THE EFFECTS OF X-IRRADIATION ON STEROID
BIOTRANSFORMATIONS BY TESTICULAR TISSUE

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ABSTRACT

A number of parameters of testicular and body function were investigated after various dosages of x-irradiation to ascertain: a) what relationship they have to the radiation syndrome and testicular repression and regeneration of the rat; and b) how sensitive these parameters are to radiation. Changes in androgen synthesis were not well correlated with either body or gonad weights, hematocrit values or testicular histology. Lipid peroxidation, catalase activity, metabolism of testosterone, prostaglandins, cyclic nucleotides and serotonin metabolism were all related to the direct effects of radiation on the male gonad. Indirect effects on the testis appear to be mediated by serotonin and the pineal gland. The pineal gland appeared to be responsible for variations in androgen synthesis and radiosensitivity of the testis through its secretory products - melatonin and arginine vasopressin. These compounds have the capacity of inducing endocrine rhythms by affecting: a) the hypothalamus-pituitary axis; b) the liver; and/or c) the gonad directly.

Prior to the initiation of this project, a series of investigations were undertaken by the principal investigator. These studies showed that in vitro androgen (androstenedione and testosterone) synthesis by mouse testes was elevated for twelve days after 540 R x-irradiation (1). This was followed by a return to normal by 20 days with a second increase in androgen synthesis noted 30 days after the insult. This latter increase was associated with a proliferation of interstitial cell mass. Corresponding changes were also noted in the intermediates of androgen synthesis. Similarly in vitro androgen synthesis by rat testes was also inhibited 20 days after 450 x-irradiation, but this change was ameliorated by the addition of a NADPH-generating system to the incubation mixture (2). Moreover, localized irradiation to the head of rats resulted in a diminution of androgen synthesis that was also alleviated by the addition of the NADPH-generating system to the incubation mixture. These data show that there are humoral effects of radiation that originated from the head (3). In addition, the irradiation defect, whether direct or indirect, is at least partly due to energy abstraction or transfer reactions (enzymatic mechanisms). During the course of the above studies, an increase in high-polar steroid intermediates was observed after irradiation. One of these compounds was isolated and characterized as $17\alpha,20\alpha$ -dihydroxyprogesterone (4).

While food deprivation and irradiation were both deleterious to rat testes, a difference was observed between the response of the two treatments and their effects on androgen synthesis in vitro (5). This difference was particularly evident with respect to the response of the testes to a NADPH-generating system. These data show that the biochemical lesions after x-irradiation are not due specifically to inanition.

Internally deposited radionuclides were also shown to inhibit in vitro androgen synthesis by canine testicular tissue (6). The damage observed in this investigation, however, was not reversed by the addition of a NADPH-generating system to the incubation mixture.

At the onset of the present grant, direct effects of x-irradiation on in vitro androgen synthesis by rat testicular tissue were observed (7). Androgen synthesis was increased with 858 R, but this increase resulted from a diminished rate of metabolism of testosterone. Exposure of the tissue to 4,050 R resulted in a depressed androgen synthesis due to a decrease in biotransformation of the precursors into androstenedione and testosterone, as well as a decreased metabolism of testosterone. A rather marked seasonal difference in the biotransformation of pregnenolone and progesterone into androgens was also observed in this study.

Under the auspices of this grant, an investigation was undertaken to ascertain if the in vitro synthesis of androgens by seminiferous tubules was affected by direct x-irradiation of rat testes with 1,500 R. The data (Fig. 1 & 2) show similar results when the tubules were compared with the minced tissue, except the rate of synthesis was somewhat lower for the teased-tubules. On the basis of the above, a dose-response curve was undertaken to ascertain how sensitive the following parameters of body function are to whole-body x-irradiation: 1) the biosynthesis of androgens from pregnenolone - 7α - ^3H and progesterone - 4 - ^{14}C , by minced tissue and teased-tubular preparations; 2) the metabolism of testosterone by minced tissue; 3) testicular weight and histology; 4) Hematocrit; and 4) body weight. Several additional parameters relating to testicular function were added to the schedule at later intervals: 1) monoamine oxidase activity (MAO) (250 and 150 R); 2) pineal hydroxyindole-O-methyl transferase activity as a measure of pineal

function (350 and 50 R); 3) lipid peroxidation (350, 250 and 150 R) as TBA chromogens (i.e.) thiobarbituric acid reacting substances produced primarily by the oxidation of unsaturated fatty acids derived from membrane phospholipids; and 4) glutathione reductase (50 R).

