CHANGES IN THE TESTES, EPIDIDYMIS AND PROSTATE GLAND OF THE RAMS INFECTED WITH *TRYPANOSOMA CONGOLENSE*

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SUMMARY

Ten rams inoculated intravenously with *Trypanosoma congolense* developed marked progressive anomalies in their reproductive organs during the infection period lasting 79 days. The testes became atrophic which was associated with marked decline in testicular weights and seminiferous tubular diameter and the development of varying degrees of non-inflammatory degeneration lesions in tubular germinal cells. The normal tall columnar prostate epithelium atrophied to low cuboidal and the cauda epididymis showed marked decline in sperm reserve. The changes in the testes, prostate and cauda epididymis in *T. congolense* infected rams elosely resembled those induced artificially in these organs by elevation of testicular temperature by scrotal insulation of uninfected rams. These findings suggest that the reproductive disorders induced by *T. congolense* in males might In part be a pyrexic effect induced by the infection which interferes with the thermoregulation of the gonads and associated tissues.

INTRODUCTION

It is well established that trypanosome-infected animals and man subsequently suffer from various kinds of reproductive disorders (Kaaya and Odour-Okelo, 1980; Akpavie *et al.*, 1987; Ikede *et al.*, 1988 Omeke and Anuora, 1992; Boly *et al.*, 1994; Mutayoba, 1995; Mutayoba *et al.*, 1994; 1995). Infected males display some alteration of sexual behaviour (Sekoni *et al.*, 1988) and a decrease of quantitative and qualitative semen parameters (Kumi-Diaka *et al.*, 1989; Sekoni *et al.*, 1990; Adeyemo *et al.*, 1990). However, the mechanisms involved in the impairment of sexual function during trypanosomiasis infection are not well understood (Mutayoba, 1995).

Several previous studies have shown that reproductive disorders induced by trypanosome infection depends on nature of infecting trypanosome species (Ikede *et al.*, 1988; Sekoni, 1994). Direct tissue damage by invading trypanosomes is probably one of the most important mechanism by which trypanosomes of the *T. brucie*-group cause deleterious effects on reproductive function. This is due to their tendency to localize extravascularly in interstitial spaces of several organs including the gonads, pituitary and hypothalamus where they provoke marked inflammatory changes. Several reports have also shown that *T. vivax* do invade and cause inflammation in tissues especially the heart, lymph nodes, cerebrospinal fluid (Masake, 1980; Emery *et al.*, 1980; Whitelaw *et al.*, 1988). However, *T. vivax* infections of the tissue appear to differ from those caused by *T. brucei*-group in that they do not localize throughout the

body of the host including the reproductive organs (Losos, 1986).

Although *T. congolense* induces progressive reproductive anomalies in infected host, the parasite does not usually leave the circulatory system after patency (Murray and Dexter, 1988, Abebe *et al.*, 1993). The parasite is only known to undergo an early extravascular phase in the skin and lymph nodes (Emery and Moloo, 1981) and has occassionally been shown to disseminate in the cerebro-spinal fluid (Masake *et al.*, 1984). Hence the mechanism by which *T. congolense* induces reproductive disorders still remain equivocal.

Animals suffering from trypanosomiasis develop chronic intermittent pyrexia with rises in temperature of between 1-5°C above normal rectal temperature depending on the virulence of infecting trypanosome strain and the virulence of the host (Losos, 1986). Such a rise in body temperature has been suggested to exert either a direct or an indirect effect on the function of the gonads and other associated tissues in infected animals (Ikede *et al.*, 1988; Omeke and Onuora, 1992). In recent studies Mutayoba (1995) has shown that *T. congolense* induces a rise in scrotal temperature of infected rams which could possibly interfere with testicular function frequently observed during the course of infection.

The aims of this study were to investigate the changes in the testes, cauda epididymis and prostate gland of the rams infected with *T. congolense*. Similar studies have only been described in *T. congolense* infected bulls and bucks (Ikede *et al.*, 1988; Sekoni, 1994). Similarly, the changes in the above organs was compared to those induced by artificial elevation of testicular temperature by scrotal insulation to assess whether the gonadal lesions occuring during trypanosome infection might in part be a pyretic effect.

MATERIALS AND METHODS

Animals

Twenty six Scottish Blackface rams aged eight months and weighing approximately 32 kg were used in these studies . Nineteen of these rams (10 infected and 9 controls) were also used to study the effects of trypanosomiasis on adrenal function and their management before and during infection as been described elsewhere (Mutayoba *et al.*, 1995). Ten rams were infected intravenously with approximately 4 x 10^5 *T. congolense* isolate 57/10 originally imported from the International Livestock Research Institute (ILRI), Kenya as IL-1180 (Dwinger *et al.*, 1987) and 10 rams served as uninfected controls. The changes in haematology, bodyweight, rectal temperature and parasitaemia levels in these rams have already been described (Mutayoba *et al.*, 1995). Scrotal insulation was performed on the remaining 6 non-infected rams on the equivalent of day 14 post-infection.

