

Cadmium Toxicity and Its Effect on the Testes of an Australian Marsupial (*Trichosurus vulpecula*)

R. W. Rose, R. Swain, and W. K. Whitten

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

The selective toxic effects of cadmium on the testes of eutherian mammals have been well documented since the original demonstrations by PARIZEK (1956, 1957, 1960). A single Subcutaneous or intraperitoneal injection of cadmium (as CdCl_2) results in testicular necrosis in most eutherian species. Doses of 0.02 - 0.04 mmol/kg body weight CdCl_2 (2.0-4.5 ppm Cd) may induce complete degeneration of the testes yet produce no visible morphological changes in other organs. Some comparative work has also been carried out on other vertebrate groups, but studies of the effects of cadmium salts on the testes of non-eutherian mammals (monotremes and marsupials) are limited to two brief reports. CHIQUOINE (1964) noted that in the American opossum (*Didelphis marsupialis*), no testicular damage occurred within 72 h after an injection of 10 or 20 mg CdCl_2 /kg body weight. This study must be considered a preliminary one since only four animals of unspecified age and breeding status were used. Subsequently CHIQUOINE & SUNTZEFF (1965) observed testicular necrosis when 0.1 mg CdCl_2 was injected directly into the testes, but no experimental details were given.

The present paper reports the results following the injection of cadmium into another marsupial, the common Australian brush-tail possum (*Trichosurus vulpecula*). Sperm are found throughout the year in this species but breeding is mainly limited to the winter months with a secondary peak in spring.

MATERIALS AND METHODS

$\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ of AR purity was dissolved in 0.154 M sodium chloride at such concentrations that the dose per kg body weight for intraperitoneal injection was contained in 1.0 mL and for intratesticular injection in 0.1 mL.

Twenty-two adult (2.4-4.6 kg) male possums were trapped during the winter. They were housed in an unheated animal room and provided with adequate food and water. Soon after capture they were allotted to one of four treatment groups as indicated in Table 1. The animals were injected, using 23 G needles for intraperitoneal injections and 26 G for intratesticular injections. They were observed at 1, 6 and 24 h after injection and if still alive were killed at 24 h or one week after injection. The animals were killed with chloroform and the scrotum with its contents removed before

the heart stopped beating. The testes were promptly cut into segments and fixed in buffered formalin. Tissue from both central and peripheral locations were taken from the gonads which had been injected intra-testicularly. The specimens were dehydrated, embedded in paraffin, sectioned at 6 μ m and stained with haematoxylin and eosin. They were examined through a light microscope.

RESULTS

The results are presented in Table 1 from which it is clear that no testicular damage was observed from systemically administered cadmium but it was evident when cadmium was injected directly into that organ. The central areas of these testes appeared hyperaemic 24 h after injection but a slight reddening was noticed in only one of the control testes.

Histological examination of the central areas of the cadmium injected testes showed necrosis within the seminiferous tubules. Sertoli cells and spermatozoa were absent and only a few layers of spermatogonia were observed. Inflammatory exudate with many erythrocytes was present between tubules. The peripheral parts of the testes were normal and indistinguishable from controls. Some gross physical damage was obvious in the injected control testes at the site of injection but the adjacent tissue showed no visible cytological damage.

TABLE 1

Group	No. of Animals	Dose of CdCl ₂ (mg/Kg)	Route of injection	Time till slaughter, h	Results
1	6	4.48	I.P.	24 ¹	Testes normal
2	8	2.24	I.P.	24(4) ² 168(4)	" "
3	6	saline only	I.P.	24	" "
4	2	0.10 mg ³	Intra-testicular into one testis, saline into the other.	24	Necrosis at site of injection of CdCl ₂ Some local damage in controls.

- 1 1 died soon after injection, 4 killed at 6 h in extremis
- 2 1 died soon after injection.
- 3 Total dose.

DISCUSSION

These findings clearly indicate that the testes of I. vulpecula are not affected when toxic doses of cadmium are given intraperitoneally. This is in marked contrast to the observation that doses of about one fiftieth of a toxic dose produce striking effects in most eutherian mammals, but is in agreement with the statement that the American opossum is insensitive (CHIUQUINE 1964; CHIUQUINE & SUNTZEFF 1965).

Previous work on the sensitivity of scrotal tissue to cadmium salts suggested that species with scrotal testes - e.g. rats (PARIZEK 1957), hamsters (PARIZEK 1960), mice (CHIUQUINE 1964), rhesus monkeys (GIROD 1965), gerbils (RAMASWAMI & KAUL 1966), rabbits (CAMERON & FOSTER 1963), and calves (PATE et al., 1970) - were sensitive to cadmium whereas species with abdominal testes were insensitive - e.g. armadillos, frog, pigeon, rooster, (CHIUQUINE 1964), and shrew (DRYDEN & McALLISTER 1970). Initially the only exceptions to this hypothesis were the opossum (CHIUQUINE 1964; CHIUQUINE & SUNTZEFF 1965), for which few experimental data were supplied, and a few laboratory strains of mice (CHIUQUINE & SUNTZEFF 1965). Later some (non-mammalian) species with abdominal testes were also found to be sensitive - brook trout (SANGALANG & O'HALLORAN 1972, 1973; SANGALANG & FREEMAN 1974), quail (RICHARDSON et al. 1974), and pigeon (SARKA & MONDAL 1973).

The scrotal Eutheria are characterised by the possession of a specialised testicular vasculature used to cool the testes and their accessory tissues below body temperature (the pampiniform plexus). The possibility that the primary site of damage in cadmium-sensitive species was the vascular supply was reinforced by the works of CHIUQUINE (1964), PAUFLE & FOOTE (1969), PARIZEK (1960) and STEVENS (1980). The marsupials do not possess the pampiniform plexus, although CHIUQUINE (1964) believed that they did, on the basis of observations reported by HARRISON (1949); instead they possess a rete mirabile, (BARNETT & BRAZENOR 1957; HEDDLE & GUILER 1973) whose function is also to provide the testes with blood at below body temperature (SETCHELL 1977). It is possible that the division of the testicular arteries into complex networks of capillaries in marsupials provides a more effective blood-tissue cadmium barrier than the spirally arranged, but undivided testicular arteries of Eutheria. In that case the insensitivity of the American opossum and the Australian brush-tail possum, two distantly related animals belonging to separate families, is possibly true of marsupials as a group.

However one must be careful in interpreting these findings because of the reported insensitivity of some inbred strains of mice. This latter observation has been confirmed by STEVENS

(1980) who obtained evidence that it was dependent on a single gene. He also noted that injection of zinc salts would protect the animal against cadmium toxicity. Thus a thorough understanding of this problem will only be reached when it is possible to control effectively genetic and environmental factors which may influence the response.

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