HEMATOCRITS AND BODY WEIGHT

Hematocrits were depressed by 450 R x-irradiation from 4 through 22 days after the insult (Fig. 3), but this effect had disappeared by and was not evident with 250 R. Body weights were slightly reduced by 450 and 350 R of x-irradiation and for roughly 19 days after 250 R, but not at any time for either 150 or 50 R (Fig. 4).

TESTICULAR WEIGHT

The gravimetric and histologic response of rat testes to x-irradiation was typical of that expressed by other workers (for reviews see refs. 12, 14), i.e., the greatest loss of weight and germinal epithelia occurred with the higher dosages of radiation and was dose-dependent (Fig. 5). Generally, the cellular and weight loss was maximal by approximately 52 days after the insult.

As the dosage of radiation increased, damage to the testis was evident at an earlier date and the recovery period was prolonged. Also the extent of damage was increased as the dosage of radiation increased. At 150 and 50 R recovery of the testis was complete (i.e., testicular weight was equal to that of the control animals by 80 days after irradiation, but with 250 R testicular weight was not equal to the control rats by 153 days after treatment.

ANDROGEN SYNTHESIS

Whole-body x-irradiation with 450 R increased testosterone synthesis 24 hrs., after the insult for both the minced and teased-tubular preparations (8), but this entity was diminished from 4 through 32 days for both groups (8, see Figs. 6 and 7). A distinct pattern was observed for both the control and treated group that was fitted to a mathematical expression and checked for goodness of fit ($R^2 = 0.904$ and 0.0875 for the teased-tubular control and irradiated groups, respectively - see Figs. 8 and 9). The above data suggested that some factor(s) other than x-irradiation was responsible for driving the pattern in both groups of animals. The rise and fall in testosterone noted for both the treated and control groups on days 16-32 was more distinct and larger for the teased-tubular preparations than it was for the minced tissue preparation. Although not published with the above data, two other progesterone metabolites have since been identified as 5α -dihydroprogesterone (5α -pregnane-3,20-dione) and tetrahydroprogesterone (5α -pregnan-20-one-3 α -ol - unpublished data) and quantified. Generally, tetrahydroprogesterone was high on day-1 for the control group; it declined on day-4, and reached a peak on day-13 just prior to the increase in testosterone synthesis observed for both the control and irradiated animals (compare Fig. 10 with Figs. 6 and 7). On the other hand, 5α -dihydroprogesterone for the control animals was high on day-1; it decreased on day-4, but rose to a peak on day-7, and declined until day-13 where it generally increased throughout the observation period (Fig. 11). This latter metabolite reached a peak just prior to the rise in testosterone noted above. Thus 5α -dihydroprogesterone and tetrahydroprogesterone were inversely related to each other and were only causally related to testosterone synthesis. When one looks at the residual progesterone and 17α -hydroxyprogesterone steroid

fractions (8), one does not see these marked fluctuations indicating that the excess substrate progesterone, not converted into androgens, is metabolized into other compounds: either 5α -dihydro- and tetrahydroprogesterone or $17\alpha,20\alpha$ -dihydroxyprogesterone. Moreover, something appears to regulate the above described process by modulating the conversion of 5α -dihydroprogesterone into tetrahydroprogesterone and 17α -hydroxyprogesterone and $17\alpha,20\alpha$ -dihydroxyprogesterone. Of significance is the fact that the 5α -reductase and the 3α - and 20α -reductase enzymes for progesterone and 17α -hydroxyprogesterone reside in the seminiferous tubules. Since progesterone is antispermatogenic, and inhibits rabbit testicular capsular (9) and rat seminiferous tubular contractions (10), it is possible that progesterone is metabolized to remove these intermediates from the seminiferous tubules that would otherwise interfere with normal testicular function. That the testis increases the rate of metabolism of progesterone into compounds other than testosterone when androgen synthesis is diminished after an irradiation insult is pointed out by our previous observation (4) and those of other workers (11) that 20α -reductase for progesterone is increased after x-irradiation when androgen synthesis is diminished.

Throughout the investigation of the dose-response curve for the sensitivity of the biosynthesis of testosterone and androstenedione from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C , some basis rhythm was observed at each treatment level. With 350 R, androstenedione (Fig. 12) produced by the teased-tubular preparations showed a marked increase that reached a maximum at between 10-13 days after treatment for both the control and the irradiated groups. As was noted for 450 R of x-irradiation, testosterone biosynthesis was elevated 24 hrs after the insult, and this was more marked for the teased-tubular preparations (Fig. 13) than was observed for the

minced preparations (Fig. 14). With the exception of day-19 for the minced preparation and days 4 and 7 for the teased-tubular preparations, irradiation diminished testosterone biosynthesis. The reduction in androgen synthesis by x-irradiation was less for 350 R than it was for 450 R.