Scrotal insulation and estimation of scrotal circumference

Scrotal insulation consisted of a pouch fashioned to fit the scrotum and made up of 0.5-1.0 cm thick cotton wool laid over a cotton gauze and wrapped with an elastic adhesive bandage (Elastoplast, Smith & Nephew Ltd, Hull, U.K.) The pouch was held around the neck of the scrotum with the same bandage. The scrotal circumference was measured at two week intervals in all control and infected rams. This was done by

restraining the rams in a sitting position by one assistant while another assistant squeezed and aligned the two testes together in the distal end of the scrotum. Scrotal circumference was measured in the midline region of the two testes using a cotton tape and a centimetre ruler.

Histopathology

The testes, epididymis and prostate glands were carefully harvested from experimental animals dying from infection or killed at the end of the study period, trimmed and weighed. Sections made from the cauda epididymis and prostate were fixed in 10% buffered neutral formalin and those made from the testes were fixed in Bouins fluid. All sections were processed for routine histology and paraffin embedded sections were sectioned at 5 μm and stained with either Ehrlich's haematoxylin and eosin (H&E) and Martius scarlet blue (MSB). The slides were examined by light microscopy and the seminiferous tubular diameter was measured using a binocular micrometer (Graticules Ltd, Tonbridge, Kent, U.K. The tubular diameter was estimated from the mean of two measurements taken perpendicular to each other and 50 tubules cut transversely were measured per animal.

RESULTS

Clinical observations

The detailed clinical observations of control and *T. congolense*-infected rams has been described by Mutayoba *et al.* (1995). Briefly, all ten infected rams became parasitaemic within 5 - 7 days after infection. The first peak of parasitaemia occurred within 9-15 days after infection and parasitaemia fluctuated thereafter. Following patency, infected rams developed intermittent pyrexia and progressive decline in packed cell volume and liveweight gain. All scrotal-insulated rams and control rams maintained good health throughout the study period. Five control and five infected rams were killed on day 28 post-infection and the rest were killed on day 79 when the study ended. Scrotal-insulated rams were killed on day 58 of the study.

Testicular changes

The mean testicular circumference (TC) (Fig. 1a) and testicular circumference/liveweight (TC/LW) ratios (Fig. 1b) at the onset of infection were not significantly different between the control and infected groups. The TC values started to decline in infected rams within 12 days post infection and from day 37-79 mean TC values were significantly lower ($P<0.05$) than in control rams. TC/LW ratios followed a similar trend. No gross testicular lesions were observed in rams killed on day 28 post infection but the testes of infected rams which survived up to day 79 were soft and smaller in size. Table 1 shows the mean weights of the paired testes (including epididymides) of infected and control rams killed on day 79. A nonsignificant decrease in mean testicular-epididymal weight and apparent increase in testes-epididymal/liveweight ratio was observed in infected rams when compared to control values.

Histologically, compared with the controls, the mean diameter of the seminiferous tubules (Table 2) of infected rams was significantly larger ($P<0.05$) on day 28 post-infection and significantly smaller ($P<0.001$) on day 79 after infection. The testes of infected rams killed on day 28 post-infection showed an apparent increase in intertubular fluid but there was no overt changes in seminiferous spermatogenic activity.

