Chapter 7

Effects of Testosterone and Cortisol on the Renal Morphology of Male *Antechinus stuartii*.

7.1 Introduction

The seasonal studies on *A. stuartii* have demonstrated that there are changes in renal structure and function in males (Chapter 3, 4). These changes in both structure and function are coincident with the period of the life cycle where the plasma concentrations of testosterone and then cortisol rise in males (Bradley *et al.* 1980, Lee *et al.* 1977, McDonald *et al.* 1981).

In the seasonal study, glomerular filtration rate (GFR) declined significantly in males in July and August, and there was a decline in GFRs of females prior to breeding (Chapter 3). Concentrations of urinary electrolytes were unchanged throughout the year in both sexes, although urea was significantly higher in females than males (Chapter 3). Plasma electrolytes were significantly different between groups, with plasma sodium and chloride being higher in males than females and higher in July and August than in February and May. Plasma potassium was significantly higher in February and May than in July and August (Chapter 3).

There were also significant renal structural changes in males from July and August (Chapter 4). While gross morphology was relatively unchanged in both sexes, there was considerable hypertrophy of the glomeruli, proximal tubules, distal straight tubules, distal convoluted tubules, cortical collecting ducts, and medullary collecting ducts. Many of these changes were found only in males from July and August, a time when endogenous plasma testosterone and cortisol levels are high (Bradley *et al.* 1980, McDonald *et al.* 1981).

The seasonal decline in GFR in male *A. stuartii*, and some of the paradoxical effects of electrolyte balance, were mimicked by the administration of testosterone, with some of the effects provoked by the actions of cortisol (Chapter 6). Treatment with testosterone

caused a significant decline in GFR, and urinary electrolyte concentrations were significantly reduced with cortisol treatment, although this was reversed by the addition of testosterone (Chapter 6). Water consumption was significantly increased in animals treated with cortisol, and urine volume also tended to increase, indicating that there were significant effects of cortisol on water and electrolyte balance. However, addition of testosterone reversed some of these effects (Chapter 6). Plasma sodium increased non-significantly in animals treated with cortisol, and plasma potassium increased in males treated with testosterone plus cortisol. While plasma sodium was higher in males in the seasonal study the plasma potassium was the inverse of what was seen in the seasonal study.

However, in general, the results obtained for the testosterone plus cortisol treatments were similar to those found for the seasonal study for males in July and August. It would appear that many of the seasonal changes in renal function and excretion are induced by the hormone testosterone, interacting with cortisol.

The seasonal changes in renal function were, in some part, explained by the administration of testosterone and cortisol. The significant changes in the renal structure of male *A. stuartii* are coincident with both the changes in renal function and the seasonal hormonal cycle. Therefore, it was decided to examine the kidneys of the males treated with either saline, testosterone only, cortisol only or testosterone plus cortisol. This chapter will explore the relationship between testosterone and cortisol administration and renal structure in *A. stuartii*.

7.2 Materials and methods

7.2.1 Animals

Animals were captured and maintained as described in Chapter 2 and Appendix II. They were held in captivity for 6-7 weeks after capture and had depot intramuscular hormone 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).

7.2.2 Histology

All animals were sacrificed and tissues processed as outlined in Chapter 2. Renal nomenclature follows Kriz and Bankir (1988).

7.2.3 Morphometrics

Kidney size, RMT, PMT were measured as described in Chapter 4. Kidney size and mass were compared to predicted values as outlined in Chapter 4. To be consistent with the seasonal study, the body mass used for predicted kidney mass was that recorded at the time of the final GFR measurement of each individual. Morphometry and PAS-Alcian blue analyses were made as defined in Chapter 4. The following counts and measurements were made:

i) Number of glomeruli per mm^2.

ii) Volumes of both superficial glomeruli and juxtamedullary glomeruli.

iii) Diameter of perpendicular cross-sections of proximal tubules (μm). Counts were made for proximal convoluted tubules (outer cortex tubules) and for proximal straight tubules (in the outer stripe of the outer medulla).

iv) Diameter of perpendicular cross-sections of distal straight tubules (or thick ascending limb) and of distal convoluted tubules in the outer stripe (μm).

v) Diameter of perpendicular cross-sections of outer cortex collecting tubules and of collecting tubules in the outer medulla (μm).

vi) Epithelial cell volumes (μm^3) of cells from the proximal tubules.

vii) Epithelial cell volumes (μm^3) of cells from the thin loops of Henle.

viii) Epithelial cell volumes (μm^3) of cells from the distal straight tubules.

ix) Epithelial cell volumes (μm^3) of cells from the cortical collecting tubules.

x) Epithelial cell volumes (μm^3) of cells from the collecting ducts.

7.2.4 Statistical analysis

The above data were analysed by two-way analysis of variance using testosterone and cortisol as the grouping variables, followed by Fishers PLSD pairwise tests where appropriate. Significance levels were $P < 0.05$ (Zar 1984)

7.3 Results

7.3.1 Gross morphology

The paired kidney masses were not significantly different between groups, although the interaction was close to significance (testosterone, NS, cortisol $P=0.10$, interaction $P=0.06$, Table 7.1). This indicated that there was a trend for kidney mass to increase with the administration of cortisol, although this appeared to be negated by the addition of testosterone (Table 7.1). The paired kidney masses of the testosterone only treatments were significantly lower than the mass predicted, and the paired kidney masses of the cortisol only treatments were significantly greater than the values predicted ($P<0.05$, Table 7.1). The paired kidney mass as a percentage of body mass was significantly different between treatments (testosterone $P<0.05$, cortisol $P<0.0005$, interaction NS). Males treated with testosterone had a significantly smaller percentage of body mass taken up by kidney mass (Fisher's PLSD test $P<0.025$) and cortisol treated males had a significantly greater percentage of body mass taken up by kidney mass (Fisher's PLSD test $P<0.0002$).

