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Prostaglandin F2αAND Aspirin Effect on the Ovaries and Adrenal Glands of the Immature Female Rat

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PROSTAGLANDIN $F_{2\alpha}$ AND ASPIRIN EFFECT ON THE OVARIES AND ADRENAL GLANDS OF THE IMMATURE FEMALE RAT

By

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BIOGRAPHY

Martin A. Sidor was born in Chicago, Illinois on July 23, 1946. He attended Forest View High School in Arlington Heights, Illinois, and graduated in June of 1964. He entered the University of Illinois, in Urbana, Illinois, where he majored in veterinary medicine and graduated with a Bachelor of Science degree in June of 1968, and a Doctor of Veterinary Medicine degree in June of 1970.

In July of 1970, the writer was accepted into the Research in Laboratory Animal Medicine, Science and Technology program at the Hines Veterans Administration at Hines Hospital, Maywood, Illinois. While a resident investigator at Hines Hospital, he began his graduate study in September of 1971 in the Department of Anatomy at Loyola University Stritch School of Medicine, Maywood, Illinois.

ABSTRACT

A series of experiments were undertaken to observe the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), acetylsalicylic acid (aspirin), follicle stimulating hormone (FSH) and luteinizing hormone (LH) on estrogen biosynthesis in the ovary as revealed histochemically; also pursued were the histologic morphology of the ovary, and the gravimetric changes of the ovaries, adrenal glands and body weights of immature, female rats when given the aforementioned during the period of developing reproductive competency.

Five major groups of thirty 22 day old female rats (one control group and four treatment groups) were given subcutaneous injections, twice daily, of either 75 micrograms of $PGF_{2\alpha}$, five milligrams of aspirin, 0.25 Armour Unit of FSH or 0.125 Armour Standard Unit of LH. Sub-groups of ten rats in each major treatment category were necropsied at 27, 33 or 37 days of age. Body weights, ovarian weights and adrenal gland weights were recorded and statistically analyzed. Ovarian sections were prepared for histologic morphology, and for histochemical analysis, i.e., dehydroepiandrosterone-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity, which is indicative of the precursors of estrone and estradiol-17β biosynthesis in the ovary. DHA-3β-HSD activity in the ovary, and adrenal gland weights were used as indices of the metabolic activity in these endocrine organs.

The results indicated that systemic administration of $PGF_{2\alpha}$ or aspirin in the immature female rat caused a significant increase of steroidogenesis in the ovary and the adrenal gland, and that this steroidogenic effect decreases over time when these compounds are chronically administered. Steroidogenesis in the ovary and adrenal gland of the LH- or FSH-injected rats was consistently less than comparable control values. It was postulated that $PGF_{2\alpha}$ and aspirin, a specific prostaglandin antagonist, exert their effects at different levels of hormone action; i.e. at the pituitary level, or at the ovarian or adrenal gland level, to produce generalized steroidogenesis in the rat.

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INTRODUCTION

In recent years, prostaglandin compounds have attracted much research interest in their role as the second messenger system in hormone action. Their intimate relationships with various reproductive parameters is well documented. One current problem which is being investigated, and reported herein, is the primary site of prostaglandin action. Several investigators have been trying to determine if prostaglandins exert their modifying action at the pituitary level or at the level of an end organ. Recent studies, using the pituitary-ovarian axis, and using prostaglandin compounds and their antagonists, have indicated that prostaglandins may exert their effects at both the ovarian and pituitary level (Orczyk and Behrman, 1972; Kuehl, Humes, Tarnoff, Cirillo and Ham, 1970).

It should be pointed out that much of the research to date on prostaglandins and their relationships with reproductive function has been accomplished in _in vitro_ systems. _In vivo_ experimentation with prostaglandins has been limited to the short-term effects of prostaglandins. This situation has been partly due to expense and limited availability of prostaglandin compounds. Only recently have prostaglandin compounds been synthetically produced (Bagali, 1970; Pike, 1970). Also, _in vitro_ systems and short-term _in vivo_ experiments allow a rigid control of variables, thus increasing the validity of observed results. Research with prostaglandins is relatively new and such rigid experimental systems

have clearly established their importance in the biochemistry of a living system. Unfortunately, the aforementioned studies do not allow observations of the "dynamics" of prostaglandins. The true effects of any compound on the body is the sum total of its interactions with all other compounds, and in the end, must be evaluated from this standpoint in order to ascertain its true significance. For example, Horton and Marley (1969) found that long-term administration of PGE_1 increased fertility in mice as measured by litter size. Such a study illustrates the effect of a compound from the standpoint of total body interaction.

Another area of prostaglandin research which has been neglected is the response of the sexually immature animal to prostaglandin compounds. To date, most work has been accomplished on their effects in the mature or gravid female, owing chiefly to the fact that prostaglandin compounds have great potential in the area of fertility control, and as relatively safe and effective abortifacient compounds (Duncan and Pharriss, 1970).

A series of experiments was designed to investigate the effects of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on the sexually immature rat ovary and adrenal gland when given over a period of weeks during the critical stage of sexual maturation. Also, the effects of acetylsalicylic acid (aspirin), a specific prostaglandin antagonist, was studied. In addition, by concurrently dosing other rats with luteinizing hormone (LH) and follicle stimulating hormone (FSH), the similarities and differences of $PGF_{2\alpha}$ and

aspirin response as opposed to gonadotropin response on immature rats was compared. End-points for comparison were gravimetric data of the ovary and adrenal gland, and histologic morphology and enzyme histochemistry (dehydroepiandrosterone-3β-hydroxysteroid dehydrogenase) data on the ovary.

REVIEW OF THE LITERATURE

A group of biologically active compounds, known as prostaglandins, were first alluded to in 1913 when Battez and Boulet found that extracts of human prostate had strong depressor action on blood pressure when injected into the dog. The true significance of their findings was not recognized. Lacking sophisticated techniques for isolation and characterization of biological compounds, this was not considered a startling finding. Extracts of just about any organ would elicit the same response due to the ubiquitous occurrence of such compounds as acetylcholine and histamine.

In 1930, two New York gynecologists, Kurzrok and Lieb, reported the actions of human seminal fluid on isolated human uterine muscle strips. They observed that seminal fluid produced both relaxation and contraction of uterine smooth muscle, and that this action was in some way correlated with past histories of pregnancy or sterility. This particular study is considered to be the first indication of differentiated, biologically active compounds in semen. Unfortunately, these actions were again attributed to acetylcholine, and no further work was done to characterize the active principle.

Approximately four years later, two independent researchers co-discovered the existence of biologically active substances in seminal fluid that were pharmacologically different from known compounds previously described. Goldblatt (1933), from England, described a depressor sub-

stance in seminal fluid, but unfortunately reported his discovery, as a brief abstract, in the journal Chemistry and Industry, little read by biologists of that time. Without knowledge of Goldblatt's work, von Euler in Sweden, in 1934, reported essentially the same findings as Goldblatt, working with human seminal fluid. Subsequently, Goldblatt (1935) continued his studies and described a variety of actions of seminal fluid, including the finding that seminal fluid sensitized the seminal vesicle of the guinea pig to adrenaline. This furthered the belief that the active principle was a new, but as yet, uncharacterized, biological compound.

Dr. von Euler in 1935, with the help of Hugo T. Theorell, a chemist at the Karolinska Institute, used electrophoresis to separate the different fractions of seminal fluid and was able to characterize the active principle as an acidic lipid. Since the compound occurred in extracts of the prostate and seminal vesicles, and was a new and previously unknown chemical, the name prostaglandin was coined by von Euler. To quote Professor von Euler, "The ether and water soluble substance, which has the effects of lowering blood pressure and stimulating various isolated smooth muscle organs, is provisionally named prostaglandin" (von Euler, 1935).

Shortly after publishing his second paper in 1935, Dr. Goldblatt died. Thus, all research on prostaglandins, for the next decade, was conducted in von Euler's laboratory at the Karolinska Institute. Dr. von Euler, in 1936, was able to show that the acidic lipid was hydroxylated, and in 1939, concluded that the active principle was a lipid soluble, fatty acid that

probably contained a double bond and a hydroxyl group.

Unfortunately, the Second World War halted any serious attempts at further elucidating the chemistry and physiology of prostaglandin. After the war, von Euler was preoccupied with his recent discovery of norepinephrine and passed on his work to Dr. Bergstrom, a physician in biochemistry at the Karolinska Institute. Dr. Bergstrom, in 1949, reported that further purification and characterization of sheep vesicular gland extracts showed that, indeed, the active principle was composed of unsaturated hydroxy acids.

It was another seven years before any further work on prostaglandin, then considered a single chemical entity, was attempted. This circumstance was primarily the result of inadequate technology in the early 1960's needed to isolate and identify minute amounts of unstable compounds. Another factor was that the intestinal smooth muscle test used to assay their activity was so unspecific that many scientific people did not believe that prostaglandins were a new class of biologically active compounds.

In 1956, an intensive investigation, led by Dr. Bergstrom, was initiated. Frozen sheep vesicular glands were collected in Iceland, Greenland and Norway. Eventually, enough active compound was extracted to obtain prostaglandin factor in pure crystalline form. This 'Prostaglandin Factor' was termed PGF. Bergstrom and Sjovall (1957), his co-worker, also reported that at least one other active acidic factor was present in the extracts of sheep vesicular glands.

In the next few years, further investigation by Bergstrom and his co-workers (1962), using new isotopic, mass spectrometric, and chromatographic techniques, finally resulted in the isolation and elucidation of the structures of three of the primary prostaglandins, today known as Prostaglandin E, Prostaglandin $F_{2\alpha}$ and Prostaglandin $F_{2\beta}$. But even at this point, further biological testing was at a disadvantage since prostaglandin compounds could not be produced efficiently in adequate quantities. Finally, in 1964-65, investigators at Karolinska Institute, Sweden, Unilever Research Laboratories in the Netherlands, and at the Upjohn Company in Kalamazoo, Michigan, (U.S.A.), independently discovered how to synthesize prostaglandins enzymatically by incubating essential fatty acid precursors with sheep vesicular glands (von Dorp, Beerthuis, Nugteren and Vonkeman, 1964; Bagali, 1970; Bergstrom, Ryhage, Samuelsson and Sjovall, 1964). Finally, total synthesis of prostaglandins and their analogues was accomplished (Bagali, 1970; Pike, 1970).

To highlight a few of the recent advances in prostaglandin research, one of the most startling findings is the ubiquitous occurrence of these compounds. Although first isolated from seminal fluid and extracts of male accessory sex glands, physiologic amounts have been recognized in female sex organs, lung tissue, iris, the central and peripheral nervous system, liver, pancreas, intestine, skeletal muscle and kidney (von Euler and Eliasson, 1967). In fact, they are probably found in physiologic

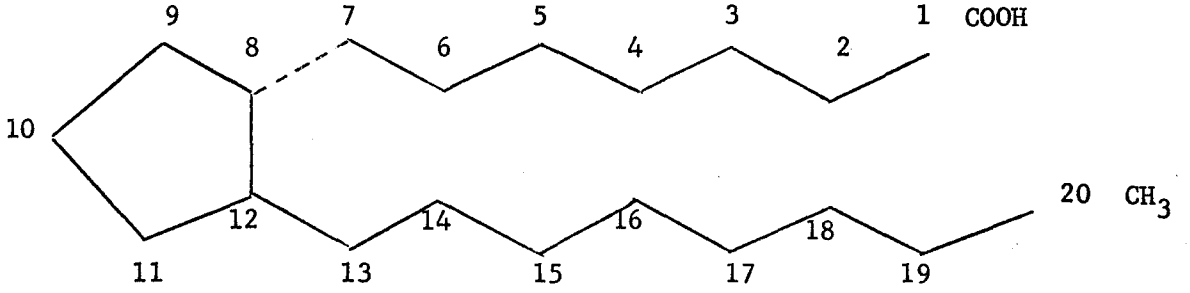
amounts in numerous tissues of the body. It has recently been recognized that prostaglandins are not strictly a mammalian compound. Prostaglandins have been identified in the skin and spinal cord of the frog, and more surprising, in living coral from the sea.