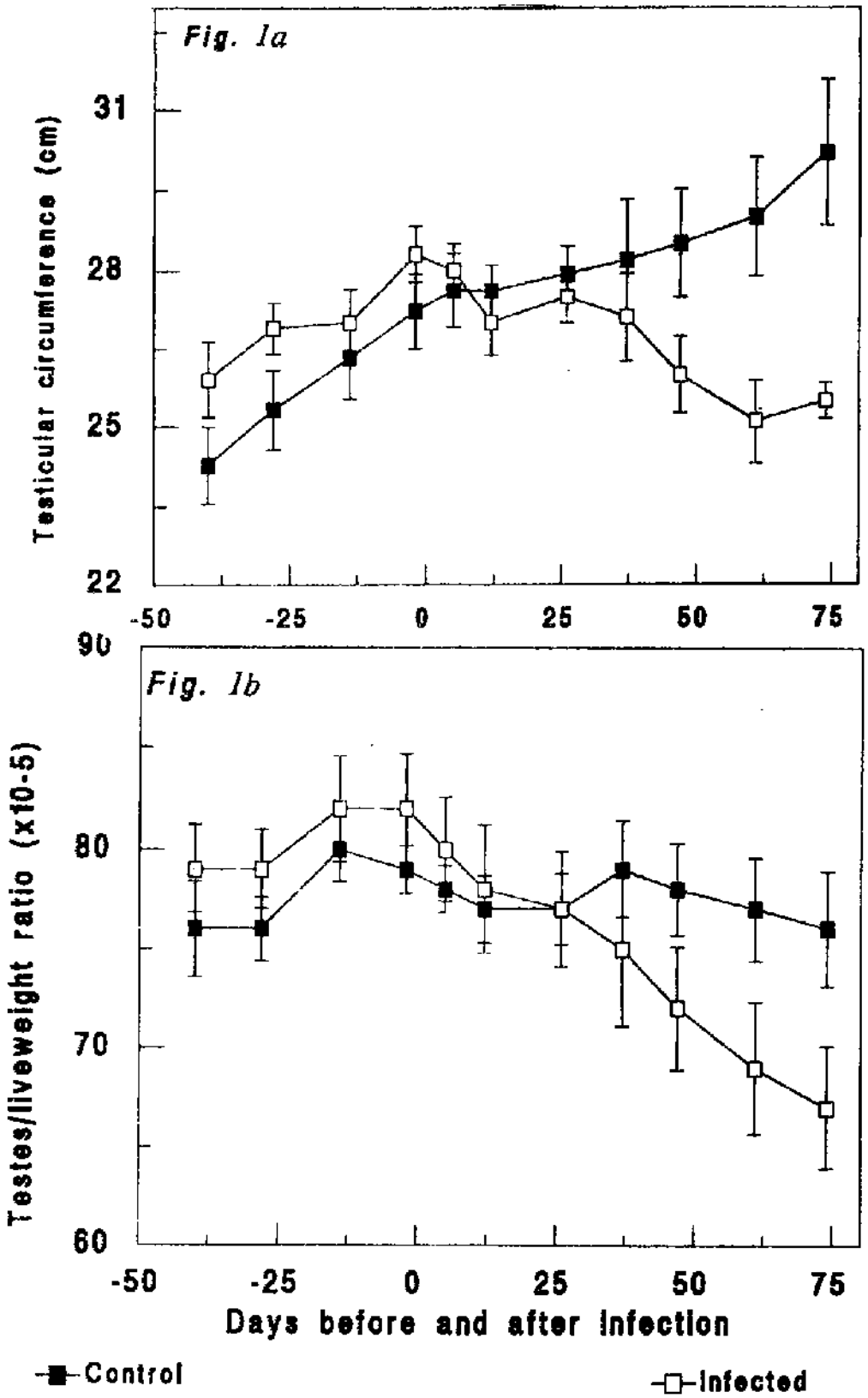

Fig 1: Mean (±s.e.m) changes in testicular circumference (Fig 1a) and testes circumference/liveweight ratio (Fig 1b) in uninfected (n=10, control) and *T. congolense*-infected (n=10, infected) rams. Five control and 5 infected rams were killed on day 28 post-infection.

