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Effects of Prostaglandin F2α on the Thyroid, Pituitary, Uterus, Vaginal Canalization, and Ovarian Progesterone Biosynthesis of Immature Rats

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EFFECTS OF PROSTAGLANDIN F₂-ALPHA ON THE THYROID, PITUITARY, UTERUS, VAGINAL CANALIZATION, AND OVARIAN PROGESTERONE BIOSYNTHESIS OF IMMATURE RATS

by

Farol N. Tomson

A Thesis Submitted to the faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

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BIOGRAPHY

Farol N. Tomson was born in Webster City, Iowa, on January 27, 1943. He attended East High School in Waterloo, Iowa and graduated in June of 1961. After graduating from the University of Iowa, Iowa City, Iowa, in 1966 with a Bachelor of Arts degree in General Science, he entered the College of Veterinary Medicine, Iowa State University, Ames, Iowa. In 1970, he graduated with his Doctor of Veterinary Medicine (D.V.M.) degree.

The writer began his graduate study in September, 1971, in the Department of Anatomy at Loyola University Stritch School of Medicine, Maywood, Illinois.
ABSTRACT

A series of in vivo experiments was undertaken for the purpose of determining what effects prostaglandin $F_{2\alpha}$ compound would have on a number of endocrine organs and reproductive structures of immature female rats. The pituitary, thyroid, uterus, vaginal canalization, ovarian progesterone biosynthesis and body weights were carefully analyzed.

The procedures included the chronic administration of $\text{PGF}_{2\alpha}$ (0.075 mg, BID) in 30 twenty-two day old female albino rats. On days 27, 33 and 37, ten rats were necropsied and their body and organ weights were recorded. Vaginal canalization was noted at this time. All tissues were formalin-fixed while the ovaries were quick frozen in liquid nitrogen for progesterone biosynthesis determination, by way of semi-quantitatively assessing the histochemical detection and intensities of pregnenolone-3B-hydroxysteroid dehydrogenase.

Other experimental groups that paralleled the prostaglandin group included a prostaglandin antagonist (aspirin), and two adenohypophyseal gonadotropins, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The controls included a non-injected group and an alcohol (aspirin vehicle) injected group.

The results indicated that the basic biological responses of immature rats to $\text{PGF}_{2\alpha}$ administration included increased ovarian progesterone biosynthesis as determined by enzyme histochemical techniques, and premature sexual development as witnessed by early vaginal canalization.
Both of these responses were significant and were related to the exogenous prostaglandin compound. No significant differences could be detected either gravimetrically or histologically among the thyroid glands, pituitary glands and uteri of the prostaglandin group versus the control groups.

The aspirin group revealed stunted growths and delayed sexual development, but aspirin toxicity, however slight, could not be ruled out.

The selected gonadotropins, FSH and LH, yielded variable responses which raised doubts about their purity. FSH, however, did show a progressively decreasing amount of ovarian progesterone biosynthesis activity, while the LH injected group could not be distinguished from the normal control group.
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INTRODUCTION

Prostaglandins are hormone-like substances that are widely distributed in human and animal tissues. They are believed to be intracellular, metabolic regulators that stimulate or inhibit the action of many hormones. Their remarkably wide range of pharmacologic effects has excited worldwide scientific interest. On the horizon now are potential medical uses of prostaglandins for numerous and diverse clinical problems, e.g. labor induction, therapeutic abortion, contraception and hypertension control.

Prostaglandins are 20-carbon acidic lipids whose metabolic precursors are the essential fatty acids. There are fourteen known prostaglandins; they are widely distributed in small amounts throughout the animal kingdom. Although very rapidly metabolized, prostaglandins are one of the most potent chemicals ever discovered, one millionth of a gram producing marked effects.

A rather confusing combination of Greek and Roman letters is used to identify these compounds, i.e. A, E₁, F₂. Of the fourteen prostaglandins, thirteen occur in man, with the highest concentration in human seminal fluid. Their wide range of physiologic effects can be shown by prostaglandin E₂, the most thoroughly studied, which not only stimulates smooth muscle, lowers blood pressure, inhibits gastric secretions and inhibits platelet aggregation, but also inhibits lipolysis and acts as a nasal vasoconstrictor, bronchodilator, causes sedation, produces optical miosis, and increases intraocular pressure. In general, the E and A groups of prostaglandins have vasodepressor effects while the
\( \text{PGF}_2 \) group tends to raise blood pressure. Both groups will induce labor and are used for therapeutic abortions.

There has been much interest in prostaglandin research in recent years mainly due to larger supplies of a purer substance. The greater interests into the effects of prostaglandins at the present time seem to be focused on the female reproductive systems of mature mammals. Little interest, however, has been given to the effects of prostaglandins on immature mammals. Therefore, it seemed pertinent to initiate some studies and experimental trials which could provide more detailed information concerning the nature of the effects of prostaglandins during the period of transition from immaturity to maturity.

For determining a possible action site of prostaglandins, a prostaglandin antagonist, acetylsalicylic acid (aspirin), was used in one experimental trial group. Two gonadotropins, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), were used in additional experimental groups to differentiate any similarity between their responses and the prostaglandin responses.

The organs selected for study were the pituitary, thyroid and uterus. Vaginal canalization was used as the index of puberty and frozen ovaries were processed to study possible progesterone biosynthesis activity.

There are no classical monographs on this subject to refer to, and this thesis is intended to add new lines of information so that we can better understand the nature of \( \text{PGF}_2 \). This study is the first such attempt to record the chronic effects of prostaglandins on the selected
organ systems of developing immature rats by thorough assessments of the sequelae of the changes over short time intervals. Such a study can make a significant contribution to the knowledge of prostaglandin research because it measures the total animal response of the animal to chronically administered prostaglandins.
The proliferation of prostaglandin research is a fairly recent development. Three Swedish investigators did the pioneer work: Professor U.S. von Euler, coiner of the term "prostaglandin"; Sune Bergstrom and Bengt Samuelsson. Isolation and determination of the structure of PGE\textsubscript{1} and PGF\textsubscript{1}, eleven years ago by Bergstrom (1962), gave a great impetus to research work.

During the first decade after prostaglandins were discovered in 1930 by Kurzrok and Lieb, all research was conducted in a single laboratory, i.e. that of Professor von Euler. After World War II, Bergstrom took on the job of attempting to isolate, identify and purify the substances. In 1957, the Bergstrom team isolated PGE\textsubscript{1} and PGF\textsubscript{1} in pure crystalline form and during the next five years developed the advanced chromatographic techniques to precisely define the chemical structure of their compounds.