Mysterious substances isolated in the past have recently been identified as prostaglandins. For example, "irin", first reported by Dr. N. Ambache in 1957; "darmstoff", described in 1949 by Dr. W. Vogt as being a biologically active substance produced in the intestines; Dr. V.R. Pickles' (1957) "menstrual stimulant", which is reseased by the endometrium during menstruation; and "medullin", isolated from rabbit kidney in 1965 by Dr. James Lee, have all been recognized as prostaglandins (von Euler and Eliasson, 1967). There is recent evidence that a prostaglandin may be the "long-searched-for" mediator of the inflammatory response (Donovan and Traczyk, 1962).

The biggest reason for the "explosion" of prostaglandin research and interest in these chemicals by scientists in universities, clinics and in pharmaceutical companies around the world is their potential therapeutic usage. Already the medical uses of prostaglandins include induction of labor, therapeutic abortion, contraception, treatment of dysmenorrhea, male sterility, prevention and therapy of peptic ulcer and thrombosis, control of hypertension, bronchodilation in asthma and nasal congestion. Another broad field of investigation in pharmaceutical companies is prostaglandin antagonists which may have widespread medical

usage. At the 1970 New York Academy of Sciences Conference on Prostaglandins, a paper on SC-19220, a dilienzo-x-azepine hydrazide derivative, a specific inhibitor of $PGF_{2\alpha}$ was presented by Sanner (published in 1971). Possible therapeutic uses of prostaglandin inhibitors include inflammation, premature labor and dysmenorrhea.

At the present time, there are 14 naturally occurring prostaglandin compounds, 13 of which have been found in man. Prostaglandins are abbreviated as PG and naming of the different PG's is based on a hypothetical molecule, prostanoic acid.



Prostanoic Acid

From the above structure, the chemical nature of prostaglandins are C-20 fatty acids containing a cyclopentane ring (Bergstrom, Ryhage, Samuelsson and Sjovall, 1962). The different structures of the cyclopentane ring subdivide the prostaglandins into four major groups: PGE, PGF, PGA and PGB.

The degree of unsaturation of the alkyl and carboxylic side chains is denoted by a subscript numeral after the group designation. Thus, subscript 1 denotes a double bond between C-13, 14; subscript 2 denotes two double

bonds between C-13, 14 and C-5, 6; and subscript 3 indicates three double bonds which include the two already mentioned and a third in the C-17, 18 position.

A further classification using subscript alpha or beta denotes the stereo position of substituents on the cyclopentane ring. If substituents are on the same side of the ring as the carboxyl group, they are in the alpha position; and those on the same side as the alkyl group are in the beta position (Anderson, 1971).

The biosynthesis of the natural prostaglandins involves the precursors C-20 essential fatty acids. These essential fatty acids undergo cyclization and introduction of molecular oxygen in the enzyme microsomal fractions of the cell. The primary precursors are Eicosartrinoic Acid (linolenic acid), Eicosatetraenoic Acid (arachidonic acid) and Eicosapentaenoic acid (Bergstrom, Carlson and Weeks, 1968). These three precursor fatty acids form the six primary prostaglandin compounds (PGE_1, PGE_2, PGE_3, $PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGF_{3\alpha}$). Thus, all the primary prostaglandins are in the PGE and PGF groups. All six are designated as primary because none are a precursor of the others. By a series of enzymatic steps the other known naturally occurring prostaglandins (PGA_1, PGA_2, PGB_1, PGB_2, and their 19-hydroxy analogues) are derived from the six primary prostaglandins (Speroff and Ramwell, 1970).

The mechanisms of action of the prostaglandins is, at this point, a matter of controversy. Pharmacologically, the prostaglandins mimic actions

of hormones and neuro-hormones, modify hormonal actions, or inhibit the hormonal actions depending on the tissue and specific prostaglandin studied. It is known that the PG's are biologically active in extremely small amounts. Dosage is measured in nanograms of prostaglandin per gram tissue.

The theory which is backed up by the most scientific data is that prostaglandins exert their effect by either inhibiting or increasing the rate of cyclic AMP metabolism intracellularly. It is also thought that hormones have this same mechanism of action of exerting their effect through cyclic AMP and adenyl cyclase systems; but prostaglandins, unlike the hormone concept, display an extraordinary lack of tissue specificity and modify cyclic AMP levels in many tissues (Butcher and Baird, 1968). Recent evidence suggests that prostaglandins probably mediate their action on cyclic AMP formation by modifying calcium levels within the cell (Shio, Shaw and Ramwell, 1971).

The need for an intact cell membrane for PG effects to be exerted, and the fact that prostaglandins bind to plasma proteins and cell membranes in a manner analagous to that of free fatty acids, suggest that possibly the site of action is in the plasma membrane itself (Shio, Shaw and Ramwell, 1971). Another avenue of prostaglandin research in the area of action mechanisms is the effects of prostaglandins in modifying cyclic AMP control of RNA, and possibly DNA. Previous evidence shows that exogenous cyclic AMP modifies RNA and protein synthesis (Postan and Perlman, 1970).

The implication toward cancer research is obvious.

The mechanism of prostaglandin release is another area which is receiving considerable interest. Studies have shown that mammalian cells apparently will secrete prostaglandin with a variety of mild, to severe, but non-specific stimuli. Examples include release of PGE_1 into splenic venous blood of the dog when the splenic nerve was stimulated (Davis, Horton and Withrington, 1968); PGE_2 and $PGF_{2\alpha}$ release in lung tissues during anaphylaxis in guinea pigs (Piper and Vane, 1969); release of PG's by the mere infusion of colloidal suspensions into the dog isolated spleen (Gilmore, Vane and Wyllie, 1969). One important feature of prostaglandin release has definitely been established: the sequence is prostaglandin biosynthesis followed by release, rather than prostaglandin storage followed by release. Lungs, adrenal glands, stomach, intestine and spleen have all been shown to release more prostaglandin when stimulated than they contained (Ramwell and Shaw, 1970). The theory relating to the specific mechanisms of prostaglandin release which is favored today was first advanced by Bennett in 1967, who proposed that PG release, in general, was associated with distortion or activation of cell membranes (Bennett, Friedman and Vane, 1967).

Piper and Vane (1971) have worked extensively on this problem and have forwarded two possibilities which suggest the local hormonal action of prostaglandins and their function as a basic "defense mechanism" of the body. The first theory examines prostaglandin release as a function of

the cellular need to resist change. The example of smooth muscle stretching correlated with an active increase in tension is given. They feel this physiological response is mediated by local prostaglandin release which prevents over-distention and rupture or distortion of muscle fibers. Another example related to the fact that PGE_2 inhibits noradrenaline release in the spleen, and that PGE_2 is released in the spleen following splenic contraction mediated via nerve stimulation with noradrenaline release. Thus, a negative feedback mechanism exists whereby the muscle tissue can reduce a stimulus that causes its contraction. Both examples, according to Piper and Vane, 1971, show that prostaglandins can both reduce the stimulus and minimize the potential injury. The other theory suggests that prostaglandin release facilitates tissue adaptation to disturbing stimuli. For example, smooth muscle storage organs, such as the bladder and stomach, will stretch without an increase in the muscle cell tension. Prostaglandins have a relaxing effect on these tissues.

Prostaglandin Action and the Ovary

The highest concentration and greatest number of different prostaglandins have been found in human seminal fluid. Indeed, the discovery of prostaglandins now known to be seemingly ubiquitous in the body were first isolated and identified in human seminal fluid. Since that time, pharmacologically active prostaglandins have been demonstrated in menstrual fluid, umbilical cord, amniotic fluid, decidua, proliferative and secretory

(progravid) endometrium, semen and vesicular glands (Speroff and Ramwell, 1970). Since they occur in all reproductive tissues, and were first discovered in secretions of the male reproductive tract, most investigation of prostaglandins has been in conjunction with their role in reproductive physiology.

Research on prostaglandin and the ovary have centered around three central theses. One is the role of prostaglandin in promoting luteolysis. Another area of research concerns the relationships, if any, between cyclic AMP, gonadotropin and prostaglandins. Finally, steroidogenesis of different hormones and prostaglandins has been investigated.

It has been known for several years that hysterectomy in different pseudo-pregnant laboratory species will result in a prolonged life-span of the corpus luteum. Thus, it was speculated that a substance, luteolysin, was produced in the uterus and caused this luteolytic effect. Current research into the problem has resulted in three candidates for "luteolysin", $PGF_{2\alpha}$, $PGF_{1\alpha}$, and PGE_1, which have been found to cause irreversible destruction of luteal tissue, in vivo, in the guinea pig (Blatchley and Donovan, 1969; Gutknecht, Cornette and Pharriss, 1970), rabbit, rat and hamster (Gutknecht, Pharriss and Wyngarden, 1971), and rhesus monkey (Kirton, Pharriss and Forbes, 1970).

Pharriss, of Alza Corporation in Palo Alto, California, has summarized his and other's investigations of this problem (Pharriss, 1971). He summarizes five theories of possible mechanisms of $PGF_{2\alpha}$ in luteolysis: 1)

direct feedback on the pituitary gland; 2) "anti-gonadotropic" effect; 3) stimulation of uterus to produce luteolysin; 4) direct toxicity on the corpus luteum; and 5) constriction of the utero-ovarian vein. The most plausible mechanisms seem to be gonadotropin antagonism and restriction of ovarian blood flow. It is known that $PGF_{2\alpha}$, injected IV, will reduce utero-ovarian venous blood flow without affecting flow in the kidney (Pharriss, 1971). It is further speculated that, although the mechanism of luteolysis is, as yet, unclear, it is known that prostaglandins cause pregnancy termination in laboratory animals, and that this is probably a result of luteolysis.

Investigation into steroidogenesis of ovarian hormones and prostaglandins has resulted in conflicting information in light of the in vivo luteolytic role of $PGF_{2\alpha}$. Several scientists have shown that in vitro incubation of luteal slices with $PGF_{2\alpha}$ causes a stimulation of progesterone synthesis (Pharriss, Wyngarden and Gutknecht, 1968; Speroff and Ramwell, 1970). It may be that in vivo luteolytic characteristics of PG's exert a chronic action on local hemodynamics while in vitro PG's exert cellular changes. It is known that steroidogenesis is promoted in the adrenal gland perfused with PGE_1, PGE_2, $PGF_{1\alpha}$, (Flock, Jessup and Ramwell, 1969). Also, urinary cortisol levels are increased when $PGF_{2\alpha}$ is administered intravenously in man (Wentz, Jones and Bledsoe, 1973).

