CHAPTER IV

DISCUSSION

There were evidences that IUD increased PGE level in the uterus of many species (132,133,134,135). It has been suggested that IUD may act by releasing prostaglandins (130). In the present study, the prostaglandin extracts from both the control and IUD horns failed to show any detectable band on TLC that is corresponding to PGE or PGF (Figure 8). This may be due to the detection limit of the technique itself. Thaler-Dao et al. (67) who studied the prostaglandin biosynthesis by the rat uterus during estrous cycle reported very low (range in nanogram) $PGF_{2\alpha}$ and PGE_2 production in vitro. In addition, loss due to extraction process could also reduce the already minute amount of prostaglandins in the extracts. Both reasons would resulted in too low amount of prostaglandins for measurement by TLC. To increase the sensitivity of the detection technique, radiochromatographic technique was employed. In vitro conversion of ^{3}H-arachidonic acid by crude microsome of the uterus was studied by TLC. Still, only very small amount of the label was incorporated into the fractions corresponded to the standards PGE and PGF (Figure 9); with PGF at the basal level and PGE just slightly above the basal level. Since changes in the products produced by prostaglandin synthetase under different conditions had been reported by Takeguchi et al. (150) and many authors have described synthetase preparations that produced only PGE in the presence of glutathione or some other cofactors (17), thus the observed higher incorporation into PGE may not reflect the in vivo

situation of prostaglandin production, in rat uterus. Thaler-Dao (67), Ham et al. (68) and Van Orden et al. (151) have earlier showed that rat uterus contained higher amount of endogenous PGF than PGE at every stage of the estrous cycle. In their results prostaglandins were quantitated by radioimmunoassay technique. The very low incorporation observed in the radiochromatogram can be explained as follows : a) low activity of the prostaglandin synthetase in rat uterus; b) the labeled products were lost during the extraction process as evidence from the observation that only 8% of ^{3}H-arachidonic acid was recovered in the thin-layer plate (data not shown); and c) the presence of endogenous arachidonic acid in the homogenate (Figure 8) might dilute the radio-active pool available for the enzyme. Flower and Ramwell (17) reported that the addition of mepacrine, an anti-malarial drug, could prevent the release of arachidonic acid from phospholipid pool within homogenate. This study did not include mepacrine in the incubation system, however.

The amount of prostaglandins produced by prostaglandin synthetase as measured by radiochromatographic method was too little to discriminate any difference, if there is any, between the control and IUD horns. Thus, another quantitative method was employed. This assay method based on the action of prostaglandin synthetase to convert L-epinephrine into adrenochrome (138). It could be shown that the microsome from rat uterus does contain the enzyme of interest (Figure 10 and 11). The K_m for arachidonic acid and V_{max} of the enzyme at estrus were 1.50 μM and 0.94 mmole adrenochrome per min, respectively. Although wide biological variation between animals existed, the tendency pointed toward indication that the presence of an IUD enhanced the prostaglandin synthetase at all stages of the

estrous cycle (Figure 20, 21 and 22). This finding agrees with the observations by Chaudhuri (152) and Saksena et al. (127) who reported a rise in the PGF level in the IUD horn of rat and hamster. Similar effect was reported in sheep (132), mice (133), rabbit (126) and baboons (135). The increase of prostaglandin synthetase activity may directly associate with the increase of prostaglandin level in the IUD horn. Thus, the elicited prostaglandin release by IUD may play an important role in the contraceptive mechanism. Such idea was supported by the consistent demonstration that IUD increased the uterine PGF content of pregnant rat on Day 4 of pregnancy (127). At that time, IUD also decreased progesterone secretion of the ovary (153). PGF was a luteolysin in many animals (57). Spilman and Duby (132) suggested that $PGF_{2\alpha}$ mediated tha anti-fertility effect of IUD by its luteolytic effect. Apisitpaisarn (137), in addition, also observed a decrease of progesterone receptor in the IUD-bearing horn of cycling rat.

The present study also demonstrated that prostaglandin synthetase activity fluctuated during estrous cycle. The activity was lowest during estrus. At other stages (proestrus, diestrus 1 and diestrus 2) the enzyme activity was about the same (Figure 22). Thaler-Dao et al. (67) also found that $PGF_{2\alpha}$ and PGE_2 synthesis in rat uterus is higher at proestrus than at estrus. Similarly, Van Orden et al. (150) and Ham et al. (68) reported highest endogenous $PGF_{2\alpha}$ and PGE_2 concentration at proestrus. Their findings, however, showed very low level of prostaglandin of both types at diestrus 1 and 2. Consistently, Bauminger and Lindner (154) had shown that the prostaglandin synthetase in the rat ovary increased 1.7 fold during the evening on the day of proestrus and reached a peak in the early

