Appendix I

Cellular Aspects of Prostaglandin
Synthesis and Testicular Function

MASTER

LeGrande C. Ellis, Jay M. Johnson and James L. Hargrove

Department of Zoology and Division of Biochemistry
Utah State University, Logan, Utah 84321

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.

leg

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.

It is somewhat surprising that investigations on prostaglandin synthesis and the relationship of prostaglandins to testicular function were neglected for so long since evidence for the existence of this class of hormone was first associated with human seminal fluid (1). Investigations in our laboratory on prostaglandin synthesis and their actions on the testis were initiated after preliminary studies showed that lipid peroxidation had a direct relationship to androgen synthesis (2) and possibly served some endocrine function in the testis. Reports by other workers (3, 4) that lipid peroxidation was involved in prostaglandin synthesis in other organs, added impetus to our studies.

Prostaglandins have now been isolated from rat testes (5) and the enzymes for inactivation of prostaglandins (e.g., 15-hydroxy prostaglandin dehydrogenase and prostaglandin- Δ^{13} -reductase) have been observed in swine (6) and rat (7) testes. We herewith report on: the presence of prostaglandin synthetase activity in rat testes, the pituitary and adrenocortical control of prostaglandin synthetase activity, and the relationship of androgen synthesis to lipid peroxidation and prostaglandin synthesis. We also present information about the action of prostaglandins on contractions of the rabbit testicular capsule, and cellular implications of lipid peroxidation and prostaglandin synthesis as they relate to aging and to changes in radiosensitivity of the rat testis.

MATERIALS AND METHODS

Androgen synthesis was measured by using radioactively labeled steroid precursors (pregnenolone-7 α -³H, progesterone-4-¹⁴C, and androstenedione-4-¹⁴C, New England Nuclear Corp.) that were rigorously purified by paper

chromatography (8, 9) prior to use. The appropriate steroid precursors were incubated with either minced or homogenized rat testicular tissue as described in the legends and footnotes of the figures and tables. The resulting steroid metabolites were extracted, purified and quantified, as described in detail elsewhere (8, 9).

Lipid peroxidation was measured by the TBA procedure (2-thiobarbituric acid assay) of Kitabachi (10) with the following minor modifications: 1) after incubation of the testicular tissue, 0.5-ml aliquots of the incubation mixtures were transferred to 15-ml conical centrifuge tubes, 2) 0.5 ml of 0.75% TBA and 0.25 ml of 35% TCA were added and mixed, 3) after boiling the samples for 15 min and centrifugation, the chromogen was read at 350 mμ in a spectrophotometer (Beckman Inst. Co.). ✓

Prostaglandin synthetase activity was measured by using arachidonic acid, norepinephrine, reduced glutathione and minced rat testicular tissue, as outlined by other workers (11).

The effects of prostaglandins on contractions of the smooth muscle of the rabbit testicular capsule were ascertained with a sensitive myography transducer (Statham Co.), a polygraph (Gilson minipolygraph) and a muscle warmer with oxygenized Tyrodes solution at 35°C (12, 13).

RESULTS

Adding NADPH to incubations of testicular tissue markedly increased lipid peroxidation and also enhanced the biotransformation of androstenedione into testosterone and other high-polar intermediates (Fig. 1). NADH similarly increased lipid peroxidation, but to a lesser extent than did NADPH (0.420) O. D. units for 4 mmolar NADPH vs. 0.264 O. D. units for 4 mmolar NADH per ✓

0.5 ml of incubation media). Similarly, 20 μ l of linoleic acid increased lipid peroxidation from 0.261 to 0.469 O. D. units per 0.5 ml of incubation media.

Adding low concentrations of ascorbic acid to the incubation mixture (Fig. 2) increased lipid peroxidation, but higher concentrations markedly inhibited this phenomenon. Ascorbic acid at higher concentrations also inhibited the conversion of androstenedione into testosterone. Hydrogen peroxide at low concentrations increased lipid peroxidation, but decreased it when added at higher concentrations (Fig. 3), while the conversion of androstenedione into testosterone was diminished with from 1-5 μ l of 30% H_2O_2 . The appearance of high-polar intermediates was increased when 1-100 μ l of H_2O_2 were added to the incubations.