No increase in testosterone biosynthesis was observed for the teased-tubular preparations for either 250 (Fig. 15) or the 150 R levels of treatment on day-1 after irradiation (Fig. 16). Slight increases were, however, noticed for the minced preparations at this time (Fig. 17). There was a more noticeable increase in androgen synthesis by teased-tubular preparations from rats exposed to 150 R (Fig. 18). An increase in androgen synthesis was observed by teased-tubular preparations from 7-22 days after treatment followed by a decrease 25 through 31 days post-treatment when the irradiated animals were compared with the control rats (Fig. 15). Testosterone biosynthesis was diminished from 4 through 13 days after treatment for the minced tissue preparations from rats exposed to 250 R (Fig. 17). It was elevated 16 through 19 days of treatment, but was similar to the control animals for the rest of the observation period. Androstenedione remained essentially unchanged for both the minced and teased-tubular preparations after 250 R irradiation (data not shown). There was a distinct increase in androstenedione production by both groups of animals for the minced tissue preparations 13 through 31 days after treatment that corresponded with the increase in testosterone production for the control rats (data not shown).

With 150 R of x-irradiation testosterone production by the teased-tubular preparations was, with the exception of days 4 and 28, less than that observed for the control animals (Fig. 16). Testosterone production by minced tissue preparations from rats irradiated with 150 R was higher on

days 1,7,10,16,28 and 31, but was lower on days 19,22,25 and 50 after treatment when compared with the control animals (Fig. 18) with the two groups vasculating around each other.

In the above studies, there was no clear-cut evidence suggesting that androgen synthesis was related to spermatogenic losses or repletion. It appears that there is a natural endogenous rhythm for testosterone synthesis in the testes and that x-irradiation altered this basic pattern through some basis mechanism(s). At higher dosages of radiation, 350-450 R, there did appear to be a reduction in testosterone synthesis during the observation period, but at lower levels this was not evident.

METABOLISM OF TESTOSTERONE

An increase in the conversion of testosterone into metabolites other than androstenedione was observed for testicular tissue from rats exposed to 450 R of x-irradiation (ref. 8 and Fig. 19) concomitant with a reduction in the conversion into androstenedione (ref. 8 and Fig. 20). Metabolism of testosterone into compounds other than androstenedione was increased on day-1, 10 through 16 days and 22-31 days after exposure to 350 R of whole body x-irradiation (Fig. 21). The conversion of testosterone into androstenedione was unaffected by this treatment (Fig. 22). The conversion of testosterone into androstenedione was unaffected by either 250 or 150 R (data not shown). The conversion of testosterone into compounds other than androstenedione was, however, affected by both 250 (data not shown) and 150 R (Fig. 23). An increased metabolism was noted for 150 R from day-16 through 50 (Fig. 23).

The above data show that the metabolism of testosterone into androstenedione is inhibited or diminished at higher dosages of irradiation

(450 R) while the metabolism of this androgen into other steroid metabolites is enhanced for 450 and 350 R. At all dosages studies (450,350,250 and 150 R) the metabolism of testosterone into other metabolites was enhanced approximately 13 days after the insult. This parameter of testicular function was more closely related to the loss and return of germinal elements of the testis than was androgen synthesis. Since this response was observed for all of the dosages of x-irradiation studied, it would appear important to the radiation syndrome.

PINEAL FUNCTION

The above studies showed seasonal rhythms as well as rhythms within the study period (usually 30 days). Furthermore there reportedly are similar rhythms after radiation that have been reported for the thyroid and adrenal (for recent reviews see 12,13). Since melatonin and serotonin are both radioprotective and are known to affect the thyroid, adrenal and testis (12-14), and the pineal gland was shown to respond histologically to x-irradiation (15), investigations were undertaken to ascertain to what extent the pineal gland contributes to the radiation syndrome of the testis. Indeed, HIMOT activity was inhibited 24 hrs. after 450 R of whole-body x-irradiation (16). This effect was maximal 1 hr. after 350 R, but the activity of this enzyme was increased from 4 through 19 days after treatment (17). At the suggestion of the referees (upon grant renewal) the organ culture method of measuring the synthesis of melatonin from tryptophan was investigated for 250 R, but this assay was found to be inadequate to measure in vivo changes in melatonin synthesis. HIMOT was measured for 17 days after 50 R (Fig. 24). HIMOT activity was decreased one day following x-irradiation, but was increased 4 through 14 days.

