J. Anat. (1987), **154**, pp. 173–186
With 17 *figures*
Printed in Great Britain

Seasonal changes in the reproductive tract of the male marsupial bandicoot, *Isoodon macrourus*

R. TODHUNTER AND R. T. GEMMELL

Department of Anatomy, University of Queensland, St Lucia 4067, *Brisbane, Australia*

(*Accepted* 9 *December* 1986)

INTRODUCTION

Bandicoots in the wild show evidence of annual cycles in their reproductive activity (Heinsohn, 1966; Gordon, 1971; Stoddart & Braithwaite, 1979; Gemmell, 1982). The ease of determining the reproductive state of the female marsupial by examination of the pouch contents, has allowed for a greater understanding of the role of the female in seasonal breeding. Bandicoots in Queensland produce young throughout the year; however the majority of births occur in late winter and spring. The number of births correlates best with the rate of change of temperature, although rainfall and day-length may also have some effect on the breeding period (Barnes & Gemmell, 1984).

There is scant information concerning seasonal changes in the reproductive tract of the male bandicoot. Heinsohn (1966) reported that large quantities of sperm were present in the bandicoot epididymis throughout the year in the bandicoots *Perameles gunnii* and *Isoodon obesulus* and no seasonal variation was noted in the appearance of the testes. Similar results were obtained for *Isoodon macrourus* by Gordon (1971) and these findings in the bandicoot are in accord with results obtained from other species of marsupial. Sperm were produced throughout the entire year in *Didelphis marsupialis* and *Philander opossum* (Hartman, 1928; Biggers, 1966), the possum *Trichosurus vulpecula* (Tyndale-Biscoe, 1955; Gilmore, 1969), the tammar wallaby *Macropus eugenii* (Hearn, 1975; Setchell, 1977), Bennett's wallaby *Macropus rufogriseus* (Catt, 1977), and in the kangaroos *Macropus rufa* and *Macropus robustus* (Sadlier, 1965). Although there was a lack of seasonal variation in sperm production in the marsupials, there was a significant seasonal change in the male accessory sex glands. In the possum, the size of the prostate increased significantly in March, with the majority of births occurring in April and May (Gilmore, 1969). Woolley (1966) observed in the dasyurid *Antechinus stuartii*, that the testis, epididymis, prostate and Cowper's gland all increased in weight prior to the mating season. Similarly, the prostate weight of the tammar wallaby increased several months prior to the mating season (Catt, 1977). Inns (1982) reported that although the weight of the testis and epididymis did not vary throughout the year, the weight of the prostate and Cowper's gland did, both of these latter glands increasing prior to the mating season in the tammar wallaby. There is no comparable information available for the bandicoot. However a recent study has demonstrated that, although there was no evidence of a seasonal variation in the size of the testes, plasma testosterone concentrations fluctuated from 0·1 to 70·0 ng/ml and a seasonal cycle was observed, with a nadir in concentrations in March and a peak in September (Gemmell, Johnston & Barnes, 1985). The peak in testosterone concentration coincided with the period of the year when the majority of births occurred.

In this present study, the morphology of the reproductive tract of the male bandicoot *I. macrourus* was compared during the breeding and non-breeding periods to determine whether structural changes accompany the seasonal hormonal concentrations observed previously (Gemmell *et al.* 1985).

MATERIAL AND METHODS

All bandicoots used in this study were bred in captivity and were housed in large enclosures, 900 square metres, with adult females. Information concerning the maintenance of the colony has been published previously (Gemmell, 1982).

Tissues were obtained from nine adult *I. macrourus*. Three animals were sampled during March, two during July and three in September. One castrated male was sampled in May (Table 1). All animals were killed with an intracardiac injection of Valabarb (V.R. Laboratories, Sydney, Australia) and the reproductive tracts were fixed immediately by perfusion with a solution of paraformaldehyde and glutaraldehyde in cacodylate buffer at pH 7·2 (Gemmell, Stacy & Thorburn, 1974). The weights and dimensions of the testes, epididymides and prostate were recorded for each animal.

Tissues were subsequently processed for examination with the light and electron microscope. Testicular and prostatic tissues were dehydrated in ethanol, embedded in celloidin and paraffin and 7 μm sections cut. These sections were stained with either Mayer's haemalum and eosin (H & E), periodic acid–Schiff (PAS), tetra-azotised dianisidine (TAD) or ninhydrin–Schiff solution. The stained sections were examined with the light microscope. Tissues for ultrastructural examination were postfixed in buffered osmium tetroxide, dehydrated, and embedded in Araldite. Sections one μm thick were stained with toluidine blue and examined with the light microscope. Thinner sections, 50 nm thick, were stained sequentially with uranyl acetate and lead citrate and examined with a Zeiss 10 transmission electron microscope.

In five bandicoots, the percentage volume of testis containing Leydig cell tissue was calculated from 50 randomly selected areas across each testis section viewed with a 121 point grid lattice. The number of times Leydig cell tissue was seen under a point in the grid was expressed as a percentage of the total number of points over all areas (Williams, 1977).

RESULTS

Gross morphology of reproductive tract

The three bandicoots (Numbers 1 to 3) sampled during the 'non-breeding' period did not differ markedly in testicular size and weight from the five bandicoots (Numbers 4 to 8) sampled during the 'breeding' period (Table 1). The 'non-breeding' period refers to the months March to June when a reduction in the number of births of *I. macrourus* was observed (Gemmell *et al.* 1985). The testes and epididymides weights expressed per 100 g body weight varied from 0·3 to 0·5 in both groups of bandicoots (Table 1). This similarity was not observed in the weight and dimensions of the prostate gland (Figs. 1, 2). The ratio of prostate to body weight per 100 g was from 0·2 to 0·3 in the 'non-breeding' group whereas the ratio was from 0·4 to 1·3 in the 'breeding' group (Table 1). There was a large increase in prostate weight and size in bandicoots during the breeding season. The size and weight of the prostate obtained from the castrated bandicoot were similar to those from the three bandicoots sampled in the 'non-breeding' period.

