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The Effects of X-Irradiation on Steroid BioTrans-
Formations by Testicular Tissue

Progress Report

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ABSTRACT

A dose-response curve (450 to 50 R of whole-body x-irradiation) relating androgen synthesis by minced and teased-tubular preparations, metabolism of testosterone, lipid peroxidation, catalase activity, and melatonin synthesis by the pineal gland to histological changes in the testis has been completed. All phenomena were affected by irradiation in a dose-dependent manner, and all were related to damage and repair of the germinal epithelium. Changes in lighting schedules were associated with changes in melatonin synthesis and changes in radiosensitivity of the testis. Melatonin has been shown to inhibit androgen synthesis, stimulate the inactivation of testosterone, and stimulate MAO activity of the testis. It inhibited MAO activity of the hypothalamus and pituitary and is thought to regulate trophic hormone release as a result of increasing serotonin levels in the brain. An increased 5 α -reductase of the testis and liver by melatonin and other radiation products is thought to be responsible for decreasing androgen levels in the gonad and increasing steroid metabolites that induce fever in the general radiation syndrome. Serotonin synthesis and metabolism has been implicated in the radiation syndrome and radioprotection of the testis and will be studied in detail in subsequent investigations.

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PERSONNEL AND EFFORT REPORT: On July 15, 1972, the principal investigator suffered a mild coronary occlusion. The recuperation period was spent, for the most part, working with students and Miss Phyllis Taylor (laboratory technician) in calculating data and submitting papers for publication. As a result, we now have a rather substantial number of manuscripts accepted for publication this year and a comparable number submitted for publication and currently being reviewed by the referees. Since the University allowed the principal investigator 100% of his time for research fall quarter (September through December) and his commitment to the project was only 50% during this time, no reduction in effort was made to the project during this period. The principal investigator will spend 50% of his time on the project during May and full-time during June and July as indicated in the proposal.

Miss Phyllis Taylor will complete her time commitment to the project as stipulated in the budget. Mr. Ronald L. Urry and Mr. Louis E. Buhrley were both paid from the project for their time devoted to the project during August and September of 1972. They have contributed to the project during the academic year and will continue their services during May through July 1973. Mr. Urry is currently being supported on an NDEA Fellowship, while Mr. Buhrley is currently on a graduate teaching assistantship. Mr. Buhrley will not be compensated for his contribution to the project this summer due to a lack of University and Grant funds. Mr. James L. Hargrove and Mr. Rodney R. Seeley have contributed approximately 50% of their time to the project from August 1972 to April 30, 1973, and will continue at this rate from May 1 to July 31. Mr. Hargrove is being supported by an NDEA Fellowship while Mr. Seeley is being supported by an NSF Fellowship. Mr. Bill Barnett worked full-time on the project during August and was paid from the project. He has since left the program to work with another staff member due to a lack of funds in the project and by the University. Mr. Greg R. Hanson has been working part-time on the project since September 1972 through the Work-Study program. He will continue through June 1, 1973. Mr. Reed Morrill has worked part-time on the project since September and will continue through May 15, 1973. He is being paid by the Logan High School Cooperative Vocational Teaching Program. Dr. John L. Frehn will arrive from Illinois State University at Normal on May 14 and will work part-time on the project through August 14, 1973. He will be on sabbatical leave and will be sponsored by the U.S. National Institutes of Health (Fellowship No. 1 FO3 HD 50435-01). Mr. Earl C. Downey devoted part of his time to the project from August 1, through December 31, 1972. He is currently financing his own way through graduate school as a male surgical assistant at the Logan LDS Hospital.

PROGRESS REPORT: Two papers were presented at the Fall meetings of the American Physiological Society held August 27 to September 1, 1972, at Pennsylvania State University, University Park, Pennsylvania entitled,

"Direct in vitro effects of melatonin on steroid biotransformation by rat testicular tissue" by L. C. Ellis and Ronald L. Urry and "Factors affecting testicular monoamine oxidase (MAO) activity: aging, irradiation, hypophysectomy, adrenalectomy, FSH, LH, prolactin, hCG, estrogen, testosterone, and melatonin" by R. L. Urry and L. C. Ellis. Three scientific papers will be presented at the American Association for the Advancement of Science meetings in Salt Lake City, Utah, June 11-15, 1973: a) Alterations in rat testicular, hypothalamic, pineal, and pituitary monoamine oxidase (MAO) activity after pinealectomy, melatonin administration, and changes in photoperiod by R. L. Urry and L. C. Ellis; b) Prostaglandin E₁, isoproterenol, epinephrine and testosterone effects on rabbit testicular capsular contractions in vivo and in vitro. R. R. Seeley, J. L. Hargrove, R. T. Sanders, and L. C. Ellis; and c) Regulation of rabbit testicular capsular motility in vitro by endogenous prostaglandin-like substances by J. L. Hargrove, R. R. Seeley, and L. C. Ellis.