The impetus for further biological work resulted from the elegant isolation and chemical identification achieved by Bergstrom, Sjovall, Samuelsson and their co-wokers. Bergstrom and Sjovall (1957) isolated two compounds which behaved differently on partition between ether and an aqueous phosphate buffer. The one more soluble in ether was called prostaglandin E (PGE), the other, more soluble in phosphate buffer was called prostaglandin F (PGF). These compounds were assigned the empirical formulae $C_{20}H_{34}O_5$ and $C_{20}H_{36}O_5$ respectively. Total chemical synthesis
of all the major natural prostaglandins is now possible as a result of
the elegant methods devised by E. Corey, et al. (1969). These have
already proven feasible for commercial production.

It is clear that within the next decade thousands of prostaglandin
analogues will be synthesized. One can, therefore, anticipate that
compounds with more specific and more potent biological activities, as
well as antagonists to prostaglandins, will become available for both
clinical and research use. Prostaglandin antagonists, like aspirin and
indomethacin, are anti-inflammatory drugs which can block prostaglandin
synthesis (Smith and Willis, 1971; Vane, 1971).

The basic question in prostaglandin research is to define the
normal function of this wide array of substances. This remains, for the
most part, not answered, but many theories exist; facilitating ejaculation
(von Euler, 1967), a menstrual "stimulant" (Pickles, 1959), a role in
parturition (Karim, et al., 1969), and an anti-hypertensive renal
endocrine function (Lee et al., 1971).

Major contributions to knowledge of the metabolic pathways of the
prostaglandins came from Hamberg and Samuelsson who, in 1966, isolated
and identified the eight naturally occurring metabolites of the six
primary prostaglandins. Steinberg and his co-workers (1963) made the
original observation that PGE₁ inhibits hormone-induced lipolysis. The
conclusions that were made included the possibilities that PGE₁ inhibits
the enzyme adenyl cyclase, and that this inhibition is an indirect action (Ramwell and Shaw, 1970).

On the other hand, there is abundant evidence that on many tissues, PGE compounds, although affecting adenyl cyclase, do not inhibit the related hormone but instead mimics that hormone's action. Thus PGE₂, like corticotropin, stimulates corticosteroidogenesis (Flack et al., 1969). Kaneko et al., (1969) has shown PGE₁ to mimic the action of thyroid stimulating hormone (TSH). PGE₁ also stimulates insulin secretion with cyclic AMP acting as the mediator. It may be concluded that interactions between PGE compounds and adenyl cyclase may underlie many of the effects produced by prostaglandins but no hypothesis has yet explained the mode of action of the PGF or PGA compounds.

It has been hypothesized that prostaglandins are probably synthesized in all or in most cells and in all species and that the primary mechanism of action of prostaglandins is on the plasma membrane and its association with adenyl cyclase and adenosine 3', 5'-monophosphate (Cyclic AMP) (Butcher, et al., 1967). Cyclic AMP,, described as a "second messenger system" - the hormone being the first messenger - is believed to mediate most, if not all, hormone actions. Cellular regulation of the response of a tissue to hormonal stimulation by prostaglandins has also been indicated, since exogenous prostaglandins can modify the hormonal responses of the tissue (Shio, et al., 1971).
Prostaglandins can increase cyclic AMP levels in many tissues in what appears to be a nonspecific manner. No qualitative differences have been observed between the groups of prostaglandins in their ability to increase the intracellular accumulation of cyclic AMP, but Butcher, et al., (1967), has found the PGE group to be generally more potent than the PGF series.

Studies by Ranwell and Shaw (1970) regarding the effect of tissue stimulation on endogenous prostaglandins have indicated that more prostaglandins are released than can be extracted, suggesting the activation of a biosynthetic process rather than the release from a preformed store. It has, therefore, been hypothesized that prostaglandins interact with cyclic AMP, thereby mimicking certain hormones - oxytocin, ACTH, follicle stimulating hormone.

It is becoming clearer that prostaglandins are involved in the response of a tissue to hormonal stimulation but the determination of the exact role awaits elucidation of whether the biosynthesis and release of prostaglandins results from a direct effect of the hormones or whether it is secondary to changes in cyclic AMP levels.

I. Circulation of Prostaglandins

Prostaglandins are released into the circulation from different organs, but a very high proportion of PGE and PGF compounds are metabolized on one circulation through the lungs.
Fifteen minutes after the intravenous injection of PGF$_2$ in mice, Green et al., (1967), showed most of the compound was concentrated in the liver, kidneys and subcutaneous connective tissue. No significant amounts could be demonstrated in the other organs including the endocrine glands. PGF$_2$ does reach the uterine venous blood, however, under physiological conditions, and has been identified as simulating the action of luteolysin (Poyser, et al., 1970; Blatchley et al., 1971; Bland, et al., 1971). PGF$_2$ is, although stable in blood, is 95% removed from the circulation on one passage through the lungs; furthermore, 80% of the prostaglandins are removed by the liver via the portal system (Ferreira and Vane, 1967).

II. Prostaglandin Actions on the Thyroid.

Very little has been done regarding the effects of prostaglandins on the thyroid gland. In canine thyroid slices, PGE$_1$ mimics the actions of thyroid stimulating hormone (TSH), (Onaya and Solomon, 1970; Zor et al., 1960). It is suggested, therefore, that PGE$_1$ is functional in the secretion of thyroid hormone which is initiated by TSH (Grimley, et al., 1969). It should be mentioned that these were in vitro studies and only speculations can be made on the in vivo environment.

III. Prostaglandin Actions on the Uterus.

Extensive studies of prostaglandins and the uterus have progressed to therapeutic applications of the prostaglandins and clinical trials.
Karim (1971) has performed clinical trials in Uganda and has reported that PGE$_2$ and PGF$_2\alpha$ administered orally will induce labor in the later stages of pregnancy in humans without serious side effects. Investigations concerning prostaglandins and the uterus have attempted to determine their role in the pregnant and non-pregnant uterus.

The non-pregnant uterus is stimulated by PGF$_{1\alpha}$ and PGF$_{2\alpha}$ compounds which are found in menstrual fluids; while the PGE compounds, found in high concentrations in seminal fluids, inhibit myometrial activity (Bygdeman and Hamberg, 1967). This general statement must be modified in view of investigations which show that the stage of the ovarian cycle is important. Human myometrium is more sensitive to inhibition by PGE at midcycle near ovulation (Eliasson, 1963) and more sensitive to PGF$_{2\alpha}$ stimulation just before menstruation (Pickles, et al., 1965) and during pregnancy (von Dorp, et al., 1964). This is probably related to the changing levels of the ovarian steroids. It is suggested, therefore, that the physiologic role of PGE in semen is to facilitate sperm migration and subsequent capacitation and fertilization.

