# CHAPTER I

## INTRODUCTION

### Biological significance of prostaglandins (PGs)

Prostaglandin (PG) is the generic name for a family of biologically and pharmacologically active fatty acids. They are 20 carbon-containing fatty acids, consisting of a cyclopentane ring which basic structure is called prostanoic acid (Figure 1). Prostaglandins, like steroids, include a family of many compounds with very similar structure

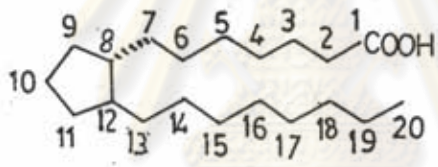


Figure 1 Prostanoic acid numbering scheme.

but with very diverse actions. They are named according to their ring substituents and the number of additional side-chain double bonds, which have the cis-configuration. An example of the stereochemistry of different prostaglandin of the 2-series ($C_{20:2}\ \Delta^{5,13}$) is shown in Figure 2.

The first clue to the existence of prostaglandins appeared in 1930 when Kurzork and Lieb (2) reported that fresh human seminal fluid could produce both relaxation and strong contraction when applied to isolated strips of the human uterus. In 1933 and 1934 Goldblatt (3) in England and von Euler (4) in Sweden independently studied smooth muscle-stimulating and vasodepressor activity by extracts of the

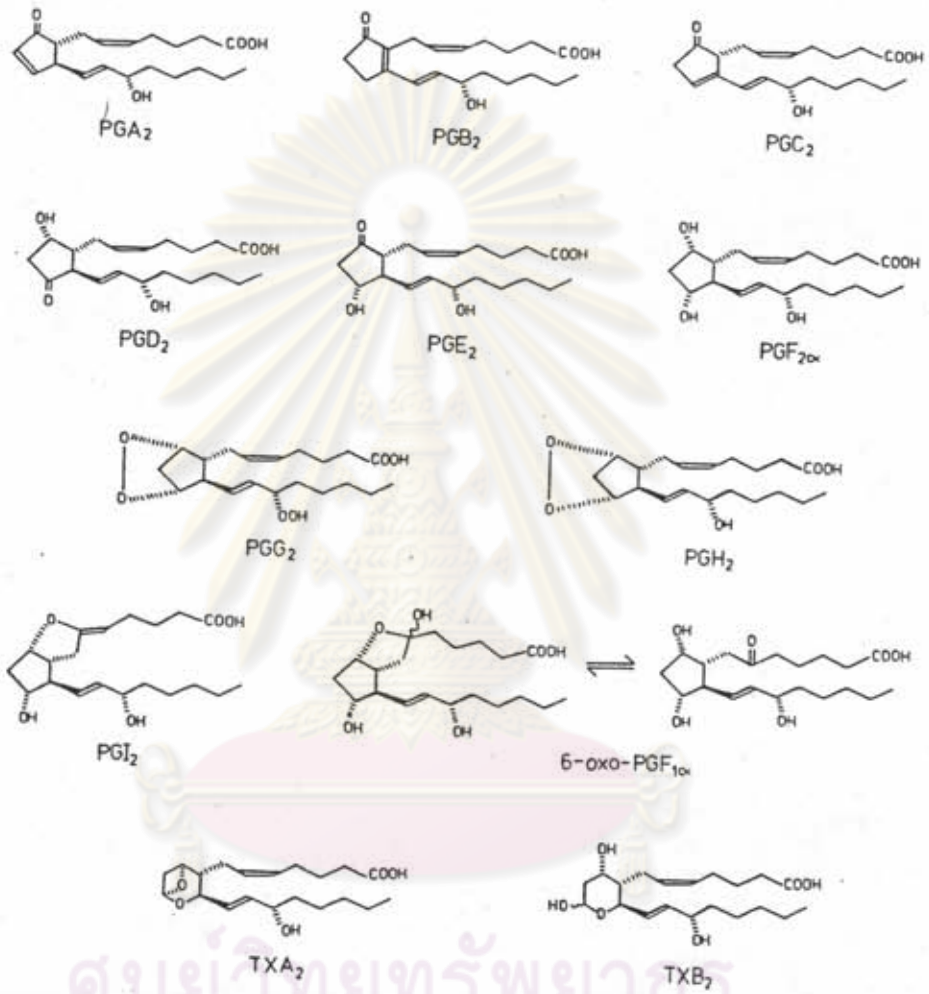


Figure 2 Prostaglandins of the 2-series : chemical structure (1).