The mean kidney sizes did not differ significantly between groups (testosterone NS, cortisol $P= 0.12$, interaction NS, Table 7.1). The mean kidney sizes when adjusted for body mass (kidney size index) were significantly larger in all groups treated with cortisol, and smaller with testosterone (testosterone $P<0.05$, cortisol $P<0.02$, interaction NS, Table 7.1). The mean kidney size of males treated with testosterone only was significantly smaller than the predicted kidney size ($P<0.05$, Table 7.1). There were no differences between groups in RMT, PMT or MT (Table 7.1).

7.3.2 Light microscopy

The unipapillar kidneys were divided into cortex and outer and inner medulla with the outer medulla of a similar thickness to the cortex. The gross morphology of the kidneys of saline treated males was similar to that seen in males from this time of year in the seasonal study (Figure 7.1a, see Chapter 4). There were several pathological changes noted in the hormone treatment groups. The glomeruli of males treated with testosterone appeared hypertrophied and many juxtamedullary glomeruli contained a proliferation of proximal tubule-

Table 7.1 Gross renal morphology of *Antechinus stuartii* from testosterone and cortisol experiments.

Treatment	Paired kidney mass (g)	Predicted kidney mass (g)	Paired kidney mass (% of body mass)	Mean kidney size (mm)	Predicted kidney size (mm)	Kidney size index	Relative medullary thickness	Percentage medullary thickness (%)	Medullary thickness (mm)
Saline	0.324 ± 0.010	0.346 ± 0.011	1.094 ± 0.036 **c**	6.087 ± 0.069	6.245 ± 0.069	0.471 ± 0.010 **a**	7.75 ± 0.45	79.50 ± 0.83	4.698 ± 0.257
Testosterone	0.377 ± 0.020	0.422 ± 0.013 *	0.990 ± 0.034 **d**	6.370 ± 0.120	6.665 ± 0.067 *	0.422 ± 0.005 **b**	7.45 ± 0.24	79.59 ± 0.96	4.663 ± 0.193
Cortisol	0.407 ± 0.035	0.311 ± 0.021 *	1.383 ± 0.078 **a**	6.388 ± 0.137	6.223 ± 0.122	0.488 ± 0.017 **a**	7.66 ± 0.37	80.66 ± 0.83	4.484 ± 0.180
Testosterone and cortisol	0.371 ± 0.012	0.359 ± 0.024	1.218 ± 0.081 **b**	6.395 ± 0.052	6.309 ± 0.143	0.477 ± 0.021 **a**	7.33 ± 0.36	80.25 ± 1.30	4.651 ± 0.221
Testosterone Cortisol Interaction	NS P=0.10 P=0.06	-	P<0.05 P<0.0005 NS	NS P=0.12 NS	-	P<0.05 P<0.02 NS	NS NS NS	NS NS NS	NS NS NS

Table 7.1 Gross morphology of the kidneys of *A. stuartii* treated with either saline, testosterone only, cortisol only or testosterone plus cortisol. Data are means and standard errors of the mean. Two-way ANOVA results are below values in each column. Asterisks indicate that the actual value is significantly different from the predicted value ($P<0.05$), and different letters in a column indicate that the values are significantly different from one another ($P<0.05$). Kidney size index is the kidney size divided by the body mass, expressed as a proportion of one, and arcsine transformed to approximate normality (Zar 1984).

Figure 7.1 Cortex of the kidney of males treated with a) saline, b) testosterone only, c) cortisol only, and d) testosterone plus cortisol. Scale bars are 50 μm.

Note the hypertrophied proximal tubules in b) and d) and the distended, thin-walled distal tubules in c). The glomeruli are hypertrophied in b), c), and d). Arrows indicate glomeruli, and asterisks indicate the distal tubules.

A
B
C
D

like cells in the Bowman's capsule. There was also considerable hypertrophy of glomeruli in animals treated with cortisol, and this was often associated with the presence of precipitated material between the glomerular tuft and the Bowman's capsule.

The proximal tubules were hypertrophied in animals treated with testosterone (Figures 7.1b, 7.1d). Precipitate was noticed in the distal tubules and the collecting ducts of many individuals treated with cortisol only, and this was often associated with a 'disintegrated' appearance of the tubules, often with extremely distended lumina of the tubules (Figure 7.1c). Some areas of the cortex in some individuals were extensively disrupted by damaged tubular tissue (Figure 7.1c).

7.3.3 Morphometry

i) The numbers of glomeruli per mm^2 of the cortex were significantly different between groups (testosterone $P<0.0001$, cortisol $P<0.0001$, interaction $P<0.002$, Table 7.2, Figure 7.2). There were significantly fewer glomeruli per mm^2 in animals treated with testosterone only, cortisol only, or testosterone and cortisol (Figure 7.2). The numbers of glomeruli per mm^2 in the saline treated animals were similar to those found in the seasonal study in May (Chapter 4).

ii) The volumes of the superficial glomeruli were significantly different between groups (testosterone NS, cortisol $P<0.0001$, interaction $P<0.05$, Table 7.2). Both cortisol only and testosterone only administration significantly increased glomerular volume, although the effects did not appear to be additive (Table 7.2, Figure 7.3a). The administration of cortisol caused a significant increase in the volume of the juxtamedullary glomeruli (testosterone $P=0.15$, cortisol $P<0.0001$, interaction NS, Table 7.2, Figure 7.3b). Values obtained for saline treated males were similar to values obtained in the seasonal study, and values obtained for cortisol only and for testosterone plus cortisol treatments were similar to values obtained for males in July and August (see Chapter 4). The juxtamedullary glomeruli were significantly larger than the superficial glomeruli for all individuals (Paired t-test, $P<0.0001$).

iii) The diameters of the proximal convoluted tubules from the

Table 7.2 Glomerular numbers per mm^2 and glomerular volumes of *A. stuartii* from testosterone and cortisol experiments.