The mechanism of prostaglandin mediation of cyclic AMP, and the relationship between cyclic AMP and hormonal actions has been previously

described. This cyclic AMP-prostaglandin relationship may very well be the underlying mechanism in all observed prostaglandin actions involving the ovary. It has been shown that the effect of LH on steroidogenesis is mediated via cyclic AMP (Marsh, 1968). Recent papers on prostaglandins and the ovary have established a relationship between luteolysis and ovulation and $PGF_{2\alpha}$ in the utero-ovarian blood of guinea pigs pre-treated with estrogen (Blatchley, Donovan, Poyser, Horton, Thompson and Los, 1971).

Prostaglandin interaction and interrelationships with gonadotropins has also proven to be a fruitful area of investigation. Significant in these investigations is the work by Pharriss (1971). Using immature rats, in vivo experiments showed that $PGF_{2\alpha}$ inhibited the effects of PMS and HCG by decreasing ovarian weight, and ovulation. Labhsetwar (1971) in studying the in vivo effects of $PGF_{2\alpha}$ in the adult female rat found a luteolytic activity but could not pinpoint the location of primary prostaglandin activity (i.e., central or local). Horton and Marley (1969), using mice as the experimental animal, found that long-term administration of PGE_1 increased fertility as measured by litter size.

Orczyk and Behrman (1972) found that administration of the prostaglandin antagonists, aspirin and indomethacin, blocked ovulation in the rat and postulated that prostaglandins play a functional role in regulating the release of LH necessary for ovulation in the rat. Luehl, Humes, Tarnoff, Cirillo and Ham (1970), using the prostaglandin antagonist prostynoic acid, and $PGF_{2\alpha}$ and LH, postulated that prostaglandin compounds exert

their effect at both the pituitary and ovarian level. A study by Behrman, Orczyk and Greep (1972) determined that the effects of aspirin and indomethacin, both antagonists of prostaglandin compounds, accomplish their biopharmacologic effects at different levels of hormone action. Aspirin blockage of ovulation was reversed by LH administration, but the ovulation blockage by indomethacin could not be reversed by either LH or gonadotropin releasing hormone.

It should be emphasized that very little work has been accomplished on the effect of prostaglandins, or their antagonists, on the reproductive maturation of an immature animal. In view of this, experimentation was instituted to observe the effects of $PGF_{2\alpha}$ and acetylsalicylic acid, a prostaglandin antagonist, on the immature rat ovary and adrenal gland when given over a prolonged period of time.

MATERIALS AND METHODS

I. Animals and Housing

A total of 190 immature (21 day old), Sprague-Dawley derived female rats (Locke-Erickson Laboratories, Inc., Maywood, Illinois) were separated into nineteen experimental groups. Rats were housed five to a unit in clear plastic "shoe-box" type caging on San-i-cel bedding (Paxton Laboratories, Paxton, Illinois). Caging was cleaned and sterilized once weekly. Feed and water were given ad libitum. Feed was a standard laboratory rat diet (Purina Lab Chow). They were all housed in the same room which was environmentally controlled, i.e. temperature: 72° F. ± two degrees, constant humidity 53% ± 5%, 12 hour light-dark cycle.

II. Treatment Categories

Five different treatment categories were defined in this experiment on the basis of the type of drug or hormone given. All drugs or hormones were delivered subcutaneously using a 1.00 cc tuberculin syringe and a 26 gauge needle. All drugs and hormones were given twice daily (BID), one injection at 8 a.m. and the other at 4 p.m.

A. Controls. Two major groups of controls were designated. One group of controls was injected with 0.25 cc of 10% ethanol. This group was necessary to establish any variable that may be introduced using alcohol as a delivery vehicle for acetylsalicylic acid in another test group. Another control group was not injected with any substance and was compared

to the alcohol controls and other treatment groups. One subgroup of 10 non-injected controls was necropsied at 22 days in order to establish base-line values. Subgroups of ten rats from the non-injected control group and ten rats from the alcohol-injected control group were necropsied on days 27, 33 and 37, giving a total of 70 rats.

B. The prostaglandin group was divided into three subgroups of ten rats. All were injected with 0.075 mg of $PGF_{2\alpha}$ twice daily (0.075 cc) from day 22 through the day prior to necropsy. The three subgroups were necropsied on days 27, 33 and 37, respectively. The prostaglandin compound was delivered frozen at a concentration of 10 mg/ml in phosphate buffer (G.D. Searle Company, Skokie, Illinois). This was diluted down to a concentration of 0.1 mg/ml with phosphate buffer solution at a pH of 7; and then divided into 1.0 cc aliquots and frozen at -60° C. until use (Karim, Devlin and Hillier, 1968). Previous work by Labhsetwar (1972) has established that 75 micrograms BID, given subcutaneously is a physiologic dose in the rat. A total of thirty rats was assigned to this group.

C. Another treatment group was given acetylsalicylic acid (aspirin) which was put into solution with 10% ethanol and injected at a dosage of 5.0 mg twice daily (0.25 ml, BID). This dosage level is active in the rat (Behrman, Orczyk and Greep, 1972). As in the prostaglandin group, three subgroups of ten rats each were necropsied on days 27, 33 and 37, respectively, and received injections of aspirin from day 22 until the day necropsied, thus giving a total of thirty rats.

D. The LH (Luteinizing Hormone) (Schwarz-Mann, Orangeburg, New York) injected rats were given 0.125 Armour Unit (LH-ovine, Armour 277-80, equivalent to N.I.H. LH S-1) (Riddle, Bates and Dykshorn, 1933) of LH twice daily from day 22 until the day of necropsy. Three subgroups of ten rats were sacrificed on days 27, 33 and 37, respectively, for a total of thirty rats in this major treatment category. The LH was received lyophilized and was reconstituted with physiologic saline to deliver a dose in 0.05 cc.

E. The FSH (Follicle Stimulating Hormone) (Schwarz-Mann, Orangeburg, New York) was also received lyophilized and reconstituted with physiologic saline and injected at a dosage of 0.25 Armour Standard Unit (FSH-porcine, Armour 264-151X, equivalent to 0.5 N.I.H. FSH S-1) (Steelman and Pohley, 1953) twice daily (0.05 cc, BID). In this treatment category, also, three subgroups of ten rats were necropsied on days 27, 33 and 37 (i.e. a total of thirty rats), and were on a treatment schedule identical to the LH group.

The age of the experimental rats (21 to 37 days of age) was chosen as an appropriate interval to study the effects of these compounds on the immature female rat during the period of vaginal canalization and the development of reproductive competence (Kasprow, 1969).

III. General Procedures

After completion of the different dosage regimes, all rats were

necropsied after recording body weight to the nearest gram on a direct reading torsion balance and euthanizing by ether anesthesia followed by decapitation. The ovary and adrenal glands were dissected free from adhering fat and other tissue and weighed to the nearest 0.2 mg on a Roller-Smith type torsion balance. After weighing, the ovaries and adrenals were either fixed in neutral formalin for histological study or quick frozen in liquid nitrogen and stored at -60° C. for histochemical analysis. In each subgroup of ten animals, the ovaries and adrenal glands of five rats were formalin-fixed; the ovaries and adrenal glands from the other five rats were frozen.

The formalin-fixed adrenal glands and ovaries were then washed, dehydrated in alcohol, cleared with xylene and embedded in paraffin. The adrenal glands and ovaries were cut at six microns and stained with Harris hematoxylin and eosin for histological examination. Photomicrographs were taken using Polaroid type 107 film and a Wild microscope. Both 40X and 100X magnification photomicrographs were made of each ovary for comparison purposes.

The ovaries that were frozen were sectioned at eight microns and incubated for 3¼ hours in a media containing 1.0 ml of 2.0 (10^{-4}) $\bar{M}$ dehydroepiandrosterone (DHA), 1.0 ml propylene glycol, 1.0 ml of 5.0 (10^{-4}) $\bar{M}$ nicotinamide-adenine dinucleotide (NAD) and 1.0 ml of 1.6 (10^{-4}) $\bar{M}$ nitro blue tetrazolium chloride (Nitro-BT), all in 4.0 ml of phosphate buffer

solution (pH-7.4). A set of companion ovarian sections were incubated in media without DHA and served as control groups. Polaroid photomicrographs of the incubated sections were used to measure semi-quantitative reactions. This particular technique allows the localization and semi-quantification of the enzyme dehydroepiandrosterone-3B-hydroxysteroid dehydrogenase (DHA-3β-HSD), which is indicative of active estrogen biosynthesis (Kalvert and Bloch, 1968). The reaction was rated on a scale of 0 to +3, depending on the degree of monoformazan or diformazan precipitate deposited on ovarian tissue sections. Plate I illustrates the semi-quantitate scale used.

EXPERIMENTAL RESULTS

A. Control Female Rats

In an effort to provide some rigid base-lines for rigid comparisons, a number of controls were studied *in extenso*, thus providing highly standardized bases for comparison, *i.e.*, controls at 22, 27, 33 and 37 days of age, *cf.* Tables I and V, and accompanying photomicrographic documentation.

Body weights, ovarian weights and adrenal gland weights of the non-injected controls and the alcohol-injected (vehicle for aspirin) controls were essentially identical (Tables I-IV). Adrenal gland weights, when compared on a milligrams per 100 grams body weight basis, showed a marked linear decrease from day 22 to day 33, of approximately 40% (from 20.5 mg% to 12.7 mg%),* and then plateaued (Table I and Text Figure 2). This weight decrease of adrenal glands in immature rats, before vaginal opening is consistent with the findings of Kasprow (1969).

Semi-quantitative histochemical estimates of average dehydroepiandrosterone-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity in ovarian sections of the non-injected control groups revealed little DHA-3β-HSD activity in the 27 and 33 day old groups. DHA-3β-HSD activity in the 37 day old non-injected controls was significantly greater (approximately 33% greater) and more variable ($+2.00 \pm 0.71$), ** than the 27 day old and 33 day

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* All organ weights are expressed in milligrams per 100 grams body weight (mg%), unless otherwise indicated.

** Average and standard deviation from the mean. Represents average of estimates derived from the follicular, luteal and interstitial components of the ovaries.

TABLE I

GRAVIMETRIC DATA ON THE BODY, OVARIES AND ADRENAL GLANDS OF THE NON-INJECTED CONTROL GROUPS OF 22, 27, 33 AND 37 DAYS OF AGE

Age in Days	n(a)	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
22	10	g, mg(b)	41 ± 5.3(c)	15.4	8.5
		range	36-51	10.3-18.9	5.5-11.6
		mg%(d)		37.6 ± 4.3	20.5 ± 4.9
		range		28.6-42.2	14 8-26.6
27	10	g, mg	59 ± 7.5	22.7	9.4
		range	51-73	16.8-30.6	8.3-13.1
		mg%		38.2 ± 5.1	15.7 ± 1.9
		range		32.2-47.8	13.4-18.5
33	9	g, mg	92 ± 6.4	26.0	11.8
		range	84-102	20.9-33.6	8.0-15.3
		mg%		28.2 ± 5.0	12.7 ± 2.4
		range		22.7-38.0	8.7-15.2
37	10	g, mg	123 ± 12.3	35.1	16.3
		range	111-149	23.5-57.2	11.7-23.0
		mg%		28.3 ± 7.0	13.3 ± 1.9
		range		19.8-40.9	9.9-15.0

a Number of rats in group.

b Body weights expressed in grams, organ weights are combined weights of both ovaries/adrenal glands expressed in milligrams. Data represents average values

c Standard deviation from the arithmetic mean.

d Organ weights expressed in milligrams per 100 grams body weight.

old subgroups (+1.50 ± 0.55 and +1.40 ± 0.55, respectively; cf. Table V, Text Figure 3 and Plate II, figures 5 and 6). It should be noted that values of DHA-3β-HSD activity represent averages of estimates or enzyme activity derived from the follicular, luteal and interstitial tissue components of the ovaries (Kovarik, 1972).

