Chapter 8

The Effect of Testosterone and Cortisol on the Reproductive Tract of Male *Antechinus stuartii*

8.1 Introduction

The seasonal changes in the reproductive tract of male *A. stuartii* were described in Chapter 5. While the spermatogenic cycle had been examined thoroughly by Kerr and Hedger (1983), the changes to the accessory reproductive tract had been described only cursorily by other authors. Organ mass, and histology based on three frozen samples, form the basis of knowledge of the accessory reproductive tract prior to the present study (Bradley *et al.* 1980, Rodger and Hughes 1973, Woolley 1966).

The seasonal changes found in Chapter 5 included some similarities with the previous study by Kerr and Hedger (1983), where the spermatogenic cycle was found to be the same for all males at each time of year, and with few associations of germ cells. The Leydig cells, however, were found to differentiate between February and May, and to continue to enlarge and proliferate throughout the year. These changes were not as well defined in the study by Kerr and Hedger (1983) although Taggart *et al.* (1993) and Woolley (1975) state that the morphology of the Leydig cells remains relatively unchanged from February.

The changes in the epididymis described in Chapter 5 also demonstrated some similarity with previous studies (Taggart and Temple-Smith 1989, 1992). The initial specialisation of the epididymis occurred in May, confirming the study by Taggart and Temple-Smith (1992). The morphometric studies in Chapter 5 elaborated on the epididymal morphometry in the earlier studies by Taggart and Temple-Smith (1989, 1992), and found that epithelial height and cell volume increased significantly through the year, with differences between the caput and caudal end becoming more pronounced as the year progressed. However, the present study began in February, after many of the post-natal differentiations observed in other studies

occurred. By the beginning of the present study both the caudal and caput regions had developed, without any obvious development of the epididymal epithelium. The later differentiation of the epididymis seen in the present study, and confirmed by the morphometry, suggests that the epididymis is stimulated by the actions of androgens, as these changes parallel the rise in androgens seen in other studies (Bradley *et al.* 1980, Moore 1974).

The plasma concentrations of androgens found in other studies also parallel the changes seen in the accessory reproductive tract. The prostate and Cowper's glands are differentiated in July (Chapter 5), after spermatogenesis has ceased and androgens have reached their 8-fold increase in plasma concentration (Bradley *et al.* 1980, Kerr and Hedger 1983). The present study found that there was an increase in cellular differentiation and secretory activity from July, and that this continued through to August, when maximal activity was observed in conjunction with the mating season.

The strong correlation between plasma androgen concentrations seen in other studies and the increase in prostate and Cowper's gland weights (Bradley *et al.* 1980, Woolley 1966), is also correlated with the changes in microanatomy seen in Chapter 5. This would confirm the findings of other marsupial studies that plasma testosterone concentrations are correlated with increases in accessory glands, although not necessarily with spermatogenic activity (Cook *et al.* 1978, Curlewis 1991, Gemmell *et al.* 1986, Jones *et al.* 1988).

The previous studies suggest that testosterone has a marked effect on accessory gland growth, but that this can be independent of spermatogenic activity. The evidence of past studies lends credence to this phenomenon also occurring in *A. stuartii* males, with the unusual exception that spermatogenesis has ceased, indeed the germinal epithelium has collapsed, before the breeding period. However, the cessation of spermatogenic activity is not associated with a collapse in the interstitium of the testes but with a maintenance of cellular density and volume (Taggart *et al.* 1993, Woolley 1966, this study). The uncoupling of these events suggests that, firstly, testosterone concentration has little effect on spermatogenic activity, and secondly that testosterone is important for accessory gland development independent of the spermatogenic cycle.

The two previous chapters concerned the influence of testosterone and cortisol on the renal structure and function of male *A. stuartii*. They found that the administration of testosterone, with or without cortisol, significantly affected renal morphology and renal function. When testosterone administration was coupled with cortisol administration, many of the observed changes mimicked those seen in males in July and August in the seasonal study.

The present chapter will examine the effects of testosterone and cortisol administration on the reproductive tract in male *A. stuartii*. There are two aspects under consideration in this study. The first is to determine whether the mechanisms of spermatogenesis and accessory gland development are separate, as suggested by the seasonal study, and in conjunction with findings from other studies on *Antechinus* (Kerr and Hedger 1983, Wilson and Bourne 1984, Taggart and Temple-Smith 1989, Woolley 1966). The second aspect is to confirm parallelism in hormone action by observing the effects of the hormone administration on a more 'traditional' target site, the accessory reproductive tract. Because the non-testicular reproductive tract is sensitive to testosterone in most marsupial species where this has been assessed (Curlewis and Stone 1985, Jones *et al*. 1984, Temple-Smith 1984), the assessment of the reproductive tract will act as a general indicator of the efficacy of the hormone administration. Therefore this chapter will address the changes in the reproductive tract associated with testosterone and cortisol administration.

8.2 Materials and methods

8.2.1 Animals

Animals were captured, housed, and maintained as described in Chapter 2 and Appendix I. They were held in captivity for 6-7 weeks after capture and had depot intramuscular injections twice, two weeks apart, of either saline, testosterone only, cortisol only, or testosterone plus cortisol (see Chapter 6). GFR and urine output were measured twice, once before hormone injection, and once about 30 days after hormone injection (see Chapter 6).

8.2.2 Histology

All animals were sacrificed and tissues processed as outlined in Chapter 2. The testes and accessory reproductive tracts were stained as outlined in Chapter 5.

8.2.3 Morphometry

The testes, bulbourethral glands, prostates and paired adrenal glands of all animals were weighed after death. The scrotal sacs were dissected away from the testes before weighing of the testes. Data are presented as actual mass, and as a percentage of body mass. Bulbourethral gland definitions are from Chapter 5, Figure 5.5. Morphometric observations were the same as for Chapter 5. Data were analysed by Two-way ANOVA for cortisol and testosterone, followed by pairwise Fisher's PLSD tests (Haycock *et al.* 1992, Zar 1984). Percentage data were arcsine transformed prior to analysis, however percentage values are presented in the tables (Zar 1984). Scrotal width at capture was subtracted from scrotal width at death and the differences were assessed by Two-way ANOVA for cortisol and testosterone.

8.3 Results

8.3.1 External morphology

At the end of the experiment, the eternal genitalia of *A. stuartii* treated with saline remained similar to those seen in other males from early June in other studies (McAllan *et al.* 1991, Woolley 1966). The scrotum was retractile and unpigmented, and the sternal gland was evident in one individual. No bulbourethral glands were observed on palpation of the urogenital region. In testosterone only males, the sternal gland was large and secretory in all individuals. The scrotum was losing fur in two individuals, darkly pigmented in another individual, and was retractile in all but one individual. All testosterone only males had mature erectile penises, evident within ten days of hormone injection. The bulbourethral glands were palpable within the same period of time and, by the end of the experiment, were large and observable as two large masses, one on each side of the urogenital sinus.

In cortisol only males some minor changes were observed in some individuals. The sternal gland was observed in two individuals and an immature penis was seen in one of these males. The scrotal sac was not retractile and losing fur in some individuals and was black under the fur in another of these individuals. One cortisol only male had small palpable bulbourethral glands. In all testosterone plus cortisol males the sternal gland was large and secretory. The scrotal sacs of all but one male were dark, losing fur and imperfectly retractile. All testosterone plus cortisol males had mature erectile penises, evident within ten days of hormone injection. The bulbourethral glands were palpable within the same period of time and, by the end of the experiment, were moderate to large and observable in some individuals as two masses, one on each side of the urogenital sinus.

The remainder of the results will be divided into two sections, i) testis, and ii) accessory reproductive tract.

8.3.2 Testis

8.3.2.1 Testes mass and (scrotal) width

The differences in the width of both testes (scrotal width) were significantly smaller in the groups treated with cortisol (Testosterone NS, Cortisol $P<0.05$, Interaction NS, Table 8.1, Figure 8.1). The testes mass did not differ between groups (testosterone NS, cortisol $P=0.14$, interaction NS), or when adjusted for body mass (testosterone NS, cortisol NS, interaction NS, Table 8.1).