Scope of investigations undertaken:

Objectives for 1972-73: Objectives 1-3 were to complete the 25 R level of a dose-response curve to ascertain: a) what specific steps in the biosynthesis of androgens are affected by irradiation; b) what specific metabolites are produced after irradiation and how they relate to testicular and other body functions after irradiation; and c) how sensitive are the above parameters to irradiation. After evaluating the response of the testes to the 50 R level of the dose-response curve, we decided not to run the 25 R level, but to spend this time in tying up loose ends of other work that needed to be completed in order to publish the data via the scientific journals as indicated below.

Objective 4 was to isolate and identify the high-polar intermediates that we have observed after irradiation and with melatonin. We have now characterized two major metabolites of progesterone as 5 α -pregnane-3,20-dione (5 α -dihydroprogesterone) and 5 α -pregnan-20-one-3 β -ol (tetrahydroprogesterone). Our data show that two steroids (progesterone and testosterone) appear to be metabolized by the same enzyme (5 α -reductase) and that this enzyme is located in the seminiferous tubules. Moreover, this enzyme is stimulated by melatonin. These data will soon be completed and ready to submit for publication.

Objective No. 5 was to ascertain if catalase activity were either FSH, LH, prolactin, or testosterone dependent. The animals have been purchased and are currently being treated to ascertain the above. This aspect of the problem will be completed by June 1973.

Objective No. 6 was to administer a glutathione oxidizing compound into rats to ascertain if this treatment will alter radiosensitivity of the testis. The compound (diamide) has been purchased and this investigation is scheduled to be run during June and July 1973. In support of this concept, when Dr. John W. Harris was here as a visiting lecturer,

we mentioned this concept to him. He has now increased the radiosensitivity of anoxic bacteria and Chinese hamster cells (confidential unpublished data) to irradiation. In addition, the radiosensitivity of anoxic tumor cells (ascites tumor cells) was increased in vivo by diamide and the shoulder of the survival curve was completely removed.

Pineal gland--Through a series of investigations, supported by this grant, we have shown that the pineal gland responds diphasically to whole-body x-irradiation. Activity of this gland was diminished during the first 24 hrs, but was increased from 1 through 19 days after treatment. These data were submitted for publication to the following journals: Nature, Proceedings of the Society for Experimental Biology and Medicine, and Radiation Research. Essentially, the same criticism was given by all three journals--i.e., that no one, but us has shown that the pineal gland is affected by irradiation and no one has observed effects on the pineal gland other than changes in lighting schedules. They recommended that other assays be used to evaluate pineal function and that other evidence should be presented in order to prove these mediated effects. As a result, we irradiated animals this year and used organ culture methods to substantiate our claims. Moreover, we have shown that histamine inhibited pineal gland HIOMT activity in vitro and in vivo after injections of this exogenous amine. These new data have been incorporated into the manuscript which has now been resubmitted for publication (see appendix I). We have also found that inanition (feed deprivation) can activate the pineal gland, and is, in part, responsible for the reduction in testicular function observed during partial starvation (1). This manuscript was submitted for publication to endocrinology last year and was returned with the comment that animals should be pinealectomized and then submitted to inanition in order to prove that the pineal gland was involved in the testicular regression we noted. Also, we should use another assay (n-acetyl transferase) in order to say that the pineal gland was activated (even though pineal weights were increased and HIOMT activity was increased) by deprivation of feed intake. We have purchased the rats, pinealectomized them, and now have them on treatment. This investigation will be completed before August 1, 1973.

Other workers have just recently reported at the Federation of American Societies for Experimental Biology Meetings in Atlantic City, April 15-20, 1973, that insulin induced hypoglycemia increases pineal gland n-acetyl transferase activity (2), and these workers have attributed this increase to hormones from the adrenal medulla. Thus, our concept of mediated effects on the pineal gland are being vindicated. Our data, however, suggest that the adrenal medulla is not involved in the radiation response since the secretion of this gland has been shown by others to be decreased after irradiation (3). In addition, other workers have now shown that nervous activity of the brain after radiation could be the factor responsible for increasing melatonin synthesis after radiation (See appendix I). We feel that this work for the most part concludes the aims and goals of our studies involving the pineal gland and irradiation.

