidna is indeed very good at learning by visual discrimination, to a level at least comparable to that of the rat.

The primary optic pathways from eye to the thalamus have been elucidated by Campbell & Hayhow (1971). There is almost complete decussation of the fibres via the optic chiasma, only about 1% of fibres going to the ipsilateral side. This may confer a modicum of binocular vision, since the eyes are directed forward to a small degree. The path of visual afferents from thalamus to the cortex has not yet been determined.

Cartilaginous ear trumpets carry sound waves from the pinnae, downward, medially and dorsally to the tympanic membrane. The stapes, standing on a membrane in the fenestra ovalis, conveys the sound impulses to the cochlea where the mechanical energy is transduced to electrical energy in the organ of Corti. The cochlea is not coiled as in the metatherian and eutherian middle ear but the organ of Corti is similar, but not identical, to that in other mammals (Chen & Anderson 1985). Uncoiled cochleas are also found in Mesozoic mammals.

The electrical potentials (cochlear microphonics) produced by the organ of Corti can be measured and are an indication of the sensitivity of the auditory apparatus to different sound frequencies. The CM audiogram for the Echidna's cochlea shows maximum response close to sound at 5 kHz (Griffiths 1968, 1978). This frequency is low compared to the range of frequencies found to stimulate maximally the cochleae of other mammals, but is probably ideal for detecting sounds emitted by termites and ants underground.

The ear ossicles of *Tachyglossus* are peculiar in that the *incus* is firmly ankylosed to the *malleus*, a process which in turn is tightly attached to the petrosal bone (Aitken & Johnstone 1972). This implies that the ear would be sensitive to sounds conducted by the body. Johnstone (Griffiths 1968) found that tapping the body at various points elicited very small CM potentials, but when the snout was tapped a large potential was observed. Echidnas probably get auditory information via mechanoreceptors in the snout when this organ is resting on or buried in the ground.

The snout has several tactile functions. Iggo, McIntyre & Proske (1985) have shown by electrophysiological techniques that the leathery skin of the dorsal surface of the snout is densely packed with specialised sensory end organs innervated by trigeminal nerve fibres. Four kinds of mechanoreceptors and two kinds of thermoreceptors are present. The latter are either cold sensitive, excited by a fall in skin temperature, or warmth sensitive, fired persistently at

temperatures above the normal  $T_b$  of 32°C. Iggo *et al.* (1985) conclude that the snout provides detailed information about the immediate physical environment.

The olfactory organs, unlike those in the Platypus, are very well developed. The area covered by the olfactory epithelium is enormous; it consists of seven vertical endoturbinas dependent from the cribriform plate; a large number of ectoturbinas (ethmoturbinas); and sets of nasoturbinas and maxilloturbinas. The cribriform plate is pierced with a large number of pores through which the branches of the olfactory nerves pass to the olfactory bulbs (Griffiths 1978 for description of olfactory pathways in the brain).

There is no published information on physiological or behavioural studies of olfaction, however, it is likely that sense of smell is of importance to echidnas in at least three ways. Tachyglossids are solitary animals for most of the year but may form groups in the breeding season (Griffiths 1978); olfaction probably plays a part in bringing individuals together. Olfaction probably assists in detection of ants and termites, since to humans, at least, both kinds of organisms possess distinctive odours. The sense of smell is probably the one special sense that an Echidna has at the time of hatching, since fully developed olfactory sense receptors are present in the cavum nasum. This development presumably enables the hatchling to find the milk areola since other senses are lacking (the malleus is still part of the jaw and the eyes are covered with a thin layer of skin-like epithelium).

Two slits forming a V are located at the dorsal surface of the hind end of the dental pad of the tongue. These lead down into deeply seated circumvallate papillae in the trenches of which taste buds occur (Oppel 1899). In addition, sets of foliate papillae furnished with taste buds are located laterally and posterior to the dental pad. These arrangements would appear to be well suited for taste since the receptors occur where the prey is being crushed.

## Reproduction

In their reproductive processes, more than in any other aspect of their biology, monotremes exhibit that mingling of reptilian and mammalian characters already described.

## Female System

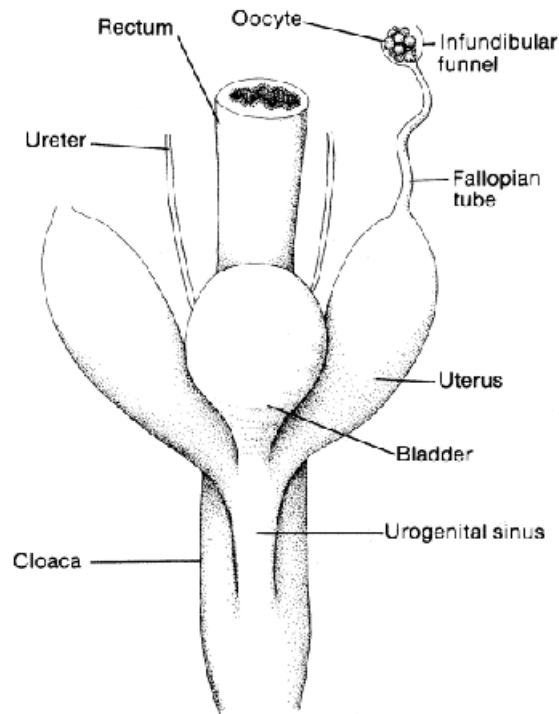
The anatomy of the monotreme reproductive system was made known to biologists on 17 December 1801 when Everard Home (1802a) read a paper to the Royal Society on the anatomy of a quadruped then named *Ornithorhynchus paradoxus*. He described both the male and female reproductive organs and concluded from the study of the latter that the animal would prove to be 'oviviparous' since the organs of generation resembled those of ovoviparous lizards. On 3 June 1802 he read another paper to the same society (Home 1802b) on Shaw's 'ant-eating Porcupine' of New Holland (*Tachyglossus aculeatus*). He found that the reproductive organs of the specimen, a male, 'bore a close resemblance to those of the *paradoxus*'. Home was close to the truth: 82 years later W.H. Caldwell showed that they were oviparous. The discovery was communicated by telegram to a meeting at Montreal of the British Association for the Advancement of Science on 2 November 1884 (Moseley 1884). On the same day Haacke (1885) demonstrated a pouch egg of *T. aculeatus* to the Royal Society of South Australia.

There are paired ovaries with ripe follicles up to 5 mm in diameter, so that at the height of the breeding season each ovary resembles a bunch of tiny grapes and looks very like the ovary of a sauropsid (Fig. 15.12). Each ovary is enclosed by a membranous infundibular funnel which leads to a convoluted Fallopian tube.

In their lower regions, Fallopian tubes exhibit tubal glands opening into the lumen of the oviduct (C.J. Hill 1933). Each Fallopian tube leads through a junctional region into a uterus, the mucosa of which exhibits a profuse array of convoluted uterine glands. The paired uteri open separately into the anterior end of a long urogenital sinus leading to the cloaca.

**Figure 15.12** Female reproductive organs of the Short-beaked Echidna (After Griffiths 1984; with permission of Churchill Livingstone; © ABRS)

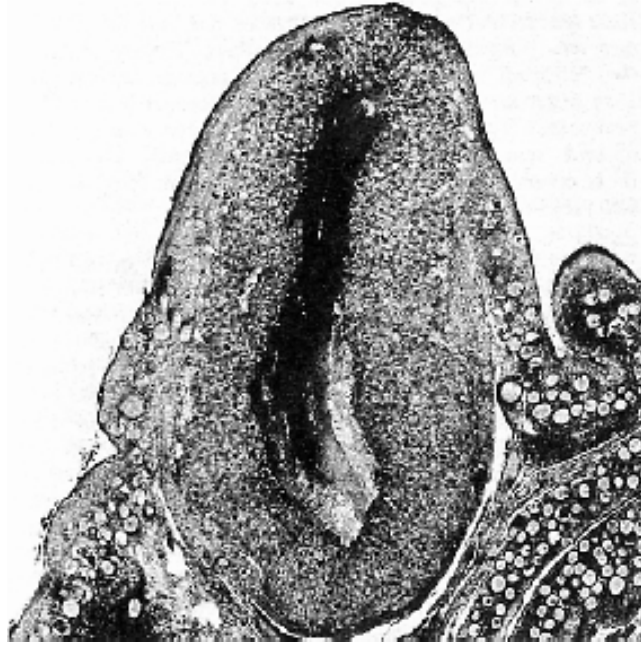
[G. Scott]



The germinal epithelium of the ovary is thrown into folds (Fig. 15.13) consisting of either flattened cuboidal or columnar epithelium. Interspersed throughout the germinal epithelium are small oocytes 0.042–0.06 mm in diameter, each of which is surrounded by a thin flattened follicular epithelium. Flynn & Hill (1939) give a definitive account of the development of these oocytes to the relatively huge yolk-laden egg about 4.0 mm in diameter. Preparations from both Platypus and Echidna ovaries were used to give a sequential account of ‘monotreme’ oogenesis, in which three phases are distinguishable. The oocytes, measuring 0.06–0.15 mm in diameter, are first located in the cortical folds of the ovary and are enclosed in a follicular epithelium of cuboidal cells beneath which is a zona pellucida, a striate layer and an egg membrane; the nucleus lies to one side of the oocyte (Fig. 15.14). At the second phase, the oocyte has a diameter of 0.2–0.5 mm and exhibits a cortical zone filled with lipid droplets; yolk sphere primordia appear among the droplets and their multiplication is at the expense of the lipid. The cortical or peripheral region remains free of yolk which occupies a large yolk zone (Fig. 15.14).