'''' = bond below the plane

TX = thromboxane

seminal vesicle. von Euler (5) in 1935, named the active principle "prostaglandin" because he thought it was secreted by the prostate gland, but it was later found that the seminal vesicles were the source of the prostaglandins in seminal plasma (6). Eventually, it was established that prostaglandins are also commonly found in almost all tissues, e.g. placenta, fetal membranes (7), endometrium, myometrium (8), kidney, lung, small intestine, brain (9), leucocytes (10) and ovarian follicle (11). At least 14 prostaglandins occur in nature. The best known prostaglandins are $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$. They are the parent compounds of other biologically active prostaglandins. Many attentions had been paid to these forms. PGEs are vasodilators and depress adrenergic vasoconstrictor responses, whereas $PGF_{2\alpha}$ is a vasoconstrictor and enhances adrenergic neurotransmission. In most cases, prostaglandins of F-type often display biologic activities opposite to those of PGEs.

Prostaglandins biosynthesis and metabolism

Prostaglandins are not stored to any extent in the tissues (12). They are synthesized and released as required or on demand following a variety of mechanical, hormonal, chemical or immunological stimuli, including simple handlings (13). Prostaglandins are synthesized from cyclization of three C-20 essential fatty acids, namely, 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid); 5,8,11,14-eicosatetraenoic acid (arachidonic acid) and 5,8,11,14,17-eicosapentaenoic acid. Each will serve as the precursor for prostaglandins of the 1, 2 and 3 series respectively (14) (Figure 3). The most abundant precursor in mammals is arachidonic acid. Thus, most research efforts has been focused on the prostaglandin of the 2-series. The levels of

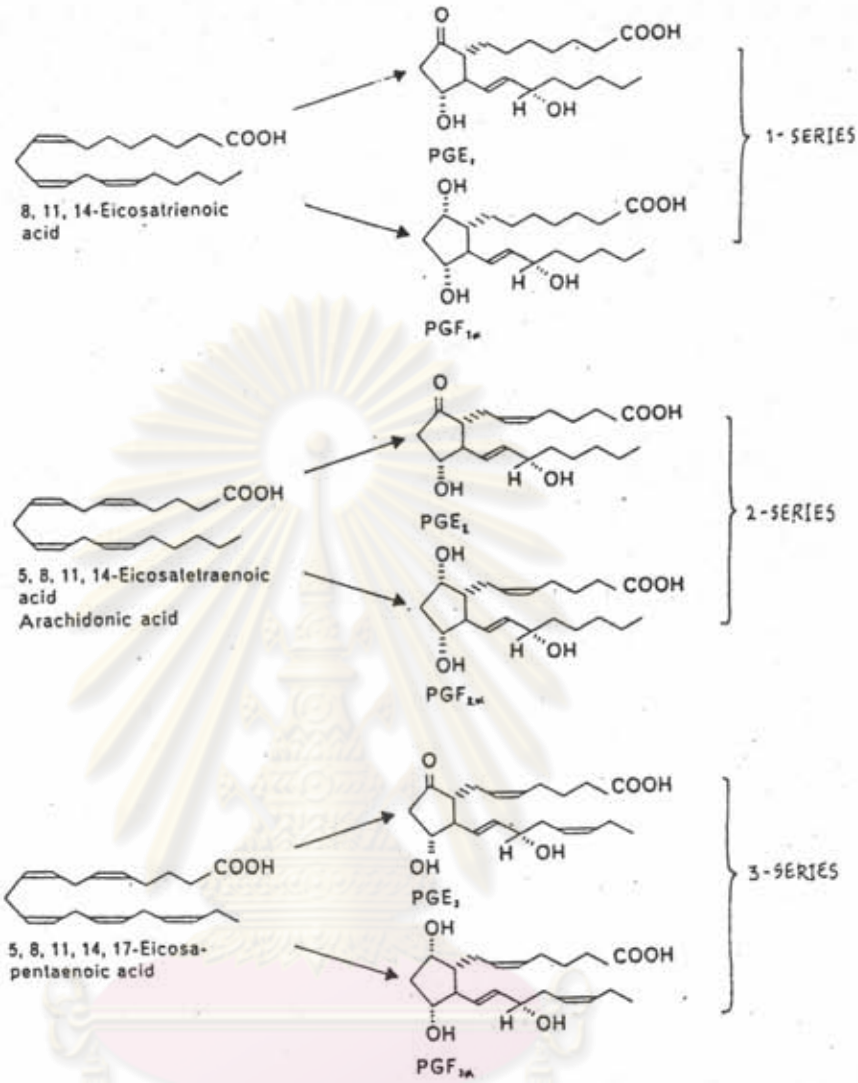


Figure 3 The primary prostaglandins and the 3 primary precursors (14).