8.3.2.2 Testicular morphology

All four groups showed similar seminiferous tubule morphology. There were late spermatids and, in two testosterone only males and one cortisol only male there were also mature sperm in the seminiferous tubules. However, all individuals in all groups had maturing sperm in various stages of spermiogenesis, and also developing spermatocytes lining the basement of the tubules.

The intertubular tissue of the testes of all individuals was plentiful and in most individuals was full of polyhedral Leydig cells. In many individuals from all groups the Leydig cells were strongly eosinophilic. However, the interstitia of the testosterone only males were more variable than for other groups, with some individuals having

Table 8.1 Measurements of the reproductive tracts and adrenal glands of male *A. stuartii* from testosterone and cortisol experiments.

Treatment	Testes (scrotal) width differences (mm)	Testes mass (g)	Testes mass (% of body mass)	Prostate mass (g)	Prostate (% of body mass)	Bulbourethral gland mass (g)	Bulbourethral gland (% of body mass)	Adrenal mass (g)	Adrenal mass (% of body mass)
Saline	1.3 ± 0.2 **a**	0.36 ± 0.02	1.21 ± 0.07	0.035 ± 0.003 **c**	0.11 ± 0.01 **c**	0.045 ± 0.005 **c**	0.15 ± 0.02 **c**	0.0065 ± 0.0008 **a**	0.021 ± 0.002 **a**
Testosterone	1.15 ± 0.1 **a**	0.41 ± 0.03	1.09 ± 0.04	0.291 ± 0.021 **a**	0.77 ± 0.05 **a**	0.456 ± 0.023 **a**	1.2 ± 0.06 **a**	0.0062 ± 0.0005 **a**	0.016 ± 0.002 **b**
Cortisol	0.5 ± 0.4 **b**	0.33 ± 0.06	1.11 ± 0.16	0.043 ± 0.005 **c**	0.15 ± 0.02 **c**	0.053 ± 0.008 **c**	0.18 ± 0.02 **c**	0.0052 ± 0.0006 **b**	0.018 ± 0.002 **a**
Testosterone and Cortisol	0.1 ± 0.9 **b**	0.32 ± 0.04	1.01 ± 0.09	0.157 ± 0.012 **b**	0.52 ± 0.06 **b**	0.287 ± 0.056 **b**	0.91 ± 0.13 **b**	0.0039 ± 0.0005 **b**	0.013 ± 0.002 **b**
Testosterone	NS	NS	NS	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	NS	$P<0.025$
Cortisol	$P<0.05$	$P=0.14$	NS	$P<0.0001$	$P<0.05$	$P<0.01$	$P=0.11$	$P<0.01$	$P=0.075$
Interaction	NS	NS	NS	$P<0.0001$	$P<0.002$	$P<0.005$	$P<0.02$	NS	NS

Table 8.1 Measurements of the reproductive tracts and adrenal glands of male *A. stuartii* treated with either saline, testosterone only, cortisol only or testosterone plus cortisol. Data are means and standard errors, two-way ANOVA results are below values in each column. Different letters in a column indicate that the values are significantly different from one another. Significance values of the percentage data are from analyses where the percentage values were normalised by arcsine transformation of the square roots of the data before analysis.

Figure 8.1

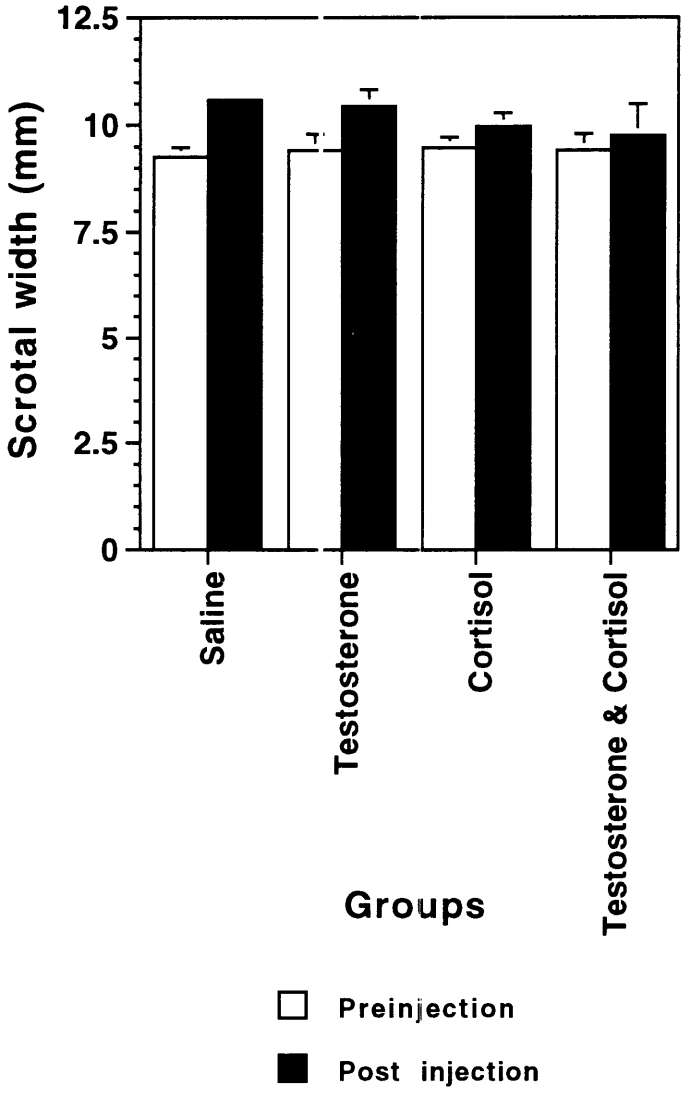

Figure 8.1 The changes in the scrotal width of *A. stuartii* treated with saline, testosterone, cortisol or testosterone plus cortisol. Values are means ± standard errors of the mean.

hypertrophied cells that had proliferated throughout the enlarged intertubular space. Other individuals had interstitial tissue that was indistinguishable from the controls and in some individuals the interstitial material was reduced. This variability was not observed in individuals from other groups.

8.3.2.3 Epididymal morphology

The epididymides of saline and cortisol only males were similar to those of the seasonal study in May, with the epithelial cells of the caput end remaining simple and columnar in appearance, and the epithelial cells of the caudal end were more columnar. However, there was considerable hypertrophy of the epididymal epithelia in both groups treated with testosterone, and the appearance was similar to those of males in August.

8.3.2.4 Epididymal morphometry

The caput epithelial height did not differ significantly between groups (testosterone NS, cortisol NS, interaction $P=0.17$, Figure 8.2a). Similarly, the caput epithelial volume did not differ significantly between groups (testosterone $P=0.14$, cortisol NS, interaction $P=0.15$, Figure 8.2b).

The caudal epithelial heights were significantly increased by the administration of testosterone (testosterone $P<0.0001$, cortisol NS, interaction NS, Figure 8.3a). The caudal epithelial volumes were significantly increased by the administration of testosterone (testosterone $P<0.0005$, cortisol NS, interaction NS, Figure 8.3b).