Exposure of rats to 350 R decreased testicular weights 25 days after treatment (17). Placing the animals in the dark for 25 days after irradiation resulted in a deleterious effect that was superimposed upon the radiation effect. When rats were placed in either constant light or constant dark for 6 days prior to and 19 days after x-irradiation with 450 R, it resulted in a radioprotective effect on the testis (17).

Localized x-irradiation to the heads of rat (450 R) resulted in an increased HIMOT activity while body-only irradiation with the same dose decreased HIOMT activity, indicating that two methods of control are operative with respect to the response of the pineal gland to irradiation (18). The inhibitory effect of localized irradiation of the body appears to be due to histamine as this biogenic amine inhibited melatonin synthesis both in vivo and in vitro (19).

Both melatonin and serotonin inhibited steroid biotransformations in vitro by rat testicular tissue (19). Melatonin, one of two substances produced by the pineal gland, specifically affected the 5α -reductase system for testosterone (19). Exogenous melatonin also affected the Δ^4 -reductase (primarily 5α -reductase) of liver and hypothalamus as did also changes in photoperiod that are known to affect melatonin synthesis (20). Serotonin, melatonin and arginine vasotocin, a second compound produced by the pineal gland, were also found to affect the Δ^4 -reductase and the glucuronide conjugation system of the liver for testosterone (13, 21, 22). Of the three, serotonin had the least effect on Δ^4 -reductase activity.

Although feed deprivation affects steroid biotransformations and inhibits androgen synthesis and the effects of radiation differ from that of feed deprivation (see above), severe feed deprivation does enhance melatonin synthesis by the pineal gland (23).

The above data show that the pineal gland and its secretory products, melatonin and arginine vasotocin, are capable of inducing endocrine rhythms that would be common to the adrenal, thyroid and testis. These effects could originate at the following levels: a) hypothalamus, b) liver, or c) the gland itself (adrenal, thyroid or testis). In this respect, the data from our laboratory indicate that the pineal gland is a transducer mediating the effects of irradiation, stress and some hormones on various other organs and systems of the body (for a recent review see ref. 23).

MONOAMINE OXIDASE (MAO) ACTIVITY

The above studies indicated that serotonin could be an important determinant of testicular function. Other workers also observed deleterious effects of serotonin on the male gonad (for review see ref. 24 & 25). Methods were worked out in our laboratory to assay the inactivation of serotonin through the monoamine oxidase (MAO) system (26). The bulk of the activity was observed to reside in the seminiferous tubules and was subject to inhibition by x-irradiation (26). The activity was high in the testis at birth, declined to a low at 57 days of age and then rose to a high at the height of sexual development (approx. 105 days of age) and declined with senescence and old age (24). Photoperiod and social stress (crowding) were observed to affect testicular MAO activity (27). Testicular MAO activity was found to be enhanced days 7-10, but diminished 19 through 153 days after 250 R of whole body x-irradiation (28). MAO activity was depressed at 56 and 74 days after 50 R of irradiation, and this decrease corresponded with a reduction in testicular weight (Fig. 25). Testicular MAO activity was diminished by hypophysectomy, but increased by HCG or a combination of FSH, LH, and prolactin (29). Pargyline (an inhibitor of MAO activity) increased

the serotonin content of rat testes (29). MAO activity was found to be located primarily in the walls of the seminiferous tubules, but some activity was also present in the germinal cells and interstitial cells of the adult male rat (30). Furthermore, the MAO activity in the tubules was positively correlated with sexual development of the male. Further evidence for a positive role for MAO with respect to testicular function was observed in house sparrows and ground squirrels where MAO increased with sexual development with both species of animals (31).

Melatonin has been found to alter MAO activity and pinealectomy, and changes in photoperiod have been shown to alter MAO activity of the pituitary (32). One of the actions of melatonin and serotonin now appears to be through the control of prostaglandin synthesis and MAO activity (13,33,34). Of significance is the recent observation that excess serotonin and hydroxy-indole acetic acid (the principal metabolite of serotonin) excretion are both associated with infertility in man (see ref. 25 for recent review) indicative of the importance of testicular MAO to normal testicular function.

CATALASE ACTIVITY

Catalase activity was observed to be localized primarily in the interstitial cells of rat testes and was diminished by hypophysectomy (35). It was also increased in activity from 4 through 31 days after 150 R of whole body irradiation (35) indicative of either a substrate induction or some mechanism for increasing this enzyme activity.