Numerical subscript = number of double bonds found in the nonring moieties

α subscript = the hydroxyl group at C-9 is on the same side of the molecule as the aliphatic side chain containing the carboxylic acid group.

free arachidonic acid in tissues are usually very low (15,16). It is mainly stored in phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (17,18,19). Cholesterol ester and triglycerides are also potential sources of arachidonic acid (20,21,22). On stimulation, arachidonic acid must be released from the bound sources. Phospholipase $A_2$, cholesterol esterase or triglyceride lipase (15,20,22) may be the enzymes responsible for the liberation of arachidonic acid in situ (Figure 4). These enzymes are found to be under hormonal control in some tissues (23).

The enzymatic oxidation of arachidonic acid could occur by two major pathways : the cyclooxygenase pathway which produces prostaglandins and thromboxanes and the lipoxygenase pathway which produces leukotrienes and hydroxy fatty acids (24) (Figure 4). The present study would mainly projected on the cyclooxygenase pathway.

The seven enzymes that catalyze the cyclooxygenase pathway, like fatty acid synthetase, are believed to cluster together to form a multienzyme complex. The enzyme complex is named prostaglandin synthetase. It is a membrane-bound enzyme and is located at the endoplasmic reticulum, nuclear membrane (25,26) and plasma membrane (27,28,29). The biosynthesis of prostaglandins mediated by prostaglandin synthetase is illustrated in Figure 5. It can be divided into 3 major steps : oxygenation and cyclization of fatty acid precursor, reduction and isomerization. The rate limiting enzyme is prostaglandin endoperoxide synthetase (30). The type of prostaglandin product which predominate in the tissue would be determined by the relative rate of the enzymes reductase and isomerase. Many factors, e.g. substrate concentration, availability and concentration of reducing agents and

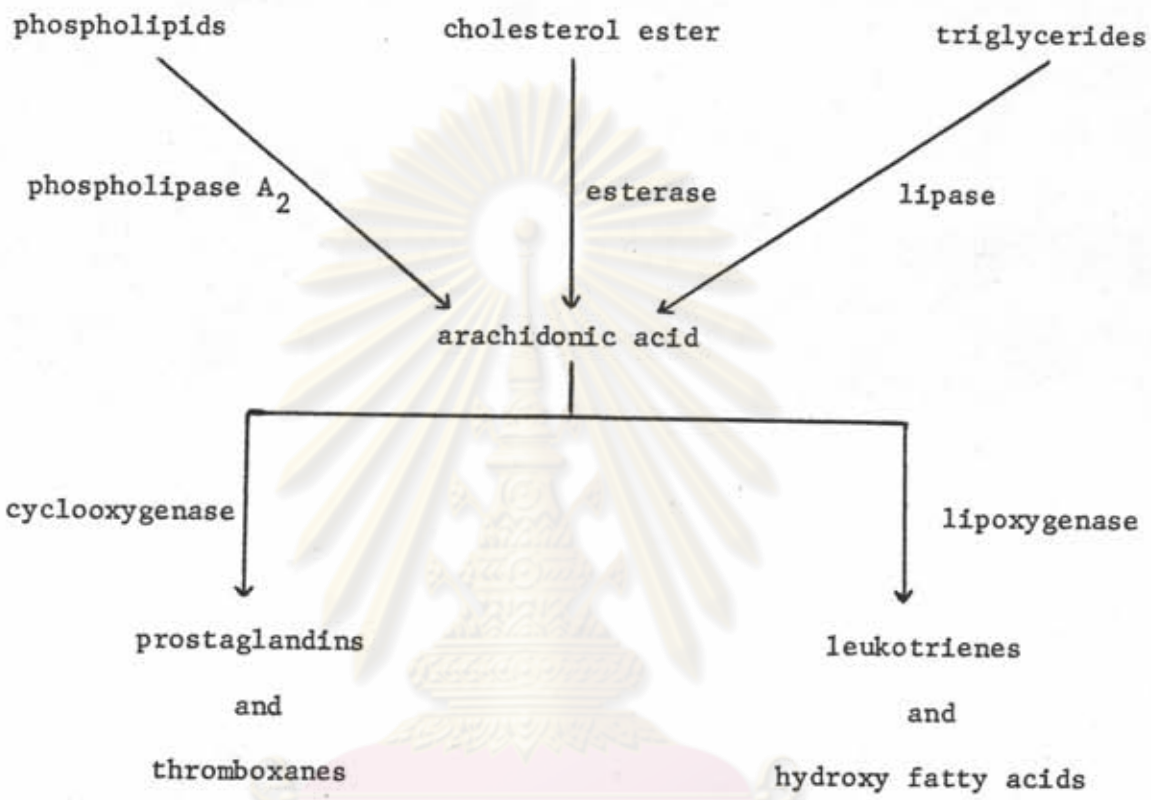


Figure 4 Enzymatic conversion of arachidonic acid (24).