8.3.3 Accessory reproductive tract

8.3.3.1 Prostate

The prostate mass changed significantly with the administration of testosterone and cortisol (testosterone $P<0.0001$, cortisol $P<0.0001$, interaction $P<0.0001$, Table 8.1). Testosterone significantly increased prostate mass but interacted with cortisol such that prostate mass of testosterone plus cortisol treated males was less than that of testosterone only males but greater than cortisol only and saline treated males (Table 8.1). The same relationship was observed when prostate mass was adjusted for body mass (testosterone

Figure 8.2a

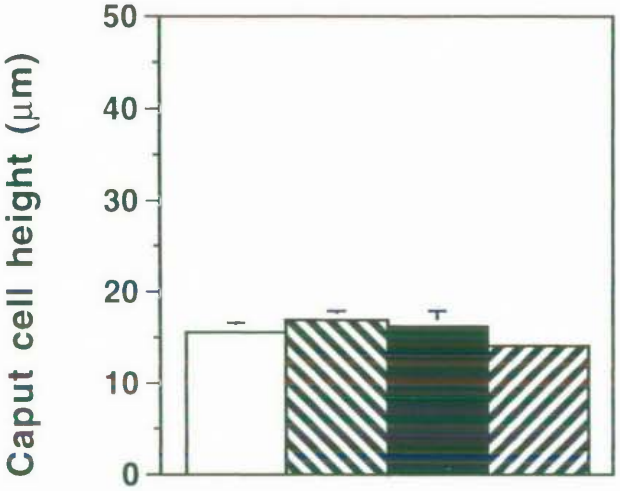

Figure 8.2b

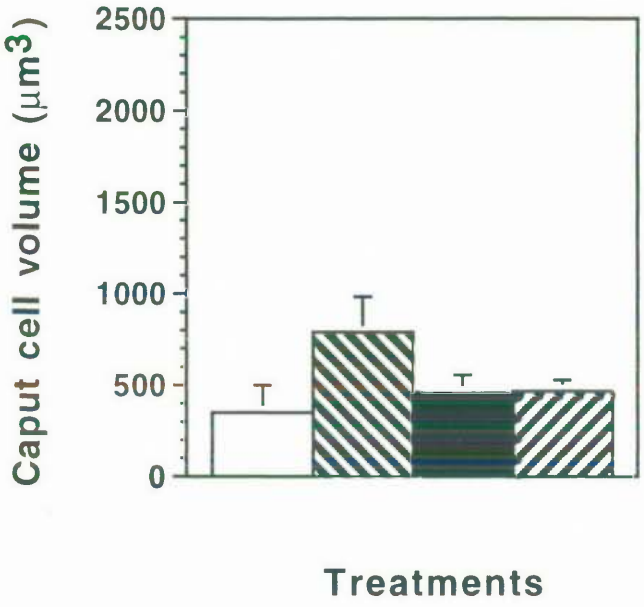

Figure 8.2 The epithelium of the caput section of the epididymis, a) epithelial cell height (µm) and b) epithelial cell volume (μm^3). Values are means ± standard errors of the mean.

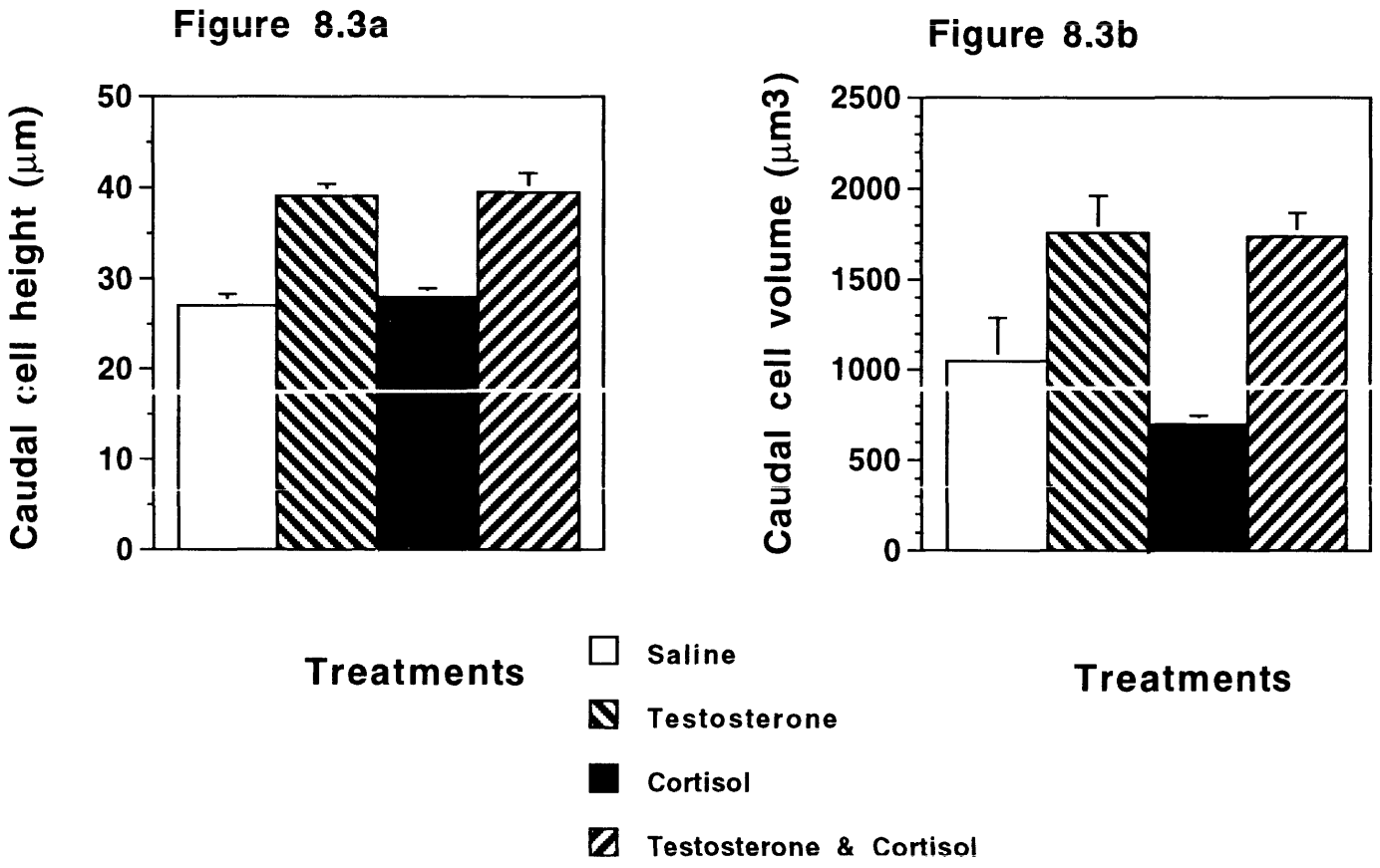

Figure 8.3 The epithelium of the caudal section of the epididymis, a) epithelial cell height (μm) and epithelial cell volume (μm^3). Values are means ± standard errors of the mean.

$P<0.0001$, cortisol $P<0.05$, interaction $P<0.002$, Table 8.1).

The prostates of saline treated males were macroscopically similar to those seen in males from May in the seasonal study, where there was a translucent anterior portion and a more opaque posterior portion. The microscopic anatomy was also similar, with no differentiation of the secretory tubules along the length of the prostate (Figure 8.4a). The same was also true of the prostate of males treated with cortisol only (Figure 8.4b).

However treatment with testosterone, either alone or with cortisol, produced significant alterations in the morphology of the prostate (Figure 8.5, Figure 8.6). The anterior portion was translucent, while the posterior portion was white and opaque. The internal segmentation was pronounced, with the anterior portion containing tubules of simple cuboidal epithelium with enlarged lumina full of secretory material (Figure 8.5a, 8.5b). As for the seasonal study, the secretory material of the anterior portion was PAS positive, but the epithelium stained with neither PAS nor Alcian blue. The posterior portion had tubules that were aligned towards the urethral centre of the prostate and consisted of columnar epithelia that secreted material into small lumina (Figure 8.5b, 8.5c, 8.5d). Again the staining with Masson's trichrome and PAS-Alcian blue was similar to the seasonal study, with differential staining of the tubular epithelium.

The morphology of the prostates of males treated with testosterone plus cortisol was similar to that of the testosterone only treated males, although the hypertrophy was not as marked (Figure 8.6). The morphological differentiation and staining were also similar (Figure 8.6).

