CHAPTER III

RESULTS

1. Measurement of prostaglandin synthesis by radiochromatographic assay

1.1 Solvent system for the separation of prostaglandins from arachidonic acid metabolites by TLC

A suitable solvent system which would separate and give good resolution of the desired products, namely prostaglandins of the E and F series, from other metabolites of ^{3}H-arachidonic acid or other microsomal lipids must first be established before quantitation of prostaglandins is possible. In this attempt, several common solvent systems employed in many laboratories were examined as reported in Table 1. Standard compounds were used and the procedure of TLC is referred to Materials and Methods.

It can be seen from the results in Table 1 that all solvent systems studied can separate PGE from PGF, but they do not enable separation of homologs (e.g. PGE_1 from PGE_2 or $PGF_{1\alpha}$ from $PGF_{2\alpha}$). It also showed the solvent systems S_1 and S_2 give better resolution of PGE and PGF than system S_3-S_5. Although S_1 and S_2 are similarly efficient in the separation of PGE from PGF, S_1 can separate the commercially available arachidonic acid into 2 spots (R_f values = 0.77 and 0.86) whereas S_2 gives only one spot of arachidonic acid (R_f value = 0.98). S_1 also required less time and less expensive solvents

Table 1 Thin-layer chromatography of prostaglandins, arachidonic acid and phospholipids by various solvent systems.

Solvent system	Ref. No.	R_f value							
		AA	PGE_1	PGE_2	$PGF_{1\alpha}$	$PGF_{2\alpha}$	PE	PS	PC
S_1 = ethyl acetate : acetone : acetic acid = 90:10:1	141	0.77 0.86	0.57	0.58	0.35	0.34	0	0	0
S_2^* = organic phase of ethyl acetate : 2,4,4-trimethylpentane : acetic acid : water = 11:5:2:10	141	0.98	0.74	0.71	0.51	0.51	0	0	0
S_3^{**} = benzene : p-dioxane : acetic acid : formic acid = 82:14:1:1 and acetone : dichloromethane = 60:40	143	0.78	0.71	0.72	0.57	0.57	-	-	-
S_4^{**} = organic phase of ethyl acetate : 2,4,4-trimethylpentane : acetic acid : water = 11:5:2:10 and acetone : dichloromethane = 60:40	†	0.89	0.58	0.57	0.43	0.45	-	-	-

Solvent system	Ref. No.	R_f value							
		AA	PGE_1	PGE_2	$PGF_{1\alpha}$	$PGF_{2\alpha}$	PE	PS	PC
S_5** = benzene : p-dioxane : acetic acid = 80:20:2 and organic phase of ethyl acetate : 2,4,4-trimethylpentane : benzene : acetic acid : water = 100:30:20:10:100	69	0.70	0.42	0.40	0.31	0.31	-	-	-

* The TLC plates were developed twice in the same dimension.

** The TLC plates were developed in the first solvent and redeveloped in the second solvent in the same dimension but only ran for $\frac{2}{3}$ of the solvent front.

- = Not determined.

† = Newly established method by the author.

for running the chromatography. Considering all these advantages, the solvent system S_1 was chosen for further experiments with TLC.

The separation by solvent systems S_1 and S_2 of some major phospholipids which might be found in the microsome preparation was also investigated. All phospholipids tested remained at the origin of the chromatogram in both systems (Table 1).

1.2 Prostaglandins levels in the control and IUD uterine horns

Firstly, attempts to see if there is any different in the levels of prostaglandins in the control and IUD uterine horns were carried out. Homogenate of whole rat uterus were extracted for arachidonic acid and prostaglandins by ethyl acetate (144). Figure 8 showed that no prostaglandins bands appeared in both the control (4.28 mg protein) and IUD (4.43 mg protein) horns, only the arachidonic acid band was observed. The stain of the arachidonic acid band from the IUD horn was slightly intenser than that of the control horn which may be due to the fact that the control horn is slightly smaller.

1.3 Comparison of prostaglandins synthesis between control and IUD uterine horns

In vitro prostaglandins synthesis by crude microsomal enzymes was compared between the control and IUD horns. ^{3}H-arachidonic acid, their precursor, was used as tracer compound, and the procedure was described in Materials and Methods. In these studies, only rats at estrus stage were used.

A typical result of the chromatogram and radioactive dis-

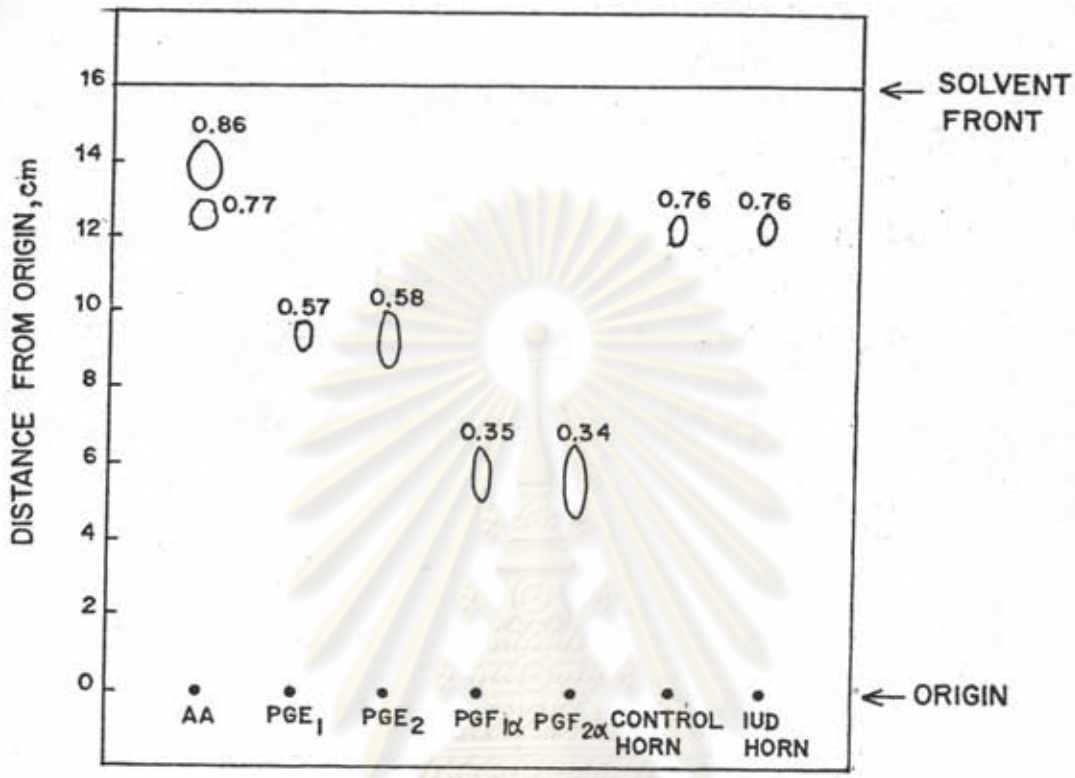


Figure 8 Thin-layer chromatography of tissue extracts from control and IUD horns of the estrus rat.

The control horn was 4.28 mg protein/gm wet weight of uterine horn and IUD horn was 4.43 mg protein/gm wet weight of uterine horn. The number above the iodine-stained spot indicates the R_f value. Homogenates from whole uteri of control and IUD horns were prepared, extracted and chromatographed. In this experiment the homogenates were not incubated with ^{3}H-arachidonic acid and external standards were not added to the samples. The solvent system employed for chromatography was solvent system S_1.

tribution is shown in Figure 9 A and B respectively. Only 29-30% and 21-26% of the label appeared in PGE and PGF respectively. The amount of incorporation into both compounds by the enzyme from either the control and IUD horns are comparatively the same. Figure 9 B also shows that the spot corresponded to arachidonic acid is the slower mobile spot (R_f value = 0.77 in S_1).

To confirm if there is really no significant different in prostaglandin synthetase activity between the control and IUD horns or that the unobserved difference was due to the detection limit of the radiochromatographic technique, the enzyme activity (the oxygenase enzyme) would also be measured by spectrophotometric means. This is based primarily on the method described by Takeguchi and Sih for bovine seminal vesicle (138).

2. Measurement of prostaglandin synthetase by spectrophotometric assay

2.1 Optimization of spectrophotometric assay conditions for prostaglandin synthetase from rat uterus

It is clearly shown from the absorption spectra in Figure 10 that the present enzyme system produces a product with maximum absorption at 480 nm. Figure 11 shows that this chromogen is an enzyme-catalyzed product. The characteristic maximum absorption of the compound and dependency on enzyme catalysis for its formation corresponded to the reported substrate-dependent formation of adrenochrome by prostaglandin synthetase (141). Thus, it is evident that the uterine microsome fraction contains the enzyme of interest. The optimum assay condition for this enzyme complex in rat uterus was then calibrated in

Figure 9 Thin-layer radiochromatogram of labeled prostaglandins E and F formed by crude prostaglandin synthetase prepared from control (Δ——Δ) and IUD (·---·) uteri of estrus rat.

The crude enzyme (600 µg protein) was incubated with 1 mM ^{3}H-arachidonic acid (1 x 10^{5} dpm) at 37°C for 5 min in 500 µl of 50 mM Tris·HCl buffer, pH 8.2, containing 5 mM each of L-epinephrine and reduced glutathione. The procedures of tissue extraction and chromatography were described in Materials and Methods. The solvent system S_1 was employed for TLC chromatography. The insert (Figure 9A) shows the iodine-stained chromatogram on TLC plate. Figure 9B shows the radioactivity of Figure 9A. The efficiency of the radioactive counting system is 35-40%.

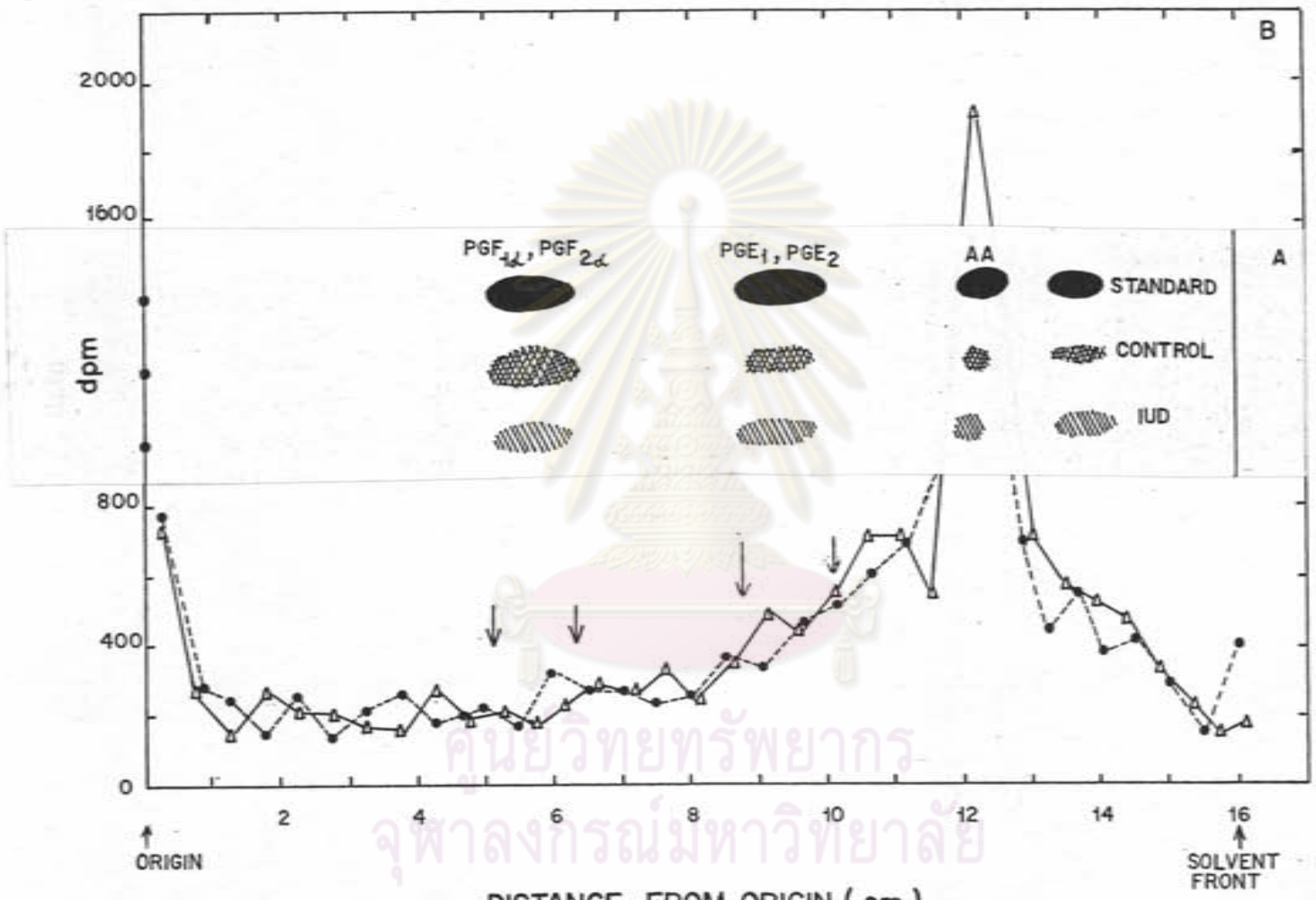


Figure 9

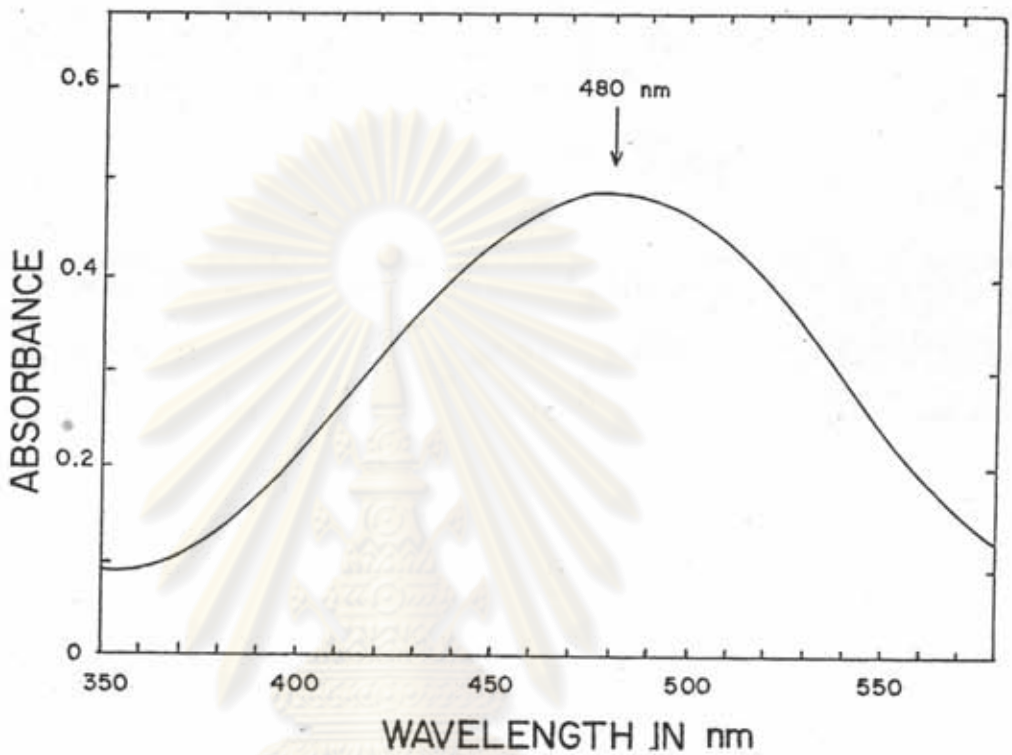


Figure 10 Absorption spectra of adrenochrome from L-epinephrine by uterine microsomal enzyme.