GLUTATHIONE REDUCTASE ACTIVITY

Glutathione reductase activity was assayed for rat testicular tissue after 50 R of whole-body x-irradiation. There was an increase in activity

for both groups of animals with time after irradiation, but no effect was observed for radiation at this dosage (Fig. 26).

PHOSPHOLIPASE A₂, LIPID PEROXIDATION AND PROSTAGLANDINS

It is generally concluded (for recent reviews see ref. 12,14), and our data described above are consistent with these findings, that the humoral or mediated effects (including the hypothalamus-pituitary axis and the pineal gland) may affect the endocrine responses of the testis, alter the radiosensitivity of the testis to radiation (protect or potentiate cellular loss) or cause some loss or gain of cellular elements; but the main effects of ionizing radiations on the gonad result from direct effects on the testis. The investigations presented below are oriented towards ascertaining what these direct effects of ionizing radiations on the testes are.

Lipid peroxidation, as measured by the TBA method, was not appreciably altered by 350 R (Fig. 27) on a per mg of tissue basis. Some effects were observed with 250 R, but maximal effects were evident with 150 R of whole-body x-irradiation. Maximal effects were observed with 150 R of whole-body x-irradiation. When the total testicular tissue was taken into account and lipid peroxidation was expressed as "total activity" (Fig. 28), the effect was more noticeable at 350 R and was minimal at 150 R. As far as direct effects of lipid peroxidation on the testis are concerned, one would expect that the concentration per unit of tissue would be most meaningful and, in this case, that would be on a per incubation basis (Fig. 27). Our data (Table 1) show that lipid peroxidation is increased in vitro by x-irradiation and is dose-dependent. Of particular significance is the observation that this increased activity is associated with a release of lysosomal acid phosphatase. Moreover, unsaturated fatty acids are the precursors of lipid peroxidation,

and they (the products of lipid peroxidation) are capable of altering steroid biotransformations in vitro (Fig. 29) and in vivo (33) and more particularly testosterone production. In addition, phenylmethyl sulfonyl fluoride (a cholesterol esterase inhibitor) markedly reduced both diene conjugation (the first step in lipid peroxidation) and the production of TBA chromogens (Fig. 30). The addition of cholesterol arachidonate (a cholesterol ester) to the incubation mixture increased TBA chromogens (Fig. 31), but not diene conjugation (Fig. 32). The cholesterol esterase inhibitor did inhibit the diene conjugation in the presence of cholesterol arachidonate (Fig. 32). Phenylmethyl sulfonyl fluoride did, however, inhibit diene conjugation. The phospholipase inhibitor, dimethyl DL-2, 3-distearoyl-2'-hydroxyethyl-ammonium acetate, inhibited lipid peroxidation (TBA chromogen formation), but had no significant effect on diene conjugation (Fig. 33). Through a series of investigations (33,36-39), we have found that lipid peroxidation in vitro and in vivo is controlled primarily through lysosomal phospholipase A₂ and the release of polyunsaturated fatty acids (primarily arachidonic acid) from membrane phospholipids. The amounts of polyunsaturated fatty acid is rate limiting in the process of lipid peroxidation since the enzymes for lipid peroxidation develop along with the enzymes for steroid biotransformations (36). In general, cyclic AMP inhibited lysosomal release while cyclic GMP increased enzyme release (34,37,38). In addition, substances that inhibit phosphodiesterase also inhibit lysosomal enzyme release through cyclic AMP accumulation.

Ascorbic acid was shown to have a biphasic response on lipid peroxidation (36). The stimulatory effect can now be shown to be due to a direct effect on lipid peroxidation (Fig. 34) while the inhibitory results from its action on phosphodiesterase and cyclic AMP accumulation. Vit. E

inhibited lipid peroxidation (36) primarily through its action on phosphodiesterase. Vit. A stimulated lipid peroxidation (33) primarily through its action on guanyl cyclase. The effects of Zn^{++} on lipid peroxidation (37) appear to be on the phosphodiesterase system and cyclic AMP accumulation. LH and testosterone (37) appear to exert their effects on lipid peroxidation through cyclic GMP. Indomethacin aspirin, cortisol, epinephrine and norepinephrine all stabilized the lysosomes and prevented the release of testicular lysosomal enzymes (37,38). They appear to do so through the cyclic AMP-phosphodiesterase systems. Thus, a number of substances that have been known to affect testicular function, but of which no mechanism of action was known, now have a mechanism of action that has now been elucidated for them.

