COO-1602-46

THE EFFECTS OF X-IRRADIATION ON STEROID BIOTRANS-
FORMATIONS BY TESTICULAR TISSUE

Progress Report

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, 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.

LeGrande C. Ellis

Department of Biology

Utah State University

Logan, Utah 84322

August 1, 1973 -- July 31, 1974

MASTER

PREPARED FOR THE U.S. ATOMIC ENERGY COMMISSION UNDER CONTRACT NO. AT(11-1)-1602

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

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

Adult male rats were irradiated with 450 R of whole-body irradiation. Animals were sacrificed 1 and 24 hours later. MAO activity, endogenous levels of 5-HT, 5-HT synthesis, and the uptake of 5-HT-¹⁴C by their testes at these two time intervals were ascertained. MAO activity was increased at both time intervals while endogenous 5-HT remained unchanged. The amount of 5-HT-¹⁴C taken up by the testes was not significantly altered by irradiation, whereas the synthesis of 5-HT from tryptophan-¹⁴C was diminished. 5-HT, melatonin, AET and Nordihydroquaretic acid were effective in inhibiting specific steps in androgen biotransformations, lipid peroxidation and malenaldehyde formation. Intratesticular implants of silastic capsules containing C-AMP resulted in reductions of testicular weight, stability of lysosomes, and reduced contractility of the seminiferous tubules. Intratesticular injections of either C-AMP or caffeine diminished testicular weight, but they were not as effective as a combination of the two.

PERSONNEL AND EFFORT REPORT:

The principal investigator consigned an equivalent of one month's time at 100% effort during August of 1973. He also devoted a minimum of 50% of his effort to the project from September 1973 to April 1974 and plans to do so during May 1974. Another month's equivalent time at 100% effort will be spent on the project during June 1974 as specified in the contract.

Miss Phyllis Taylor has put 100% of her effort into the project from August, 1973 to April 1974 and will also devote 100% of her effort to the project from April through July 1974. Dr. Ronald L. Urry completed his Ph. D. in November and left to accept employment at the University of Rochester in the Division of Urology. Dr. Rodney R. Seeley completed his Ph. D. in August 1973 and is now working in the Biology Department of Idaho State University at Pocatello, Idaho. Mr. James L. Hargrove is currently supported on an NDEA Fellowship and has contributed time to the project. He plans to complete his Ph. D. degree in August 1974. Mr. Earl C. Downey devoted part of his time to the project, but is self supported. He plans to complete the M. S. degree in August 1974. Mr. Randall J. Merrill has devoted part of his time to the project during the year and is self supported. Mr. Sidney O. Alozie completed his M. S. degree in April 1974 and will work towards his Ph. D. degree in the Department of Food Sciences at Utah State University. He contributed some time to the project and was self sustained. Mr. Dean K. Sorenson has contributed a substantial effort to the project. He, too, is self supported. Mr. Bradley Torgeson contributed time to the project through the work study program. Of the above personnel, only Miss Taylor received any financial assistance in the form of wages or salary.

PROGRESS REPORT:

Three papers were presented at the American Association for the Advancement of Science meetings in Salt Lake City, Utah, June 11-15, 1973 entitled: 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 E_1 , isoproterenol, epinephrine and testosterone effects on rabbit testicular capsular contractions in vivo and in vitro" by 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. A scientific paper entitled "Prostaglandins: bimodal effect on catecholamine-induced contractions of rabbit testicular capsules in vitro and in vivo" by J. L. Hargrove and L. C. Ellis was presented at the Utah Academy of Sciences meeting held at Weber State College, Ogden, Utah, October 26, 1973. Similarly a scientific paper entitled "Factors affecting the integrity of rat testicular lysosomal

membranes and enzyme release" by D. K. Sorenson and L. C. Ellis; and another entitled "Vitamin E deficiency, lipid peroxidation and mitochondrial swelling of rat liver preparations" by S. O. Alozie, R. M. Johnson, J. C. Street and L. C. Ellis were presented at the Utah Academy of Sciences meeting held at Brigham Young University, Provo, Utah, March 29, 1974.

