ACTION OF CADMIUM ON SOME TESTICULAR ENZYMES OF THE DESERT GERBIL MERIONES HURRIANAE JERDON

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Summary. The active male gerbils were administered with a single sub-lethal dose of 0.56 mg of cadmium chloride per 100 g of body weight in order to study the histological and enzyme histochemical changes after 12 and 24 hr. The testis autopsied after 12 hr showed a red colour due to hyperaemia. The spermatocytes, spermatids and the spermatozoa were displaced from their normal positions. Twenty-four hours after administration, the testis showed an intense red colour due to haemorrhage. The regular succession of the cells in the seminiferous epithelium was upset and the nuclei became pycnotic or showed fragmentation. The interstitium was oedematous and haemorrhagic and the Leydig cells showed necrosis. The histochemical results revealed inhibition of adenosine triphosphatase and 5-nucleotidase activities. In the majority of the tubules, succinic dehydrogenase activity was inhibited in the spermatogonia but the damaged cells showed an increased activity. An increase was also observed in the distended interstitium for sudanophilic lipids.

INTRODUCTION

The earlier studies of Blume (1934), Simon, Potts & Gerard (1947) and Green & Neurath (1953) on inhibition of various enzymes by cadmium, have revealed certain explanations concerning the relationships between metabolic and functional phenomena in the living matter. Later on, the destructive effects of cadmium on the testis were reported by Pařízek & Zahor (1956), Pařízek (1957) and Chiquoine (1964). Gunn, Gould & Anderson (1963) also showed that the characteristic necrosis caused by cadmium could be seen in cryptorchid rats, and in the rats deficient in vitamin E (Mason, Brown & Nesbit, 1964). Kar & Kamboj (1965) showed that zinc and selenium salts failed to protect the rat testis from the effects of cadmium chloride. However, when administered subcutaneously at a remote site, these salts prevented the noxious effects of locally injected cadmium chloride. Mazzanti, Lopez & Berti (1964), after the administration of fluoroacetamide, noted the changes in the mature germinal elements of the testis but not in those elements which showed active mitosis.
Ramaswami & Kaul (1966) observed weight increase in gerbil testis 24 hr after a single, sub-lethal intramuscular injection of cadmium. After 24 hr, the necrosis was evident in the Leydig cells and the germinal epithelium. On the other hand, selenium-injected gerbils did not show any change up to 48 hr and thereafter reduction in the weight of the testis, and atrophy of the Leydig cells and seminiferous cells were observed. They reported, however, that the combined dose of cadmium and selenium caused severe necrosis after 1 week.

While the histological details of the necrotic testis due to cadmium have been extensively studied, there is practically no information about the fate of different enzymes associated with the cell inclusions during the different stages of spermatogenesis. The aim of the present investigations has been to study the increase or decrease of certain enzyme activities both in the germinal elements as well as the interstitium after the administration of this metallic compound.

MATERIAL AND METHODS

The active male gerbils were given, subcutaneously, a single, sub-lethal dose of 0·56 mg of cadmium chloride per 100 g of body weight. The controls were injected with distilled water. The animals were killed by quick exsanguination after 12 and 24 hr. The testes were removed and chilled at 4° C, mounted on cryostat for frozen sections and cut at 16 µ. The calcium method of Padykula & Herman for adenosine triphosphatase, and the calcium method of Pearse & Reis for 5-nucleotidase as described by Pearse (1961) were carried out in the fresh frozen sections. The α-naphthol technique of Gomori (1952) for non-specific esterase and staining technique of Nachlas et al. for succinic dehydrogenase as described by Pearse (1961) were brought into practice. The gross lipids were stained by Sudan Black B technique.

RESULTS

Histology

The distilled water injections did not bring about any change in the testes of the control animals. The testis showed all the stages of spermatogenesis (Pl. 1, Fig. 1).

The cadmium-treated testis after 12 hr showed a red colour due to hyperaemia. The spermatocytes, the spermatids and the spermatozoa were displaced but not the spermatogonia.

Twenty-four hours after the injection, the testis showed an intense red colour due to haemorrhage. The regular succession of the cells in the seminiferous epithelium was upset and the nuclei became pycnotic or showed fragmentation. The interstitium was more oedematous and haemorrhagic, and the Leydig cells showed necrosis (Pl. 1, Fig. 2).

Histochemistry

5-Nucleotidase. In the control testis, the basement membrane showed negative reaction. The spermatogonia and the spermatocytes exhibited a very dull reaction to adenosine 5-phosphate (Pl. 1, Fig. 3). The spermatids displayed a
Fig. 1. Photomicrograph of the control testis showing normal spermatogenesis. H. & E. x 320.

Fig. 2. Photomicrograph of the testis 24 hr after the administration of cadmium. H. & E. x 320.

Fig. 3. Fresh frozen section of the control testis showing the localization of 5-nucleotidase activity. x 80.

Fig. 4. Fresh frozen section of the testis 24 hr after the administration of cadmium. Note the inhibition of 5-nucleotidase activity. x 80.

Fig. 5. Fresh frozen section of the control testis showing the distribution of ATPase activity. x 80.

Fig. 6. Fresh frozen section of the testis 24 hr after the administration of cadmium. Note the decreased ATPase activity. x 80.

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Fig. 7. Fresh frozen section of the control testis showing the non-specific esterase activity in the interstitium. ×80.

Fig. 8. Reduced esterase activity in the testis operated 24 hr following the administration of cadmium. ×80.