Table 1. *Size and weight of the testis, epididymis and prostate gland of the male bandicoot Isoodon macrourus*

		Non-breeding			Breeding period					Castrate
Bandicoot number		1	2	3	4	5	6	7	8	9
Killed		28 May	28 Mar.	28 Mar.	23 July	27 July	18 Sep.	18 Sep.	18 Sep.	3 May
Body weight (g)		1618	2424	1376	2750	1616	1545	1980	1980	1620
Right testis dimensions	length × breadth (mm)	22 × 17	23 × 18	23 × 17	23 × 18	21 × 16	23 × 18	23 × 17	23 × 17	—
Prostate dimensions		32 × 24	33 × 28	25 × 19	50 × 33	37 × 34	48 × 34	37 × 31	35 × 29	27 × 20
Testes and epididymides weight (g)		7·9	6·6	5·6	8·9	6·9	8·3	7·9	8·5	—
Prostate weight (g)		4·9	7·4	2·5	16·8	11·3	19·7	12·5	7·6	2·7
Testes and epididymides weight	per 100 g body weight	0·5	0·3	0·4	0·3	0·4	0·5	0·4	0·4	
Prostate weight		0·3	0·3	0·2	0·6	0·7	1·3	0·6	0·4	0·2

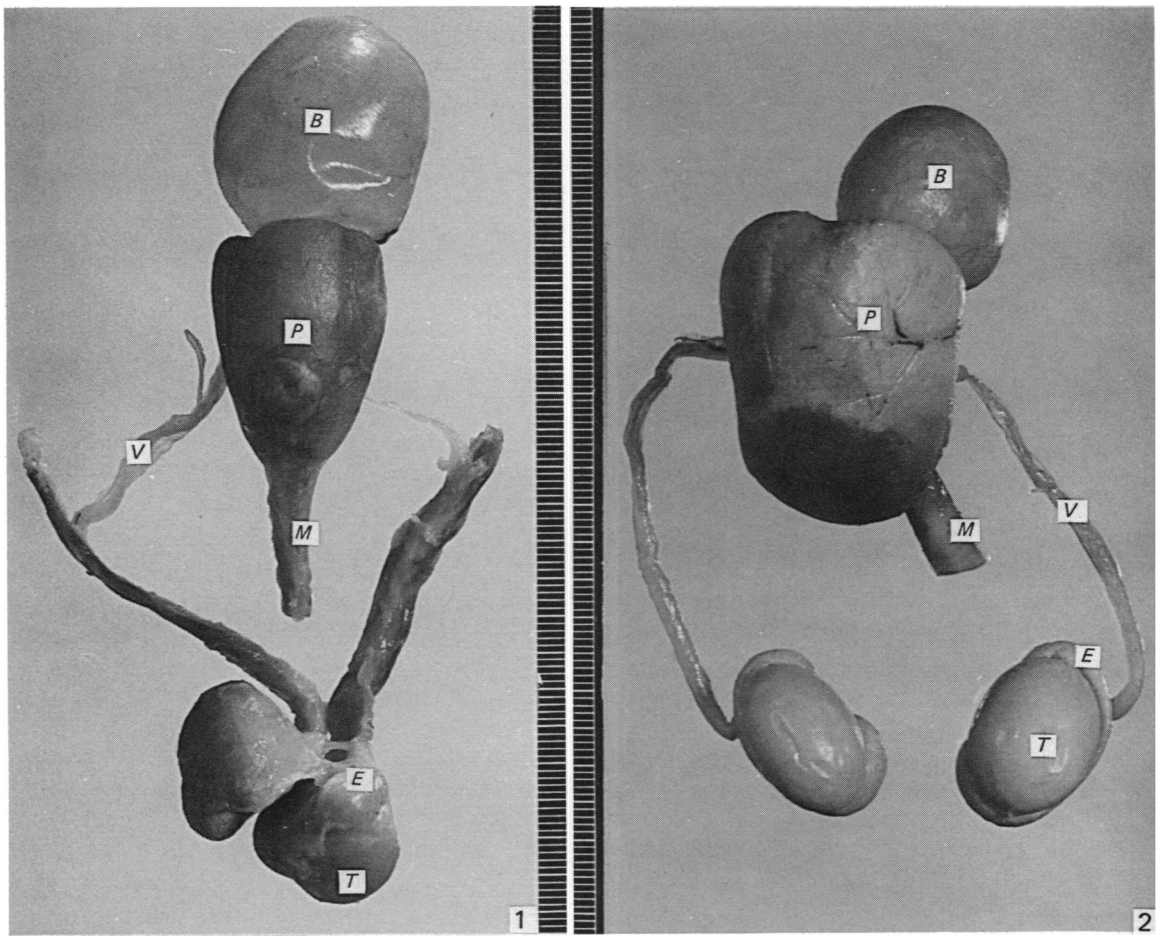


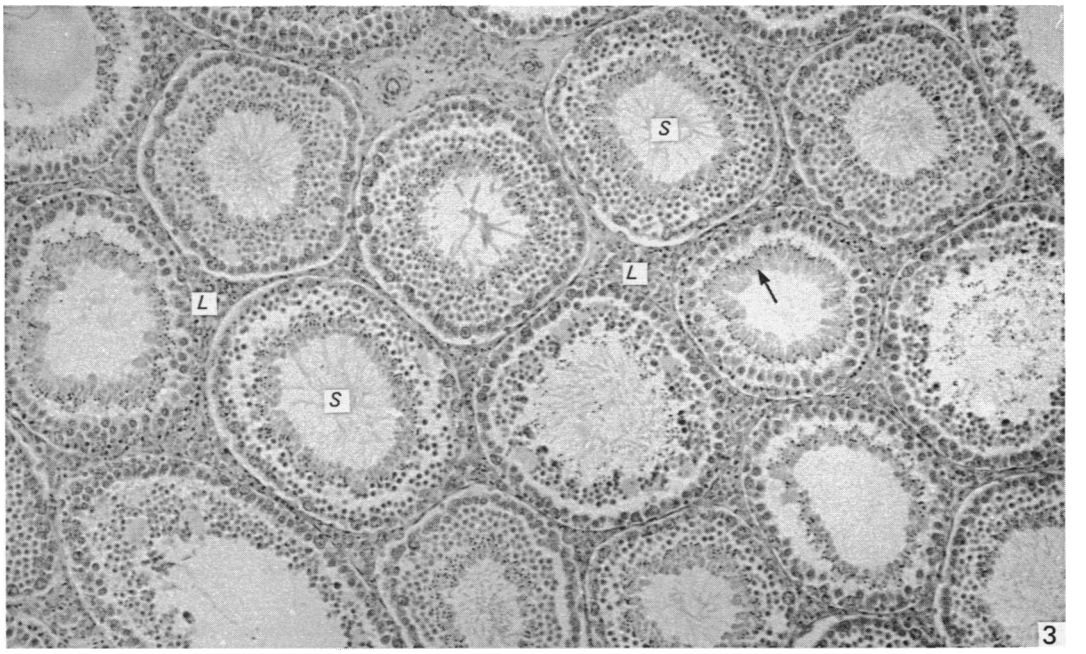
Fig. 1. Ventral view of the male reproductive system of the bandicoot during the non-breeding season.

Fig. 2. Ventral view of the male reproductive system of the bandicoot during the breeding season. *B*, bladder; *P*, prostate; *V*, vas deferens; *M*, membranous urethra; *T*, testis; *E*, epididymis. Each ruled division is 1 mm.

Sperm was present in the testis and epididymis of all eight bandicoots. The volume percentage of Leydig tissue was 8·6, 8·7 and 8·9 in bandicoots 1, 2 and 3 respectively, and 21·1 and 21·4 in bandicoots 7 and 8. Although there was no apparent difference in testicular size and weight between the two groups, the volume of Leydig tissue was greater in the 'breeding' group (Figs. 3, 4).

