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Effect of Cadmium on the Reproductive Organs of the Male Potoroo *Potorous tridactylus* (Macropodidae)

S. L. Bryant and R. W. Rose

Department of Zoology, University of Tasmania, Box 252C, G.P.O. Hobart, Tas. 7001.

Abstract

Low (2·28 mg/kg) or high (4·56 mg/kg) doses of cadmium chloride were administered, either intraperitoneally or subcutaneously, to adult male potoroos (*P. tridactylus*). After 7 days, the testes, caput epididymis and spermatic cords of the high-dose groups all displayed a degree of cellular damage; however, the damage was not as extensive as that which occurs in some eutherian species after only 24 h. This time differential might be attributed to structural differences between the testicular blood supply in eutherians and marsupials.

Introduction

Cadmium has a destructive and seemingly selective effect on the reproductive system of a number of animal species (Parizek 1960; Dryden and McAllister 1970; Sangalang and O'Halloran 1972). Chiquoine (1964) and Chiquoine and Suntzeff (1965) found an absence of testicular damage in a number of animals, including the American opossum *Didelphis marsupialis*, after administering high doses of cadmium. A specific investigation of the effects of cadmium on the Australian brushtail possum *Trichosurus vulpecula* by Rose *et al.* (1981) found that the testes were not affected, even after lethal doses of cadmium were administered. These three papers led to the suggestion by Rose *et al.* (1981) that the reproductive system of marsupials as a group may be relatively insensitive to cadmium.

Cadmium acts principally on the blood vessels of the testes and proximal region of the caput epididymis, increasing their permeability and reducing blood flow (Harkonen and Kormano 1970). The vascular injury from cadmium is most prominent in the venous system (Gunn and Gould 1970). The testicular artery of the eutherian pampiniform plexus is extremely convoluted and is surrounded by numerous branches of the testicular vein. The marsupial rete mirabile in contrast, however, is characterized by parallel branches of the testicular artery which intermingle with a similar number of branches from the spermatic vein (Harrison 1948). Rose *et al.* (1981) suggested that the positioning of testicular vessels in marsupials may result in a more 'effective blood-tissue barrier to cadmium' than the spirally arranged pampiniform plexus.

The potoroo, *Potorous tridactylus*, is a small macropodid marsupial found widely in Tasmania. It is a fully protected animal hence only small numbers are available for experimentation. The male of the species is fertile throughout the year (Hughes 1964) and the testes are carried in an external scrotal sac. According to Barnett and Brazenor (1958) the potoroo, in relation to body size, has the lowest rete vessel number of all marsupial species. If the marsupial testicular rete does afford some protection against cadmium, this protection should be minimal in the potoroo.

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Materials and Methods

In all, 12 adult male potoroos (Pt), weighing between 0·910 and 1·625 kg, were housed individually in an unheated animal room. Their diet comprised bread, fruits in season, dog chow and water. For comparative purposes, dosages of cadmium were equivalent to those used by Chiquoine (1965) and Rose *et al.* (1981) and were apportioned according to body weight. Cadmium chloride ($CdCl_2 . 2H_2O$) was dissolved in 0·154 M NaCl; a low dose of 2·28 mg/kg or a high dose of 4·56 mg/kg was given either intraperitoneally or subcutaneously. Control males were injected with 0·9% (w/v) saline.

Seven days after injection, animals were killed with chloroform; testes, epididymides and spermatic cords were removed immediately. They were fixed for 1 week in cold 7·5% (v/v) buffered formalin; the testes were bisected only after the first 24 h of fixation so as to minimize tubule distortion (Chiquoine 1964). Tissues were wax-embedded, sectioned at 8 μm and slides routinely stained with Ehrlich's haematoxylin and eosin.

Results

Testicular damage was assessed according to the following parameters: percentage of tubules with spermatozoa ($n = 40$), regularity of basement membrane, presence and configuration of germinal epithelia and the extent of erythrocyte infiltration. Spermatic cords also were examined for evidence of blood coagulation, i.e. erythrocyte infiltration. The condition of the testes after animals had been treated with cadmium chloride were defined as: normal, partially damaged, or damaged.