Serotonin, MAO activity and radiation--We have shown through a series of investigations, supported by this grant, that MAO (monoamine oxidase) activity is altered after irradiation. These data were submitted for publication, but the manuscript was returned to us because in the opinion of the referees, no one has shown this enzyme to be of physiological importance to the testis. They specifically requested that we show that it is of importance, to indicate its control, and to show that changes in activity of this enzyme results in alterations in the levels of serotonin in the testis and that the altered levels in turn affect testicular function. On this basis, we have now shown that testicular MAO activity was age dependent, positively related to age changes in androgen synthesis, and maximal in activity during the mature or adult stage, but was diminished prior to maturation and again after senescence was evidenced in this organ (appendix II). We have shown that this enzyme is gonadotrophin dependent, primarily FSH, and is also activated by melatonin in vitro and in vivo. Moreover pargyline, an MAO blocking agent, was found to inhibit this enzyme in the seminiferous tubules in vivo and in vitro in organ culture. In addition, when MAO activity of the seminiferous tubules was blocked by pargyline, they failed to grow in organ cultures while the control tubules did (unpublished data). FSH increased the MAO activity of seminiferous tubules in organ cultures over the non-treated tubules.

Similarly, reserpine (a serotonin depleting agent) and p-chloro-phenylalanine (an inhibitor of serotonin synthesis) when injected into mature rats, both decreased testicular weight, but reserpine was the most effective (Table 1). Both compounds significantly reduced testicular serotonin levels. These data are being analyzed to ascertain what effects they had on MAO activity. We also have preliminary data indicating that the tubules, capsule, and interstitial elements of the testis all are capable of synthesizing serotonin from tryptophan-3-¹⁴C (unpublished data).

We also studied MAO activity of the hypothalamus and pituitary as it related to melatonin and changes in pineal gland activity (appendix III). We have found that melatonin inhibited this enzyme in the above two tissues and thus explains how melatonin and serotonin may control the synthesis and release of such trophic hormones as FSH, LH, Prolactin, TSH, and ACTH. Indeed, it now appears that melatonin mediates its effects on trophic hormone synthesis and release through MAO and serotonin levels in the brain (see appendix III for references and discussion). The importance of this finding is evidenced by the recent observation that serotonin induced radioprotection after intracerebral injections of this compound. Thus, as these workers postulated, serotonin may mediate its radioprotective action through the hypothalamus. Our data would corroborate this conclusion and would suggest that melatonin might do the same, but through serotonin. We now feel that serotonin and melatonin act panoramically as endocrine transducers by affecting directly both the hypothalamus and the gonads. In this respect, Dr. John L. Frehn,

who worked with us last year, will be in our laboratory May 14 through August 14. Dr. Frehn and Mr. Downey have now shown direct effects of melatonin on the 5 α -reductase activity for testosterone in the liver and the testes (unpublished confidential information). We also have preliminary data that show that H₂O₂ and lipid peroxides act similar to melatonin in that they activate the 5 α -reductase enzyme, while inhibiting androgen biosynthesis (unpublished confidential information). At the same time, the conjugation of steroids is inhibited by both melatonin and serotonin and probably H₂O₂ and lipid peroxides. Thus, irradiation through an increase in circulating serotonin and the localized production of peroxides (H₂O₂ and lipid peroxides) effectively reduces the excretion of steroids and at the same time increases the amount of 5 α -reduced steroids. These 5 α -reduced steroid metabolites have pyrogenic activity (5,6) and as such can induce pyrexia as one of the frank symptoms of the radiation syndrome. Thus, our data are capable of showing in biochemical terms how radiation affects the mammalian organism whether it be on stem-cell renewal or the gross symptoms of the radiation syndrome.

Prostaglandins--Five papers were published this year on prostaglandins and testicular function (see publication list ref. nos. 5-9). One additional paper has been submitted for publication (see manuscripts submitted for publication ref. no. 7 and Appendix IV), and the investigations for several more will be completed by the end of the contract year. In addition, Mr. Louis E. Buhrley is currently studying the relationship of prostaglandins to contractility of the seminiferous tubules. He has found that prostaglandins induced strong rhythmic contractions of this tissue in vitro. Through this series of investigations, we have been able to show that both the rabbit and rat testes contain significant quantities of endogenous prostaglandins. Moreover, the contractile structures are dependent on prostaglandins for their activity. We know from our studies, that the basic mechanism for prostaglandin synthesis is present in the testes (7,8). We have also shown that prostaglandin synthetase activity is present in the testis and is responsive to gonadotrophins (8,9). Although other workers do not appear to be aware of the role of prostaglandins with respect to contractile structures in the testis, interest has recently been manifest in the effects that radiation may have on the seminiferous tubules (10).

These studies now conclude the dose-response curve and the involving of the pineal gland in the radiation syndrome. Studies will now be aimed at ascertaining the involvement of serotonin in the testicular response to irradiation next year (1973-4) and finally the involvement of prostaglandins in the testicular response during 1974-5.

