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

Progress Report

LeGrande C. Ellis

Departments of Biology and Chemistry
Utah State University
Logan, Utah 84322

August 1, 1974 - July 31, 1975

MASTER

Prepared for the U.S. Energy Research and
Development Administration under Contract
AT(11-1) - 1602

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISTRIBUTION OF THIS DOCUMENT UNLIMITED

Reg

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Abstract

Our data show that x-irradiation of testicular tissue either in vivo or in vitro labilized the lysosomal membranes with a release of both acid phosphatase and phospholipase A₂ resulting in an increased lipid peroxidation. The results from these investigations suggest that the lipid endoperoxides and malonaldehyde are responsible for mediating the effects of radiation on steroid biotransformations. Estradiol, testosterone, 5 α -dihydrotestosterone, prolactin, acetylcholine, cGMP, H₂O₂, PUFA, ethanol and vitamin A increased lysosomal fragility and initiated enzyme release while ATP, cAMP, vitamin E, theophylline, indomethacin, caffeine, cortisol, epinephrine, NADPH, NDGA, FSH and Zn⁺⁺ decreased both phenomena.

An increase in catalase activity was consistently observed after irradiation and by cAMP indicative of an increase in testicular cAMP content following irradiation. Seminiferous tubules were found to be dependent on prostaglandins for their contractions.

"This report was prepared as an account of Government-sponsored work. Neither the United State, nor the Energy Research and Development Administration nor any person acting on behalf of the Commission.

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed on this report may not infringe privately owned rights; or
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of, any information, apparatus, method, or process disclosed in this report.

As used in the above, 'person acting on behalf of the Commission' includes any employee or contractor of the Administration or employee of such contractor, to the extent that such contractor prepares, disseminates, or provides access to any information pursuant to his employment or contract with the Administration or his employment with such contractor."

PERSONNEL AND EFFORT REPORT:

The principal investigator consigned an equivalent of one month's time at 100% effort during August 1974. He devoted a minimum of 50% of his effort to the project from September 1974 to April 1975 and plans to do so during May 1975. A second month's equivalent time at 100% will be spent on the project during June and July 1975 as specified in the contract.

Miss Phyllis Taylor resigned her position effective June 30, 1974 in order to accept another position at a higher salary - she had other offers here on our campus with a substantial increase in salary. After a two month search for an adequately trained individual, Mrs. Kaye Wessman was hired as Research Technician.

Three graduate students, Dr. James L. Hargrove, Dr. Dean K. Sorenson and Dr. Louis E. Buhrley, completed their Ph. D. degrees this year. Mr. Robert A. Palmer will complete his Ph. D. this summer. A fifth student, Mr. Randy Merrill expects to complete his M. S. degree in August. Mr. Earl C. Downey, a masters candidate who was working on the project last year was accepted into and is now attending medical school. He left the project in September and his work is currently being prepared for publication. A seventh student, Mr. Craig Farr, joined our group in September and is working on a project directly related to the project. Dr. James L. Hargrove completed his dissertation in February, 1975. He has accepted a postdoctoral research fellowship to work in Dr. Greenberg's laboratory, Department of Pharmacology, Medical School, Minneapolis, Minnesota. None of the above students have been supported by the grant, but their work has been directly related to the goals and objectives of the program.

Two work-study students contributed time to the project. They are: Mrs. Deborah Love and Mr. Larry Weight. With the exceptions noted above with respect to personnel changes, the contract requirements were satisfied. No equipment was purchased with funds from the grant.

PROGRESS REPORT:

Two papers were presented at the Utah Academy of Science Meeting in Salt Lake City, Utah, March 28, 1975. The first paper was entitled "The control of catalase activity in rat testis" and was authored by D. K. Sorenson, L. C. Ellis and L. E. Buhrley. The second paper, indirectly related to the project, was entitled "Seasonal Variation in Uinta ground squirrel testicular monoamine oxidase activity" and was authored by R. A. Palmer and L. C. Ellis.