Control (n = 3)

Control animals injected with saline had testes which corresponded to control non-injected males (Bryant and Rose, unpublished data) and were histologically similar to those of other marsupials and eutherians. On average, 76% of tubules maintained spermatozoa release. Seminiferous tubules were round with oval lumina (Fig. 1*a*). Basement membranes were regular and intact and spermatogonia were concentrically organized. The Leydig cells of the interstitial tissue were polygonal in shape and had a large spherical nuclei with one eccentric nucleolus. Tubules in the proximal end of the caput epididymis were normal and contained sperm. The arteries and veins of the rete mirabile were not engorged with blood (Fig. 2*a*).

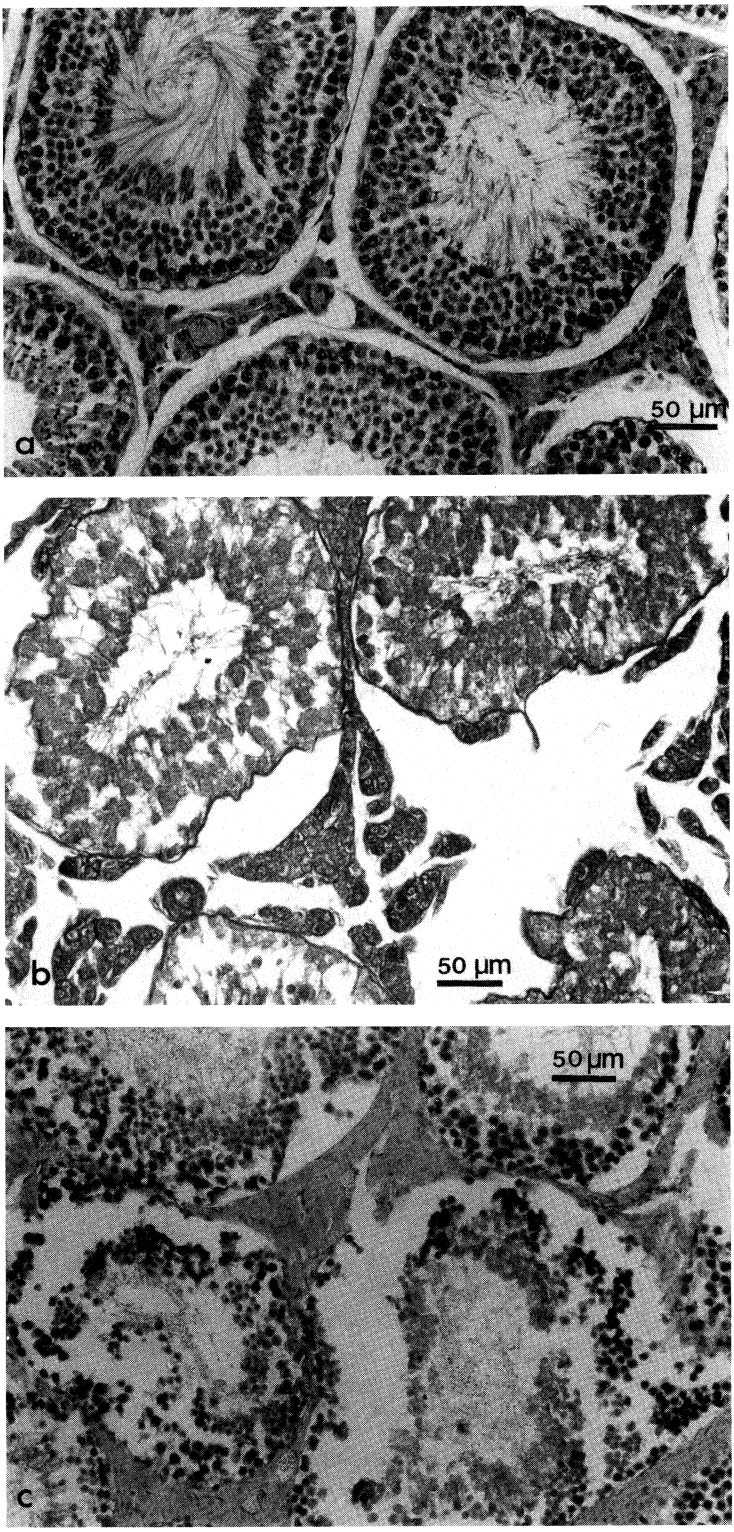
Cadmium—Low Dose (n = 2)

There was no change to the histological structure of the testes in low-dose animals (injected intraperitoneally) after 7 days. Basement membranes were regular and intact, germinal cells maintained normal concentric configuration and a high percentage of tubules (90%, 73%) contained spermatozoa. In both animals, the lining of the initial segment of the epididymis had degenerated and contracted, and sperm were absent. The arterial vessels of the rete mirabile were normal. Although some cellular disorganization was observed in the caput epididymis, the testes of this group were considered to be undamaged.

