III Results

III.1 Isolation and Purification of HCG and β - HCG Subunit.

Crude HCG was obtained from pooled urine from choriocarcinoma pataients by saturated ammonium sulfate precipitation, then the contaminated substances were removed by 80% ethanol precipitation. Purifications of HCG and β - HCG subunit were performed by chromatography on DEAE Sephadex A.50, Biogel P-60 and 8 M urea-DEAE Sephadex A.25 column, respectively as described in section II.5.1.

Total protein, total activity, specific activity, purification fold and % yield of each step in the isolation and purification of HCG and β - HCG subunit are summarized in Table III.1.

The total protein and total activity contents in initial pooled chriocarcinoma urine (3.2 L) were 2508 mg and 480,000 i.u. detected by agglutination assay, respectively. The specific activity was 192 i.u./mg protein.

After precipitation with saturated ammonium sulfate , the precipitate was redissolved in 0.01 M phosphate buffer, pH 7.0 and the insoluble precipitate was removed by centrifugation. The supernate was dialysed, brown - coloured crude HCG solution (55 ml) contained 1012 mg of total protein and 220,000 i.u. of total activity. Its specific activity and purification fold were 220 i.u./mg protein and 1.1, respectively while the HCG yield was 46 %. This crude HCG solution was further

precipitated by 80 % ethanol.

After ethanol precipitation , centrifugation and dialysis , 80 ml of crude HCG solution contained 770 mg of protein and 200,000 i.u. of HCG. The specific activity was increased to 260 i.u./mg protein. The HCG yield and purification fold were 42% and 1.3, respectively.

In the first purification step , the crude HCG solution was applied on a DEAE Sephadex A.50 column. Elution profiles of DEAE Sephadex A.50 chromatography are shown in Figure III.1. The equilibrated peak contained HCG level less than 50 i.u./ml. After the application of sodium chloride concave gradient , fractions number 110-130 contained HCG level between 1,000-2,000 i.u./ml and these fractions were pooled , dialysed and lyophilized. Two ml of the lyophilizate contained 54 mg of protein and 150,000 i.u.of HCG in total content. The specific activity and purification fold were increased to 2,770 i.u./mg protein and 14.8 , respectively. The HCG yield was 31% . This partially purified HCG was further applied on a Biogel P-60 column.

Elution profiles of Biogel P-60 chromatography are shown in Figure III.2. The first eluted peak , fractions number 11-16 contained HCG level more than 4,000 i.u./ml and these fractions were pooled , dialysed and lyophilized. Two ml of the lyophilizate contained 27 mg of protein and 135,000 i.u.of HCG in total content. The specific activity and purification fold were increased to 5,000 i.u./mg protein and 29.1, respectively. The HCG yield was 28%, the specific activity of this

highly purified HCG was 9250 i.u./mg protein detected by commercial ELISA test (Roche) (1^{st} I.R.P for the immuoassay). This HCG preparation showed one major protein band when subjected to native - polyacrylamide gel electrophoresis as shown in Figure III.4. The highly purified HCG preparation was further treated for subunit dissociation by 10 M urea and isolated of subunits in a 8 M urea - DEAE Sephadex A.25 column.

Elution profiles of 8 M urea - DEAE Sephadex A.25 chromatography are shown in Figure III.3. The first peak emerged from the column after elution with equilibrating buffer while the second peak was eluted by equilibrating buffer containing 1 M sodium chloride and 0.2 M glycine. Protein containing fractions under each peak was pooled , dialysed and lyophilized. Two ml of the second - peak lyophilizate totally contained 8 mg of protein and 60,000 i.u.of β - HCG. The β - HCG yield was 12%. Its specific activity and purification fold were increased to 7,500 i.u./mg protein (2^{nd} I.S for the immunoassay) and 29 , respectively. This β - HCG subunit preparation showed 14,350 i.u./mg protein detected by Commercial β - HCG ELISA (Roche) (1^{st} I.S. for the immunoassay). It showed a single protein band corresponding to β - HCG subunit prepared from standard HCG when subjected to SDS - polyacrylamide gel electrophoresis as shown in Figure III.5.

Table III.1 Isolation and Purification of HCG and β - HCG Subunit.

