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Radiation effects on an Energy Generating Enzyme in Chick Testis

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ABSTRACT: The present investigation was undertaken to study the radiation induced damages in chick testes. The study conducted on 7 days old chicks for a maximum period of 60 days. Histopathological changes along with radiation induced alterations in the energy generating enzyme such as adenosinetriposphate, succinicdehydrogenase and lactate dehydrogenase profile of the testis have been considered for the study. The result revealed that the testes undergo degenerative changes after radiation. Histological lesions in testicular architecture were more severe. It is expected that the metabolism of the testes will also be disturbed which is evident from biochemical profile of the irradiated tissue.

Keywords: chicks, gamma radiation, testes

INTRODUCTION

Energy generating enzyme such as adenosine triphosphatase succinic and lactate dehydrogenase are affected by irradiation. Adenosine triphosphatase breaks down ATP to meet the immediate energy requirements. Dehydrogenases play a crucial role in supplying energy needed for various metabolic functions in somatic and germ cells. They form a group of enzymes of mitochondrial and cytoplasm origin which facilitate many oxido-reductions responsible for generating ATP. Lactate and succinic dehydrogenases are important oxidoreductases linked to the processes of spermatogenesis and androgenesis these are the key enzymes of tricarboxylic acid cycle. Lactate dehydrogenase is involved in the conversion of lactate pyruvate. Succinic dehydrogenase has been shown to be the histochemical 'marker' for mitochondria in cells. Cells exhibiting intense succinic dehydrogenase reaction suggest high turnover energy in the cells.

Changes in adenosine triphosphatase after irradiation have been reported an increase in several mammalian systems (Ord and Stocken, 1961). The studies made by Daltan and Hamilton (1964), Pora et al 1970 and James et al (1976) indicate variation in the activity of cytochrome oxidase and adenosine triphosphatase after irradiation. Gupta and Bawa (1971) have reported decrease in adenosine triphosphatase activity in rat testis up to 15 days post irradiation following 720 R partial body γ-irradiation. Nehru et al (1991) have described initial increase in adenosine triphosphatase activity followed by a significant decrease in the testis of rat in the later post irradiation period. Shah *et.al.* (1973) have reported significant decrease in succinic dehydrogenase concentration in irradiated rat tissues.

Lactate dehydrogenase is a cytoplasmic marker enzyme which is a well known indicator of damages induced by several factors including radiation (Chandra and Kale, 1998, Wahl *et.al*, 1998). Lactate dehydrogenase is responsible for metabolism and biosynthesis of energetic macromolecules for different essential function (Khan *et.al*, 2001). Azab *et.al,* (2003) found that whole body Gamma irradiation of rats with a short dose of 6Gy produces remarkable increase of serum LDH activity1,7.and 14 days post exposure. Said and Hanafy, (2006) showed that rat exposed to1Gy whole body gamma irradiation exhibited increase LDH activity in the serum in 1,7, and 14 days post exposure .Any interference in the activity of LDH enzyme leads to biochemical impairment and lesions of the tissue and cellular function In the present work an attempt has been made to highlight the radiation induced changes in enzyme system associated with energy production in the cells. Histological study has been used to support the biochemical parameters to understand the response of testis to gamma irradiation.

MATERIALS AND METHODS

Newly hatched white leghorn male checks (*Gallus domesticus*) were procured from Government Hatchery, Sunder Nagar (H.P.) these were maintained in the animal house under optimal hygienic conditions. Seven days old male chicks were divided into two groups the animals of group A served as control whereas, the chicks in group B were exposed to a whole body dose of 5.6 Gy gamma radiations at the dose rate of 0.22 Gy per second. The chicks were scarified by cervical dislocation on days 1,3,5,7,14,28,35 and 60 post irradiation.

Testes were excised from sacrificed animals were fixed in Bouin's fluid and processed for histological studies paraffin embedded tissue sections of 5u thickness were cut and stained with Harris hematoxylin and eosin. Biochemical estimation of adenosine triphosphatase was done according to method of Kielly (1969). Method employed for the quantitative determination of succinate dehydrogenase activity was that of Nachlas *et al*., (1960). The quantitative determination of lactate dehydrogenase activity was done by the hydrazine method of Wotton (1974).