Cadmium—High Dose (n = 7)

Seven animals were injected with a high dose of cadmium. In the two animals which were injected subcutaneously with cadmium, the testes were classified as partially

Fig. 1. Testes of *P. tridactylus* after exposure to cadmium. (*a*) Control: round seminiferous tubules with concentrically organized spermatogonia. Lumina contain spermatozoa. (*b*) High dose—subcutaneously: partly damaged testes illustrated by irregular basement membranes and disorganization of spermatogenic cells with vacuoles appearing. (*c*) High dose—intraperitoneally (Pt 17): the damaged testes show a disintegration of basement membranes, enabling scattering of spermatogenic cells and infiltration by erythrocytes and necrotic material.


a
50 µm
b
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c
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damaged. The seminiferous tubules had contracted basement membranes, causing some loss of basal spermatogonia. Necrotic material and numerous vacuoles were also present within the tubules (Fig. 1*b*). A percentage of tubules (70%, 40%) were maintaining spermatozoa release although the lumina had contracted. The interstitial tissue was

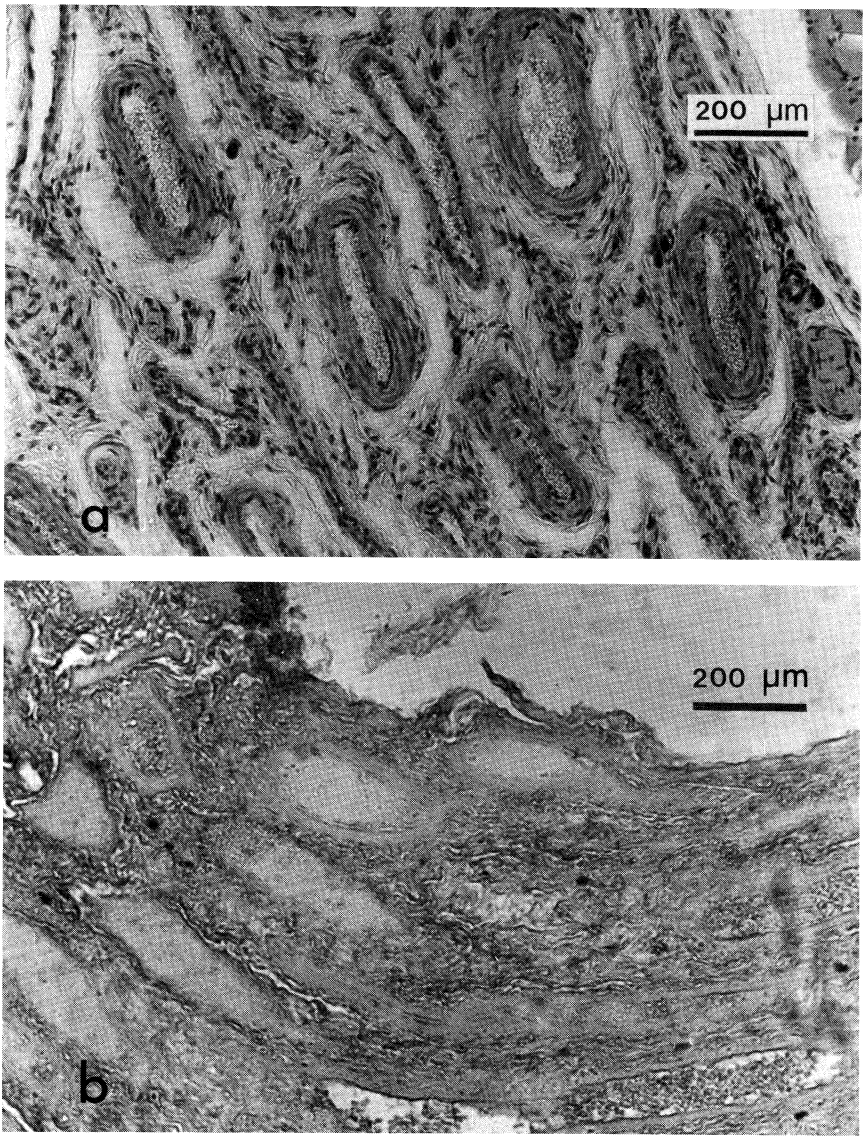

Fig. 2. Arterial vessels of the spermatic cord of *P. tridactylus*. (*a*) Control: the arteries and veins are not dilated but are flattened. (*b*) Cadmium—high dose (Pt 17): thrombosis and congestion is evident in the arteries and veins, which is presumed to reduce blood flow.

normal. Motile sperm were detected in urine samples at 7 days; however, sperm was not observed in the histological sections of the initial segment of the caput epididymis.

Of the five animals injected intraperitoneally with a high dose of cadmium, three died within 24 h. The testes of these animals were normal, except for slight post mortem

deterioration. The testes of the two surviving animals (Pt 17, Pt 61) showed some external haemorrhaging at the junction of testis and testicular artery, but no overall discoloration.

The testes of Pt 17 (potoroo with the lowest body weight, 0·910 kg), were classified as damaged, the damage being concentrated mainly within the seminiferous tubules and the caput epididymis. Basement membranes of seminiferous tubules had either contracted or fragmented, allowing germinal cells to disperse and, with erythrocytes, fill the widened intertubule spaces (Fig. 1*c*). Tubule lumina had either disappeared or were irregular in shape and contained eosinophilic necrotic material. Only 20% of the seminiferous tubules contained spermatozoa. Erythrocytes had also invaded the caput epididymis and infiltrated the epithelium. The caput tubules showed marked degeneration, dilation and atrophy of the epithelium. Thrombosis was evident in the rete arterial vessels supplying the testes (Fig. 2*b*).