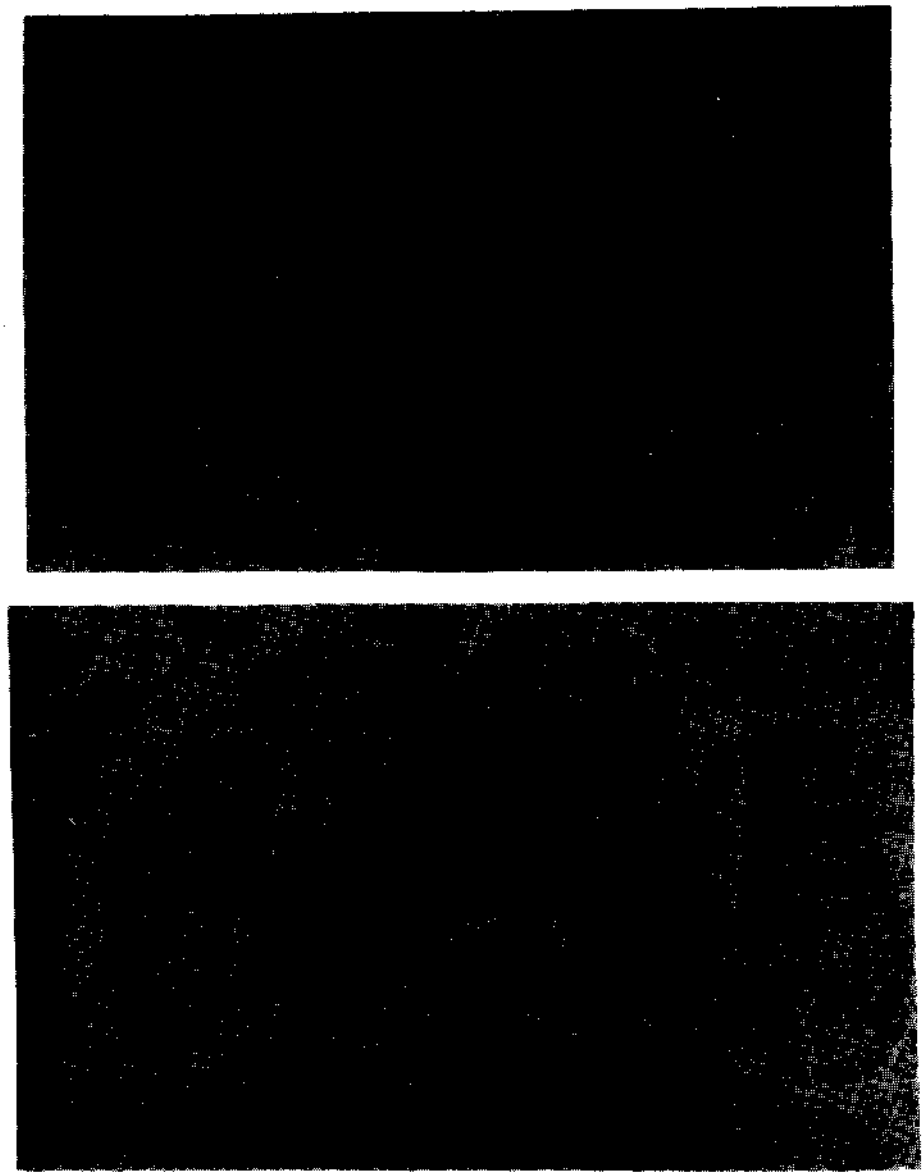

Fig. 2a: Testis of infected ram killed on day 79 post-infection showing generalized atrophy of the seminiferous tubules. Note several tubules with 2-3 layer thickness x50 HE.

Fig.2b:Testis of an infected rams dying of infection on day 58 post-infection showing degeration of the seminiferous germ cells and increased intertubular spaces x50 HE

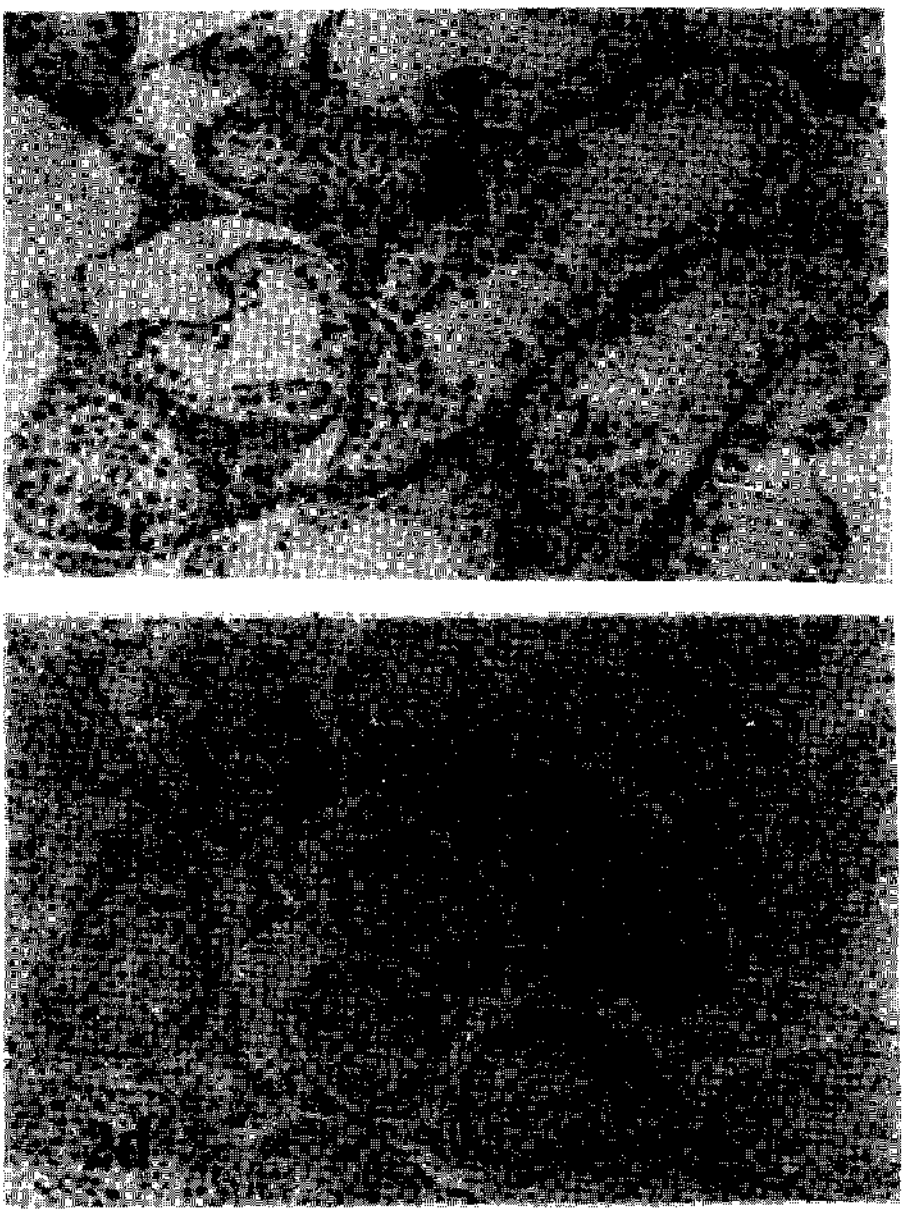

Fig 2c: Testis of an infected ram killed on day 79 post-infection showing marked degeneration of seminiferous epithelium, tubular collapse and in some tubules only spermatogonia and sertoli cells are evident x50 HE.

