THE DESTRUCTIVE EFFECT OF CADMIUM ION ON TESTICULAR TISSUE AND ITS PREVENTION BY ZINC

BY J. PAŘÍZEK

From the Czechoslovak Academy of Sciences, Laboratory of Physiology and Pathophysiology of Metabolism, Prague

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SUMMARY

The subcutaneous administration of cadmium salts (cadmium chloride or lactate) to male rats and mice leads to acute destruction of the testes, with destruction of the seminiferous epithelium and interstitial tissue. These changes in turn evoke castration phenomena, but the atrophied accessory sex organs retain the ability to react to testosterone propionate.

Within 20 days after the injection of cadmium, proliferation of fibroblasts in the interstitial spaces under the albuginea begins and is accompanied by an extensive formation of new blood vessels. Later, new Leydig cells appear; this is followed by a gradual return of the endocrine function of the testes. The spermatogenic epithelium of the seminiferous tubules, on the other hand, does not regenerate even 133 days after the injection of cadmium.

The simultaneous administration of a large dose of zinc salts protects the testes completely against cadmium damage. The mechanism of interaction between these physico-chemically related metals and the theoretical and practical significance of these observations will be studied further.

The attention of various authors has been focused recently on the effects of cadmium salts on various physiological functions (for example, nerve conduction and myocardial contractility: Košťojanec [1954]; Kleinfeld, Greene & Stein [1955]). Inhibition of various enzyme systems by cadmium [Krebs, 1930; Blume, 1934; Barron & Kalnitsky, 1947; Simon, Potts & Gerard, 1947; Green & Neurath, 1953] may explain certain relationships between metabolic and functional phenomena in living matter. While studying certain aspects of protein metabolism, rats were treated subcutaneously with cadmium salts. It was found that administration of even small doses of cadmium salts caused marked macroscopic damage of the testes which occurred within a few hours of injection [Pařízek & Záhoř, 1956]. A more detailed investigation of testicular destruction caused by cadmium was therefore undertaken.

MATERIALS AND METHODS

Animals

Adult male rats (Wistar substrain Konárovice), weighing 180–240 g, and male mice (H strain), weighing 20–30 g, were used. They were kept in an air-conditioned animal room at 21.5–22.5°C, and fed a diet composed of:

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>622.6</td>
</tr>
<tr>
<td>Dried milk</td>
<td>108.2</td>
</tr>
<tr>
<td>Casein</td>
<td>163.3</td>
</tr>
<tr>
<td>Lucerne</td>
<td>32.7</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>16.4</td>
</tr>
<tr>
<td>Margarine</td>
<td>47.2</td>
</tr>
<tr>
<td>Fish oil</td>
<td>7.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The animals were provided with food and water ad lib.
Experimental procedure

0.02 M Cadmium chloride, prepared from Merck’s cadmium carbonate, pH > 2.5, was injected subcutaneously into the interscapular region of each animal.

Some of the cadmium-treated animals received testosterone propionate (Agovirin Spofa) intramuscularly into the thigh. The hormone was diluted with olive oil so that 1 ml. of solution contained 1 mg of hormone. Control animals were also given cadmium, and were injected with an equal amount of pure olive oil.

In a third group of cadmium-treated rats, the remnants of the destroyed testes were removed surgically, through the scrotum, on the 52nd day, under ether anaesthesia. The epididymis was left in place. Control rats were also anaesthetized, their scrotas opened, and the testes manipulated, but left in situ.

A fourth group of cadmium-treated rats received injections of 0.2 M zinc acetate (Lachema p.a.) under the skin of the back just above the tail; control rats received injections of 0.4 M sodium acetate (Lachema p.a.) or 0.2 M calcium or copper acetate.

Organ weights and histological study

All the animals were killed by decapitation. The organs were weighed immediately with the exception of the seminal vesicles and prostate, which were weighed after fixing in Bouin’s fluid for 24–36 hr.

For histological study all organs were fixed in Bouin’s fluid, mounted in paraffin blocks, sectioned, and stained with haematoxylin and eosin. Castration phenomena in the pituitary gland were demonstrated by the Hotchkiss-McManus PAS reaction [Pearse, 1954] as described by Pearse [1952a, b], and also by the Wilson & Ezrin [1954] modification of this method.