The bandicoot prostate was easily divisible into two morphological and functional distinct zones, as described by Rodger & Hughes (1973), namely a dorsal and a ventral prostate (Fig. 5). The majority of cells within the dorsal prostatic tubules were high columnar cells with apical spherical nuclei (Figs. 6, 7, 9, 10). The cells of the ventral prostate were low columnar or cuboidal cells with central or basal nuclei (Figs. 6, 8, 13). These two zones within the bandicoot prostate differed in their epithelial morphology and their luminal secretions.

The dorsal prostate was shown by histochemical investigation to produce a carbohydrate secretion containing the enzyme acid phosphatase and this region failed to



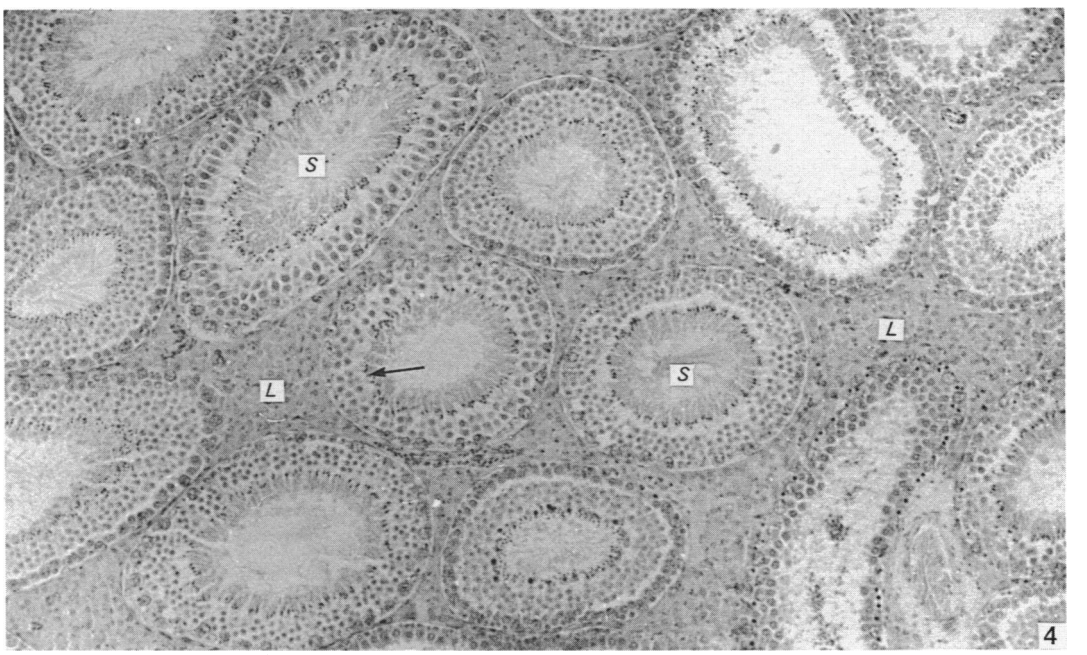


Fig. 3. The testis of bandicoot 1 obtained during the non-breeding season. Only small areas of Leydig cell tissue (*L*) were observed between the seminiferous tubules (*S*). Sperm were still seen within the tubules indicating that spermatogenesis was occurring. × 100.

Fig. 4. The testis of bandicoot 8 obtained during the breeding season. A dramatic increase in the amounts of Leydig cell tissue (*L*) between the seminiferous tubules (*S*) could be seen and sperm were again visible in the tubules. × 100.

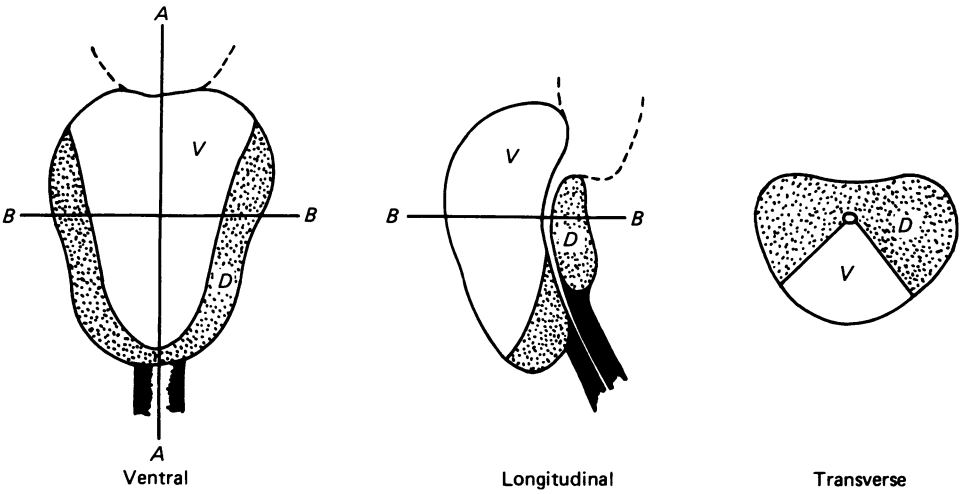
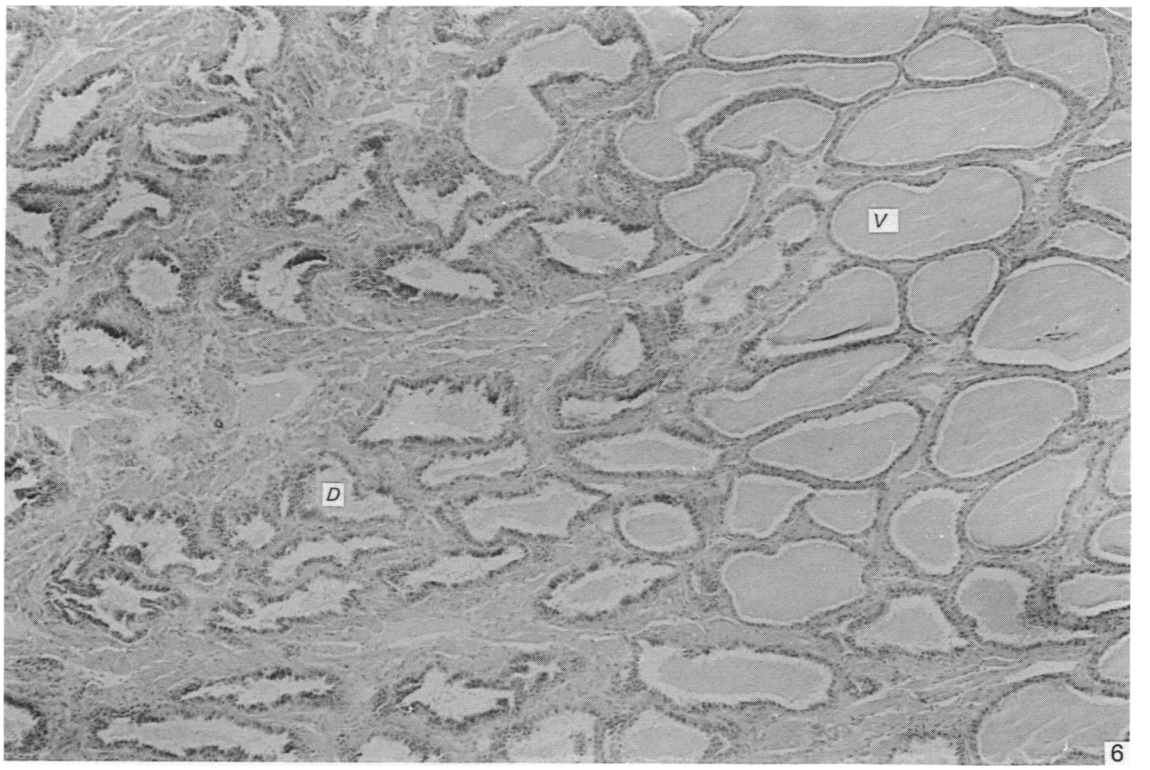
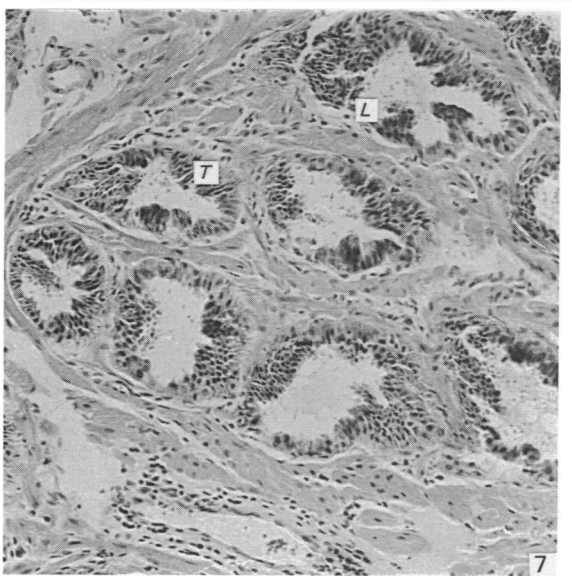