8.3.3.2 Cowper's or Bulbourethral Glands

The bulbourethral gland mass changed significantly with the administration of testosterone and cortisol (testosterone $P<0.0001$, cortisol $P<0.01$, interaction $P<0.005$, Table 8.1). Testosterone significantly increased bulbourethral gland mass but interacted with cortisol such that bulbourethral gland mass of testosterone plus cortisol treated males was less than that of testosterone only males,

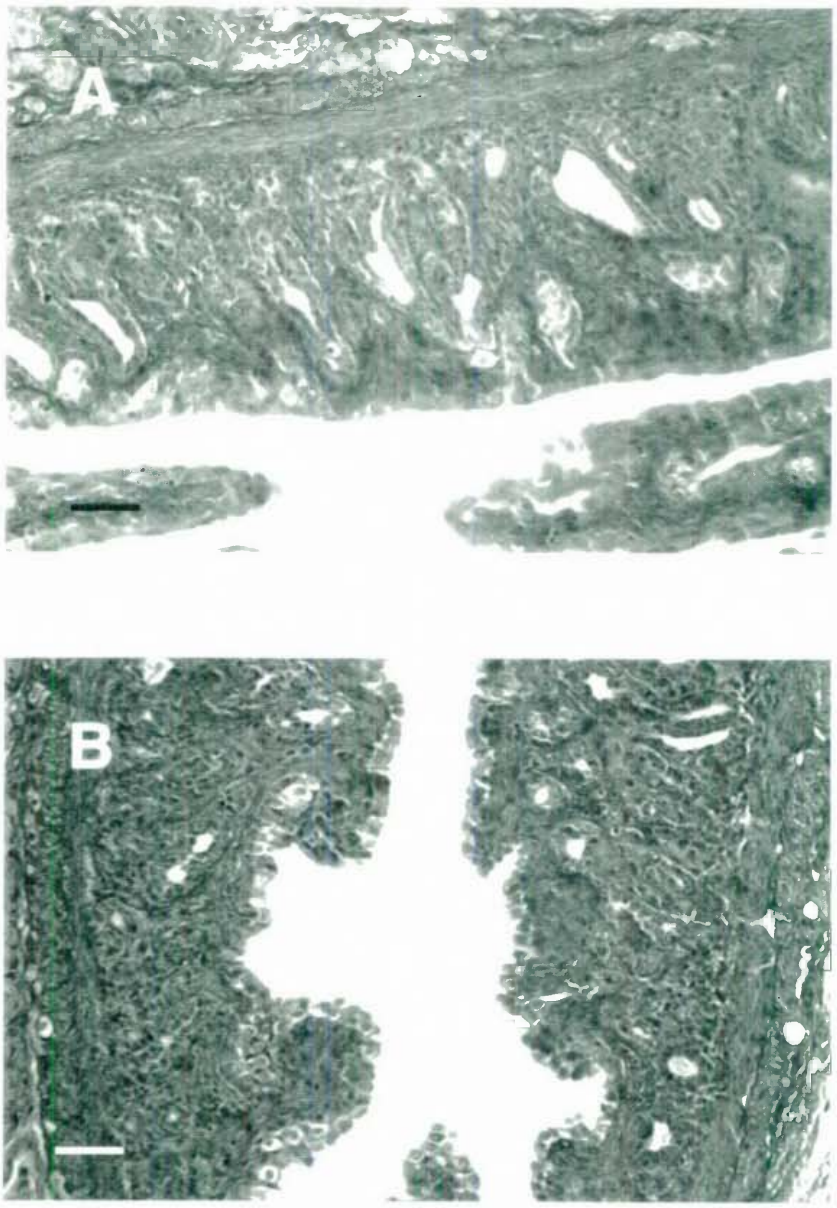

Figure 8.4 The prostate of A) a saline treated male and B) a cortisol treated male. The views include both the anterior and posterior segments of the prostate. Bars are 50 μm.

Figure 8.5 (FACING PAGE) Prostate of a testosterone only treated male. Scale bars are 50 μm

A) The anterior portion of the prostate.

B) Junction between the anterior (left) and posterior (right) portion of the prostate. The arrow indicates the junction between the two tubule forms.

C) The posterior prostate. The arrow indicates a duct opening into the urethral lumen at the base of the image. The posterior prostatic tubules can be seen and urethral cells line the lumen.

D) The posterior prostate. Secretory material can be seen in the lumina of the tubules.

A
B
C
D

Figure 8.6 (FACING PAGE) Prostate of a testosterone plus cortisol treated male. Scale bars are 50 μm

A) The anterior portion of the prostate.

B) Junction between the anterior (top) and posterior (bottom) portion of the prostate. The arrow indicates the junction between the two tubule forms.

C) The posterior prostate. The arrow indicates a duct opening into the urethral lumen at the base of the image. The posterior prostatic tubules can be seen and urethral cells line the lumen.

D) The posterior prostate. Secretory material can be seen in the lumina of the tubules.

A
B
C
D

but greater than cortisol only and saline treated males (Table 8.1). A similar relationship was observed when bulbourethral gland mass was adjusted for body mass testosterone $P<0.0001$, cortisol $P=0.11$, interaction $P<0.02$, Table 8.1).

The macroscopic morphology of the bulbourethral glands was similar in the saline and cortisol only treated males. Gland IV (Figure 8.7c, Figure 8.8c) was the only one that was easily identified, however histologically there was some differentiation of the other bulbular structures (Figure 8.7a, 8.7b; Figure 8.8a, 8.8b). The glands I, II, and III were small associations of tubules with simple cuboidal epithelium. At this point (early June) they were discrete units, but had no internal secretory specialisations (Figure 8.7a, 8.7b; Figure 8.8a, 8.8b). The urethral bulbs were small and indistinguishable from those of males from May in the seasonal study.

The bulbourethral glands of the testosterone only males were both macroscopically and histologically hypertrophied (Figure 8.9). All bulbourethral glands were easily identified on dissection and gland III, in particular, was considerably enlarged. The muscular layers of the urethral bulb had increased in size (Figure 8.11a). The secretory differentiation seen in the seasonal study in males from July and August was again observed in glands I, II, and III. In glands I and II the simple columnar epithelia lined tubules whose lumina were extensively expanded by secretory material (Figure 8.9a, 8.9b). The secretory material in gland I was PAS positive and did not stain with Alcian blue, stained bright red with Masson's trichrome and was colloidal-like in appearance (Figure 8.9b). In gland II the crystalline material observed in the seasonal study was again present, and stained red and blue with Masson's trichrome and was PAS positive and Alcian blue negative (Figure 8.9b).

Gland III consisted of branching tubules that drained into common ducts that led to the urethral opening (Figure 8.9c). The secretory cuboidal epithelium of the tubules of gland III stained weakly with PAS and the luminal surface stained with Alcian blue pH 1.0 and 2.5. The secretory material in the lumina of the tubules was PAS positive. Gland IV had a similar structure to those of males from saline and cortisol only treated groups, although the layers of striated muscle and the transitional epithelium lining the gland were enlarged.

Figure 8.7 (FACING PAGE) The bulbourethral glands of a saline treated male. Scale bars are 50 μm

A) Bulbourethral gland III (centre) with associated skeletal muscle (upper right). To the upper left is part of gland IV.

B) Bulbourethral gland I or II (centre) with associated skeletal muscle (upper right). It was not possible to distinguish glands I and II.

C) Bulbourethral gland IV demonstrating luminal lining, and skeletal muscle capsule. Parasites and their eggs are easily observed in the lumen.

