RADIATION EFFECTS ON TESTES
II. INCORPORATION OF $^{65}$Zn AFTER PARTIAL BODY GAMMA-IRRADIATION OF RATS

G. S. GUPTA AND S. R. BAWA

Department of Biophysics, Panjab University, Chandigarh-14, India

(Received 25th February 1974)

Summary. The decrease in the uptake of $^{65}$Zn by irradiated testes (720 R) was followed by recovery after 30 days. After a dose of 2000 R, uptake of $^{65}$Zn was systematically reduced over a period of 74 days. Studies following the administration of testosterone and FSH to the irradiated rats confirm that the incorporation of zinc in the non-germinal cells of the testes is under the control of pituitary gonadotrophins.

INTRODUCTION

The rôle of zinc in the normal function of the male genital tract has been studied by Gunn & Gould (1958), Millar, Elcoate, Fischer & Mawson (1960), Miller, Fischer, Elcoate & Mawson (1958) and Gunn, Gould & Anderson (1961). The uptake of $^{65}$Zn by irradiated testes has been studied by Shikita & Tamaoki (1963). It is reported that the uptake of $^{65}$Zn by the testes and male sex accessory organs is under the control of pituitary gonadotrophins and testosterone (Gunn et al., 1961). Zinc plays an important rôle in spermatogenesis and its most probable site of action is the primary spermatocyte (Pařízek, Boursnell, Hay, Babicky & Taylor, 1966). Timm & Schulz (1966) found high levels of zinc in spermatogonia, spermatocytes and sperm heads. Sertoli and Leydig cells also contain zinc (Gunn & Gould, 1970). In view of the fact that it has been reported that zinc deficiency can cause malignancy in the testis but is also capable of inhibiting tumour growth (Gunn & Gould, 1970), it was considered worthwhile to study the incorporation of $^{65}$Zn by irradiated testes after partial body $\gamma$-irradiation and the response to hormone therapy.

MATERIALS AND METHODS

Radiation procedure and treatment of tissue

Ninety-two normal white albino male rats (160 to 220 g) were irradiated with a single surface dose between 720 and 6000 R from a shielded cobalt-60 source in the radiotherapy unit. The rats were anaesthetized with Nembutal and the body area of each rat inferior to the penis was irradiated (70 to 80 R/min) at a distance of 65 cm from the source (Gupta & Bawa, 1971a). Twenty-eight rats were sham irradiated and acted as controls. The animals were killed by decapitation.
Hormone therapy

Twenty-five rats (200 to 220 g) were allotted to five groups with five rats in each group. Group I rats were sham irradiated and injected with oil vehicle; Group II rats were sham irradiated, and injected with testosterone propionate (TP); Group III rats were irradiated and injected with oil; Group IV rats were irradiated and injected with TP; Group V rats were irradiated and injected with oil and FSH (NIH-FSH-P1).

The scrotum of rats in Groups III, IV and V were irradiated at 2000 R. After 14 days, each rat in Groups II and IV was injected subcutaneously on alternate days with 0.7 mg TP dissolved in 0.2 ml ground-nut oil. The rats in Group V received FSH in 0.2 ml water (2 mg/kg) and 0.2 ml oil. Hormone treatment was continued for 12 days. The rats in Groups I and II, receiving 0.2 ml ground-nut oil, acted as controls. The rats were killed 26 days after irradiation.

Administration of $^{65}$Zn

Before any of the rats in Groups I to V were killed, $^{65}$Zn in the form of zinc chloride (sp. act. = 500 μCi/g zinc) was administered intraperitoneally (50 μCi/kg). The activity was stabilized for 72 hr according to the method described by Singh, Nath & Chakravarti (1970). The activity was determined in 2.5-ml homogenates digested in KOH on a well-type NaI scintillation medical spectrometer.

RESULTS

Complete absence of germ cells and shrunken testicular tubules was observed 26 days after irradiation at 2000 R (see Gupta & Bawa, 1974a, Plate 1). The Sertoli cells seemed to be intact but the interstitial tissue showed marked

![Text-Fig. 1. The uptake of $^{65}$Zn by rat testes after irradiation at (a) 720 R and (b) 2000 R. The values are expressed as a percentage of the control values. Each point is the mean of the results of two experiments. There were four control rats and six irradiated rats. ▲, $^{65}$Zn activity/mg protein; △, $^{65}$Zn activity/g tissue; ○, $^{65}$Zn activity/testis.](image-url)
hyperplasia. Though hyperplasia was detectable after 30 days at 720 R, it was marked at the same interval after 2000 R.

The percentage differences for $^{65}$Zn uptake between normal and irradiated rat testes on different days are shown in Text-figs 1 and 2. At a sub-lethal dose, $^{65}$Zn activity declined for the first 30 days (Text-fig. 1a), but later showed an upward trend. At a lethal dose, however, the $^{65}$Zn-uptake by the irradiated testes declined continuously up to 74 days (Text-fig. 1b). The pattern of $^{65}$Zn incorporation/mg testicular protein was similar to that of $^{65}$Zn/g fresh tissue (Text-figs 1 and 2). Text-figure 2 reveals that the declining ability of the irradiated testis to incorporate $^{65}$Zn was dose-dependent up to 6000 R and the overall pattern seemed to bear an exponential relationship. The effect of radiation castration on the weights of the ventral prostate and seminal vesicles is shown in Table 1. Administration of TP reduced the uptake of $^{65}$Zn in

Table 1. Weights of the accessory organs of rats after partial body $\gamma$-irradiation of testes and hormone administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean wt ± S.E. ventral prostate (g)</th>
<th>Mean wt ± S.E. of seminal vesicles (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Sham irradiated + oil</td>
<td>0.20 ± 0.04</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>Sham irradiated + TP</td>
<td>0.34 ± 0.05</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>III</td>
<td>Irradiated + oil</td>
<td>0.15 ± 0.03</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td>Irradiated + TP</td>
<td>0.32 ± 0.05</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>V</td>
<td>Irradiated + FSH</td>
<td>0.16 ± 0.03</td>
<td>0.20 ± 0.03</td>
</tr>
</tbody>
</table>

TP, testosterone propionate.
normal and irradiated testes and FSH increased its uptake in irradiated testes (Text-fig. 3).