RESULTS

(1) Effect of cadmium on the rat testis

Macroscopic changes in the testes appeared within the first few hours following treatment with cadmium chloride. They first became swollen, dark red or purple. Their weights then decreased rapidly and they became small, hard and yellowish in colour (Pl. 1, figs. 1, 2). Doses as small as 0.02 m.mol. of cadmium chloride/kg body weight produced testicular damage. When a tenth of this dose was given, the testes remained undamaged 10 days later. Cadmium lactate, when given instead of the chloride, caused similar changes. Mercuric chloride injected in an equimolar amount did not cause any testicular damage [Pařízek & Záhoř, 1956].

In Pl. 1, figs. 3–6 and Pl. 2, figs. 7, 8, the time sequence of histological changes produced by 0.04 m.mol. of cadmium chloride/kg body weight is shown. Within the first 6 hr after injection of the salt, hyperaemia and interstitial oedema appeared, accompanied by changes in the seminiferous tubules affecting particularly the sperm cells and their immediate precursors. Forty-eight hr later, there was total destruction of all tubular epithelium, while in the interstitial tissues there were haemorrhages, vascular thromboses and a slight inflammatory reaction. After 10 days the whole testis was replaced by masses of eosinophilic material through which scanty basophilic nuclear residue was distributed.

During the following days, while the central portion of the testis remained necrotic,
proliferation of fibroblasts, numerous blood vessels and islands of Leydig cells appeared under the tunica albuginea.

(2) Castration phenomena after cadmium application

Such complete destruction of the testis naturally greatly decreases or even abolishes its endocrine activity. This leads, as is shown in Text-fig. 1, to a rapid loss of weight of the seminal vesicles and prostate. In the pituitary gland castration changes developed. When testosterone propionate was given, beginning on the 10th day after cadmium administration (one injection of hormone every other day, seven doses of 200 μg each), the weight of the seminal vesicles and prostate again increased (Table 1). This shows that the weight loss of these organs was due mainly to hormone

Table 1. Weight of seminal vesicles together with coagulating glands and weight of prostate, when testosterone propionate was given, starting 10 days after cadmium injection (means ± S.E.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Seminal vesicles + coagulating glands (mg/100 g body weight)</th>
<th>Prostate (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (olive oil)</td>
<td>6</td>
<td>367.1 ± 36.5</td>
<td>124.1 ± 12.5</td>
</tr>
<tr>
<td>Cadmium + olive oil</td>
<td>6</td>
<td>43.2 ± 3.3</td>
<td>33.8 ± 6.0</td>
</tr>
<tr>
<td>Cadmium + testosterone propionate</td>
<td>6</td>
<td>381.5 ± 56.4</td>
<td>75.9 ± 3.6</td>
</tr>
</tbody>
</table>
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deficiency and not so much to a direct toxic effect of cadmium. The possibility that cadmium acts directly on the prostate, however, requires further study.

These castration phenomena are not permanent. By the 52nd day after cadmium was given, the weight of the prostate and seminal vesicles had again increased. This growth continued during the following month, paralleling the proliferation under the tunica albuginea. The adrenal glands, which had increased in weight in the first days after cadmium injection, returned to normal again within this time (Text-fig. 1). Castration changes in the pituitary gland did not disappear. In cases where the testicular remnants were extirpated on the 52nd day, another fall in weight of the prostate and seminal vesicles took place within 14 days (Table 2).

Table 2. Endocrine effect of surgical removal of testicular remnants 53 days after cadmium injection. Weight of seminal vesicles together with coagulating glands and weight of prostate 2 weeks after operation, 67 days after cadmium treatment (means ± s.e.)

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>Seminal vesicles + coagulating glands (mg/100 g body weight)</th>
<th>Prostate (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium controls</td>
<td>5</td>
<td>236·1 ± 27·7</td>
<td>66·3 ± 5·7</td>
</tr>
<tr>
<td>Cadmium + surg. castration</td>
<td>6</td>
<td>48·8 ± 9·0</td>
<td>44·2 ± 2·6</td>
</tr>
<tr>
<td>Cadmium + sham operation</td>
<td>5</td>
<td>173·0 ± 31·9</td>
<td>54·9 ± 11·1</td>
</tr>
</tbody>
</table>

(3) Effect of cadmium on the mouse testis

Similar destruction of the testes of mice was observed after cadmium (Table 3).

Table 3. Effect of cadmium on the mouse testis (with resulting castration phenomena). Weight of the testis and weight of seminal vesicles with coagulating glands 40 days after subcutaneous injection of 0·04 m.mol. cadmium chloride/kg body weight (means ± s.e.)