The third phase involves growth from 0.6 mm to the fully developed diameter of 4 mm. Hughes (1984) refers to membrane-bound yolk vesicles that contain a granular substance. Possibly these are the yolk primordia of Flynn & Hill (1939). In the centre of the yolk zone is the latebra, a flask-shaped area relatively free of yolk and consisting of alveolar cytoplasm. The neck of the flask ascends towards the nucleus where it expands to form the primordium of the germinal disc, a saucer-shaped area immediately below the nucleus (Fig. 15.14). The monotreme latebra strikingly resembles that of the sauropsidan egg (Semon 1894c). At the end of phase three the follicular epithelium is well developed, consisting of two cell layers throughout and exhibiting secretion granules, the precursors of the follicular fluid which Flynn & Hill (1939)

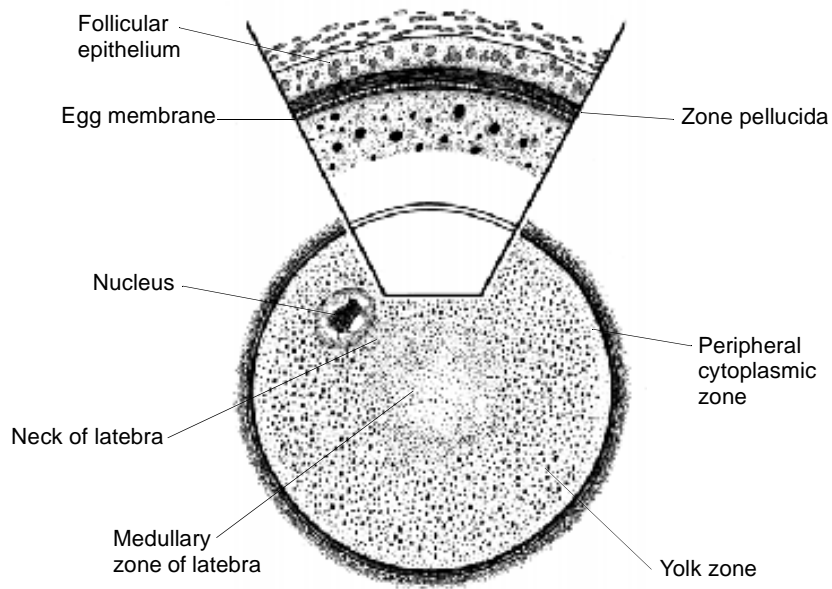




**Figure 15.13** Sagittal section of a young corpus luteum from the Short-beaked Echidna showing cavity filled with coagulum. Note immature oocytes in cortex of ovary. x 18 (From Griffiths 1978; with permission of Academic Press)

consider to be the homologue of the liquor folliculi of the Graafian follicles of other mammals. At this time the first maturation spindle appears in the germinal disc, the nucleus divides and the first polar body is given off in the ovary. The ovum is now shed into the infundibulum, where the second polar body is thrown out; at this stage the germinal disc contains the female pronucleus. The egg passes to the Fallopian tube where fertilisation occurs; the spermatozoan penetrates the egg and is transformed into the male pronucleus. The fertilised egg is then invested with a putative mucoïd coat (Griffiths 1978) apparently identical to those observed in marsupials (McCradly 1938; Hughes 1984) and rabbits (Braden 1952).

After deposition of the mucoïd layer, the egg is passed to the junctional region of the Fallopian tube where the anlagen of the basal and rodlet layers of the egg shell are laid down. The egg then passes to the uterus where conjugation of the two pronuclei takes place, followed by meroblastic cleavage, as in the eggs of reptiles and birds (Caldwell 1887). The first cleavage furrow divides the germinal disc into a larger and a smaller area and the second furrow is laid down at right angles, so that the four-celled stage consists of two large and two small blastomeres sitting on top of a sphere of yolk. Further division of the blastomeres leads to formation of the blastodisc. The cells at the margin of the disc spread peripherally over the surface of the yolk mass, leading to almost complete enclosure of the yolk by a unilaminar blastocyst. Some of these cells become endoderm by sinking below the general level of the outside layer where they form a network. The interstices of the network are infilled by mitotic division of its cells. In this way, a layer of endoderm comes to lie immediately below the outer layer, at this stage termed ectoderm. This bilaminar blastocyst is almost complete except for a small area of naked yolk located at the lower pole; this is soon covered over by the yolk navel, a crumpled plug of ectoderm, endoderm and yolk. Thus, the yolk is now enclosed in what may be termed a yolk sac.



**Figure 15.14** Section of an *Echidna* oocyte 0.65 x 0.60 mm in diameter.  
(After Flynn & Hill 1939; with permission of the Zoological Society of London;  
© ABRS) [S. Collin]

The blastocyst commences cell division. The deficiency in yolk is compensated for by absorption of a nutritive fluid secreted by the uterine glands. The egg has a diameter of about 5.7 mm when the yolk navel is established; at this stage the first sign of an embryo, the primitive streak, appears at the pole opposite the yolk navel. This is followed by the differentiation of mesodermal somites anterior to the streak. At the four-somite stage the egg has reached a diameter of about 11.5 mm; Hughes (1984) observed five pairs of somites in an egg of 11.7 mm diameter.

Extra-embryonic mesoderm spreads peripherally between ectoderm and endoderm during the somite stages of development, forming trilaminar omphalopleure (Luckett 1977). This process continues until the whole of the yolk sac is enclosed by omphalopleure.

At the final stage of intrauterine development the egg has a diameter of about 15 mm and the embryo exhibits 19 pairs of somites. A crescentic thickening of extraembryonic ectoderm anterior to the neural plate marks the site of the development of the proamniotic head fold (Luckett 1977).

The egg shell has been forming while the above events have been taking place. In its definitive form it consists of three layers: basal, rodlet and protective or granular, which exhibit two zones (J.P. Hill 1933). The basal layer is first laid down when the fertilised egg arrives in the tubal gland region of the Fallopian tube (C.J. Hill 1941). At its first appearance, it is a thin transparent membrane 1.3  $\mu\text{m}$  in thickness, applied to the mucoid coat. When fully formed, it is 3.6  $\mu\text{m}$  thick and exhibits a laminated structure (Hughes & Carrick 1978).

C.J. Hill (1941) and J.P. Hill (1933) expressed the view that the basal layer is derived from secretory globules elaborated in the cells of the tubal glands. *In utero*, however, the egg increases progressively in size from 4–5 mm diameter to 16.5 x 15.0 mm in size and during that sojourn the basal layer exhibits growth as well, not mere mechanical stretching. C.J. Hill (1941) considered that the material for the growth of the basal layer *in utero* also comes from the tubal

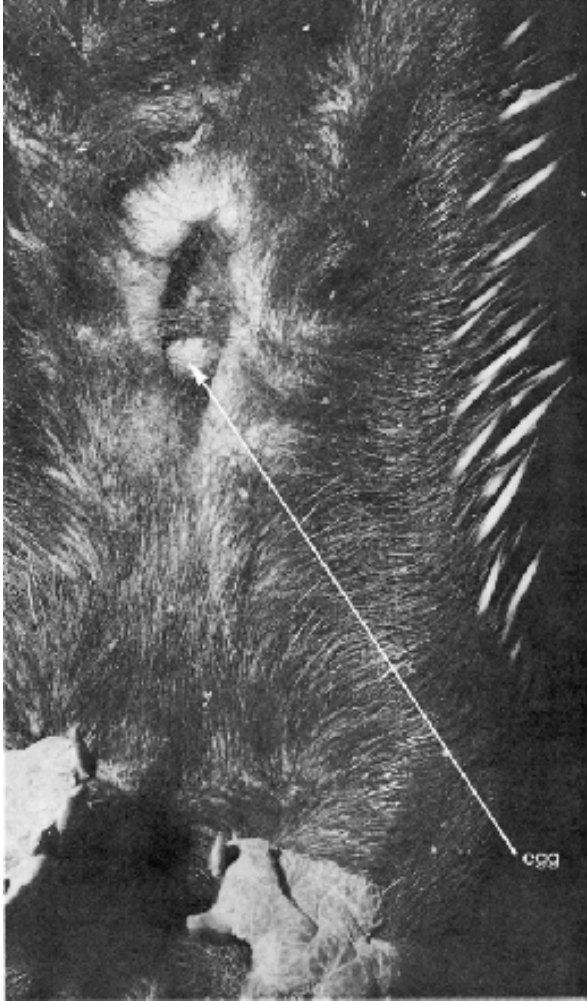
gland secretion stored in the lumina of the glands. Hughes & Carrick (1978), however, identified secretion products in tubal and uterine glands of *Platypus* which exhibit the disulphide groups characteristic of precursors known to give rise to ovokeratins in the shell membranes of other vertebrates. It would appear from this that the basal layer of the uterine egg shell grows by intussusception of uterine ovokeratin precursors.

During the passage of the egg through the junctional region, a second homogeneous layer, a product of both tubal and uterine glands, is deposited onto the basal layer (C.J. Hill 1941); this is the anlage of the rodlet layer, but the main secretion forming the rodlet layer is elaborated in the glands of the uterus. This second layer soon differentiates into a matrix and a series of close-set radially arranged rod-shaped bodies. At this stage the egg has a diameter of about 6.5 mm and differentiation of somites is taking place within. In the shell of the uterine egg of 10 mm diameter the rodlets have attained their definitive length of 0.019 mm and tend to occur in groups of three or four, separated by spaces filled with clear matrix. The rodlet layer grows with uptake of uterine nutritive secretions; this growth is achieved at the expense of the secretion in tubal and uterine glands.

The third layer appears when the egg has a diameter of about 11.8 mm (J.P. Hill 1933) the embryo exhibiting four to five pairs of somites. It is made up of granules aggregated to form rounded masses investing the outer surface of the rodlet layer. The precursor of this layer is a coarse granular secretion elaborated by the uterine glands (C.J. Hill 1941) until the egg reaches its full size of about 15 mm. The third layer exhibits inner and outer zones: the inner is made up of loosely arranged coarse granules, the spaces between them communicating with the inter-rodlet spaces. The outer zone exhibits irregularly oblong masses apparently formed by fusion of coarse granules. Clefs, fissures and irregular channels in this zone open to the exterior at pores (J.P. Hill 1933; Hughes & Carrick 1978; Hughes 1984). J.P. Hill summed up the properties of the monotreme egg shell: 'the 2-layered shell is well adapted by its structure (a) to increase in surface area *pari passu* with increase in size of the egg and (b) to transmit to the interior of the egg the nutritive uterine secretion which is necessary to supplement the yolk content of the same.....'. Laying down of the robust outer layer is completed only after the full complement of the uterine secretions is taken up. This third layer protects the embryo during the 10.5 days (see below) of incubation in the mother's pouch (Fig. 15.15). Here the pores of the outer layer of the egg shell permit respiration.