B. Prostaglandin $F_{2\alpha}$ -Injected Female Rats.

Body weights and ovarian weights of the Prostaglandin $F_{2\alpha}$ -injected rats showed no significant variation from the weights of the 27, 33 and 37 day old non-injected controls. Average ovarian weights, on a mg% basis, were 37.3 at 27 days, 29.9 at 33 days and 26.0 at 37 days (Tables II-IV and Text Figure 1). Adrenal gland weights were significantly greater than control values in the 27 and 33 day subgroups ($P = <0.01$ and <0.10, respectively;*** cf. Tables II-IV and Text Figure 2). In the 27 day old $PGF_{2\alpha}$ subgroup, there was an approximate 33% increase in average weight of the adrenal glands over control values. The average weight of the adrenal glands at 27 days of age was 19.0 mg% in the $PGF_{2\alpha}$ -injected rats compared to 15.7 mg% average adrenal gland weight in the 27 day old non-injected control rats. At 37 days of age, the average adrenal gland weight when compared to controls was not significantly different ($P = <0.20$).

Average DHA-3β-HSD, as estimated semi-quantitatively in ovarian sections, revealed elevations and patterns similar to the changes in average

*** P = probability of differences when compared to the non-injected control values ("student's" t-distribution).

TABLE II

GENERALIZED AND SUMMARIZED GRAVIMETRIC DATA ON THE BODY, OVARIES AND ADRENAL GLANDS OF THE 27 DAY OLD TREATMENT GROUP

Treatment	n (a)	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
I. Alcohol (0.25 cc of 10% ethanol, BID)	10	g, mg (b)	60 ± 7.6 (c)	22.4	9.4
		range	50 - 71	17.1-31.0	7.0-12.9
		mg% (d)		37.5 ± 5.0	15.7±2.0
		range		31.1 - 46.3	13.2 - 18.6
		P (e)			
II. Prostaglandin $F_{2\alpha}$ (75 micrograms, BID)	10	g, mg	55 ± 5.5	20.7	10.5
		range	49 - 69	16.9 - 28.7	9.2 - 14.3
		mg%		37.2 ± 4.4	19.0 ± 1.1
		range		16.9 - 28.7	16.4 - 20.7
		P	<0.20		<0.001
III. Aspirin (5.0 milligrams, BID)	8	g, mg	54 ± 4.5	20.9	10.0
		range	47 - 62	16.2 - 24.0	8.7 - 12.3
		mg%		38.9 ± 4.8	18.9 ± 2.7
		range		29.5 - 45.6	15.5 - 22.4
		P	< 0.20		<0.01

- Contuned -

TABLE II (continued)

Treatment	n	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
IV. Follicle Stimulating Hormone (0.025 Armour Unit, BID)	10	g, mg	68 ± 8.1	26.9	9.4
		range	56 - 84	20.9 - 41.7	7.8 - 11.4
		mg %		39.0 ± 8.6	13.8 ± 1.8
		range		30.8 - 57.9	11.1 - 16.0
		P	<0.02		<0.05
V. Luteinizing Hormone (0.125 Armour Standard Unit, BID)	10	g, mg	65 ± 3.6	32.0	9.6
		range	60 - 71	16.5 - 41.8	8.0 - 11.3
		mg%		48.8 ± 11.2	14.8 ± 1.9
		range		27.5 - 64.3	11.3 - 16.9
		P	<0.01	<0.02	

[a]Number of rats in group.

[b]Body weights expressed in grams, organ weights are combined weights of both ovaries/adrenal glands expressed in milligrams. Data represents average values.

[c]Standard deviation from the arthmetic mean.

[d]Organ weights expressed in milligrams per 100 grams body weight.

[e]Probability of differences of average body weights in grams or average ovarian/adrenal gland weights in mg% when compared to non-injected control values ("student's t-distribution). Probabilities of <0.20 or less reported.

weights of adrenal glands in the $PGF_{2\alpha}$ subgroups at comparable days of age (Table V). There was high DHA-3β-HSD activity at day 27 ($P=<0.05$) and at day 33 ($P=<0.10$) when compared to control values; but at day 37, enzyme activity was similar to control values (Table V and Plate III, Figures 7 and 8). Average semi-quantitative estimates of enzyme activity were +2.40 $\pm$ 0.55 at 27 days of age, +2.20 $\pm$ 0.80 at 33 days of age and +1.75 $\pm$ 0.49 at 37 days of age for the $PGF_{2\alpha}$ subgroups.

Morphologic examination of histologic sections revealed corpus luteal formation at day 37 in the $PGF_{2\alpha}$ treatment category. This finding is inconsistent with the short-term effects of $PGF_{2\alpha}$ administration, i.e., $PGF_{2\alpha}$ will cause irreversible destruction of luteal tissue in vivo (Blatchley and Donovan, 1969).

C. Aspirin-Injected Female Rats.

There was a marked similarity between the effects of acetylsalicylic acid (aspirin) and $PGF_{2\alpha}$ on the ovarian weights, adrenal gland weights and average DHA-3β-HSD activity in the ovaries. Adrenal gland weights were increased significantly at day 27 over control averages ($P=<0.01$). Average adrenal gland weight was 18.9 mg% in the 27 day aspirin subgroup compared to 15.7 mg% in the 27 day non-injected control subgroup (Tables I and II, and Text Figure 2). At days 33 and 37, adrenal gland weights were comparable to those of the controls. Average adrenal gland weight was 13.9 mg% at both 33 and 37 days of age in the aspirin-injected rats, compared to

TABLE III

GENERALIZED AND SUMMARIZED GRAVIMETRIC DATA ON THE BODY, OVARIES AND ADRENAL GLANDS OF THE 33 DAY OLD TREATMENT GROUP

Treatment	n[a]	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
I. Alcohol (0.25 cc of 10% ethanol, BID)	10	g, mg[b]	93 ± 7.4[c]	26.2	11.6
		range	80 - 105	19.3 - 33.1	8.2 - 15.1
		mg%[d]		28.5 ± 6.3	12.5 ± 2.2
		range		20.8 - 41.4	8.8 - 15.6
		P[e]			
II. Prostaglandin $F_2\alpha$ (75 micrograms BID)	10	g, mg	90 ± 11.2	26.5	13.2
		range	76-103	19.5	9.8 - 19.6
		mg%		29.9 ± 8.8	14.7 ± 1.9
		range		22.1 - 49.1	12.6 - 18.0
		P			<0.10
III. Aspirin (5.0 milligrams BID)	7	g, mg	89 ± 12.7	25.6	12.4
		range	63-98	20.3 - 45.0	10.6 - 17.4
		mg%		28.4 ± 6.4	13.9 ± 1.5
		range		20.4 - 40.2	11.8 - 15.5
		P			

- Continued -

TABLE III (contined)

Treatment	n	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
IV. Follicle Stimulating Hormone (0.025 Armour Unit, BID)	10	g, mg	103 ± 12.2	39.9	12.5
		range	89 - 125	29.4 - 50.6	9.9 - 18.9
		mg %		38.4 ± 5.5	12.1 ± 1.3
		range		32.0 - 46.6	9.9 - 14.1
		P	<0.05	<0.001	
V. Luteinizing Hormone (0.125 Armour Standard Unit, BID)	9	g, mg	101 ± 16.2	44.1	13.2
		range	64 - 117	29.4 - 55.6	10.5 - 15.3
		mg%		43.8 ± 6.2	13.3 ± 2.5
		range		36.4 - 54.5	9.5-18.3
		P	<0.20	<.001	

[a]Number of rats in group.

[b]Body weights expressed in grams, organ weights are combined weights of both ovaries/adrenal glands expressed in milligrams. Data represents average values.

[c]Standard deviation from the arthmetic mean.

[d]Organ weights expressed in milligrams per 100 grams body weight.

[e]Probability of differences of average body weights in grams or average ovarian/adrenal gland weights in mg% when compared to non-injected control values ("student's"t-distribution). Probabilities of <0.20 or less reported.

12.7 mg% at 33 days and 13.3 mg% at 37 days for the controls (Tables I-IV and Text Figure 2). At 37 days of age, ovarian weight was not increased significantly over control values, 30.9 mg% compared to 28.3 mg% for controls. Interestingly enough, all rats were cycling at this period, and such variations in ovarian weights can be expected normally, as previously shown earlier by Kasprow (1969).

Average DHA-3β-HSD activity in the ovaries of the aspirin treated rats increased markedly and significantly (approximately a 67% increase, $P=<0.025$) at day 33 when compared to controls, but was unchanged from control values at days 27 and 37. Average semi-quantitative estimates of DHA-3β-HSD activity was $+1.50 \pm 0.58$ at 27 days, $+2.50 \pm 0.91$ at 33 days and +2.00 at 37 days for the aspirin treated rats. Comparable control values were $+1.50 \pm 0.58$ at 27 days, $+1.40 \pm 0.55$ at 33 days and $+2.00 \pm 0.71$ at 37 days (Table V and Plate IV, Figures 9 and 10, and Text Figure 3). Histologic sections of the ovaries of the aspirin treatment group revealed luteal formation at 27 days of age, while no control ovaries showed corpus luteum formation until 37 days.

In the 37 day old aspirin subgroup, gross body weight was significantly less ($P=<0.05$) than comparable control body weights. Average body weight of the 37 day control rats was 123 grams, while the 37 day aspirin-injected subgroup had an average body weight of 110 grams (Tables I and IV). Chronic salicylate intoxication could account for this weight difference.

TABLE IV

GENERALIZED AND SUMMARIZED GRAVIMETRIC DATA ON THE BODY, OVARIES AND ADRENAL GLANDS OF THE 37 DAY OLD TREATMENT GROUP

Treatment	n[a]	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
I. Alcohol (0.25 cc of 10% ethanol BID)	10	g, mg[b]	122 ± 13.9[c]	35.8	15.8
		range	109 - 155	23.3 - 61.0	12.0 - 21.4
		mg%[d]		28.9 ± 71.	13.0 ± 1.9
		range		20.0 - 39.4	10.3 - 15.9
		P[e]	<0.01		
II. Prostaglandin $F_{2\alpha}$ (75 micrograms BID)	9	g, mg	120 ± 15.6	30.1	15.4
		range	88 - 139	21.0 - 44.4	12.3 - 21.4
		mg%		26.0 ± 7.2	12.9 ± 1.7
		range		18.1 - 37.5	10.1 - 15.4
		P			
III. Aspirin (5.0 milligrams BID)	8	g, mg	110 ± 12.1	34.2	15.2
		range	85 - 122	20.0 - 47.8	11.8 - 17.3
		mg%		30.9 ± 7.6	13.9 ± 0.8
		range		19.6 - 39.5	12.7 - 14.9
		P	<0.05		

- Continued -

TABLE IV (Continued)

Treatment	n	Item	Body Weight	Ovarian Weight	Adrenal Gland Weight
IV. Follicle Stimulating Hormone (0.025 Armour Unit, BID)	10	g, mg	127 ± 8.1	43.1	16.9
		range	115 - 142	28.6 - 52.4	12.7 - 22.8
		mg%		34.0 ± 6.8	13.2 ± 1.7
		range		24.0 - 43.1	11.0 - 16.1
		P		<0.10	
V. Luteinizing Hormone (0.125 Armour Standard Unit, BID)	10	g, mg	126 ± 9.3	48.4	16.1
		range	110 - 144	32.3 - 63.0	13.7 - 20.6
		mg%		38.4 ± 6.1	12.9 ± 1.8
		range		29.3 - 48.1	10.5 - 16.0
		P		<0.005	

[a]Number of rats in group.