A paper entitled "Age changes in testicular radiation sensitivity associated with changes in androgen synthesis and lipid peroxidation" by L. C. Ellis was presented at the Fifth International Congress of Radiation Research held July 14-20, 1974, at Seattle, Washington. The principal investigator also presented a paper entitled "Control of rat testicular 5 α -reductase activity for testosterone and progesterone: blinding, x-irradiation, melatonin, prolactin, LH and FSH" at the Fourth International Congress on Hormonal Steroids (Appendix I) held in Mexico City, September 2-7, 1974. He was asked and did present a paper in the symposium "Prostaglandin

and Steroidogenesis" entitled "Mechanisms and interactions in testicular steroidogenesis and prostaglandin synthesis" (Appendix II). As requested by the program chairman, the principal investigator chaired a session entitled "Steroid catabolism: Androgens II". The abstract of the first paper was published in J. Steroid Biochemistry 5 (4): 317 (1974) while the manuscript for the symposium is currently being edited and will be published at a later date in J. Steroid Biochemistry, Vol. 6.

Drs. L. C. Ellis and Russel J. Reiter organized a symposium on the pineal gland entitled "New Aspects of Pineal Function: Its Endocrine Role" that was held in Tuscon, Arizona, December 28, 1974, in connection with the Annual Meeting of the American Society of Zoologists, Division of Comparative Endocrinology. Two papers were presented from this laboratory summarizing what has been observed with respect to radiation effects on the pineal gland and the role the pineal gland and its secretions have on various organs after irradiation. Also included in these manuscripts are our serendipitous findings that relate to the general functioning of the pineal gland of the mammalian organism. These two papers are: a) "Peripheral and CNS effects of the pineal gland: target organs common to tissues and species"; authored by L. C. Ellis and b) "Factors other than light affecting the pineal gland: hypophysectomy, testosterone, dihydrotestosterone, histamine, estradiol, cryptorchidism, irradiation and stress" authored by R. L. Urry, K. A. Dougherty, J. L. Frehn and L. C. Ellis. These manuscripts will be published in the fall issue of the American Zoologist. Dr. Ellis is now writing his manuscripts and editing the other manuscripts.

Dr. L. C. Ellis was requested by the American Physiological Society to organize a symposium entitled "Physiology of the pineal gland". Dr. Ellis organized the symposium, conducted it and is now preparing the manuscript for publication in the Federation Proceedings later this year.

Dr. A. D. Johnson and W. R. Gomes asked the principal investigator if he would review his current work in Volume IV of "The Testis", a new volume to up-date Volumes I, II, and III. Two chapters will be contributed to this book from our laboratory. The first entitled "Prostaglandins" will cover the role of prostaglandins as they relate to testicular function. Heavy emphasis will be placed on irradiation effects and it will be authored by L. C. Ellis and J. L. Hargrove. The second chapter entitled "Biogenic Amines" will cover the role of the biogenic amines as they relate to testicular function especially after x-irradiation. It will be authored by R. L. Urry and L. C. Ellis. These two chapters will be submitted for publication, fall 1975.

The journal "Prostaglandins" is publishing a series entitled "The Future of Medicine". Dr. L. C. Ellis has been asked to write a review of prostaglandins as they relate to testicular function based on our work. This group also invited the principal investigator to their annual meeting in Vail, Colorado, March 12-15, 1975 which he attended.

The above information attests to the positive manner in which the work sponsored by this project has been accepted by the scientific community and the contributions this project has made to the understanding of male reproduction, particularly the radiation effects.

