MASTER

TECHNICAL PROGRESS REPORT

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

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The principal investigator's full effort was applied to this research project during August 1970, and one-half effort was spent on the project during the period from September through April 30, 1971. One-half-time effort will also be spent in May 1971, with full-effort being given in June and July 1971. A one week vacation was taken in August and a second week's vacation is planned in June. Mrs. Jeanine Griffin resigned her position June 30, 1970, to accompany her husband to California where he will continue his education. Mrs. Cheryl Billings was hired in July 1970, to fill this vacancy. Mr. Ronald Urry worked on the project as a graduate research assistant during August and half of September. He and Mr. Lewis Burhley will work as graduate research assistants this June and July. Mr. Thomas Partington and Mr. Jeffery Johnson worked on the project through the "Work Study" program from September through December. Mr. Blake Thornley worked on the project through the Logan High School Cooperative Vocational Teaching Program during March and April and will continue through May 1971. Mr. Jay Johnson has worked as an hourly diener and Mrs. Lynda Johnson has worked hourly as a typist.

In 1969, I wrote a review article for a book entitled "The Testis." Our current work on melatonin synthesis and synthesis of androgens by teased-tubular preparations, and the initial study in the dose-response determinations were reviewed in terms of current literature and work that has been done by us and by others. The book was published in three volumes (I), my chapter appears as chapter 9 in volume III and is included with this document as Appendix I.

The data obtained from the highest level of the dose-response curve (450 r) has been submitted to the Journal of Radiation Research (2) and should be

published in late 1971. A copy of the manuscript is attached here as Appendix II. We found particular compounds that were produced predominantly by teased-tubular preparations that have not been reported in the literature. Radiation produced changes in these compounds. Our on-going investigations to positively identify the compounds have eliminated several possible steroid intermediates as candidates. In an aging study undertaken by Dr. Mario H. Baptista, these two metabolites were observed and were found to change during aging in the rat. As soon as the two compounds are identified, we will publish data on their relationships to aging and radiation effect.

Data obtained from the 350 r level of the dose-response curve for melatonin synthesis was published this year (3) with other data collected on the radiomimetic and radioprotective aspects of constant light and constant darkness on the rat testis as possibly mediated through the pineal gland. This manuscript constitutes Appendix III.

Dr. August Jaussi did some work on the effects of irradiation on melatonin synthesis using localized irradiation to the head, localized irradiation to the body, and in vitro irradiation. A manuscript was prepared and sent to Nature for publication. The manuscript was returned to us with a note indicating that the editors felt that the data were too technical for their readers. The data have been submitted to the Proceedings of the Society for Experimental Biology and Medicine, and a copy (4) is included with this progress report as Appendix IV.

Some data collected by Dr. Mario H. Baptista on androgen synthesis and observations made on the metabolism of serotonin by testicular minced and teased-tubular preparations by Dr. August W. Jaussi were pooled to constitute

a manuscript on the relationship to aging of monoamine oxidase and androgen synthesis. A copy of the manuscript, which was submitted for publication in *Endocrinology* (5), is included with this progress report as Appendix V.

The data obtained from the 250 r level of the dose-response curve have been analyzed and some significant changes in monoamine oxidase were observed after radiation. As was observed during the aging study (5), the minced preparations had less activity than did the teased-tubular tissue. The data from teased-tubular preparations were found to correlate better with radiation effects than similar data collected from minced preparations. These data implicate serotonin as a possible mediator of radiation effects on the testis. The information will be prepared for publication during the next year. Additional studies have been undertaken in order to ascertain what controls the levels of the enzymes in the testes that function in the inactivation of serotonin. Injections of serotonin, hypophysectomy, gonadotrophin injections into hypophysectomized animals, and starvation all have been tested for possible implication in the changes in monoamine oxidase activity associated with radiation. To date, the data indicate that the activity of the enzyme is under gonadotrophic control, as was suggested by the aging studies (5). Our results are currently being readied for publication.

In 1969, evidence was obtained that steroid biotransformations are directly inhibited by melatonin and serotonin in vitro (1,6). These data were prepared for publication in *Endocrinology*. The manuscript was returned to me with a request that the mechanisms of inhibition be determined through the use of kinetic studies. We did the work and the revised manuscript has been resubmitted for publication (7). A copy of the manuscript is included

with the progress report as Appendix VI. The data substantiate further physiological significance of serotonin and melatonin relative to the normal functioning of the rat testis and changes that occur after an irradiation insult to the testis.

In order to better understand the mechanism for the control of pineal function, we studied melatonin synthesis in pineal glands of hypophysectomized rats. We primarily wanted to ascertain if changes in pineal function after irradiation could be ascribed to alterations in either pituitary or gonadal function. The resultant data have been prepared for publication (8) and appear with the progress report as Appendix VII.