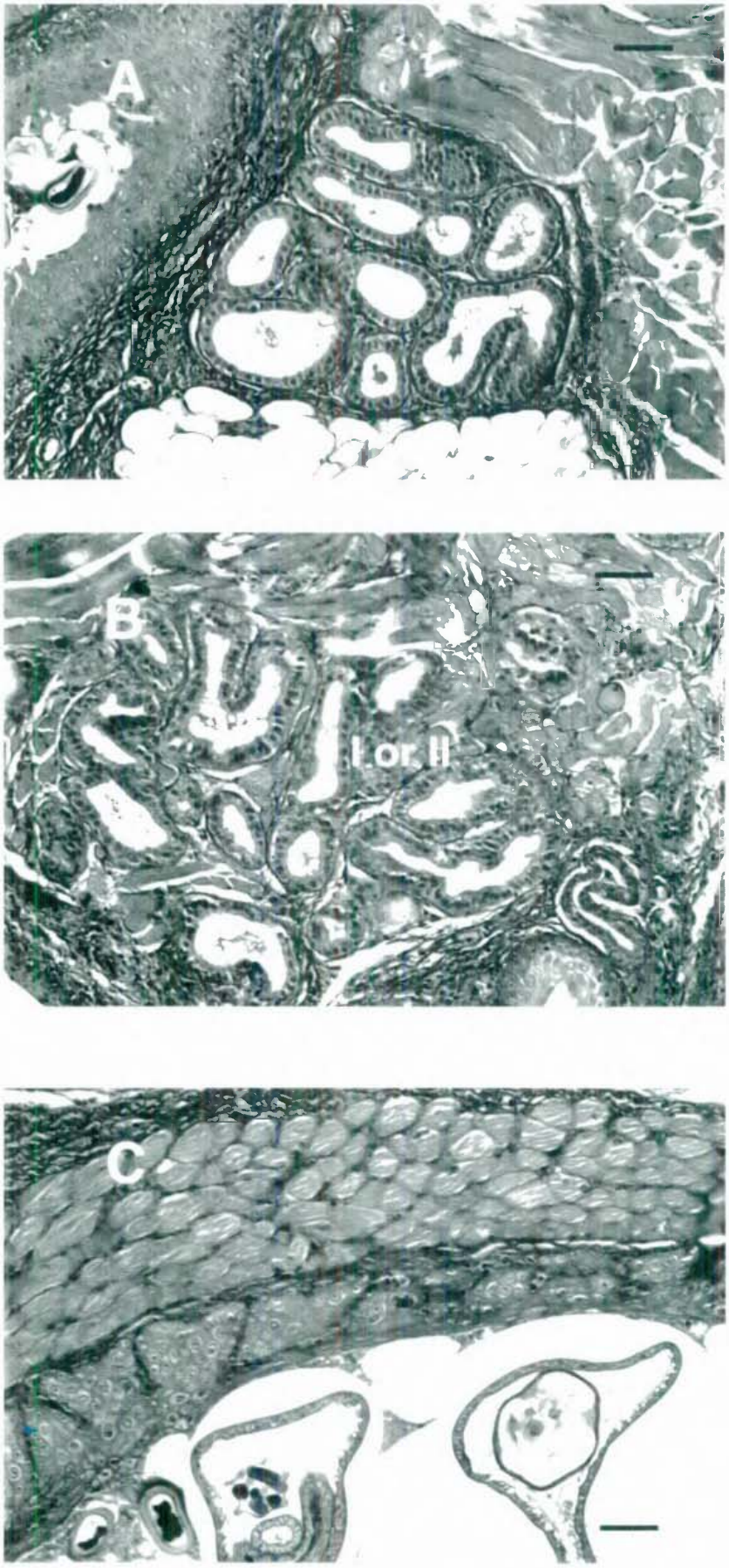
A
B
I or II
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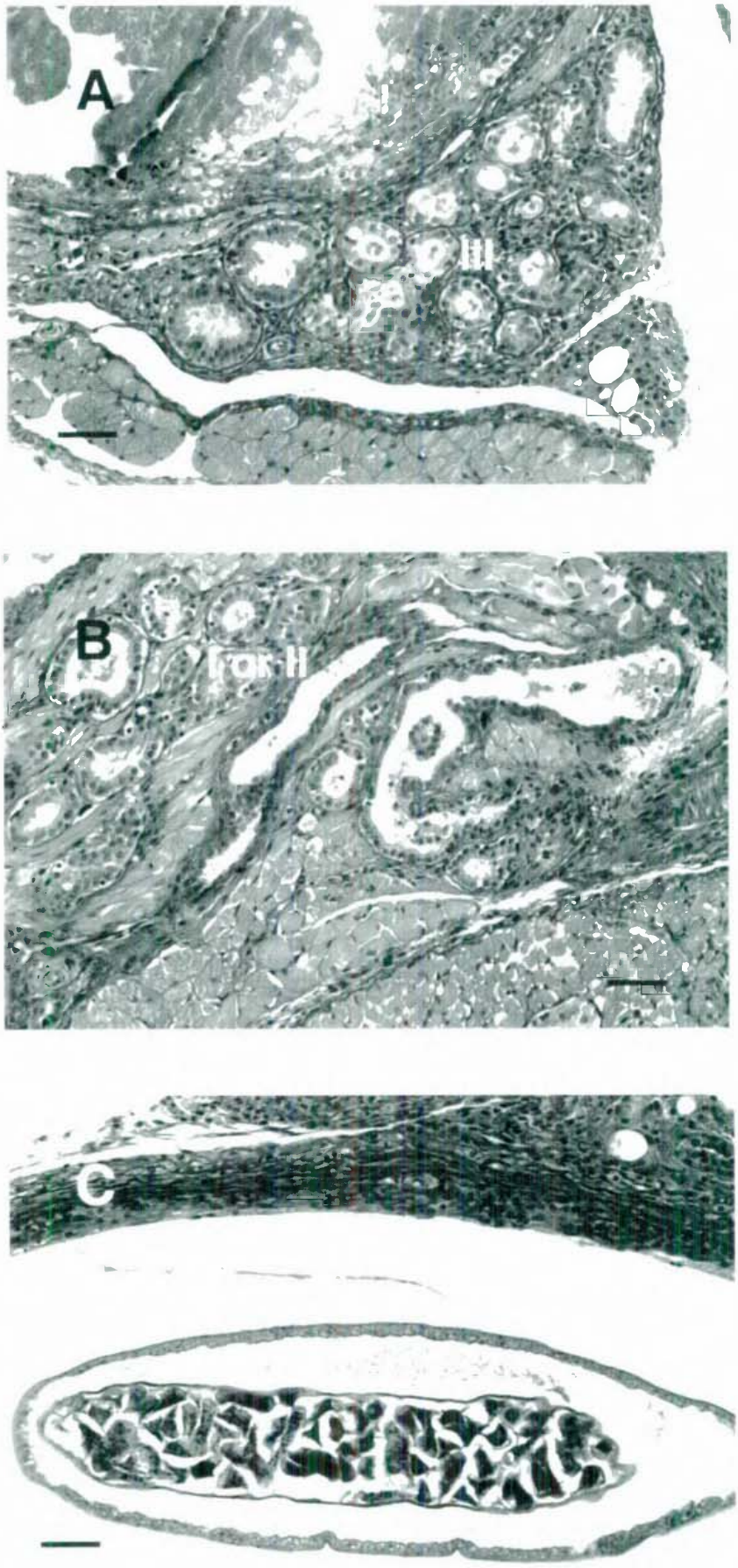
Figure 8.8 (FACING PAGE) The bulbourethral glands of a cortisol only treated male. Scale bars are 50 μm.

A) Bulbourethral gland III (centre) with associated skeletal muscle (upper right). To the upper left is part of gland IV.

B) Bulbourethral gland I or II (centre) with associated skeletal muscle (upper right). It was not possible to distinguish glands I and II.

C) Bulbourethral gland IV demonstrating luminal lining, and skeletal muscle capsule. A large parasite is easily observed in the lumen.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The bulbourethral glands of the males treated with testosterone plus cortisol were also easily distinguished macroscopically. Their histological appearance was very similar to that of the males treated with testosterone only (Figure 8.10). However, there were some differences. While the cytostructure of all glands was similar to that of the testosterone only group, there was often less secretory material located in the lumina of the tubules (Figure 8.10a, 8.10b). The staining affinities were the same as for the testosterone only males. Gland IV was similar in structure to that of males treated with testosterone only (Figure 8.11b).

8.3.4 Adrenal glands

Paired adrenal mass was significantly lowered by administration of cortisol (testosterone NS, cortisol $P<0.01$, interaction NS, Table 8.1). When expressed as a percentage of body mass, paired adrenal mass was significantly lowered by the administration of testosterone (testosterone $P<0.025$, cortisol $P=0.075$, interaction NS).