Dr. L. C. Ellis was asked to attend the Fourth International Congress on Hormonal Steroids to be held in Mexico, September 2-7, 1974, and present a paper entitled "Mechanisms and interactions in testicular steroidogenesis and prostaglandin synthesis" to be presented in a symposium on "Prostaglandins and Steroidogenesis". He has also submitted an abstract entitled "Control of rat testicular 5 α -reductase activity for testosterone and progesterone: blinding, x-irradiation, melatonin prolactin, LH, and FSH" to be presented in one of the regular sessions at this meeting.

Dr. Ellis and Dr. Russell J. Reiter from the Department of Anatomy, The University of Texas at San Antonio, Texas, have organized a symposium entitled "New Aspects of Pineal Function" to be held December 28, 1974, in connection with the Annual meeting of American Society of Zoologists, Division of Comparative Endocrinology. The tentative program calls for two papers to be presented from this laboratory: a) one entitled "Peripheral and CNS effects of the pineal gland: target enzymes common to tissues and species"; and b) the second entitled "Factors other than light affecting the pineal gland: hibernation, season, starvation, x-irradiation, hypophysectomy, histamine and estrogens".

An abstract has been submitted to the Fifth International Congress of Radiation Research to be held July 14-20, 1974 at Seattle, Washington, entitled, "Age changes in testicular radiation sensitivity associated with changes in androgen synthesis and lipid peroxidation" by L. C. Ellis.

SCOPE OF INVESTIGATIONS UNDERTAKEN:

Objective number 1 for the contract year 1972-73 was accomplished as outlined in the proposed technical program. In this respect, MAO activity (Table 1) was increased at both time intervals similar to a trend that was observed for rats 24 hours after exposure to 250 R of whole-body x-irradiation (1). The amount of endogenous serotonin (5-HT) in the testes was not changed by irradiation at either 1 or 24 hours after treatment. In this respect, the testis is similar to the liver and spleen, but different than the brain or gut with reference to changes in 5-HT content after irradiation (see reference 8). Of significance in the observation by these workers that the increase in blood 5-HT and urinary 5-HIAA observed after irradiation originates from the intestinal tract.

The second objective was accomplished as outlined in the renewal proposal. The amount of radioactivity labeled 5-HT taken up by the testes from irradiation animals was somewhat less than for the control animals, but the difference was not statistically significant due to a large variation among the control animals (835 ± 316 vs $3,214 \pm 2,632$

c/m/0.3 g tissue, respectively). Samples are currently being extracted and analyzed to ascertain if there are differences in 5-HT- ^{14}C and 5-hydroxyindole acetic acid- ^{14}C (%-HIAA) in the testes.

We incubated aliquots of rat testicular tissue with tryptophan- ^{14}C and have established that this organ does convert tryptophan to 5-HT (Table 1). Moreover, 5-HT was rapidly converted to 5-HIAA with less than 5% of the total amine being present as 5-HT at the end of the incubation period. A MAO inhibitor was used in preliminary studies to reduce the 5-HIAA formed, but this compound markedly reduced the total conversion of tryptophan to 5-HT. On this basis, we deemed it inadvisable to use the inhibitor in this type of study. Our data (Table 1) indicate that 5-HT synthesis (5-HT + 5-HIAA) was reduced at 24 hours after treatment, although the amount of 5-HT isolated 1 hour after treatment did approach significance.

Objective number three is currently being completed. Some of the compounds have been assayed while others are currently being evaluated. To date, we have found that androgen synthesis, lipid peroxidation, malonaldehyde production, and prostaglandin synthesis share many of the same respiratory enzymes. These enzymes are LH dependent and increase in quantity as the gonad develops with age. There is a lysosomal phospholipase A_2 present in the testis. The amount of lipid peroxidation, malonaldehyde and prostaglandin production appears to be dependent on the amount of polyunsaturated free fatty acid (PUFA) present in the incubation medium. Moreover, the amount of PUFA in the media is regulated by the amount of phospholipase A_2 released from the lysosomes during incubation. Furthermore, the release of phospholipase from the lysosome is inhibited by 3',5'-cyclic AMP (C-AMP) and this latter compound is increased by LH. In this respect, we have found that LH both in vivo and in vitro stabilizes the lysosome thereby reducing lipid peroxidation and increasing androgen synthesis. PUFA in the incubation media compete for NADPH as the source of energy for steroid biotransformation while at the same time produce lipid peroxides and malonaldehyde that either stimulate or inhibit steroid biotransformations.

