FAILURE OF ^{109}Cd TO TRAVERSE SPERMATOGENIC PATHWAY

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Wetterdal (1958) showed that zinc, an element essential for spermatogenesis, is incorporated into developing spermatogenic elements within the testis. Cadmium, physico-chemically similar to zinc, causes selective destruction of the testis; administration of zinc prevents cadmium damage (Parizek, 1957; Kar, Das & Mukerji, 1960; Gunn, Gould & Anderson, 1961). Parizek (1960) suggested that cadmium exerts testicular injury by displacing zinc from its natural sites in seminiferous tubules and that the haemorrhagic reactions, so characteristic of cadmium injury, are a secondary effect. More recent evidence indicates that the vascular endothelium of the testis is the primary site of damage and that necrosis of parenchyma follows secondarily (Gunn, Gould & Anderson, 1963a; Chiquoine, 1964; Mason, Brown, Young & Nesbit, 1964; Niemi & Kormano, 1965; Waites & Setchell, 1966). The following experiments were initiated using radio-isotopes to determine whether cadmium, like zinc, enters the spermatogenic pathway. (Radio-isotopes used were ^{109}Cd (1.7 mc/mg) and ^{65}Zn (1.9 mc/mg) from Nuclear Science & Engineering Corporation, Pittsburgh, Pennsylvania.)

The animals used were CD-1 mice from The Charles River Mouse Farms, Inc. and CD rats from The Charles River Breeding Laboratories, Inc., North Wilmington, Massachusetts.

Fifty 12-week-old male mice (40 g) received a single subcutaneous injection of 10 μC of ^{109}Cd (equivalent to 5.9 μg of cadmium/mouse or 0.0014 m-mole/kg) to which had been added the minimal dose of cadmium chloride (0.012 m-mole/kg) needed to produce testicular necrosis. Mice were killed at times varying from 1 hr to 6 weeks (five mice each time period). The testis and the entire epididymal tract from one side were removed and placed in individual counting-vials containing Bouin's fixative. Radio-active content was measured in a scintillation detector and tissues were subsequently processed by routine histological techniques. Since this dose of cadmium produces massive haemorrhage in the testis, which could impede further progress of the metal into the tubules, fifty additional mice received the 10 μC of ^{109}Cd with no additional carrier cadmium, a dose well below the damaging level.

The results (Table 1) show that with 0.0134 m-mole/kg of cadmium maximal uptake was attained in testis between 4 hr and 1 day, by which time there was widespread vascular injury. ^{109}Cd uptake diminished from 2 days to 1 week,

during which period there was clean-up of necrotic debris, and then was maintained constant from 2 to 6 weeks. Tissue ^{109}Cd levels (in % administered dose) were slightly lower with the carrier than with the tracer dose (a reflection of dilution effect). With the tracer dose there was no damage and the maximal ^{109}Cd level attained in the testis by 1 day was maintained constant for 6 weeks. Neither experiment gave any indication that there was any prominent transport of cadmium in the epididymis as a consequence of its concentration in the testis, as occurs with zinc.

TABLE 1
 ^{109}Cd UPTAKE IN TESTIS AND EPIDIDYMISS OF MICE: COMPARISON MADE BETWEEN A DOSE OF CADMIUM WHICH WAS DAMAGING TO THE TESTIS AND A NON-INJURIOUS TRACER DOSE

Time following injection	^{109}Cd uptake (% administered dose/organ)			
	Damaging dose of Cd (0.0134 m-mole/kg)		Tracer dose of Cd (0.0014 m-mole/kg)	
	Testis	Epididymis	Testis	Epididymis
1 hr	0.039	0.008	0.029	0.012
4 hr	0.050	0.015	0.062	0.017
1 day	0.069	0.022	0.079	0.024
2 days	0.050	0.023	0.080	0.027
1 week	0.042	0.022	0.081	0.028
2 weeks	0.031	0.027	0.074	0.026
3 weeks	0.034	0.025	0.083	0.027
4 weeks	0.029	0.023	0.079	0.029
5 weeks	0.032	0.023	0.081	0.028
6 weeks	0.033	0.022	0.080	0.028

In two groups of fifty 20-week-old male rats (450 g) the vasa efferentia were severed unilaterally, thus interrupting the sperm transport pathway on one side (technique in Wetterdal, 1958). Immediately afterwards one group received 10 μC of ^{109}Cd ; the other group received 2 μC (1.1 μg) of ^{65}Zn . Neither dose produced any injury to testis or epididymis. Five rats from each group were killed at times varying from 1 hr to 6 weeks. Radio-active uptake by testis and caput, corpus and cauda epididymidis from the operated side was compared with that of tissues from the intact side.

The results are summarized in Text-fig. 1. On the intact side ^{65}Zn cumulated in the testis reaching a peak at 1 week; as testicular levels fell, ^{65}Zn rose in temporal sequence in caput, corpus (not shown) and cauda epididymidis. As Wetterdal (1958) indicated, the distinct peaks of ^{65}Zn in the epididymis at 2 and 3 weeks reflected the transport of radio-isotope by products of the testis since these peaks failed to appear on the side in which the vasa efferentia were severed. Microscopic examination confirmed the absence of spermatozoa in the epididymis from the operated side. In sharp contrast to ^{65}Zn , ^{109}Cd showed no prominent peaks in either testis or epididymis; neither was there any difference in ^{109}Cd uptake on the side in which the sperm transport pathway was interrupted.

It has been brought out that a number of substances are prevented from passing the testicular capillary barrier into the seminiferous tubules (Kormanov,

1967; Setchell, 1967). Whether cadmium is actively excluded from penetration into the tubules or whether it merely has a natural predilection for binding to sites within the capillary endothelium is a moot question. Autoradiographs have shown that ^{109}Cd is confined in intertubular areas (Berlin & Ullberg, 1963) and Skold (1961) demonstrated the localization of cadmium within the endothelial walls of testicular capillaries.

TEXT-FIG. 1. Distribution of tracer doses of ^{65}Zn (upper graphs) and ^{109}Cd (lower graphs) in rat testis and epididymis; comparison made between operated side (in which vasa efferentia were severed) (○) and non-operated side (●). (a) Testis; (b) caput epididymidis; (c) cauda epididymidis.

Two elements which are definitely known to penetrate the blood-testis barrier are zinc (Wetterdal, 1958) and selenium (Gunn, Gould & Anderson, 1967), both of which prevent cadmium injury to the testis (Kar *et al.*, 1960; Mason & Young, 1967). Radio-active studies show, however, that, whereas most of the concentration of ^{65}Zn and ^{75}Se occurs in the seminiferous tubules, a very definite portion is associated with non-tubular areas (Gunn, Gould & Anderson, 1963b). Since cadmium does not enter the spermatogenic pathway, and produces its typical vascular injury even in testes devoid of spermatogenic elements (Gunn *et al.*, 1963a; Mason *et al.*, 1964), then the protection offered by zinc and by selenium is probably not related to concentration of these elements in seminiferous tubules but, more likely, to their association with interstitial tissue, blood vessels in particular (Gunn & Gould, 1967).

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