D- α -tocopherol markedly reduced lipid peroxidation (Fig. 4), but had only transient effects on the conversion of androstenedione into testosterone and high-polar intermediates. Melatonin at low concentrations reduced the conversion of pregnenolone and progesterone into testosterone, but increased the production of lipid peroxides (Fig. 5).

Hypophysectomy (Table 1) decreased testicular weights and lipid peroxidation, but when a combination of growth hormone and ACTH were injected into hypophysectomized animals, there was an increase in both testicular weight and lipid peroxidation. When minced testicular tissue and teased-tubular preparations were evaluated for androgen synthesis from pregnenolone and progesterone, and for lipid peroxidation (Table 2), both phenomena had the same distribution with respect to the seminiferous tubules and the parenchyma of the testis.

In an aging study (Fig. 6), we determined that a positive relationship existed with respect to testicular development (size), androgen synthesis, and lipid peroxidation. A marked increase in lipid peroxidation was observed between 28 and 34 days of age. This increase was associated with a decrease in testosterone but not androstenedione synthesis. The testosterone: androstenedione ratio changed from less than one prior to 20 days of age to greater than one after 24 days of age. Incubation of rat testicular tissue with PGA_1 (Fig. 7), resulted in an accumulation of progesterone and 17^α -hydroxyprogesterone and a decrease in androstenedione, testosterone and total androgens. Lipid peroxidation was increased by PGA_1 , while PGE_1 and PGF_1 promoted an accumulation of 17^α -hydroxyprogesterone, and PGE_1 decreased testosterone production. Both PGE_1 and PGF_1 diminished lipid peroxidation.

PGE_1 decreased the rate and overall tone of autorhythmic smooth muscle preparations (Fig. 8). Moreover, response was dose dependent (Fig. 8, 9) with contractions effaced with higher concentrations of the compound. The solvent (EtOH, Fig. 9c) when added to the preparation in concentrations greater than those used in this study (Fig. 9), had only minor effects on the rate of contractions. PGE_1 also decreased rate and amplitude of the contractions without altering tone (Fig. 8b). In dose-dependent reactions, (Fig. 10, 11) PGF_1^α increased overall tone, increased the rate of contractions, and increased the rhythmicity of the contractions. PGF_1^α also increased the overall tone of the preparation in the absence of contractions (Fig. 12) and initiated rhythmic contractions in quiescent preparations. Spontaneous contractions of the rabbit testicular capsule have been observed in this laboratory in late winter, spring and early summer. During late summer and fall, contractions were absent (Fig. 12) or very minimal (Fig. 10a).

Adding PGF_1^α to an inactive preparation induced large and rhythmic

contractions (Fig. 12a). Occasionally, bursts of contractions were observed (Fig. 12a). When such preparations were repeatedly flushed (three times) with fresh oxygenated Tyrodes solution, the contractions stopped, but were reinitiated if small quantities of PGE_1 were added to the medium. On the other hand, when PGE_1 was similarly flushed from the chamber, large rhythmic contractions reappeared.

DISCUSSION

The following observations suggest that the testis is a dual endocrine organ: a) both lipid peroxidation and steroid biotransformations were NADPH dependent (Fig. 1); b) these phenomena closely followed one another during the early aging process (Fig. 2); c) they both had the same relative distribution in the testis with respect to the seminiferous tubules and the parenchyma (Table 2); d) the activity of each of the two phenomena was diminished by hypophysectomy (Table 1 for lipid peroxidation and unpublished observations for androgen synthesis); e) prostaglandin synthesis is now known to involve lipid peroxidation (3, 4); f) prostaglandin synthetase activity is diminished following hypophysectomy (Table 3) as were lipid peroxidation and androgen synthesis; g) prostaglandins have been isolated from rat testis (5); h) rat testes can metabolize prostaglandins (6, 7); i) and prostaglandins appear to modulate rabbit testicular capsular contractions (Figs. 8-12). Moreover, prostaglandins now appear to be needed for testicular function in regulating the contractions of the testicular capsule. Of possible significance is the observation (Fig. 7) that, of the prostaglandins tested, only PGA_1 appreciably modified lipid peroxidation or androgen synthesis. These effects were probably due more to end-product inhibition than to physiological effects, since large quantities of the prostaglandins were required to observe an effect.