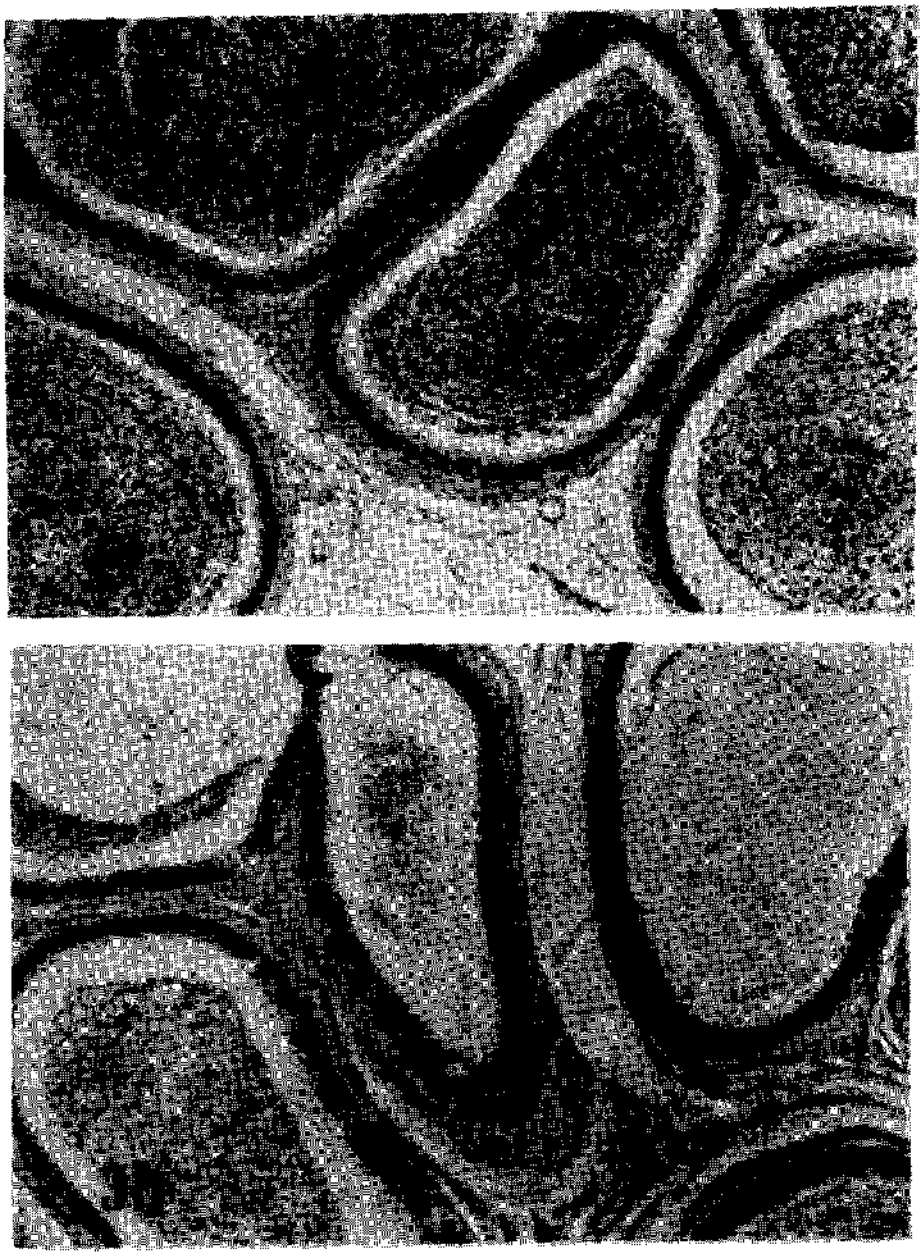

Fig. 3a: Cauda epididymis of a controll ram showing high concentration of spermatogonia (sperm reserve) stored in the tubules. x10 HE.

Fig. 3b: Cauda epididymis of infected rams killed on day 79 post-infection showing marked reduction in sperm reserve in the tubules (Fig 3b) and aspermic tubules (Fig 3c) x10 HE.