Fig. 9. Fresh frozen section of the control testis showing the distribution of sudanophilic lipids. ×80.

Fig. 10. Sudanophilic lipids in the testis 24 hr after the administration of cadmium. Note an increased activity in the distended interstitium. ×80.

Fig. 11. Fresh frozen section of the control testis showing the distribution of succinic dehydrogenase activity. ×80.

Fig. 12. Succinic dehydrogenase activity 24 hr after the administration of cadmium. Note an increased activity in some of the tubules. ×80.

(Facing p. 511)
Testicular enzymes of the desert gerbil

moderate reaction. The maximal enzymatic activity was shown by the spermatozoa. The interstitial cells were enzyme-negative. In the cadmium-treated testis after 12 hr, the reaction was almost similar to that of the control. After 24 hr, irregular distribution of the enzyme was observed. No reaction could be observed in the spermatogonia and the spermatocytes (Pl. 1, Fig. 4). The spermatids showed a reduced activity as indicated by the intensity of the reaction. In some of the tubules, the enzyme activity was quite inhibited.

*Adenosine triphosphatase.* In the control testis, the basement membrane displayed a positive reaction to ATP. The spermatogonia and the spermatocytes revealed a feeble reaction. Positive reactions were shown by the spermatids and the spermatozoa (Pl. 1, Fig. 5). The Leydig cells showed negative reaction. The testis, after 12 hr, did not indicate any difference in the intensity of the reaction. After 24 hr, the distribution of the enzymatic activity in the seminiferous tubules was irregular. In the majority of tubules the activity was inhibited (Pl. 1, Fig. 6).

*Non-specific esterase.* In the control testis, the localization of the enzyme was mainly restricted to the Leydig cells (Pl. 2, Fig. 7). However, in some of the tubules, the Sertoli cells also exhibited positive reaction. In the testes after 12 and 24 hr, the necrosed Leydig cells showed a conspicuous decrease of esterase activity (Pl. 2, Fig. 8).

*Lipids.* In the control testis, the sudanophilic lipids were located in the various spermatogenetic cells. The reaction in the spermatogonia and the spermatocytes was minimal in contrast to the reaction observed in the spermatids. Both coarse and fine droplets occurred within the seminiferous tubules. The coarse particles varied in size and were located irregularly at the periphery of the tubules near the basement membrane. The minute particles were widely scattered, existing both at the periphery and toward the centre of the tubules and were concentrated within the cytoplasm of the spermatids. The Leydig cells contained maximal sudanophilic lipid contents (Pl. 2, Fig. 9). In the testis after 12 hr, a decrease was noticed in the spermatogonia. However, no change was observed in the spermatocytes and the spermatids. After 24 hr the lipid contents varied in the seminiferous tubules. In some of the tubules, the distribution was similar to that in the control testis, while in others, inhibition of lipid contents was noticed. The lipid contents increased fairly in the interstitium (Pl. 2, Fig. 10).

*Succinic dehydrogenase.* The enzymatic activity was located in the mitochondrial region of the seminiferous epithelium and occurred chiefly in the younger cell types. Reactivity was also observed in the cytoplasm of these cells, whereas the nuclei were practically devoid of it. The Sertoli cells too, gave positive reaction. The middle piece of the sperm heads exhibited maximum enzyme activity. The interstitial cells showed a very faint activity (Pl. 2, Fig. 11). In the testis after 12 hr, the distribution of succinic dehydrogenase did not reveal any marked difference. The testis after 24 hr revealed erratic succinic dehydrogenase activity. In some of the tubules, the activity was inhibited in the spermatogonia whereas the spermatids and the spermatozoa did not indicate any variation in the stainability (Pl. 2, Fig. 12). In the majority of the tubules, the damaged cells showed an increased activity.
DISCUSSION

Several attempts have been made to understand the toxic effects of cadmium on the various enzymes and it has been reported by Barron & Kalnitsky (1947) and Simon et al. (1947), on the basis of their studies in different tissues, that cadmium brings about inhibition due to its combination with the sulphydryl groups of the protein moiety of the enzymes and forms mercaptides, an effect which can be reversed by the addition of dithioles. It is known that large numbers of enzymes contain sulphydryl groups and these groups may play some role in connection with the nuclear division, a process which is so markedly disrupted in the spermatogenic cells by cadmium, although the evidence is not entirely conclusive. It has further been observed by Sanadi, Langley & White (1959) that cadmium is a potent inhibitor of the enzymic oxidation of α-ketoglutarate. Jacob, Jacob, Sanadi & Bradley (1956) observed that cadmium ions in very low concentrations could completely uncouple phosphorylation associated with the oxidation of succinate and citrate in the mitochondria.

There is, however, no information about the effects of cadmium on the enzyme systems of the testis. Simon et al. (1947) in other tissues, concluded that succinic dehydrogenase was most readily inhibited by cadmium impairing the physiological activity of this enzyme. But the studies on the cadmium-treated testes of gerbils, operated after 12 and 24 hr showed an increase in the succinic dehydrogenase activity in the damaged cells as indicated by the intensity of the reaction. Furthermore, our results indicated marked inhibition in the 5-nucleotidase and ATPase activities. Cadmium chloride which also affected Leydig cells of the experimental animals, showed gradual inhibition of nonspecific esterase activity. The increase in the sudanophilic lipids in the distended interstitium could be due to the haemorrhagic effect produced by this metallic compound. From the results it is thus obvious that cadmium brings about its toxic effects on certain enzyme activities rendering the cellular components functionless.

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