[b]Body weights expressed in grams, organ weights are combined weights of both ovaries/adrenal glands expressed in milligrams. Data represents average values.

[c]Standard deviation from the arthmetic mean.

[d]Organ weights expressed in milligrams per 100 grams body weight.

[e]Probability of differences of average body weights in grams or average ovarian/adrenal gland weights in mg% when compared to non-injected control values ("student's" t-distribution). Probabilities of <0.20 or less reported.

D. Follicle Stimulating Hormone-Injected and Luteinizing Hormone-Injected Female Rats.

Follicle Stimulating Hormone (FSH) at the dose levels used in this experiment caused a significant increase in average body weights of the 27 day old ($P=<0.02$) and 33 day old ($P=<0.05$) FSH rats when compared to those of the controls. Body weight averages were 68 grams compared to 59 grams, and 103 grams compared to 92 grams for controls, respectively (Tables I-III). The 37 day old FSH subgroup was also heavier than controls (127 grams vs. 123 grams for controls), but the difference was not statistically significant. Luteinizing Hormone (LH) also caused significant body weight increases in the 27 day old LH subgroup ($P=<0.01$). Average body weight was 65 grams compared to 59 grams for the 27 day control subgroup (Tables I and II). Body weight increases in the 33 and 37 day LH subgroups was also recorded, but was not significantly changed from control values. Average body weight for the 33 and 37 day LH subgroups was 101 and 126 grams respectively (Tables III and IV). An explanation of these weight increases in both the FSH and LH groups could be possible contamination of the gonadotropin preparations with Growth Hormone (GH).

The adrenal glands of the FSH and LH groups showed weight decreases when compared to control values at 27, 33 and 37 days of age. The weight of the adrenal glands of the 27 day FSH subgroup was significantly less ($P=<0.05$) than average control values. The average adrenal gland weights of the 27, 33 and 37 day FSH subgroups were 13.8 mg%, 12.1 mg% and 13.2 mg%,

TABLE V

GENERALIZED AND SUMMARIZED DATA BASED ON THE SEMI-QUANTITATIVE HISTOCHEMICAL ESTIMATES OF AVERAGE DEHYDROEPIANDROSTERONE-3β-HYDROXYSTEROID DEHYDROGENASE (DHA-3β-HSD) ACTIVITY IN OVARIAN SECTIONS OF THE CONTROL AND TREATMENT GROUPS

Item	Control	Prostaglandin $F_2\alpha$	Aspirin	Follicle Stimulating Hormone	Luteinizing Hormone
27 day old					
n[a]	4	5	4	5	4
avg.±S.D.[b]	+1.50±0.58	+2.40±0.55	+1.50±0.58	+1.20±0.45	+1.40±0.55
P[c]		<0.05			
33 day old	5	5	4	5	4
n	+1.40±0.55	+2.20±0.80	+2.50±0.91	+1.20±0.45	+1.50±0.58
avg.±S.D.		<0.10	<0.025		
P					
37 day old					
n	5	4	4	5	5
avg.±S.D.	+2.00±0.71	+1.75±0.49	+2.00±0.82	+1.40±0.55	+1.20±0.45
P				<0.20	<0.05

[a]Number of rats in group.

[b]Average and standard deviation from the mean. Represents average of estimates of DHA-3 -HSD activity derived from the follicular, luteal and interstitial components of the ovaries.

[c]Probability of differences when compared to the non-injected controls ("student's" t-distribution). Only probabilities of <0.20 or less reported.

respectively, 14.8 mg%, 13.3 mg% and 12.9 mg% for the LH subgroups, respectively, and for the non-injected control subgroups, 15.7 mg%, 12.7 mg% and 13.3 mg%, respectively (Tables I-IV and Text Figure 2).

Histologic sections of the ovaries of the FSH group revealed multiple follicular development with early luteinization when compared to control sections. Also in this experiment, the FSH group showed statistically significant increases in average ovarian weights in the 33 day old subgroup ($P=<0.001$). Average control weight for the 33 day ovarian weights was 28.2 mg%; while comparable FSH ovarian weight average was 38.4 mg%. The 27 day and 37 day average ovarian weights were comparable to control values, i.e. 39.0 mg% vs 38.2 mg% for control ovaries at 27 days, and 34.0 mg% vs 28.3 mg% for the controls at 37 days. (Tables I-IV and Text Figure 1). Velardo (1958) reported that FSH, when given subcutaneously in immature rats, resulted in ovarian weight increases and luteinization when given over a period of days.

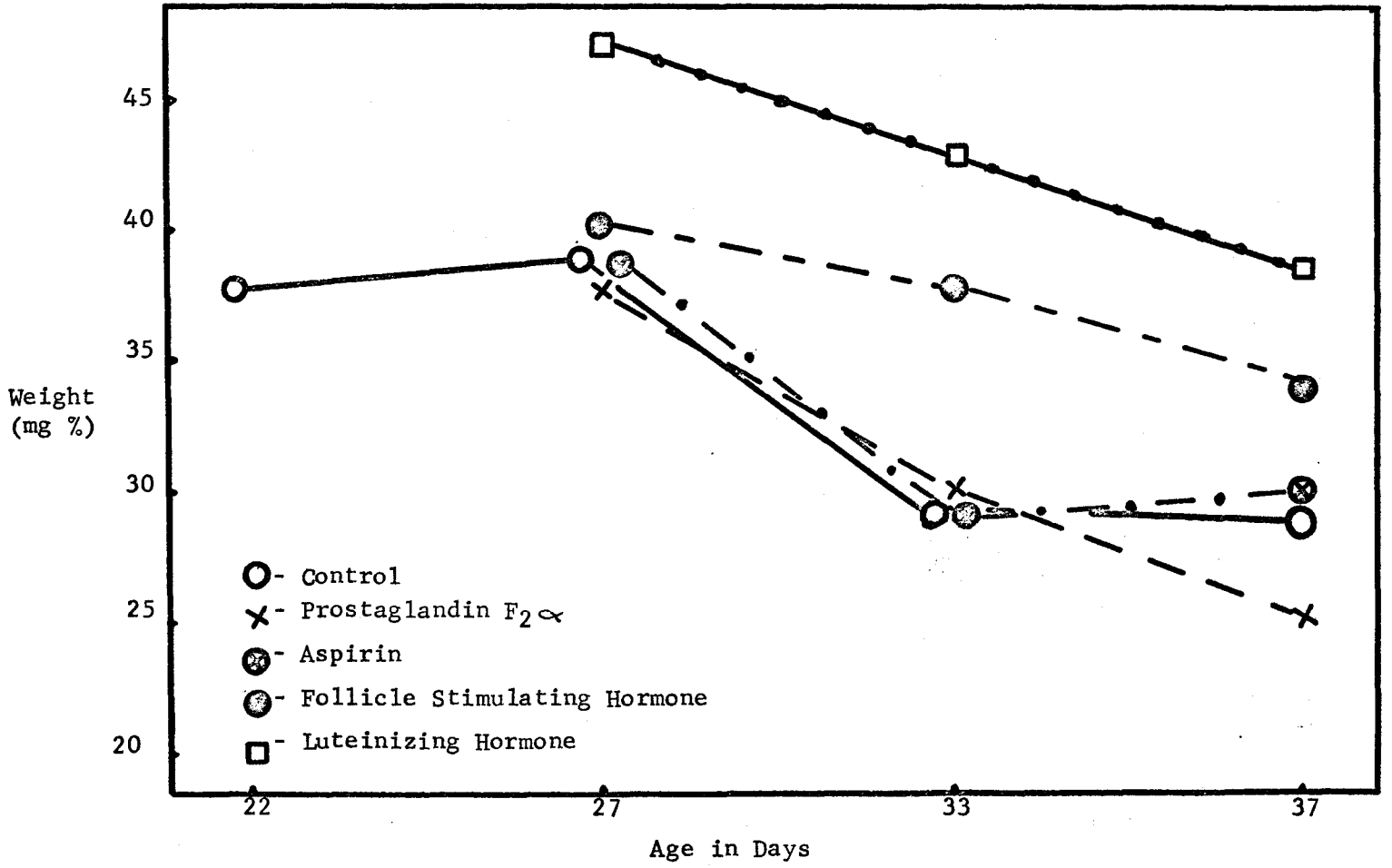
Ovarian weights were greatly increased in the LH group when compared to the non-injected control animals. The average ovarian weights of the LH subgroups was of a greater magnitude than comparable FSH values. This weight increase was significant, when compared to controls, in the 27- ($P=<0.02$), 33- ($P=>0.001$) and 37- ($P=<0.005$) day old LH subgroups. Recorded ovarian weight averages were 48.8 mg%, 43.8 mg% and 38.4 mg%, respectively (Tables I-IV and Text Figure 1). Also, histologic sections

of the ovaries in the LH group revealed luteinization at day 27, much earlier than comparable control sections which did not reveal luteinization until day 37. Pure LH preparations will cause formation of corpora lutea in immature female rates if prior growth of graafian follicles is stimulated by FSH (Greep, van Dyke and Chow, 1942).

Average DHA-3β-HSD activity was decreased when compared to control values at 27, 33 and 37 days of age for both the LH and FSH subgroups. This decrease was significant ($P=<0.05$) in the 37 day LH subgroup. The average semi-quantitative estimates of enzyme activity were $+1.20 \pm 0.45$, $+1.20 \pm 0.45$ and $+1.40 \pm 0.55$ for the 27-, 33- and 37-day old FSH subgroups, respectively, and were $+1.40 \pm 0.55$, $+1.50 \pm 0.58$ and $+1.20 \pm 0.45$ for the 27-, 33- and 37-day old LH subgroups, respectively. Very little diformazan deposits were observed in any ovarian sections from either the FSH or LH groups. Most ovarian sections in both the FSH and LH groups showed only monoformazan deposits (Table V, Text Figure 3 and Plate V, Figures 11 and 12).