Figure 5 Enzymes and cofactors of prostaglandin and thromboxane biosynthesis (30).

(1) Prostaglandin endoperoxide synthetase

(2) Endoperoxide-D isomerase

(3) Endoperoxide-E isomerase

(4) Endoperoxide reductase

(5) Prostacyclin synthase

(6) Thromboxane A isomerase

(7) Not designated

MDA = malondialdehyde

HHT = 12L-hydroxy-5,8,10-heptadecatrienoic acid

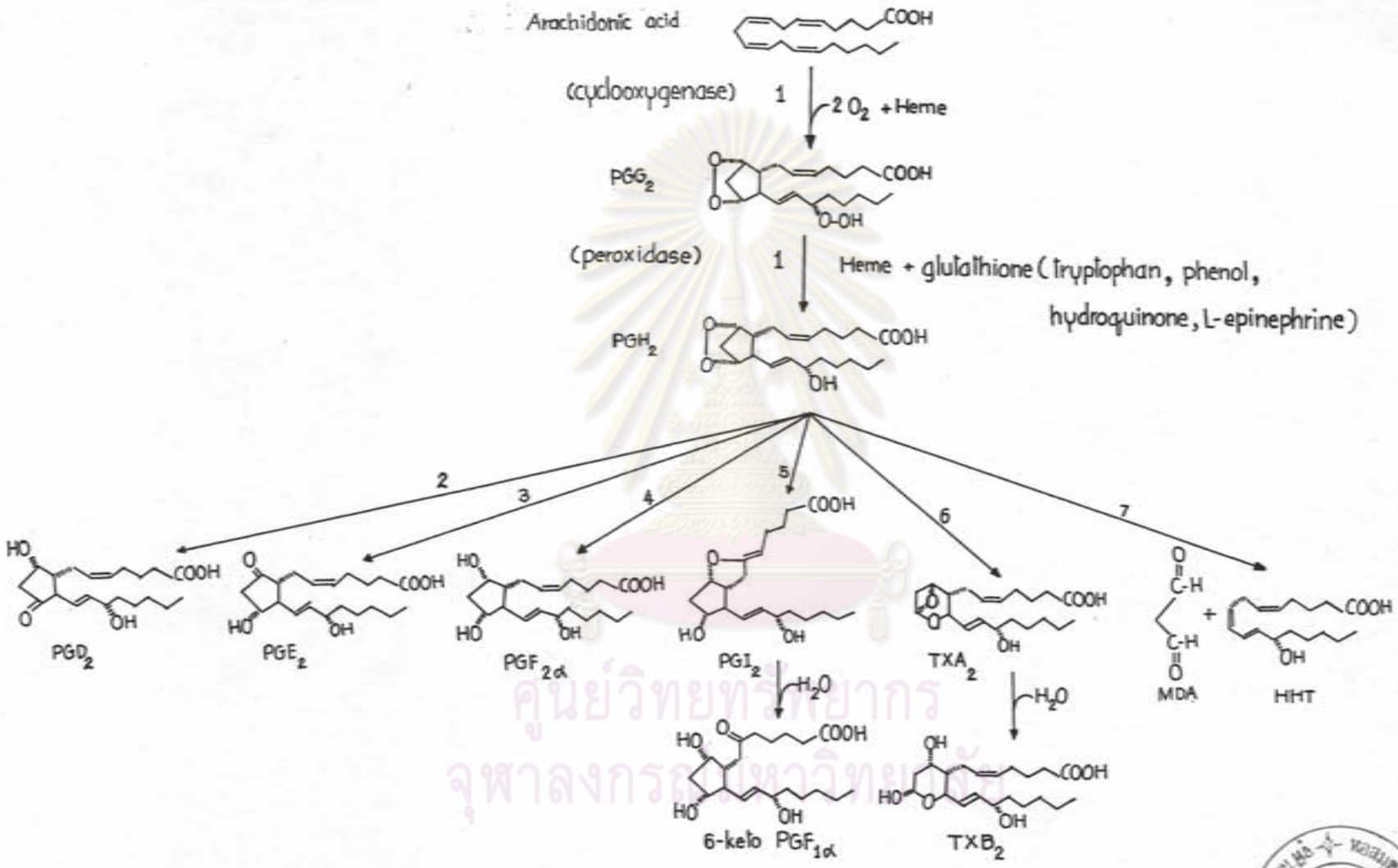


Figure 5

several other physiologic mechanisms, are known to influence which prostaglandin is formed at any one time.