Fig. 5. A diagrammatic representation of the segmentation of the bandicoot prostate. *D*, dorsal prostate; *V*, ventral prostate.

stain with either TAD or the ninhydrin–Schiff reaction, indicating a lack of protein in the secretion. With all stains used on the luminal contents (H & E, PAS, TAD, ninhydrin–Schiff and acid phosphatase stains) 'globules' of various sizes were noted amongst a matrix of coarse granular and filamentous substance (Figs. 9, 10, 11). In the dorsal prostatic tubular epithelium two cell types could be distinguished on the basis of their morphology and their cytochemical reactivity. The two cell types have been designated as either tall or low cells, referring to tall columnar cells and cuboidal cells respectively. In the samples obtained during the breeding season the majority of cells were tall. In transverse sections through the tubules, only a few low cells were seen in each tubule (Fig. 9). In general, the tall cells stained lightly and evenly with PAS, although areas of moderately PAS-positive material could sometimes be seen in their apical cytoplasm as could areas which stained for acid phosphatase. The low cells also stained lightly with PAS and for acid phosphatase. The two protein stains (TAD and ninhydrin–Schiff reaction) both yielded negative results for the secretion from and within the cells of the dorsal prostate, although some of the globules of the secretion did stain to some extent with TAD. On ultrastructural examination many differences could be noticed between the two cell types. The low cells possessed many of the 'typical' cellular features – endoplasmic reticulum, mitochondria etc. – and they also showed interdigitations of the basal cytoplasmic membrane (Fig. 11). Tall cells, on the other hand, lacked most of the common cellular organelles; instead the cytoplasm was filled with a pale staining material, presumably secretory product (Figs. 10, 11). Small mitochondria were visible, as were aggregations of microtubules. Occasionally 'swirls' of endoplasmic reticulum were seen in the basal cytoplasm and the large apical nuclei were often deeply indented. The low cells were far more common and the tubules were dilated during the breeding season when compared to the non-breeding season. Microvilli and small electron-lucent apical vesicles were usually present as were Golgi zones and agranular endoplasmic reticulum. Tight junctions and desmosomes strongly attached adjacent cells to each other. The tall cells of the dorsal prostate were smaller in volume during the non-breeding season and the cytoplasm was devoid of secretory product (Fig. 12).

The secretion produced by the cells of the ventral prostate was, in general, much





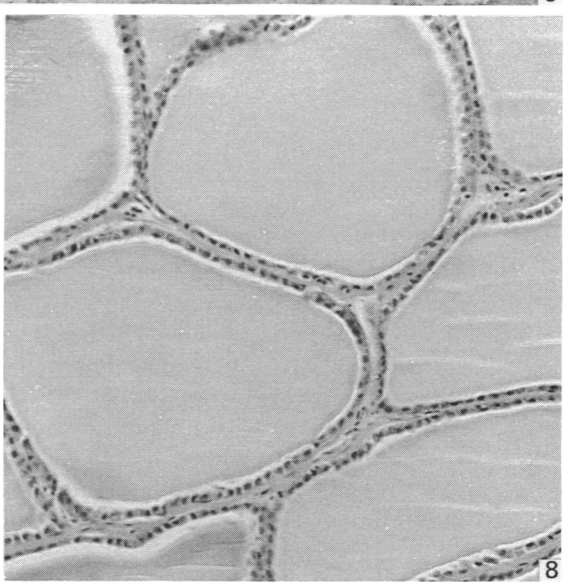
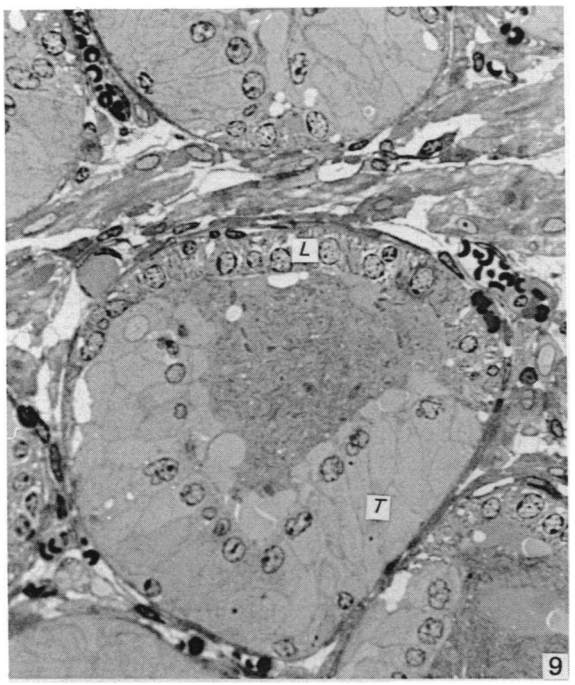


Fig. 6. The junction between the dorsal (*D*) and ventral (*V*) prostate of the bandicoot. × 60.