SCOPE OF INVESTIGATIONS UNDERTAKEN:

Objective number one for current year either has been or is being accomplished as outlined in the proposed technical program. When testicular

tissue was irradiated in vitro (Table I), the amount of enzyme release was dose-dependent and correlated well with our previous data on androgen synthesis (1). Our data show that there is a lysosomal phospholipase A₂ in the testis (2,3) that acts on membrane phospholipids to release polyunsaturated fatty acids (PUFA - primarily arachidonic acid) that are in turn converted into endoperoxides and finally prostaglandins (3,4). Radiation resulted in the release of this enzyme. Moreover when we added arachidonic acid to the incubation media containing testicular tissue, lysosomal integrity was lost and the enzymes were released into the media (2). Similarly, H₂O₂ labilized rat testicular lysosomes and resulted in the release of the enzymes, particularly acid phosphatase, into the media (2). In vivo studies are currently being undertaken to ascertain to what extent the lysosomes are perturbed in vivo by x-irradiation as outlined in the proposed research. Many additional substances were studied as to their effect on lysosomal integrity (2,3). Generally speaking estradiol, testosterone and 5 α -dihydrotestosterone, prolactin, acetylcholine, cGMP, PUFA, H₂O₂, melatonin, ethanol and Vitamin A increased lysosomal fragility and enzyme release while ATP, cAMP, Vitamin E, theophylline, indomethacin, caffeine, cortisol, epinephrine, NADPH, NDGA, FSH, and Zn⁺⁺ decreased both phenomena (2).

Rat testes were also implanted with cGMP filled silastic capsules and this also markedly altered the ultrastructural and cellular contents of the seminiferous tubules as did cAMP (Confidential information). Our data suggest that both cyclic nucleotides are necessary for spermatogenesis, but that an excess of either compound is detrimental to the testes and spermatogenesis. Similarly, the lysosomes apparently play an important role in spermatogenesis and must be stabilized at certain stages, but labilized at other stages - particularly the later stages (2).

The second objective was accomplished by adding linoleic acid to testicular tissue in vitro and the effects of this lipid on androgen synthesis was ascertained (Fig. 1). These data show that lipid peroxidation does alter steroid biotransformations in vitro. The data are consistent with the release of lysosomal enzymes (Table I) and the concept that malonaldehyde and lipid peroxides are mediators of radiation effects. Moreover, when we added arachidonic acid to incubation media containing testicular tissue, lysosomal integrity was lost and the membrane bound enzymes were released into the media (2). The release of phospholipase A₂ was sufficient to produce enough lipid peroxides and malonaldehyde to alter the steroid biotransformations that we have previously noted for in vitro effects of x-irradiation on steroid biotransformations (Fig. 1 and ref. 1).

We plan to purchase kits for cAMP in order to initiate the studies outlined in objectives 3 and 4. These investigations will be initiated this summer and carried over into the next year.

Pineal Gland and Irradiation

The research in this area has been essentially completed and no major investigations are envisioned in this area. The only work undertaken this year was to complete theses and dissertations and to fulfill editorial request for material submitted for publication. We anticipate several publications this next year resulting from completed theses and material that is currently being prepared for publication. Our work will be thoroughly reviewed in the two pineal symposia. When the manuscripts are

completed, they will be forwarded to ERDA. In summary, we have found that the pineal gland does respond to irradiation. It can alter the response of the testes to irradiation. Two products are formed by the pineal gland that appear to act on target enzymes, particularly monoamine oxidase activity (MAO) in the hypothalamus, liver, pancreas, pituitary and testes. The gland appears to initiate changes in blood levels of the thyroidal, adrenal and testicular hormones. Three possible sites of action have been delineated to explain this common rhythm - the hypothalamus-pituitary axis, the liver Δ^4 -reductase and glucuronosyl transferase system, and the glands themselves. The cyclic activity of the pineal gland appears to be due initially to the peripheral release of histamine and its action on HIOMT activity and secondly to the release and action of adrenal hormones (medullary and cortical) and an increased nervous activity of the CNS. Melatonin also affects catalase activity (2) and inhibits smooth muscle contractions of seminiferous tubules (5). This action on smooth muscle appears to be mediated through an inhibition of prostaglandin synthesis (6).