Some of the enzymes of the prostaglandin synthetase complex has been purified and characterized. Since sheep and bovine seminal vesicles posses high enzyme activity, much understanding have been gained from studies in these tissues.

Prostaglandin endoperoxide synthetase is a heme enzyme which converts arachidonic acid into $PGG_2$ and $PGH_2$ has been purified from bovine (31) and sheep vesicular glands (32,33,34). The purified enzyme is a dimer of two identical subunits (MW 72,000 daltons each). Each subunit can bind 2 hemin molecules, but binding of 1 mole of hemin per subunit was sufficient for maximum enzyme activity (32). The catalytic active residue is serine (34,35). This enzyme has two distinct activities : cyclooxygenation and peroxidation. The cyclooxygenase catalyzes the formation of $PGG_2$ and the peroxidase catalyzes the formation of $PGH_2$. Each activity utilizes different substrate and cofactors (31,36). $PGG_2$ is required for the peroxidase activity (37, 38). Peroxidase also required reducing cofactor such as glutathione, tryptophan or phenol. Studies on the kinetics of the cyclooxygenase showed it to be a suicidal enzyme (37). Egan et al. (39) reported that during the conversion of $PGG_2$ to $PGH_2$ an oxidizing agent is liberated which can deactivate the peroxidase as well as the cyclooxygenase activities.

Aspirin and several related compounds inhibit the production of prostaglandins. They act by inhibiting the cyclooxygenase enzyme. It appears that the aspirin-like compounds exert their effects by acetylating the serine residue at the active site (34,35). In addi-

tion, some synthetic analogs of the essential fatty acids and arachidonic acid can competitively bind to the active site and inhibit prostaglandin synthesis.

$PGH_2$ is the precursor of another six enzymes in the prostaglandin synthetase complex. Isomerization will produce PGE, PGD and thromboxane whereas reduction will produce PGF (20).

The physiological functions of prostaglandins depend on their turnover rate. Rapid turnover in matter of minutes is observed in liver, lung and in other tissues (20,30,40). Prostaglandins is metabolized into a ketone compound by prostaglandin dehydrogenase (PGDH) which converts the hydroxyl group at C-15 to the corresponding ketone. PGDH is also found widely distributed in mammalian tissues. Alam et al. (41) reported that PGDH is activated by estrogen and progesterone in decidual and myometrial tissues of pseudopregnant rat. The major urinary metabolite is formed through two steps of β-oxidation and Ω-oxidation to a dicarboxylic acid (14,40).

Prostaglandins in reproduction

Since the first discovery of the action of prostaglandins in human semen in 1930, apprehensive reports on the connection of prostaglandins with both male and female reproduction are accumulating. The knowledge is only rudimentary and it is still uncertain. Though, what direct role prostaglandins do play and if it is a single or multiple mechanism. The vast family of prostaglandins coupled with their very diverse biological actions which the effect produced being dependent upon the tissues and species have made interpretations very difficult.

A. Male reproduction

The source of the prostaglandins in male is the Leydig cells of the testes. At least 13 different prostaglandins and their 19-hydroxylated derivatives had been identified in human semen with the most abundant being $PGE_1$ and $PGE_2$ (42). More recent, it was discovered that the true major prostaglandins in human semen are their more potent 19-hydroxylated products (43). Interestingly, $PGE_1$ and $PGE_2$ are about equal amounts (44). The high concentration of $PGE_1$ is unusual since $PGE_1$ is present in much lower quantities than $PGE_2$ in most tissues and secretions. Studies in bull seminal vesicles showed unusual high level of ω-dihomo-γ-linolenic acid in this tissue. They claimed that the concentration of this fatty acid may be responsible. If a similar situation exists in the human, this may account for the high concentration of PGE in seminal plasma. There are several evidences which also demonstrated that the level of prostaglandins in semen is under the control of testosterone (23).