8.4 Discussion

The administration of testosterone and cortisol significantly altered the accessory reproductive tract of male *A. stuartii*. Under the influence of testosterone the prostate and the bulbourethral glands became differentiated and hypertrophied, and the lumina were full of secretory material. The addition of cortisol to the testosterone diminished the response to testosterone. However, some parts of the reproductive tract remained unaltered by hormone administration, notably the seminiferous tubules. The epididymis was segmentally sensitive to the effects of testosterone, with only the caudal end responding to hormone administration.

The adrenal mass was also affected by testosterone and cortisol administration with lower mass in adrenals of the cortisol treated males. This contrasts with seasonal studies on *A. stuartii*, where adrenal mass continues to rise in males during the mating period (Barnett 1973, Moore 1974). This suggests that hormonal feedback mechanisms were still intact in the present study, as organ mass has decreased, indicating glandular inactivity because of exogenous concentrations of testosterone and cortisol.

Figure 8.9 (FACING PAGE) The bulbourethral glands of a testosterone only male. Scale bars are 50 μm.

A) Gland I.

B) Gland II.

C) Gland III has some secretory material within the branching lumina of the tubules.

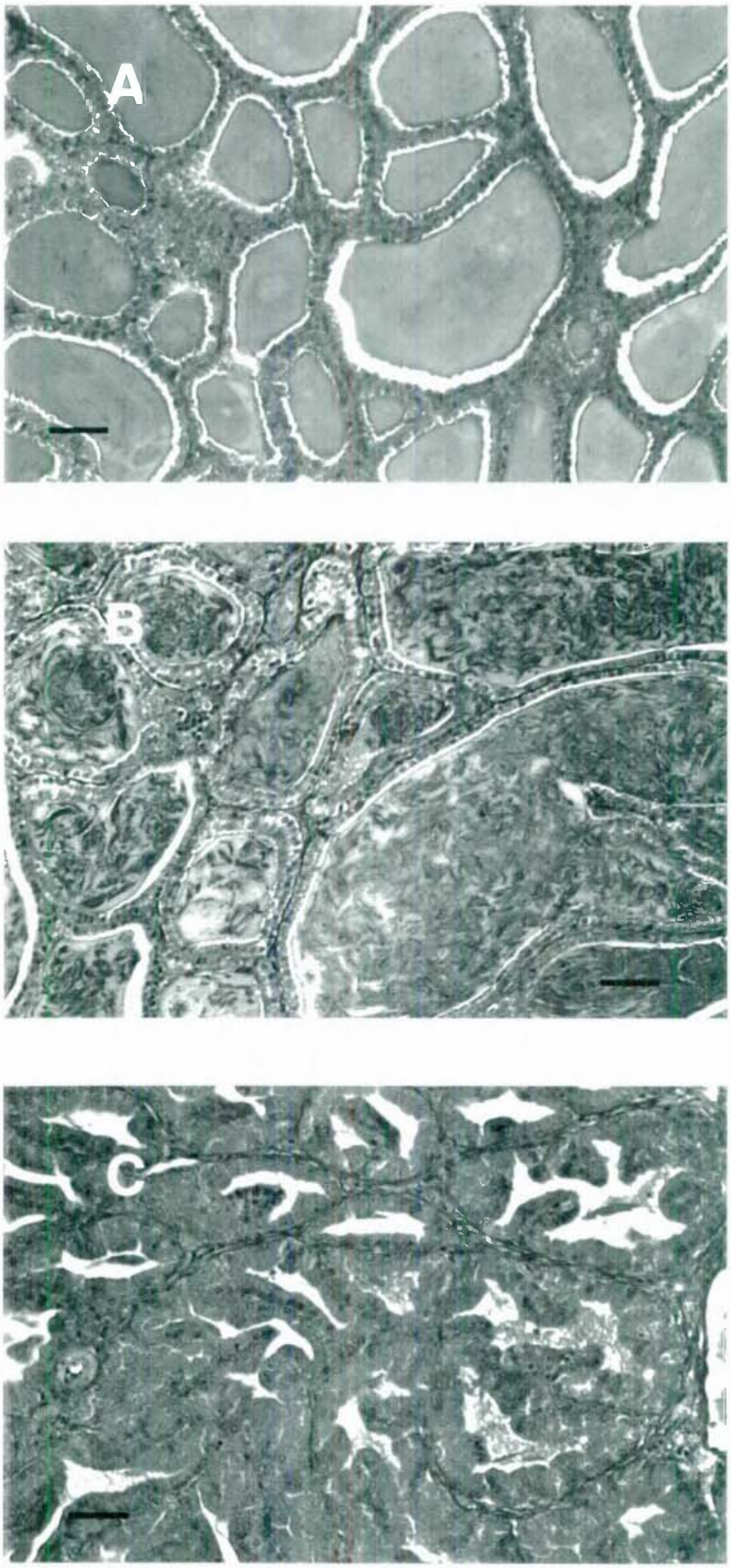
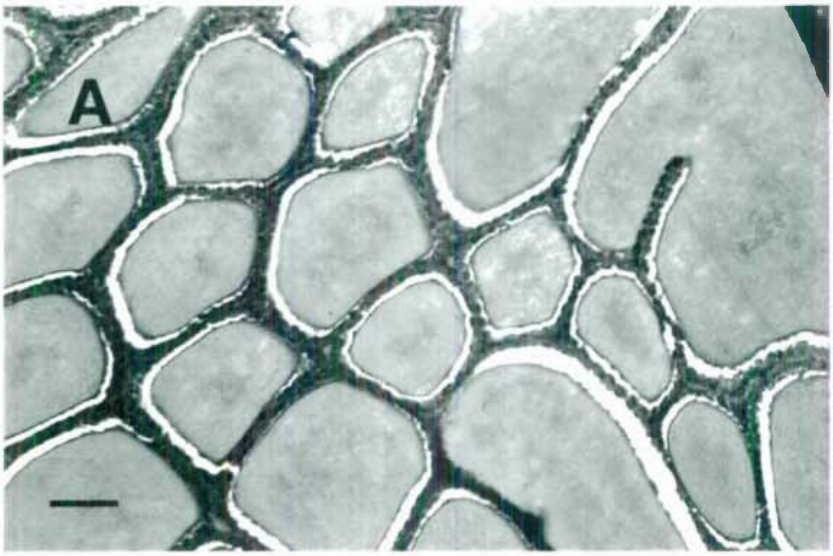

A
B
C

Figure 8.10 (FACING PAGE) The bulbourethral glands of a testosterone plus cortisol male. Scale bars are 50 μm.

A) Gland I.

B) Gland II.