Fig. 7. Dorsal prostatic tubules of the bandicoot during the breeding season. Both tall cells (*T*) and low cells (*L*) could be distinguished in the epithelium. × 100.

Fig. 8. Ventral prostatic tubules of the bandicoot during the breeding season. A very homogeneous secretion was seen in the lumen of the tubules and the epithelium consisted of cuboidal cells. × 100.



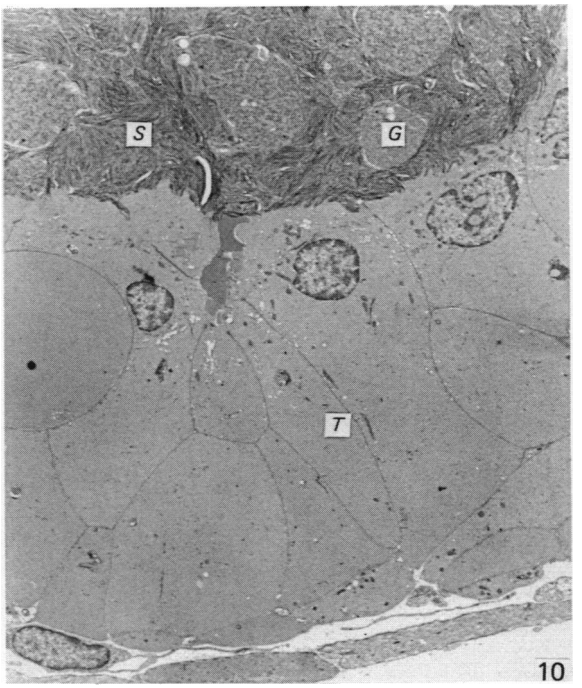
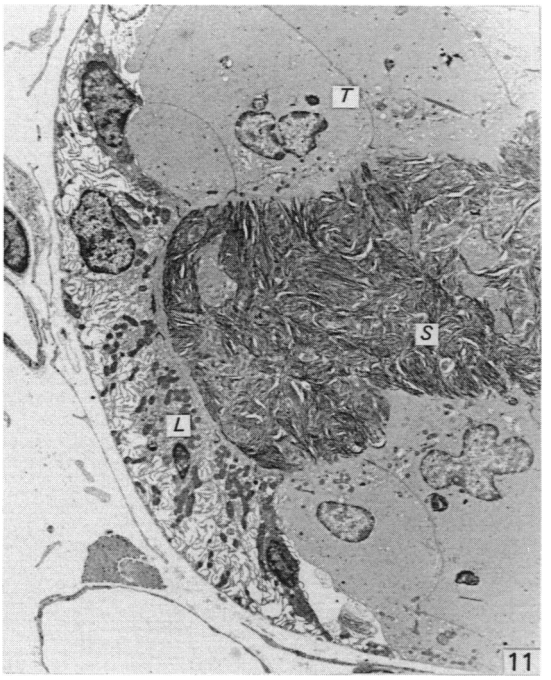


Fig. 9. Dorsal prostatic tubules of the bandicoot during the breeding season. Both tall cells (*T*) and low cells (*L*) could be seen in the epithelium. × 1500.

Fig. 10. Tall cells (*T*) of the dorsal prostate during the breeding season demonstrating an apparent lack of most cellular organelles. Several cytoplasmic globules (*G*) could be seen in the luminal secretion (*S*). × 6000.

more homogeneous than that produced from the dorsal prostate (Figs. 6, 8, 13). In the lumen of some tubules, however, more granular secretion was seen and the size of the granules varied from fine to quite coarse granules in some tubules. This granularity was most evident in the sections stained with toluidine blue (Fig. 13). The secretion from the ventral cells stained intensely with PAS, moderately with both protein stains (TAD and ninhydrin–Schiff reaction) and was negative for acid phosphatase, indicating that the ventral prostate was producing a mucoprotein secretion which lacked acid phosphatase activity. The cells of the epithelium of the ventral prostate tubules were all low columnar or cuboidal and they stained moderately and evenly with PAS and the protein stains. The secretion from the cells of the ventral prostate was isolated in secretory vacuoles or granules (Figs. 14, 15). These secretory granules were of several types. Some of the granules were electron-lucent, some had a granular component in a lucent matrix and still others showed an electron-dense core. All forms may simply be different stages of the same type of secretory granule. Occasionaly the granules were seen discharging their contents into the lumen of the tubule (Fig. 14). Granular endoplasmic reticulum was evident and a prominent Golgi apparatus was often noted and other features typical of secretory cells were seen. The cells of the tubules which showed a very granular secretion were similar in appearance to the cells of other tubules except they were taller and showed larger electron-lucent secretory granules (Fig. 15). Very few microscopic morphological differences were noted in the ventral prostate between seasons other than an obvious dilation of the tubules in the breeding



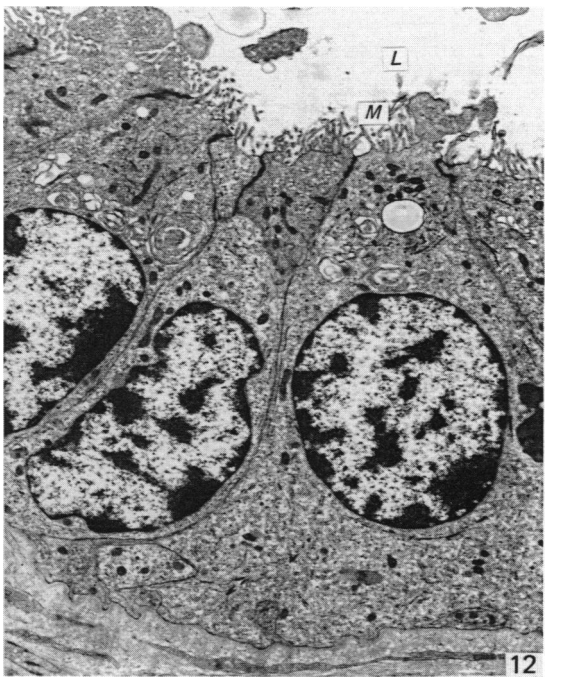


Fig. 11. Low cells (*L*) of the dorsal prostate during the breeding season. Many mitochondria and basal interdigitations were present in the low cell. The fibrillar nature of the secretion (*S*) in the lumen of the tubule and several tall cells (*T*) were also observed. ×4500.