Ascorbic acid and D- α -tocopherol (Figs. 2, 4) had rather pronounced effects on lipid peroxidation while demonstrating only minor effects on steroid biotransformations. On the other hand, H_2O_2 and melatonin obviously increased lipid peroxidation and inhibited the biotransformation of steroids. An increase in high-polar steroid intermediates was noted with additions of H_2O_2 (Fig. 3), but not melatonin (14). A number of other compounds have been assayed for their effects on steroid biotransformations and lipid peroxidation in our laboratory. Those tested to date are: 1) serotonin, histamine and heparin, which are associated with the inflammatory response (15); 2) melatonin (for a recent review, see ref. 16), NDGA (nordihydroguaiaretic acid, 17) AET (18), which are radioprotective agents; and NDGA, which has been reported to have anticancer properties (17).

Of importance is the observation that antioxidants may alter androgen synthesis, (14 and unpublished data) lipid peroxidation (Figs. 1, 4, 5, unpublished data), and prostaglandin synthetase activity (19). Moreover, the type of prostaglandin synthesized (E or F series) now appears to be determined by the amount and nature of the cofactors (antioxidants) added (20). In this respect, melatonin and serotonin have been observed to have a marked effect on androgen synthesis with serotonin acting to regulate the androstenedione: testosterone ratios during aging and the development of the testes (14). Thus, antioxidants appear to be very important in regulating the flow of electrons and protons through the cells, thereby determining whether the energy is used in steroid hydroxylations, prostaglandin synthesis or malonaldehyde production. A summary of what is currently known concerning the utilization of NADPH by rat testicular tissue with respect to androgen synthesis, prostaglandin synthesis and malonaldehyde production is illustrated in Fig. 13. This scheme shows that the P_{450} step is required for lipid peroxidation and

hence prostaglandin synthesis, but recent data indicate that this step is not mandatory (11).

Lipid hydroperoxides have recently been shown to inactivate sulfhydryl-containing enzymes (21). Moreover, malonaldehyde may react with amino acids and proteins to yield conjugated Schiff's base (22), which could markedly alter cellular structure and affect biochemical reactions or pathways. In addition, lipid endoperoxides, as intermediates of prostaglandins, are free radicals and may induce the production of other free radicals, which could also have pronounced effects on cellular function. Recent investigations now indicate that the radiosensitivity of the rat testis increases *in utero* at the time when androgen synthesis is initiated and again at 30 days of age when androgen synthesis and lipid peroxidation is reinitiated with sexual development (for review, see ref. 2).

Apparently, lipid peroxidation, the concomitant free radicals produced, and/or malonaldehyde synthesis may affect androgen synthesis. At 30 days of age (Fig. 6), there was a marked increase in lipid peroxidation with a concomitant reduction of testosterone production. Androstenedione production was increased, but the androstenedione:testosterone ratio was altered from less than one prior to 20 days to greater than one after 24 days of age. It is significant that ascorbic acid, D- α -tocopherol, serotonin, melatonin, and unsaturated fatty acids, interact through a common metabolic scheme that involves the synthesis of either androgens or prostaglandins. Perhaps it is more than happenstance that ascorbic acid; D- α -tocopherol, and unsaturated fatty acids are needed for normal testicular development in the rat and that melatonin and serotonin are deleterious to the testis (14).

The observations that adrenalectomy diminished prostaglandin synthetase activity (Table 3) while TSH and ACTH increased lipid peroxidation in

hypophysectomized animals suggest that the adrenal cortical system may influence the testis in part through regulating prostaglandin synthesis.

One particular area where prostaglandins appear to function in the testes is through regulating contractile mechanisms (Figs. 8-12). Our data now suggest that prostaglandins must be present for normal contractions of the testicular capsule and for oxytocin to act on the capsule (unpublished observations). In aging studies of the rabbit testicular capsule, autorhythmic contractions were observed as early as one month postpartum when the testes weighed less than 0.2 g. Preparations from all age levels responded to prostaglandins, but the contractions were smaller in amplitude and tension than those observed in mature animals (unpublished observations). Seasonal differences have also been observed in these autorhythmic contractions. From August to November, 30% (N = 17) of the preparations totally lacked spontaneous contractions even after several rinses of the bathing media. The rest of the year the contractions were autorhythmic (unpublished data).