C) Gland III has some secretory material within the branching lumina of the tubules.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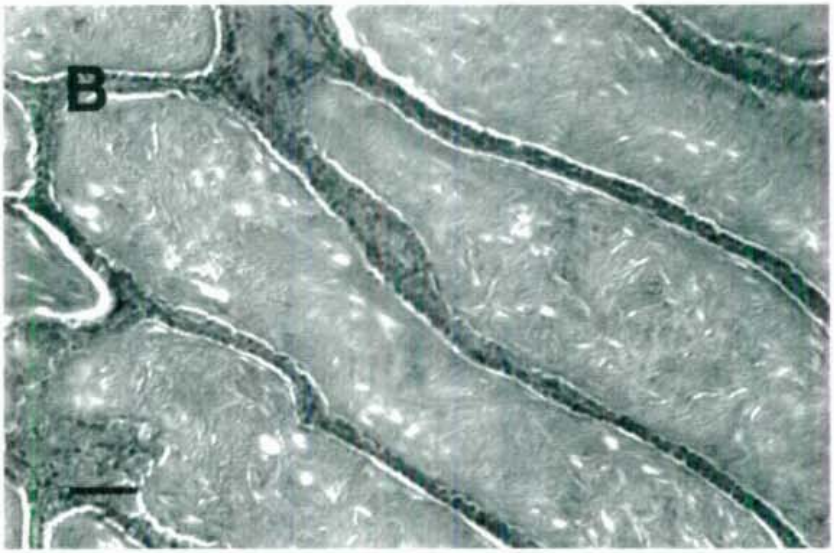
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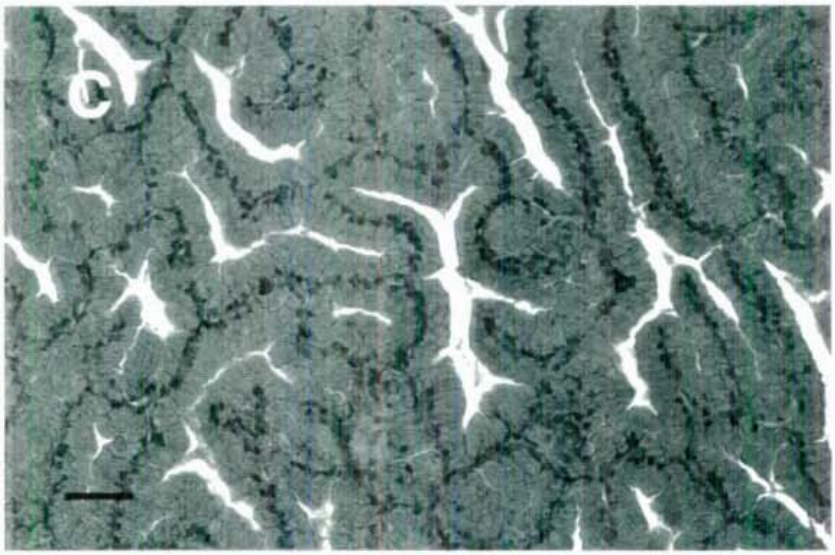
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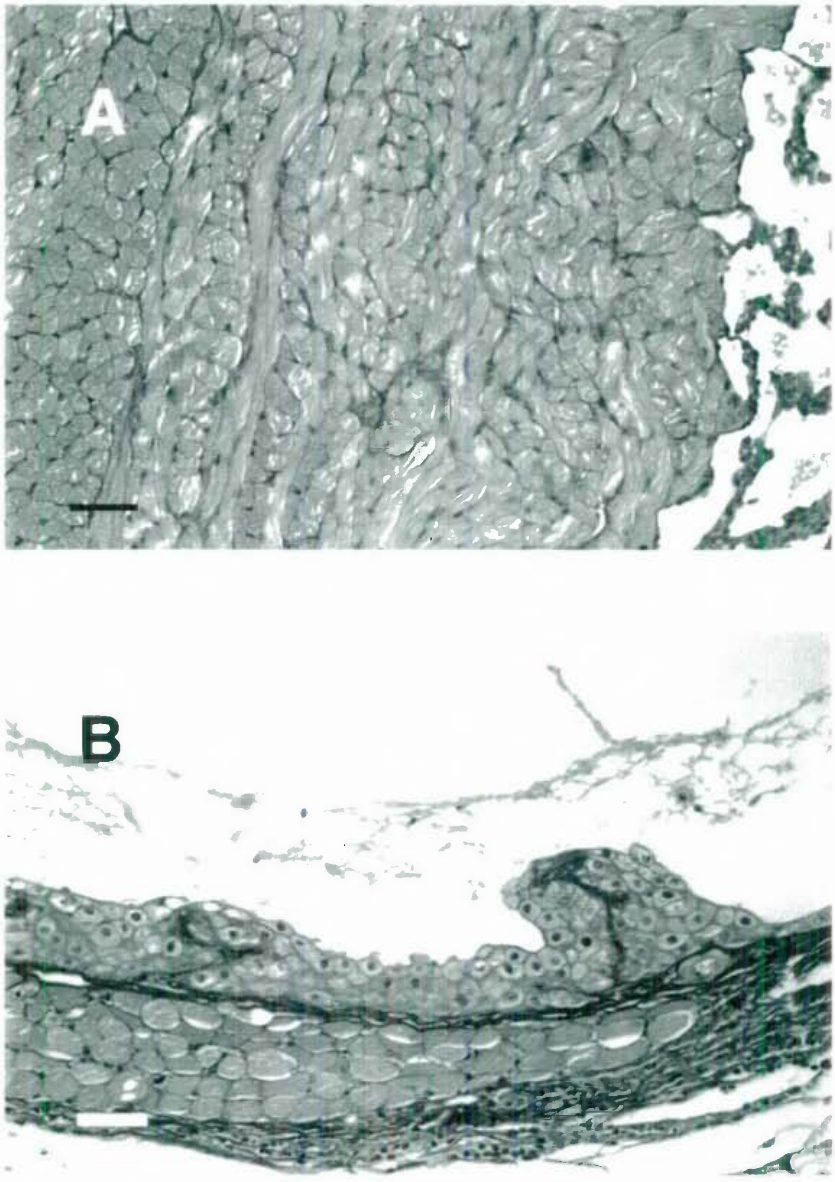

Figure 8.11 a) The urethral bulb of a male treated with testosterone only. b) Gland IV of a male treated with testosterone plus cortisol. Bars are 50 μm.

This study confirms many hypotheses concerning reproductive activity in male *A. stuartii*. The accessory glands were sensitive to the presence of testosterone and the testes were, in general, not responsive. Testicular mass did not change with hormone treatment, although testicular width decreased with cortisol treatment. Glucocorticoids, not mineralocorticoids, have been shown to diminish testosterone release from the testes in rats and the protection of the Leydig cells from the actions of corticosterone appears to be mediated by 11-β-hydroxysteroid dehydrogenase, the enzyme active in allowing corticoid specificity in the medullary region of the kidney (Monder *et al.* 1994a, Monder *et al.* 1994b, Monder *et al.* 1994c).

However, in *A. stuartii*, the testicular morphology and mass did not alter with either cortisol or testosterone treatment, suggesting that the decrease in scrotal width is extragonadal. On dissection, the scrotal tissues of cortisol treated groups did not contain as much surrounding fat and retractor muscles as was observed in males without cortisol treatment. The external appearance was also more degenerated in many of the cortisol treated males. Some of the metabolic actions of cortisol include hepatic gluconeogenesis, hyperglucagonaemia and centripetal obesity (Tepperman and Tepperman 1987). The widespread effects of cortisol may also be affecting the scrotal tissue surrounding the testes, thus diminishing scrotal width in cortisol treated males. The testicular mass and scrotal width did not differ between saline and testosterone only treated males, supporting the hypothesis of the relative insensitivity of the testes to the presence of testosterone in *A. stuartii*.

The similar testicular mass and morphology in all males, and their insensitivity to hormone treatment, concurs with some other studies on marsupials, and differs from some studies on placentals. In other mammals, testicular mass and morphology often correspond positively with plasma testosterone concentrations (Zaime *et al.* 1992). In marsupials, however, this is not always the case. Androgen concentrations are not correlated with testicular size in the koala (*Phascolarctos cinereus*, Cleva *et al.* 1994), the tammar (*Macropus eugenii*, Inns 1982), the white-belly opossum (*Didelphis albiventris*, de Queiroz *et al.* 1995) and in *Antechinus* (*A. minimus*, Wilson and Bourne 1984, *A. stuartii* Kerr and Hedger 1983), but do correlate with

scrotal width in the eastern quoll (*Dasyurus viverrinus*, Bryant 1986). Moreover the spermatogenic activity of many marsupials is not associated with seasonal androgen cycles (Curlewis 1991, Gemmell *et al.* 1985, Inns 1982, Kerr and Hedger 1983, Todhunter and Gemmell 1987, Wilson and Bourne 1984).

The present study confirms the pattern of independence of spermatogenic activity and peak androgen concentrations seen in many marsupials. Testosterone administration had no effect on the spermatogenesis of *A. stuartii*, even though it is believed to be essential for promoting spermatogenesis in many mammals (McLachlan *et al.* 1996). Testosterone secretion occurs from the Leydig cells and is stimulated by luteinising hormone (LH, Rommerts 1990) although follicle stimulating hormone (FSH) may be necessary for the maturation of Leydig cells (Kerr and Sharpe 1985). Early germ cell development and later spermiogenesis is dependent on the secretion of testosterone in other mammals (McLachlan *et al.* 1996). However, low testicular concentrations of testosterone may be ten-fold higher than peripheral concentrations and still be sufficient to maintain testicular activity (Rommerts 1988, Zirkin *et al.* 1989). The testicular lymph of rats can have 15-35 times higher concentrations of testosterone than the venous drainage, and the concentration gradients are higher from the Leydig cells to the peripheral circulation (Rommerts 1988). Moreover spermatogenesis can be maintained on an 80% reduction of testosterone concentrations in other mammals (Zirkin *et al.* 1989). This suggests that spermatogenic activity in marsupials, and especially in *A. stuartii*, may be locally regulated by the mature Leydig cells.