Malenaldehyde has long been considered to be the substance formed from the peroxidation of polyunsaturated fatty acids, but recent evidence now suggests that lipid endoperoxides the same or similar to the endoperoxide intermediates in the synthesis of prostaglandins themselves undergo acid or thermal catalyzed decomposition to give malonaldehyde and that gives rise to TBA chromogens (40,41). While some of the lipid endoperoxides may be transformed into either malonaldehyde or prostaglandins (40,41), some are biologically and biochemically active, some of which are more active than are the prostaglandins (PGs) (42). Moreover, even lysolecithin, a biproduct of the action of lysosomal phospholipase A_2 , also has regulatory activity on the guanylate and adenylate cyclase systems (43).

Of importance is the fact that lipid peroxidation exerts radiomimetic effects on a variety of model systems (44). Typical biochemical changes in proteins exposed to peroxidizing lipid are similar to those of ionizing radiation and include: a) loss of enzymes activity (45); b) destruction of

individual amino acids (46), particularly methionine, lysine, histidine, cystine, and tryptophan; and c) polymerization crosslinking, or scission (47,48), depending on the nature of the protein (45). Indeed, it has been proposed that reactions of free radicals arising during lipid peroxidation are as important as those of peroxide breakdown products, such as malonaldehyde (49). Malonaldehyde is a particularly damaging substance since it forms Schiff's base reactions with proteins and is capable of inhibiting many biochemical reactions (for rev. see 33, 34, 36).

As indicated above and in Fig. 34, lipid peroxidation and PG synthesis are intimately related to testicular function and pathology. A series of investigations were undertaken to ascertain what physiology roles PGs normally serve in the testis. These studies have periodically been reviewed and have been published elsewhere (33,34,36,39). Our data indicate that PG's are needed for contraction of the testicular capsule of most species (9,50-53) and the seminiferous tubules of all species tested (see also ref. 10, 49).

PGE₁ was found to inhibit testicular capsular contractions at high concentrations (24), while both PGE's and PGF's stimulated contractions at low concentrations (9, 53). Pregnenolone, progesterone and testosterone at 10^{-6} M inhibited testicular capsular contractions (9, 55), but testosterone at lower concentrations 10^{-7} and 10^{-8} M stimulated contractions of the seminiferous tubules (Farr and Ellis - unpublished data, not shown). This effect of testosterone occurs concomitantly with an increase in the release of lysosomal enzymes (37), specifically phospholipase A₂ activity. PG's were found to be necessary for the induction of contractility by Ca⁺⁺, serotonin or acetylcholine, but not epinephrine (57). Our data (58,59) indicate that cyclic AMP was responsible for the inhibition of testicular

capsular contractions with higher concentrations of PGE's. Similar observations have been made for rat seminiferous tubules (10, 33).

Of significance is the recent observation and finding that the lysosomal-enzyme release is associated with the testicular damage resulting from cryptorchidism (34). Also, contractility of the seminiferous tubules increases initially followed by a decrease in activity with an accumulation of cyclic AMP (34). Our data (33) indicate the CAMP and cyclic GMP are both deleterious to the testicular germinal epithelium and cause a loss of testicular weight. PG's themselves can also cause denuding of the seminiferous tubules and a loss of the germinal epithelium (for review see ref. 34). The similarities between cryptorchidism and x-irradiation damage to the testis (34) lead us to speculate that they both share some basic mechanisms of action. In this respect, recent evidence has shown that PG's do increase in the liver and brain after whole-body irradiation (59). Moreover, cycle AMP increases in intestinal mucosa and especially brain, the latter with a progressive long-lasting increase (60).

Radiation induced changes in membrane permeability have been intensively studied and shown to be related to damage of the protein components (for review see ref. 61). Moreover, the loss of proliferative capacity of cells by ionizing radiation varies inversely with sulfhydryl content of the cell membrane (62). Lastly, superoxide dismutase, an enzyme capable of reducing lipid peroxidation and PG formation results in a radioprotective effect on phospholipid membranes (61).

Histochemical studies from our laboratory now show that PG's are formed in the corpus and cauda epididymides, but not the cauput epididymidis (63). The vas deferens, however, produced more PG's than did the testes or the epididymis. The testis produced only small amounts of PG's, but physical

trauma resulted in excessive production of PG's by the seminiferous tubules. Our data are in accord with recent observations that aspirin will ameliorate traumatic damage to the testis (see ref. 34). Recent observations showing that PGs restore spermatogenesis in fatty acid deficient animals suggests a physiological need for PGs, but the fact that aspirin enhances spermatogenesis even in normal mice suggests that even under normal physiological conditions PGs may retard spermatogenesis (for a recent review of this topic see ref. 34).