The pregnant uterus, in contrast to the non-pregnant uterus, responds differently to the prostaglandin compounds. Both PGE$_2$ and PGF$_{2\alpha}$ are spasmogenic on upper uterine muscle strips, but inactive on lower muscle segments (Embrey and Morrison, 1968). Additional follow-up studies by Karim (1968) showed that PGF$_{2\alpha}$ was consistently found in venous blood of patients in labor, but absent in women who needed surgical deliveries or those women not in labor. The source of this PGF$_{2\alpha}$, according to
As the investigation and research continues into the effects of prostaglandins on the uterus, interesting speculations have arisen, all of which are still under serious consideration.

Kirton et al. (1970) has proven the successful role of PGF$_{2\alpha}$ as a contraceptive when given after ovulation in primates. They have shown this effect to be caused by the luteolytic properties of PGF$_{2\alpha}$ and have hypothesized this to be the specific factor which normally causes the corpus luteum to regress each month if no pregnancy occurs. This has opened a new approach to female contraception whereby prostaglandins may be administered once each month as compared to the conventional birth control pills (Karim and Sharma, 1971).

Bygdeman and Samuelsson (1966) have investigated PGE's role into human male infertility and have suggested that 8% of male infertility might be due to PGE deficiency. PGE's role in preventing gastric and duodenal ulcers in rats and dogs has been strongly suggested by Shaw and Ramwell (1968). When administered as aerosols, PGE$_1$ will act as a bronchodilator according to Jackson (1970) with this decongestant, such effect lasting up to 14 hours.

The PGA group has been selectively researched into the area of hypertensive effects because they are not quickly metabolized and inactivated in contrast to the PGE and PGF groups. Lee et al., (1971) has shown the marked reduction in blood pressure in hypertensive patients by the infusion of PGA.
A final comment to be made concerning the effects of prostaglandins on the endocrine and reproductive systems is that many statements and facts are being made, but that no acceptable proven hypothesis governing prostaglandin action thus far has been established. Investigations have only resulted in speculations. The intensity of research into this area will undeniably shed new light on their true significance. Many questions remain unanswered.
MATERIALS AND METHODS

I. Animals and Housing.

A total of 190 immature (21 day old) Sprague-Dawley derived female rats (Locke-Erickson Laboratories, Maywood, Illinois) were separated randomly into 19 experimental groups. Rats were housed five to a unit in clear plastic "shoe-box" shaped cages (10.5 X 19 X 8.5 inches) on Sanicell bedding (Paxton Laboratories, Paxton, Illinois). Feed (Purina Lab Chow) and water were provided ad libitum. They were all housed in the same environmentally controlled room where the temperature (72±2° F), humidity (43±5%) and a 12 hour light cycle remained constant.

II. Experimental Groups.

1. All injections were given subcutaneously using a tuberculin syringe and a 26 gauge needle; they were given twice daily (8 a.m. and 4 p.m.). Two major control groups were defined: a non-injected normal control group and an alcohol-injected control group injected with 0.25 cc of 10% ethanol. This was necessary because alcohol was the vehicle for the acetylsalicylic acid. Ten non-injected controls were necropsied at 22 days to establish baseline values. Then, successive groups of 10 rats from both control groups were necropsied at ages of 27, 33 and 37 days, i.e. after 5, 11 and 15 days of treatment.

2. The prostaglandin group was injected with 0.075 mg of PGF$_2$α twice daily (0.075 cc, BID) from day 22 through the day prior to termination on days 27, 33 and 37. The PGF$_2$α was delivered frozen at a concentration
of 10 mg/ml in a phosphate buffer from G.D. Searle Co., Skokie, Illinois. This was further diluted to a concentration of 0.1 mg/ml with a phosphate buffer solution and then dispensed into 1.00 cc aliquots and refrozen at -60° C until used.

3. The aspirin (acetylsalicylic acid) was dissolved with 10% ethanol and injected at dosages of 5 mg (0.25 cc) BID. The injections were started on day 22 and continued up to the termination on days 27, 33 and 37.

4. The LH (Schwarz-Mann, Orangeburg, New York) injected rats were given 0.125 Armour Unit (LH-ovine, Armour, 277-80 equivalent to NIH-LH-S1) BID from day 22 until time of sacrifice on days 27, 33 and 37. The LH was received lyophilized and was reconstituted with sterile saline to deliver a dose of 0.05 cc.

5. The FSH (Schwarz-Mann, Orangeburg, New York) injected rats were given 0.25 Armour Unit (FSH-porcine, Armour, 264-15lx equivalent to 0.5 NIH-FSH-S1), BID in a 0.05 cc dose. The injections began on day 22 and continued up to termination on days 27, 33 and 37.

The age of the immature rat and this determination of the necropsy dates were made to coincide with the specific growth periods of the reproductive system as described by Kasprov (1969).

III. Procedures.

Rats were euthanized and necropsied after recording body weight to the nearest gram on a direct reading torsion balance and killing by ether
anesthesia followed by decapitation. Vaginal openings were recorded at time of necropsy. The pituitary, thyroid, ovaries and uterus were dissected free and weighed to the nearest 0.2 mg on a Roller-Smith type torsion balance. In cases where the uterus was distended with fluid, incisions were made to drain the fluid and the uterus was blotted dry with bibulous paper. After weighing, half of the thyroids, uteri and ovaries were fixed in buffered formalin and the other half were quick frozen in liquid nitrogen and stored at -60°C. The pituitaries were fixed in a Bouin's-Holland solution.

The formalin-fixed tissues were then washed, dehydrated, cleared and embedded in paraffin blocks. Sections were taken at six microns and stained with the standard Harris hematoxylon and eosin stain. Photomicrographs were taken using a Wild microscope and Polaroid 107 type film at 100X magnifications for comparison.