The reaction mixture contained the crude enzyme (100 µg protein), L-epinephrine (6.0 mM) with 0.5 mM arachidonic acid in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. Enzyme activity was measured after the reaction mixture was incubated for 5 min. The absorbancy was scanned automatically in a Beckman 25 Spectrophotometer.

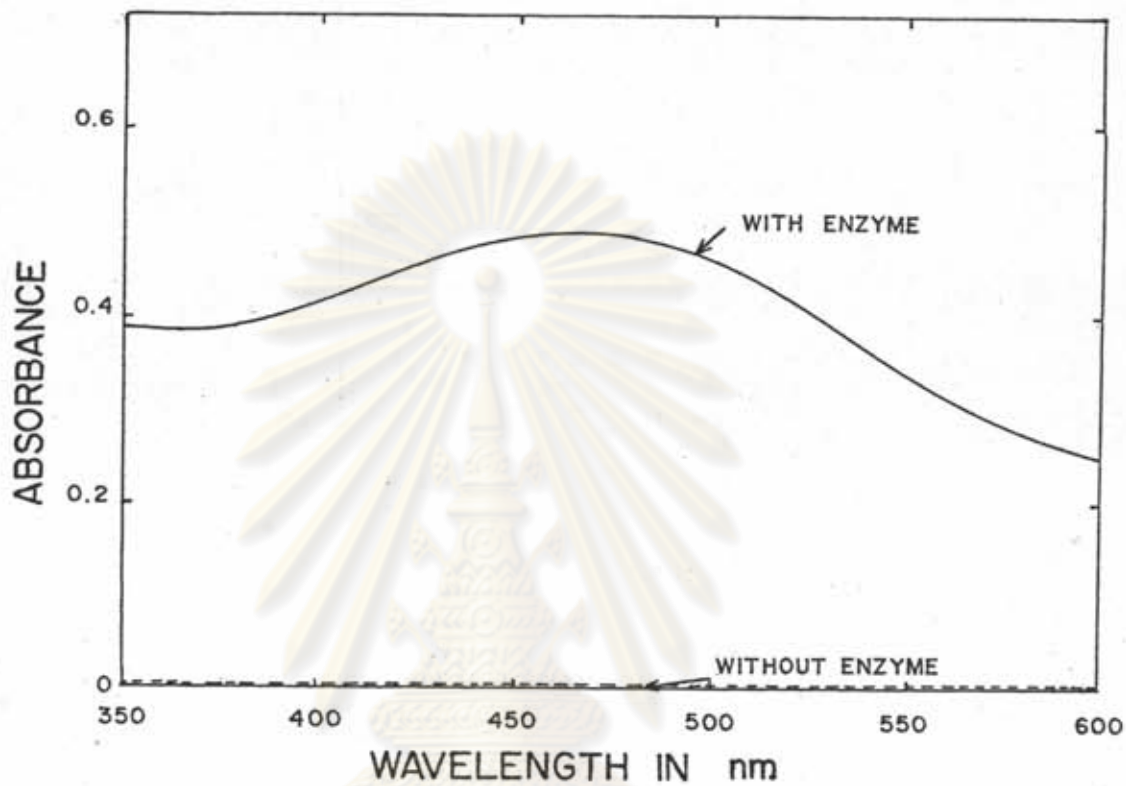


Figure 11 Formation of adrenochrome from L-epinephrine.

The incubation was performed with or without 500 µg microsomal enzyme. The assay mixture contained 6.0 mM L-epinephrine, 0.5 mM arachidonic acid in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. Enzyme activity was measured as described in Materials and Methods. This experiment was repeated twice.

normal rat at estrus stage.

2.1.1 The effect of preincubation time

The incubation mixture was preincubated for various times and the absorbancy at 480 nm was then measured immediately. Figure 12 shows the results of 3 separate experiments. It could be seen that preincubation of different enzyme preparations gave varying saturation time and maximum absorption. In most of the later experiments, though, the absorbancy after 5 min of preincubation was close to that of Experiment III. Moreover, there is evidence that the prostaglandin cyclooxygenase is irreversibly self-deactivated during the oxygenation of arachidonic acid (39). Therefore, preincubation of the enzyme mixture for 5 min before adding arachidonic acid to the assay system was chosen.

Addition of exogenous arachidonic acid after preincubation would markedly increase the absorption at 480 nm (Figure 13). An example from 6 experiments showed that the absorption increased from 0.189±0.036 to 0.821±0.070. The absorbancy before the addition of arachidonic acid was lower by 23%.

2.1.2 The effect of incubation time

The incubation time of the enzyme solution was also varied. Figure 14 showed that there is a time-dependent formation of adrenochrome during the first 10 min of incubation after which saturation is reached. All subsequent assays were then incubated for 12 min.

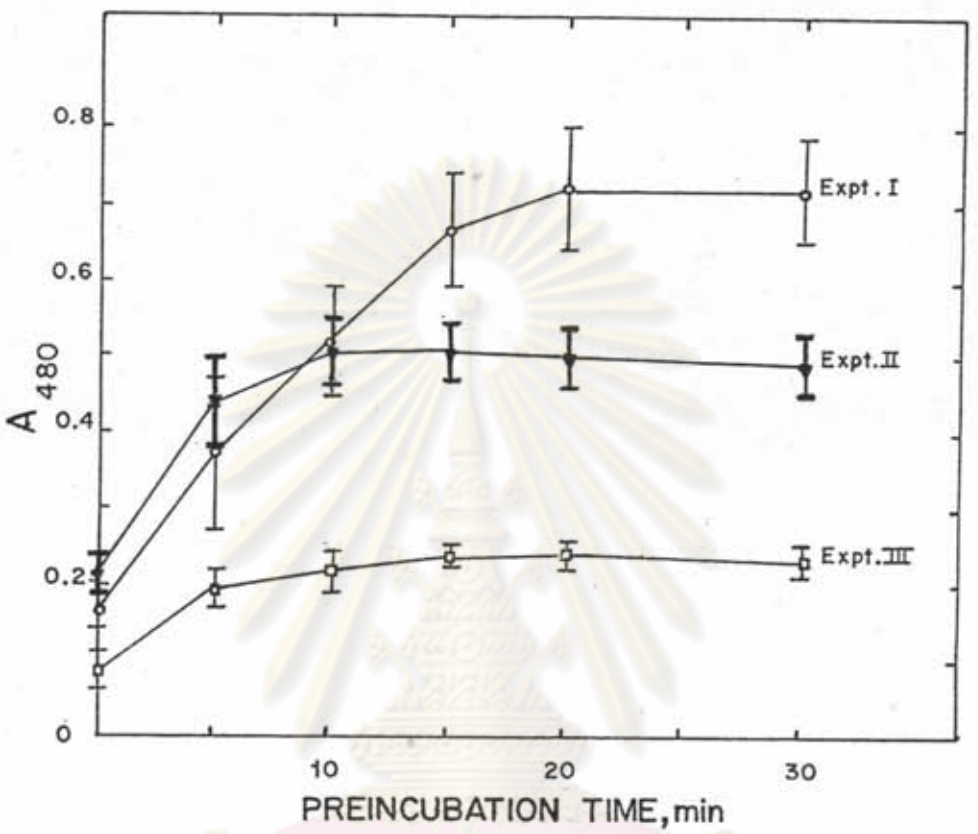


Figure 12 The effect of preincubation time on adrenochrome formation.

The crude enzyme (100 µg protein) was preincubated with L-epinephrine (6.0 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2, at 25°C for various periods. Absorbancy at 480 nm was immediately measured after each preincubation time without the addition of exogenous arachidonic acid. The vertical bar represents the mean ± S.E.M. from 3 experiments.

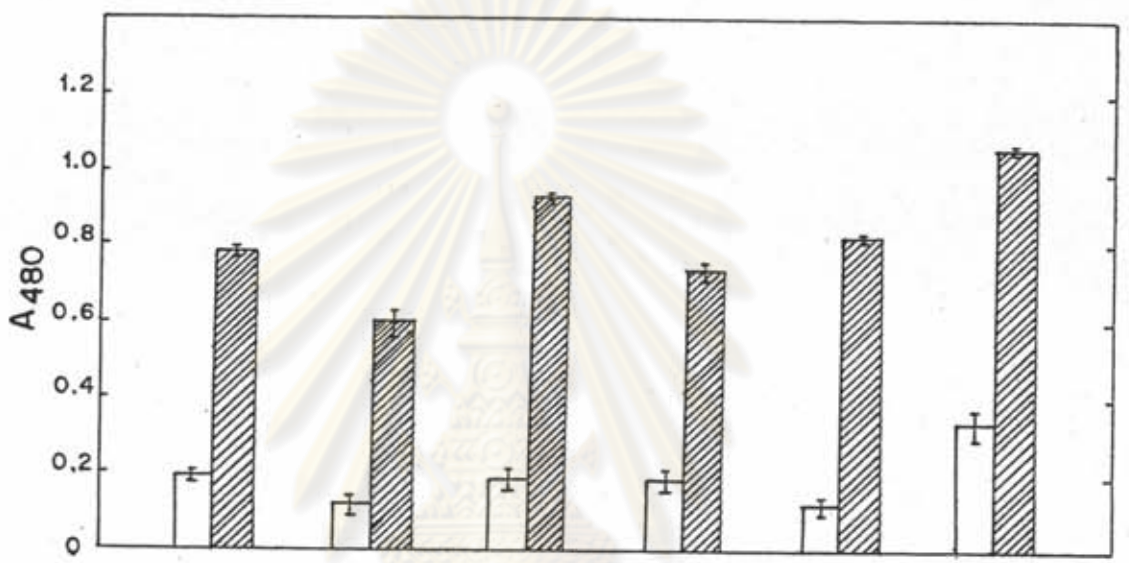


Figure 13 The absorbancy at 480 nm of the assay mixture before (□) and after (▨) the addition of exogenous arachidonic acid.

Enzyme activity of the shaded bars were measured by standard procedure as described in Materials and Methods, whereas the opened bars were measured immediately after preincubation for 5 min.

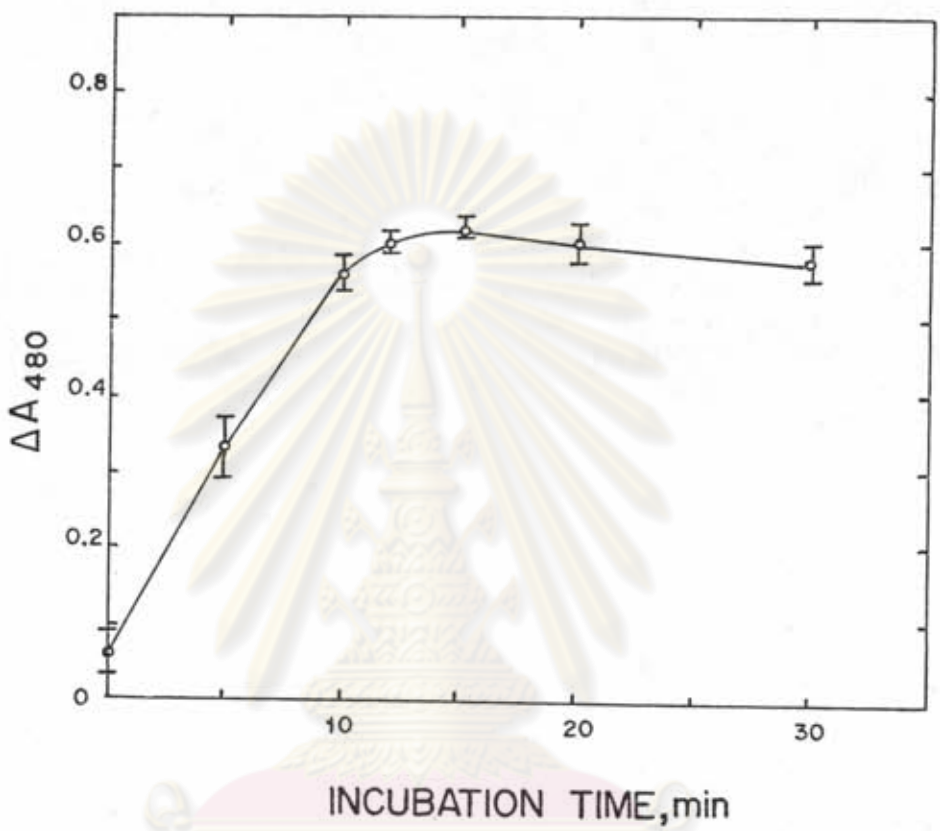


Figure 14 The effect of incubation time on the rate of adrenochrome formation.

The reaction mixture contained the crude enzyme (100 µg protein), L-epinephrine (6.0 mM) and arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. The assay condition was described in Materials and Methods. Each bar represents the mean±S.E.M. from 3 experiments.