The processes of secretion of the uterine nutritive fluid are known only for *Ornithorhynchus* (C.J. Hill 1933, 1941). The fluid is formed in the superficial and middle regions of the uterine glands; the secretion precursors consist of fine granules which are passed to the apices of the cells of the glands. The apices project into the lumina of the glands and are cut off as small vesicles (Hughes & Carrick 1978) whereupon the granules liquefy and the fluid passes through rodlet and basal layers of the shell to the yolk sac. As the egg swells it comes into intimate contact with the uterine endometrium which no doubt facilitates passage of the fluid into the yolk sac. The latter may now be considered to be a placenta equivalent to the marsupial yolk sac placenta. During absorption of the fluid, the yolk liquefies and mingles with the uterine fluid. Nothing is known of the composition of the uterine secretion but it may prove similar to that of the marsupials Tammar Wallaby, *Macropus eugenii* and *Didelphis marsupialis*, which has been characterised by Renfree (1977).

Information on the gestation period in *Tachyglossus* is sketchy. The longest period observed is 27 days (Broom 1895) but the evidence is equivocal; other observations show that the period is at least 17 days and there is some evidence that monotremes can store sperm in the uterus (Griffiths 1984). Whatever the length of gestation, it is certain that it is under the control of a corpus luteum



**Figure 15.15** An Echidna egg in the pouch at eighth day of incubation; note presence of tumescent lips of the pouch, which before egg-laying is a sign of pregnancy. (From Griffiths 1972; with permission of the Australian Museum)

(CL). This forms in the ovary when the egg is shed into the oviduct; six stages in development of the CL have been described by Hill & Gatenby (1926), mainly in *Platypus* ovaries. Only two of the corpora lutea in the series are known from the Echidna; one referred to stage 1 and the other to stage 6 which came from the ovary of an animal that recently had laid an egg. Griffiths (1978, 1984), however, described a young CL (Fig. 15.13) from an Echidna carrying an egg which exhibited basal and rodlet layers in its shell and 16 blastomeres within. The uterine endometrium resembles that of metatherians and eutherians during the luteal phase. The CL observed was a young one intermediate between stages 1 and 2 (of Hill & Gatenby 1926); it contained a large central cavity filled with a dense coagulum and the luteal cells were large and well formed. Nothing is known of the secretion of progesterone in echidnas, but plasma progesterone levels have been determined in female *Platypus* (Hughes & Carrick 1978).

The mechanism of transfer of the egg to the pouch is a mystery, although some observations on pregnant captive echidnas may provide clues. Just before egg-laying it was noticed that when these animals were picked up, the cloaca everted and practically reached into the pouch. If they were allowed to curl up, the everted cloaca reached into the pouch (CSIRO 1969) suggesting that the egg may be deposited therein by this contortion of the body. Luckett (1977) found that the embryo in the newly laid egg still had the 19 pairs of somites found at the termination of uterine development, by which stage the head had begun to invaginate into the yolk sac lying below. The greater part of embryogenesis takes place during the 10–11 days of incubation in the pouch.



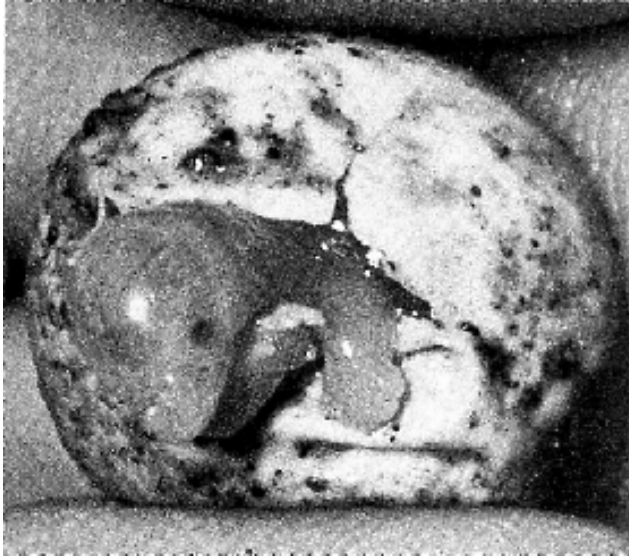
Semon (1894b, 1894c) recognised 14 stages of development during incubation of pouch eggs (termed 40–53) and 46–53 in pouch young. Stage 40 is an embryo 7 mm long with lobate forelimbs, buds for hind limbs, prominent pharyngeal pouches, about 39 pairs of somites and a tail. Luckett (1977) examined 38 embryos of the Hill collection housed in the Hubrecht Laboratory, Utrecht, giving an account of the early development of the amnion and allantois. The latter is tiny even at the 27 somite stage, suggesting that supply of nutrients (stored uterine secretions) and oxygen to the embryo is via the vascular yolk sac invested with trilaminar omphalopleure. The end result is that at stage 45, just before hatching, the embryo lies between a large vascular yolk sac to its left and an equally large allantois which together fill the available space (Semon 1894b).

Both yolk sac and allantois are in contact with porous egg shell. Since the allantois is highly vascular (its arterial and venous vessels passing to the embryo via the allantoic stalk dependent from the undersurface of the embryo), its presumed function is to provide gaseous exchange between the atmosphere in the pouch and the embryo. The arterial supply of the yolk sac typically consists of two branches from the dorsal aorta which communicate with a rete mirabile on the surface of the sac. The venous drainage of this rete coalesces into two large veins which unite and pass as a single vein into the embryo via the yolk sac stalk. The yolk sac is replete with stored liquid nourishment, derived from the secretions of the uterine glands, which are being absorbed by the rete capillaries and transported to the embryo. At this stage (45), the latter has enormous pentadactyl forelimbs, hind limbs still at the bud stage and a fore-shortened snout with terminal mouth ready for suckling. The snout also bears a caruncle and an egg tooth for tearing open the egg shell.

The development of various organs is described by Alexander (1904, organs of balance and hearing); Bresslau (1920, mammary glands); Gaupp (1908, chondrocranium); Hochstetter (1896, blood vessels); Maurer (1899, derivatives of the pharyngeal pouches) and Ziehen (1905, brain).

The anlage of the egg tooth appears at stage 43 as a median conical papilla which arises at the anterior end of the snout, projects downwards over the mouth and is covered with epidermis. At stage 44, a portion of the epidermis shows differentiation of its cells to form an enamel organ. Dorsal to the tooth anlage is a median condensation of mesenchyme, which forms bone and which unites with mesenchyme of the premaxillae; this in turn forms bone, giving the tooth a solid foundation. Just before hatching (stage 45), the tooth has a sharp point and is invested with enamel, which overlays a dental pulp rich in capillaries (Seydel 1899; Hill & de Beer 1949). At stage 45 and at hatching the lungs are in an embryonic condition compared with those of eutherians at birth (Narath 1896). True alveoli are not present but the bronchi end blindly in modified bronchioles which function as respiratory chambers for some time after hatching. These chambers, 'geräumige Luftkammern', are lined with respiratory epithelium but become ordinary respiratory bronchioles when true alveoli later arise from them.

The incubation period of the egg in the pouch has been determined with reasonable accuracy in four specimens of *T. aculeatus*. The most accurate determination of incubation time is 10.25 days (Griffiths 1978, 1984). This egg was laid at about 12:15 hours on the 17th September and had diameters of 15 x 15 mm; the temperature of the pouch was 32°C. At 16:00 hours on the 10th day the egg was found to have a dimple on one side and on removal from the pouch, movements could be detected under the shell which was occasionally distorted from the round to a pear shape. By 18:10 hours the hatchling had broken the shell, presumably by tearing it with its egg tooth and caruncle, and was struggling with a writhing motion of its head and forelimbs to escape the shell (Fig. 15.16). Incubation was deemed to be finished at this point. This span agrees well with the times for the other incubations recorded and reasonably well with the 12 days for an incubation observed in Tara, Queensland (Griffiths 1978).



**Figure 15.16** Young Echidna emerging from egg after ca. 10 days six hours incubation. (From Griffiths 1972; with permission of the Australian Museum)