In male, the possible roles of various prostaglandins in semen, testes and spermatogenesis has been suggested. A very good review of prostaglandins in male was presented by Poyser (23). Over-production and oversensitivity to prostaglandins may cause male infertility. In the rat and mice, prolonged treatment of $PGF_{2\alpha}$ and $PGE_2$ reduces their fertility although the effect was not observed with short treatment (23). The decreased fertility was correlated with disrupted spermatogenesis, less testosterone production by the Leydig cells and atrophy of the accessory sexual glands. In other experiments, it was shown that infertility and impotency may be associated with high plasma level of 15-methyl $PGF_{2\alpha}$ (23). Indomethacin or

removal of the prostaglandin implant could reverse these trends. Some discrepancy were observed with species difference, however. In bulls, monkeys and rabbits, $PGF_{2\alpha}$ increases plasma testosterone and LH output whereas $PGE_2$ has no effect on or decreases testosterone level (23,45,46).

There are reports that infertility in man may be related to low prostaglandins levels. Bygdeman et al. (47) found that in certain infertile patients with clinically unexplained cause, their seminal PGE levels were lower than normal. Kelly et al. (48) also reported that infertile men with abnormally high sperm counts contained lower levels of 19-hydroxy PGE. It is possible that prostaglandins may facilitate sperm penetration and sperm transport in the female reproductive tract (49,50,51). Evidences are that semen invariably causes the uterus to contract (52,53,54). There is also substantial supports that PGE and PGF did increase the number of sperm detected in the Fallopian tubes and the percentage of fertilized eggs (23). Most results did not suggest, however, that prostaglandins will facilitate ejaculation mechanism as is widely postulated.

Whether there is any significant correlation between prostaglandins and male fertility remain to be elucidated.

B. Female reproduction

In a series of reports in the 1960s, Pickles and associates had demonstrated that dysmenorrhea was linked to elevated levels of $PGE_2$ and $PGF_{2\alpha}$ (55). Since then, much interest has been initiated in the role of prostaglandins in female reproduction. As in the case of male, differences amongst species are observed.

Pharriss and Wyngarden (56) were the first to note the luteolytic actions of the prostaglandins. $PGF_{2\alpha}$ depresses progesterone output from the ovary and causes regression of the corpus lutea in subprimates such as sheep, cow, pig, mare, bitch, guinea-pig, rat, hamster, rabbit and mouse (57). It is now recognized that $PGF_{2\alpha}$ directly acts on corpora lutea. Attempts to demonstrate that prostaglandins are also luteolysis in primates have failed so far (58). $PGF_{2\alpha}$, if given together with estradiol does result in premature menstruation in monkey (59). It also inhibits progesterone production by cultured human granulosa cells (60). If the granulosa cells are primed with LH and FSH before exposure to $PGF_{2\alpha}$, the inhibitory effect is much less.

It was found that $PGE_2$ mimics the action of the LH in the ovary in being able to stimulate <u>in vitro</u> ovum maturation, ovulation, luteinization, cAMP accumulation and steroidgenesis (61). If this has any physiologic importance is still uncertain. It is thought that only in ovulation do ovarian prostaglandins have an obligatory role. Like steroids, the levels of PGE, PGF and the activity of prostaglandin synthetase do exhibit a cyclic pattern during the ovulation cycle in mammals (61) although the effective prostaglandin is different amongst species. Extensive studies in rat and rabbit showed peak PGE and PGF in the Graafian follicles around the time of ovulation (62,63). The information concerning ovarian prostaglandin levels during ovulation in other species is more limited. Most evidences, however, all indicate a trend of increased ovarian prostaglandins during or prior to ovulation and that prostaglandins are under steroidal control (64). A question arise, how do prostaglandins bring about ovulation? Prostaglandins may cause contraction of the theca external cells of

the follicle, initiate the synthesis of enzymes involved in the weaking of follicular cell wall and increase the pressure within the follicle (65).

A number of physiologic changes during pregnancy also appear to intimately associate with prostaglandins actions. The action ranges from initiating blastocyst implantation, maintenance of pregnancy and induction of labour. There are evidences that estradiol stimulates an increase in prostaglandin production by rat uterus (66, 67,68). The increase in plasma concentration of estradiol early on Day 4 (69) may be responsible for the increase in uterine prostaglandin synthesizing capacity. This sequence of events is perhaps necessary for blastocyst implantation in the rat. Kennedy (70) proposed prostaglandins as mediators of the uterine vascular response during the initiation of implantation in rats. PGE, PGF and $PGI_2$ levels in the dye site in the rat uterus were higher than elsewhere in the uterus on the evening of Day 5 (70,71). PGE levels are similarly high in the dye site in the hamster (72). Kennedy (73,74) found that $PGE_2$, but not $PGF_{2\alpha}$ or $PGI_2$, increased endometrial vascular permeability, suggesting that $PGE_2$ is the mediator of this process during implantation. Treatment with indomethacin, an inhibitor of prostaglandin synthesis (75,76,77), in rat (70,77), hamster (72), mouse (78) and rabbit (79, 80) delayed implantation. However, $PGE_2$ and $PGF_{2\alpha}$ only partially overcome the reduction in number of implanted blastocyst in indomethacin treated rabbit (71). Similar result was observed in mice (81). In mice, inhibition of implantation by indomethacin could be overcome by exogenous $PGF_{2\alpha}$ and histamine (82). In nonpregnant rat or mouse, artificially induced decidualization is reduced by indomethacin administration (83,84)