When cyclic AMP, caffeine, or a combination of the two were injected into irradiated testes, there was a reduction in weight of the ipsilateral testis that was superimposed on the radiation effect. In this respect, C-AMP was more active than caffeine and the combination more effective than C-AMP (Table 2). In addition, when silastic implants containing C-AMP were placed in rat testes they reduced testicular weight and lysosomal acid phosphatase activity (Table 3). There was also a reduction in both the frequency and the depth of contraction of the seminiferous tubules (Table 4). Although not shown, dibutyryl C-AMP was more effective than C-AMP in respect to inhibiting the above phenomena.

One of the objectives for the 1972-73 contract year was to assess the effects of sulfhydryl depletion on the radiosensitivity of the testis. Diamide injections of 0.05 ml of 5.0 m molar into rat testes resulted in a weight loss, but 0.03 ml of 0.1 m molar did not. The lower dose did, however, increase pituitary weights. Injections of this glutathione sulfhydryl depletor had no noticeable affect on the radiosensitivity of the rat testes when either 0.05 or 0.15 ml of 5.0

m molar solution was injected directly into the testes. This lack of effect of diamide on testicular radiosensitivity can best be explained by the short period of time that diamide acts (within seconds, see ref. 9) and the high level of glutathione reductase normally found in rat testes (4). It is possible that if ischemia were present or if there was a reduction of glutathione reductase, then the change in radiosensitivity would be manifest.

SYNTHESIS AND METABOLISM OF 5-HT BY RAT TESTES AFTER IRRADIATION

Through a series of investigations supported by this grant, we have shown that rat testes synthesize 5-HT from tryptophan and in turn metabolizes it to 5-HIAA by the enzyme MAO. We have shown that testicular MAO activity increases with age and reproductive development (23). It increases immediately after irradiation and then remains depressed until regeneration of the testis occurs (Table 1 and ref. 4). The observation that 5-HT synthesis (Table 1), endogenous levels of 5-HT and uptake of 5-HT-¹⁴C were not altered by irradiation, however, suggests that 5-HT synthesis and metabolism may not be very important during the first 24 hours after the radiation insult. Our data do not rule out an importance of 5-HT on the hypothalamus and gonadotrophin secretion or effects later than 24 hours after irradiation on the gonads. We plan to prepare the 5-HT synthesis data for publication later this year.

Our data do indicate that the effects of melatonin on the pituitary and hypothalamus are mediated through MAO activity and 5-HT levels in these organs. This data is currently being prepared for publication and will be reviewed in the symposium to be held in Tucson, Arizona, December 27, 1974. We feel that this work for the most part concludes the aims and goals of our studies involving MAO activity, 5-HT uptake and 5-HT synthesis after irradiation.

PINEAL GLAND AND IRRADIATION

We feel that this work is essentially completed and do not envision any new major investigations in this area. These data will be reviewed in a symposium organized and chaired by the principal investigator entitled "New Aspects of Pineal Function" to be held in Tucson, Arizona, December 27, 1974.

LYSOSOMAL PHOSPHORYLASE ACTIVITY, LIPID PEROXIDATION, PROSTAGLANDIN SYNTHESIS, ANDROGEN SYNTHESIS, AND CYCLIC-AMP INTERACTIONS AFTER IRRADIATION

Our previous data on this subject have been reported at the symposium on "Radiation Biology of the Fetal and Juvenile Mammal" held in Richland, Washington, May 5-8, 1969 (5), the symposium on "Prostaglandins in Cellular Biology" held in Carmel, California,

October 24-27, 1971 (6), Federation of American Societies for Experimental Biology held in Atlantic City, April 9-14, 1972 (7), Utah Academy of Science Meetings (appendixes I-III), and will be reviewed at the Fourth International Congress on Hormonal Steroids" held in Mexico City, September 2-7, 1974 (see appendix III) and the "Fifth International Congress of Radiation Research" held in Seattle, Washington, July 14-20, 1974 (see appendix VII).