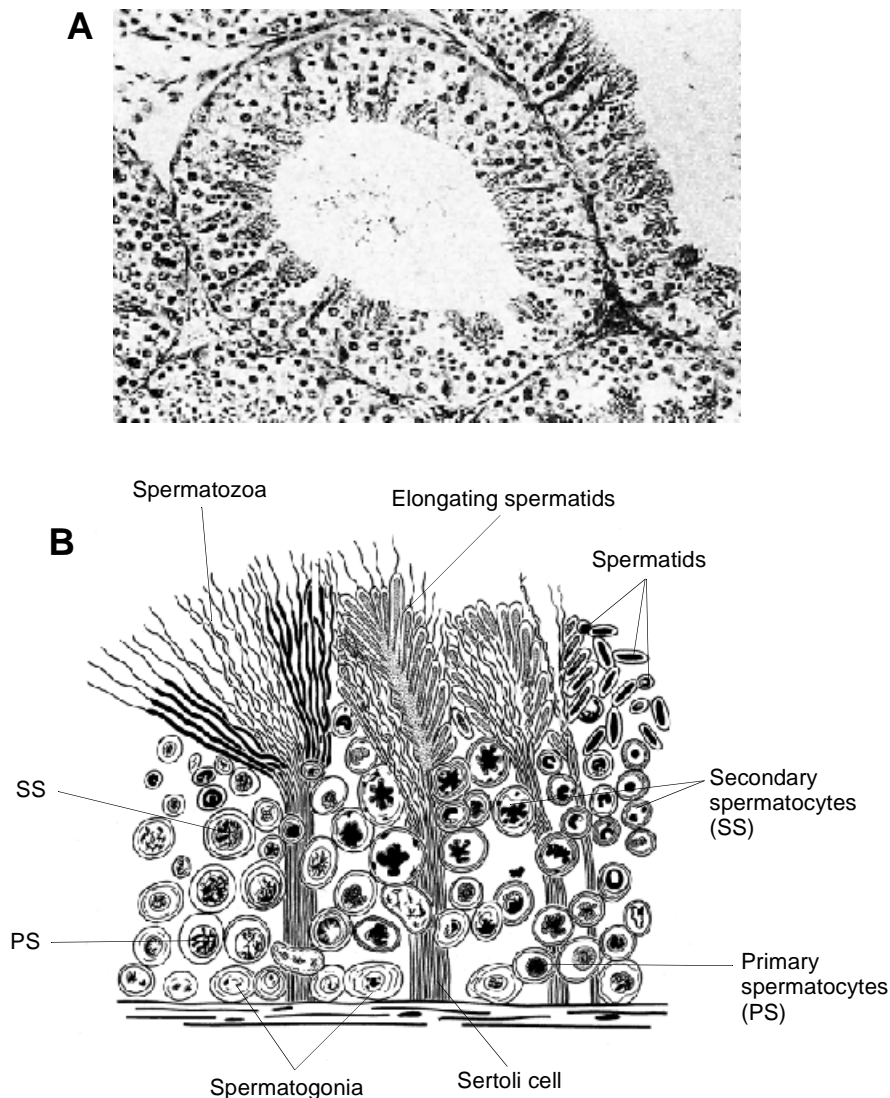
### Male System

Monotremes are testicond, the ovoid testes being suspended from the dorsal wall of the abdominal cavity by mesorchial folds of peritoneum, posterior to the kidneys. The seminiferous tubules pass to the anterior end of the testis where they concentrate to join seven ductuli efferentes passing to an epididymis, which is differentiated into a large caput and small cauda (Djakiew & Jones 1981) connected by a vas deferens (Fig. 15.8). The fine cytology and function of the epididymis is described by Bedford & Rifkin (1979) and by Djakiew & Jones (1981, 1982, 1983). The short vas deferens conveys the sperm from the cauda to the anterior end of a long medial urogenital sinus from the posterior end of which a muscular penis protrudes ventrally; the urethra of the penis communicates with the lumen of the urogenital sinus; and passes spermatozoa only in monotremes (Home 1802a, 1802b).

The penis is about 70 mm long, dorso-ventrally compressed and with a bifid glans. It lies within a thin-walled preputial sac located ventral to the cloaca. In the ventral wall of the latter is an opening through which the penis, upon erection, is forced into the cloaca and to the exterior through the cloacal sphincter (Fig. 15.8). Paired Cowper's glands (Home 1802a, 1802b), communicate by short ducts with the penile urethra. There is no definite prostate gland but disseminate urethral glands are present.

Along with the changes in weight and tubule diameter, testis histology changes seasonally. The following description applies equally to all monotremes. In the resting phase of summer and autumn the tubules have no lumina and consist of a peripheral ring of spermatogonia each of which has a very large nucleus with a collection of elongated Sertoli cells interspersed between the spermatogonia. Each Sertoli cell stretches from the tunic to the centre of the tubule and since their nuclei are elongated and radially arranged, this confers a 'cartwheel' appearance on the tubule seen in cross section. Spermatogenesis, in general, starts in May: division of spermatogonia gives rise to a generation of primary spermatocytes. By late June, the tubules have greatly increased in size, exhibit primary and secondary spermatocytes and spermatids and the first signs of a lumen. By the end of July and mid-August the tubules are ripe, exhibiting spermatozoa in the now expanded lumen (Fig. 15.17a). The definitive study of spermatogenesis in the ripe tubules was made by Benda (1906a), summarised by Griffiths (1968). The usual processes of mitotic division of spermatogonia result in a population of primary spermatocytes (Fig. 15.17b). These divide by meiosis

and the secondary spermatocytes so formed give rise to spherical spermatids located between the very long Sertoli cells which now stretch from the basement membrane of the tubule to the lumen. Each spermatid, including its nucleus, commences to elongate and to exhibit a flagellum. Numbers of spermatids then burrow down into the cytoplasm of a Sertoli cell; groups of up to 20 spermatids, occasionally at different stages of development, can be seen in the one Sertoli cell (Fig. 15.17B). Carrick & Hughes (1982) presented details of spermiogenesis in all three genera of monotremes. The end result is a filiform spermatozoan, with a very long coiled head (Fig. 15.17B) and cytoplasmic droplet, resembling that of a sauropsidan. These are then shed into the lumen of the tubule from where they pass to the epididymis. At first they are immotile, but on passing along the epididymis they lose the cytoplasmic droplet and become motile (Bedford & Rifkin 1979; Djakiew & Jones 1983). The latter authors noted that in the tail of the epididymis, the spermatozoa assemble into cone-shaped bundles reminiscent of the sperm rouleaux formed by guinea-pig spermatozoa during maturation.



**Figure 15.17** A, Ripe tubules of testis of Short-beaked Echidna showing spermatozoa with the coiled heads embedded in the elongated Sertoli cells. B, Section of portion of seminal epithelium in testis ripe tubule. Note coiled heads of spermatozoa and different stages of developing spermatids in the one Sertoli cell. (After Benda 1906a; Griffiths 1978; with permission of Academic Press; © ABRS) [S. Collin]

Testes weights of south-eastern Australian *Tachyglossus aculeatus* show marked seasonal changes. From October to March the testes weigh 1–3 g/kg body weight and the mean diameters of the testicular tubules range from 70–150  $\mu$  (Griffiths 1978). In early April testes weights range from 1–8 g/kg. Testes in early April and May exhibit spermiogenesis. Tubules are at their greatest diameters in August and September, the highest testes weight recorded being 17g/kg and the highest mean tubule diameter 370  $\mu$ . After this, testes suddenly regress occurs (Griffiths 1978) and by the end of September, they have shrunk to bean-sized organs (Semon 1894a).

In monotremes the male is the heterogametic sex and in *T. aculeatus* the sex chromosome system takes the form  $X_1X_2Y/X_1X_1X_2X_2$  (Murtagh & Sharman 1977). In both sexes, however, there are six chromosomes found at mitosis, termed a-f, which have no homologous partners. At meiosis in the male, these are included with the three sex chromosomes in a multivalent of nine:  $X_1Y_1X_2$  f.e.d.c.b.a. and at metaphase two kinds of sperm are produced  $X_1X_2$  e.c.a and Y f.d.b (Murtagh 1977).

Since the Short-beaked Echidna rarely breeds in captivity, information on genetics is confined to karyology of the gametes and to electrophoretic analysis of a few of their proteins. The chromosome number is  $2n = 63$  / male, 64 / female (Bick & Jackson 1967). The chromosomes range in size from macrochromosomes to microchromosomes. The latter occur in the chromosome complements of Sauropsida, but not in those of mammals other than the Monotremata. Echidnas collected from various parts of south-eastern Australia exhibit two haemoglobins, HbI and HbII, as do New Guinean and Western Australian specimens. Polymorphs of both kinds of haemoglobin have been detected by Cooper *et al.* (1973) who assumed, by analogy with other polymorphs studied in animals for which genetic data were available, that the polymorphism of Echidna haemoglobin is under genetic control. HbI and HbII have three polymorphs, each designated A, B and C. The results to date indicate that these forms are distributed between subspecies as shown in Table 15.1

A hybrid group exhibiting all three HbI polymorphs was found in southern Victoria, extending into South Australia. Another hybrid group, also exhibiting three HbI polymorphs, occurs north of Sydney as far as Narrabri and Port Macquarie.

**Table 15.1** Distribution of each of the three alleles at each haemoglobin locus in different subspecies of *Tachyglossus aculeatus*.

	HbI	HbII
<i>T. a. setosus</i>	B	A, AB, C
<i>T. a. aculeatus</i>	B	A
<i>T. a. multiaculeatus</i>	A, AC	A
<i>T. a. acanthion</i>	A	A
<i>T. a. lawesii</i>	A	A (one sample)

Amino-acid sequencing of  $\alpha$  and  $\beta$  chains of HbIA, HbIB and HbIIA and of myoglobin in Short-beaked Echidnas and a similar study of Platypus globins offered no support for the concept of a constant rate of mutation of proteins independent of species. Echidna myoglobin is composed of 153 residues (Castello, Jones & Lehmann 1978; Whittaker, Fisher & Thompson 1978). The primary amino-acid sequences exhibited nine differences from those of Platypus, compared to some 20 to 25 differences from known marsupial and



eutherian myoglobin. Castello *et al.* (1978) suggested that the Tachyglossidae and Ornithorhynchidae diverged from a common ancestry more recently than did the Eutheria and Metatheria from one another.

Hosken & Boettcher (1978) prepared *Tachyglossus* HbIA, HbIB and HbIIA in pure form and studied their oxygen binding parameters. The principal finding was that HbII has an oxygen affinity markedly higher than the polymorphs of HbI. *Tachyglossus* blood contains 20% HbIIA, suggesting that either there has not been sufficient time for the effect of genetic load to select either for or against HbIIA; or, alternatively, HbIIA has some specialised function essential to *Echidna* respiratory function.

Other proteins for which genetic variation has been detected in the *Echidna* are phosphoglycerate kinase (Cooper *et al.* 1974; VandeBerg & Cooper 1979), thought to be autosomally inherited and lactate dehydrogenase (LDH) (Baldwin & Temple-Smith 1973; Holmes, Cooper & VandeBerg 1973). The testis-specific LDH found in Eutheria and Metatheria was not detected in the spermatogenetically active testes of Monotremata by Baldwin & Temple-Smith, but was found by Holmes *et al.* (1973) in testes thought to be spermatically inactive by the former authors.