Treatment	Glomerular number / mm^2 of cortex	Superficial glomerular volumes $\mu m^3 \times 10^5$	Juxtamedullary glomerular volumes $\mu m^3 \times 10^5$
Saline	17.33 ± 0.18 **a**	1.69 ± 0.09 **c**	3.51 ± 0.14 **b**
Testosterone	12.98 ± 0.26 **c**	2.07 ± 0.08 **b**	3.90 ± 0.21 **b**
Cortisol	14.13 ± 0.49 **b**	2.69 ± 0.21 **a**	4.89 ± 0.41 **a**
Testosterone plus Cortisol	12.40 ± 0.45 **c**	2.42 ± 0.11 **a**	5.36 ± 0.29 **a**
Testosterone Cortisol Interaction	P<0.0001 P<0.0001 P<0.002	NS P<0.0001 P<0.05	P=0.15 P<0.0001 NS

Table 7.2 Glomerular numbers per mm^2 and glomerular volumes of *A. stuartii* from testosterone and cortisol experiments. Data are means and standard errors of the mean. 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 ($P<0.05$).

Figure 7.2

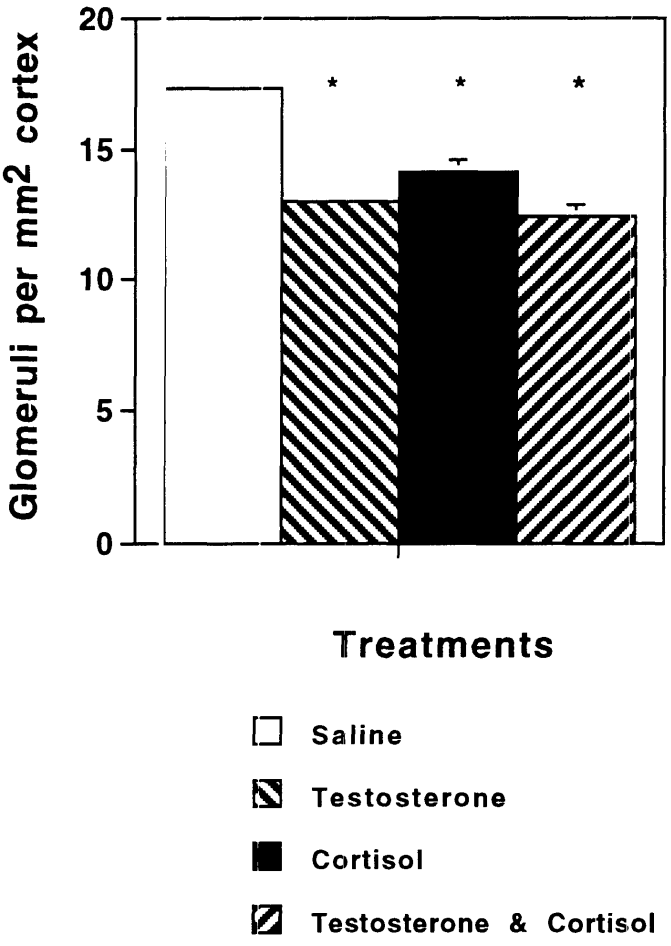


Figure 7 2 The number of glomeruli per mm^2 of renal cortex. Values are means± standard errors of the mean. Asterisks indicate means that were significantly smaller than the saline treated males.

Figure 7.3a

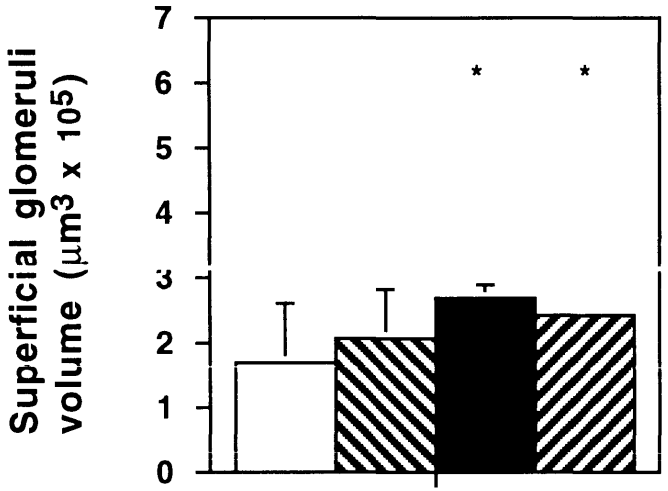
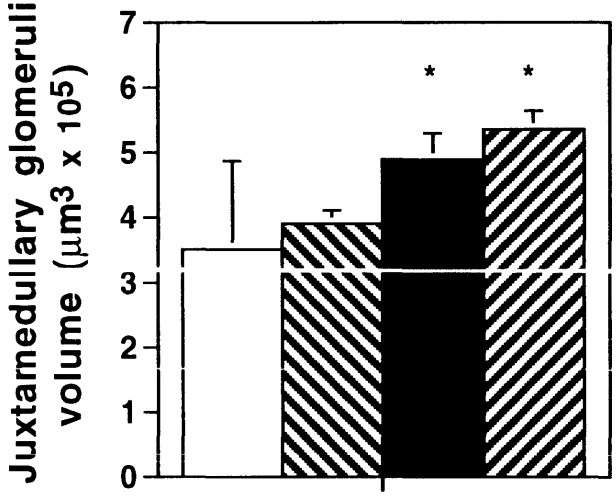


Figure 7.3b



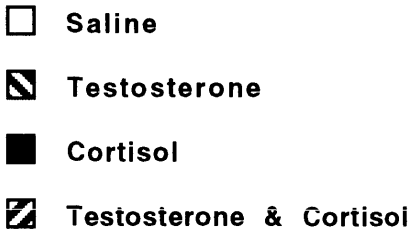


Figure 7.3 Volumes (μm^3) of a) superficial glomeruli, and b) juxtamedullary glomeruli. Values are means ± standard errors. Asterisks indicate means that were significantly larger than those from other treatment groups.

cortex were significantly increased with the administration of testosterone (testosterone P=0.05, cortisol P=0.12, interaction NS, Table 7.3). The diameters of the proximal straight tubules from the outer stripe were significantly increased with the administration of testosterone (testosterone P<0.05, cortisol P=0.12, interaction P=0.14, Table 7.3).

iv) The diameters of the distal straight tubules differed significantly between treatment groups (testosterone P<0.0005, cortisol NS, interaction P<0.05, Table 7.3). Testosterone significantly increased the diameters of the tubules, although this was reduced by the addition of cortisol to the administered testosterone (Table 7.3). The values obtained in this study were higher than those obtained for the seasonal study (Table 7.3, Chapter 4).