Several months work has been devoted to developing thin-layer techniques for the separation of high-polar steroid intermediates for use in evaluating the effects of malinaldehyde and lipid peroxides on steroid biotransformations. These methods are now ready for use in these studies.

Catalase Activity

We have shown that catalase is consistently increased by irradiation (7,8). It is increased by melatonin and cAMP, but decreased by serotonin (8). Our data suggest that irradiation increases lipid peroxidation and prostaglandin synthesis. As a result of an increase in prostaglandin E's there is an accumulation of cyclic AMP that in turn increases catalase activity (3).

Prostaglandin Synthesis

Our studies to date indicate that the testicular capsule (2,5) and seminiferous tubules (3,4,9) both require prostaglandins for their contractility. Contractility of the seminiferous tubules is stimulated by testosterone, 5 α -dihydrotestosterone, estradiol and cAMP (unpublished observations). All of these agents labilize lysosomes and increase lipid peroxidation (2) and unquestionably prostaglandin synthesis. cAMP and PGE₁ inhibit seminiferous tubular contractions (5,9) indicative of the importance of these compounds to tubular function.

PAPERS PUBLISHED AND ACCEPTED FOR PUBLICATION DURING THE YEAR

1. Frehn, J. L., R. L. Urry and L. C. Ellis. Effect of melatonin and short photoperiod on Δ^4 -reductase activity in liver and hypothalamus of the hamster and the rat. *J. Endocrinol.* 60:507-515 (1974).
2. Seeley, R. R., J. L. Hargrove, R. T. Sanders and L. C. Ellis. Rabbit testicular capsular contractions - prostaglandins, cyclic adenosine 3', 5'-monophosphate and epinephrine. *Biochem. Pharm.* 23:29 69-2976 (1974).
3. 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. *Acta Endocrinol.* 76:392-402 (1974).

4. Ellis, L. C. Control of rat testicular 5α -reductase activity for testosterone and progesterone. *J. Steroid Chem.* 5:317 (1974).
5. Hargrove, J. L., R. R. Seeley and L. C. Ellis. Rabbit testicular contractions: bimodal interaction of prostaglandin E_1 with other agonists. *Am. J. Physiol.* 228:810-814 (1975).
6. Urry, R. L., K. A. Dougherty and L. C. Ellis. Alterations with age in rat seminiferous tubule monoamine oxidase activity when compared with whole testicular tissue. *Proc. Exptl. Biol. Med.* 148:805-807 (1975).
7. Alozie, S. O., L. C. Ellis, R. M. Johnson and J. C. Street. Effects of Vitamin E deficiency on GSH-induced swelling of rat liver mitochondria. *Proc. Soc. Exptl. Biol. Med.* 148:929-932 (1974).
8. Urry, R. L. and L. C. Ellis. Monoamine oxidase activity (MAO) of the hypothalamus, and pituitary: alterations after pinealectomy, changes in photoperiod, or additions of melatonin in vitro. *Experientia* - In Press 1975.
9. Urry, R. L., J. L. Frehn, K. L. Dixon, D. F. Balph and L. C. Ellis. Seasonal variations in testicular monoamine oxidase in the house sparrow (Passer domesticus) and Uinta ground squirrels (Spermophilus armatus). *Experientia* - In Press 1975.
10. Hargrove, J. L., D. Nesbitt, M. J. Gaspar, and L. C. Ellis. Indomethacin and aspirin induced rat uterine contractions in vitro. *Am. J. Obs. Gyn.* In press 1975.
11. Buhrley, L. E. and L. C. Ellis. Contractility of rat testicular seminiferous tubules in vitro: prostaglandin F_1 and indomethacin. *Prostaglandins* - In press 1975.
12. Ellis, L. C., D. K. Sorenson and L. E. Buhrley. Mechanisms and interactions in testicular steroidogenesis and prostaglandin synthesis. *J. Steroid Chem.* In press 1975.
13. Urry, R. L., K. A. Dougherty, J. L. Frehn and L. C. Ellis. Factors other than light affecting the pineal gland: hypophysectomy, testosterone, dihydro-testosterone, histamine, estradiol, cryptorchidism, irradiation and stress. *Am. Zool.* - In press 1975.
14. Ellis, L. C. Peripheral and CNS effects of the pineal gland: target enzymes common to tissues and species. *Am. Zool.* - In press 1975.