as is the change in endometrial vascular permeability (73). Decidualization of the uterus induced in mice by sesame oil or trauma is associated with a rapid 10 fold increase in uterine $PGF_{2\alpha}$ levels (83). In rat increased PGE and prostaglandin synthetase activity was observed (85).

Prostaglandins have been implicated in numerous studies as having important roles in parturition (86-93). At least $PGE_2$ and $PGF_{2\alpha}$ are involved in reducing and maintaining uterine activity during human labour because there are increases in the levels of $PGE_2$ and $PGF_{2\alpha}$ within the amniotic cavity (91,92). In the rat, in vitro studies have shown that the production of uterine PGF increased with gestational age reaching a peak at parturition (87,91,92,94). Wilson and Frienkel (87) showed that the uterine concentrations of PGE and PGF in vivo and net production in vitro increased as labour approaches. In addition, indomethacin delayed parturition and prolonged labour when administered to rats during the final days of pregnancy (95,96). Similarly, treatment of pregnant rats with $PGF_{2\alpha}$ antibody delayed parturition (88). In many species, except primates and guinea-pig, a decline in plasma progesterone level is a prerequisite for parturition. In goats (97), the fall in ovarian progesterone output occurring 24 to 36 h before parturition is associated with an increase in the $PGF_{2\alpha}$ level in uterine venous plasma. Plasma progesterone level in pregnant rat gradually fell from day 15 and then sharply declined after day 19. This abrupt fall is associated with an increase in $PGF_{2\alpha}$ level in uterine venous plasma (98,99). The administration of $PGF_{2\alpha}$ to pregnant goat (97), rat (100) and rabbit (101) caused progesterone level to fall and induced premature parturition. Indomethacin treatment of rabbit during the last part of pregnancy delayed the decline in

plasma progesterone level and parturition was delayed (102). Due to its luteolytic action, $PGF_{2\alpha}$ could induce abortion during pregnancy. Higher doses of $PGF_{2\alpha}$ was needed to induce luteolysis in pregnant than in the non-pregnant animals (93,96).

At term the soften of the cervix is an essential prerequisite for its efficient dilation during childbirth. This is thought to depend upon changes in the interaction between cervix glycoprotein, glycosaminoglycan and collagen at that time (103). In vitro, $PGI_2$ and $PGE_2$ production in sheep by and output from the cervical increased markedly during normal parturition (104). Administration of $PGE_2$ or $PGF_{2\alpha}$ to pregnant rats near term increased cervical extensibility (105). In women treated with $PGE_2$ prior to the induction of labour, softening and dilation of the cervix occurred in most patients (106,107). Consequently $PGE_2$ is now preferred for inducing labour because of its cervical dilation.

The myometrium is usually quiescent during pregnancy due to the influence of progesterone and / or relaxin. There appears also specific binding sites for prostaglandins in the myometrium. These binding sites are under the influence of steroid hormones (108,109, 110). The administration of $PGE_2$ and $PGF_{2\alpha}$ will initiate uterine contraction and cause expulsion of the foetus when administered at any stage during human pregnancy. Large doses of prostaglandins are usually required during mid-pregnancy than are required at term. $PGE_2$ is more potent than $PGF_{2\alpha}$ (111). The major prostaglandin synthesis by the myometrium is $PGI_2$, and its release from the rat myometrium in vitro is stimulated by oxytocin (112). As contraction of the rat uterus produced by oxytocin are potentiated by $PGI_2$, any interaction

between prostaglandins and oxytocin on the myometrium may specifically involve $PGI_2$ (113).