The diameters of the distal convoluted tubules were significantly increased by both testosterone and cortisol treatment (testosterone P<0.01, cortisol P<0.0001, interaction P<0.001, Table 7.3). Tubular diameters from males treated with testosterone were not as large as those from males treated with cortisol, and the tubules from the testosterone plus cortisol animals were intermediate between the testosterone only and the cortisol only value (Table 7.3). The values for the saline treatment were similar to values found for males in May in the seasonal study (Table 7.3, Chapter 4). Values for the testosterone plus cortisol treated males were similar to those found for males from August, but higher than males from July (Table 7.3, Chapter 4).

v) The diameters of the cortical collecting ducts were significantly increased by the administration of cortisol and testosterone, with values from males treated with testosterone only, cortisol only, and testosterone plus testosterone significantly higher than those from males treated with saline (testosterone NS, cortisol P<0.05, interaction P<0.01, Table 7.3). The values of the saline treated males were similar to those from animals in May in the seasonal study. The values of the testosterone plus cortisol treated males were similar to those of males from August, but slightly higher than those of males from July in the seasonal study (Table 7.3, Chapter 4).

The diameters of the collecting ducts from the outer medulla

Table 7.3 Tubular diameters of renal components in *Antechinus stuartii* from testosterone and cortisol experiments.

Treatment	Diameter of proximal tubules (cortex μm)	Diameter of proximal tubules (outer stripe μm)	Diameter of distal straight tubules (μm)	Diameter of distal convoluted tubules (μm)	Diameter of collecting ducts (cortex μm)	Diameter of collecting ducts (outer medulla μm)
Saline	34.2 ± 0.9 **b**	26.9 ± 0.7 **b**	26.0 ± 0.5 **c**	24.8 ± 0.8 **c**	21.7 ± 0.4 **b**	23.5 ± 1.0 **b**
Testosterone	37.5 ± 0.9 **a**	30.2 ± 0.4 **a**	29.0 ± 0.3 **a**	29.3 ± 0.5 **b**	24.5 ± 0.4 **a**	24.6 ± 0.6 **b**
Cortisol	37.0 ± 1.4 **b**	29.7 ± 1.3 **b**	27.3 ± 0.5 **b**	31.1 ± 0.6 **a**	25.0 ± 0.9 **a**	27.8 ± 0.5 **a**
Testosterone plus Cortisol	38.0 ± 0.6 **a**	30.2 ± 0.9 **a**	28.3 ± 0.5 **a,b**	30.4 ± 0.6 **a,b**	23.9 ± 0.6 **a**	27.0 ± 0.6 **a**
Testosterone Cortisol Interaction	P=0.05 P=0.12 NS	P<0.05 P=0.12 P=0.14	P<0.0005 NS P<0.05	P<0.01 P<0.0001 P<0.001	NS P<0.05 P<0.01	NS P<0.0001 NS

Table 7.3 Tubular diameters of renal components in *Antechinus stuartii* from different treatment groups. Data are means and standard errors of the mean. 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 (P<0.05).

were significantly increased by the administration of cortisol (testosterone NS, cortisol $P<0.0001$, interaction NS). The values obtained for the saline treated males were not significantly higher than those for males from May in the seasonal study, and values for males treated with cortisol only were higher than those obtained from males from July and August, although those treated with cortisol plus testosterone were similar to those from August (Table 7.3, Chapter 4).

vi) The proximal tubule cell volumes were significantly increased by the administration of testosterone (testosterone $P<0.001$, cortisol NS, interaction NS, Table 7.4, Figure 7.4). The values obtained from males from both groups treated with testosterone were similar to values obtained from males in July and August in the seasonal study (Table 7.4, Chapter 4).

vii) There were no significant differences found between treatments in the cellular volume of the thin loops of Henle (testosterone NS, cortisol NS, interaction NS, Table 7.4). The cellular volumes were all smaller than those found in the seasonal study (Table 7.4, Chapter 4).

viii) The distal straight tubule cell volumes were significantly increased by the administration of testosterone (testosterone $P<0.0005$, cortisol NS, interaction NS, Table 7.4, Figure 7.5). The cellular volumes were all larger than those found in the seasonal study (Table 7.4, Chapter 4).

ix) The cortical collecting duct cell volumes were significantly increased by the administration of testosterone (testosterone $P<0.005$, cortisol NS, interaction NS, Table 7.4, Figure 7.6). The cellular volumes were all larger than those found in the seasonal study (Table 7.4, Chapter 4).

x) The collecting duct cell volumes were significantly decreased by the administration of testosterone (testosterone $P<0.01$, cortisol NS, interaction NS, Table 7.4, Figure 7.7). The cellular volumes of all males were similar to those found in May in the seasonal study (Table 7.4, Chapter 4).

7.4 Discussion

The present study demonstrates that the renal structure of male *A. stuartii* is significantly modified by the administration of cortisol and

Table 7.4 Cellular volumes (μm^3) of renal components in *Antechinus stuartii* from testosterone and cortisol experiments.