Comparison of Monotreme Reproductive Processes with those of Sauropsida and of Metatheria

Considered alone, the fact that the monotremes are testicond may not seem very significant, since many other mammals share this 'reptilian' character. But when combined with the filiform nature of the spermatozoa, like those of Sauropsida, that groups of up to 20 developing spermatids occur in the one Sertoli cell and that sections of quiescent and ripe tubules are strikingly like those in sauropsidan testes (Matthews & Marshall 1956), it is understandable that Griffiths (1984) is of the opinion 'that the monotreme testis is as reptilian in character as the interclavicle of the pectoral girdle'.

Reproduction in female monotremes resembles in many ways that of oviparous reptiles: both have large yolk-laden eggs in which a latebra is present, cleavage is meroblastic and a yolk navel forms; the laid eggs have a well-developed protective shell and the newly hatched young has an egg tooth or teeth. The ovarian follicle of the monotreme, however, elaborates a secretion not found in the sauropsidan follicle, which Flynn & Hill (1939) considered to be a homologue of the liquor folliculi of the Graafian follicles in other mammals. In monotremes, uni- and bilaminar blastocysts are formed before the appearance of any sign of an embryo, unlike the circumstances of embryo formation in Sauropsida. Further, the sauropsid egg contains nutrient in the form of yolk sufficient to produce independent viable young. Even the eggs of placental viviparous reptiles contain enough yolk to allow development to proceed and give rise to fully formed young (Thompson 1977). On the other hand, monotremes depend for their development largely on uptake of uterine secretions. The egg shell of the oviparous reptile does not grow by intussusception *in utero*, but is laid down as a complete entity round the mass of yolk. This shell consists of two layers, both of which are made of long fibres arranged parallel to one another (Giersburg 1922), a structure which bears no resemblance to the granular aggregations in the protective layer of the monotreme's egg shell.

According to Hill (1910), Flynn & Hill (1947), Hill & de Beer (1949) and Luckett (1976, 1977) the reproductive processes of marsupials are slightly modified monotreme reproductive processes. The eggs of marsupials are relatively large (0.2–0.3 mm diameter) and their cytoplasm, surrounded by a *zona pellucida*, contains a substance referred to as yolk by Caldwell (1887), McCrady (1938) and Lyne & Hollis (1977). The eggs of all metatherians acquire a coat of acid mucopolysaccharide in the Fallopian tube, as in the monotremes.

While mucoid coats are not confined to these groups and are known to occur in at least some eutherians, the marsupial egg resembles that of the monotremes in that the mucoid layer is invested with a shell of putative keratin. J.P. Hill (1910, 1933) considered this to be a homologue of the basal layer of the monotreme egg shell. In the Opossum (*Didelphis virginiana*), the egg shell is in the form of loosely intertwining keratinous fibres (Krause & Cutts 1983c) which in section have some similarity to the laminated structure seen in the monotreme basal layer with the difference that the latter is more densely packed. In the shells of the Australian marsupials *Isodon macrourus* (Northern Brown Bandicoot), *Perameles nasuta* (Long-nosed Bandicoot) and *Trichosurus vulpecula* (Common Brushtail Possum), the fibres are more closely packed than those in the *Didelphis* egg shell (Hughes 1977; Lyne & Hollis 1977).

Boyd (1924), Hartman (1916) and McCrady (1938) have all shown that the shell in *Didelphis* is laid down in the fallopian tube. Hughes (1984) stated that the egg shell of *Trichosurus* is laid down exclusively in the uterus; despite the fact that as well as those of *Didelphis*, eggs with shells have been observed in the fallopian tubes in the Eastern Quoll (*Dasyurus viverrinus*) by J.P. Hill (1910), the Quokka (*Setonix brachyurus*) by Sharman (1961b) and in *Trichosurus* by Hughes (1974). Hughes (1984) advanced the novel notion that, since the *Trichosurus* egg shell and the structurally utterly different protective layer of the monotreme egg shell are laid down in the uterus, 'it is the outer matrix layer of the monotreme egg shell (not the inner component as reported in the earlier literature) which is homologous with the shell membrane of *Trichosurus* and presumably other marsupials'. The sets of gland cells producing the keratinous shells, however, must be considered to be the homologues; where they carry out their functions in the reproductive tract is immaterial. Even if the *Trichosurus* egg shell is exclusively laid down in the uterus, it is not inconceivable that during the course of evolution of viviparity from the oviparity of ancient marsupials (see below) that uterine cells responsible for the elaboration of protective layers became vestigial and finally disappeared and that those responsible for formation of the basal layer in the oviduct migrated to the uterus in the ancestors of *Trichosurus*.

Following its arrival in the uterus, the marsupial egg undergoes holoblastic cleavage and extrusion of yolk from the blastomeres into intercellular spaces occurs. Hughes & Carrick (1978) denied that the substance is yolk, but whatever its nature, its removal from the blastomeres leads to a greatly reduced volume of cytoplasm within the shell. Increase in the number of blastomeres by mitosis is followed by their migration outwards to the shell where they flatten themselves against it. This eventually leads to the formation of a hollow unilaminar blastocyst surrounded by the shell and containing aggregates of 'yolk', as in the monotremes. A bilaminar blastocyst is formed by processes practically identical to those in the monotreme egg.

This bilaminar blastocyst, surrounded by its keratinous egg shell, absorbs uterine secretions, grows (in the Tammar Wallaby, *Macropus eugenii*, the egg shell diameter of some 15 mm equals that of the *Tachyglossus* egg; Renfree 1982) and develops a primitive streak as in the monotremes. The shell persists intact for 10 days of the 13 day gestation period in *Didelphis* and in *Macropus eugenii* the shell starts to break down at the 17th day of a 27 day gestation period; hence Luckett's (1976) conclusion that the marsupials are virtually ovoviviparous. After this 'hatching' in the uterus, a yolk sac placenta takes over the function of uptake of nutrients. At this stage, the embryo has about 20 pairs of somites so that the greater part of organogenesis takes place in the space of 10-11 days as happens during the 10-11 days of incubation of the egg in *Tachyglossus*. A vestige of an egg tooth appears during the ontogeny of the Brush Tail Possum and the Koala, *Phascolarctos cinereus*. The new-born pouch young of marsupials also exhibit caruncles identical to those of monotremes, as

can be seen in Fig. 15.18. Comparison of these two neonates shows how remarkably alike the newly-hatched monotreme and the new-born marsupial are. Both have relatively enormous forelimbs, rudimentary hind limbs and anatomically well-developed olfactory organs. The neonate and the hatchling are similar in many other ways (Griffiths 1968): the weight of an *Echidna* at hatching is 378 mg and the weights of new-born of various wallabies range from 320–400 mg; chondrocrania are at similar stages of development; kidneys exhibit *mesonephroi*; lungs are non-alveolar, exhibiting brochioles functioning as respiratory chambers; and organs of hearing are rudimentary, the ossicles being still joined onto the posterior end of Meckel's cartilage (the membranous labyrinths, however, are well developed and are probably functional).

From the foregoing, the suggestion is that ancient marsupials were oviparous, that their embryos were encased in eggshells that had to be torn open by the action of egg tooth and caruncle and that what emerged from the egg resembled the hatchlings of extant monotremes. Perhaps the fact that marsupial blastocysts (like those of the Tammar Wallaby, *Macropus eugenii*) take about 17 days to produce an embryo of about 20 somites 'is an echo of times when marsupials had to store enough uterine fluid in their large blastocysts to tide them over an incubation period outside the uterus' (Griffiths 1978).

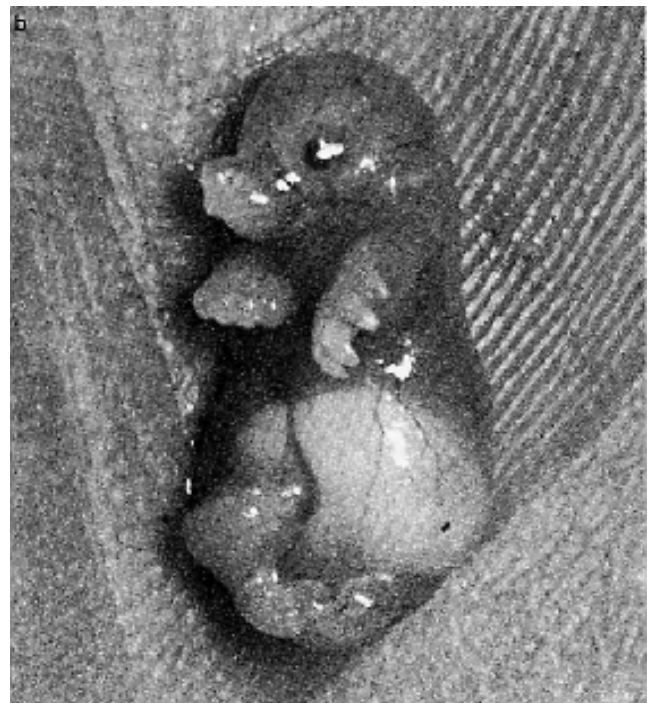
### Lactation

As pointed out above, the anatomy and physiological and reproductive processes of monotremes exhibit an intriguing melange of reptilian and mammalian characters. Monotreme lactation, on the other hand, is as sophisticated and highly evolved as that of any eutherian.

**A**



**B**



**Figure 15.18** **A**, Young *Echidna* shortly after hatching showing relatively enormous development of the forelimbs and resemblance to a marsupial neonatus. **B**, Remnants of foetal membranes are still adherent to the abdomen of the *Echidna*. Milk has been ingested and is visible through (A, from Griffiths *et al.* 1969a; with permission of the Zoological Society of London; B, from Griffiths 1978 with permission of Academic Press)

At its emergence from the egg, the hatchling of about 1.47 mm crown-rump measurement and 378 mg in weight (Griffiths 1968) makes its way to one of the milk areolae, situated at the dorso-lateral surface of the pouch, where it clings to a sparse pelage of hairs at the surface of the areola (Fig. 15.18). The means of orientation to the areola is not certainly known, but since the olfactory epithelium lining the cavum nasum is well developed and exhibits differentiated olfactory sense cells (Griffiths, McIntosh & Coles 1969; Griffiths 1978); olfaction probably plays a part.