The finding by Bydgeman et al. (114) that prostaglandins contract human uterine smooth muscle was followed by the use of these agents in terminating pregnancy. Initially, $PGE_1$, $PGE_2$ and $PGF_{2\alpha}$ were used to induce labour at term (115,116,117). $PGE_1$ and $PGE_2$ are more potent than $PGF_{2\alpha}$. The efficacy of prostaglandins and oxytocin for induction of labour was similar. But the use of prostaglandins often produced excessive side effects. Clinically, prostaglandins were used to induce first and second-trimester abortion (118) and to terminate pregnancy in the conditions where oxytocin is ineffective (119). Another possible mechanisms in prostaglandin-induced abortion are the cervical dilation effect of $PGE_2$ and the vasoconstriction effect by $PGF_{2\alpha}$. The latter action is associated with a reduction on plasma progesterone, but this is likely to be an indirect luteolytic action brought about as a result of strong uterine stimulations dislodging the blastocyst and thus removing the trophic influence of the conceptus. Successful abortions were preceding by a decline in plasma progesterone levels which indicates that a decrease in progesterone secretion is required (120).

In conclusion, it is demonstrated that prostaglandins involve in many physiological processes of female reproduction but their role is still not entirely clear. Many important findings have led to major advances in clinical medicine and animal husbandry and to further understand the events in the reproductive cycle.

Rationale

The intrauterine device (IUD) prevents fertilization and embryogenesis in many species. The exact nature of the mechanism is not known. Evidences showed that the antifertility effect by IUD may be a multiple mechanism and IUD may act differently in different species. IUD prevents fertilization in the sheep by affecting sperm transport mechanism (121) and may stimulate phagocytosis and / or cytolysis of the sperm (122). IUD also affects ovulation of Indian water buffaloes (123), interferes with the development of the corpora lutea of many mammals (124,125) and blocks blastocyst implantation in the rabbits (126), rats and hamsters (127). It is generally believed that IUD elicits inflammation of the endometrium (128,129). The infiltrating macrophages or their lysate could destroy sperm, blastocyst or render the endometrium unfavorable for implantation. Since prostaglandins are believed to be mediators of inflammatory responses, it is plausible to propose that prostaglandins may play a major role in the contraception effect of an IUD. Chaudhuri suggested that one of the antifertility action of the IUD could involve prostaglandins (130). Later, it was found that the presence of an IUD is associated with the increased $PGF_{2\alpha}$ in the uterus of rats, hamsters (127), guinea-pig (131), sheep (132), mice (133), rabbits (134) and baboons (135). In guinea-pig (131) and sheep (132), IUD stimulates the release of $PGF_{2\alpha}$ and causes premature luteal regression. In support of these evidences, Myatt et al. (136) and Apisitpaisarn (137) reported that IUD causes the decrease of progesterone receptor in rat uterus.

Research aim

From the above evidences, it is interesting to elucidate whether IUD acts by mediating de novo prostaglandin production in utero. In this thesis, a hypothesis that IUD enhances the activity of prostaglandin synthetase in the rat uterus is proposed. To test this hypothesis, the activity of the enzyme in the control and IUD uteri during the estrous cycle will be compared. The enzyme activity will be measured by a) quantitating the prostaglandin products of $^{3}H$-arachidonic acid by radiochromatographic method; and b) spectrophotometric measurement of the enzyme-dependent formation of adrenochrome from L-epinephrine by the modified method of Takeguchi and Sih (138). The reaction is shown in Figure 6. In this thesis, some factors which could influence the enzyme properties will also be studied.

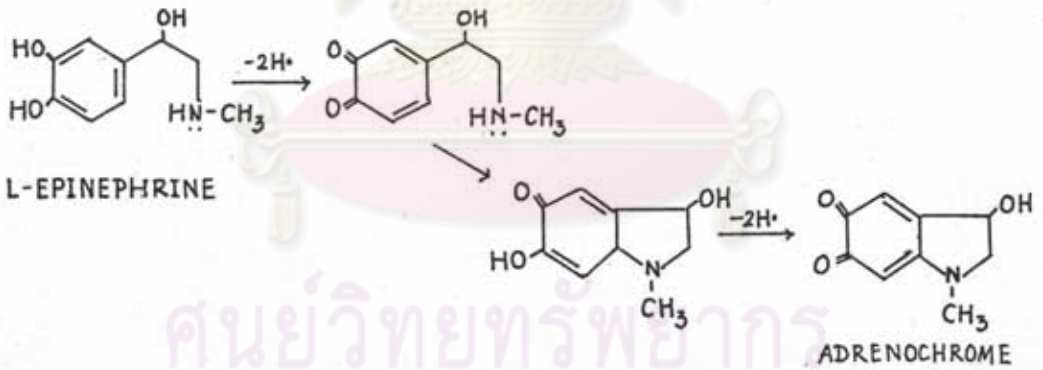


Figure 6 The autooxidation of L-epinephrine to adrenochrome (138).