RESULTS AND DISCUSSION

A. Histology

Normal testis. The paired testes of the foul are internal and each is attached by as short mesorchium close to the dorsal body wall at the anterior end of the kidney. The testis is contained within its connective tissue capsule, the tunica albuginea which is very thin and it does not give off septa to divide the testis into separate lobules. In normal chick testes during early stages, from days 1 to 7, testes is covered by a thick outer capsule. Below the capsule are present seminiferous tubule lined with single layer of spermatogonial cells.

Spermatogonia are usually present at the periphery of the tubule. Each samniferous tubule is covered with a thin layer of basement membrane. The interstitial cells are loosely arranged (Fig. 1A). From 14 days onwards the population of the germ cells increase and basement membrane of the tubule become thin.

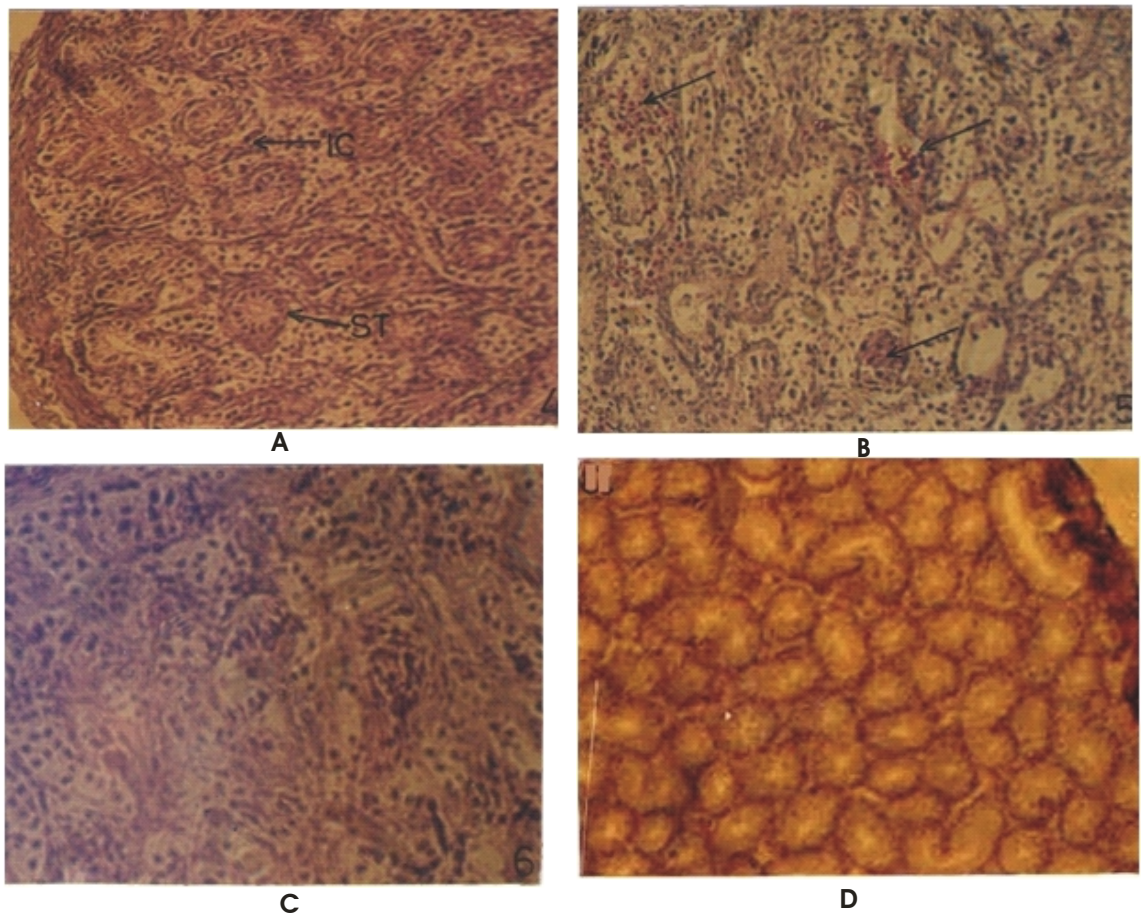

Fig. 1. (A). Photomicrograph of normal chick testis at 5 day stage showing thick outer capsule the seminiferous tubules are lined with a single layer of spermatogonial cells. Note the loosely arranged interstitial cells (IC) between the tubule (X-150). **(B).** Photomicrograph of testis at 14 day stage after exposure to 5.6GYdose of Gamma radiation. Note the infiltration of erythrocytes in the tissue and reduced population of cell in shrunken tubule (X-150). **(C).** Photomicrograph of testis at 14 day stage after exposure showing shrunken condition of cells and exfoliation of germ cells (x-150). **(D).** Photomicrograph of normal chick testis at 35 day stage showing compact arrangement of the tubules. Note the reduced interstitial tissue between the tubules(X-150).