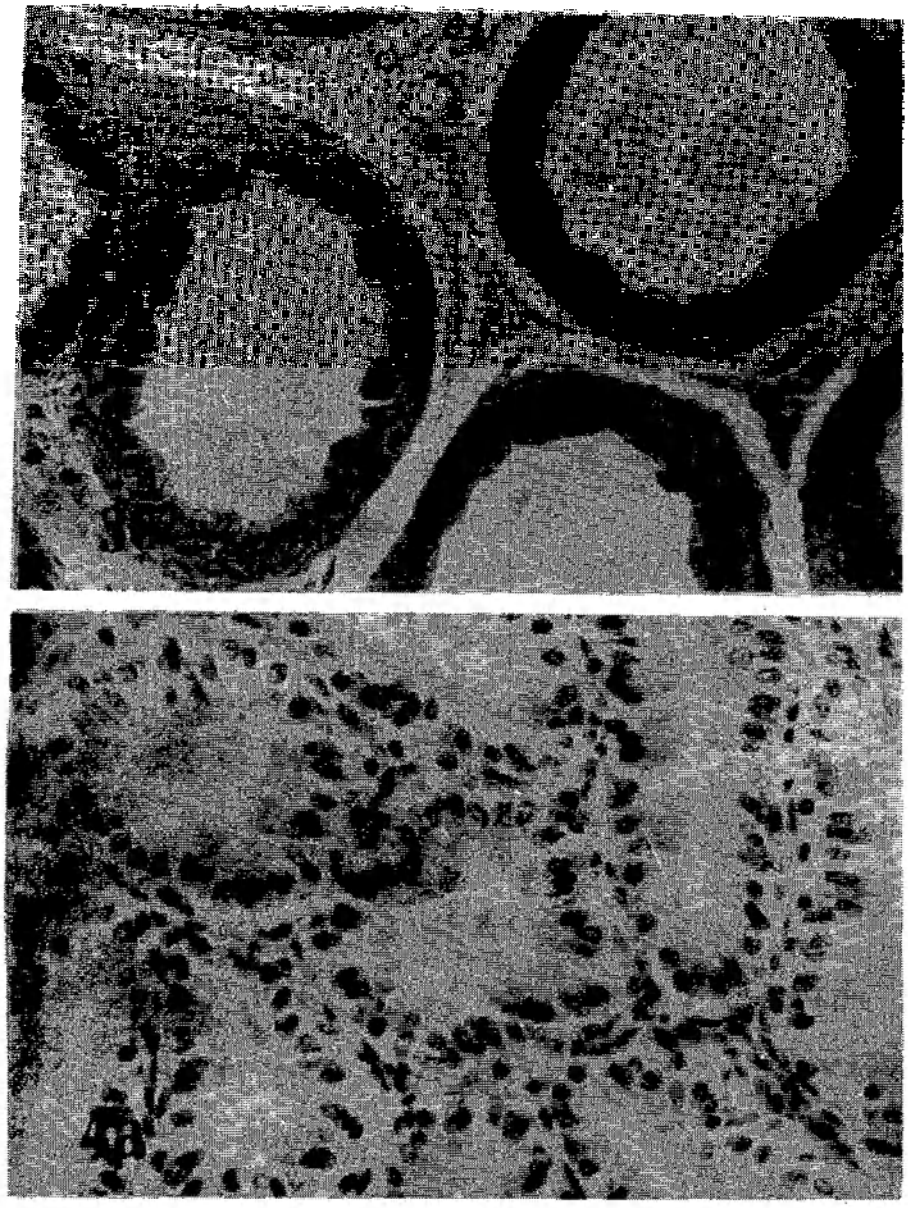

Fig 3c: Cauda epididymis of infected rams killed on day 79 post-infection showing marked reduction in sperm reserve in the tubules (Fig 3b) and aspermic tubules (Fig 3c) x10 HE.

Fig. 4a: Prostate gland of a control ram showing normal tall columnar follicular epithelium and sparse connective tissue. X50 HE.