Treatment	Proximal tubule cell volume (μm^3)	Thin loops of Henle cell volume (μm^3)	Distal straight tubule cell volume (μm^3)	Cortical collecting duct cell volume (μm^3)	Collecting ducts cell volume (μm^3)
Saline	990.4 ± 53.0 **b**	13.6 ± 1.3	483.4 ± 31.3 **b**	303.5 ± 14.2 **b**	839.4 ± 51.2 **a**
Testosterone	1281.1 ± 91.9 **a**	15.3 ± 1.3	632.5 ± 43.6 **a**	418.1 ± 31.2 **a**	729.1 ± 58.4 **b**
Cortisol	885.0 ± 82.8 **b**	13.9 ± 2.4	485.0 ± 29.7 **b**	313.7 ± 36.6 **b**	827.6 ± 55.7 **a**
Testosterone plus Cortisol	1189.5 ± 70.4 **a**	12.6 ± 1.8	639.6 ± 33.7 **a**	416.9 ± 43.3 **a**	622.0 ± 29.9 **b**
Testosterone Cortisol Interaction	$P<0.001$ NS NS	NS NS NS	$P<0.0005$ NS NS	$P<0.005$ NS NS	$P<0.01$ NS NS

Table 7.4 Cellular volumes (μm^3) of renal components in *Antechinus stuartii* from different treatment groups. Data are means and standard errors of the mean. 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 ($P<0.05$).

Figure 7.4

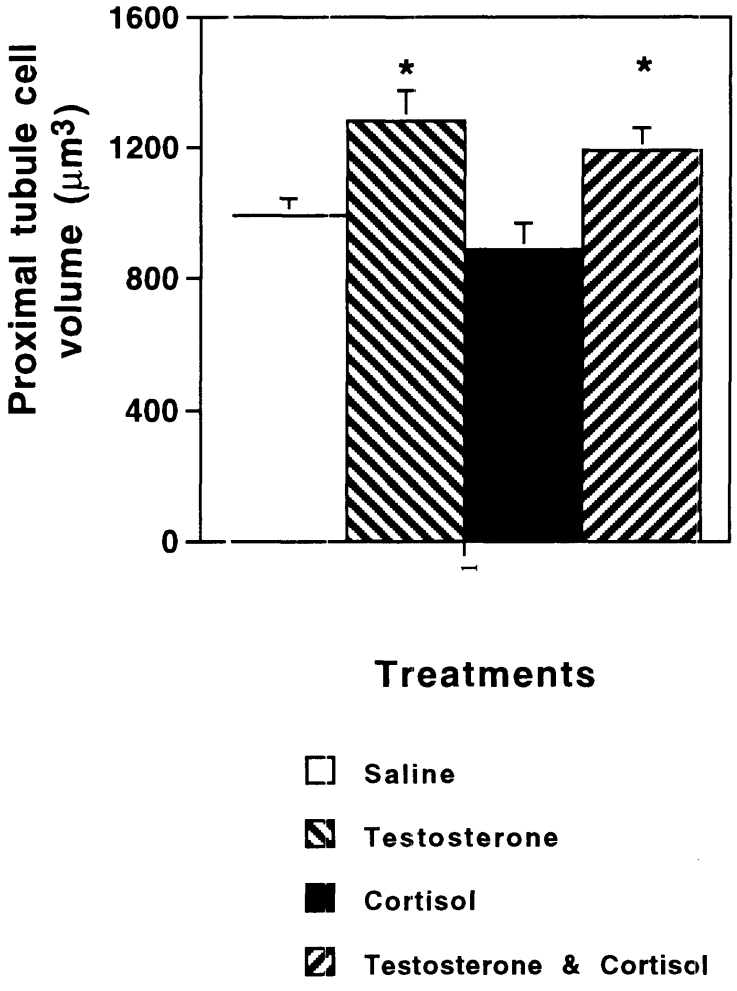


Figure 7.4 Proximal tubule cell volumes (μm^3). Values are means ± standard errors. Asterisks indicate means which were significantly larger than those from other treatment groups.

Figure 7.5

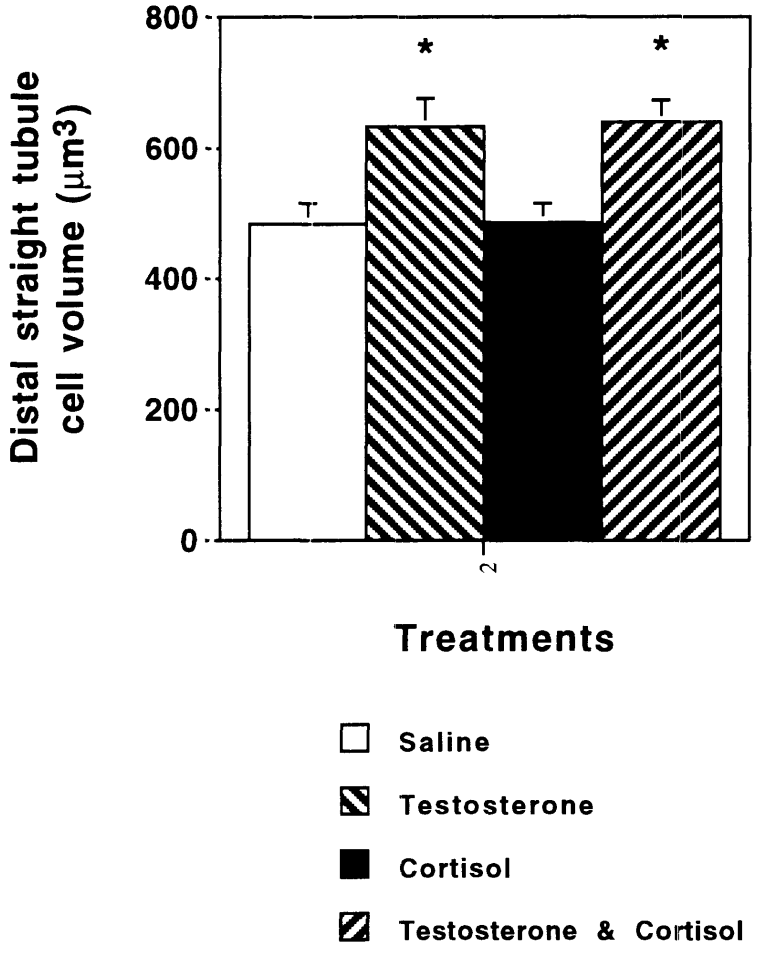


Figure 7.5 Distal straight tubule cell volumes (μm^3). Values are means ± standard errors. Asterisks indicate means which were significantly larger than those from other treatment groups.