Clegg, Hall and Pickles (23) have described two effects of prostaglandins on the uterine wall: an immediate, direct effect on the membrane, and an "enhancement" noticed after several rinses, which is assumed to result from an effect of prostaglandin inside the cell. Our data appear to support the conclusions of these workers and suggests that the mechanism of action of the prostaglandins is the same for both preparations.

REFERENCES

1. Kurzrok, R. and C. C. Lieb (1930). Biochemical studies of human semen II. The action of semen on the human uterus. Proc. Soc. Exptl. Biol. Med. 28:268-272.
2. Ellis, L. C. and M. H. Baptista (1969). A proposed mechanism for the differential sensitivity of the immature rat testis. In "Radiation Biology of the Fetal and Juvenile Mammal" (M. R. Sikov and D. D. Mahlum, eds.) pp. 963-974, Proc. Ninth Annual Hanford Biology Symposium, Richland, Washington. May 5-8, 1969. U. S. Atomic Energy Commission, Division of Technical Information.
3. Samuelsson, B., E. Granstrom, and M. Hamberg (1966). In "Prostaglandins" (S. Bergstrom and B. Samuelsson, eds.) pp. 31-44, Proc. Second Novel Symposium, Stockholm, Interscience Publ. New York.
4. Euler, U. S. and R. Eliasson (1967). "Prostaglandins" pp. 1-159, Academic Press, New York.
5. Carpenter, M. P. and B. Wiseman (1970). Prostaglandins of rat testes. Fed. Proc. 29:248.
6. Anggard, E., C. Larsson, and B. Samuelsson (1971). The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin - Δ^{13} -reductase. Acta. Physiol. Scand. 81:396-404.
7. Nakano, J., B. Montague, and B. Darrow (1971). Metabolism of prostaglandin E. in human plasma, uterus and placenta, in swine ovary and in rat testis. Biochem. Pharm. 9:2512-2514.
8. Ellis, L. C. and D. L. Berliner (1963). The effects of ionizing radiation on endocrine cells. 1. Steroid biotransformations and androgen production by testes from irradiated mice. Radiation Res. 20:549-563.

9. Ellis, L. C. and D. L. Berliner (1965). Sequential biotransformation of 5-pregnenolone- 7α - ^3H and Progesterone-4- ^{14}C into androgens by mouse testes. *Endocrinology* 76:591-599.
10. Kitabachi, A. E. (1967). Inhibition of steroid C-21 hydroxylase by ascorbate: alterations of microsomal lipids in beef adrenal cortex. *Steroids* 10:567-575.
11. Takeguchi, C., E. Kahno and C. J. Sih (1971). Mechanism of prostaglandin biosynthesis. I. Characterization and assay of bovine prostaglandin synthetase. *Biochemistry* 10:2372-2376.
12. J. L. Hargrove, J. M. Johnson and L. C. Ellis (1971). *Proc. Exptl. Biol. Med.* 136:958-961. Prostaglandin E_1 induced inhibition of rabbit testicular contractions in vitro.
13. Johnson, Jay M., J. L. Hargrove and L. C. Ellis (1971). Prostaglandin $\text{F}_{1\alpha}$ induced stimulation of rabbit testicular contractions in vitro. *Proc. Soc. Exptl. Biol. Med.* 138:378-381.
14. Ellis, L. C. (1972). Inhibition of rat testicular androgen synthesis in vitro by melatonin and serotonin. *Endocrinology* 90:193-204.
15. Garattini, S. and L. Valzelli (1965). In "serotonin" pp. 148-168, Elsevier, Amsterdam.
16. Ellis, L. C. (1970). Radiation Effects, In "The Testis" pp. 333-376, (eds. A. D. Johnson, W. R. Gomes, and N. L. Vandemark) Vol. III, Academic Press, New York.
17. Burk, D. and M. Woods (1963). Hydrogen peroxide, catalase, glutathione peroxidase, quinones, nordihydroquaiaretic acid, and phosphopyridine nucleotides in relation to x-ray action on cancer cells. *Radiation Res. Suppl.* 3, 212-246.