The ovaries that were frozen were sectioned at eight microns and incubated for 3½ hours in a media containing pregnenolone, propylene glycol, nicotinamide-adenine dinucleotide (NAD) and nitro blue tetrazolium chloride (Nitro-BT), all in a phosphate buffer solution (pH 7.4) (Wattenberg, 1958). A set of companion ovarian sections were incubated in media without pregnenolone and served as control groups. Polaroid photomicrographs were taken of the incubated sections to determine and measure the semi-quantitative reactions.
This particular technique allows for the localization and semi-quantitation of the enzyme pregnenolone-3β-hydroxysteroid dehydrogenase (P-tita0118•3p-HSD), which is indicative of active progesterone biosynthesis. The intensity of activity was rated on a scale of 0 to +5, depending on the degree of monoformazan and diformazan precipitate on the ovarian tissue, (Plates I-III).
EXPERIMENTAL RESULTS

I. Control Groups.

Gravimetric analyses of body, thyroid, pituitary and uterine weights of the immature female rats revealed a linear progression of increasing weights. The body, pituitary and uterine weights show a greater than 100% increase in size from day 22 to day 33, for body weights, 41 mg to 92 mg; a 100% increase for pituitary weights, 2.2 mg to 4.4 mg; and a greater than 300% increase for uterine weights, 20.2 mg to 85.2 mg (Table I). The thyroid gland increased approximately 80% during this same time period, i.e. 4.7 to 8.4 mg.

As would normally be expected the mg% values of the thyroid and pituitary glands began to decline as the rats passed the 27th day; the gland growth slowing while the body growth continued, 11.5 to 7.9 and 5.4 to 4.2 respectively. The uterine mg% values became rather constant past the 27th day, i.e. 92.0 at 33 days and 92.5 at 37 days. The standard deviations of these uterine weights increased considerably as the animal matured because of the beginning hormonal activity of their reproductive systems, and such variations can be expected in this age range (Kasprow, 1969).

From the tabular data, one observes the fact that up to day 33, 100% of the animals were sexually immature, as manifested by closed vaginas. Thereafter, by day 37, 100% became sexually mature. This five day range of vaginal canalization is acceptable as a normal range when the animals are of an inbred strain and housed together (Locke, 1971).
The pregnenolone-3β-HSD activity displayed by the ovaries of the control groups was of minimal intensity at 27 days with a 75% increase in activity intensities by day 33 (Table V, Text Figure V). This progesterone biosynthetic capability remained constant from day 33 to day 37. It is interesting to recall that the uterine mg% weight also remained constant from day 33 to day 37.

II. Prostaglandin Group.

The body weights of the chronically injected prostaglandin rats were always lighter than the controls, but the differences were not significant, i.e. 55 gm vs 59 gm at 27 days; 90 vs 92 at 33 days; 120 vs 123 at 37 days (Tables I-IV). Gravimetric analysis of the thyroid glands from days 22 to 27 showed no significant differences from the control, i.e. 6.7 mg vs 6.9 mg at day 27; 8.4 vs 8.4 at day 33; 10.0 vs 9.6 at day 37. The same was true for their mg% values (Text Figure I, and Tables I-IV).

The pituitary weights were almost identical with those of the control groups as were the mg% values, i.e. 3.0 mg vs 3.2 mg at day 27; 4.3 vs 4.4 at day 33; 5.2 vs 5.2 at day 37, (Text Figure II, and Tables I-IV).

The uterine weights of the prostaglandin injected rats were comparable at day 27 and became progressively lighter than the controls, i.e. 34.2 mg vs 34.0 at day 27; 75.9 vs 85.2 at day 33; 107.9 vs 114.2 at day 37, (Text Figure III and Tables I-IV). No statistical significance could be detected. Needless to say, the large standard deviation values due to the sexual maturation and uterine responses may have hidden or masked any real growth differences which may have, in fact, existed. Analyzing uterine weights during first ovulations may prove an interesting field of
endeavor. The histological observations on all these tissues failed to show any detectable differences from the normal morphology of the controls.

The most significant finding of the PGF$_{2\alpha}$-injected immature female rats was the premature sexual maturation which was evidenced by 90% of the rats by day 33, (Text Figure IV). In rats, the vaginal opening occurs near the time of the first ovulation and is used as an index of puberty. On day 33, none of the control vaginas were open and this difference was significant using the student's t-test ($P<0.001$), (Table III). By day 37, all the rats in the prostaglandin and control groups were open.

The tabulated data of the semi-quantitative histochemical estimates of P-one-3β-HSD activity of the PGF$_{2\alpha}$ rat ovaries displays a significantly greater intensity of activity on days 27 and 37, than on day 33 when it fails to show a similar difference, i.e. 4.0 vs 1.8 at day 27; 2.3 vs 3.0 at day 33; 4.2 vs 3.0 at day 37, (Text Figure V, Figure V).

This early progesterone biosynthesis is interesting because it may provide a clue as to how subcutaneously administered PGF$_{2\alpha}$ can exert its effects on the biological response of premature sexual maturity as witnessed by the early vaginal canalizations.

III. Aspirin Group.

Gravimetric analyses of the body weights revealed a consistently lighter weight which was becoming progressively lighter than the controls, i.e. 54 gm vs 59 at day 27; 89 vs 92 at day 33; 110 vs 123 at day 37, (Tables I-IV). The general appearance of these rats towards day 37
suggested the possibility of an aspirin toxicosis because of the roughened dry hair coat, mild emaciation and dehydration. But despite this general appearance and smaller weight size, the endocrine gland weights were comparable to the normal controls if not larger.

The thyroid gland was variable in its weight comparisons, but none were significantly different from the control thyroids, i.e. 6.0 mg vs 6.9 at day 27; 8.9 vs 8.4 at day 33; 9.2 vs 9.6 at day 37. The uterine weights of the aspirin injected rats were all lighter than the controls but again no statistical significance could be detected because of the large values of the standard deviations, i.e. 26.6 mg vs 34.0 mg at day 27; 70.9 vs 85.2 at day 33; 109.3 vs 114.2 at day 37, (Tables I-IV, Text Figure I).

The pituitary gland, on the other hand, was somewhat larger than those of the controls on day 27 (3.4 mg vs 3.2 mg) and this small difference was significant (P<0.05) (Table II). This was an interesting finding because aspirin, a prostaglandin antagonist, blocks the biosynthesis of endogenous prostaglandins, and any blockage of prostaglandins would have decreased the weight of an end organ, unless a negative feedback mechanism was operating. This larger value could also be regarded as part of an aspirin toxicity phenomena which would render it meaningless for our purposes. The successive values of the pituitary weights on days 33 and 37 were 4.5 mg and 5.8 mg compared to the control values of 4.4 mg and 5.2 mg respectively; neither values were significantly different than those of the controls, (Tables I-IV, Text Figure II).
A delayed sexual maturity was observed in the aspirin-treated rats as witnessed by the failure of vaginal canalization by day 37. Only 75% of the rats displayed open vaginas by day 37 compared to 100% of the control rats, (Text Figure IV). This lag in sexual maturity may have been caused by either of two initiating factors. Either the possible aspirin toxicity was effective enough in its manifestations to stunt or retard normal growth because of its toxicity, or the aspirin used did, in fact, block the prostaglandins which are involved with the production of and biological responses to the ovarian steroids. To establish this fact, further experimental trials must be done; one can only speculate with the results obtained so far.