Figure 7.6

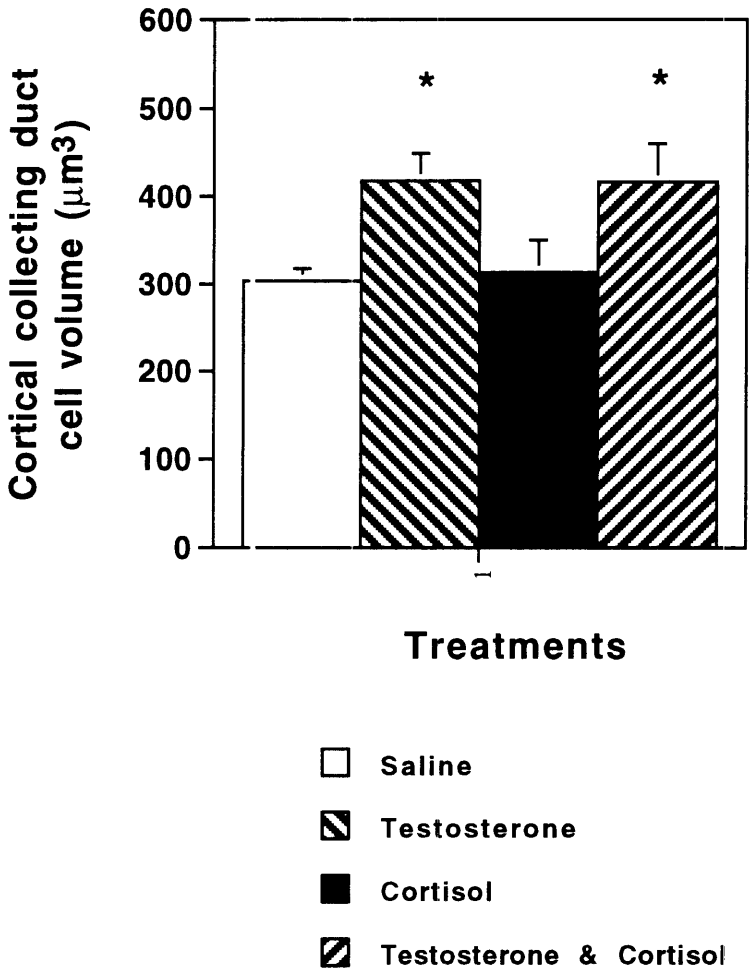


Figure 7.6 Cortical collecting duct cell volumes (μm^3). Values are means ± standard errors. Asterisks indicate means that are signifcantly larger than those from other treatment groups.

Figure 7.7

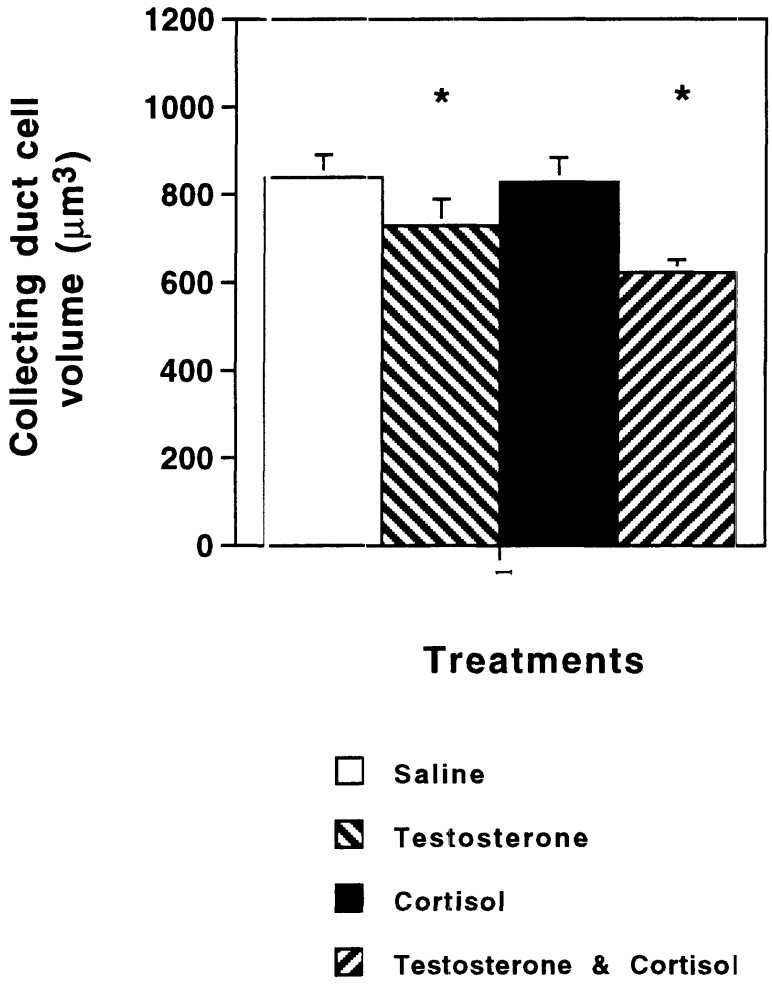


Figure 7.7 Collecting duct cell volumes (μm^3). Values are means ± standard errors. Asterisks indicate that the means were significantly smaller than those from other treatment groups.

testosterone in doses that mimic the naturally occurring hormone changes. Both testosterone and cortisol affected renal morphology and, in the main, their actions involved hypertrophy of renal tissue. Many of these modifications parallel the changes seen in the renal structure of males from July and August in the seasonal study.