The testes of the second surviving animal (Pt 61), were classified as partially damaged. This may be due to the animal receiving two single low doses of cadmium (24 h apart), instead of the usual single high dose, in an attempt to prevent death. Some 38% of the seminiferous tubules contained sperm, although tubule lumina were contracted. Basement membranes of the tubules were intact but irregular and some loss and disorganization of later stages of spermatogonia had occurred. Vacuoles were also present within the tubules. Sperm were absent from the initial segment of the epididymis and the tubules were contracted; however, the remainder of the tissue appeared to be histologically normal. Massive erythrocyte infiltration into the testes, epididymis or spermatic cord was not evident.

Discussion

An important aspect arising from the present study, as with that of Rose *et al.* (1981), is that marsupials appear to be highly sensitive to cadmium and death may result from dosages which in some eutherians damage only the testes. In contrast to previous findings, however, the reproductive system of the potoroo is sensitive to cadmium toxicity and testicular and epididymal damage results when high, but non-lethal doses, are administered.

An absence of testicular damage in the potoroo, 7 days after an intraperitoneal low dose of cadmium chloride, agrees with the findings of Rose *et al.* (1981) in the brushtail possum. In contrast, and as predicted, a high dose of cadmium chloride given intraperitoneally or subcutaneously in the potoroo, causes deterioration of the seminiferous tubules and interrupts spermatogenesis in animals able to survive for 7 days. Cadmium injected either subcutaneously, or as two single doses, appears to reduce the degree of injury, as well as prevent death (when compared to one high intraperitoneal dose). This finding agrees with those of Gunn and Gould (1970).

There are noticeable differences between the extent of damage in the testes of the potoroo and of that recorded for the rat over a similar time period (Parizek 1957, 1960). There was severe haemorrhaging in rat testes 24 h after treatment with cadmium. By 7 days, the rat's testes had diminished in size and the tissue replaced by eosinophilic material, interspersed with basophilic nuclear residue. In the potoroo, 7 days after a high dose of cadmium, the testes also showed damaged and cellular disorganization. Some haemorrhaging had occurred, but not to the extent of that seen in the testes of the rat. Oedema and ischaemic effects, which are considered responsible for degeneration in eutherian testes, due to the interference of cadmium with testicular vascularization

and breakdown of the blood–testis barrier (Waites and Setchell 1966; Setchell and Waites 1970; Aoki and Hoffer 1978), were not pronounced in the testes of the potoroo.