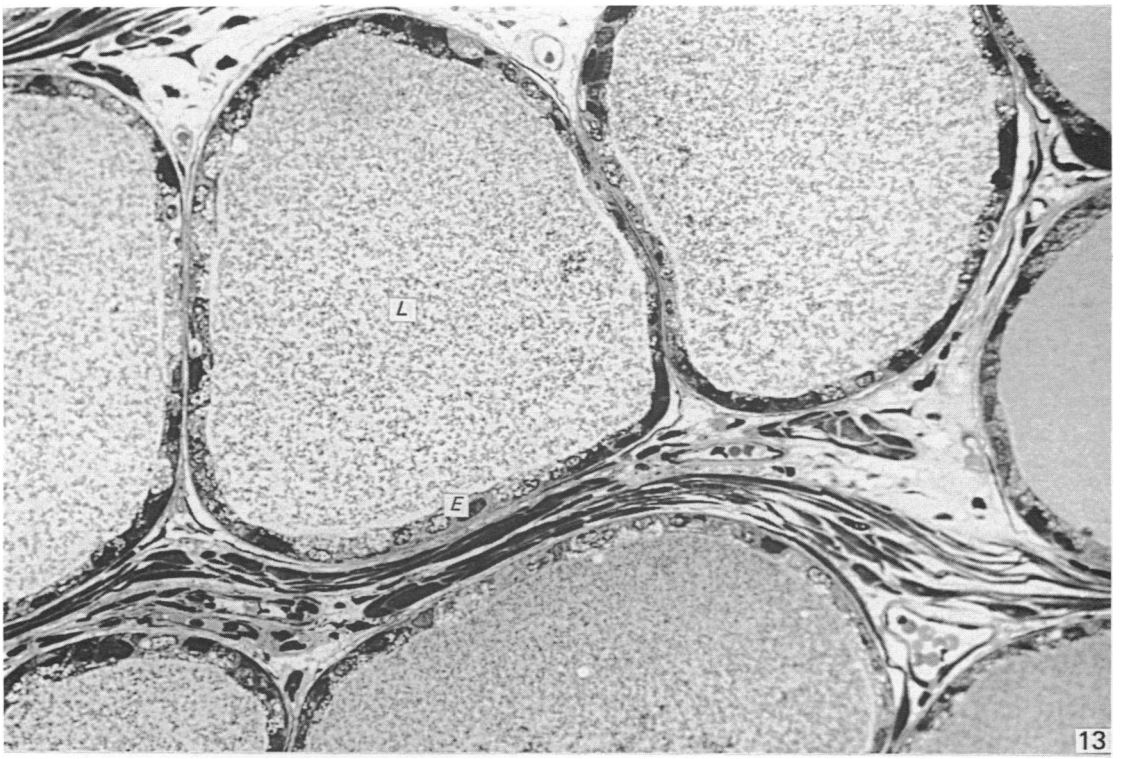
Fig. 12. Tall cells of the dorsal prostate during the non-breeding season. Most cellular organelles remained obscured by secretory product. Cytoplasm occasionally extruded into the lumen (*L*) of the tubule and microvilli (*M*) lined the apical surface of the cells. ×1100.

months. The other major difference was that the nuclei of these cells in the non-breeding season often had large, moderately electron-dense bodies within them.

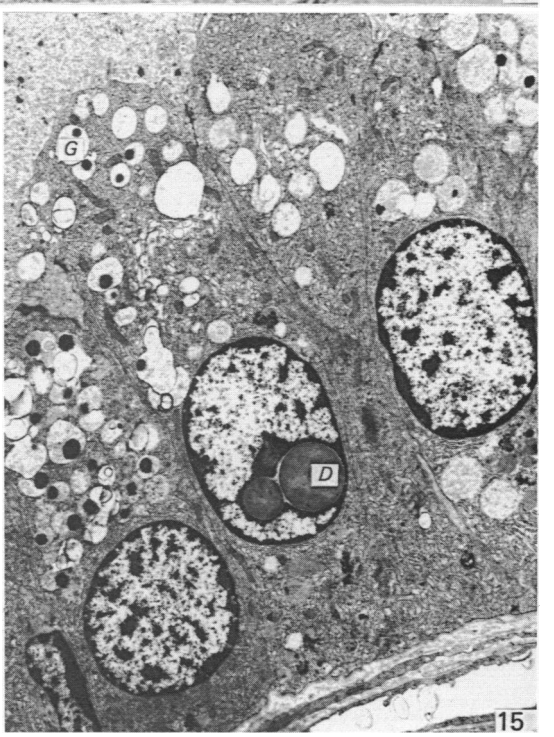
The prostate tissue of the castrated bandicoot showed similar properties to those found in the intact animals taken during the non-breeding season. Both dorsal and ventral zones were distinguished in the prostate of the castrated bandicoot in the same arrangement as described previously for the intact animals. The staining reactions of both zones were also similar except that the lumen of many tubules, particularly the dorsal tubules, were devoid of secretion. The cells of the dorsal prostate were similar to those seen in the non-breeding animals. The cells had the same morphology as the tall cells of the intact animals during the non-breeding season. Apical cytoplasm could still occasionally be seen discharging into the lumen (Fig. 16). The ventral cells of the castrated bandicoot were little different from those of the intact animals except that fewer secretory granules were seen (Fig. 17).

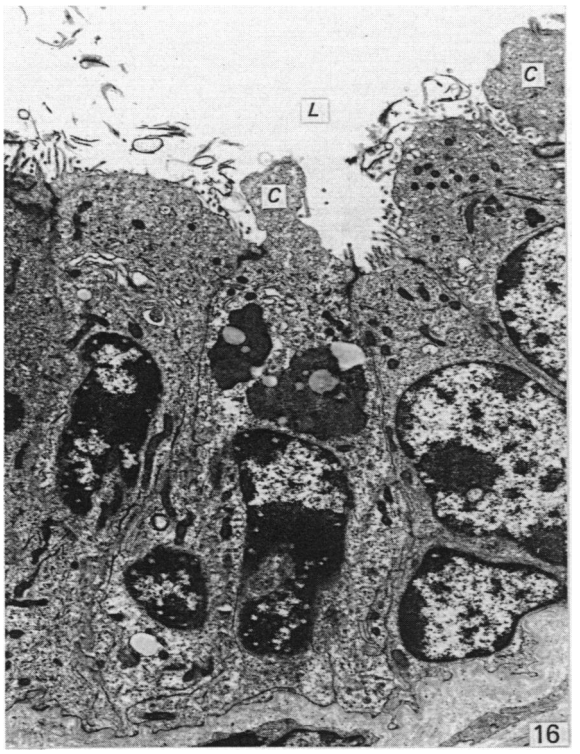
DISCUSSION

Although there is no apparent outward change occurring in the reproductive tract of the male bandicoot between the breeding and the non-breeding period, that is the body weight and scrotal size do not alter with season (Gemmell *et al.* 1985), there are changes in the internal structure of the testis and the prostate gland. The increase in Leydig cell volume correlates well with the increase in plasma testosterone con-