The semi-quantitative analyses of the pregnenolone-3β-HSD intensity in the ovaries of the aspirin injected rats revealed a variable linear relationship between the aspirin and the control groups. The 27 day value was greater than the control (2.3 vs 1.8); the 33 day values were equal at 3.0; while the 37 day value was less than that of the control, i.e. 2.5 vs 3.0 respectively. None of these aspirin-elicited values were of statistical significance, (Table V, Text Figure V).

IV. The Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) Groups.

Gravimetric analyses of the FSH and LH groups yielded significant differences in the pituitary, thyroid and uterus, body weight and vaginal canalization, but the purity of these compounds must be questioned because of the possible contamination with growth factors. The increases in body
weights in the FSH and LH groups ranged from 68 g and 65 g vs 59 g at 27 days; 103 and 101 vs 92 at 33 days; 127 and 126 vs 123 at 37 days, (Tables I-IV). The 27 day differences were statistically significant (P=0.01 and 0.02) after five days of injections while the 37 day variations were not significantly different. This sudden increase in body size and the resulting fall back into the normal range of the control groups cannot be explained if these substances were pure compounds.

The thyroid data shows the FSH and LH group mg% values to be consistently larger than the controls but analysis showed that these differences were not significant. i.e. 6.8 mg and 7.3 mg vs 6.9 mg at day 27; 9.3 and 10.0 vs 8.4 at day 33; 10.7 and 10.4 vs 9.6 at day 37, (Tables I-IV, Text Figure I). The pituitary glands showed a similar relationship with the values being larger but not significantly larger, i.e. 3.5 mg and 4.1 mg vs 3.2 mg at day 27; 4.9 and 4.9 vs 4.4 at day 33; 6.2 and 6.7 vs 5.2 at day 37, (Tables I-IV, Text Figure II). These similar weight comparisons of the thyroid and pituitary glands to the controls might possibly be explained by the presence of growth hormone factors in the FSH and LH preparations.

The uterine weights were all significantly larger than the controls, which should be expected because of the gonadotropic effects on the ovaries and their resulting effects on the uteri, i.e. 49.4 mg and 124.5 mg vs 34 mg at 27 days; 149.9 and 204.1 vs 85.2 at day 33; 147.3 and 161.2 vs 114.2 at day 37, (Tables I-IV, Text Figure III).
Premature sexual maturation as seen in the PGF$_2\alpha$-injected rats was also present in the FSH and LH injected groups; and this was a statistically significant difference from that of the control (P=0.001), (Table III, Text Figure IV). One hundred per cent of the rats in each group displayed open vaginal canalization at the time of necropsy at day 33. These gonadotropic hormones are directly responsible for the early activity of the ovary which in turn initiates the uterine and vaginal development via the ovarian steroids. This activation of ovarian steroidogenesis may be the same method by which the PGF$_2\alpha$ group exerts its effect on premature sexual maturity.

Analyzing the semi-quantitative histochemical estimates data of the enzyme converting ovarian precursor of progesterone (P-one-3\beta-HSD), one finds seemingly increased levels of intensities in the 27 day FSH and LH groups when compared to the controls, i.e. 3.2 and 2.3 vs 1.8, (Table V, Text Figure V). These differences, however, were not statistically significant.

The FSH injected rat ovaries show decreased P-one-3\beta-HSD intensities for the 33 and 37 day intervals as compared to controls, i.e. 2.3 vs 3.0 and 1.4 vs 3.0 respectively, while the LH injected rat ovaries showed similar P-one-3\beta-HSD intensities, as the control groups, i.e. each had values of 3.0, (Table V, Text Figure V).
DISCUSSION

While studying the biological effects of the prostaglandins, one is impressed with their wide distribution and their unparalleled ability to modify hormone actions, an effect now known to correlate with changes in cyclic AMP levels within the different cell types. Cyclic AMP is now considered the key intracellular mediator in tissue responses to many hormones (Robison et al., 1971). According to the current concept, the hormones are released from their tissue or origin and travel via the circulation to the target tissue where they stimulate adenyl cyclase to catalyze the conversion of adenosine triphosphate (ATP) to cyclic AMP, which in turn induces the target tissue to respond. The concept is complicated by the multiplicity and diversity of the actions reported for cyclic AMP and by the actions of other cyclic mononucleotides (Robison et al. 1971).

Also, the prostaglandins can mimic many of the responses usually attributed to cyclic AMP. These interrelationships are neither simple nor well understood.

Depending on the tissues or organs involved, prostaglandins either increase or decrease the synthesis of cyclic AMP by adenyl cyclase or the degradation of cyclic AMP by phosphodiesterase. As an oversimplified generalization, prostaglandins decrease cyclic AMP in adipose tissue and toad bladder (Lipson and Sharp, 1971), and increase cyclic AMP in most other tissues. Studies with rat brown fat, and with canine, rabbit and human fat, indicate species differences are involved which further complicate the picture (Bizzi, et al., 1968; Hinman, 1972). Prostaglandins E
and F have already been reported to stimulate cyclic AMP formation in the pituitary, thyroid and uterus but this work was done in vitro while in vivo results are still forthcoming (Ramwell and Shaw, 1970).

The earlier sexual maturity and increased progesterone biosynthesis in the ovaries revealed that the injected PGF$_2\alpha$ did, indeed, have biological effects on these immature rats. These experiments, however, revealed no biological effects, either gravimetrically or histologically, on the pituitaries, the thyroid glands and the uteri. This in vivo experiment evaluated the response of the animal in total equilibrium with all functioning body systems.

Despite the high rate of deactivation of injected prostaglandins by the lungs and liver, enough PGF$_2\alpha$ did circulate to influence the ovarian steroidogenesis by day 27. Further investigations into the histochemistry of the pituitary, thyroid and uterus would be of value to add to the in vivo knowledge of prostaglandin effects on immature animals.