hours on the day of estrus, at about the time of ovulation. The ovarian prostaglandin and enzyme levels then declined to basal value by late morning in the day of estrus. The same result was obtained by assessing the concentrations of PGE and PGF in the Graafian follicles. In contrast, Poyser and Scott (155) showed that there was no daily variation in the PGE_2 and $PGF_{2\alpha}$ levels in the uterus and ovary when assayed between 10:00 and 12:00 h on each day of the estrous cycle. Variation was observed in the concentration of 6-keto $PGF_{1\alpha}$ (which they claimed is the major prostaglandin synthesized by the uterus) with higher level on estrus than on diestrus. They pointed out that the reason for such discrepancy may lie in the design of experiments, i.e., timing of sample collection, type and preparation of tissue, utilization of endogenous or exogenous arachidonic acid, cofactors or factors within the incubation system, etc. Whether uterine prostaglandin production varies within the ovulation cycle and whether it has any physiological significance remain to be elucidated. To confirm this, the author would like to suggest that it will be relevant to also study the production of 6-keto $PGF_{1\alpha}$, the major prostaglandin synthesized by rat uterus as claimed by Poyser and Scott (155). More sensitive method such as radioimmunoassay or gas chromatography-mass spectrometry should also be employed for direct quantitation of prostaglandins in further study.

Eventhough it is still not conclusive about the true pattern of variation in prostaglandin production <u>in utero</u> of cycling rat, evidences by this study and by other authors seemed to suggest that prostaglandin synthetase might be controlled by steroid hormones. Several studies have shown that estradiol could stimulate prostaglandin

production in rat uterus (67,68,156,157). How estrogen acts on the enzyme is not known. Recently, Dey et al. (158) proposed that estrogen regulated PGE_2 and $PGF_{2\alpha}$ production in rat uterus by stimulating phospholipase A_2 which is responsible for the release of arachidonic acid from phospholipids. They also suggested that progesterone might modulate the enzyme activity by regulating the amount of available arachidonic acid for the enzyme.

Increasing amount of microsomal protein could decrease the activity of prostaglandin synthetase (Figure 16). The inhibition might be a result of increased protein concentration which nonspecifically inhibit the enzyme as supported by the inhibitory effect of BSA (Figure 23). There is evidence that BSA can bind to fatty acid (159). Such binding may either interfere with the available arachidonic acid in the system or disturb the membrane bound enzyme. The present study does not rule out, however, if any intrinsic component(s) of the microsome is responsible for the inhibition.

The cytoplasm may also contain factor(s) which affects the enzyme. The cytosol isolated from rat liver (147), rabbit kidney medulla (148,160) and bovine seminal vesicle (150) were found to inhibit prostaglandin synthetase. In contrast, the isolated cytoplasm of human fetal membrane, uterine decidua vera and placenta (149) showed stimulatory effect on prostaglandin biosynthesis. If the crude microsome preparation from rat uterus was contaminated with these factors from cytoplasm, the higher amount of microsome could mean an increase in the inhibition (or activation) of the enzyme. Studies on the addition of CF to the microsome indicated that there

were both inhibitor(s) and activator(s) of the enzyme in the CF (Figure 24 and 25). The inhibitory effect was more pronounced when higher amount of CF was added (Figure 24B, C and D) suggested that the inhibitor might be more potent than the activator. Heat inactivation and dialysis experiments (Figure 24 and 25) indicated that the inhibitor was heat-labile and undialysable, large molecule—probably a protein. Similar properties of the inhibitor was reported in the cytoplasm of rabbit kidney medulla (148,160). Whether the inhibitor is a specific protein within the CF or just any protein which at high concentration can nonspecifically inhibit the enzyme (as in the case of BSA) could not be concluded from this study. The activator in CF appeared to be heat stable and small molecule. These results are another indication that prostaglandin production in the uterus is regulated by factors in the cytoplasm.

The observed effect of CF was not due to the spontaneous oxidation of L-epinephrine into adrenochrome since the reaction by CF alone was very low in comparison to the enzyme catalyzed reaction (Figure 24). Moreover, the same experiment showed that boiling of the CF abolished the spontaneous production of adrenochrome whereas boiled CF still could stimulate the enzyme activity.

In conclusion, the present study showed that

a. TLC failed to detect any endogenous prostaglandins in the uterine horn. The result may be due to the detection limit of the technique. Increasing the sensitivity of detection by using radiochemical substrate demonstrated basal levels of prostaglandin produced by rat microsome. The result from the radiochromatogram could not discriminate the extent of enzyme activity between the control and IUD horns.

b. An optimum condition for the measurement of prostaglandin synthetase activity in rat uterus by spectrophotometric method was established.

c. IUD increased prostaglandin synthetase activity in that uterine horn at all stages of the estrous cycle.

d. Prostaglandin synthetase activity fluctuated during the estrous cycle. The enzyme activity was least at estrus, while comparatively the same activity was observed at proestrus, diestrus 1 and diestrus 2.

e. Activator and inhibitor of the enzyme were present in the cytoplasm of rat uterus. The activator was small, heat-stable molecule. The inhibitor was heat-labile macromolecule – probably protein(s) in nature. The inhibitor may be a specific protein within the CF or any protein which at high concentration could nonspecifically inhibit the enzyme.

f. High amount of protein could inhibit the enzyme activity by non-specific type of inhibition.