L
E
13


G
N
G
14


G
D
15



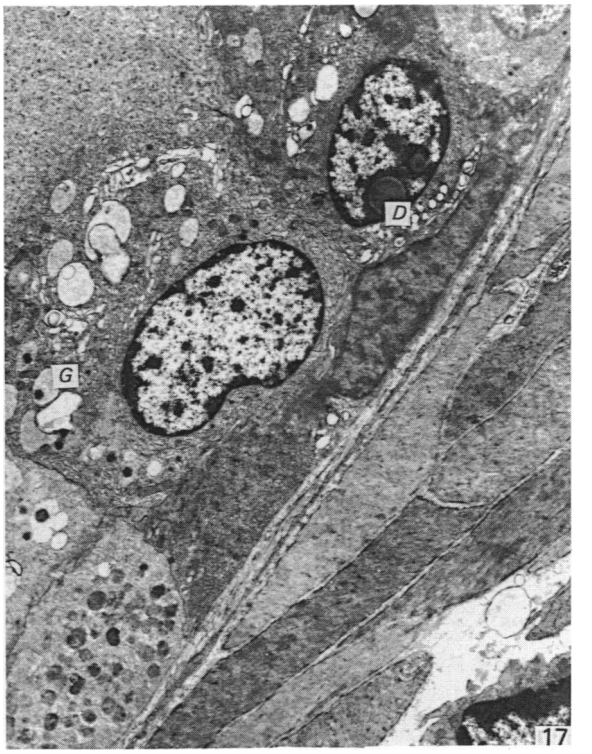


Fig. 16. The cells of the dorsal prostate of the castrated bandicoot. Some cytoplasm (*C*) is seen extruding into the lumen (*L*). ×7000.

Fig. 17. The cells of the ventral prostate of the castrated bandicoot. Fewer secretory granules (*G*) are seen than in the same cells of the intact bandicoot in the breeding season. Nuclear inclusions (*D*) similar to those seen in the ventral prostate cells of the non-breeding bandicoots are seen. ×6000.

centration previously observed during the breeding season (Gemmell *et al.* 1985) and it is probable that this increase in steroid hormone concentration causes an enlargement in the size of the prostate. The bandicoot prostate displays the same cyclic pattern in size with respect to season as observed in the possum *T. vulpecula* (Gilmore, 1969). Spermatogenesis occurs throughout the year in the possum and although no obvious changes occur in the testis and epididymis, the prostate shows a sixfold increase during the breeding season (Gilmore, 1969). Curlewis & Stone (1985) confirmed this result, reporting that although mean epididymal weight does not change outside and during the breeding period, prostatic weight increases from $6{\cdot}75 \pm 2{\cdot}015$ g to $30{\cdot}75 \pm 3{\cdot}648$ g. In the bandicoot, the prostate increased from $4{\cdot}9 \pm 1{\cdot}4$ g (mean ± S.E., $n = 3$) to $13{\cdot}6 \pm 2{\cdot}1$ g ($n = 5$), about a threefold increase. Other marsupials that display a seasonality in the size of their accessory glands are the

Fig. 13. Tubules of the ventral prostate during the breeding season, stained with toluidine blue. Note the granular secretion in the lumen (*L*) and the thin cuboidal epithelium (*E*). ×900.

Fig. 14. The cells of the ventral prostate showing flattened elongate nucleus (*N*) and various flocculent secretory granules (*G*). A number of secretory granules showed an electron-dense core. ×9200.

Fig. 15. Cells of an atypical ventral prostatic tubule during the non-breeding season show a high columnar appearance and many large lucent secretory granules (*G*). Moderately electron-dense deposits (*D*) were seen in the nucleus of some cells. ×6000.

opossum *Didelphis virginiana* (Chase, 1939), the dasyurid *Antechinus stuartii* (Woolley, 1966) and the native cat *Dasyurus viverrinus* (Fletcher, 1985). In all three marsupials the prostatic size increases during the breeding season.

Just as Leydig cell volume increases during the breeding season with *I. macrourus*, Gilmore (1969) has reported similar results with *T. vulpecula*. These changes in Leydig cell volume correlate well with the seasonal variation in plasma testosterone for both the bandicoot (Gemmell *et al.* 1985) and the possum (Gemmell, Cepon & Barnes, 1986).

The bandicoot and the koala *Phascolarctos cinereus* differ from the majority of marsupials in that they both possess a heart-shaped prostate, the remaining marsupials having a carrot-shaped prostate (Rodger & Hughes, 1973; Temple-Smith, 1984; Fletcher, 1985). The bandicoot prostate is unusual in that it is composed of two segments, the dorsal and ventral portions. The segmentation of *P. cinereus* has not been reported. All the other marsupial species that have been examined possess three zones within the prostate (Rodgers & Hughes, 1973; Temple-Smith, 1984; Fletcher, 1985) with the exception of the honey possum *Tarsipes rostratus* which has only two zones, an anterior and a posterior portion (Woolley & Scarlett, 1984). However, *Tarsipes* has a carrot-shaped prostate and the form of segmentation differs from that observed in the bandicoot and is most similar to the dasyurid type (Rodger & Hughes, 1973; Woolley & Scarlett, 1984).

During the breeding season, the volume of the dorsal prostate appears to increase proportionately more than that of the ventral portion. In addition, the lumen of the tubules of the dorsal prostate of the castrated bandicoot has little secretion, whereas secretion is present in the ventral prostate. These results suggest that the increase in plasma testosterone concentrations observed during the breeding season are required to activate the cells of the dorsal prostate.

The mode of secretion of the cells of the dorsal prostate is difficult to ascertain. It is possible that the low cells of the dorsal prostate are simply immature representatives of the tall cells which become filled with secretory product and eventually break down and release their cellular contents in the lumen of the tubule. Cellular debris can sometimes be seen within the luminal secretion. Also during the non-breeding season, when secretion is reduced, the tall cells are hard to distinguish from the low cells. Another alternative form of secretion utilised by the cells of the dorsal prostate is apocrine secretion. Apocrine secretion has been described in a segment of the prostate of the opossum *D. virginiana* (Hruban *et al.* 1965; Martan & Allan, 1965) and another American marsupial *D. marsupialis* (Hardin, 1965). With the bandicoot, many instances were noted where the apical cytoplasm of the tall cells was seen to be extruding into the lumen as is observed with apocrine secretion. Whereas the method of secretion of the dorsal prostate was difficult to ascertain the cells of the ventral prostate displayed the normal granular form of secretion seen in many secretory glands.

In conclusion, it is apparent from the changes in plasma testosterone, volume of Leydig cells and the size and secretory state of the prostate that the male bandicoot reproductive tract is influenced by season. However, the effect of these seasonal changes on the male reproductive tract and the subsequent changes, if any, to the birth rate has still to be ascertained. With respect to the accessory glands of the marsupial, there are now numerous studies of the morphology of the male marsupial reproductive tract (Rodger & Hughes, 1973; Temple-Smith, 1984); however, further information is required concerning the chemistry and function of these accessory glands before a further understanding of the role of the prostate in marsupial reproduction can be obtained.

SUMMARY

Reproductive activity is seasonal in bandicoots. To determine the seasonal changes in the reproductive tract of the male bandicoot, the morphology of the testis and the prostate was examined during the breeding and non-breeding season.