A factor to consider is that, although the body weights tripled in size from day 22 to day 37, the standard daily dose per rat remained constant at 0.075 mg BID and the delivered dosage per gram of body weight became progressively smaller. This was unavoidable because of the limited supply of PGF$_2\alpha$ compound. Because of this, more significance might be given to the 27 day increased ovarian progesterone biosynthesis seen in the prostaglandin injected group. The increased progesterone biosynthesis undoubtedly was responsible for the premature sexual development
Had the dosage of PGF$_2\alpha$ per gram of body weight been constant during this growth period, perhaps more obvious biological responses would have been seen. This provides another area for future investigation.

The dramatic effect of premature sexual development in immature rats raises several areas for speculation. Knowing that PGF$_2\alpha$ can mimic hormone actions via increasing cyclic AMP levels in end-organ cell units, one can only deduce from this experiment which hormone or hormones were mimicked. Undoubtedly, small amounts of estrogen(s) play a significant role in early ovarian development which also commences the ovarian-pituitary cycle (Velardo, 1958), but its exact location and source remains uncertain. Therefore, the daily administration of PGF$_2\alpha$ may have indeed mimicked the small amount of endogenous estrogen(s) needed to start the maturity of the female reproductive systems. This is supported by other investigators who report that all prostaglandins, including F$_2\alpha$, increase steroidogenesis (Speroff and Ramwell, 1970) and comment on their wide variability in responses. These results were based on in vitro luteal steroidogenesis and they seemingly contradict the luteolytic effects of PGF$_2\alpha$ when large doses were given in vivo to rats (Pharriss and Wyngarden, 1969). Pharriss and Hunter (1971) tried to solve this apparent contradiction, but their interpretations of the in vitro versus in vivo experiments to resolve this problem were difficult and confusing. While higher doses of PGF$_2\alpha$ cause antagonism to luteolysis, lower doses would bring about luteolysis. They also stated that PGF$_2\alpha$ administered did not stimulate ovulation but that uterine growth was stimulated when used in
conjunction with Pregnant Mare Serum (PMS). Their conclusions were based on the assumption that increased ovarian estrogen secretion altered the ratio of estrogen to progesterone thereby stimulating myometrial growth.

Velardo (1960), proved experimentally that both FSH and LH are needed to induce ovulation; thus, might one imply that PGF$_2$$\alpha$ could have, indeed, mimicked several endogenous hormones to bring about this early maturation?

The semi-quantitative enzyme histochemical analysis of the ovaries of rats injected with PGF$_2$$\alpha$ provided some clues to clarify which hormone or hormones the prostaglandin is mimicking. On day 27, the PGF group showed the highest reading of all the experimental and control groups, i.e., 4.0. This was more than twice the activity as seen in the control ovaries, 1.8. The effects of this increased progesterone biosynthesis by the ovaries is two-fold, (1) progesterone regulates the secretion of FSH and LH from the pituitary (Burrows, 1949; Velardo, 1958), thus indirectly affecting further ovarian development; and (2) progesterone has specific effects on the uterus.

The gravimetric and enzyme cytochemistry of in vivo prostaglandin rat ovaries deserves more scientific attention to determine if such an effect was caused exclusively by an increase in progesterone biosynthesis. The effects of prostaglandin F$_2$$\alpha$ on the uterus were not significant either gravimetrically or histologically. Obviously, enzymatic histochemical analysis of these uteri will answer some of these perplexing questions.
To complicate things more, other investigators are reporting a decline in progesterone secretion following PGF$_{2\alpha}$ infusion using in vivo sheep ovaries (Cahmley et al., 1972) and those of pregnant hamsters (Guthknecht, et al., 1971). They were measuring plasma progesterone levels rather than ovarian biosynthesis, so in fact these results may still be compatible. But this does point out the confusion that results from analyzing data from in vitro vs in vivo studies as suggested earlier. Perhaps these different effects may represent two different mechanisms of action.

Pharriss and Wyngarden (1969) have proposed that PGF$_{2\alpha}$ produces its biological effects by venoconstriction producing a local ischemia via reduced ovarian blood flow. Therefore, one major difference explaining the discrepancies between the in vitro and in vivo results is the effect of local hemodynamics. Relatively simple experiments could easily be devised to answer this question.

It appears that the prostaglandins are leading us to the common meeting ground where cellular communication and metabolism are being investigated. The cell membrane is indeed coming to be recognized as a most important crossroad for a great number of vital activities. How prostaglandins mediate this cell-to-cell communication remains yet to be explored, but to be sure, the role of prostaglandins with the formation of cyclic AMP is becoming increasingly important.
SUMMARY AND CONCLUSION

A. When prostaglandin $F_{2\alpha}$ was administered twice daily in 0.075 mg doses beginning at day 22 in immature female rats, the first significant observation was made on day 27 when the activity of P-one-3$\beta$-HSD in the ovaries was more than twice that present in control ovaries.

B. Premature sexual development as witnessed by early vaginal canalisations was produced by the administered $PGF_{2\alpha}$ and was observed by day 33 of the experiment. The previous high levels of progesterone biosynthesis undoubtedly played a role since early ovarian steroids are responsible for ovulation and puberty. The exact role of this prostaglandin was speculated as being directly responsible via end-organ response or indirectly responsible via pituitary gonadotropins.

C. By day 37, the ovarian progesterone biosynthesis was intensified again, but the manifestation revealed by the histochemical assessments could be ever so much more revealing when specific stages of estrous cycles could be pursued. Further investigations into the effects of prostaglandin and the different stages of estrus is therefore suggested, as an extension of these original observations.

D. No gravimetric or histologic differences were noted on the pituitary, thyroid and uterus as they pertained to $PGF_{2\alpha}$ administration. There were some minor weight variations but were not statistically significant.
E. Aspirin was used as a prostaglandin antagonist to determine if any contrasting data would be obtained as it pertains to PGF$_2\alpha$. The concentration of injectable aspirin was determined and as the experiment progressed, the body weights failed to keep up with the controls, and with the presence of rough hair coats, a status of aspirin toxicity was suspected. This has undoubtedly interfered with some of the reportable data obtained. The delay of sexual maturity was the most obvious contrasting phenomenon by the aspirin-administered rats. This delay could not be explained adequately by antagonizing prostaglandin because of the suspected toxicity present. This may prove a fruitful area for further research.

F. Both FSH and LH groups by day 27 had significantly larger body weights which were not in accord with pure FSH and LH responses. This complicated the other results observed in these groups ie. uterine responses and ovarian progesterone biosynthesis. Having a pure compound of FSH and LH would have enabled a reliable pattern of organ growth and development to which the prostaglandin group could be compared. Further experimental trials using such compounds in conjunction with and without prostaglandins would be revealing.