Some investigators consider cadmium acts specifically on the heat-regulating mechanisms of the testes (Maekawa and Hosoyama 1965); others believe that it interferes directly with the permeability of the testicular vasculature, causing intravascular coagulation and poor venous drainage (Gunn and Gould 1970). Some inherent difference between the rete mirabile and the pampiniform plexus may be responsible for the reduction in damage (or delay of onset) in the testes of marsupials as compared with those of eutherians.

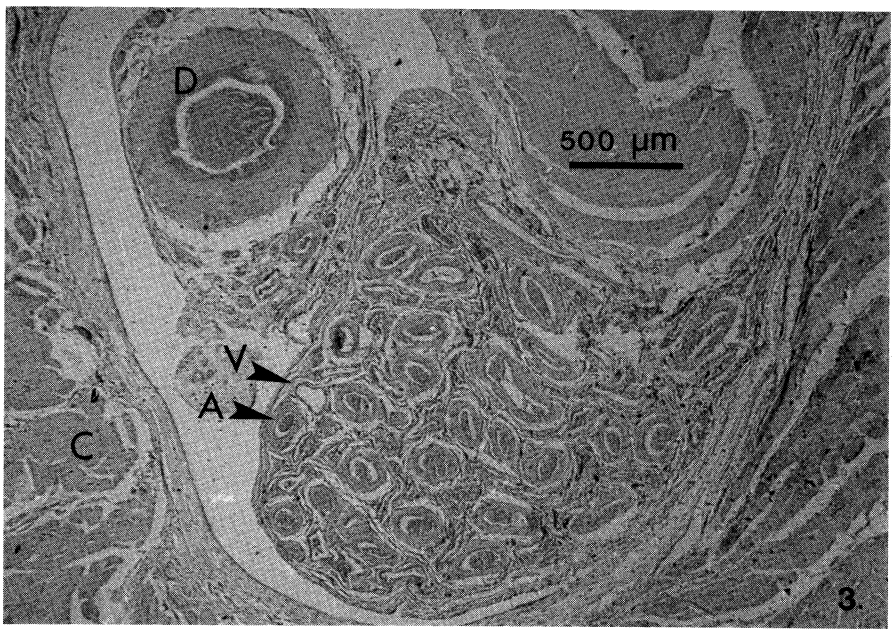

Fig. 3. Midlength cross-section of the spermatic cord of *P. tridactylus*. The parallel positioning of the arteries and veins is a characteristic feature of the marsupial testicular rete mirabile. 27 arteries (*A*) and 29 veins (*V*) are illustrated in this section of the cord. The ductus deferens (*D*) is heavily invested by smooth muscle and has a small arterial supply. A thick diffuse cremaster muscle (*C*) ensheathes the cord.

Because of its parallel branching, the testicular rete mirabile of marsupials has a large cross-sectional area. By virtue of this lowered resistance, the rete may be less susceptible to the effects of intravascular coagulation than would the highly coiled, but unbranched, system of eutherians. The close contact between arteries and veins in the marsupial rete may increase its effectiveness as a countercurrent heat-exchange unit, compensating for small changes in temperature (which might be caused by cadmium).

Barnett and Brazenor (1958) determined the number of vessels in the testicular rete mirabile of the potoroo as six arteries and six veins; however, Fig. 3 illustrates a section midway along the spermatic cord containing 27 arteries and 29 veins. They also stated the rete mirabile of the brushtail possum has 49 arteries and 56 veins, which suggests a greater degree of branching and hence a larger cross-sectional area than in the potoroo. A direct comparison between cadmium susceptibility in the potoroo and the possum is difficult, since Rose *et al.* (1981) were unable to maintain possums for longer than 24 h after a high-dose treatment. Although the potoroo has a far more extensive rete

mirabile than previously stated, its testes still appear to be more susceptible to cadmium than any other of those marsupials examined to this date.

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