Within a matter of hours of hatching the young imbibes milk, which can be seen in the stomach through the transparent body wall (Fig. 15.18A). The mammary glands supplying the milk consist of 100 to 150 club-shaped lobules, bound together by connective tissue, which increase in size during pregnancy and incubation. Each lobule is expanded distally and since they all converge to the relatively small areola, the whole gland is fan or half-moon shaped and measures about 50 x 25 mm. Increase in size of the gland contributes to the swollen tumescent appearance of the lips of the pouch (Fig. 15.15). Internally, the lobules consist of a series of blindly ending thick-walled convoluted tubules lined by a secretory epithelium. The lumina of the tubules, filled with milk, communicate with those of ducts which lead to the exterior at the milk patch. The histology of the milk patch resembles that of the human nipple (Griffiths 1978). Even at the time of hatching some of the tubules exhibit blindly ending sac-like outgrowths, the beginning of alveoli.

By the time the pouch young reach a weight of 90 g and a snout-rump length of 100 mm, the mammary glands have increased to a size of 60–80 x 30 mm and comprise innumerable thin-walled alveoli, as do the fully lactating mammary glands in Metatheria and Eutheria.

The secretory cells lining the alveoli exhibit microvilli at their luminal surface and their bases abut onto myoepithelial cells and a limiting membrane. The cell nuclei are large and rounded, the endoplasmic reticulum is well developed and the Golgi apparatus is particularly so. Casein granules (the characteristic protein of milk) are present in the Golgi complex and in the lumen of the alveolus. Fat droplets are present in the apical cytoplasm of the cell and can be observed ballooning out of the apical cell membrane and passing into the lumen (Fig. 15.19). All these structures and formative processes of milk constituents are to be found in the milk secretory cells of all mammals (Griffiths *et al.* 1973). The myoepithelial cells mentioned above are of particular interest, since they also occur in the mammary glands of all other mammals. These cells are filamentous and form a network over the surface of the alveoli; their cytoplasm contains muscle filaments which contract under the influence of oxytocin. The contraction deforms the alveoli, leading to increase in intra-alveolar pressure and to expulsion of the milk into the ducts and thence to the exterior. Oxytocin or, in marsupials, the related hormone mesotocin, is reflexly secreted in response to the stimulus of sucking. Oxytocin injected into lactating monotremes elicits this response.

As in Eutheria and Metatheria, imbibition of milk by *Tachyglossus* is by sucking (CSIRO 1974; Griffiths 1965b, 1978). At least this is the case for large pouch young; how hatchling *Tachyglossus* imbibe milk is not established, although the structure of their tongues is known to be different from that of the adult. The latter condition has been observed in a suckling of estimated age 68 days. Mechanisms of sucking in hatchling and advanced suckling are thus quite different (Griffiths *et al.* 1969; Griffiths 1978). In the advanced pouch young, the sucking action is vigorous and involves movements of the body which result in butting action reminiscent of the sucking movements of piglets (CSIRO 1974). The young are retained in the pouch until they start to grow spines when, understandably, the mother has to eject them. This occurs at a mean age of 53 days (range 40–63; Griffiths personal observation). She does this in a burrow





**Figure 15.19** Electron micrograph of the alveolar lining of the fully lactating mammary gland of the Short-beaked Echidna. Bl = basal lamina; blv. = blood vessels; c = casein granule; E = endoplasmic reticulum; F = fat globule; g = Golgi apparatus; m = mitochondria; Me = myoepithelium. Magnification x 8750. (From Griffiths *et al.* 1973; with permission of the Zoological Society of London) [Preparation by Dr G.I. Schoefl]

(Semon 1894a) where she continues to suckle them. The suckling process following ejection of the young has been observed in the laboratory (CSIRO 1974; Griffiths 1965b, 1978): the female approaches the young, which is usually asleep, and wakes it by nudging it under her body with snout and a forelimb until it lies under her belly. The young rolls over onto its back, hangs on to the belly hair with its powerful forelimbs and thrusts its snout into the pouch. Milk secretion follows and the young sucks its milk from one of the areolae. The snout, like that of the adult, is flattened on the undersurface where the V-shaped mouth is situated. This flattened surface is well adapted for sucking milk from the flattened but protuberant nipple-like areola. The sucking process and milk secretion are strikingly efficient: intakes of milk equal to 10–20% of the body weight in a matter of 30–60 minutes are common (Griffiths 1965b; Green *et al.* 1985); the record absolute intake was 97 g in 24 hours by a suckling initially weighing 681 g. The daily weight variations of the young indicate that they sometimes do not take milk for 3–4 days at a time.

After ejection from the pouch, the young continues to be suckled in the burrow for a matter of months (Griffiths 1978). Green *et al.* (1985) recorded a burrow young 50 days old weighing 206 g, and the same individual at 104 days weighing 566g. Although at the latter stage it had a well-developed pelage and spines, it could not walk in a coordinated manner and was manifestly incapable of looking after itself. As its faeces contained no insect remains, and since the mother was still in full lactation, it was deemed to be subsisting entirely on milk.

Duration of parental care in the wild is about 200 days (see below), but a young Short-beaked Echidna raised by its mother in the laboratory was still being suckled at 6 months of age (Griffiths 1978), finally weaned on to termites at 7 months and capable of independent foraging at about 9 months. After weaning, the mammary glands shrink and regress from the alveolar state to tightly-coiled solid duct tissue, lacking lumina and embedded in a pad of fat (Griffiths *et al.* 1969a). Similar events take place in the mammary glands of the

fox, *Vulpes vulpes*, and in those of the Platypus. The solid duct system persists through the winter months but in all the above species, growth and differentiation occurs towards the end of July, resulting in glands with lobules filled with loosely packed ducts which then exhibit lumina. If pregnancy occurs in *Tachyglossus*, the glands attain the condition at hatching described above.

Marston (1926) first showed that the milk was like that of other mammals in that it contained casein, lactose and triglyceride. Since that time the constituents of Echidna milk have been precisely defined by the results of numerous studies on milk obtained from females injected with oxytocin. The milk at hatching has been obtained only once (Griffiths *et al.* 1969a). Compared with mature milk (see below) this is dilute, containing about 12% solids, 1.25% fat, 7.85% protein and, by subtraction, 2.85% carbohydrate and minerals. Of the latter, only the iron content (8.3 µg/ml) was determined. The ratio of oleic acid to palmitic acid is about unity (32.1 : 28.9). Mature milk of *Tachyglossus*, on the other hand, is very concentrated, containing 48.9% solids (Griffiths *et al.* 1984). Of these, fat accounts for 31.0%, protein 12.4%, hexose 1.6%, sialic acid 0.7% and minerals 0.5%. The latter includes an iron content of 33 µg/ml (range 12.6–47.8) which is very high relative to that found for the milk of Eutheria.

Like that of other mammals, the milk fat consists of small amounts of free fatty acids, cholesterol esters, phospholipids, but some 95% triglycerides. (Griffiths 1965b). The major fatty acids in the triglycerides of mature milk of wild echidnas are oleic (61%), palmitic (16%), palmitoleic (6%), linoleic (5%) and stearic (4%) acids. Unlike the milk at hatching, the ratio of oleic acid to palmitic acid is therefore 3.8 : 1. The mature milk triglycerides contains very little in the way of polyunsaturated fatty acids, unlike the triglyceride of Platypus milk, which contains large amounts of those acids (Griffiths *et al.* 1973; Griffiths *et al.* 1984). It is noteworthy that the anatomically very similar young of echidnas and Platypuses are raised on such dissimilar milks.

In *Tachyglossus*, the milk, unlike that of eutherians in which lactose is the principal sugar, contains little free lactose; the principal carbohydrates are fucosyllactose and sialyllactose (Messer & Kerry 1973). The principle carbohydrate of Platypus milk, on the other hand, is difucosyllactose (Messer *et al.* 1983), accompanied by thirteen other oligosaccharides (Amano, Messer & Kobata 1985).

The nature of the synthetase system for the formation of the lactose moiety of the oligosaccharides is a matter of uncertainty (Griffiths 1978; Griffiths *et al.* 1985). In those eutherians that have been studied, the synthetase system of the mammary glands also occurs in the milk. It consists of galactosyl transferase, which is located in the fat globule membrane, and the modifier protein,  $\alpha$ -lactalbumin, located in the whey (Kuhn 1983).

The whey in *Tachyglossus* milk exhibits lactose synthetase activity, but the presence of  $\alpha$ -lactalbumin has not been demonstrated unequivocally. The notion that lysozyme, which does occur in Echidna milk and which in its amino acid sequence resembles that of  $\alpha$ -lactalbumin, acts in the capacity of the latter (Hopper & McKenzie 1974) has still to be substantiated. Lysozyme has been isolated in pure form from the whey of Echidna milk and its amino acid sequence has been determined (Teahan 1986).

The whey also contains a very large amount of the iron-binding protein  $\beta_2$  globulin or transferrin (Griffiths 1968; Jordan & Morgan 1969). This is not synthesised in the mammary gland, but is derived from the blood serum (Griffiths *et al.* 1985). Other blood serum proteins, albumin and immune  $\gamma$ -globulin are also found in the whey (Jordan & Morgan 1969), as are at least three other milk-specific proteins. The whey of mature milk in *Tachyglossus* is reddish-pink, as it is in rabbit milk (Jordan, Kaldor & Morgan 1967). The colour can be intensified by the addition of iron and is due to the presence of

the  $\beta_1$ -globulin or transferrin which, when bound to iron, takes on a red colouration (Jordan & Morgan 1969); its amino acid sequence has been determined (Teahan 1986).