This area of research is the most neglected, but yet promising area of research and will be the area of major thrust for the succeeding years. In this respect we published two papers on prostaglandins and the control of rabbit testicular contractions (see ref. no. 1 & 2 under papers published), and have two additional papers submitted for publication (see ref no. 1 & 2 under papers submitted for publication). We have another paper in this area being prepared for publication. We have also collected data on the role of prostaglandins and C-AMP in regulating the contractility of the seminiferous tubules. These data will be prepared for publication this next year.

PAPERS PUBLISHED DURING THE YEAR:

1. Seeley, R. R., J. L. Hargrove, R. T. Sanders, and L. C. Ellis, Response of rabbit testicular capsular contractions to testosterone, Prostaglandin E_1 , and isoproterenol in vitro and in vivo. Proc. Soc. Exptl. Biol. Med. 144:329-332 (1973).
2. Hargrove, J. L., R. R. Seeley, and L. C. Ellis. Contractions of rabbit testes in vitro: permissive role of prostaglandins for the actions of calcium and some smooth-muscle stimulating agents. Prostaglandins 3:469-480 (1973).
3. Ellis, L. C., A. W. Jaussi, G. R. Tait, and R. L. Urry. In vitro and in vivo effects of x-irradiation and histamine- PO_4 on rat and bovine pineal HIOMT activity and melatonin synthesis. Life Sciences 13:835-845 (1973).
4. Buhrlay, L. E., and L. C. Ellis. Calatase activity in rat testicular preparations: distribution and changes in activity induced by hypophysectomy and x-irradiation. Fertil. Steril. 24:956-961 (1973).

MANUSCRIPTS SUBMITTED FOR PUBLICATION:

1. 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. submitted for publication - 1974.
2. Seeley, R. R., J. L. Hargrove, R. T. Sanders, and L. C. Ellis. Rabbit testicular capsular contractions: prostaglandins, cyclic AMP, and epinephrine. Biochem. Pharmacol. In press, 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. In press, 1974.
4. 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. Jour. Endocrinol. In press, 1974.
5. Urry, R. L. and L. C. Ellis. The effects of partial feed deprivation on rat testicular monoamine oxidase activity, testosterone metabolism, pineal hydroxyindole-O-methyl transferase activity and N-acetyltransferase activity. Endocrinology - submitted for publication, 1974.
6. Urry, R. L. and L. C. Ellis. Monoamine oxidase activity of the hypothalamus, and pituitary: alterations after pinealectomy, melatonin administration, and changes in photoperiod. Brain Res. Submitted for publication, 1974.
7. Urry, R. L., T. Oldfield, R. T. Sanders and L. C. Ellis. Alterations in testicular capsular contractions with various pharmacological agents: differences in response between the house sparrow (Passer domesticus) and the Uinta ground squirrel (Spermophilus armatus). Gen. Comp. Endocrinol., Submitted for publication, 1974.

REFERENCES

1. Urry, R. L. and L. C. Ellis. Inhibition of rat testicular monoamine oxidase activity after 250 R of whole-body x-irradiation. *Experientia* 29:1223-1225, 1973.
2. 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
3. Frehn, J. L., R. L. Urry, D. F. Balph and L. C. Ellis. Photoperiod and crowding effects on testicular serotonin metabolism and lack of effects on melatonin synthesis in Uinta ground squirrels (Spermophilus armatus). *J. Exptl. Zool.* 183:139-144, 1973.
4. Urry, R. L. and L. C. Ellis. Inhibition of rat testicular monoamine oxidase activity after 250 R of whole-body x-irradiation. *Experimentia* 29:1223-1225, 1973.
5. 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. Mahlen, eds.) Proc. 9th Annual Hanford Biology Symposium pp. 963-174, Richland, Washington, U.S. Atomic Energy Commission, Division of Technical Information, 1969.
6. Ellis, L. C., J. M. Johnson, and J. L. Hargrove. Cellular aspects of prostaglandin synthesis and testicular function. In *Prostaglandins and Cellular Biology and the Inflammatory Process*. (P. W. Ramwell and B. B. Pharriss, eds.), pp. 385-398. Plenum Press, 1972.
7. Ellis, L. C. Rat testicular prostaglandin synthesis and its relationship to androgen synthesis. *Fed. Proc.* 31:295 (1972).
8. Veningo, T. S., J. Kerkstro, and J. Wagenaar. Origin of radiation released serotonin in rabbits and mice. *Acta Radiologica* 12:454-464 (1973).
9. Harris, J. W. and J. A. Power. Diamide: a new radiosensitizer for anoxic cells. *Radiat. Res.* 55:97-109 (1973).