G. Thus, in sum, the hallmark noted effects of PGF$_2\alpha$ in these experiments are two in number: (a) PGF$_2\alpha$ induced early vaginal canalization, and (b) PGF$_2\alpha$ elicited comparatively high intensities of the progesterone precursor in the maturing ovary.
LITERATURE CITED


### VAGINAL OPENINGS AND THE GRAVIMETRIC COMPARISONS OF BODY, THYROID, PITUITARY AND UTERINE WEIGHTS OF THE NORMAL CONTROL GROUPS OF ALBINO RATS

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<th>Item</th>
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<th>Thyroid Wt.</th>
<th>Pituitary Wt.</th>
<th>Uterus Wt.</th>
<th>Vaginal Openings(c)</th>
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<td>50.0+7.6</td>
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### TABLE I (continued)

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*a* Body weights are shown in grams (g), organ weights in milligrams (mg).

*b* Arithmetic mean followed by the standard deviation.

*c* Figure before diagonal represents number of rats with vagina open at time of necropsy; figure after diagonal represents number of rats in age group.
<table>
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<th>Group</th>
<th>Item</th>
<th>Body Wt.</th>
<th>Thyroid Wt.</th>
<th>Pituitary Wt.</th>
<th>Uterus Wt.</th>
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aBody weights are shown in grams (g), organ weights are shown in milligrams per 100 grams body weight (mg%).

bAverage weight ± the standard deviation.

cFigure before diagonal represents number of rats with vagina open at time of necropsy; figure after diagonal represents number of rats in age group.
<table>
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<td>8.9±1.0</td>
<td>4.8±0.5</td>
<td>143.1±39.1</td>
<td>89.5-197.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>7.3-10.9</td>
<td>4.0-5.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| LH    | g, mg| 101±16.2 | 10.0        | 5.9          | 204.1      | 9/9            |
|       | range| 64-117   | 7.8-11.7    | 4.0-7.1      | 145.6-290.4|                 |
|       | mg% | 10.0±1.2 | 5.9±0.7     | 210.4±77.9   | 136.2-259.3|                 |
|       | range| 8.9-12.2 | 4.6-7.1     |              |            |                 |
|       | Probability | <0.001 |            |              | <0.001     | <0.001         |

aBody weights are shown in grams (g), organ weights are shown in milligrams per 100 grams body weight (mg%).

bAverage weight ± the standard deviation.

cFigure before diagonal represents number of rats with vagina open at time of necropsy; figure after diagonal represents number of rats in age group.
### TABLE IV

VAGINAL OPENINGS AND THE GRAVIMETRIC COMPARISONS OF BODY, THYROID AND UTERINE WEIGHTS OF 37 DAY OLD GROUPS TO CONTROL GROUPS OF ALBINO RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Item</th>
<th>Body Wt.</th>
<th>Thyroid Wt.</th>
<th>Pituitary Wt.</th>
<th>Uterus Wt.</th>
<th>Vaginal Openings&lt;sup&gt;(c)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>g, mg&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>122±13.9&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>9.7</td>
<td>5.2</td>
<td>117.4</td>
<td>10/10</td>
</tr>
<tr>
<td>Control</td>
<td>range</td>
<td>109-155</td>
<td>7.2-11.9</td>
<td>4.1-7.1</td>
<td>51.3-221.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg%</td>
<td>8.0±1.0</td>
<td>4.3±0.8</td>
<td>96.2±33.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>6.4-9.3</td>
<td>3.1-5.3</td>
<td>42.4-164.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>g, mg</td>
<td>120±15.6</td>
<td>10.0</td>
<td>5.2</td>
<td>107.9</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>88-139</td>
<td>8.0-13.6</td>
<td>4.2-6.1</td>
<td>62.8-143.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg%</td>
<td>8.6±1.9</td>
<td>4.3±0.7</td>
<td>80.3±29.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>5.8-11.3</td>
<td>3.3-5.8</td>
<td>53.7-132.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>g, mg</td>
<td>110±12.1</td>
<td>9.2</td>
<td>5.2</td>
<td>109.3</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>85-122</td>
<td>6.8-10.8</td>
<td>4.1-6.2</td>
<td>36.5-167.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg%</td>
<td>8.4±1.0</td>
<td>4.8±0.6</td>
<td>103.2±43.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>7.0-10.1</td>
<td>4.0-5.6</td>
<td>41.7-162.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Continued -
<table>
<thead>
<tr>
<th>Group</th>
<th>Item</th>
<th>Body Wt.</th>
<th>Thyroid Wt.</th>
<th>Pituitary Wt.</th>
<th>Uterus Wt.</th>
<th>Vaginal Openings</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>g, mg range</td>
<td>127±8.1</td>
<td>10.7</td>
<td>6.2</td>
<td>147.3</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>mg% range</td>
<td>115-142</td>
<td>8.5-12.0</td>
<td>5.0-7.0</td>
<td>113.3-207.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>8.6±1.4</td>
<td>4.9±0.4</td>
<td>&lt;0.02</td>
<td>117.0±28.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>6.9±1.5</td>
<td>4.2±0.7</td>
<td>&lt;0.02</td>
<td>79.8-163.9</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>g, mg range</td>
<td>126±9.3</td>
<td>10.4</td>
<td>6.7</td>
<td>161.2</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>mg% range</td>
<td>110-144</td>
<td>8.6-11.3</td>
<td>5.1-8.0</td>
<td>138.7-195.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>8.3±0.6</td>
<td>5.4±0.8</td>
<td>&lt;0.01</td>
<td>128.4±11.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td>7.6-9.3</td>
<td>3.9-6.2</td>
<td>&lt;0.01</td>
<td>105.9-147.4</td>
<td></td>
</tr>
</tbody>
</table>

aBody weights are shown in grams (g), organ weights are shown in milligrams per 100 grams body weight (mg%).

bAverage weight ± the standard deviation.

cFigure before diagonal represents number of rats with vagina open at time of necropsy; figure after diagonal represents number of rats in age group.
### TABLE V
SUMMARIZED DATA BASED ON SEMI-QUANTITATIVE HISTOCHEMICAL ANALYSIS OF PREGNENOLONE-3B-HSD ACTIVITY IN THE OVARIAS OF ALBINO RATS

