TECHNICAL PROGRESS REPORT

MASTER

Submitted to the U. S. Atomic Energy Commission

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

CONTRACT NO.: AT(11-1)-1602

Principal Investigator: LeGrande C. Ellis

Institution and Department Where Research Was Carried Out:

Department of Zoology
College of Science
Utah State University
Logan, Utah 84321

LeGrande C. Ellis

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The principal investigator's full effort was applied to the research project during August 1971, and one-half effort will have been spent on the project during the period from September through May 30, 1972. Full effort will be given the project during June and July 1972. Mrs. Cheryl Billings spent her full time on the project during the 1971-72 year, but she resigned her position effective April 21, 1972, to have her baby in early May. Miss Phyllis Taylor has been hired to replace Mrs. Billings effective June 1, 1972. Mr. Ronald Urry and Louis Burhley both worked on the project as research assistants during the summer months and have assisted during the winter months, although they were not paid by the grant during the academic year. Mr. Michael Jones has worked part-time on the project during the academic year through the Work-Study program. Miss Karen Brackett worked part-time during the academic year through the Logan High School Cooperative Vocational Teaching Program. During the academic year, Dr. John L. Prehn, Jay M. Johnson, James L. Hargrove, Rodney R. Seeley and Robert A. Palmer contributed to the project, but they were not funded from the grant.

In October 1971, I was invited to present a paper at the Alza Conference Series on "Prostaglandins in Cellular Biology and the Inflammatory Process" entitled "Cellular aspects of prostaglandin synthesis and testicular function" (Appendix I). The book based on the conference is scheduled for publication by Plenum Press in May 1972. In April 1972, I presented a paper at the 56th Annual Meeting of the Federation of American Societies for Experimental Biology entitled "Rat testicular prostaglandin synthesis and its relationship to androgen synthesis" (1, c.f., Appendix II). I was also invited to submit a short review article to Research in Reproduction entitled "Prostaglandins and the dual endocrine role of the testis" (Appendix III).

In the above three papers, we reviewed the biochemical mechanisms involved in androgen and prostaglandin synthesis and the role that anti-oxidants and radioprotective agents play in regulating these two systems. Lipid peroxidation was also reviewed as it relates to the two above phenomena and to changes in radiosensitivity of the testis with age and development of the male gonad.

The data from the highest level of our dose-response curve (450 R) for androgen synthesis was published this last year (2, c.f., Appendix IV). Our data from the 350 R level of the dose-response curve for melatonin synthesis and a discussion of the relationship of the changes in melatonin synthesis as it relates to changes in androgen synthesis was published in 1970 (3). Our data showed that various lighting regimes before or after irradiation modify the severity of damage manifest by the testis after treatment. We have now shown that melatonin synthesis is decreased from 1-24 hr after irradiation; it increases the 4th to the 19th day after treatment and returns to control levels on the 22nd day. A series of investigation has shown us that melatonin synthesis, using the organ culture techniques, is elevated 1 hr after head irradiation and that injections of histamine or the addition of histamine to the incubation media *in vitro* will inhibit this synthesis (4). On the other hand, localized irradiation to the body results in an decrease in melatonin synthesis 1 hr after treatment. Thus, the changes in melatonin synthesis after irradiation can now be accounted for by either a direct effect on the brain or a mediated effect from the body, probably by histamine.

We attempted to pinealectomize rats, irradiate them, and then place some in constant darkness to ascertain if the pineal gland *per se* is responsible for the previously noted deleterious effects of constant darkness on the testis after irradiation (3). The rats used in this investigation became sick, due to a nutritional imbalance they experienced before we purchased them. The investigation is being redone.

A description of our work on the direct effects of melatonin and serotonin on androgen synthesis *in vitro* was published this year (5, c.f., Appendix V). We can now account for the changes in steroid biotransformations (2) after irradiation on the basis of changes in serotonin and melatonin and their affects on this parameter of testicular function. We have reported that endogenous serotonin can be taken up by the testis (3, 6) to be metabolized by the interstitial cells and walls of the seminiferous tubules (6, 7) by monoamino oxidase (MAO). Moreover, this enzyme was positively correlated with androgen synthesis, the development of the testis, and the later senescence of this organ (6). Although the enzyme activity paralleled changes in androgen synthesis, it was not affected by testosterone, but appeared to be controlled primarily by FSH (8, Appendix VI). The enzyme activity decreased 42-80 days after irradiation with 250 R, but was equal to that of the control animals by 153 days after treatment (Figs. 1 and 8). However, both the control and treated animals exhibited much lower MAO activity at 152 days of age than they did at either 126 or 134 days of age, which we have shown to be due to the senescence of the testis (6).