The milks in the Platypus (Griffiths 1983) and in marsupials (Green 1984) are also rich in iron. This high iron content is thought to be related to the small size of the hatchlings and neonates (Griffiths 1978). In general, the milks of eutherians, except that of the rabbit, have very little iron. Unlike the tiny neonates of monotremes and marsupials, newborn eutherians are relatively large and their substantial liver contain enough iron to sustain them until they can obtain it from their definitive diets. As well as iron, levels of sodium, potassium, calcium, magnesium, zinc and copper have been determined in Echidna milk (Griffiths *et al.* 1984).

Estimates of milk intake of sucklings of captive and wild Short-beaked Echidnas show a mean body weight increment of  $0.41 \pm 0.10$  g/ml of milk consumed (Green *et al.* 1985). This rate of growth is similar to that found in fast-growing species such as the guinea pig (0.4 g/ml) and the rabbit (0.3-0.6 g/ml) but higher than those of slower growing species such as cattle (0.10 g/ml), sheep (0.13 g/ml) and elk (0.22 g/ml). There is a wide discrepancy in growth rates of young echidnas, positively correlated with the body weight of the mother, that has been found to hold good for other mammals (Green *et al.* 1985).

### Comparison of the Mammary Glands and Lactation of the Monotremata, Metatheria and Eutheria

The lobular structure of the mammary glands is identical in marsupials and monotremes (Eggeling 1905; Griffiths *et al.* 1973). Although the marsupials have teats, before eversion they closely resemble the tachyglossid areola.

At hatching and at birth, the mammary glands of the two taxa consist of thick-walled convoluted tubules which later in lactation sprout thin-walled alveoli (Griffiths *et al.* 1969a; Griffiths, McIntosh & Leckie 1972; Findlay 1982). As well as similarity in structure, the mammary glands in *Tachyglossus* and marsupials elaborate dilute milks for the hatchling and new-born (Griffiths 1978; Griffiths *et al.* 1984) which progressively become richer as lactation proceeds. The period of lactation in both taxa, during which the young live on milk alone, is in the order of months. The proximate composition of milk in *Ornithorhynchus* at hatching, however, is as yet unknown.

The mature milks of all mammals contain casein micelles, lipid in the form of globules bounded by a unit membrane and carbohydrate in the form of lactose or of oligosaccharides containing lactose. The mammary glands and lactation of prototherians are thus as sophisticated and highly evolved as those of the Metatheria and Eutheria, but have more in common with the former.

## NATURAL HISTORY

### Life History

In south-eastern Australia the breeding season commences in June (Flynn & Hill 1939). Indirect evidence, from regressions of growth curves of pouch young for Kangaroo Island echidnas, *Tachyglossus aculeatus multiaculeatus*, indicates the same pattern (Green *et al.* 1985; Griffiths 1978). Knowing the date of capture, an approximate date of hatching can be assigned to the pouch young. For seventeen such pouch young taken between 12th September and 1st November during the years 1965 to 1984, it has been estimated that dates of hatching ranged from 4th August to 3rd September. If a tentative gestation period of 20 days is accepted and 10–11 days incubation period (see below) this would indicate copulation takes

place at Kangaroo Island at times ranging from the end of June to the beginning of August. In agreement with this, Haacke (1885) found an egg in the pouch of a Kangaroo Island animal on 25th August 1894 which had been removed from the island on 3rd August. Recently, a Kangaroo Island specimen laid an egg on or about the 18th August 1985 (Griffiths personal observation).

Breeding seems to occur later on the mainland. Semon (1894a) obtained uterine eggs on 23rd July from echidnas living at the Burnett River, Queensland, but none before that date; an egg was laid by an individual caught at Orange, New South Wales as late as 22nd October (Griffiths *et al.* 1969a; Griffiths 1978).

Like Platypus (Grant, Griffiths & Leckie 1983), *Tachyglossus* does not breed every year. On Kangaroo Island, for example, of 14 adult females captured between 26th October and 3rd November 1973, only two were lactating (Griffiths unpublished data).

The Echidna is a long-lived mammal; in captivity at the Philadelphia Zoo one specimen lived for 49 years on a diet of horse meat, baby cereal, boiled eggs, peanut oil and a supplement of vitamins, aminoacids and minerals.

Information on the longevity of *Tachyglossus* in the wild is sparse. Griffiths (1968, 1978) released 67 marked individuals in three different habitats in the Australian Capital Territory. Twenty-five of these were recovered at times ranging from 3 months through 3, 5 and 6–16 years after marking. The latter was an adult male weighing 3250 g at time of marking and was probably at least 3 years old. It was released in forested mountainous country at an altitude of about 1000 metres. It would seem from this that *Tachyglossus* can live in a natural habitat for at least 20 years. Another long-lived Echidna, a male, was marked by Augee, Ealey & Price (1975), released in Flinders Chase, Kangaroo Island and recaptured 10 years later.

It has not proved possible to age echidnas, as has been done for platypuses (Grant this Volume), but an educated guess may be made about onset of sexual maturity in males. Two males, the ages of which as pouch young were reasonably accurately estimated, were both completely weaned and capable of foraging in the wild at 7–7.5 months of age, in March (Griffiths unpublished observation). At this age, their weights were 1700 and 1600 g, respectively. At Kangaroo Island small males weighing as little as 2250 g in early August were found to be sexually mature (Djakiew & Jones 1983). These could have been 2–3 years of age.

### Ecology and Behaviour

No systematic, comprehensive study of the ecology of the Short-beaked Echidna has been conducted, but opportune observations and planned studies of different aspects of their ecological behaviour have been made. Possibly the first ecological observation on the species was published by Shaw (1792) when he wrote that a specimen had ‘been found in the midst of an anthill for which reason it was named by its first discoverers the ant-eating porcupine’. Griffiths & Simpson (1966) reported extensive Echidna burrowings in the mounds of the meat ant, *Iridomyrmex detectus*, in the Mt Tidbinbilla region of the Australian Capital Territory. The burrowing process was different from the vertical subsidence usually shown by echidnas and involved delving with head and shoulders directed down into the mound. The damage to the mound was of three types: small round conical holes 25–80 mm deep made by a thrust of the snout; large ‘Echidna-sized’ holes characterised by a broad working face, conical in the lower part and ending in a snout hole; deep, well-formed burrows with a flat floor and arched roof (Fig. 15.20). The two latter types of excavation penetrated to the brood chamber and the greatest depth observed was about 1 metre. Occurrence of attacks on these mounds was strictly seasonal, lasting from late July into October. The attacks ceased when the winged virgin female ants and



the males leave the mounds for the nuptial flight. Lipid accounts for 47% of the dry weight in the female ants, which are gigantic compared with the males and workers. Griffiths & Simpson (1966) concluded that echidnas attack meat ant mounds only in the springtime to ingest the lipid-rich virgin queens found only at that time.



**Figure 15.20** Burrow passing deep into a meat-ant mound, made by a foraging Echidna. (From Griffiths & Simpson 1966; with permission of CSIRO Editorial and Publications Service)

The diet of the Short-beaked Echidna consists largely of ants and termites, but occasionally items like earthworms, scarab larvae and moth larvae (*Xyleutes*) are eaten. Tachyglossids cannot digest the chitinous exoskeleton of their prey so these pass out in the faeces in a recognisable form (Griffiths 1978) which persists even in old scats. The organs identified in these studies of ingesta are the heads of ants, jaws of worker and soldier termites and nasuses of soldier termites. In general, as judged by frequency of occurrence in scats, termites are preferred in arid areas. In temperate south-eastern Australia, ants are apparently preferred, but considerable amounts of termites are eaten at times. In Tasmania, echidnas are myrmecophagous. This cannot be ascribed to preference, but rather to the occurrence of few species of termite, none of which are mound builders. Only species living in rotten wood and forest litter are available.

Why termites are preferred in arid areas where ants are abundant is not known, but may be due to the fact that termites contain more water (up to 80%) than ants (64%).

In south-eastern Australia echidnas exhibit a seasonal food preference. They attack the mounds of an ant, *Iridomyrmex detectus*, during August, September and October of each year and ingest worker, male and female ants (Griffiths & Simpson 1966).

The Echidna moves about anytime of the day or night (Griffiths 1968, 1978); Augée *et al.* (1975) showed that activity periods of the Short-beaked Echidna at Flinders Chase, Kangaroo Island, correlate with ambient temperature: on days when air temperatures reach 40°C, activity periods shift into the evening. During winter, when air temperature seldom rises above 18°C, the periods of activity are random compared with those of other seasons, although activity during early morning is rare. One adult was observed active during a very cold winter night. During rainy periods throughout the year the animals are inactive

and shelter under thick tussocks of the native iris, *Orthroanthus multiflorus*, or in burrows underneath the mounds of the termite *Nasutitermes exitiosus*. Such a burrow has been recorded as a nursery refuge (see above). In general, however, *Tachyglossus* shelters in shallow depressions in leaf litter at the bases of shrubs and trees.

Animals at Flinders Chase have a definite home range, the diameter of which is of the order of 800 m, but exhibit no territoriality (Augee *et al.* 1975). Over periods ranging from 3–25 months, the maximum distance between sightings of marked individuals in the above study was 347 m. Griffiths (1968, 1978) found similar patterns: eight marked animals were recaptured at intervals ranging from 1–3 years at distances ranging from practically nil to 800 m from the place of release; one animal marked 16 years previously was recaptured about 100 m from the place of release. There were exceptions to this apparently sedentary behaviour: one animal moved 5.5 km in 7 days; another, 18 km in about 5 months and yet another was located 9.6 km away from where it was released 5.5 years previously.

Recently, the diameter of home ranges of lactating echidnas has been found in the Mt Tidbinbilla area to be about 800 metres. One animal was nursing a 1.3 kg burrow-young of estimated age about 200 days. The intervals between sucklings were 5–10 days and between sucklings she travelled minimal distances (determined by radio-tracking) of 2.14–2.25 km (M. Griffiths, F. Kristo, B. Green, A. Fogerty, K. Newgrain unpublished data).