This contrasts with the epididymides and accessory reproductive tract, which rely on significantly higher concentrations of testosterone to maintain cellular activity. The reliance of the accessory reproductive tract of mammals on testicular activity is well known (Heller 1930, 1932, Leydolph 1930, Moore *et al.* 1930, Rubin 1944, Wells 1936).

In *A. stuartii* the epididymis was differentially sensitive to testosterone administration. The caput end of the epididymis did not significantly change in epithelial height or volume, although the values for this time of year (early June) were intermediate between the May and July values (Chapter 5). Taggart *et al.* (1993) suggest that the epithelial cells of the caput epididymis are more sensitive to androgen

levels than the cells of the caudal regions. This was based on the early development of the epididymis, where the anterior end develops earlier in association with greater blood supply to the anterior end, delivering more androgens to this end of the epididymis (Taggart *et al.* 1993). However, the present study suggests that the caput end of the epididymis is less sensitive to testosterone administration than the caudal end, where significant increases in epithelial height and volume were directly related to testosterone administration (Figure 8.3). Previous studies suggest that the morphology of the caudal end makes sperm storage unlikely in the male, although it may serve to limit the number of sperm released on ejaculation, to maximise the number of successful matings of the males (Taggart and Temple-Smith 1989, 1990, 1994, Taggart *et al.* 1993). The results of the present study suggest that the development of the caudal end is under control of testosterone in preparation for the mating period.

In other marsupials the epididymal mass can change seasonally, although the response to testosterone is less clear. The epididymides of the brush-tail possum, *T. vulpecula*, and *A. stuartii* have a small, but significant, seasonal mass increase in the breeding season (Curlewis and Stone 1985). Other marsupials demonstrate no seasonal change in epididymal mass (Curlewis 1991, de Queiroz *et al.* 1995, Fletcher 1985, Inns 1982, Jones 1989). However segmental differences in sensitivity to androgen administration and also in 5α-reductase activity are present in *M. eugenii* (Jones *et al.* 1988).

The conversion of testosterone to dihydrotestosterone (DHT) by 5α-reductase is believed to be an important mechanism of testosterone activity in the reproductive tract of mammals and, in the prostate, absence of 5α-reductase causes cell death (Rittmaster *et al.* 1995). High activity of 5α-reductase has been reported in the epididymides of marsupials (Cook *et al.* 1978, Curlewis and Stone 1985, Jones *et al.* 1988), and in the tammar (*M. eugenii*) this is not associated with sensitivity to testosterone. Paradoxically there is much evidence to suggest that the prostate is far more sensitive to the actions of testosterone, even though there is less 5α-reductase and little conversion of testosterone to DHT (Cook *et al.* 1978, Curlewis and Stone 1985, Jones *et al.* 1988). This has perplexed some authors and there is some agreement that the prostate may be supported by

androgens transported along the excurrent ducts of the testes into the urethra (Cook *et al.* 1978, Jones 1989, Temple-Smith 1984). However, in hamsters the testes are the main source of circulating DHT, and conversion of testosterone to DHT in peripheral organs appears to be negligible (Aerchl and Nieschlag 1995). Perhaps DHT is converted from testosterone by 5α-reductase in the epididymides of marsupials and then transported to the accessory reproductive tract by the excurrent ducts or venous drainage.

Testosterone administration to castrate tammars caused an overshoot of the mass of Cowper's glands and prostate compared to intact males (Jones *et al.* 1988). The epididymis of the same males did not return to intact levels with testosterone administration, although the epithelial heights of the caudal end were closer to values for intact males (Jones *et al.* 1988). The greater sensitivity to testosterone of the caudal end of the epididymis in *A. stuartii* was also evident in the present study, as was the pronounced sensitivity of the prostate and Cowper's glands to testosterone administration.

Close association between testosterone concentration and prostate mass and activity has been observed in other marsupials (Cook *et al.* 1978, Inns 1982, Wilson and Bourne 1984), and in *A. stuartii* (Bradley *et al.* 1980). The present study supports this, but also includes the effect of cortisol on the reproductive tract. Like testosterone, cortisol administration did not affect the seminiferous tubules. However, in contrast to testosterone, administration of cortisol only had no significant effect on the caudal epididymis, the prostate or the bulbourethral glands. Addition of cortisol to testosterone administration caused a significant negative interaction between the two hormones, such that cortisol diminished the hypertrophic effects of testosterone only administration. The reduced effect of the combination of the two hormones produced significant correlations with the seasonal study. The masses of both the bulbourethral glands and the prostate of the testosterone plus cortisol group were similar to those found by Woolley (1966) and Bradley *et al.* (1980) for animals during the breeding season. The masses of both the bulbourethral glands and the prostate of the testosterone only group represents an overshoot of the normal weights, and in this

respect this finding is similar to that of Jones *et al.* (1988) for the tammar.

The morphology of the accessory glands reflected the changes in mass. Both the bulbourethral glands and the prostate of testosterone only males had considerably more secretory material in the lumina of the tubules than the testosterone plus cortisol males (see Figures 8.9, 8.10). The content and appearance of the prostate and the bulbourethral glands of the testosterone plus cortisol males are similar to those seen in the males from August in the seasonal study (Chapter 5).

It would appear that cortisol reduces the effect of testosterone on the secretory activity of the cells produced by testosterone. In other mammals, cortisol affects the LH frequency and amplitude in the many studies that have involved females (Dobson and Smith 1995, Loriaux and Nieman 1990, Michael and Cooke 1994, Tepperman and Tepperman 1987). Adrenal hyperactivity caused a reduction in ovarian and uterine weight and disrupted the ovarian cycle of rats (Christian 1964a, b).

Studies on male mammals have found that seminal vesicle and prostate weights are inversely related to adrenal activity (Christian 1955a, b; Andrews *et al.* 1972). ACTH administration stimulates adrenal activity and also arrests testicular development in *Peromyscus leucopus* (Christian *et al.* 1965) and *Microtus pennsylvanicus* (Pasley and Christian 1971). In rats (*Rattus norvegicus*) glucocorticoids suppress Leydig cell activity, either directly by the hypothalamic-pituitary-gonadal system, or by direct action on the Leydig cells (Monder 1994c). These findings contrast with those of the present study, where cortisol administration did not affect testicular activity.

Moreover, glucocorticoids have been found to halve the plasma concentration of testosterone in men without changing steroid binding globulin capacity and, in baboons, the testicular response to LH is attenuated by high circulating concentrations of cortisol (Loriaux and Nieman 1990, Sapolsky 1985). The present study demonstrated that cortisol affected the accessory reproductive tract, but did not disrupt testicular activity, nor did it alter the morphology of the prostate and bulbourethral glands, which were indistinguishable from those from the saline group. Cortisol only administration caused a small, but

significant rise in prostate and bulbourethral mass (Table 8.1), indicating some interaction between corticosteroids and androgen actions. However, the main effect of cortisol administration observed in the present study was to negate the hypertrophic stimulation of the accessory reproductive tract by testosterone administration.

In conclusion, it can be seen that testosterone and cortisol affect the reproductive tract of *A. stuartii*. The seminiferous tubules are insensitive to hormone action, but the further the target organs are away from the site of testosterone production the greater the sensitivity to the actions of the hormone. This pattern of hormone sensitivity is similar to other marsupials. Moreover, the morphology and morphometric changes mimic those seen in males in August, the time of reproduction in *A. stuartii*.

The present study has had two main aims, both of which were achieved. The first was to determine whether the spermatogenic cycle and accessory gland development were separate. The second aim was to determine the efficacy of the hormone administration and to observe any parallels between the hormone administration and the seasonal study.