TABLE 1. MAO activity of Rat Testicular Tissue after 450 R of whole-body Irradiation
Expressed on a per mg of Tissue basis.

Treatment	MAO Activity (c/10 min)	P value	Serotonin		Synthesis		Total (c/10 min)	P value
			5-HT (c/10 min)	P value	5-HIAA (c/10 min)	P value		
<u>1 hr. after Irradiation</u>								
Control	342,091±43,771*		5,203±839		264,769±42,341		269,972±42,080	
Irradiated	441,320±22,010	0.05>P<0.10	8,712±1,736	0.05>P<0.10	207,710±27,314	>0.20	242,661±32,689	>0.50
<u>74 hrs. after Irradiation</u>								
Control	61,971±4,671		2,280±1,969		247,490±28,009		248,915±31,474	
Irradiated	99,152±19,513	0.05>P<0.10	10,682±1,550	<0.50	117,092±15,501	<0.01	127,773±16,565	<0.01

TABLE 2. Effects of Intratesticular Injections of Caffeine, cyclic-AMP or a combination of both on Rat Testes In vivo.

Treatment	Weight of Control Testis (g)	Weight of Injected Testis (g)	Difference in ¹ Testicular wt. (g)	
Control	1.630	1.633	(+) 0.003±0.011	
Caffeine	1.254	1.228	(-) 0.026±0.035	>0.50
C-AMP	1.304	1.264	(-) 0.040±0.029	<0.40
Combination	1.388	1.316	(-) 0.072±0.027	<0.05

¹Difference between treated and untreated testes

²Standard Error of mean values

TABLE 3. Effects of Silastic Implants containing cyclic-AMP on Rat testes In vivo.

Treatment	Difference in ¹ Testicular wt. (g)	P value	Difference in Acid Phosphatase Activity (O.D. Units)	P value
Control	(-) 0.040±0.002 ²	<0.01	0.02	<0.05
2 mg C-AMP	(-) 0.118±0.004	<0.02	(-) 0.16	<0.01
5 mg C-AMP	(-) 0.223±0.064	<0.001	(-) 0.12	<0.01

¹ Difference between treated and untreated testes

² Standard Error of mean values

TABLE 4. Effects of silastic implants containing cyclic AMP on contractility of rat seminiferous tubules.

Treatment	Diameter of Tubule (μ)	P value	Frequency of Contractions (c/m)	P value	Depth of Contractions (μ)	P value
Control	354.4 $\pm$ 4.2		7.8 $\pm$ 0.2		5.57 $\pm$ 0.31	
2 mg C-AMP	374.8 $\pm$ 8.3	<0.1	4.4 $\pm$ 0.8	<0.01	2.45 $\pm$ 0.54	<0.001
Control	362.5 $\pm$ 12.7		7.9 $\pm$ 0.6		6.23 $\pm$ 0.96	
5 mg C-AMP	417.0 $\pm$ 28.3	<0.2	2.7 $\pm$ 0.4	<0.001	1.50 $\pm$ 0.18	<0.01

TABLE 5. Effects of Intratesticular Injections of diamide on Rat Testes in vivo.

Treatment	Difference in Testicular wt. ¹ (g)	P value	Pituitary weight (mg)	P value
Control	0.004±0.015 ²	>0.50	4.7±0.27	
0.1 m Molar Diamide	0.012±0.028	>0.50	7.2±0.53	<0.01
5.0 m Molar Diamide	(-) 0.048±0.016	<0.05	6.3±0.72	<0.40

¹Difference between diamide and saline injected testes of same group of animals.

²Standard error of mean values.