Although the dimensions of the testes of the bandicoot did not change with season, the volume of testicular Leydig cell tissue increased about twofold and this change within the testis was accompanied by a threefold increase in prostate gland size. The bandicoot prostate can be divided into two zones, a dorsal zone which produces a carbohydrate secretion containing the enzyme acid phosphatase, and a smaller ventral zone which produces a mucoprotein secretion lacking acid phosphatase activity.

These morphological changes within the testis and the prostate gland of the male bandicoot correlate with the seasonal variation in plasma testosterone concentrations previously reported. The testosterone concentration in the male starts to rise in May and reaches a peak in September and presumably causes the increase in prostate gland size and activity.

The authors thank the Australian Research Grants Scheme for financial assistance.

REFERENCES

BARNES, A. & GEMMELL, R. T. (1984). Correlations between breeding activity in the marsupial bandicoots and some environmental variables. *Australian Journal of Zoology* **32**, 219–226.

BIGGERS, J. D. (1966). Reproduction in male marsupials. In *Comparative Biology of Reproduction in Mammals* (ed. I. W. Rowlands), *Symposia of the Zoological Society of London* **15**, 353–363. London: Academic Press.

CATT, D. C. (1977). The breeding biology of Bennett's wallaby (*Macropus rufogriseus fruticus*) in South Canterbury, New Zealand. *New Zealand Journal of Zoology* **4**, 401–411.

CHASE, E. B. (1939). The reproductive system of the male opossum, *Didelphis virginiana* Kerr and its experimental modification. *Journal of Morphology* **65**, 215–239.

CURLEWIS, J. D. & STONE, G. M. (1985). Peripheral androgen levels in the male brush-tail possum (*Trichosurus vulpecula*). *Journal of Endocrinology* **105**, 63–70.

FLETCHER, T. P. (1985). Aspects of reproduction in the male Eastern Quoll, *Dasyurus viverrinus* (Shaw) (Marsupialia: Dasyuridae), with notes on polyestry in the female. *Australian Journal of Zoology* **33**, 101–110.

GEMMELL, R. T. (1982). Breeding bandicoots in Brisbane (*Isoodon macrourus*; Marsupialia, Peramelidae). *Australian Mammalogy* **5**, 184–191.

GEMMELL, R. T., CEPON, G. & BARNES, A. (1986). Weekly variations in weight and plasma testosterone concentrations in the captive male possum, *Trichosurus vulpecula*. *General and Comparative Endocrinology* **62**, 1–7.

GEMMELL, R. T., JOHNSTON, G. & BARNES, A. (1985). Seasonal variation in plasma testosterone concentrations in the male marsupial bandicoot, *Isoodon macrourus* in captivity. *General and Comparative Endocrinology* **59**, 184–191.

GEMMELL, R. T., STACY, B. D. & THORBURN, G. D. (1974). Ultrastructural study of secretory granules in the corpus luteum of the sheep during the oestrous cycle. *Biology of Reproduction* **11**, 447–462.

GILMORE, D. P. (1969). Seasonal reproductive periodicity in the male Australian brush-tailed possum (*Trichosurus vulpecula*). *Journal of Zoology, London* **157**, 75–98.

GORDON, G. (1971). A study of island populations of the short-nosed bandicoot, *Isoodon macrourus* (Gould). PhD thesis, University of New South Wales.

HARDIN, J. H. (1965). Cytological features of glycogen-containing cells in the third part of the prostate gland of the opossum, *Didelphis marsupialis*. *Anatomical Record* **151**, 358.

HARTMAN, C. G. (1928). The breeding season of the opossum (*Didelphis virginiana*) and the rate of intra-uterine and post-natal development. *Journal of Morphology* **46**, 142–215.

HEARN, J. P. (1975). The role of the pituitary in the reproduction of the tammar wallaby, *Macropus eugenii*. *Journal of Reproduction and Fertility* **42**, 399–402.

HEINSOHN, G. E. (1966). Ecology and reproduction of the Tasmanian bandicoots (*Perameles gunnii* and *Isoodon obesulus*). *California Publications in Zoology* **84**, 1–107.

HRUBAN, Z., MARTIN, J., SLESSERS, A., STEINER, D. F., LUBRAN, M. & RECHCIGL, M. (1965). Fine structure of the prostatic epithelium of the opossum (*Didelphis virginiana*, Kerr). *Journal of Experimental Zoology* **160**, 81–106.

INNS, R. W. (1982). Seasonal changes in the accessory reproductive system and plasma testosterone levels of the male tammar wallaby, *Macropus eugenii* in the wild. *Journal of Reproduction and Fertility* **66**, 675–680.

MARTAN, J. & ALLEN, J. M. (1965). The cytological and chemical organization of the prostatic epithelium of *Didelphis virginiana* Kerr. *Journal of Experimental Zoology* **159**, 209–230.

RODGER, J. C. & HUGHES, R. L. (1973). Studies of the accessory glands of male marsupials. *Australian Journal of Zoology* **21**, 303–320.

SADLIER, R. M. F. S. (1965). Reproduction in two species of kangaroo *Macropus robustus* and *Megaleia rufa* in the arid Pilbara region of Western Australia. *Proceedings of the Zoological Society of London* **145**, 239–261.

SETCHELL, B. P. (1977). Reproduction in male marsupials. In *The Biology of Marsupials* (ed. B. Stonehouse & D. Gilmore), pp. 411–457. London: Macmillan Press Ltd.

STODDART, D. M. & BRAITHWAITE, R. W. (1979). A strategy for utilization of regenerating heathland habitat by the brown bandicoot (*Isoodon obesulus*; Marsupialia, Peramelidae). *Journal of Animal Ecology* **48**, 165–179.

TEMPLE-SMITH, P. D. (1984). Reproductive structures and strategies in male possums and gliders. In *Possums and Gliders* (ed. A. P. Smith & I. D. Hume), pp. 89–106. Sydney: Australian Mammal Society.

TYNDALE-BISCOE, C. H. (1955). Observations on the reproduction and ecology of the brush-tailed possum, *Trichosurus vulpecula* Kerr (Marsupialia) in New Zealand. *Australian Journal of Zoology* **3**, 162–184.

WILLIAMS, M. A. (1977). *Quantitative Methods in Biology* (ed. A. M. Glauert), pp. 5–84. Amsterdam: North Holland Publishing Co.

WOOLLEY, P. (1966). Reproduction in *Antechinus spp.* and other dasyurid marsupials. In *Comparative Biology of Reproduction in Mammals. Symposia of the Zoological Society of London* **15**, 281–294.

WOOLLEY, P. A. & SCARLETT, G. (1984). Observations on the reproductive anatomy of male *Tarsipes rostratus* (Marsupialia: Tarsipedidae). In *Possums and Gliders* (ed. A. P. Smith & I. D. Hume), pp. 445–450. Sydney: Australian Mammal Society.