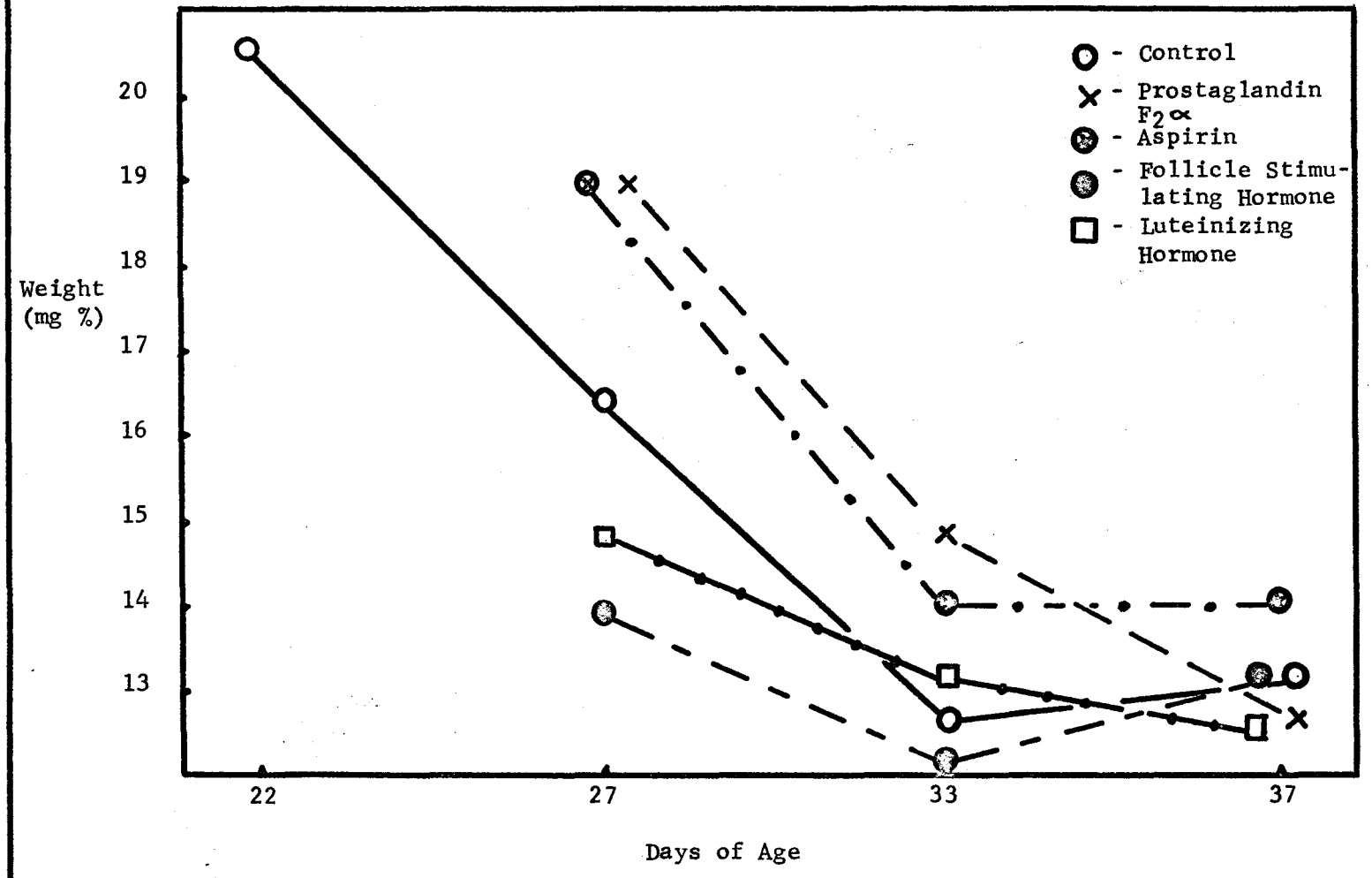
TEXT FIGURE 1

GRAPH SHOWING GRAVIMETRIC DATA ON THE OVARIES OF IMMATURE FEMALE RATS WHEN EXPOSED TO THE DIFFERENT TREATMENT REGIMES INDICATED (DATA BASED ON COMBINED WEIGHTS OF BOTH OVARIES CONVERTED INTO MILLIGRAMS PER 100 GRAMS BODY WEIGHT, i.e. MG%)



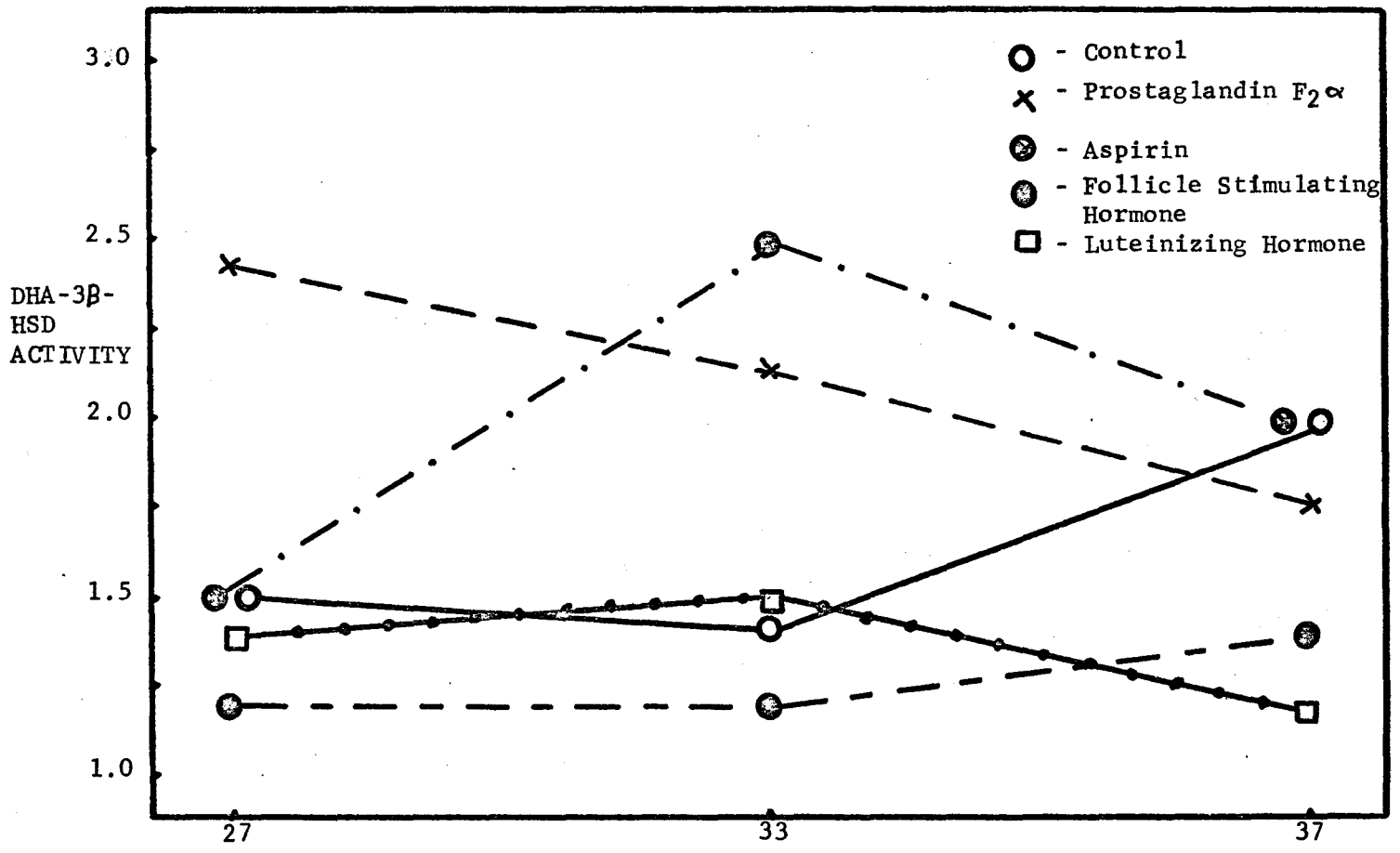
TEXT FIGURE 2

GRAPH SHOWING GRAVIMETRIC DATA ON THE ADRENAL GLANDS OF IMMATURE FEMALE RATS WHEN EXPOSED TO THE DIFFERENT TREATMENT REGIMES INDICATED (DATA BASED ON COMBINED WEIGHTS OF BOTH ADRENAL GLANDS CONVERTED INTO MILLIGRAMS PER 100 GRAMS BODY WEIGHT, i.e. MG %)



TEXT FIGURE 3

GRAPH SHOWING DATA BASED ON THE SEMIQUANTITATIVE HISTOCHEMICAL ESTIMATES OF AVERAGE DEHYDROEPIANDROSTERONE-3β - HYDROXYSTEROID DEHYDROGENASE (DHA-3β -HSD) ACTIVITY IN OVARIAN SECTIONS OF THE CONTROL AND TREATMENT GROUPS



DISCUSSION

It is postulated that prostaglandins exert their effects by regulating intracellular levels of adenosine 3', 5'-monophosphate (cyclic AMP). Cyclic AMP is considered to be the second messenger of hormone action (Sutherland and Rall, 1960; Shaw and Ramwell, 1968). Studies with prostaglandins and their effect on hormone action have revealed that prostaglandins (PG's) can serve in both a positive and a negative feedback role in hormone action (Steinberg, Vaughan, Nestel, Strang and Bergstrom, 1964). The prostaglandins, as a group, will mimic actions of hormones and neurohormones, modify hormonal actions, or inhibit the hormonal actions depending on the tissue and specific prostaglandin studied. In vivo studies compared with in vitro actions of the same prostaglandin compound sometimes reveal conflicting end results. In vitro incubation of luteal slices with $PGF_{2\alpha}$ cause a stimulation of progesterone synthesis (Pharriss, Wyngarden and Gutknecht, 1968), while Labhsetwar (1971) found that in vivo $PGF_{2\alpha}$ in the adult female rat causes irreversible luteolysis. Aspirin will inhibit prostaglandin synthesis in vitro (Vane, 1971). Prostaglandins will increase steroidogenesis in in vitro preparations of rat adrenal glands (Flock, Jessup and Ramwell, 1969) and salicylates in the in vivo system will also cause an increase in adrenal steroidogenesis (Done, Ely and Kelly, 1958).

It appears that there are several pronounced contradictions insofar as the biological actions of the prostaglandins are concerned. One explanation for these apparent contradictions is the importance of recognizing

different levels of prostaglandin action. In the case of *in vitro* $PGF_{2\alpha}$ ovarian steroidogenesis, a cellular phenomena is a possible explanation while the *in vivo* luteolytic activity of $PGF_{2\alpha}$ may represent a chronic action on local hemodynamics of the ovary (Pharriss, 1971). Aspirin increases ACTH levels in the body which results in adrenal steroidogenesis (Done, Ely and Kelly, 1958). Thus, aspirin may exert its effect at the pituitary level by pituitary stimulation of ACTH release while $PGF_{2\alpha}$ may exert its effect at the adrenal level to cause steroidogenesis, resulting in the identical end result of increased steroidogenesis.

In an effort to ascertain some critical lines of data on the biological actions of the prostaglandins, several experiments were undertaken. First, a series of controls were thoroughly assessed, so as to provide rigid baselines on comparisons. Secondly, a four part experimental procedure was undertaken: (a) Rats of 22 days of age were given 0.075 milligrams of $PGF_{2\alpha}$ twice daily (BID) and necropsied at 27, 33 and 37 days of age; (b) 5.0 milligrams of aspirin BID were similarly given, and the rats were necropsied according to the aforementioned schedule; (c) Likewise, 0.025 Armour Unit of FSH were similarly studied; and finally (d) LH (0.125 Armour Standard Unit) was assessed as per the experimental schedule. Two distinctive parameters were pursued: (1) the gravimetrics of the endocrine organs, with specific notes on reproductive events, and (2) the changing nature of DHA-3β-HSD co-incident with maturity and treatment with each of the substances named, *e.g.* $PGF_{2\alpha}$, aspirin, FSH and LH.