Some of the gross morphology observed in the hormone study was similar to the seasonal study, where the gross morphometrics, which include RMT, PMT and MT, did not change with treatments. There were, however, differences between actual and predicted values of the paired kidney mass and mean kidney size between treatments. The kidney mass of testosterone only treated males was lower than the predicted value and the opposite was seen in the cortisol only males. Testosterone plus cortisol treatment summed the two effects, and thus no differences between actual and predicted values were found. This contrasted with the seasonal study, where the kidney mass of the August males was less than the predicted value, similar to the result of testosterone only males. The observations for kidney size were not the same as for kidney mass. The changes for testosterone only males were similar, but no effects of cortisol were observed. Again, values for testosterone only males were similar to the seasonal study.

The contrasting effects of cortisol and testosterone administration are also observed in the paired kidney mass as a percentage of body mass. Testosterone reduced this ratio, cortisol increased it, and the combination of the two produced a ratio similar to that seen in males in the seasonal study. It would appear that the anabolic effects of the testosterone only treatment (see Chapter 6) on the skeleto-muscular system are not as pronounced in the kidney. There is not as much hypertrophy in the kidney compared to the increase in body mass with testosterone only treatment (see Chapter 6 for body weights in testosterone only males). This contrasts with a study by Selye (1939), who found that, in *M. musculus* treated with testosterone propionate, kidney mass increased, but body mass did not. Kochakian *et al.* (1948) found that testosterone propionate administration did not affect the body mass or kidney mass of hamsters.

There does appear to be an absolute increase in kidney mass with body mass in cortisol only treated males, which is also evident in the testosterone plus cortisol treated males. An increase in kidney mass relative to body mass has been found in other studies using glucocorticoid administration or stimulation by ACTH (Christian *et al.* 1965, Pasley and Christian 1971, Selye 1950, Selye and Bois 1956). Some of these studies found that body mass did not increase with treatment, or decreased (Christian *et al.* 1965, Pasley and Christian 1971, Selye and Bois 1956). Final body mass of cortisol treated males was not significantly different from those from the saline treated males in the present study see Chapter 6).

The gross hypertrophy of the renal tissue is, however, reflected in the differential hypertrophy of components of the nephron. Both testosterone and cortisol affect glomerular number per unit area, but the mechanisms are different for each hormone treatment. For both groups treated with testosterone, the proximal tubular diameter increased, although the superficial glomeruli were less hypertrophied than those of the cortisol only treated males. Even though glomeruli in the cortisol only males were more hypertrophied than for testosterone only males, there were more glomeruli per mm^2 in cortisol only treated males than in both groups of testosterone treated males. This reflects the unchanged proximal tubules in cortisol only males. However the glomerular numbers per mm^2 obtained for the testosterone plus cortisol males paralleled those seen in males from August and July in the seasonal study. This indicates that in male *A. stuartii*, the appearance of the cortex was selectively affected by the hormones, with the glomeruli modified by cortisol, and proximal tubule morphology modified by testosterone. Moreover, the proximal tubule-like cells seen in the Bowman's capsule appeared to be due mainly to the actions of testosterone. This finding is similar to that of other studies on *M. musculus* (Crabtree 1940, 1941b, Selye 1939).

Testosterone treatment was also the main cause of hypertrophy of the proximal tubules, as evidenced by the increase in tubular diameter, which resulted from actual cellular hypertrophy. The cellular hypertrophy as a result of testosterone administration was also seen in the cells of the distal straight tubules, and cortical collecting ducts. The hypertrophy of these cells paralleled changes seen in these parts

of the nephron of males in July and August in the seasonal study. Proximal tubule hypertrophy due to the presence of testosterone has also been observed in other studies (Crabtree 1940, 1941b, Oudar *et al.* 1991, Selye 1939).

However, not all changes were due to cellular hypertrophy. The increase in diameters of the distal straight tubules, the cortical collecting duct and medullary collecting duct were influenced by cortisol as well as testosterone. This was due to distention of the tubules. The cortical collecting duct tubular disruption and expansion of the lumina, due to the actions of cortisol only administration, are seen in Figure 7.1c. Minor expansion of this section of the nephron is seen in Figure 7.1d. The results of the combination of testosterone plus cortisol administration mimic the phenomena found for males in August and July, where the diameters of these tubules increased, but cellular hypertrophy was associated with luminal increase as well (see Figure 4.2d, Figure 7.1d).

The extensive disruption of the cortex and medulla seen in some males treated with cortisol only has been seen in other studies on stress and renal failure in mammals. All of the studies involved mammals with social stress and consequent death as part of the life cycle, and all showed tubular disintegration as well as glomerular pathology (Andrews *et al.* 1972, Andrews *et al.* 1975, Barnett *et al.* 1975, Christian *et al.* 1965, Munday and Blane 1961, von Holst 1972a). Moreover the renal failure exhibited by these mammals was often swift, with some dying within a week of persistent social stress (Barnett *et al.* 1975, von Holst 1972a). In the present study, two of the cortisol only males and one of the testosterone plus cortisol males died before the end of the experiment. All these animals exhibited signs of "die-off" pathologies described by other studies of *A. stuartii* in the wild (Barker *et al.* 1978, Bradley *et al.* 1980, Moore 1974). Other cortisol only males had gastrointestinal bleeding, bloody urine and proliferation of interna parasites, features usually seen in dying wild males (see Chapter 6, Barker *et al.* 1978, Bradley *et al.* 1980, Moore 1974). The resistance to the debilitating effects of cortisol in wild males, was generally mimicked, in the present study, by adding testosterone to the cortisol administration. This suggests that

testosterone effects on the kidney may ameliorate the damaging effects of cortisol.