MAO inactivates serotonin by the mechanism outlined below (9):

Aldehyde Dehydrogenase

Since H_2O_2 is formed during this reaction and by irradiation (see Appendix VII), catalase was investigated with respect to its distribution in the testis and changes in its activity after hypophysectomy. Our data show that this enzyme has the same distribution in the testis as the steroid-biotransforming enzymes and MAO (9). Moreover, catalase activity decreases after hypophysectomy, but increases in intact rats after 150 R of whole-body x-irradiation (9). Since serotonin is metabolized by the testis and forms H_2O_2 , we investigated H_2O_2 as to its effects on steroid biotransformations. The compound inhibited the conversion of androstenedione into testosterone, but increased the conversion of testosterone into high-polar compounds (10). Thus, H_2O_2 could very effectively reduce androgen levels in the seminiferous tubules by shutting off androgen synthesis and increasing its metabolism into other compounds. This observation is significant because we had previously proved that androgen synthesis can be inhibited *in vitro* with large doses of x-irradiation (11).

Two important endogenous compounds (melatonin and serotonin) affect androgen synthesis and are deleterious to the testis. We therefore initiated a series of investigations to ascertain how these compounds functioned in the testis. Lipid peroxidation had been observed in the testis, but no information was available as to its control or its physiological purpose (for review, see ref. 10). Since lipid endoperoxides are free radicals and have been observed to increase after irradiation (for review, see ref. 10), we studied the influence of several antioxidants on this system. The enzymes for lipid peroxidation had the same general distribution in the testis as androgen synthesis, MAO, and catalase (10, 12). In addition, lipid peroxidation decreased after hypophysectomy and paralleled the pattern of androgen synthesis with development of the testis (10, 12, 13). Ascorbic acid up to 0.4 mMolar concentration increased lipid peroxidation, but diminished it above this concentration (10, 12). Likewise, H_2O_2 at low concentration increased lipid peroxidation, but at higher concentrations was inhibitory (10, 12). D- α -tocopherol markedly inhibited lipid peroxidation, but had only a small effect on the conversion of androstenedione into testosterone (10, 12).

Melatonin increased lipid peroxidation (13), inhibited androgen synthesis (5), inhibited prostaglandin synthesis (5), and inhibited testicular contractions. Serotonin, on the other hand, decreased lipid peroxidation, inhibited androgen synthesis, increased prostaglandin synthesis, and increased contractions of the testicular capsule (12, 13). On these bases, we have concluded that the testis is a dual endocrine organ (12, 13, 14).

In looking at lipid peroxidation as it relates to development of the testis, we observed that malonaldehyde production increased at 30 days post-natally (Fig. 1), which coincided with a marked increase in radiosensitivity of the testis (15). Thus, our data indicate that free radical production, as it relates to antioxidants and androgen synthesis, is very important in understanding testicular function.

When lipid peroxidation was shown to be involved in prostaglandin synthesis, we investigated this phenomenon in the testis and found that prostaglandin synthesis was reduced by hypophysectomy (12, 13) and by adrenalectomy (12). Prostaglandins were subsequently shown to modulate contractions of the testicular capsule (16, 17) and to be obligatory for contractions *in vitro* (19).

In summary, we have identified systems (pineal gland and serotonin) that could affect the testis and androgen synthesis through a mediated response. We have observed that systems within the testis can be affected directly by irradiation (catalase, H_2O_2 production, lipid peroxidation, and possibly MAO activity). In addition, if given before irradiation, serotonin and melatonin may be radioprotective, but if given after irradiation, they are harmful (20). Thus, for the first time, radiation effects on the testes can be explained on a biochemical basis and the biochemical changes correlate well with the pathological changes. Nevertheless, much more work is needed to separate direct effects from mediated effects of radiation on the testis.

This last year, I submitted, as director of our program in reproductive physiology, a NIH training grant proposal on Environmental Influences on Reproduction. This program was approved for funding, but was not funded, due to a lack of finances.

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Fig. 1

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