CONCLUSIONS

We conclude that ionizing radiations directly affect the testes through the formation of free radicals and the release of lysosomal enzymes, particularly phospholipase A_2 , the latter of which attacks membrane phospholipids releasing polyunsaturated fatty acids that are peroxidized to form potential free radicals in the presence of oxygen. These free radicals, in turn, react with -SH groups of enzymes and protein constituents, DNA and specific amino acids to greatly alter cellular biochemistry. Malonaldehyde is released and further alters cellular biochemistry by forming Schiff's bases. PGs accumulate and have direct effects on the germinal epithelium as well as causing cyclic AMP and/or cyclic GMP to accumulate and further alter cellular kinetics within the testis. Alteration of membrane -SH groups and loss of lipid components perturbs the membranes thereby increasing the permeability cells causing them to rupture and die while changes in sulfhydryl content and accumulations of cyclic nucleotides act in concert with the above changes to prevent cellular proliferation with the resultant maturation depletion of germinal epithelia that is characteristic of the radiation syndrome of the testis. Moreover, catalase activity is increased

to inactivate H_2O_2 and other peroxides formed during or after irradiation. Serotonin is increased in the blood after irradiation and the decrease in testicular MAO enables serotonin to express a deleterious effect on the germinal epithelium of the gonad.

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LIST OF PUBLICATIONS

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Table 1. Dose-Dependent release of Lysosomal
Acid Phosphatase from Rat Testicular
Tissue In Vitro.¹

Treatment	Acid Phosphatase Activity	P Value	TBA	P Value
	O. D. Units		O. D. Units	
Control	0.117 ± 0.009*		0.205 ± 0.006*	
858 R	0.136 ± 0.008	<0.05	0.250 ± 0.014	<0.02
4050 R	0.192 ± 0.007 [†]	<0.001	0.296 ± 0.008 [†]	<0.001

* S.E.M. Values.

[†] p < 0.001 when compared with 858 R group.

¹ Taken from Progress report for the 1965-66 fiscal year (C00-1602-56).

Fig. 1.

Biotransformation of 5-pregnenolone-7α-³H and progesterone-4-¹⁴C into androgens by rat testicular minced tissue 24 hrs. after localized x-irradiation of the gonads with 1500 R of x rays when 12 weeks of age (unpublished data).

Fig. 2.

Biotransformation of 5-pregnenolone-7 α -³H and progesterone-4-¹⁴C into androgens by rat teased-tubular preparations 24 hrs. after localized irradiation of the gonads with 1500 R of x rays when 12 weeks of age (unpublished data).

Fig. 3.

Average hematocrit values of rats subjected to either 450, 350 or 250 R of whole-body x-irradiation when 12 weeks of age (unpublished data).

Fig. 4.

Average body weights of rats subjected to 450, 350, 250, 150 and 50 R of x-irradiation when 12 weeks of age (unpublished data).

Fig. 5.

Average body weights (three per point) of adult male rats (12 weeks of age at the onset of the experiment) irradiated with either 450, 350, 250, 150 or 50 R of x rays (unpublished data).

Fig. 6.

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone-4- ^{14}C by rat testicular minced preparations following whole-body x-irradiation with 450 R of x rays (taken from Ellis and Van Kampen, ref. 8).

Fig. 7.

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular teased-tubular preparations following whole-body x-irradiation with 450 R of x rays (taken from Ellis and Van Kampen, ref. 8).

Fig. -8.

Testosterone values from Fig. 6 for the control groups fitted to a mathematical expression

$$(y = ab^x, c^x \cdot 2^{dx^2} \cdot 2^{ex^3})$$

with a goodness of fit $R^2 = 0.904$ (taken from Ellis and Van Kampen, ref. 8).

Fig. 9.

Testosterone values from Fig. 6 for the irradiated group fitted to a mathematical expression

$$(y = ab^x, c^x d^{x^2} e^{x^3/2})$$

with a goodness of fit $R^2 = 0.875$ (taken from Ellis and Van Kampen, ref. 8).

Fig. 10.

Tetrahydroproesterone (5α-pregnan-20-one-3α-ol) biosynthesis by rat testicular teased tubular preparations after whole-body x-irradiation with 450 R (unpublished data).