<table>
<thead>
<tr>
<th></th>
<th>No. of mice</th>
<th>Testis (mg/100 g body weight)</th>
<th>Seminal vesicles + coagulating glands (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>10</td>
<td>298·4 ± 18·4</td>
<td>322·4 ± 36·6</td>
</tr>
<tr>
<td>Cadmium</td>
<td>7</td>
<td>102·9 ± 9·5</td>
<td>109·0 ± 23·4</td>
</tr>
</tbody>
</table>

(4) Effect of simultaneous administration of zinc

Elcoate, Fischer, Mawson & Millar [1955] found that chronic nutritional deficiency of zinc leads to testicular damage. Considering that cadmium administration and zinc depletion both lead to testicular damage and that these two elements are similar physico-chemically, the question arose whether the same mechanism might not underlie both toxic effects.

Eighty, one hundred and two hundred times the amount of zinc acetate was given, together with a toxic amount of cadmium [Pařízek, 1956]. The total amount of zinc was divided into three doses, one being given 5 hr before the cadmium, the second together with it, and the third 19 hr later. The results are shown in Pl. 2, figs. 9 and 10, and Text-fig. 2. The testes were fully protected against the toxic effect of cadmium and castration phenomena did not develop. In a control group, also given acetate ions in the form of sodium acetate together with cadmium, the castration phenomena
were produced to a full extent. In other, preliminary, experiments other divalent cations, calcium and copper, were given together with cadmium, but did not prevent characteristic injury to the testes, even if the dose given was two hundred times that of cadmium.

Text-fig. 2. The protection of testes against cadmium damage by simultaneous administration of a larger amount of zinc salts in rats. Weight of the testes, seminal vesicles together with coagulating glands and prostate in mg/100 g body weight. C: normal animals and animals treated with 6.6 and 9.6 m.mol. sodium acetate/kg body weight. Cd: animals treated with 0.032 and 0.04 m.mol. of cadmium chloride/kg. Half of the rats also received the same amount of sodium acetate as in group C. Cd + Zn: animals given the same amount of cadmium as in the preceding group, together with 3.33 and 4.8 m.mol. zinc acetate/kg. Zn: animals given the same amount of zinc as the preceding group but without cadmium. The acetates of sodium and zinc were given in divided doses before, together with and after cadmium injection. The rats were killed 8 or 9 days after cadmium administration.

DISCUSSION

The biological effects of cadmium have been studied in toxicology and industrial medicine for nearly a century [Marmé, 1867]. Alsberg & Schwartz [1919] and Schwartz & Alsberg [1923] noticed 'a blue discoloration of the testicle' after parenteral application of cadmium in studying the emetic action of this cation. This seems to have escaped attention, however, and no further work appears to have been devoted by these or other authors to testicular changes, even though they are more constant and obvious than those in the liver and other organs. No report can be found on the necrosis produced by parenteral cadmium in testes either in earlier or more recent literature about cadmium [Prodan, 1932; Blume, 1934; Patty, 1949; Hunter, 1953; Friberg, 1952, 1956; Odescalchi & Scudier, 1956]. Some observations, however, corroborate
our own results, in that Johns, Finks & Alsberg [1923] found that cadmium is more
toxic for the male than for the female rat. More recently, White [1955] has reported
that cadmium is extremely toxic for sperm cells in vitro. The finding of testicular
degeneration in studying the effect of long-term cadmium feeding on teeth [Pindborg,
1950] is however morphologically completely dissimilar from the necrosis found by us.
A non-specific basis for these changes in Pindborg’s animals, with such a pronounced,
long-term, cachexia and gastrointestinal necrosis, cannot be excluded.

The testicular necrosis observed after parenteral cadmium chloride cannot be pro-
duced by any other non-specific stress. Testicular damage is also caused by salts of
cadmium other than chloride (cadmium lactate). The toxic effect therefore seems
specific to the cation.

In our present study, damage to the testis occurred with doses of cadmium smaller
than those used in our previous experiments [Pařízek & Žáhoř, 1956]. With smaller
doses changes developed more slowly and were primarily located about the lumen of
the seminiferous tubules and in the interstitial tissues. As yet it cannot be stated with
any certainty whether subcutaneous injection of cadmium affects primarily the
Leydig cells or some other structures such as spermatogenic epithelium. The latter
is more probable.