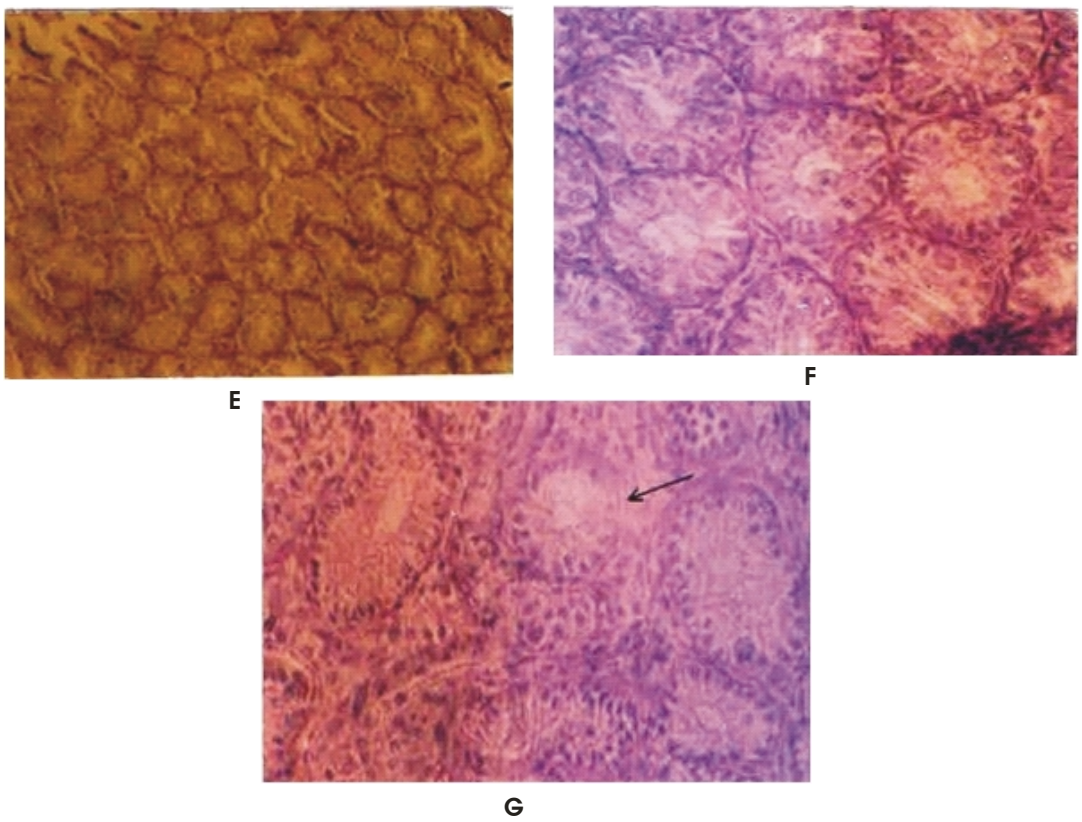

Fig. 1. (E). Photomicrograph of testis at 35 day stage after exposure showing less distorted primary spermatocytes and disorganization of germinal epithelium (x-150). **(F).** Photomicrograph of normal chick testis at 60 day stage showing compectly arranged tubules. (x-500). **(G).** Photomicrograph of testis at 60 day stage showing diameter of the tubules which is still sub normal. (x-500).

At 35 days stage, large number of spermatogonia and a few primary spermatocytes were noticed. Compact arrangement of the tubule and reduced interstitial tissue between the tubule was also observed (Fig. 1D). At 60 day stage the number of primary spermatocytes increases. A gradual increase in the cross sectional dimension of seminiferous tubule is noticed. The interstitial tissue reduces and testicular tissue becomes more compact (Fig. 1F).