In this study, DHA-3β-HSD activity in the ovary was used as an indication of specific steroidogenesis in the ovary. DHA-3β-HSD is required for biosynthesis of estradiol 17-β and estrone in the ovaries of rats (Kalvert and Bloch, 1968). Adrenal gland weights (on a mg% basis) were used as supportive of metabolic activity in this endocrine organ. Injected ACTH will cause steroidogenesis in the rat adrenal with a concomitant increase in weight; hypophysectomy will cause a decrease in steroidogenesis and, also, a decrease in adrenal weight (Kitchell and Wells, 1952). In Cushing's syndrome in man, there is hypercortisolism and enlarged, hyperplastic adrenal glands (Anderson and Cleveland, 1969). The ovarian weight is not a good indication of steroidogenesis in the ovary since ovarian weight is more dependent on morphologic components present, i.e., follicles, corpora lutea (Kasprow, 1969).

This thesis reports similar end-results of increased estrogen synthesis in the ovary, and increased adrenal weights in immature female rats, when given either $PGF_{2\alpha}$ or aspirin over a period of weeks. Possibly aspirin is working at the pituitary level and $PGF_{2\alpha}$, when given systemically, works at the end organ level to produce a generalized steroidogenic effect in the body with either compound. Another possible explanation is that aspirin only effects PG synthetase and not formed prostaglandins. In view of the apparent in vitro antagonism of aspirin and $PGF_{2\alpha}$, it is interesting to note their similarity in promoting increased steroidogenesis in the ovary and adrenal glands in the in vivo system. Also in comparing the actions

of the $PGF_{2\alpha}$ and aspirin, after approximately two weeks of chronic administration, their effects on steroidogenesis in the adrenal gland and ovary is insignificant when compared to the non-injected controls. Perhaps the in vivo system adapts to these high levels of $PGF_{2\alpha}$ and aspirin, or perhaps a drug insensitivity or tolerance phenomenon occurs. This apparent insensitivity is also illustrated in the 37 day $PGF_{2\alpha}$ group which showed luteal tissue in histologic sections of the ovary. Short-term in vivo $PGF_{2\alpha}$ administration in the adult female rat causes irreversible luteolysis (Labhsetwar, 1971). Another explanation could be deterioration of the $PGF_{2\alpha}$ and aspirin solutions, and, thus, loss of biologic activity over time. Also, one should consider the fact that dose remained constant over time while body weight increased markedly over this time period of rapid growth, thus effectively decreasing dose per unit weight levels.

Vogt (1957) and Kasprow (1969), using bilaterally ovariectomized rats injected with estradiol 17-β, reported uterine weight increases and significant increases in adrenal weights. In this study, increases in estradiol-17β synthesis in the ovary, as measured by DHA-3β-HSD activity in the ovary, resulted in concomitant increases in adrenal weights. Perhaps the same mechanism is operating in both instances to increase adrenal steroidogenesis. Estradiol-17β synthesis could directly influence adrenal steroidogenesis by providing the chemical precursors for adrenal steroid hormones

(Hechter, Solomon, Zaffaroni and Pincus, 1953). Another possibility is an estradiol-17β feedback mechanism on the pituitary to influence tropic hormone release (Vogt, 1957).

Investigations utilizing prostaglandins and prostaglandin inhibitors (aspirin, indomethacin and prostynoic acid) and comparing their modifying action on gonadotropin response have indicated that the level of prostaglandin action is an important variable when considering prostaglandin action in the in vivo system. Orczyk and Behrman (1971) reported that both aspirin and indomethacin will block ovulation in the rat. Aspirin and indomethacin reduced $PGF_{2\alpha}$ plasma concentrations and pituitary and hypothalamus $PGF_{2\alpha}$ concentrations. Utilizing gonadotropic releasing hormone and LH to reverse the effects of ovulation blockage in the rat with aspirin and indomethacin, the site of ovulation blockage of these drugs was elucidated (Behrman, Orczyk and Greep, 1972). It was determined that aspirin blocked ovulation at the pituitary level, while indomethacin probably exerted its effect at the ovarian level. LH reversed aspirin ovulation blockage, but not indomethacin ovulation blockage. Thus, prostaglandins probably act at both the pituitary and ovarian levels to influence hormonal actions on the ovary.

Evidence also suggests prostaglandin interaction at the level of the hypothalamus. PGE_1 will stimulate ACTH release when placed in the rat hypothalamus, but has no effect when placed directly in the pituitary of the rat (Hedge, 1971).

The exact relationship between prostaglandins, gonadotropins and cyclic AMP, is another area of current investigation. Prostaglandin E_1 and LH will cause cyclic AMP production when incubated with mouse ovary; this effect is blocked with the addition of prostynoic acid, a specific prostaglandin inhibitor (Kruehl, Humes, Tarnoff, Cirillo and Ham, 1970). Grinwick, Kennedy and Armstrong (1972) reported a dissociation of ovulatory and steroidogenic properties of LH by studying indomethacin effects on rabbit ovaries. Indomethacin blocked ovulation but did not prevent luteinization. Such studies indicate possible multiple interactions between gonadotropins and prostaglandins at the cellular level. It should be emphasized that there are many conflicting reports of prostaglandin action in the literature. Much work must be accomplished to establish the valid role of prostaglandin-gonadotropin interactions.

Another interesting observation was the decrease in growth rates of the aspirin-treated rats. This observation could simply reflect the generalized toxic effects of salicylates but also could indicate interference or antagonism of various pituitary hormones which influence growth. Vaginal opening was also delayed; but early luteinization of the ovary was observed in the aspirin group.

Also in this study, there was a consistent decrease in DHA-3β-HSD activity in the ovarian sections of the LH and FSH groups. Ovarian histomorphology revealed early luteinization when compared to control ovarian sections. FSH, when given without LH, causes follicular development, but

without LH, estrogen synthesis is minimal (Turner, 1971). Histologic sections of the ovaries in the FSH group revealed multiple follicular development with early luteinization. Velardo (1958) reported that FSH, when given subcutaneously in immature rats resulted in ovarian weight increases and luteinization when given over a period of days. Fevold (1941) indicated that relatively pure preparations of FSH are inefficient in inducing biosynthesis of estrogen in the immature rat ovary. Lack of estrogen synthesis was apparent in this study by the lack of DHA-3β-HSD activity in the ovary. Fevold (1941) also reported that LH, when given alone, does not stimulate estrogen synthesis. Both LH and FSH, in the proper ratio, are necessary to stimulate estrogen biosynthesis (Velardo, 1958 and 1960).

Also of note in this study was a consistent decrease in adrenal gland weight of the FSH and LH group when compared to control values. Earlier in this discussion it was noted that estradiol-17β will cause adrenal gland weight increases when injected subcutaneously in rats (Kasprow, 1969). DHA-3β-HSD activity in the ovaries of the rat is indicative of estradiol-17β synthesis (Kalvert and Bloch, 1968). In the LH and FSH subgroups, it was previously noted that DHA-3β-HSD activity was less, on the average, than control DHA-3β-HSD activity. The decrease in estradiol-17β synthesis could explain the decrease in adrenal gland weights in the LH and FSH groups.

Companion studies not reported in this dissertation did not show any significant uterine weight increases within the aspirin or $PGF_{2\alpha}$ subgroups. DHA-3β-HSD studies on these same ovaries did show significant increases

in estrone and estradiol-17β synthesis. Estradiol-17β will cause significant uterine weight increases at a dosage of 0.1 microgram (Kasprow, 1969). DHA-3β-HSD activity in the ovary apparently is a more sensitive indicator of estrogen synthesis than uterine weight changes in the rat.

Regarding the dynamic role of the prostaglandins in the body, much remains for future study. The literature is replete with contradictions and vague generalities concerning these biologically active compounds. Their ubiquitous occurrence in many tissues of the mammalian body, and the possibility of species differences somewhat tend to confuse experimental design and results. Investigations into the area of endocrine physiology of the reproductive tract and prostaglandin interaction have only resulted in speculation of the roles of prostaglandin in reproductive functions.

It may very well be that prostaglandins are the long recognized, but previously unknown, luteolytic agents, initiators of parturition and agents responsible for ovum transport and sperm capacitation. Some such evidence for the latter exists. Therapeutic applications of the PG's have been surprizingly successful up to this point. The intensity of research in the area of prostaglandin biochemistry and physiology will undoubtedly shed new light on their true significance in the mammalian body in the near future.

SUMMARY AND CONCLUSIONS

1. A series of experiments were undertaken to observe the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), acetylsalicylic acid (aspirin), follicle stimulating hormone (FSH) and luteinizing hormone (LH) on estrogen biosynthesis in the ovary as revealed histochemically; also pursued were the histologic morphology of the ovary, and the gravimetric changes of the ovaries, adrenal glands and body weights of immature, female rats when given the aforementioned drugs during the period of developing reproductive competency.

Five major groups of thirty 22 day old female rats (one control group and four treatment groups) were given subcutaneous injections, twice daily, of either 75 micrograms of $PGF_{2\alpha}$, give milligrams of aspirin, 0.25 Armour Unit of FSH or 0.125 Armour Standard Unit of LH. Sub-groups of ten rats in each major treatment category were necropsied at 27, 33 or 37 days of age. Body weights, ovarian weights and adrenal gland weights were recorded and statistically analyzed. Ovarian sections were prepared for histologic morphology, and for histochemical analysis, i.e., dehydroepiandrosterone-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity, which is indicative of the precursors of estrone and estradiol-17β biosynthesis in the ovary. DHA-3β-HSD activity in the ovary, and adrenal gland weights were used as indices of the metabolic activity in these endocrine organs.

2. Both $PGF_{2\alpha}$ and aspirin significantly increased (at the 0.05 level of predictability or less) the dehydroepiandrosterone-3β-hydroxysteroid

dehydrogenase (DHA-3β-HSD) activity in the ovary over comparable control values in the 27 and 33 day $PGF_{2\alpha}$ subgroups and the 33 day aspirin subgroup. Activities of DHA-3β-HSD in the LH- and FSH-injected rats were consistently below control levels throughout the experimental procedures. DHA-3β-HSD ovarian activity in the $PGF_{2\alpha}$ and aspirin injected rats approached control values after 16 days of continuous injections of these compounds. Average semi-quantitative ovarian DHA-3β-HSD activities on a "0" to "+3" scale were: +1.50 ± 0.58, +1.40 ± 0.55 and +2.00 ± 0.71 for the 27, 33 and 37 day old non-injected control subgroups, respectively; +2.40 ± 0.55, +2.20 ± 0.80 and +1.75 ± 0.49 for the 27, 33 and 37 day old $PGF_{2\alpha}$ -injected subgroups, respectively; +1.50 ± 0.58, +2.50 ± 0.91 and +2.00 ± 0.82 for the 27, 33 and 37 day old aspirin-injected subgroups, respectively; +1.20 ± 0.45, +1.20 ± 0.45, and +1.40 ± 0.55 for the 27, 33 and 37 day old FSH-injected subgroups, respectively; and +1.40 ± 0.55, +1.50 ± 0.58 and +1.20 ± 0.45 for the 27, 33 and 37 day old LH-injected subgroups, respectively.

3. Ovarian weights were not significantly different, when compared to controls, for the $PGF_{2\alpha}$ and aspirin treatment groups. The FSH and LH groups showed statistically significant ovarian weight increases throughout the experimental procedure. Statistically significant average ovarian weight increases were recorded in the 33 day old FSH subgroup, and in the 27, 33 and 37 day old LH subgroups.