18. Savkovic, N. (1964). Effect of chemical protective agents (cysteamine and β -aminoethylisothiuronium (AET) Cl HCl) and homologous testes-DNA on the death rate of the progeny of a first generation originating from male rates irradiated in the infantile period. *Nature* 203:1393-1394.
19. Nugteren, D. H. (1970). Inhibition of prostaglandin biosynthesis by 8 cis, 12 trans, 14 cis-eicosatrienoic acid and 5 cis, 8 cis, 12 trans, 14 cis-eicosa-tetraenoic acid. *Biochem. Biophys. Acta* 210:171-176.
20. Van Dorp, D. A. (1967). Aspects of the biosynthesis of prostaglandins. *Progr. Biochem. Pharmacol.* 3:71-82.
21. Matsuchita, S., M. Kabayashi, and Y. Nitta (1970). Inactivation of enzymes by linoleic acid hydroperoxides and linoleic acid. *Agri. Biol. Chem.* 34:817-824.
22. Chio, K. S. and A. L. Tappel (1969). Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. *Biochemistry* 8:2821-2827.
23. Clegg, P. C., W. J. Hall and V. R. Pickles (1966). The action of ketonic prostaglandins on the guinea-pig myometrium. *J. Physiol.* 183: 123-144.

Legends for Figures

- Figure 1. Lipid peroxidation and the biotransformation of androstenedione into testosterone and high-polar intermediates by rat testicular tissue in vitro. The incubation media consisted of 0.05 μ Ci of androstenedione-4- 14 C (58.0 mCi/mmol), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, 0.3 g of homogenized tissue, and increasing amounts of NADPH. The samples were incubated in air for 1 hr at 37.5°C. (Unpublished data).
- Figure 2. Lipid peroxidation and the biotransformation of androstenedione into testosterone by rat testicular tissue in vitro. The incubation mixture consisted of 0.05 μ Ci of androstenedione-4- 14 C (58.0 mCi/mmol), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l of propylene glycol, 0.3 g of homogenized tissue, and increasing amounts of ascorbic acid. The samples were incubated in air for 1 hr at 37.5°C. (From Ellis, L. C., and M. H. Baptista, ref. 2).
- Figure 3. Lipid peroxidation and the biotransformation of androstenedione into testosterone and high-polar steroid intermediates by rat testicular tissue in vitro. The incubation mixture consisted of 0.05 μ Ci of androstenedione-4- 14 C (58.0 mCi/mmol), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, 0.3 g of homogenized tissue, and from 1 to 300 μ l of 30% hydrogen peroxide. The samples were incubated in air for

1 hr. at 37.5°C. (From Ellis, L. C., and M. H. Baptista, ref. 2).

Figure 4. Lipid peroxidation and the biotransformation of androstenedione into testosterone and high-polar steroid intermediated by rat testicular homogenate in vitro. The incubation mixture consisted of 0.05 μ Ci of androstenedione-4- 14 C (58.0 mCi/mmole), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, 0.3 g homogenized tissue, and from 1 to 80 μ moles of D- α -tocopherol acid succinate. The samples were incubated in air for 1 hr. at 37.5°C. (From Ellis, L. C., and M. H. Baptista, ref. 2).

Figure 5. Lipid peroxidation and biotransformation of pregnenolone and progesterone into testosterone by a rat testicular preparation in vitro. The incubation mixture consisted of 0.5 μ Ci pregnenolone-7 α - 3 H (2.275 nmoles), 0.05 μ Ci progesterone-4- 14 C (2.275 nmoles), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, 0.3 g minced tissue and from 50 to 5,000 picomoles of melatonin. The samples were incubated for 1 hr. at 37.5°C. (Unpublished data).

Figure 6. Lipid peroxidation and the biotransformation of pregnenolone and progesterone into androstenedione, testosterone, and total androgens (androstenedione plus testosterone) by rat mince testicular tissue in vitro expressed on a per animal basis. The incubation mixture consisted of 0.5 μ Ci pregnenolone-7 α - 3 H

(2.275 nmoles), 0.05 μ Ci progesterone-4- 14 C (2.275 nmoles), 10 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, and various amounts of testicular minced tissue. The samples were incubated for 2½ hrs. at 37.5°C. (Unpublished data).