2.1.3 The effect of preincubation temperature

The effect of preincubation temperature on the enzyme activity was also studied. The result is shown in Figure 15. Figure 15 showed that the rate of reaction increased as a function of preincubation temperature. A slight delay (about 1 min) of adrenochrome formation was observed at low temperatures (25°C, 15°C, 4°C). The rate at 4°C was slowest and still did not reach maximum at 12 min. The reaction rates at 25°C and 15°C were more moderate and reached the same maximum. The rate at 25°C is slightly faster than 15°C. The reaction rate at 37°C was most rapid and reached a plateau within 2 min. Since the assay mixture did not cool to the desired temperature when arachidonic acid was added to initiate the reaction, this may indicate that this enzyme operated better at 37°C. The observed result may also due to the liberate of large amount of arachidonic acid by phospholipase A_2 during preincubation at 37°C.

Since 25°C is also easily maintained in an air-conditioned room without the use of any thermostat controlled equipment, the preincubation temperature at 25°C was chosen. The incubation temperature was also carried out at 25°C. As a thermoregulated spectrophotometer is not available in this laboratory, the study on the effect of incubation temperature is not possible.

2.1.4 The effect of protein concentration

Figure 16 showed that the activity of the enzyme increased with protein concentration up to at least 300 µg microsomal protein. Maximum activity was observed at 300-500 µg protein. A noticable decreased in the enzyme activity was always observed in every set of experiment if the protein concentration was increased to 600 µg or more.

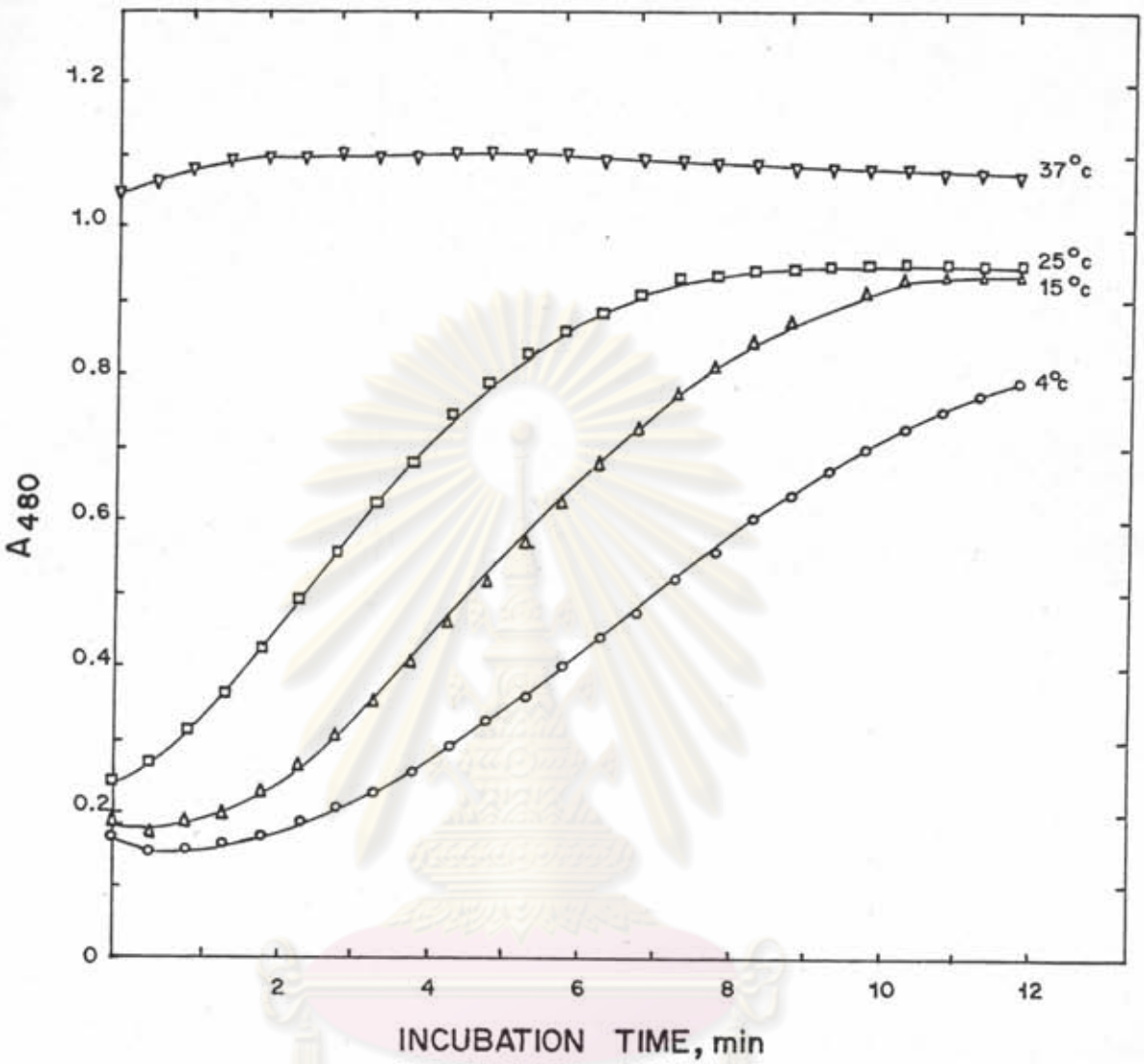


Figure 15 Effect of preincubation temperature on the rate of adrenochrome formation.

Preincubation of the assay mixture containing 100 µg protein of crude enzyme and 6.0 mM of L-epinephrine was carried out at various temperatures for 5 min prior to the addition of arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris.HCl-2% Tween 40 buffer, pH 8.2. Enzyme activity was measured for 12 min as described in Materials and Methods. This experiment was done thrice.

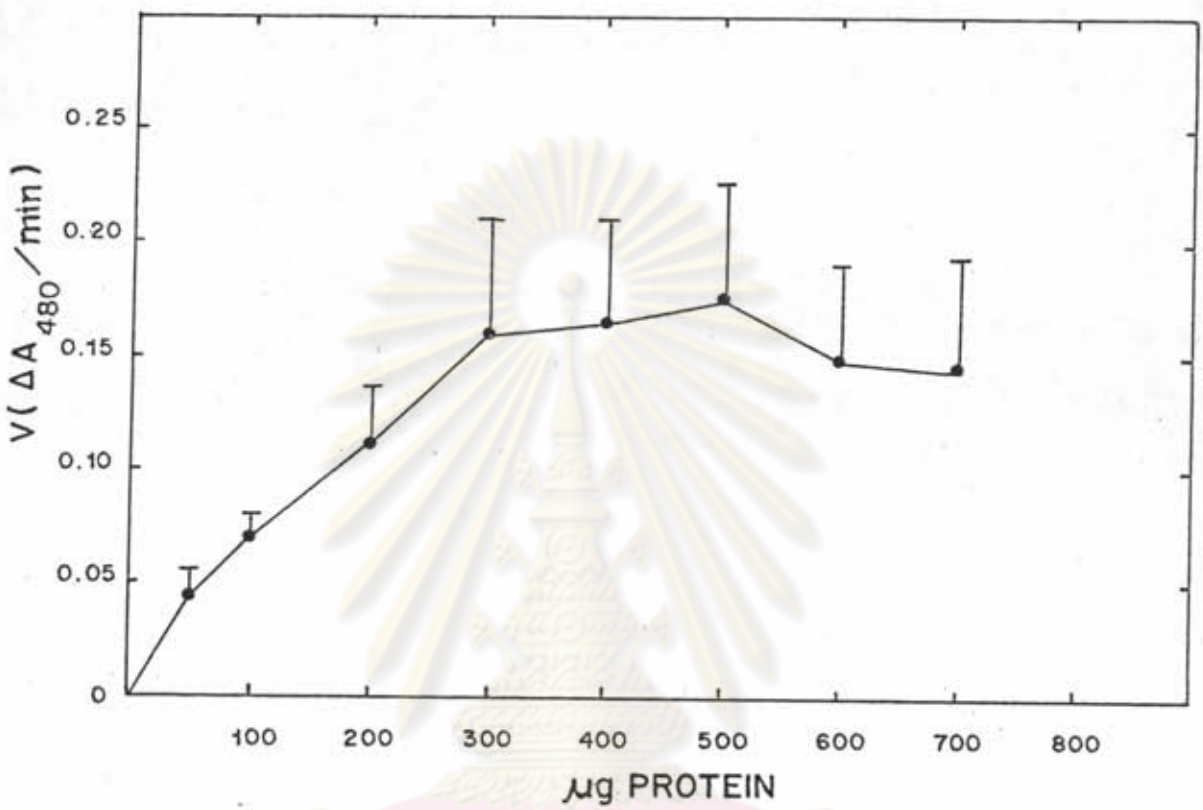


Figure 16 Crude prostaglandin synthetase activity as a function of enzyme concentration.

Varying amount of the crude enzyme (50-700 µg protein) was preincubated with L-epinephrine (6.0 mM) at 25°C for 5 min before the addition of arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. Enzyme activity was measured as described in Materials and Methods. The vertical bar represents the mean ± S.E.M. from 3 experiments.

V = initial velocity.

2.1.5 The effect of detergent

Prostaglandin synthetase is a membrane protein. In many cases, it was found to be embedded in the endoplasmic reticulum (31,33,145). This enzyme may be partially solubilized with non-ionic detergents such as cutscum, Tween 40, Tween 20, TritonX-100 (31,33,146). Rome and Lands (146) reported that 2% Tween 40 could solubilize 80-90% of the oxygenase of sheep vesicular gland. This study wanted to examine whether Tween 40 has the same effect on the enzyme in rat uterus. Comparison of the results using 0.03% and 2% Tween 40 showed that the latter concentration would increase the enzyme activity about 3 folds. Figure 17 clearly demonstrated that, at 2 percent, Tween 40 did not alter the rate and amount of adrenochrome formed by the enzyme. Since initial experiments of this study has adopted the method recommended by Takeguchi and Sih (138) in which 0.03% cutscum was added to the enzyme system, therefore 2% Tween 40 was still included in all assays.

2.1.6 The effect of L-epinephrine concentration

Figure 18 showed that the activity of the enzyme increased with increased amount of L-epinephrine and became saturated at 5.0-7.0 mM. Therefore, 6.0 mM of L-epinephrine is chosen for the assay system.

2.1.7 The effect of arachidonic acid concentration

The formation of adrenochrome as catalysed by prostaglandin synthetase is an arachidonic acid dependent reaction (141). To see if the amount of added arachidonic acid has any effect

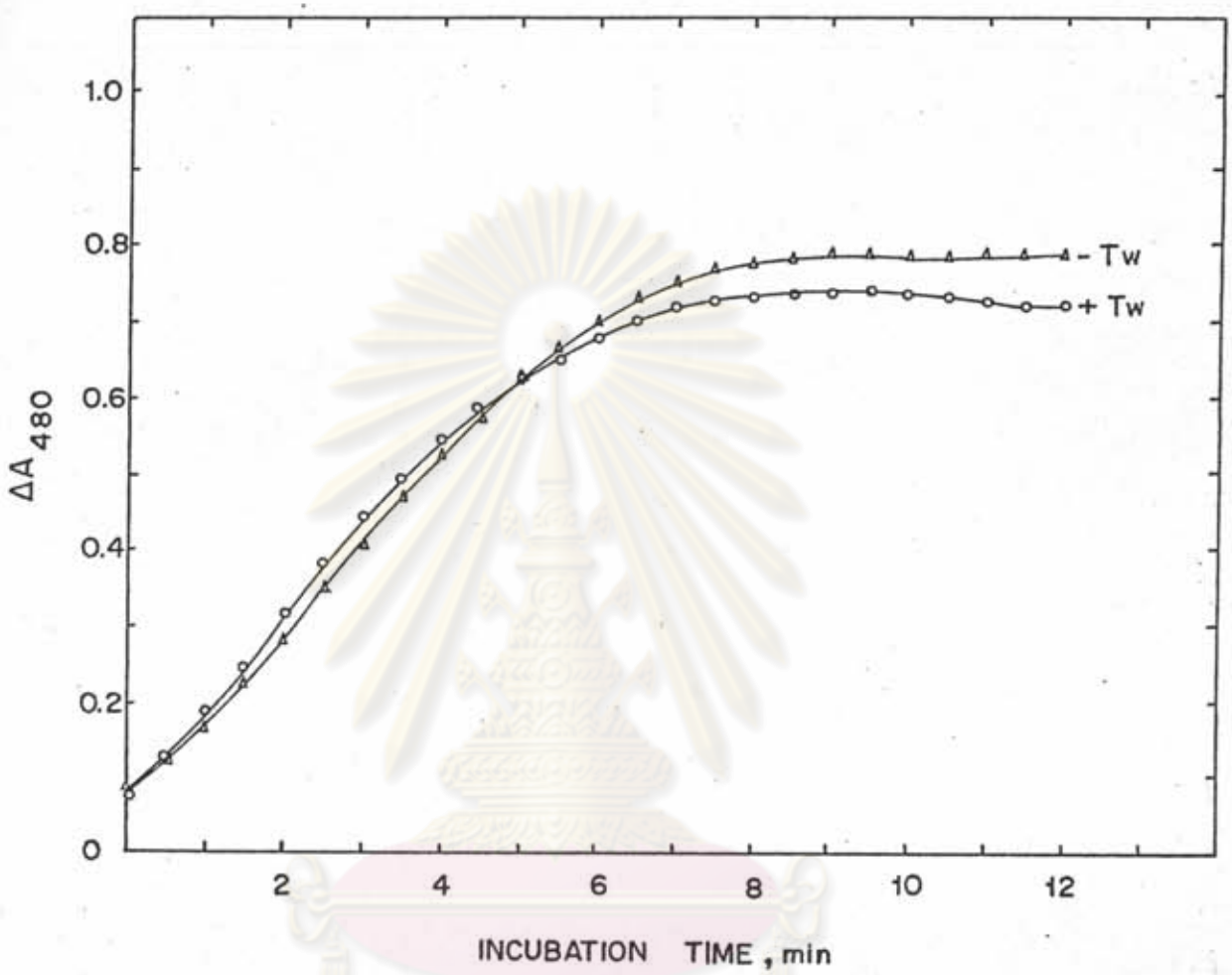


Figure 17 Effect of Tween 40 on adrenochrome formation.

The assay mixture contained 100 µg crude enzyme was incubated with or without 2% Tween 40 (Tw) in the presence of 6.0 mM L-epinephrine and 0.5 mM arachidonic acid in 0.5 ml of 50 mM Tris·HCl buffer, pH 8.2. Absorbancy at 480 nm was recorded at 25°C for 12 min. This experiment was repeated 4 times.