It was pointed out above that echidnas lack mechanisms to cope with high ambient temperatures and that to survive in habitats with these conditions, it must avoid such extremes. Davies (cited in Griffiths 1968) found evidence of continuous use of breakaway caves by echidnas in the Mileura district of Western Australia, on the Murchison River, which has an annual rainfall of 230 mm and air temperatures that range 0–44 °C in the shade. The cave environment was greatly buffered compared with the changes in temperature and relative humidity observed outside (Table 15.2). No diurnal temperature variation was detectable, but a small diurnal variation in relative humidity of 6% was noted. The animals emerge from the caves to feed; and following rain, when it is cool, their diggings are particularly conspicuous. Davies also remarks ‘The Echidna appears to have evolved a very satisfactory way of life in the Murchison. It has come to terms with the extremes of climate by avoiding them’. Other aspects of behaviour have already been discussed above.

*Tachyglossus* carries various ecto- and endoparasites. The former consist of fleas, mites and ticks (Griffiths 1968). The latter include trichostrongylid nematodes (Durette-Desset & Cassone 1983), a filarial nematode (Mackerras 1962), cestodes (Beveridge 1980a, 1983) and a piroplasmid sporozoan (Backhouse & Bolliger 1959). The role of these parasites and of others in bringing about disease and death in wild *Tachyglossus* is under investigation by Dr S. McOrist of the Department of Agriculture, Bairnsdale, Victoria, who has kindly allowed me to present data from this study (Table 15.3); McOrist & Smailes (1986) and Whittington (1988).

Most of the data are from clinical and pathological examinations of specimens presented to Victorian veterinary laboratories over the past 10 years. Toxoplasmosis, a multi-systemic protozoal disease brought about by ingestion of oocysts passed in cats' faeces, has been identified in a small number of *Tachyglossus*.

Light burdens of less than 100 individuals of the trichostrongyloid nematode genera *Nicollina* species, *Tasmanema* species and *Tachynema* species are frequently detected in animals killed by motor vehicles (see below). Heavy burdens of 500–1000 nematodes caused a pathologically significant enteritis in

**Table 15.2** Temperature and relative humidity in caves inhabited by echidnas at Mileua, W.A. and temperature and relative humidity of the open air. Data of Dr S. Davis; \*Cyclone.

15th DAY OF MONTH	CAVE TEMPERATURE (noon) C	EXTERNAL TEMPERATURE		RELATIVE HUMIDITY	
		Max.	(Min)	Cave	(External)
May	23	28	(9)	50	(28)
June	19	17	(0)	64	(50)
July	14	18	(6)	66	(50)
August	13	22	(8)	71	(20)
September	18	21	(11)	68	(42)
October	23	28	(17)	67	(42)
November	26	30	(16)	55	(36)
December	29	43	(27)	56	(25)
January	33	44	(31)	58	(35)
February	29	24*	(18)*	72*	(60)*
March	31	38	(27)	67	(32)
April	27	38	(21)	60	(46)
May	25	26	(16)	57	(44)

8% of the individuals examined. As well as this type of enteritis, an acute abscessating form was found in 4% of the individuals examined; this was probably bacterial in origin.

Acute suppurative bronchopneumonia brought about fatal lesions in 6% of specimens examined. These consist of firm consolidation of pulmonary tissue with pus in the airways attributed to the activities of a mixture of aerobic and anaerobic pyogenic bacteria. The formation of firm, fibrous granulomata forming large swellings was found in the forefeet of two echidnas. These granulomas are due to coccal bacterial colonies surrounded by fibrous inflammation. Having entered the body, bacteria may encounter a lowered immunity in some instances due to low body temperature, since effective phagocytosis of bacteria is greatly reduced at low temperatures. Other causes of death were non-specific septicaemia and granulomatous hepatitis lesions.

Infestations with the intestinal cestode *Linstowia echidnae* were regularly observed and were non-pathogenic. An apparently healthy Echidna weighing 3850 g was shown to be carrying a closely packed infestation of 546 *Linstowia echidnae* that resided throughout the gut from pylorus to cloaca (D. Spratt personal communication).

Infestations with the tick *Aponomma concolor* on the skin and in the ear holes are common and individuals infested with loads greater than 20 ticks can show anaemia. Such tick loads occur on *Tachyglossus aculeatus multiaculeatus* at Kangaroo Island; 71 engorged ticks have been found on a specimen weighing only 1400 g (M. Griffiths personal observation).

By far the leading cause of death found was injury by motor vehicles and the percentage attributed to this and given in Table 15.3 may be an underestimate.

**Table 15.3** Causes of disease and death of free-living echidnas. Data of Dr S. McCorist (pers.comm.).

FINDING	NUMBER	PERCENTAGE
Dog, fox wounds	11	22
Wire snare wound	1	2
Trichostronglylosis	4	7
Enteritis — non specific	3	6
Purulent bronchopneumonia	3	6
Toxoplasmosis	2	4
Motor vehicle injury	20	39
Septicaemia — non specific	3	6
Hepatitis — non specific	2	4
Bacterial granulomata	2	4
Total	51	100

Faecal examination of foxes and dingoes shows that these carnivores ingest *Echidna* flesh, the spines passing through undigested; curiously, the blunt ends of the spines emerge first (Griffiths unpublished observation). It seems unlikely that foxes could kill and eat echidnas, more likely they scavenge carcasses. Dingoes, however, could kill echidnas and examination of the stomach contents of foxes shows that they can kill and eat the burrow young. Predation on burrow young by feral cats occurs at Kangaroo Island and the goanna *Varanus rosenbergi* has been observed there with a naked *Echidna* young in its mouth. Apparent predation by Goannas on a burrow young has been recorded by Overton (1987).

## BIOGEOGRAPHY AND PHYLOGENY

### Distribution and Affinities within the Tachyglossidae

Two monotypic genera comprise the *Tachyglossidae*. *Zaglossus bruijnii* lives in the central cordillera of New Guinea. It differs from the Australian *Echidna* by its much greater size (up to 1 metre long and 16 kg body weight) and a very long, relatively gracile snout. It has a tongue that is grooved on the dorsal anterior third and furnished with three rows of backwardly directed, very sharp, keratinous spines. These are used for hooking and ingesting its prey, which is mainly earthworms. An *Echidna*-like *Z. bruijnii* once occurred in Australia, but became extinct in the late Pleistocene (Murray 1978). *Tachyglossus aculeatus* also occurs in New Guinea, both in coastal areas and the highlands (Van Deusen & George 1969).

The nominate subspecies of *Tachyglossus aculeatus* is found in south-eastern New South Wales and Victoria. *T. a. multiaculeatus*, a very distinctive *Echidna* (Fig. 15.1), occurs only on Kangaroo Island. *T. a. setosus* is confined to Tasmania and some Bass Strait Islands; *T. a. acanthion* is found in the arid and northern tropical regions of the mainland and is practically hairless, the pelage consisting of spines and bristles (Fig. 15.2a). *T. a. lawesii* was the name proposed for the New Guinea sub-species by Ramsay (1877); Griffiths (1978) is of the opinion that *T. a. lawesii* occurs in the rainforests of north-eastern Queensland.



## COLLECTION AND PRESERVATION

### Collection

Collecting *Tachyglossus* is a matter of opportunistic spotting of the animals while walking through open forest, spinifex-iron bark associations in arid zone, rock outcrops, snowfields or other suitable habitat, particular attention being paid to areas where characteristic fresh diggings occur. This is best done in the afternoon and evening during late spring, summer and early autumn when the animals are moving about and feeding. In winter they move about anytime of the day or night. When located, the animal is transferred to a strong hessian bag secured with strong cord.

### Preservation

Fixation in Bouin's fluid gives good results with mammary gland, testis, ovary, tongue and adrenal tissues for light microscopy. For electron microscopy, perfusion with glutaraldehyde in the anaesthetised live animal with beating heart is necessary; suitable anaesthesia can be obtained by intraperitoneal injection of 20 ml of a 2.5% solution of Avertin (Bayer). The perfusion of glutaraldehyde, preceded by perfusion with 0.9% sodium chloride, can be carried out by gravity feed via a needle into the left ventricle. If unpressurised aircraft are used for transport of the fixed tissues they must be completely sealed off from the atmosphere, otherwise nitrogen in the tissues and fixative will come out of solution and form bubbles under the reduced atmospheric pressure, ruining the preparation.

### Maintenance in Captivity

Echidnas are easily maintained, preferably at a temperature of 21–22°C. Strong, smooth-sided metal bins 450 x 450 mm square and 600 mm deep make suitable cages. Food should be placed outside the bin. An access hole 50 x 50 mm square is located centrally 100 mm above the floor of the bin. Adult non-lactating animals may be kept on a substratum of earth, hay or sawdust but on no account should these be used for housing females carrying pouch young, since this material inevitably contaminates the pouch. If this occurs two undesirable things may happen: the female will use her grooming toenail to remove the offending matter, at the same time squashing or ejecting the young or egg; or, hard particles in the pouch will abrade the delicate snout of the young and it may cease to suckle. For such animals, bran makes the best substratum. It is soft, absorbent and warm, does not cause pouch irritation and may be removed without abrasion when the pouch is cleaned before milking. Bran also provides a safe and warm substratum for the young when it is finally ejected from the pouch. When this happens, care must be taken to see that the animal house temperature is maintained at 21–22°C. At lower ambient temperatures, the young will become hypothermic and fail to digest milk properly, resulting in death from putrefaction of the gut contents. The bins should be cleaned out twice a week.

A suitable diet convenient to prepare is a custard consisting of 30 g of lactose-free milk powder (Digestelact), 200 ml water and two 50 g eggs. This is homogenised and heated in a boiling water bath with constant stirring until a smooth semi-liquid custard forms. This amount fed fresh daily will keep a large lactating Echidna about 4000 g in weight in good health for 6–8 weeks while she provides a milk suitable for growth and differentiation of the young.

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