Fig. 11.

5α-Dihydroprogesterone biosynthesis by rat testicular teased-tubular preparations following 450 R of whole-body x-irradiation with 450 R (taken from ref. 13).

Fig. 12.

Androstenedione biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular teased-tubular preparations after 350 R. of whole-body x-irradiation (unpublished data).

Fig. 13.

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular teased-tubular preparations after 350 R of whole-body x-irradiation (unpublished data).

Fig. 14

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular minced preparations after 350 R of whole-body x-irradiation (unpublished data).

Fig. 15.

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular teased-tubular preparations after 250 R of whole-body x-irradiation (unpublished data).

Fig. 16.

Testosterone production from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular teased-tubular preparations after 150 R of whole-body x-irradiation (unpublished data):

Fig. 17.

Testosterone biosynthesis from pregnenolone- 7α - ^3H and progesterone- 4 - ^{14}C by rat testicular minced tissue preparations after 250 R of whole-body x-irradiation (unpublished data).

Fig. 18.

Testosterone production from pregnenolone- 7α - ^3H and progesterone-4- ^{14}C by rat testicular minced tissue preparations after 150 R of whole-body x-irradiation (unpublished data).

Fig. 19.

Residual testosterone-4-¹⁴C recovered after incubation of this substrate with minced tissue from mature rats exposed to 450 R of whole-body x-irradiation (taken from ref. 8).

Fig. 20.

The conversion of testosterone-4- ^{14}C into androstenedione by testicular tissue from mature rats exposed to 450 R of whole-body x-irradiation (taken from ref. 8).

Fig. 21.

Residual amounts of testosterone-4-¹⁴C after incubation of this substrate with minced testicular tissue from mature rats exposed to 350 R of whole-body x-irradiation (unpublished data).

Fig. 22.

The conversion of testosterone-4-¹⁴C into androstenedione by mature rat testicular minced tissue from mature rats exposed to 350 R of whole-body x-irradiation (unpublished data).

Fig. 23.

Residual amounts of testosterone-4-¹⁴C after incubation of this substrate with minced testicular from mature rats exposed to 150 R of whole-body x-irradiation (unpublished data).

Fig. 24.

Pineal gland HIOMT activity after 150 R of whole-body x-irradiation to mature male rats (unpublished data).

Fig. 25.

Rat total testicular MAO activity after 150 R of whole-body x-irradiation to mature male rats (unpublished data).

Fig. 26.

Total glutathione reductase activity of mature rat testicular tissue after 50 R of x-irradiation (unpublished data).

Fig. 27.

Lipid peroxidation as measured by the TBA assay by mature rat testicular minced tissue after exposure to various dosages of whole-body x-irradiation expressed on a per unit of mass or per incubation (0.3 g of tissue used per incubation) basis (unpublished data).

Fig. 28.

Total lipid peroxidation as measured by the TBA assay by mature rat testicular minced tissue after exposure to various dosages of whole-body x-irradiation (Total capacity of both testes to produce TBA chromogens - unpublished data).

Fig. 29.

The effects of linoleic acid on the synthesis of testosterone from androstenedione-4-¹⁴C in vitro by mature rat testicular minced tissue (Taken from progress report for 1975-6 fiscal year - C00-1602-55).

Fig. 30.

Inhibition of *in vitro* mature rat testicular diene conjugation and lipid peroxidation by phenylethyl sulfonyl fluoride (unpublished data).

Fig. 31.

Increase in mature rat testicular lipid peroxidation (TBA chromogens) by cholesterol arachidonate (a cholesterol ester) (unpublished data).

Fig. 32.

The inhibition of diene conjugation produced by mature rat testicular minced tissue by a cholesterol esterase inhibitor ($p < 0.003$ for all cholesterol esterase inhibited samples when compared with the control samples; $p < 0.001$ for 1 mg cholesterol arachidonate and < 0.05 for 0.25 mg cholesterol arachidonate when inhibited groups were compared with non-inhibited groups - unpublished data).

Fig. 33

Inhibition of rat mature testicular lipid peroxidation (TBA chromogens) by the phospholipase inhibitor, dimethyl DL-2,3-distearoyl-oxypropyl-2'-hydroxyethyl-ammonium acetate (unpublished data).

Fig. 34.

Summary slide showing some of the relationships that exist between steroid biotransformations, lysosomal phospholipase A₂, cholesterol esterase, lipid peroxidation, malonaldehyde formation and PG synthesis (taken from refs. 33, 34, 36).