**DISCUSSION**

The decrease in the $^{65}$Zn activity at lethal doses from 2000 to 6000 R suggests total failure of $^{65}$Zn uptake by the rat testis. The results at 30 days of a sub-lethal dose of 720 R suggest that the demand for $^{65}$Zn is related to the proliferation of the interstitium (Gupta & Bawa, 1971b, 1972), incorporation of $^{32}$P into nucleic acids (Gupta & Bawa, 1974b) and a higher incidence of glycogen in the non-germinal cells (Gupta & Bawa, 1974a). Since spermatozoa are the richest source of zinc, it seems that, at a dose of 2000 R and above, decreased spermatogenic activity possibly compensates for the abrupt fall in the uptake by the interstitium. Shikita & Tamaoki (1963) have stated that the loss of $^{65}$Zn incorporation by the testis is independent of the pituitary and that its uptake 24 hr after irradiation is related to the interstitial cells.

There are two aspects of the problem concerning the uptake of $^{65}$Zn by the testis: (1) the relation of zinc to non-spermatogenic tissue and interstitial cell hyperplasia, and (2) the rôle of $^{65}$Zn in the spermatogenic cells. Concerning the first of these, zinc is known to have an inhibitory rôle in the hyperplasia of the interstitial cells that occurs following testicular injury by cadmium (Gunn & Gould, 1970). As irradiation of the testes results in a decline in NADP
isocitric dehydrogenase activity followed by a subsequent recovery (Gupta & Bawa, 1971b, 1972), it is possible that the radiation-induced hyperplasia of rat testicular interstitium is related to higher incorporation of $^{65}$Zn in NADP isocitric dehydrogenase at a late stage of radiation. Lindsey, Nichols, Sheline & Chaikoff (1969) suggested that interstitial cell hyperplasia in rat testes following irradiation is due to temporary loss of testosterone which may, in turn, stimulate secretion of pituitary gonadotrophins. According to Lindsey et al. (1969), low doses of radiation may lead to a higher incidence of tumour formation.

Regarding the second aspect of the problem, it is known that zinc deficiency in spermatogenic cells leads to alterations in lipid metabolism. There is an increase in total lipids and cholesterol of animals fed a zinc-deficient diet compared to levels in controls and zinc deficiency results in the impairment of spermatogenesis (Johnson, 1970). Due to the close relationship between zinc and oxidoreductases (Prasad, Oberleas, Wolf & Horwitz, 1967) and the transient lack of oxidoreductases in irradiated testes (Gupta & Bawa, 1971b, 1972), the associated zinc deficiency will result in accumulation of lipids. The zinc deficiency observed in the present study appears, therefore, to be related to the lipid accumulation observed by Lacy (1967) and Kochar & Harrison (1971). Macapinlac, Pearson, Barney & Darby (1968) reported that an increased catabolism of protein and RNA occurs in the testes of zinc-deficient rats rather than a decrease in their synthesis. Since RNA (Gupta & Bawa, 1970, 1974b) and $^{65}$Zn decline in irradiated testes, the possibility of catabolism of RNA due to zinc deficiency cannot be excluded.

Interpretation of the data in Table 1 reveals that the loss in the weight of the ventral prostate was due to loss in the androgen-synthesizing capacity of the testis (Ellis, 1970). The finding that testosterone enhanced the weights of ventral prostate and seminal vesicles in both normal and irradiated rats is in agreement with earlier reports (Mann, 1964), though the enhanced uptake of $^{65}$Zn by seminal vesicles after administration of TP and FSH was not as marked as that by the ventral prostate. Zinc metabolism in the ventral prostate appears to be under the positive control of testosterone and FSH.

It is uncertain whether the increased uptake of $^{65}$Zn by irradiated testes in the presence of FSH is a direct effect of FSH or due to contamination with LH. Pařízek (1960) reports that uptake of $^{65}$Zn by the testes of immature rats is increased by a single dose of 1 to 4 i.u. FSH. Gunn & Gould (1970) maintain that FSH is not effective in low doses but that if $200 \mu$g FSH is given the uptake of $^{65}$Zn can be fully restored. The effect of testosterone in reducing the uptake of $^{65}$Zn by normal and irradiated testis (Text-fig. 3) appears to be due to inhibition of the pituitary and agrees with the finding of Gunn et al. (1961). Studies following administration of TP suggest that non-spermatogenic tissue has a zinc metabolism of its own and confirm the observations of Gunn & Gould (1970).

ACKNOWLEDGMENT

The authors wish to thank Dr P. N. Chhuttani and Dr B. D. Gupta of the Institute of Postgraduate Medical Education and Research, Chandigarh, for providing irradiation facilities.
REFERENCES