MANUSCRIPTS SUBMITTED FOR PUBLICATION

1. Urry, R. L., K. R. Van Kampen, and L. C. Ellis. Testicular and pituitary alterations after administration of reserpine of p-chloro-phenylalanine in rats of different ages. *J. Reprod. Fertil.* - submitted for publication.
2. Sorenson, D. K., L. E. Buhrley and L. C. Ellis. Control of rat testicular catalase activity: ageing, adrenalectomy, hypophysectomy, pinealectomy, irradiation, photoperiod, LH, FSH, prolactin, serotonin, melatonin and 3',5'-cyclic adenosine monophosphate. *J. Reprod. Fertil.* - submitted for publication.

3. Sorenson, D. K. and L. C. Ellis. Steroid hormonal control of rat testicular lysosomal membrane integrity as related to lipid peroxidation and prostaglandin synthesis. In preparation
4. Ellis, L. C. The pineal gland as an endocrine transducer: target enzymes and factors controlling pineal function. In preparation for Fed. Proc.

REFERENCES

1. Simpson, C. G. and L. C. Ellis. The direct effects of x-irradiation on the biosynthesis of androgens by rat testicular tissue in vitro. Radiation Res. 31:139-148 (1967).
2. Sorenson, D. K. The control of lysosomal membrane integrity in testicular tissue in relation to lipid peroxidation and the possible role of lysosomes in the control of prostaglandin synthesis. Ph.D. dissertation. Utah State University, pp. 150. Logan, Utah.
3. Ellis, L. C., D. K. Sorenson and L. E. Buhrley. Mechanisms and interactions in testicular steroidogenesis and prostaglandin synthesis. J. Steroid Chem. - In press 1975.
4. Ellis, L. D., J. L. Hargrove and J. M. Johnson. Cellular aspects of prostaglandin synthesis and testicular function. In Prostaglandins in Cellular Biology and the Inflammatory Process. (Eds. P. W. Ramwell and B. R. Pharriss) pp. 385-398. Plenum Press. N.Y.
5. Buhrley, L. E. Contractions of the seminiferous tubules of the rat: effect of prostaglandins, indomethacin, cyclic AMP and theophylline. Ph.D. dissertation pp. 115, Utah State University, Logan, Utah.
6. Ellis, L. C. The pineal gland as an endocrine transducer: target enzymes and factors controlling pineal function. In preparation for Fed. Proc. 1975.
7. Buhrley, L. E. and Ellis, L. C. Catalase activity in rat testicular preparations: distribution and changes in activity induced by hypophysectomy and x-irradiation. Fertil. Steril. 24:956-961. 1973.
8. Sorenson, D. K., L. E. Buhrley and L. C. Ellis. Control of rat testicular catalase activity: ageing, adrenalectomy, hypophysectomy, pinealectomy, irradiation, photoperiod, LH, FSH, prolactin, serotonin, melatonin and 3',5'-cyclic adenosine monophosphate. J. Reprod. Fertil. - submitted for publication. 1975.
9. Buhrley, L. E. and L. C. Ellis. Contractility of rat testicular seminiferous tubules in vitro: prostaglandin $F_{1\alpha}$ and indomethacin. Prostaglandins - In press 1975.

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

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

* S.E.M. Values.

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

Fig. 1. The effect of linoleic acid on the synthesis of testosterone from progesterone-4- ^{14}C in vitro by rat testicular tissue.