Figure 7. The effects of prostaglandins A₁, E₁, and F₁α on lipid peroxidation and the biotransformation of pregnenolone and progesterone into 17α-hydroxyprogesterone, androstenedione, testosterone, and total androgens by rat testicular minced preparations in vitro. The incubation mixture consisted of 0.5 μ Ci pregnenolone-7α- 3 H (2.275 nmoles), progesterone-4- 14 C (2.275 nmoles), 5 ml of a balanced phosphate buffer (pH 7.4), 20 μ l propylene glycol, 0.3 g minced tissue and 1 mg of the respective prostaglandin. The samples were incubated in air for 1 hr. at 37.5°C. The prostaglandins used in this study were generously provided by Dr. John E. Pike of the Upjohn Co. (From Ellis, L. C., and M. H. Baptista, ref. 2).

Figure 8. Inhibition of rabbit testicular contractions in vitro with Prostaglandin E₁ (A) PGE₁ concentration was 3.6 nM and the recorder sensitivity was 0.3 g/5mm of pen deflection; (B) PGE₁ concentration was 11 nM and the recorder sensitivity was 0.3 g/5mm of pen deflection; (C) PGE₁ concentration was 18 nM and the recorder sensitivity was 0.4 g/5mm of pen deflection. (From Hargrove, J. L., J. M. Johnson, and L. C. Ellis, Proc. Soc. Exptl. Biol. Med. 136:958-961, 1971).

Figure 9. Inhibition of rabbit testicular contractions in vitro with prostaglandin E_1 and the lack of effect of the diluent (ethanol) on this phenomenon: (A) PGE_1 concentration was 21 nM and the recorder sensitivity was 1.05 g/5mm of pen deflection; (B) PGE_1 concentration was 36 nM and the recorder sensitivity was 0.59 g/5mm of pen deflection; (C) ethanol concentration was 11 μ M and the recorder sensitivity was 0.4 g/5mm of pen deflection. (From Hargrove, J. L., J. M. Johnson, and L. C. Ellis, Proc. Soc. Exptl. Biol. Med. 136:958-961, 1971).

Figure 10. Stimulation of rabbit testicular contraction in vitro with prostaglandin $F_{1\alpha}$: (A) $PGF_{1\alpha}$ concentration was 0.4 nM and the recorder sensitivity was 0.03 g/5mm of pen deflection; (B) $PGF_{1\alpha}$ concentration was 0.7 nM and the recorder sensitivity was 0.2 g/5mm of pen deflection; (C) $PGF_{1\alpha}$ concentration was 40 nM and the recorder sensitivity was 0.2 g/5mm of pen deflection; (D) $PGF_{1\alpha}$ concentration was 60 nM and the recorder sensitivity was 0.2 g/5mm of pen deflection. (From Johnson, J. M., J. L. Hargrove, and L. C. Ellis, Proc. Soc. Exptl. Biol. Med. 138:378-381, 1971).

Figure 11. Stimulation of rabbit testicular contraction in vitro with prostaglandin $F_{1\alpha}$: (A) $PGF_{1\alpha}$ concentration was 71 nM and the recorder sensitivity was 0.078 g/5mm of pen deflection; (B) $PGF_{1\alpha}$ concentration was 142 nM and the recorder sensitivity was 0.078 g/5mm of pen deflection; (C) $PGF_{1\alpha}$ concentration was 213 nM and the recorder sensitivity was 0.078 g/5mm of pen

deflection. (From Johnson, J. M., J. L. Hargrove, and L. C. Ellis, Proc. Soc. Exptl. Biol. Med. 138:378-381, 1971).

Figure 12. Initiation of rabbit testicular contractions in vitro with prostaglandin $F_{1\alpha}$: (A) $PGF_{1\alpha}$ concentration was 14 nM and the recorder sensitivity was 0.143 g/5mm of pen deflection; (B) $PGF_{1\alpha}$ concentration was 57 nM and the recorder sensitivity was 0.2 g/5mm of pen deflection. (From Johnson, J. M., J. L. Hargrove, and L. C. Ellis, Proc. Soc. Exptl. Biol. Med. 138: 378-381, 1971).