Cortisol treatment also induced the formation of precipitate in glomeruli and in the distal and collecting tubules of many individuals. The tubule expansion in these animals seems to have been due to urine accumulation in the tubules. These individuals produced more urine in the GFR experiments (Chapter 6), and it would appear that the cortisol influence on renal function also translates to a structural correlate. Precipitated material in all of the above structures has been observed in studies on the renal pathology of stress (Andrews *et al.* 1972, Andrews *et al.* 1975, Barnett *et al.* 1975, Christian *et al.* 1965, Munday and Blane 1961, von Holst 1972a). It would appear that the addition of testosterone to the cortisol administration in the present study prevents the reduction in tubular reabsorption seen in the cortisol only males. Moreover the structural similarities between the testosterone plus cortisol treated males and those in the seasonal study from August suggest that the absence of renal failure in these males may be due to the hypertrophic effects of testosterone.

However, the parallelism between the seasonal study and the hormonal study was not always observed. The cellular volumes of the thin loops of Henle were smaller in the hormonal study. This was also true of the cellular volumes of the collecting ducts of the outer medulla. Moreover the testosterone treated males had smaller cellular volumes than the other two groups, and this was the reverse trend to the seasonal study where the cellular volumes from the collecting ducts of males from August and July were larger than at other times of year. The cortical collecting duct cells were larger in all the hormonal groups than in the seasonal study, although the increase in volume in the testosterone treated males paralleled the changes seen in the males from August and July.

Why there should be these discrepancies between these two studies is unclear. Other cellular volumes and the tubular diameter dimensions were similar between the two studies, eliminating measurement error because of the different times of data collection (the morphometric analyses were completed 18 months apart). While samples were collected and processed at different times, thus not excluding batch differences, the similarity of some morphometric

observations with the seasonal study excludes this as a reason for the differences. Thus the reasons for these differences remain unexplained.

Moreover, there were some differences in cellular volumes between the two studies. Cells from the collecting duct were larger in males from July and August, although they were smaller in males treated with testosterone, and the cell volumes from males treated with cortisol only were the same as those from saline treated males. It is unclear why this should occur when so many of the seasonal changes have direct parallels with the hormonal study. The hypertrophy in cells from the proximal tubule, distal straight tubule, and cortical collecting duct in males from July and August in the seasonal study, was reproduced by administration of testosterone in the present study.

However, the general trends of the hormonal study are that the different components of the nephron are sequentially affected by either testosterone or cortisol. As the nephron is traversed from the glomerulus to the collecting duct a pattern of hormonal action along the nephron is observed. The glomerular volume is increased by cortisol action and this is coupled with a replacement of the parietal lamina of the Bowman's capsule by a proliferation of proximal tubule like cells of some glomeruli under the influence of testosterone. The cells of the proximal tubules hypertrophy with testosterone administration, thereby increasing the tubular diameter. As the nephron leaves the cortex, the proximal tubules remain influenced by testosterone although the loops of Henle are not altered by hormone action. Returning toward the cortex, the nephron is again under hormonal influence. The cells of the distal straight tubules are hypertrophied due to testosterone action and the overall diameter of the distal straight tubule is affected by the interaction with cortisol. Even though there is no cellular hypertrophy in cortisol only treated males, cortisol increases the diameter of the tubule, although this increase is less than for testosterone treated males. This suggests that the diameter increase from cortisol only administration is due to tubular distention rather than cellular activity. Cortisol does, however, reduce the cellular hypertrophy caused by testosterone administration.

As the distal tubule convolutes, both cortisol and testosterone modify the appearance of the tubule. Testosterone administration increases tubular diameter. The diameters of the distal convoluted tubules are increased more by cortisol administration. The combination of testosterone plus cortsiol gives a diameter intermediate between testosterone and cortisol treatment, indicating that the material found in the lumina of the tubules of cortisol only treated males, which causes the tubular distention, is partly reabsorbed by the cells in the males who were also treated with testosterone. Although it could be argued that there may be less filtrate produced in the testosterone plus cortisol males than in the cortisol only males (see Chapter 6) the disturbed appearance of the cells of the distal tubule (Figure 7.1c) indicates that the precipitate is more likely to be a product of malfunctioning of distal tubule cells.

While the nephron continues in the cortex as the cortical collecting duct, the hypertrophic influences of testosterone remain evident. Testosterone causes cellular hypertrophy that translates into an increased tubular diameter. The cortical collecting duct is also affected by cortisol, where the diameters are significantly increased, although there is an interaction with testosterone, which reduces the distention of the tubules. This again indicates that cortisol and testosterone interact so that the disturbance of the functional integrity of the nephron by cortisol is modified by the hypertrophic effects of testosterone, which maintain the absorptive ability of the cell. As the collecting duct leaves the cortex, the hypertrophic effects of testosterone dissipate. The cells of the collecting duct are reduced in size and the diameters are unaffected by testosterone administration. Again cortisol does not affect cell size, but significantly increases tubular diameter. The failure to absorb luminal contents must be suspected as the reason for tubular distention.

The present study indicates that the influence of testosterone on the renal tissue is on cellular hypertrophy. The actions of testosterone do not appear to impair the absorptive function of the kidney, and it is clear from the renal tissue of the testosterone plus cortisol treated males that the disruptive effects of cortisol are reduced. Cortisol only treatment appears to cause a disruption to the absorptive function, where the distended lumina are often full of material. Moreover,

cortisol only individuals with advanced renal damage showed signs of cortical and medullary disintegration that would provoke further dysfunction in tubular reabsorption.

The reduction of the destructive effects of cortisol by the addition of testosterone leads one to suspect that the sensitivity of male *A. stuartii* to testosterone is one of the reasons that male *A. stuartii* do not show the dramatic signs of social stress seen in other mammals as part of their life history (Andrews *et al.* 1972, Andrews *et al.* 1975, Barnett *et al.* 1975, Christian *et al.* 1965, Munday and Blane 1961, von Holst 1972a,b). It is possible that testosterone induced hypertrophy of renal tissue allows male *A. stuartii* to live long enough to mate before the males succumb to the cascade of stress related illnesses, of which renal dysfunction may be a part.