<table>
<thead>
<tr>
<th>Age in Days</th>
<th>Control</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Aspirin</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>1.8±0.96(a)</td>
<td>4.0±1.41</td>
<td>2.3±1.26</td>
<td>3.2±1.58</td>
<td>2.3±1.09</td>
</tr>
<tr>
<td></td>
<td>1-3(b)</td>
<td>2-5</td>
<td>1-4</td>
<td>1-5</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>5(c)</td>
<td>4</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probability</th>
<th>&lt;0.05</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>33</th>
<th>3.0±0.82</th>
<th>2.3±1.26</th>
<th>3.0±0.82</th>
<th>2.3±1.5</th>
<th>3.0±0.82</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-4</td>
<td>1-4</td>
<td>2-4</td>
<td>1-4</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probability</th>
<th>&lt;0.005</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>37</th>
<th>3.0±0.71</th>
<th>4.2±0.84</th>
<th>2.5±0.58</th>
<th>1.4±0.56</th>
<th>3.0±0.71</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-4</td>
<td>3-5</td>
<td>2-3</td>
<td>1-2</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Probability</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td>&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Arithmetic average ± the standard deviation based on semi-quantitative histochemical estimates of pregnenolone-3B-HSD activity as observed on ovarian sections. Scale ranges from +1 to +5.

<sup>b</sup>Range

<sup>c</sup>Number of rats in group.
TEXT FIGURE I

GRAVIMETRIC COMPARISON OF THYROID GLANDS OF RATS BASED UPON THE WEIGHT OF THE ORGAN PER 100 GRAMS OF BODY WEIGHT (mg%)
### Text Figure II

**Gravimetric Comparisons of the Pituitary Glands of Rats Based on the Organ Weights Per 100 Grams of Body Weight (mg%)**

<table>
<thead>
<tr>
<th>Days of Age</th>
<th>Organ Wt. mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>7.0</td>
</tr>
<tr>
<td>27</td>
<td>6.0</td>
</tr>
<tr>
<td>33</td>
<td>5.0</td>
</tr>
<tr>
<td>37</td>
<td>4.0</td>
</tr>
</tbody>
</table>

- **Control** (○ ○ ○)
- **PGF₂α** (× × ×)
- **Aspirin** (△ △ △)
- **FSH** (● ● ●)
- **LH** (■ ■ ■)
TEXT FIGURE III
GRAVIMETRIC COMPARISONS OF THE UTERINE WEIGHTS OF RATS BASED UPON THE ORGAN WEIGHT PER 100 GRAMS OF BODY WEIGHT (mg%)

<table>
<thead>
<tr>
<th>Control</th>
<th>PGF2α</th>
<th>Aspirin</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
</table>

Organ Wt. mg%

Days of Age
TEXT FIGURE IV

SUMMARY OF DATA CONCERNING VAGINAL OPENINGS OF RATS DETERMINED AT TIME OF NECROPSY, BASED ON A PERCENTAGE OF VAGINAL OPENINGS

- Square: Common 0% and 100% points
- Circle: Control
- X: PGE₂
- Triangle: Aspirin
- Circle: FSH
- Square: LH

Per Cent

Age in Days

0 27 33 37
TEXT FIGURE V

SEMI-QUANTITATIVE COMPARISONS OF PREGNENOLONE-3B-HSD ACTIVITY IN THE OVARIES OF RATS BASED ON A RELATIVE SYSTEM OF +1 TO +5

Activity of Pregnenolone-3B-HSD in Ovaries

Age in Days

Control
PGF₂α
Aspirin
FSH
LH
Introductory Note to Plates

All photomicrographs are of cross sections of ovaries. Photomicrographs were taken using Polaroid type 107 film and a Wild microscope. All photomicrographs are at 100X magnifications.
Photomicrographs of rat ovarian sections, normal H&E is portrayed in Figure 1, the control is Figure 2 and Figure 3 was incubated with pregnenolone demonstrating the appearance of pregnenolone-3β-hydroxysteroid dehydrogenase (P-one-3β-HSD) in the form of precipitated deposits which localize the sites of the biosynthesis of progesterone. These photomicrographs were semi-quantitatively evaluated on a scale of 0 to +5 depending upon the amount of precipitate formed. All photographs are at 100X.

Fig. 1. Control ovarian section stained with H&E to demonstrate the appearance of the ovarian structure (100X).

Fig. 2. Control section of ovary incubated without pregnenolone. Represents a zero reaction (100X).

Fig. 3. Represents a +1 reaction. Very little diformazan deposits visible.
Plate II

Photomicrographs of rat ovarian sections which were incubated with pregnenolone demonstrating the appearance of pregnenolone-3β-hydroxy-steroid dehydrogenase (P-one-3β-HSD) in the form of precipitated deposits which localize the sites of the biosynthesis of progesterone. These photomicrographs were semi-quantitatively evaluated on a scale of 0 to +5 depending upon the amount of precipitate formed. All photographs are at 100X.

Fig. 4. Represents a +2 reaction. Few deposits are in evidence indicating slight P-one-3β-HSD activity.

Fig. 5. Represents a +3 reaction. More discrete deposits are visible.

Fig. 6. Represents a +4 reaction. Larger areas of more dense P-one-3β-HSD activity are visible.
Plate III

Photomicrographs of rat ovarian sections which were incubated with pregnenolone demonstrating the appearance of pregnenolone-3β-hydroxy-steroid dehydrogenase (P-one-3β-HSD) in the form of precipitated deposits which localize the sites of the biosynthesis of progesterone. These photomicrographs were semi-quantitatively evaluated on a scale of 0 to +5 depending on the amount of precipitate formed. All photographs are at 100X.

Fig. 7. Represents a +5 reaction. Confluent areas of dense deposits demonstrated indicating highest activity of P-one-3β-HSD present.
Plate IV

Photomicrographs of sections of ovaries from the non-injected control rats which were incubated with pregnenolone and nicotinamide adenine dinucleotide (NAD) to localize the activity sites of progesterone biosynthesis.

Fig. 8. Ovarian section from a 33 day old non-injected control rat, showing +2 P-one-3β-HSD activity in the interstitial tissue (100X).

Fig. 9. Ovarian section from a 37 day old non-injected control rat, showing +3 P-one-3β-HSD activity in the sub-capsular interstitial tissue.
Plate V

Photomicrographs of sections of ovaries from the PGF20< injected rats which were incubated with pregnenolone to localize the sites of progesterone biosynthesis.

Fig. 10. Ovarian section from a 27 day old PGF20< injected rat showing maximum 45 P-one-3B-HSD activity in the intesititial tissue and in the theca interna of the follicles (100X).
The thesis submitted by Farol N. Tomson has been read and approved by four members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

DATE May 18, 1973

Signature of Advisor