Figure 13. Proposed scheme showing the apparent routes for the synthesis of malonaldehyde, prostaglandin E_1 (PGE_1) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and testosterone synthesis from pregnenolone. The asterisks are placed on the lipid peroxides to indicate their action on the conversion of sulfhydryl compounds to disulfide bridges. During lipid peroxidation, free radicals are formed. These free radicals are brought to the ground state by accepting protons from other organic compounds. RSH is used in this scheme to represent either enzyme protein sulfhydryl groups, glutathione sulfhydryl groups, or any organic RH_2 moiety. The chain reaction of a free radical producing another free radical in this way can be stopped by the radical reacting with sulfhydryl groups or with antioxidants so that stable free radicals are formed. Once disulfide bridges are formed from sulfhydryl groups, the sulfhydryl groups may be regenerated through the glutathione-type reductase involving glucose-6-

phosphate and NADPH as indicated at the upper-left-hand corner of the figure. NHP represents non-heme iron; P₄₅₀ represents the iron containing pigment that develops an ultraviolet absorption spectrum with maximum absorption at 450 mμ in the presence of carbon monoxide. The TBA chromogen is the pigment measured with thiobarbituric acid reagent as a measure of lipid peroxidation. Two methods are shown for the peroxidation of lipids. The first and predominant method is heat labile and enzymatic. This route involves NADPH, non-heme iron (NHP), and the P₄₅₀ pigment. The second method involves ascorbic acid, ADP, and Fe²⁺. It is heat stable but is much slower in its rate of synthesis of malonaldehyde. It is not known whether ascorbic acid acts as a proton donor in regenerating Fe²⁺ from Fe³⁺ or possible as a complex with Fe²⁺. (From Ellis, L. C., and M. H. Baptista, ref. 2).

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Figure 10.

Figure 11.

Figure 12.

TABLE 1. The effect of hypophysectomy and the combined treatment of growth hormone (STH) and adrenocorticotrophic hormone (ACTH) on lipid peroxidation by rat testicular preparations in vitro, expressed on a per animal basis.¹

Treatment	Testes Wt. (gm)	TBA O.D. Units
Control	2.40 [±] .030	0.436 [±] .016
Hypophysectomized	0.12 [±] .008 ⁺	0.012 [±] .003 ⁺
Hypophysectomized + STH + ACTH	0.16 [±] .007*	0.29 [±] .003**

*P < 0.05 when compared with the hypophysectomized group.

⁺P < 0.001 when compared with the control group.

**P < 0.01 when compared with the hypophysectomized group.

¹Unpublished data.

TABLE 2. The quantitative distribution of lipid peroxidative enzyme and steroid biotransforming enzyme capacities for rat testicular, teased-tubular and minced preparations expressed on a per animal basis.¹

Steroid Isolated	Amount of Steroid Isolated (μ moles)	TBA O.D. Units/ 30 Min. Inc.
<u>Mince</u>		
Androstenedione	0.702 $\pm$.059	
Testosterone	0.910 $\pm$.054	0.239 $\pm$.023
Total Androgens	1.612 $\pm$.057	
<u>Teased Tubules</u>		
Androstenedione	0.112 $\pm$.010	
Testosterone	0.006 $\pm$.001	0.009 $\pm$.002
Total Androgens	0.118 $\pm$.007	

¹Unpublished data

TABLE 3. Prostaglandin synthetase activity of rat testicular tissue after hypophysectomy and adrenalectomy expressed on a per animal basis.¹

Treatment	(N)	Prostaglandin Synthetase Activity (O.D. Units)	P Value
Control	6	1.166 $\pm$ 0.133	
Hypophysectomy (3 weeks)	6	0.905 $\pm$ 0.149	
Hypophysectomy (5 weeks)	6	0.323 $\pm$ 0.029	
Control	6	1.955 $\pm$ 0.158	
Adrenalectomy (10 days)	6	0.503 $\pm$ 0.130	0.001

¹Unpublished data