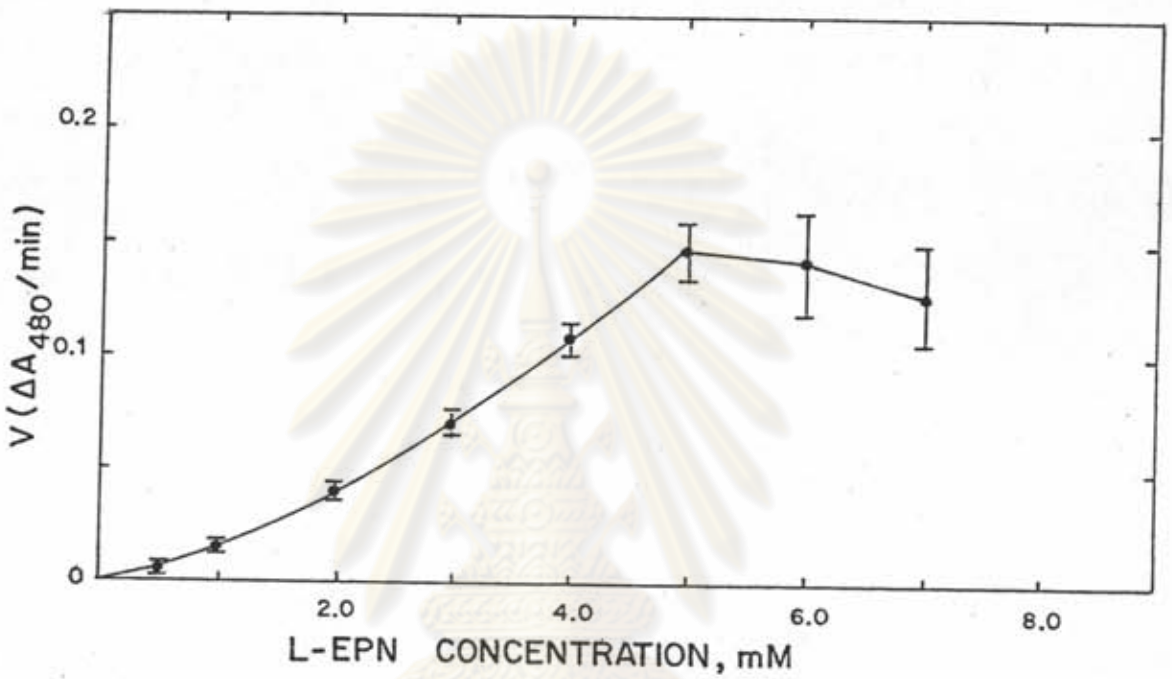


Figure 18 The activity of crude prostaglandin synthetase as a function of L-epinephrine (L-EPN) concentration.

The assay mixture contained the crude enzyme (500 µg protein) and various amounts (0.5-7.0 mM) of L-epinephrine and 0.5 mM arachidonic acid in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. This experiment was done twice. V = initial velocity.

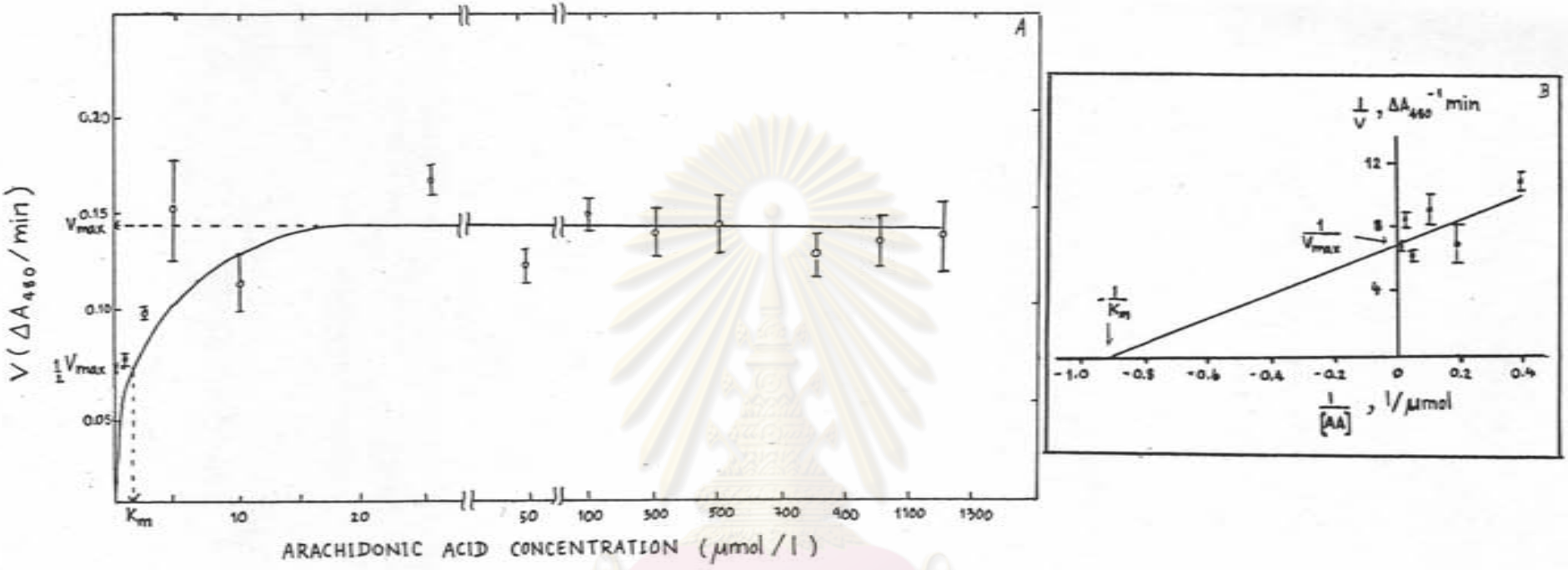


Figure 19 The effect of arachidonic acid (AA) concentration on the rate of prostaglandin synthetase which are shown in the Michaelis-Menten plot (A) and Lineweaver-Burk plot (B).

Various amounts of arachidonic acid was added to the enzyme mixture containing 500 μg microsomal protein and 6.0 mM L-epinephrine in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. Enzyme activity was measured as described in Materials and Methods. This experiment was repeated twice.

V = initial velocity.

on the rate of catalysis, the enzyme activity was assayed with varying amounts of arachidonic acid. Figure 19 showed a typical Michaelis-Menten kinetic. The reaction rate increased linearly with low concentration of arachidonic acid, slowly falled off at increasing concentration and approached a constant rate after 15.0 µM of arachidonic acid is reached. Therefore, concentration of arachidonic acid exceeding 15.0 µM could be used for the assay system.

From Michaelis-Menten plot, the calculated K_m for arachidonic acid and V_{max} were 1.50 µM and 0.94 mmole of adrenochrome per min respectively. Furthermore, when the result was transformed into Lineweaver-Burk plot, it also showed similar values of K_m and V_{max} (1.05 µM and 0.94 mmole of adrenochrome per min respectively).

From all the results obtained, an optimum condition for the assay of crude prostaglandin synthetase in rat uterus is established as follows: enzyme protein not exceeding 300 µg, L-epinephrine 6.0 mM, arachidonic acid 0.5 mM, 2% Tween 40, preincubation time 5 min, preincubation temperature 25°C, incubation time 12 min.

2.2 Effect of IUD on prostaglandin synthetase

Many investigators have demonstrated that insertion of an IUD will stimulate the release of prostaglandins into the uterine horn (126,127,134). In this study, attempts were made to see if IUD also enhance prostaglandin synthetase activity. Figure 20 (A,B,C,D) shows the enzyme activity in the control and IUD horns at various stages of the estrous cycle. Biological variation between rats was obvious. In most animals, the enzyme activity in the IUD horn is, if not equal, almost always higher than that of the control horn. It is also

Figure 20 The activity of crude prostaglandin synthetase in the control (0--0) and IUD (Δ--Δ) horns at each stage of the estrous cycle.

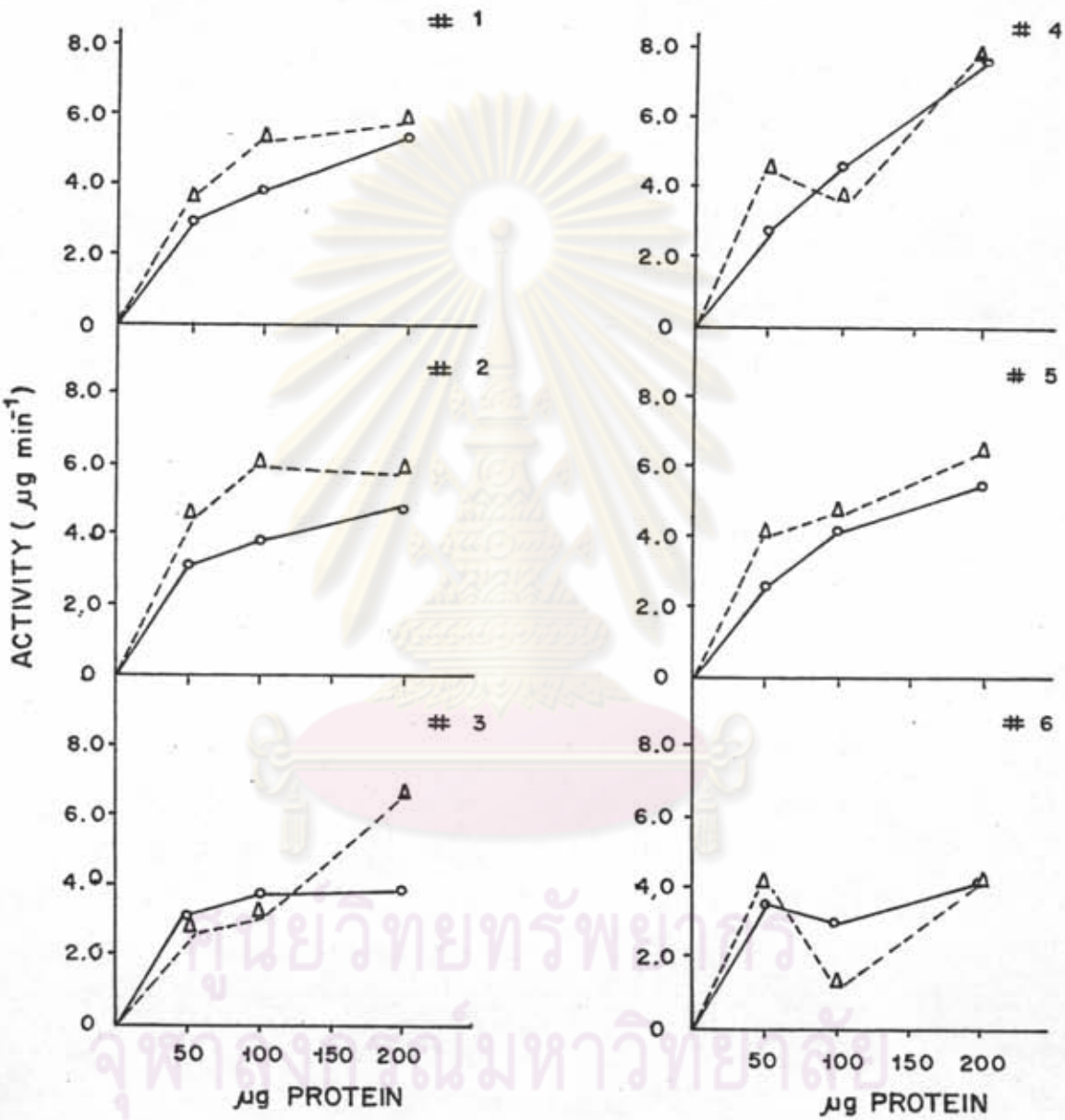
The enzyme activity was determined at various concentrations of microsome (50, 100, 200 µg protein). The activity was expressed as µg adrenochrome formed per min. The rat number is shown at the corner of each graph.