Recorded average ovarian weights, on a milligrams per 100 grams body weight (mg%) basis were: 38.2, 28.2 and 28.3 for the 27, 33 and 37 day old

non-injected control subgroups, respectively 37.3, 29.9 and 26.0 for the 27, 33 and 37 day old $PGF_{2\alpha}$-injected control subgroups, respectively; 38.9, 28.4 and 30.9 for the 27, 33 and 37 day old alcohol-injected (vehicle for aspirin) subgroups, respectively; 39.0. 38.4 and 34.0 for the 27, 33 and 37 day old FSH-injected subgroups, respectively; and 48.8, 43.8 and 38.4 for the 27, 33 and 37 day old LH-injected subgroups, respectively.

4. Histomorphologic comparisons of ovarian sections revealed early luteinization in the LH, FSH and aspirin groups, but the ovaries of the $PGF_{2\alpha}$-injected rats resembled control sections.

5. Adrenal gland weights in the $PGF_{2\alpha}$ and aspirin treatment groups were significantly increased over control values in the 27 and 33 day old $PGF_{2\alpha}$ subgroups, and in the 27 day old aspirin subgroups. This increase in average adrenal gland weights over control values paralleled DHA-3β-HSD activity in the ovary for the $PGF_{2\alpha}$ and aspirin groups. Again, as with DHA-3β-HSD activity, adrenal weights were comparable to control values after 16 days of continuous injections of either $PGF_{2\alpha}$ or aspirin. FSH- and LH-injected rats showed consistent decreases of adrenal weight, but this decrease was not statistically significant, when compared to controls.

Average adrenal gland weights, on a mg% basis, were 15.7, 12.7 and 13.3 for the 27, 33 and 37 day old non-injected controls, respectively; 19.0, 14.7 and 12.9 for the 27, 33 and 37 day old $PGF_{2\alpha}$-injected subgroups, respectively; 18.9, 13.9 and 13.9 for the 27, 33 and 37 day old aspirin-injected subgroups, respectively; 13.9, 12.1 and 13.2 for the 27, 33 and 37

day old FSH-injected subgroups, respectively; and 14.8, 13.3 and 12.9 for the 27, 33 and 37 day old LH-injected subgroups, respectively.

6. Body weights in the FSH and LH groups were consistently and significantly increased over body weights of comparable control groups. It was postulated that possible growth hormone contamination of the LH and FSH preparations could account for this weight increase. Aspirin-injected rats showed a significant decrease in body weight at 37 days when compared to controls. It was postulated that this weight decrease in the aspirin group could reflect interference with pituitary hormones which influence growth, or could simply reflect the generalized toxic effect of the salicylates.

Recorded average body weights in grams were: 59, 92 and 123 for the 27, 33 and 37 day old non-injected control subgroups, respectively; 55, 90 and 120 for the 27, 33 and 37 day old $PGF_{2\alpha}$-injected subgroups, respectively; 54, 89 and 110 for the 27, 33 and 37 day old aspirin-injected subgroups, respectively; 68, 103 and 127 for the 27, 33 and 37 day old FSH-injected subgroups, respectively; and 65, 101 and 126 for the LH-injected subgroups, respectively.

7. It was concluded, from the aforementioned observations, that both $PGF_{2\alpha}$ and aspirin, a specific prostaglandin antagonist, will increase ovarian and adrenal steroidogenesis in the immature female rat; and that this steroidogenic effect decreases over time when these compounds are chronically administered. It was postulated that aspirin and $PGF_{2\alpha}$

may exert their effects at different levels of hormone action; i.e., at the pituitary level, or at the ovarian or adrenal level.

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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 magnification.

PLATE I

Photomicrographs of sections of ovaries from rats which were incubated with dehydroepiandrosterone (DHA) showing the semi-quantitative histochemical scale of DHA-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity in ovarian sections.

Fig. 1. Control section of ovary incubated without DHA. Represents a "0" reaction (100X).

Fig. 2. Represents a "+1" reaction. Very little diformazan deposits are in evidence, and DHA-3β-HSD activity is evidenced by monoformazan deposits (100X).

Fig. 3. Represents a "+2" reaction. Discrete diformazan and monoformazan deposits are in evidence (100X).

Fig. 4. Represents a "+3" reaction. Confluent areas of diformazan deposits are in evidence (100X).

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

Photomicrographs of sections of ovaries from the non-injected contol rats which were incubated with dehydroepiandrosterone (DHA) to localize the sites of estrogen biosynthesis, i.e., DHA-3β-hydroxy-steroid dehydrogenase (DHA-3β-HSD) activity.

Fig. 5. Ovarian section from a 33 day old non-injected control rat showing moderate (+2) DHA-3β-HSD activity in the interstitial tissue and the theca interna of the tertiary follicles. The antrum shows a typically negative reaction (100X).

Fig. 6. Ovarian section from a 37 day old non-injected control rat showing strong (+3) DHA-3β-HSD activity in the interstitial tissue and in the theca interna of the follicles. Moderate (+2) DHA-3β-HSD activity is seen in the granulosa cells of the follicles (100X).

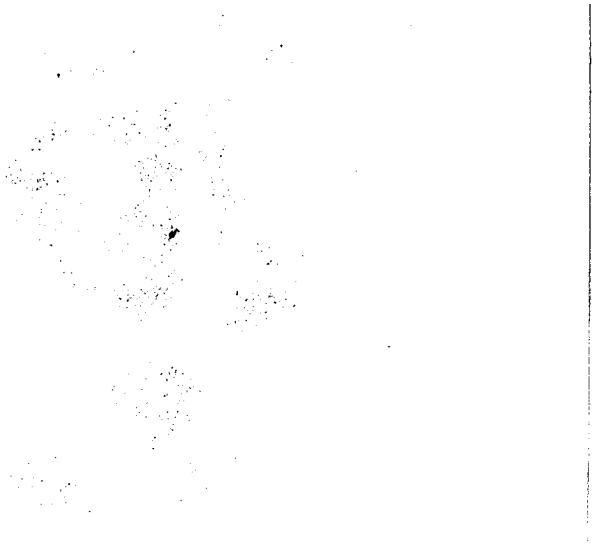

FIGURE 5

FIGURE 6

Photomicrographs of sections of ovaries from the $PGF_{2\alpha}$-injected rats which were incubated with dehydroepiandrosterone (DHA) to localize the sites of estrogen synthesis, i.e., DHA-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity.

Fig. 7. Ovarian section from a 27 day old $PGF_{2\alpha}$-injected rat showing moderate (+2) to strong (+3) DHA-3β-HSD activity in the interstitial tissue and in the theca interna of the follicles (100X).

Fig. 8. Ovarian section from a 37 day old $PGF_{2\alpha}$-injected rat showing moderate (+2) to strong (+3) DHA-3β-HSD activity in the interstitial tissue and weak (+1) to moderate (+2) DHA-3β-HSD activity in the theca interna of the follicles (100X).

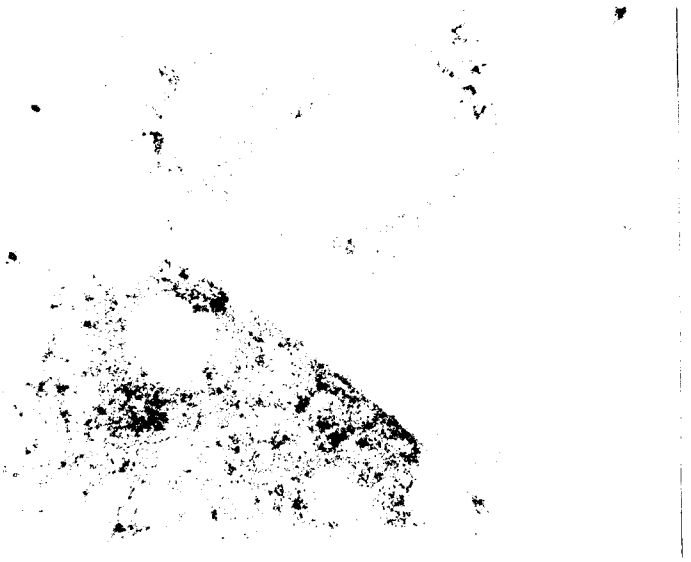

FIGURE 7

FIGURE 8

Photomicrographs of sections of ovaries from the aspirin-injected rats which were incubated with dehydroepiandrosterone (DHA) to localize the sites of estrogen synthesis, i.e., DHA-3β-hydroxysteroid dehydrogenase (DHA-3β-HSD) activity.

Fig. 9. Ovarian section from a 27 day old aspirin-injected rat showing weak (+1) DHA-3β-HSD activity in the interstitial tissue, and in the theca interna and granulosa cells of the follicle. Some diformazan deposits are in evidence in the interstitial tissue (100X).

Fig. 10. Ovarian section from a 33 day old aspirin-injected rat which shows strong (+3) DHA-3β-HSD activity in the subcapsular interstitial tissue and moderate (+2) DHA-3β-HSD activity in the theca interna of the follicles (100X).

FIGURE 9

FIGURE 10

Photomicrographs of sections of ovaries from the FSH- and LH-injected rats which were incubated with dehydroepiandrosterone (DHA) to localize the sites of estrogen biosynthesis, i.e., DHA-3B-hydroxy-steroid dehydrogenase (DHA-3B-HSD) activity.

Fig. 11. Ovarian section from a 37 day old FSH-injected rat showing weak (+1) DHA-3B-HSD activity in the interstitial tissue, and in the theca interna and granulosa cells of the follicles (100X).

Fig. 12. Ovarian section from a 37 day old LH-injected rat showing weak (+1) DHA-3B-HSD activity in the corpus luteum, interstitial tissue, and in the theca interna and granulosa cells of the follicle (100X).

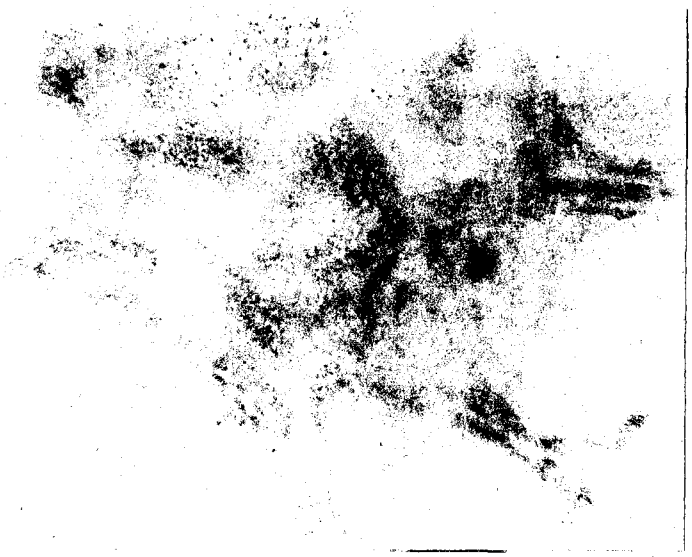

FIGURE 11

FIGURE 12

Fig. 13. Ovarian section from a 37 day old non-injected control rat cut at eight microns and stained with Harris hematoxylin and eosin. Both follicular and corpus luteal structures are evident (100X).

FIGURE 13

APPROVAL SHEET

The thesis submitted by Martin A. Sidor 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 thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis 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 17, 1973

Prof. Jayant S. Velardo

Signature of Advisor