THE EFFECT OF CADMIUM CHLORIDE ON THE BLOOD-TESTIS BARRIER OF THE GUINEA-PIG

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Cadmium exerts a selective toxic effect on the blood vessels of the testis and caput epididymidis (Gunn, Gould & Anderson, 1963; Chiquoine, 1964; Mason & Young, 1967) and investigations into the changes in blood flow and vascular permeability suggest that the primary site of action is the end-arterial bed (Niemi & Kormano, 1965; Waites & Setchell, 1966; Clegg & Carr, 1966; Gupta, Barnes & Skelton, 1967). The resulting ischaemia and interstitial oedema then cause failure of spermatogenesis (Mason & Young, 1967).

Dye studies suggest that a barrier exists at the tubular as well as the vascular level (Kormano, 1967a, b), and the presence of this barrier is essential for the production of a testicular fluid differing in composition from serum (Setchell, 1967; Setchell, Dawson & White, 1968; Johnson & Setchell, 1968). The present study shows that, in the untreated guinea-pig as in the rat, subcutaneously administered acriflavine produces weak staining of interstitial nuclei and cannot penetrate the tubular barrier but that, following CdCl_2 administration, staining of all parts of the testis occurs.

Guinea-pigs, weighing 500 to 600 g, were injected subcutaneously with a 2% CdCl_2 solution (10 mg/kg). Groups of animals were given a subcutaneous injection of 2% acriflavine solution (20 mg/kg) at a different site after 0, 7, 19, 31 or 43 hr, and 5 hr later were killed and the testes removed. Blocks were snap frozen, and freeze-dried sections were prepared and examined on the Zeiss photomicroscope. Positive controls consisted of testes from animals which had been immunized with homologous testis, and injected with acriflavine 5 hr before killing (Pl. 1, Fig. 1) (tubular permeability to acriflavine rises during the initial stages of the resulting testicular damage—Johnson, unpublished data), and a testis from a 2-day-old guinea-pig injected with acriflavine (Pl. 1, Fig. 2) (the tubular barrier is not fully developed at this age). Negative controls consisted of a testis from an animal which was injected with CdCl_2 but not acriflavine (see Pl. 1, Fig. 3 and line 6 in Table 1) and a testis from an animal injected with acriflavine only (see line 1 in Table 1). The results in the cadmium chloride-treated animals are summarized in Table 1. The staining of interstitial nuclei is shown in Pl. 1, Fig. 4 and of interstitial and degenerating intratubular nuclei in Pl. 1, Fig. 5.

Staining of interstitial nuclei became stronger within 12 hr of cadmium administration, suggesting an increased vascular permeability. After 24 hr, there was a focal breakdown of the tubular barrier but by 48 hr the whole

testis was involved. Phase-fluorescence examination of the freeze-dried sections revealed that all obviously damaged tubules contained fluorescent nuclei. The intensity of fluorescence was not correlated with the degree of damage but probably varied with the proximity of blood vessels. Some apparently normal tubules were also stained and this suggested that breakdown of the barrier occurred early in the production of damage. Other normal tubules in partially damaged testes were not stained.

TABLE 1

THE EFFECT OF CdCl_2 ON THE ACRIFLAVINE STAINING PATTERN OF THE GUINEA-PIG TESTIS

<i>Time after CdCl_2 injection (hr)</i>	<i>No. of testes examined</i>	<i>Vascular staining</i>	<i>Interstitial staining</i>	<i>Intratubular staining</i>	<i>Histology</i>
0 (negative control— acriflavine only)	10	++	— to +	—	Normal
5	4	++	± to +	—	Normal
12	10	++	++	—	Interstitial oedema; very few sites of focal damage
24	12	++	++	—/±/+	Interstitial oedema; areas with necrosing tubules and pycnotic nuclei
36	12	++	++	—/±/+/+ ++	Interstitial oedema; areas with necrosing tubules and pycnotic nuclei
36 (negative control— CdCl_2 only)	2	—	—	—	Interstitial oedema; areas with necrosing tubules and pycnotic nuclei
48	4	++	++	++	Complete degeneration

Damage within the tubules is usually regarded as due to a direct effect of ischaemia on spermatogenesis. However, experimental ischaemia of the perfused testis causes an almost immediate cessation in the flow of rete testis fluid (Linzell & Setchell, 1968) and the production of this fluid is presumably closely linked with the selective exclusion by the blood-testis barrier of serum components. This suggests a new explanation for the damage caused by CdCl_2 . The tubular barrier could be the primary site of action of the ischaemia produced by the administration of CdCl_2 , and after the break-

PLATE 1

Acridine fluorescence in guinea-pig testes.

FIG. 1. Positive control testis following immunization by homologous testis antigen.

FIG. 2. Positive control testis from 2-day-old animal.

FIG. 3. Negative control testis of animal treated with cadmium chloride but not with acridine.

FIG. 4. Testis of cadmium chloride-treated animal showing staining of interstitial nuclei.

FIG. 5. Testis of cadmium chloride-treated animal showing staining of interstitial and degenerating intratubular nuclei.

PLATE I

down of this barrier, the influx of serum components might secondarily initiate the damage to tubule contents. This secondary damage could be due to a specific cytotoxic action against germinal cells caused by the presence of a natural antibody (Spooner, 1964; Johnson, 1968), to cadmium interference with zinc metabolism in the tubule (Pařízek 1960) or to non-specific changes in the micro-environment of the cells.

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