Figure 20A = proestrus stage

Figure 20B = estrus stage

Figure 20C = diestrus 1 stage

Figure 20D = diestrus 2 stage



PROESTRUS

Figure 20A

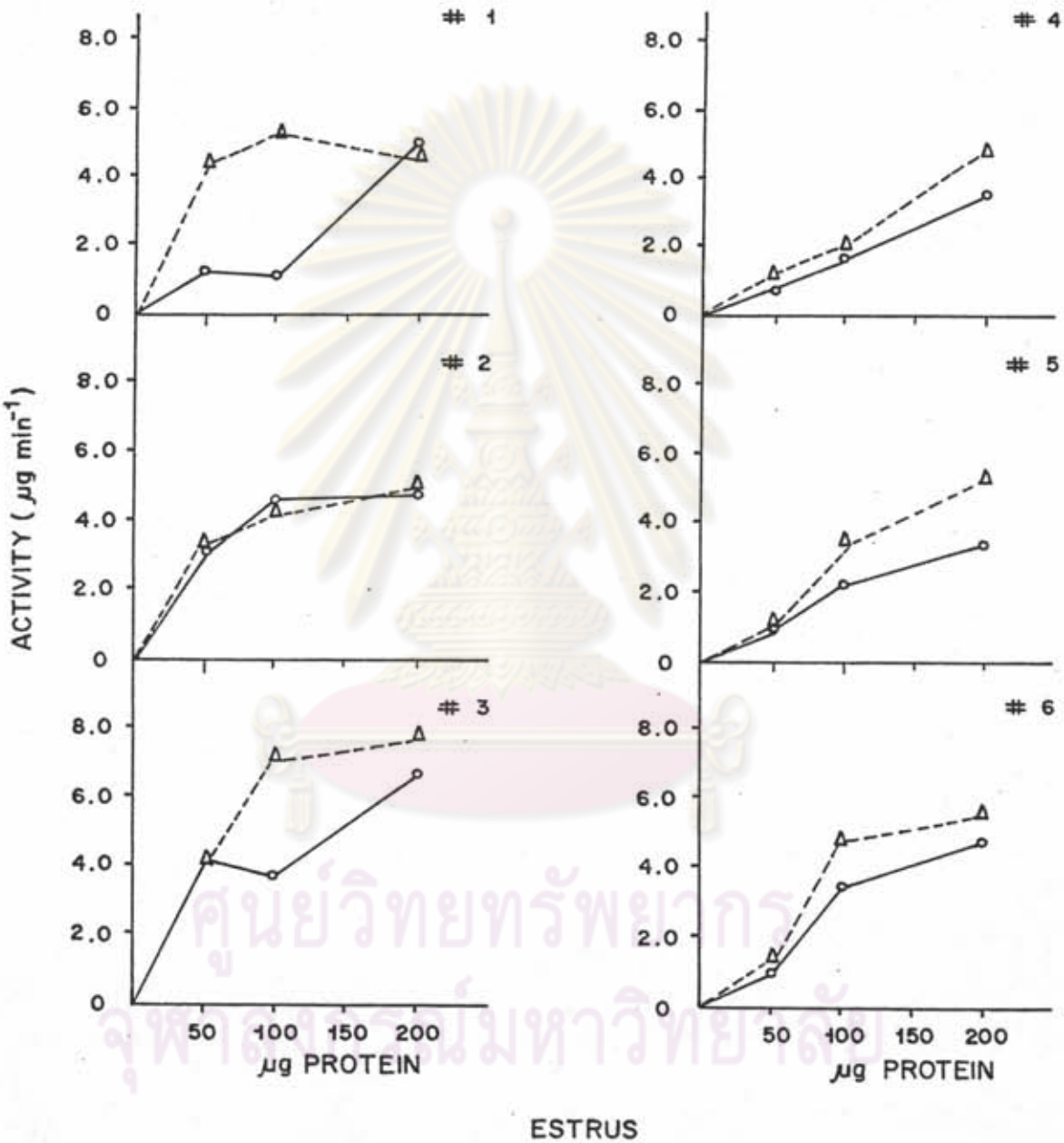
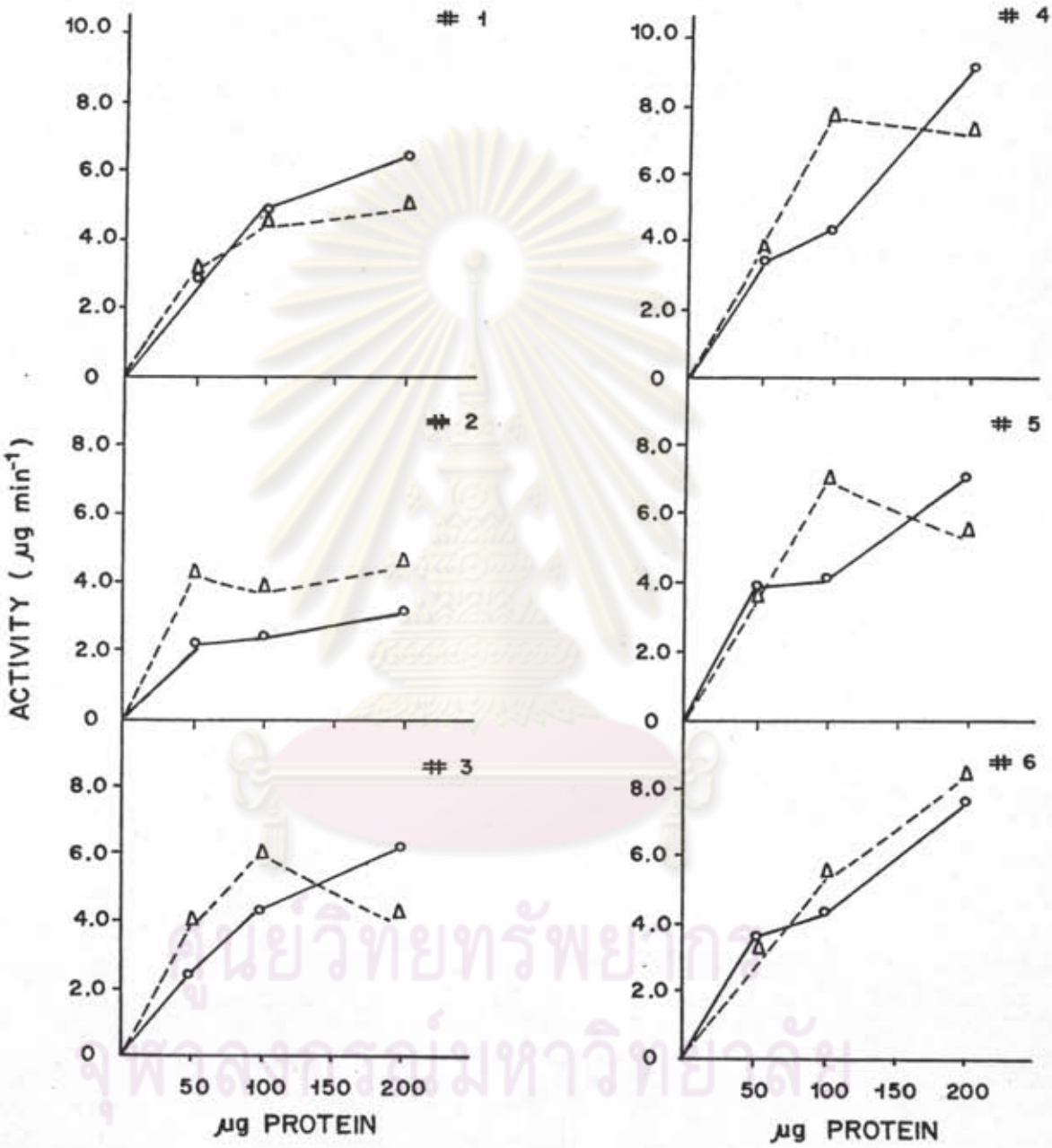
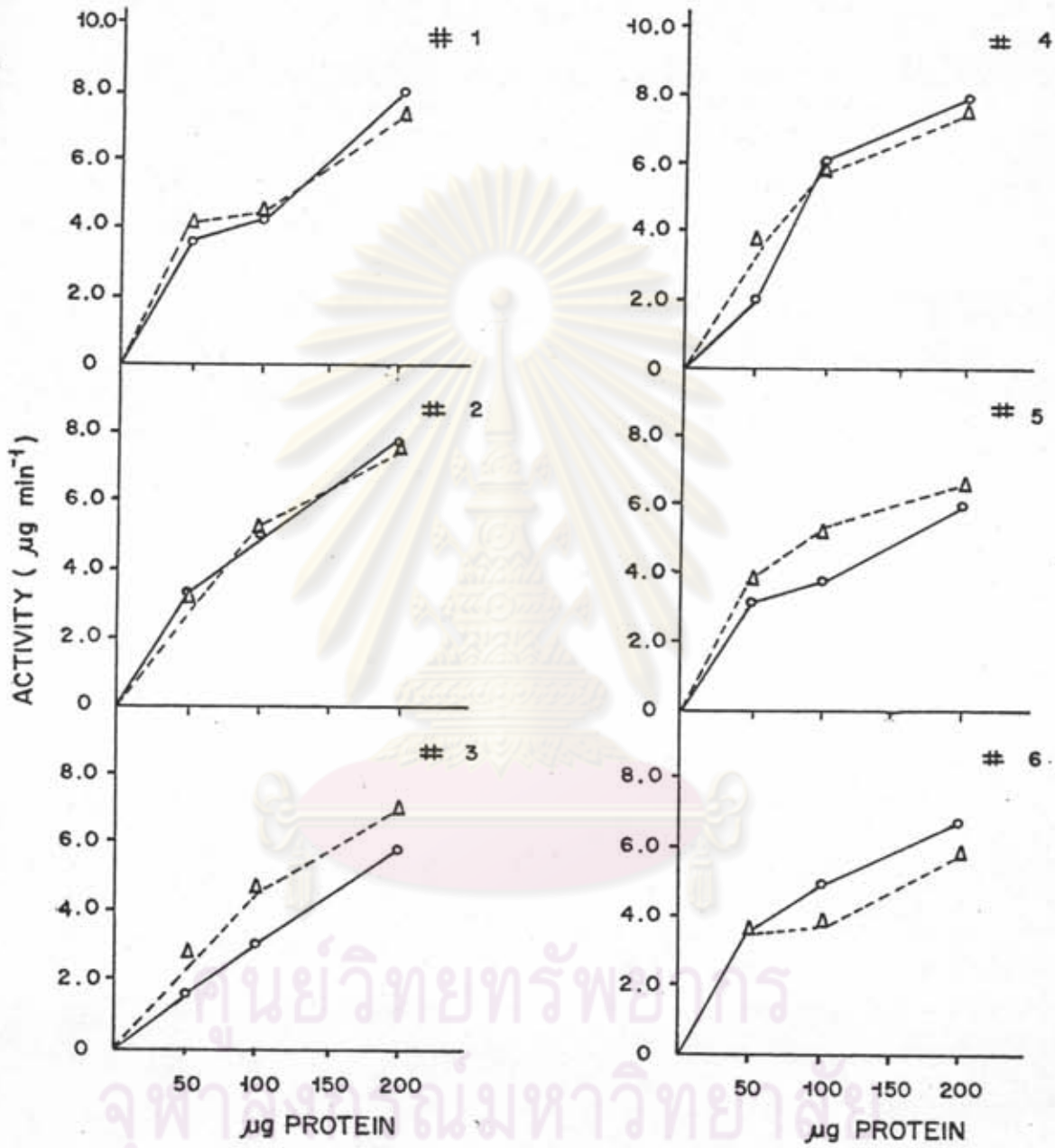


Figure 20B



DIESTRUS 1

Figure 20C



DIESTRUS 2

Figure 20D

Figure 21 The specific activity of crude prostaglandin synthetase in the control (O--O) and IUD (Δ--Δ) horns at each stage of the estrous cycle.

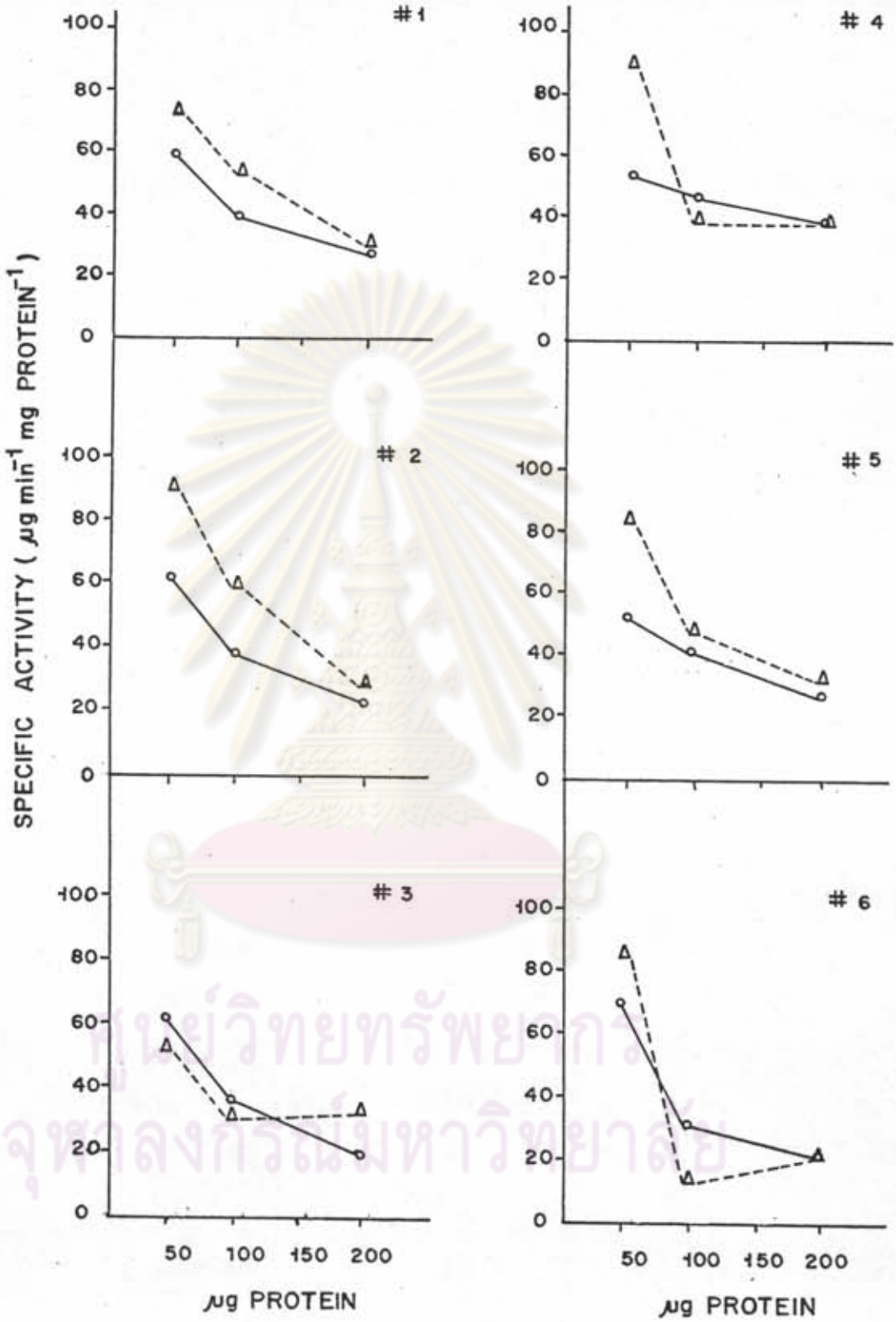
The enzyme activity was determined at various concentrations of microsome (50, 100, 200 µg protein). The specific activity was expressed as µg adrenochrome formed per min per mg protein. The rat number is shown at the corner of each graph.