For several years we have been gathering information relative to free radical formation in the rat testis under physiological conditions. Part of these data have been published in a review article with a postulate as to the physiological significance of the phenomenon (9) as it relates to the radiation syndrome. We theorized that the changes that occur in androgen synthesis at 30 days of age in the rat could account for the increase in radiosensitivity of the testis that were reported by other workers. Dr. Mario H. Baptista found (10) that a marked increase in lipid peroxidation occurred at 28-30 days of age. This rapid increase in lipid peroxidation produced an immediate inhibition of androgen synthesis. Recovery of both phenomena occurred after this time interval. These data thus seem to explain the marked increase in radiosensitivity that occurs at 30 days of age. (For a review of how free radicals and antioxidants relate to age changes in the radiosensitivity of the testis see ref. 1.) Recent preliminary investigations with vitamin-E-deficient animals show that lipid peroxidation is accelerated in these animals and that endogenous lipid

peroxides are also elevated at the time of sacrifice. Thus, free radicals do appear normally in the rat testis and are elevated under certain experimental conditions. Antioxidants such as nordihydroguaretic acid, vitamin E, ascorbic acid, serotonin and melatonin do affect the production of the lipid peroxides and may be important to the animal in regulating these processes. We cannot help but feel that the radioprotective action of some of these antioxidants can be explained on the basis of how they inhibit the flow of electrons and protons in the cell with respect to biological oxidation, the reduction of specific compounds and free radical trapping. We, therefore, postulated that prostaglandins synthesis occurred in the testis and that this phenomenon had a positive relationship to androgen synthesis (9). We have since observed a definite action of prostaglandins on the contraction of the rabbit testicular capsule (11). The relevant data have been published and reprints are included as Appendix VIII. Data that have been collected for another prostaglandin, $F_{1\alpha}$, are being prepared for publication (Fig. 1-3) and should be ready to submit to a journal in May 1971. Prostaglandin $F_{1\alpha}$ was observed to have the opposite action of prostaglandin E_1 on testicular capsular contractions. We conclude that the contraction of the testicular capsule is modulated by hormones and that different prostaglandins can either stimulate or inhibit these contractions.

Mr. Jay M. Johnson has incubated arachidonic acid with various testicular preparations to ascertain if the rat testis can synthesize prostaglandins. If so, we will evaluate the effects of irradiation on the synthesis of these compounds using the methodology we are developing. Mr. Johnson is currently assaying the tissue and we should know shortly

whether the various testicular preparations can synthesize these compounds.

As outlined in last year's proposed plan of study, we anticipate pinealectomizing rats and studying the mediated effects of constant light and darkness on the rat testis. Now that the technique of pinealectomy has been mastered, the study will be conducted in May and June 1971.

The 150 r dose level of the dose-response curve will also be completed as outlined in the proposed technical program. Thus, we are far ahead of schedule in the research that we proposed for 1971.

In addition to the described research, the principal investigator was selected to direct a training program in the area of "Environmental Influences on Reproduction." A preliminary training grant proposal was written by the principal investigator and submitted for a preliminary review by the National Institutes of Health. A final draft was submitted to NIH on October 1, 1970. An on-site visit was conducted on December 7, 1970. We just heard that the proposal will not be funded by the environmental section. A second agency of NIH had previously expressed an interest in the proposal, and we are now waiting to hear if they are still interested in the proposal.

REFERENCES

1. L.C. Ellis. Radiation Effects In "The Testis" (W.D. Johnson, W.R. Gomes, and N.L. Vandemark, eds.) Academic Press, Inc., New York, Volume III, pp 333-376, 1970.
2. L.C. Ellis and K.R. Van Kampen. Effects of ionizing radiations on endocrine cells VII. Androgen synthesis and metabolism by rat testicular minced and teased-tubular preparations after 450 r of whole-body X-irradiation. Radiation Research -- Submitted for publication.
3. L.C. Ellis, D.W. Barfuss and K.R. Van Kampen. Modification of rat testicular response to X-irradiation by various lighting regimes: I. The pineal gland and melatonin synthesis as a possible mediator of the response. Life Sciences 9: 1011-1020 (1970).
4. A.W. Jaussi and L.C. Ellis. Alterations in melatonin synthesis by rat pineal glands after localized irradiation of the head and body in vivo and in vitro irradiation. Proc. Soc. Exptl. Biol. Med. -- Submitted for publication 1971.
5. L.C. Ellis, A.W. Jaussi and M.H. Baptista. Correlation of age changes in serotonin metabolism and androgen synthesis by rat testicular minced and teased-tubular preparations in vitro. Endocrinology -- Submitted for publication.
6. L.C. Ellis. The direct action of melatonin and serotonin on testicular androgen production in vitro. J. Reprod. Fert. 18: 19 (1969).
7. L.C. Ellis. Inhibition of androgen synthesis in vitro by serotonin and melatonin. Endocrinology -- Submitted for publication.
8. R.L. Urry, D.W. Barfuss and L.C. Ellis. Alterations in melatonin synthesis by rat pineal glands after hypophysectomy. Life Sciences -- Submitted for publication.
9. L.C. Ellis and M.H. Baptista. A proposed mechanism for the differential radiosensitivity of the immature rat testis. In "Radiation Biology of the Fetal and Juvenile Mammal. Proc. Ninth Annual Hanford Biology Symposium, AEC 17, Symposium Series, pp 963-974, U.S. Atomic Energy Commission, Division of Technical Information, 1969.
10. M.H. Baptista. Mechanisms of aging in relationship to steroid biotransformations and thiobarbituric acid reactive material in rat testicular tissue in vitro. Ph.D. Thesis, Utah State University, 1969.
11. J.L. Hargrove, J.M. Johnson and L.C. Ellis. Prostaglandin E₁ induced inhibition of rabbit testicular contractions in vitro. Proc. Soc. Exptl. Biol. Med. 136: 958-961 (1971).

Fig. 1

Stimulation of rabbit testicular capsular contractions in vitro by Prostaglandin $F_{1\alpha}$ showing an increase in overall tonus and rate of contraction.

Fig. 2

Increase in overall tonus and frequency of contractions of rabbit testicular capsular contractions in vitro with Prostaglandin $F_{1\alpha}$.

Fig. 3

Initiation of contractions of rabbit testicular capsular contractions in vitro with Prostaglandin F_{1α}.