Wherever its chief site of action, cadmium eventually leads to the total destruction
of the testis, which in turn rapidly evokes castration phenomena. These phenomena
are, however, only temporary. The regenerating interstitial tissue under the albu-
ginea produces androgenic substances. This regeneration of hormone-producing
interstitial tissue, while necrosis of the germinal epithelium persists, offers a new
experimental approach to the study of the biology of Leydig cells.

According to the most widely accepted theory, the toxic effect of cadmium is due to
blocking of sulphydryl groups. It is interesting, however, that such a strong sul-
phydryl inhibitor as mercuric chloride does not affect the testes [Pařízek & Žáhoř,
1956]. Mačák, Černoč, Bartek, Wiedermann & Štantavý [1954] could not demon-
strate any decrease in sulphydryl groups in rat tissue following the administration of
cadmium.

It was demonstrated recently by Canadian authors [Elcoate et al. 1955] that
insufficient nutritional intake of zinc salts, lasting for several weeks, leads to testicular
damage. The physico-chemical characteristics of cadmium and zinc are closely
related. In our experiments, the prevention of testicular damage by cadmium
through administration of large amounts of zinc salts was shown [Pařízek, 1956].
Many authors have noted a high concentration of zinc compounds within the sperm
cells and prostate, although its physiological significance is not yet clear [Bertrand
& Vladesco, 1921; Mann, 1945; Mawson & Fischer, 1952, 1953; Dyrendahl, 1954].
Recently, it was stated that the metabolic turnover of this metal is higher in the
testis than in any other organ studied [Mawson, Fischer & Riedel, 1955]. It may be
assumed that zinc is associated with certain structures of maturing sperm cells during
spermatogenesis. Cadmium probably affects spermatogenesis. This effect of cadmium
may be counteracted with large amounts of zinc salts, suggesting competitive
inhibition between these two elements.

Further work on this problem is required to determine whether such a relationship
also exists in other organs. Okamoto [1942, 1955], for example, has discovered
that a large quantity of functionally important zinc is contained in the islets of Langerhans.

Our results seem to be the first example of an antagonistic relation between zinc and cadmium in higher animals. Such a relationship between two physico-chemically related metals may be of wide biological importance. It is interesting that White & Munns [1951] found that an inhibitory effect of cadmium on yeast growth was antagonized by the addition of zinc to the medium.

The effect of cadmium upon the testes, as we have shown, is not species specific. Further study is needed to determine whether these findings are of significance in industrial hygiene.

I wish to express my sincere thanks to Dr O. Poupa for his keen interest, valuable advice and criticism throughout this study, and in the preparation of the manuscript.

REFERENCES

Marné, W. [1867]. Z. rationelle Medizin, Leizp. and Heidelbg. 29, 125.

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EXPLANATION OF PLATES

PLATE 1

Figs. 1, 2. The destructive effect of cadmium on the testis. On the left (fig. 1), the testis of a normal adult rat; on the right (fig. 2), the testis of a rat of the same weight 133 days after subcutaneous injection of 0.04 m.mol. cadmium chloride/kg body weight.

Figs. 3–6. Time sequence of histological changes after subcutaneous injection of cadmium (0.04 m.mol. cadmium chloride/kg body weight). (Haematoxylin-eosin stain, × 92.) Fig. 3. Normal testis of an adult rat. Fig. 4. Testis of an adult rat 6 hr after cadmium injection. Interstitial oedema and early changes in the centres of some tubules. Fig. 5. 48 hr after cadmium. The convoluted tubules are completely destroyed, except for rare cells remaining near the basement membrane. Interstitial oedema, minimal cellular infiltration, vascular thrombosis. Fig. 6. 10 days after cadmium. Replacing testicular tissue are masses of eosinophilic necrotic material with small amounts of basophil nuclear residue.

PLATE 2

Fig. 7. 20 days after cadmium. Proliferation of fibroblasts and formation of a rich vascular network in the interstitial spaces under the albuginea, regeneration of interstitial tissue.

Fig. 8. 75 days after cadmium. Thickened albuginea; interstitial spaces rich in cells. No signs of regeneration in convoluted tubules.

Fig. 9. Testis 8 days after 0.04 m.mol. cadmium chloride/kg body weight.

Fig. 10. Testis after the same amount of cadmium together with 3.3 m.mol. zinc acetate/kg body weight. (Haematoxylin-eosin stain, × 92.)