Figure 21A = proestrus stage

Figure 21B = estrus stage

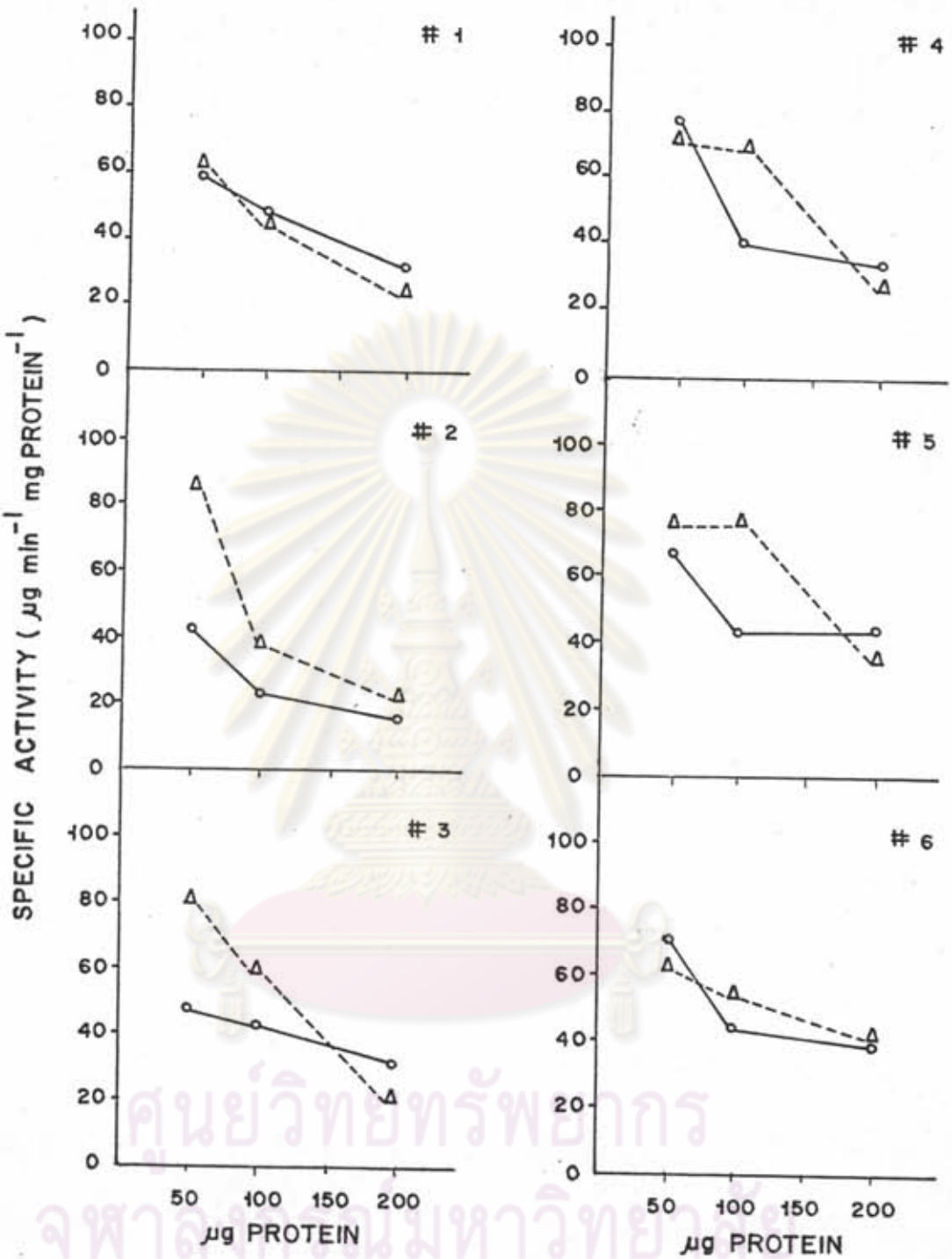
Figure 21C = diestrus 1 stage

Figure 21D = diestrus 2 stage



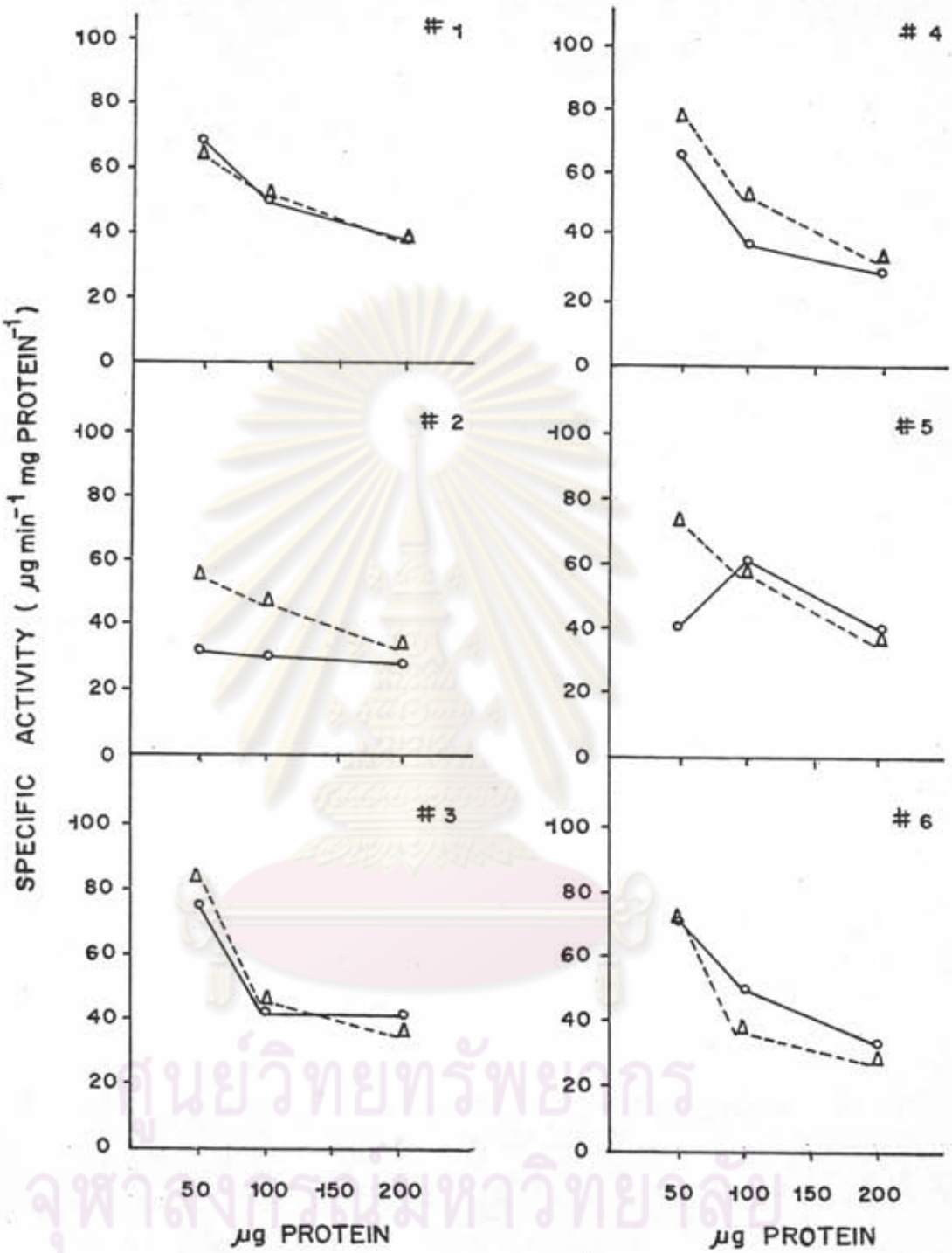
PROESTRUS

Figure 21A



DIESTRUS 1

Figure 21C



DIESTRUS 2

Figure 21D

apparent that the measurable enzyme activity did not increase in response to increasing protein concentration. The specific activity of the enzyme as shown in Figure 21 (A,B,C,D) decreased when higher amount of microsome is used. At 200 µg protein, no significant difference of the specific activity was observed at all stages. Histograms showing the specific activity of prostaglandin synthetase in the two horns were presented in Figure 22. At 50 µg protein, the enzyme activity in both the control and IUD horns was least during estrus. At other stages, i.e. proestrus, diestrus 1 and 2, the enzyme activity is about the same. No cyclic pattern is observed when higher amount of microsome was used in the assay.

2.3 Factors influencing prostaglandin synthetase

Many previous experiments have indicated that the prostaglandin synthetase activity was depressed with increasing amount of microsome. Such inhibition could arise nonspecifically from proteins present in the assay mixture or could be a result from some specific component(s). To test these assumptions, several experiments were performed. Only control rats were used in those experiments.

2.3.1 The effect of BSA

To test whether protein could nonspecifically inhibit the enzyme activity, BSA (100-600 µg) was added to a fixed amount of microsome (100 µg) and the enzyme activity was measured. An inhibitory effect by BSA was observed (Figure 23). One hundred to three hundreds µg and 400-600 µg of BSA depressed the enzyme at min 2 by 61.1% and 88.9%, respectively. At the end of incubation (min 2),

Figure 22 Histograms of the specific activity of crude prostaglandin synthetase in the control (▭) and IUD (▨) horns of the rats during the estrous cycle.

Fifty µg protein (A), 100 µg protein (B) and 200 µg protein protein (C) of the crude enzyme were used in the assay. Each bar represents the mean ± S.E.M. from 6 experiments. This data is derived from Figure 21.

* = $P<0.05$ was tested by paired comparison test.