Chicks subjected to 5.6Gy whole body gamma radiation, results in a series of physiological events. Most easily detectable changes are in testicular size and weight. Histopathological investigation revealed that damage was less marked in the early post irradiation period. The seminiferous tubules appeared shrunken. The tubules of the central region depicted more damage than those of the peripheral region. At 14 days onwards, testis exhibited high degree of damage after irradiation. Infiltration of erythrocytes in the tissue, and reduced cell population in the shrunken tubules was prominent The seminiferous tubules especially the central one, exhibited exfoliation of the germ cells (Fig. 1B,C). Detachment of sertoli cells and spermatogonia from the basal lamina was also observed by Mugunthan *et al.* (2015) in mice testis after exposure to 2G and 3G radiation. At 28 and 35 days post irradiation there was significant regression of the tubules. Distortion and disorganization of germinal epithelium was noticeable (Fig. E. G). Recovery of the tissue was evident at day 60 post irradiation but population of the germ cells and the diameter of seminiferous tubule and the number of primary spermatocytes per tubule cross section in the irradiated testis were significantly less in comparison to that of the normal testis (Fig. 1G).

Biochemical: Adenosine Triphosphatase (Table 1)

Normal chick:

An increase in the activity of adenosine triphosphatase was recorded from day 1 (0.654± 0.005) to day 3 (0.730±0.013) in the unirradiated chick testis. Decline in the value was recorded at day 5 (0.620±0.011 to 1.092±0.080). The value fell at day 28 (0.548±0.005). ATPase exhibited an elevated value at 35 and 60 day stages (1.200± 0.016 and 1.731± 0.002) respectively.

Irradiated Chick: The adenosine triphosphatase content in the testis of the irradiated chicks decreased from day 1 to 14 as compared to the normal chick testis. A significant decrease was noticed at day 1 (15.4%) and 3 (15.6%).

Table 1: Adenosine triphosphatase activity in chick testes following gamma irradiation as µg Pi released/mg fresh tissue weight/hr at 37● C± S.E.

Period in days

Dose	Group	1	3	5	7	14	28	35	60
0Gy	Normal	0.654 ±0.005	0.730 ±0.013	0.620 ±0.036	0.638 ±0.011	1.092 ±0.080	0.549 ±0.005	1.200 ±0.016	1.731 ±0.002
5.6Gy	Irradiated	0.533 ±0.018	0.616 ±0.016	0.578 ±0.007	0.570 ±0.054	0.853 ±0.040	0.577 ±0.004	1.123 ±0.019	0.980 ±0.009

b (P<0.011) indicates significant change in comparison to unirratiated control

This was followed by a slight increase in the enzyme level at day 28 (5.1%) and day 35 (6.4%) after exposure but a significant decrease in the enzyme activity was once again observed at 60 day stage (43.0%).

Succinate Dehydrogenase (Table 2)

Normal Chick: In normal chick the level of succinate dehydrogenase increases from 1 day stage to 60 day, maximum enzyme activity was observed at 60 day stage (19.016± 0.098ug/mg). In the irradiated chicks, testis exhibited increased content of enzyme during early stages. A Significant increase in the enzyme activity was noticed at 3 day (15.6%) and 5 day (57.9%) stages. Decline in the enzyme level was noticed from day 7 to 35 as compared to normal. The decrease in the activity was significant on days 14 (9.2%), 28 (16.6%) and 35(33.7%) post irradiation. At day 60, a significant increase in the enzyme level was recorded (20.4%).

Table 2: Succinate dehydrobogenase activity in chick testes following gamma irradiation as diformazan formed/mg fresh tissue weight /hr at 37● C ± S.E

Period in Days

Dose	Group	1	3	5	7	14	28	35	60
0Gy	Normal	7.151 ±0.224	8.095 ±0.077	8.319 ±0.198	11.169 ±0.060	12.833 ±0.196	14.706 ±0.211	15.565 ±0.101	19.016 ±0.098
5.6Gy	Irradiated	7.277 ±0.057	9.317 ±0.089	13.144 ±0.560	11.083 ±0.140	11.650 ±0.165	12.251 ±0.601	10.319 ±0.601	22.913 ±0.196

b (P<0.011) indicates significant change in comparison to unirratiated control

Lactate dehydrogenase: (Table 3)

Normal chick:

Lactate dehydrogenase level in the unirradiated testis decreased from 0.298+-0.028 at day 1 to 0.231+-0.003 at day 5. An increase in the value was observed at day 7(0.272+-0.002). At 14 day stage a decline in the lactate dehydrogenase activity was noticed (0.262+-0.001). Thereafter from day 28 to 60 alternate pattern of increase and decrease in the enzyme activity was observed.