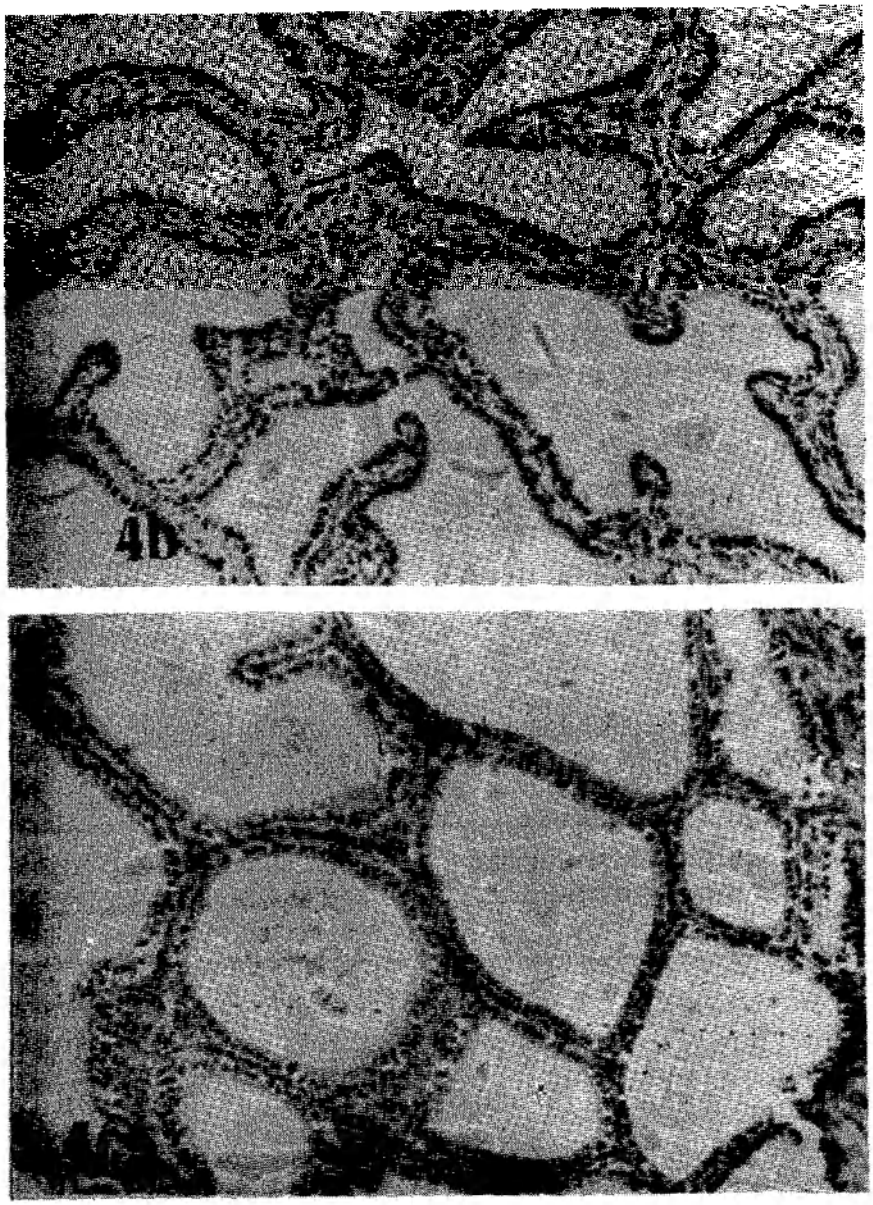

Fig: 4b and Fig 4c: Prostate gland of an infected ram (Fig 4b) and scrotal-insulated ram (Fig 4c) showing similar reductions in the height of follicular cells (low cuboidal) and increase in interfollicular connective tissue. X50 HE.

Table 1. Mean (±s.e.m.) paired testes (including the epididymides) and prostate gland weights and organ/liveweight ratios in control, *T. congolense*-infected and scrotal-insulated rams.

	Control (day 79) (n=5)	**Infected** (day 79) (n=5)	**Scrotal-insulated** (day 58) (n=6)
Testes (including epididymides) (gm)	235.5±18.3	192.5±9.4	211.5±8.2
Testes/liveweight ratio ($x10^3$)	4.96±0.1	5.2±0.4	4.8±0.5
Prostate gland (gm)	5.3±0.5	3.2±2.6**	3.0±2.1**
Prostate/liveweight ratio ($x10^4$)	1.1±0.1	0.8±0.1***	0.8±0.2***

**, ***Denotes significant differences at ($P<0.01$, $P<0.001$, respectively) when compared with control values in the same row.

Table 2: Mean (±s.e.m.) seminiferous tubular diameter and tubular diameter/testes weight ratios in control, *T. congolense*-infected and scrotal-insulated rams.

	Seminiferous tuhular diameter (μm)	Seminiferous tubular diameter/testis weight (gm)
Day 28		
Control (n=5)	78.4±0.1	NC
Infected (n=5)	83.2±1.3 ($P<0.05$)	NC
Day 79		
Control (n=5)	76.3±1.9	0.7±0.4
Infected (n=5)	64.4±0.8 ($P<0.001$)	0.7±0.2
Day 58		
Scrotal-insulated (n=6)	68.1±0.5 ($P<0.01$)	0.6±0.5

NC= Not calculated: because the testes were not weighed on day 28 post-infection.
The correlation between seminiferous tubular diameter and testicular weight on day 79 post-infection in infected and control rams was ($r=0.7$, $P<0.05$) ($n=10$).

However, infected rams killed on day 79 showed varying degrees of seminiferous tubular degeneration which included tubular atrophy and reduction in the number of germinal cell layers (Fig 2a, b) and in severely affected testes, tubules had collapsed and only few germ cells and Sertoli cells were evident (Fig 2c). But in all infected rams, no overt changes were observed in the Leydig cells and there was no inflammatory cell infiltration in the testes. Almost similar degenerative changes of seminiferous tubular epithelia were observed in scrotal-insulated rams killed on day 58 post-insulation (Fig. 2d).

Changes in the epididymis

No gross changes were observed in the epididymis of control and infected rams killed on day 28 and only small amounts of semen was observed on cut surface of the epididymis of infected rams killed on day 79 when compared with controls. Histologically, only the cauda epididymis was examined and a marked reduction in sperm reserve was evident in infected rams killed on day 79 post-infection (Fig 3b, c) when compared to controls (Fig 3a). The cauda epididymis of scrotal-insulated rams showed similar changes to those observed in infected rams.

Changes in the prostate gland

The prostate gland of infected and scrotal-insulated rams killed on day 79 and 58, respectively were generally smaller and firmer that corresponding control prostates. The prostate weights and prostate/liveweight ratio (Table 1) were significantly smaller ($P<0.01$, $P<0.05$, respectively) in the infected and scrotal-insulated rams than in control rams. Histologically, the glands of infected rams killed on 79 post-infection showed a marked proliferation of interfollicular connective tissue and atrophy of the follicular epithelium to low cuboidal (Fig 4b) when compared to the tall columnar cells observed in the controls (Fig 4a). Changes in the prostate glands of scrotal-insulated rams (Fig 4c) resembled those of infected rams.