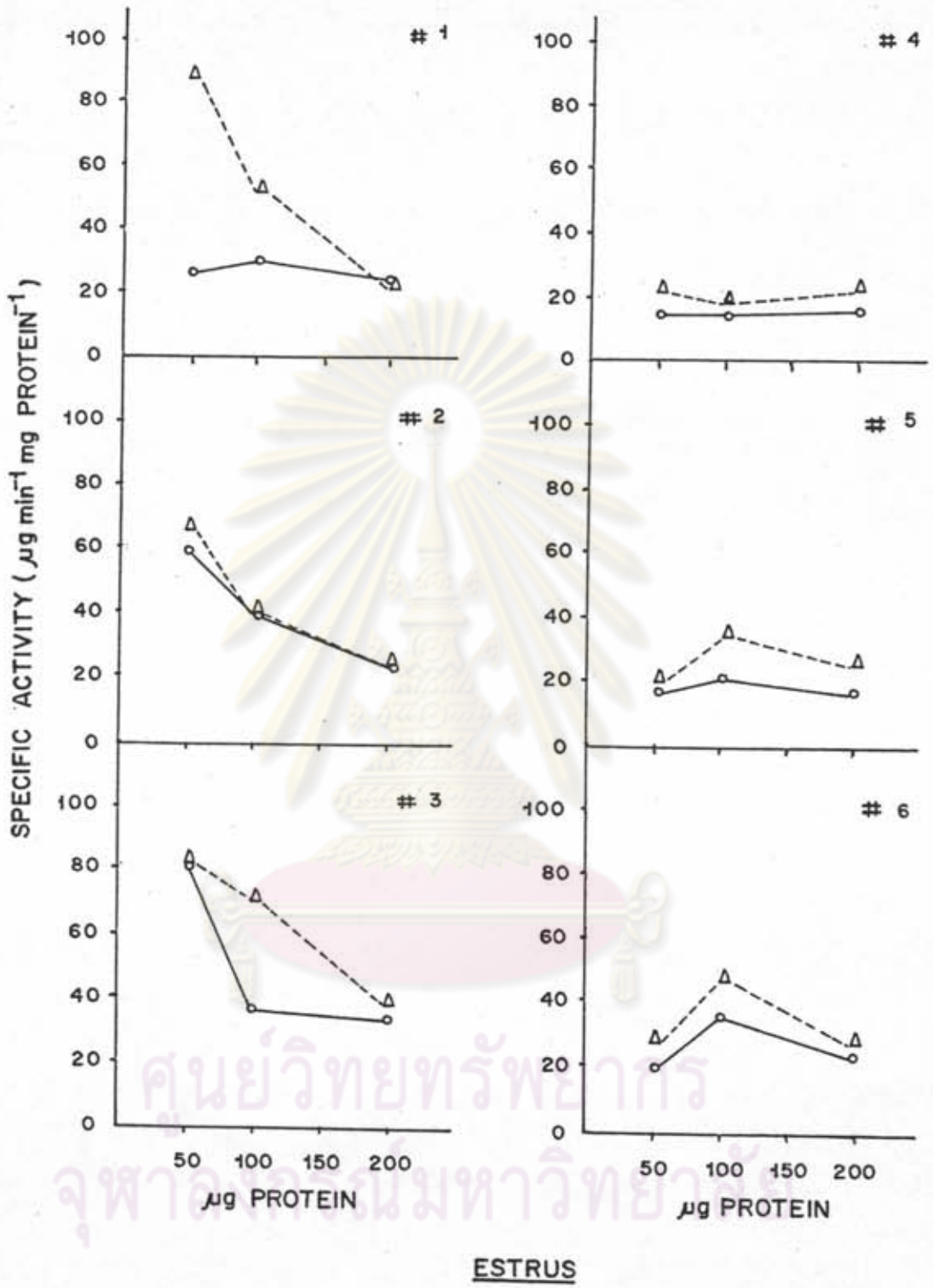


Figure 21B

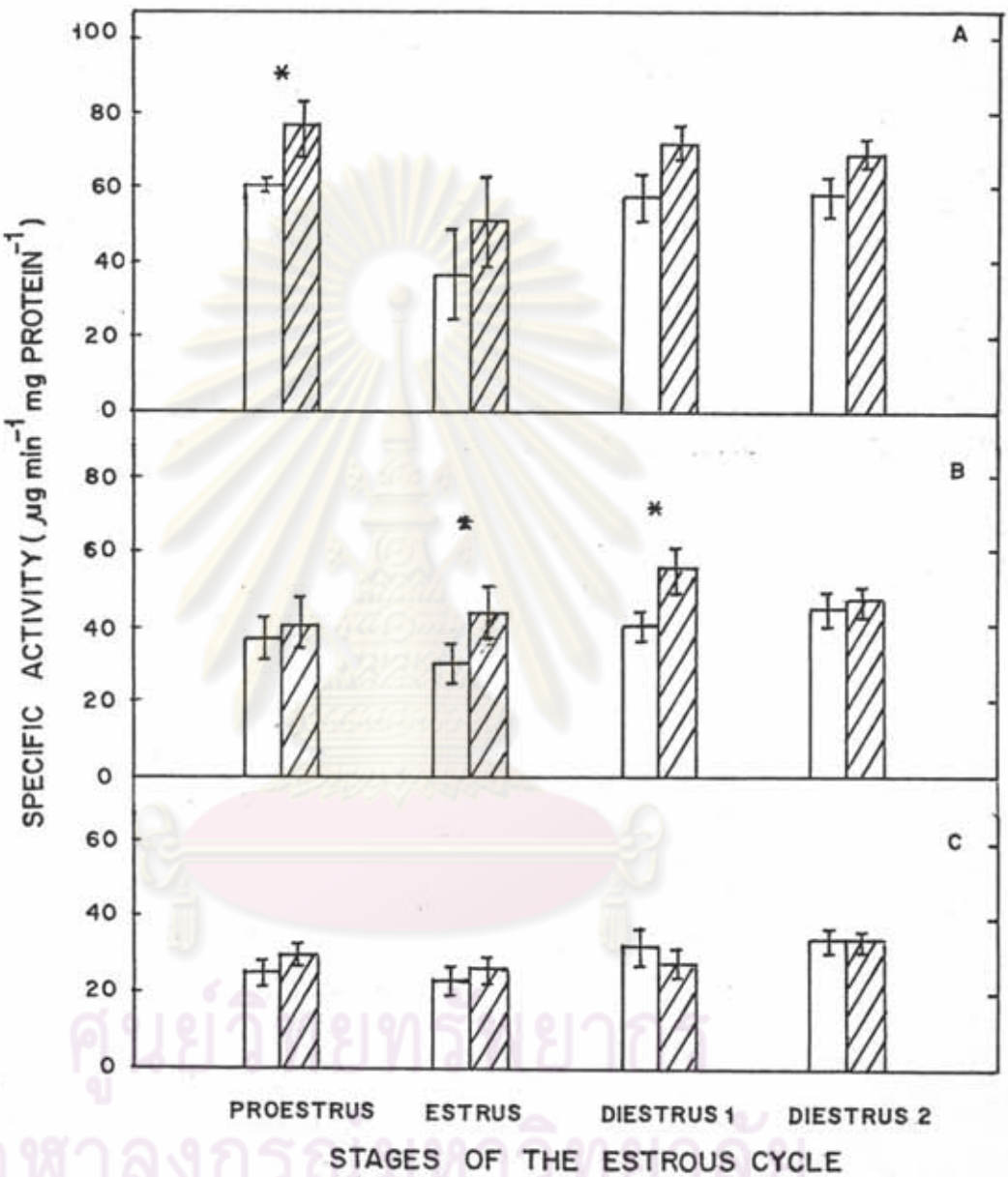


Figure 22

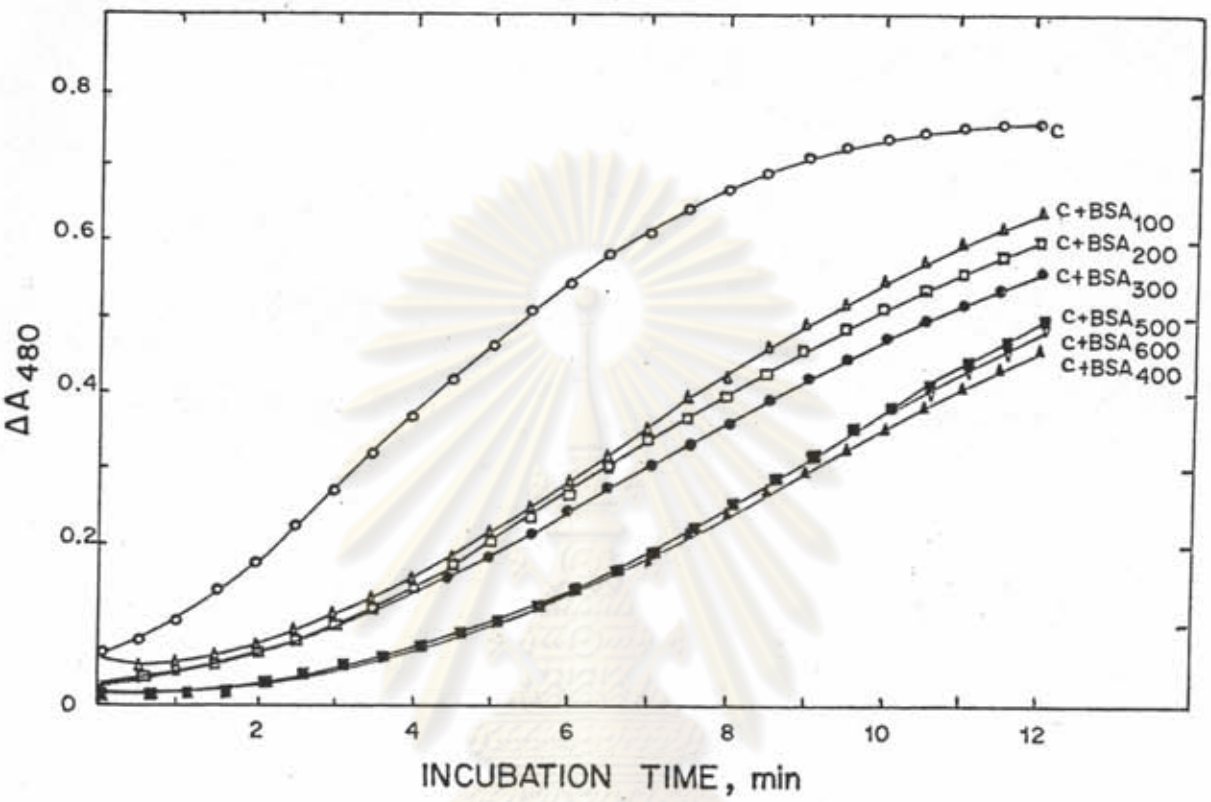


Figure 23 Effect of BSA on adrenochrome formation.

Various amounts (100-600 µg) of BSA was added to a fixed amount of crude microsome (100 µg) and the enzyme was assayed as described in Materials and Methods. This experiment was repeated twice.

C = the crude microsomal protein

BSA subscript = the amount (µg) of BSA added to the enzyme system.

the inhibition was less (19.7% and 34.8%, respectively) but was still significant.

2.3.2 The effect of cytosolic factors

Many investigators have shown that some cytosolic factors from rat liver (147), rabbit kidney medulla (148) and human uterus (149) could change prostaglandin biosynthesis by either activate or inhibit the process. This study has also examined such effect in rat uterus. Cytosol fraction was prepared by centrifugation of the rat's uterine homogenate in Tris.HCl buffer at 100,000 x g; the resulting supernatant was designated "cytosol fraction (CF)". The influence of CF was tested by incubating the microsome enzyme in the presence of varying amount of the CF. The result is shown in Figure 24. It is clearly shown that, rather than inhibited, the CF at 100 µg stimulated prostaglandin synthetase after 3 min of incubation (Figure 24A). However, the kinetic pattern in both reaction mixtures was the same. It is noteworthy that the CF alone could slowly convert L-epinephrine into adrenochrome. The extent of conversion was comparatively equal to the increased activity observed in the presence of 100 µg CF. When the CF was boiled for 5 min, centrifuged and the resulting supernatant was added to the enzyme mixture, the activity to produce adrenochrome was lost. However, supernatant from the boiled CF could still stimulate prostaglandin synthetase in the microsome and exhibited the same kinetic pattern as those of the control or the control with CF.

When higher amount of the CF was added to the microsome, the kinetic pattern changed remarkably (Figure 24B, C and D). At 300 µg, the CF depressed the enzyme activity during the first

Figure 24 Effect of the cytosol fraction on adrenochrome formation in rat uterus.

The cytosol fraction is the supernatant from 100,000 xg centrifugation of uterine homogenate. The reaction mixture contained the crude enzyme (100 µg protein), the cytosol fraction (100-1000 µg protein), L-epinephrine (6.0 mM) and arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. This experiment was repeated 3 times.

C = the crude enzyme

CF_x = the cytosol fraction containing x µg protein

$CF_{x,b}$ = supernatant from the boiled cytosol fraction which contained x µg protein