Table 3: Lactate dehydrogenase activity in chick testes following gamma irradiation as mM pyruvate reacting/g fresh tissue weight/min ± S.E.

Period in days

Dose	Group	1	3	5	7	14	28	35	60
0Gy	Normal	0.298 ±0.028	0.279 ±0.005	0.231 ±0.003	0.272 ±0.002	0.262 ±0.006	0.353 ±0.004	0.262 ±0.01	0.471 ±0.010
5.6Gy	Irradiated	0.319 ±0.012	0.292 ±0.002	0.332 ±0.003	0.293 ±0.004	0.244 ±0.006	0.262 ±0.006	0.244 ±0.006	0.244 ±0.006

b (P<0.011) indicates significant change in comparison to unirratiated control

Irradiated chick: In the irradiated chick testis an increase in the enzyme level was recorded from day 1to7 .Increase in the value was significant at days 5(43.7%) and 7 (7.7%) post irradiation .From 14 day onwards lactate dehydrogenase activity decreased. Fall in the enzyme activity was significant at 14 (6.8%), 28(25.7%) and 60(13.5%).

DISCUSSION

A series of histopathological lesions were observed in the chick testis following irradiation. Testis showed shrunken condition and the germinal epithelium were disorganized. The germ cell population was reduced but interstitial tissue remained unaffected. Hyperaemia was visible in the irradiated tissue. Reduction in testicular cell types after irradiation was noticed. Reduction in the number of spermatogonia was due to cell death as well as mitotic inhibition among stem cell. There is depilation of various cell types after irradiation. Adenosine triphosphatase is an ATP splitting enzyme associated with production of energy in the cells and with transport function the cell membrane. In the present investigation, adenosine triphosphatase activity in the chick testes showed an increase with age, from day 1 today 60 stages expect at 28 day stage.

Enzyme activity in the testes decreased following irradiation except at day 28. The decrease indicates that the ATP splitting enzymes are either not synthesized or have been rendered non-functional as a result of irradiation. It may be recalled that no testicular growth takes place during this period. As such, decrease in ATPase activity in chick testes following irradiation indicates the energy requirement metabolism occurring in animals. Mitochondria are the carriers of oxidative enzymes. Nachalas *et al* (1957), localized SDH histochimcally in testes. Turpenen *et al* 1968) found the enzyme activity to be more concentrated in the young cell types and sertoli cells.

In normal chicks, the level of succinic dehydrogensase increase from 1 day stage to 60 day stage is very much in conformity with the ATPase activities recorded. After irradiation, the enzyme activity increased at days 1 and 3 followed by significant fall from days 14 to 35 after exposure. There was a significant increase at day 60 post irradiation the portable explanation for such a response in younger animals could be that they have better capability to recover from the irradiation almost injury. In the present study the LDH activity in the normal chick testis remained almost same from 1 day stage to 35 day stage, increasing at 60 day stage of a value which was 58% higher than observed at 1 day stage. Chicks when exposed to a dose of 5.6 Gy exhibit an increase in lactate dehydrogenase activity at early intervals but from 14 days onwards the activity decreased. Usually a high activity of lactate dehydrogenase in spermatozoa and reproductive tissue ensures that locate is an oxidisabel substrate for the energy production in the tissue (Story and Kayhe, 1975). The decline in adenosine triphosphatase in irradiated testis from day 1 to 14 shows the metabolic needs of the tissue are low during period. A significant increase in SDH activity at 60 day pos 9 irradiation points to the high energy needs of the recouping tissue ionizing gradation is a therapeutic agent in the treatment of pathological conditions particularly cancer. The normal tissues which are intimately associated with turnover or are in the path of the treatment get damaged. To protect the normal tissue from the adverse radiation effects, search is on to find drugs which can potentiate and protect against radiation effects. With the increasing incidences of radiation induced reproductive disorders, there is urgent need to identify a radio protector which helps to protect testes from deleterious effects of radiation and bring about faster recovery of the damaged testicular tissue.

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