DISCUSSION

Following experimetal infection with *T. congolense*, all infected rams developed clinical manifestations of the disease within 1-2 weeks which was characterized by intermittent pyrexia and parasitaemia and anaemia and impaired growth rates as previously described by Mutayoba *et al.* (1995) in the same group of rams used in this study. The infection induced variable degrees of degenerative changes in the testes, epididymis and prostate glands which were more severe in infected rams which survived up to the end of the experimental period than those killed at an earlier time during the study period.

The testicular degenerative changes were mainly of the simple type consisting in most cases of changes in the number of seminiferous germ layers and decrease in the amount of semen stored in the cauda epididymis. The testes of infected rams killed on day 28 post-infection had increased seminiferous tubular diameter and intertubular oedema when compared to testes of control rams. The increase in intertubular fluid in the testes suggests that the infection affected the testicular blood vascular and lymphatic dynamics during this period.

The changes in testicular pathology in infected rams were progressive since the testes of infected rams killed on day 79 post-

infection showed more advanced degenerative changes than those killed on day 28. The reduction in epithelial thickness and seminiferous tubular diameter and signs of testicular atrophy were more evident in rams killed on day 79 than in those killed on day 28 post-infection. Testicular atrophy (as measured by testicular weight) was significantly correlated with seminiferous tubular diameter (see Table 2). Testes from infected rams obtained on day 79 post-infection contained small seminiferous tubules and increased intertubular connective tissue leading to an apparent increase in the number of Leydig cells in a given cross-sectional area. No overt changes in Leydig cells were observed by light microscopy. This agrees with the findings of Ikede (1979); Kaaya and Odour-Okelo (1980) and Omeke and Onuora (1992).

The marked decline in sperm reserve in the cauda epididymis of infected rams killed on day 79 reflects the progressive decline in spermatogenic activity of infected rams. Indeed, marked deterioration of semen consistency, sperm mortility and increase in the percentage of sperm with primary and secondary defects was observed in the same group of rams (Mutayoba, 1995). Since secondary sperm abnormalities are usually of epididymal origin (Setchell, 1984) it is likely that the epididymis is one of the main site at which *T. congolense* affects sperm quality as the transit of sperm through the epididymis is usually associated with significant sperm maturation changes. However, there is no available information on the effects of trypanosomiasis on the physiology of the epididymis and its influence on epididymal sperm maturation, transport and storage. The epididymal changes observed in the infected rams during this study have been described in other species of animals infected with *T. congolense* or *T. vivax* (Omeke and Onuora, 1992; Sekoni, 1994).

The prostate glands of infected rams had flattened cuboidal epithelium and showed evidence of increased amounts of interfollicular connective tissue. Moderate prostate changes have been described in cattle infected with *T. congolense* or *T. vivax* (Sekoni, 1994). The changes in the prostate gland in infected rams in the present study could have been induced by low testosterone concentration (Mutayoba *et al.*, 1994) since similar changes are observed in castrated animals (Westin *et al.*, 1993). The observed changes in the prostate epithelium of infected rams were suggestive of decreased secretory activity and this could have possibly contributed to the poor semen quality obtained in infected rams.

The mechanisms by which *T. congolense* induces infertility in animals are not well investigated and the evidence of involvement of direct trypanosome-borne and indirect trypanosome-induced factors have been suggested (reviewed by Mutayoba, 1995). One of the factor which has been suggested is the role of fever induced by *T. congolense* infection on the seminal and gonadal changes of infected animals. Trypanosome-infected rams used in this study were found to have a significant increase in scrotal temperature which paralleled similar increases in rectal temperature (Mutayoba, 1995). In the present study, the changes in the gonads and associated tissues of *T. congolense*-infected rams were compared to those induced by local elevation of testicular temperature by scrotal insulation. Scrotal-insulation did induce varying degrees of seminal and testicular pathology which were comparable to those induced by *T. congolense* infection in rams. Similarly, in both groups, degeneration of the testes was not associated

with any inflammatory response in the peritubular tissues. Furthermore, the changes in the cauda epididymis and prostate gland were quite similar between the two groups of animals. These observation suggest that pyrexia induced by *T. congolense* infection might in part contribute to the reproductive anomalies frequently observed in infected animals (Ikede *et al.*, 1988; Sekoni, 1994; Mutayoba, 1995). In infections caused by the *T. brucei* group, inflammation definitely plays a major role in the degeneration of the testicles and surrounding tissues but it would also appear that a pyretic effect may contribute to gonadal changes. This is because the degenerative changes induced by these trypanosomes which include scrotal dermatitis, periorchiditis and epididymitis are likely to interfere with the cooling of the testis, as do other scrotal lesions as eczema and ringworm (Arthur, *et al.*, 1982).

In conclusion, these studies have shown that *T. congolense* induces progressive non-inflammatory degenerative changes in the testis, cauda epididymis and prostate gland of the rams similar to those observed in other animal species. It is postulated that these changes are in part induced by trypanosome-induced pyrexia which results in the elevation of testicular temperature. The elevated testicular temperature possibly acts in conjunction with other trypanosome-borne and trypanosome-induced factors to induce infertility in infected animals.

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