Figure 24A : CF = 100 µg protein

Figure 24B : CF = 300 µg protein

Figure 24C : CF = 600 µg protein

Figure 24D : CF = 1000 µg protein

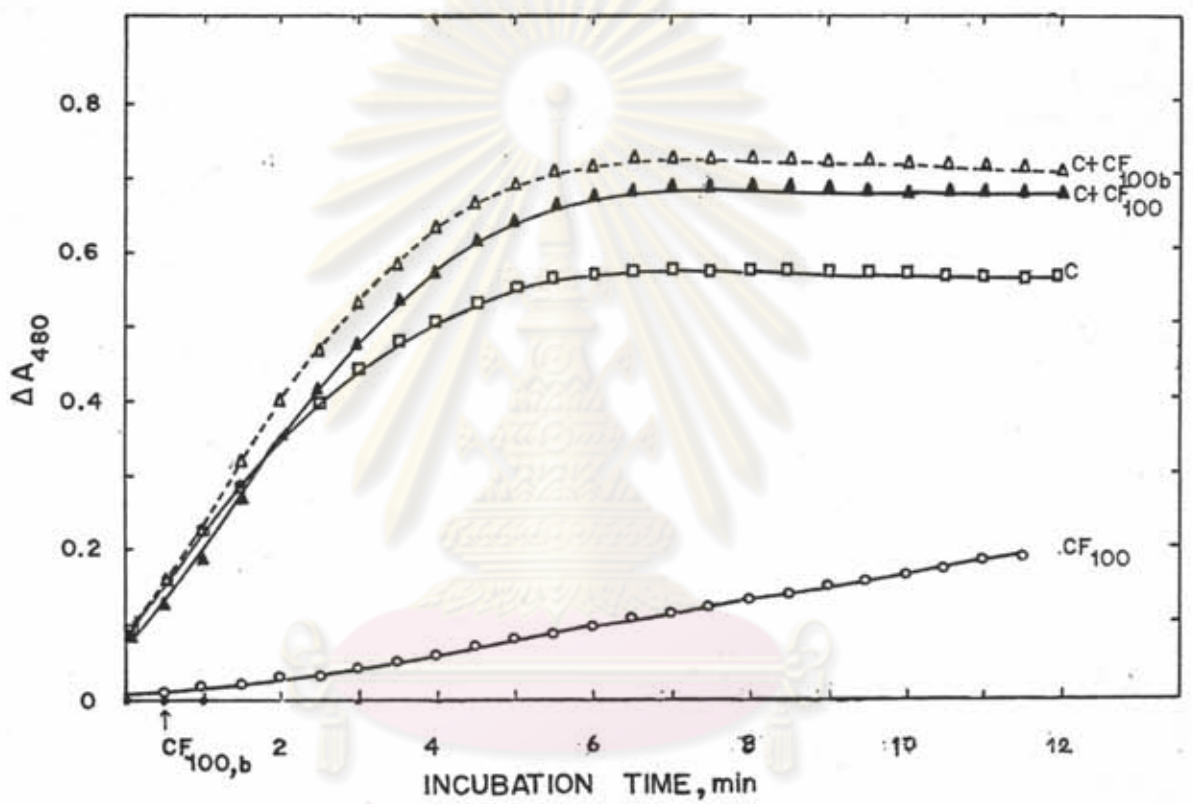


Figure 24A

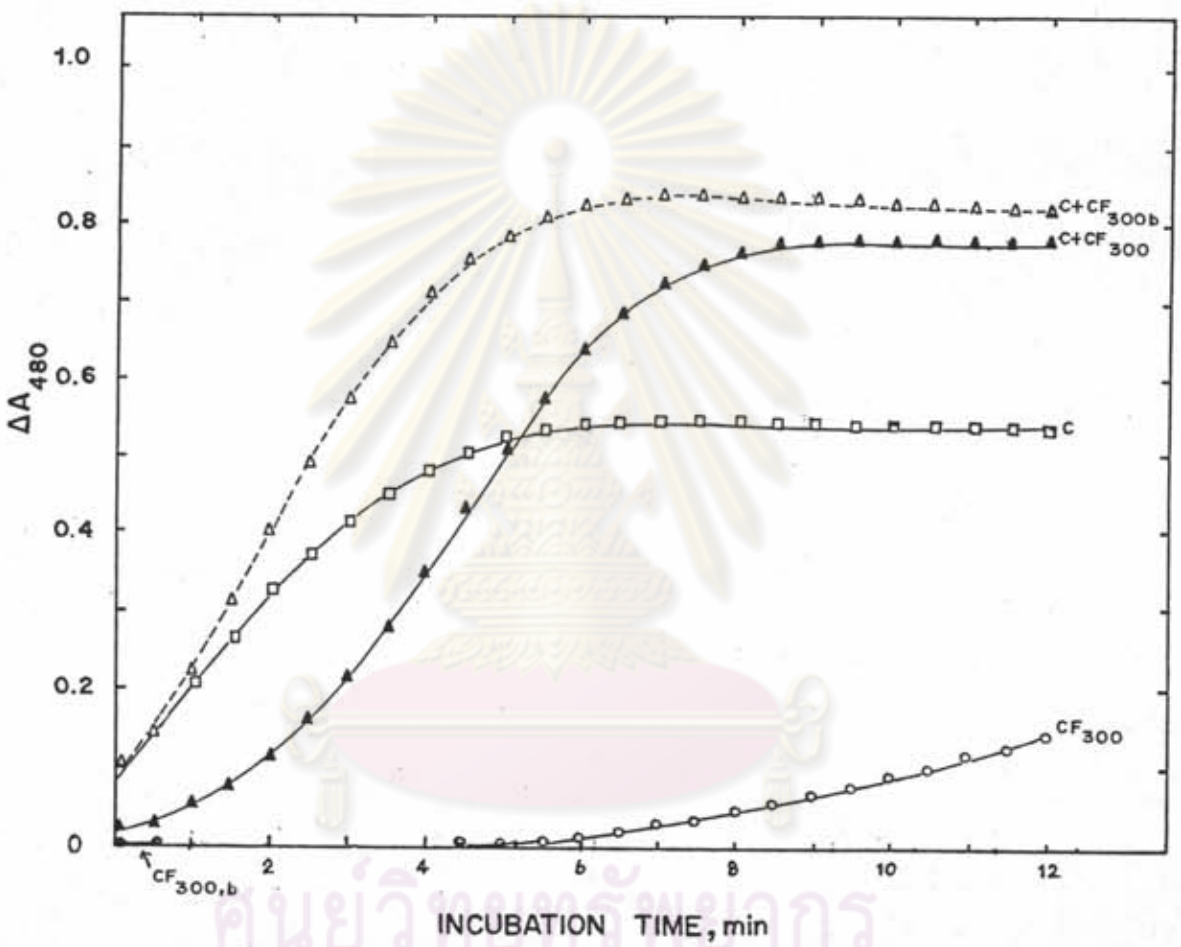


Figure 24B

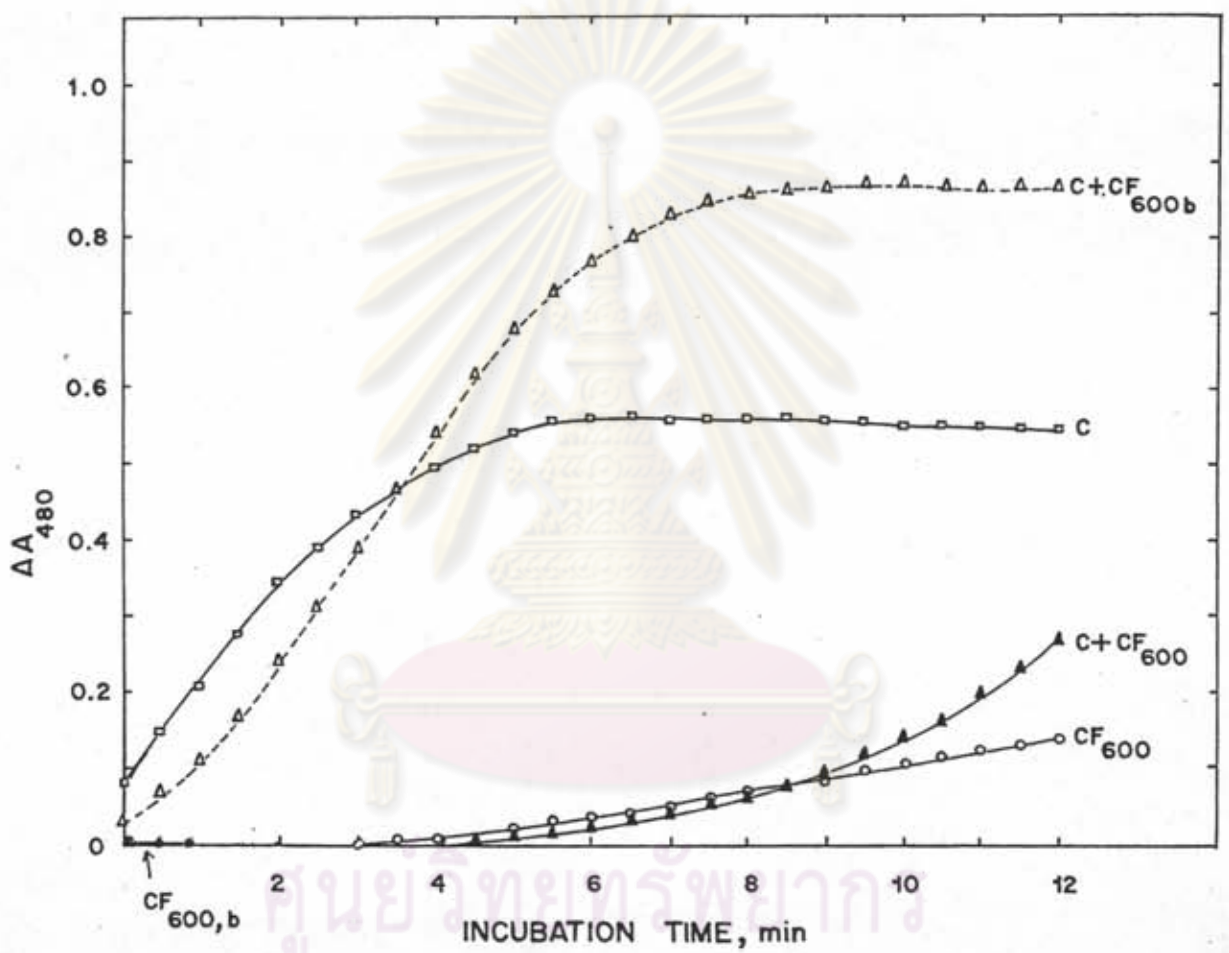


Figure 24C

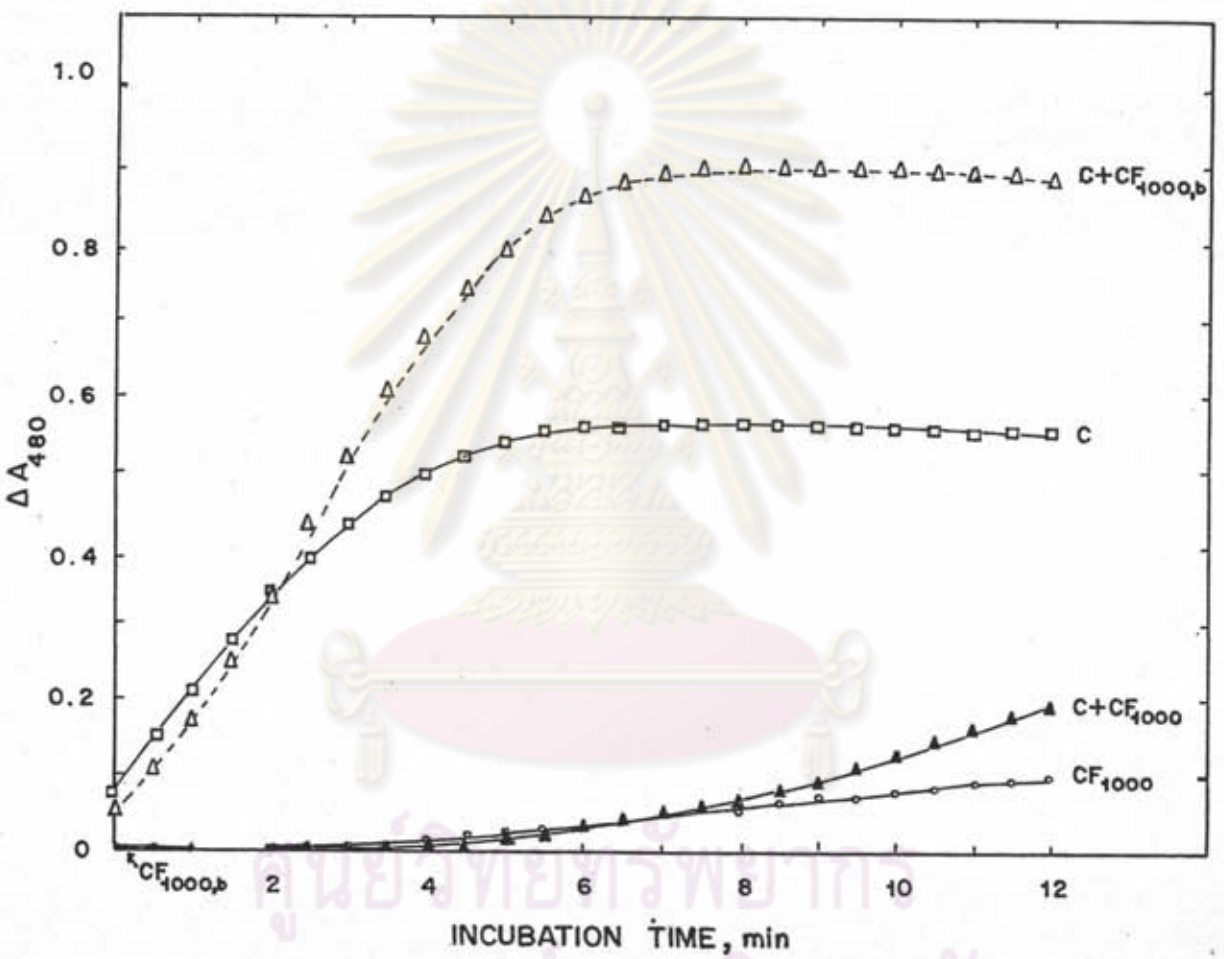


Figure 24D

4 min but became higher after 5 min (Figure 24B). Maximum stimulation (44.8%) was observed after 8 min. The extent of stimulation was always higher than that of the CF alone. At very high protein concentrations (600 and 1000 µg), the CF dramatically inhibited the enzyme activity (Figure 24C and D). Boiling of the fraction would abolish such inhibitory effect but would stimulate the enzyme instead.

To further investigate the nature of the effector(s) in the CF, the CF was dialysed against 50 mM Tris·HCl buffer, pH 8.2, at 4°C for 24 h at the volume ratio of CF : buffer = 100 : 1. The macromolecular fraction in the dialysis bag was then collected and added to the microsome enzyme. Figure 25 showed that at the amounts of CF used, both the CF and the dialysed CF would always significantly inhibit the enzyme activity over the period of study. With 300 µg CF, the degree of inhibition was less when the CF was dialysed against buffer. The enzyme activity was just above background when higher amount of CF (600 and 1000 µg) were tested. The dialysis of these CF gave contrasting effects on the enzyme. The activity in the presence of dialysed CF was higher than that of the undialysed CF when 600 µg CF was used, where as at 1000 µg, the enzyme activity was the same.

2.3.3 The effect of indomethacin

Indomethacin is a prostaglandin synthetase inhibitor. The inhibitory effect of 5, 10 and 20 mM of indomethacin on the enzyme activity is shown in Figure 26. It clearly indicates that 5, 10 and 20 mM of indomethacin inhibited this enzyme about 22, 44 and 88%, respectively. I_{50} of indomethacin was 11.3 mM.

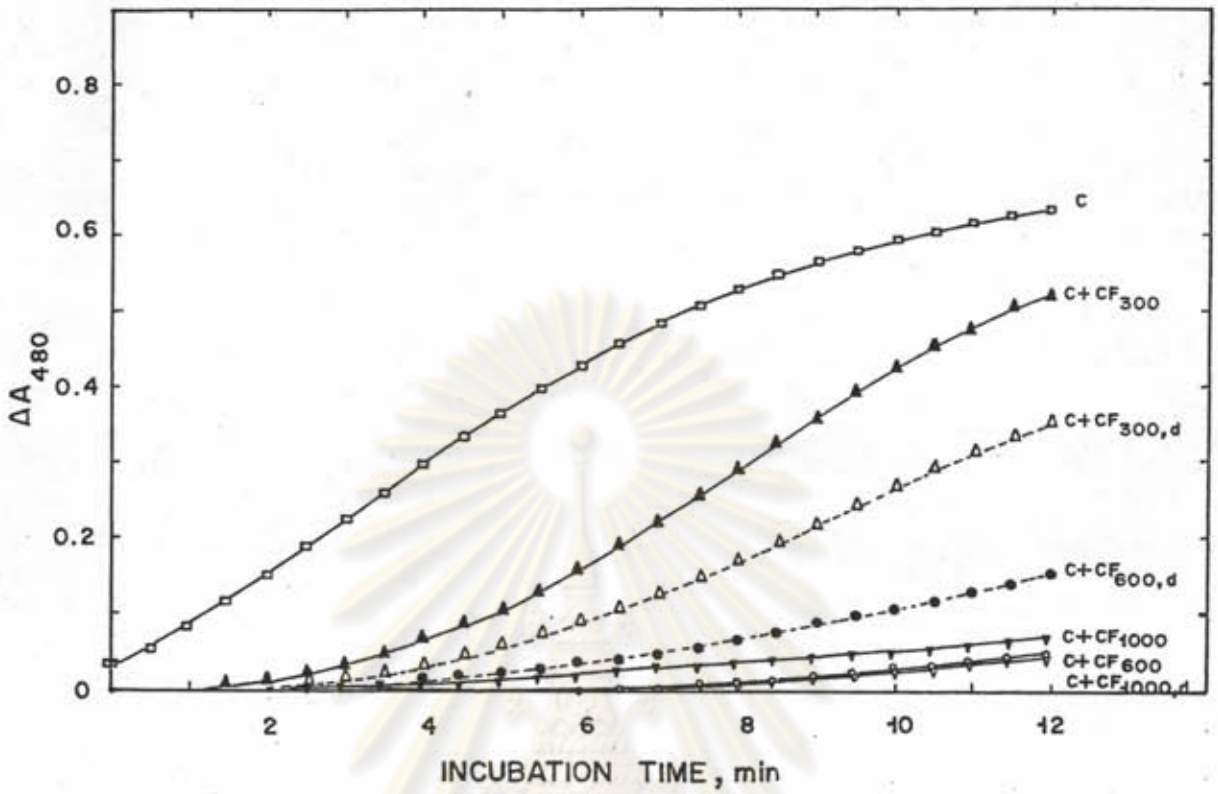


Figure 25 Effect of the cytosol fraction (undialysed and dialysed) on adrenochrome formation in rat uterus (n=2).

The reaction mixture contained the crude enzyme (100 µg protein) with or without the cytosol fraction (300, 600 and 1000 µg protein), L-epinephrine (6.0 mM) and arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2.

C = the crude microsome

CF_x = the undialysed cytosol fraction containing x µg protein

$CF_{x,d}$ = the dialysed cytosol fraction containing x µg protein

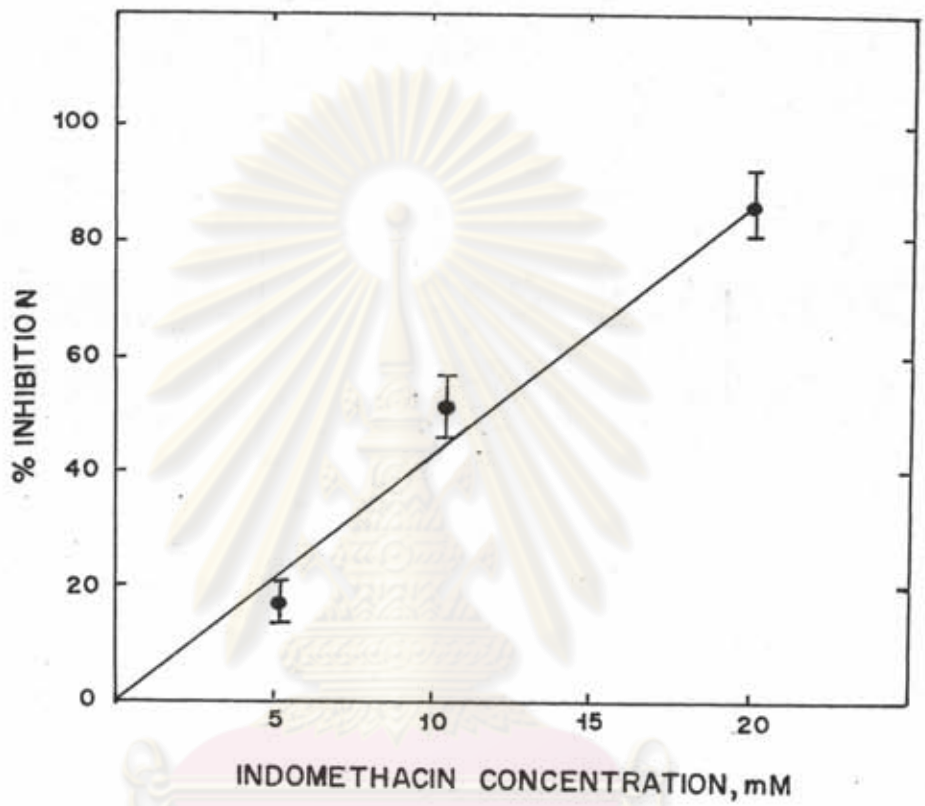


Figure 26 The inhibitory effect of indomethacin on crude prostaglandin synthetase.

The crude enzyme (100 µg protein) was preincubated with L-epinephrine (6.0 mM) in the presence or absence of indomethacin (5, 10, 20 mM) at 25°C for 5 min before the addition of arachidonic acid (0.5 mM) in 1.5 ml of 50 mM Tris·HCl-2% Tween 40 buffer, pH 8.2. The enzyme activity was measured as described in Materials and Methods. This experiments was repeated twice.