Papers published during year:

1. Ellis, L. C., A. W. Jaussi, M. H. Baptista, and R. L. Urry, Correlation of age changes in monoamine oxidase activity and androgen synthesis by rat testicular minced and teased-tubular preparations in vitro. *Endocrinology* 90:1610-1618 (1972).
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5. Ellis, L. C., J. M. Johnson, and J. L. Hargrove, Cellular aspects of prostaglandin synthesis and testicular function. In Prostaglandins in Cellular Biology (P. W. Ramwell and B. B. Pharriss, eds.) pp 385-398, Plenum Publishing Corp. New York. 1972.
6. Seeley, R. R., J. L. Hargrove, J. M. Johnson, L. C. Ellis. Modulation of rabbit testicular contractions by prostaglandins, steroids and some pharmacological compounds. *Prostaglandins* 2:33-40, 1972.
7. Hargrove, J. L., R. R. Seeley, J. M. Johnson, and L. C. Ellis. Prostaglandin-like substances: irritation and maintenance of rabbit testicular contraction in vitro. *Proc. Soc. Exptl. Biol. Med.* 142: 205-209 (1973).
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9. Ellis, L. C., J. L. Hargrove, J. M. Johnson and R. R. Seeley. Prostaglandins and the dual endocrine role of the testis. *Res. Reprod.* 4:2 (1972).

Manuscripts submitted for publication:

1. Ellis, L. C., A. W. Jaussi, G. R. Tait and R. L. Urry. Alterations in melatonin synthesis by various modes of irradiation and possible mediators of the response. *Life Sci.*--submitted for publication, 1973.

2. Urry, R. L. and L. C. Ellis. Inhibition of rat testicular monoamine oxidase activity after 250 R of whole-body x-irradiation. *Experientia*--Submitted for publication, 1973.
3. Buhrley, L. E. and L. C. Ellis. Changes in catalase activity in rat testicular preparations induced by hypophysectomy and x-irradiation. *Fertil. Steril.*--submitted for publication, 1973.
4. Urry, R. L., J. L. Frehn, and L. C. Ellis. Control of rat testicular monoamine oxidase (MAO) activity: Hypophysectomy, adrenalectomy, FSH, LH, prolactin, HCG, and testosterone. *Endocrinology*--resubmitted for publication, 1973.
5. Urry, R. L. and L. C. Ellis. Monoamine oxidase activity: Alterations after pinealectomy, melatonin administration, and changes in photoperiod. *Science*--submitted for publication, 1973.
6. Urry, R. L. and L. C. Ellis. The effects of partial feed deprivation on rat testicular monoamine oxidase activity, androgen synthesis, and pineal hydroxyindole-O-methyl transferase activity. *Endocrinology*--submitted for publication, 1973.
7. Seeley, R. R., J. L. Hargrove, R. T. Sanders, and L. C. Ellis. Comparison of in vitro and in vivo effects of testosterone, prostaglandin E_1 , and isoproterenol on rabbit testicular capsular contractions. *Proc. Soc. Exptl. Biol. Med.*--Submitted for publication, 1973.

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7. Ellis, L. C. 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*. (M. R. Sikov and D. D. Mahlem, eds.) *Proc. 9th Annual Hanford Biology Symposium* pp. 963-974, Richland, Washington, U.S. Atomic Energy Commission, Division of Technical Information, 1969.
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9. Ellis, L. C. Rat testicular prostaglandin synthesis and its relationship to androgen synthesis. *Fed. Proc.* 31:295 (1972).
10. Kormano, M. and O. Hovatta. In Vitro contractility of rat seminiferous tubules following 400 R whole-body x-irradiation. *Strahlentherapie* 144:713 (1972).

TABLE 1. The Effects of Pargyline, p-chlorophenylalanine, and reserpine on the serotonin content of rat testes.

Treatment	Serotonin Content (ng/g Tissue)	P Value	Testes Weights (g)	P Value
Control	425.2 $\pm$ 70.5		2.78 $\pm$	
Pargyline (15 mg/kg)	255.8 $\pm$ 9.1	0.05	2.62 $\pm$	
Control	244.1 $\pm$ 63.0		3.50 $\pm$ 0.08	
P-Chlorophenylalanine (20 mg/rat for 5 days)	128.7 $\pm$ 29.1	0.01	3.22 $\pm$ 0.10	0.10
Reserpine (0.05 mg/rat for 5 days)	124.3 $\pm$ 20.1	0.01	2.72 $\pm$ 0.24	0.01

* Animals were sacrificed 24 hrs after one injection.