Procedure steps.	Total protein	Total activity	Specific activity	Purification fold	Yield
	mg.	i.u.*	i.u.*/mg protein		%
Urine Sample	2508	480,000	192	1	100
$(NH_4)_2SO_4$ precipitation	1012	220,000	220	1.1	46
Ethanol precipitation	770	200,000	260	1.3	42
DEAE Sephadex A.50	54	150,000	2,770	14.4	31
Biogel P.60	27	135,000	5,000	26.0	28
8 M Urea - DEAE Sephadex A.25	8	60,000	7,500	39.6	12

*2nd I.S for the immunoassay.

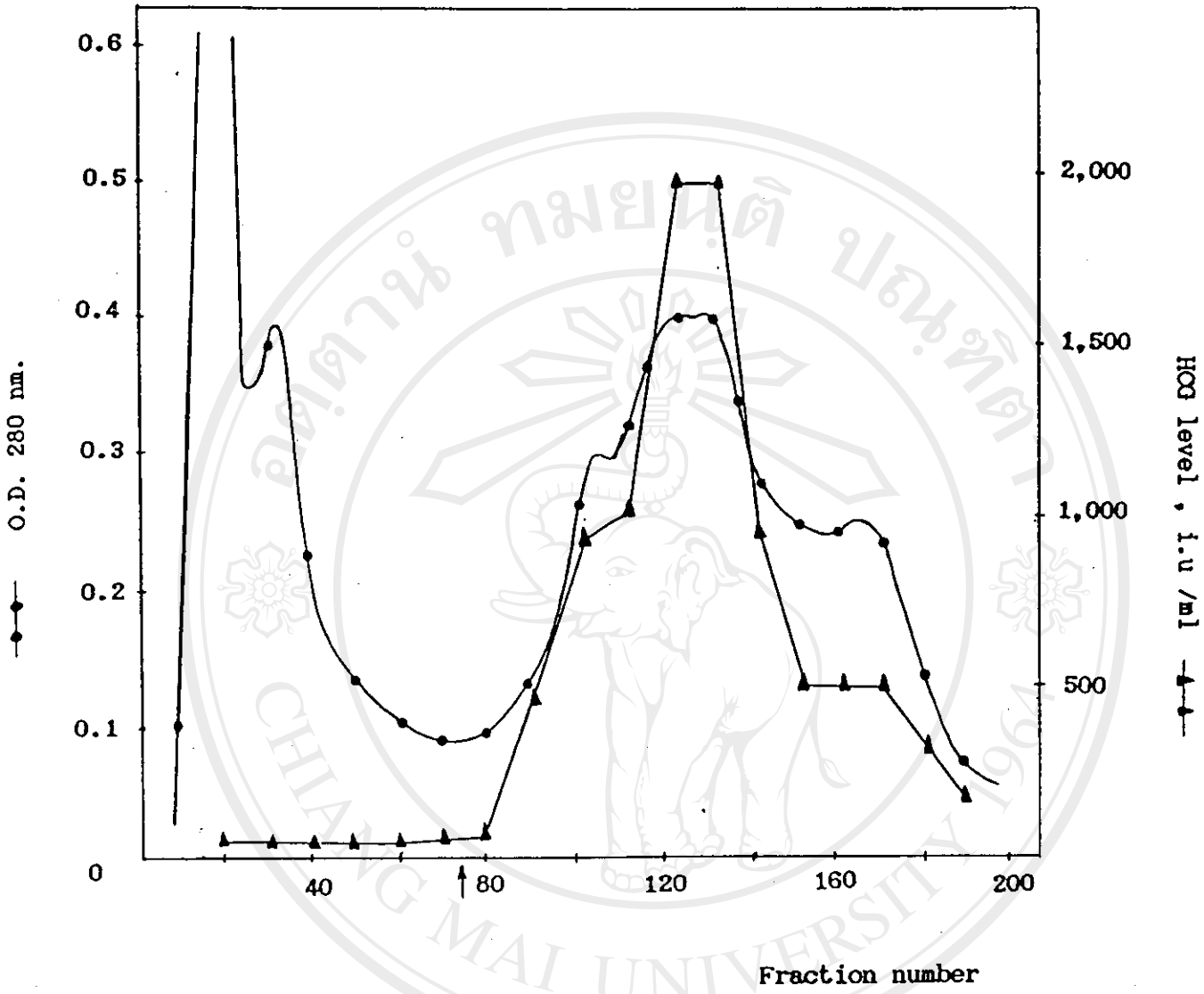

Figure III.1 DEAE Sephadex A.50 Chromatography of Crude HCG Solution.

↑ Application of NaCl Concave Gradient

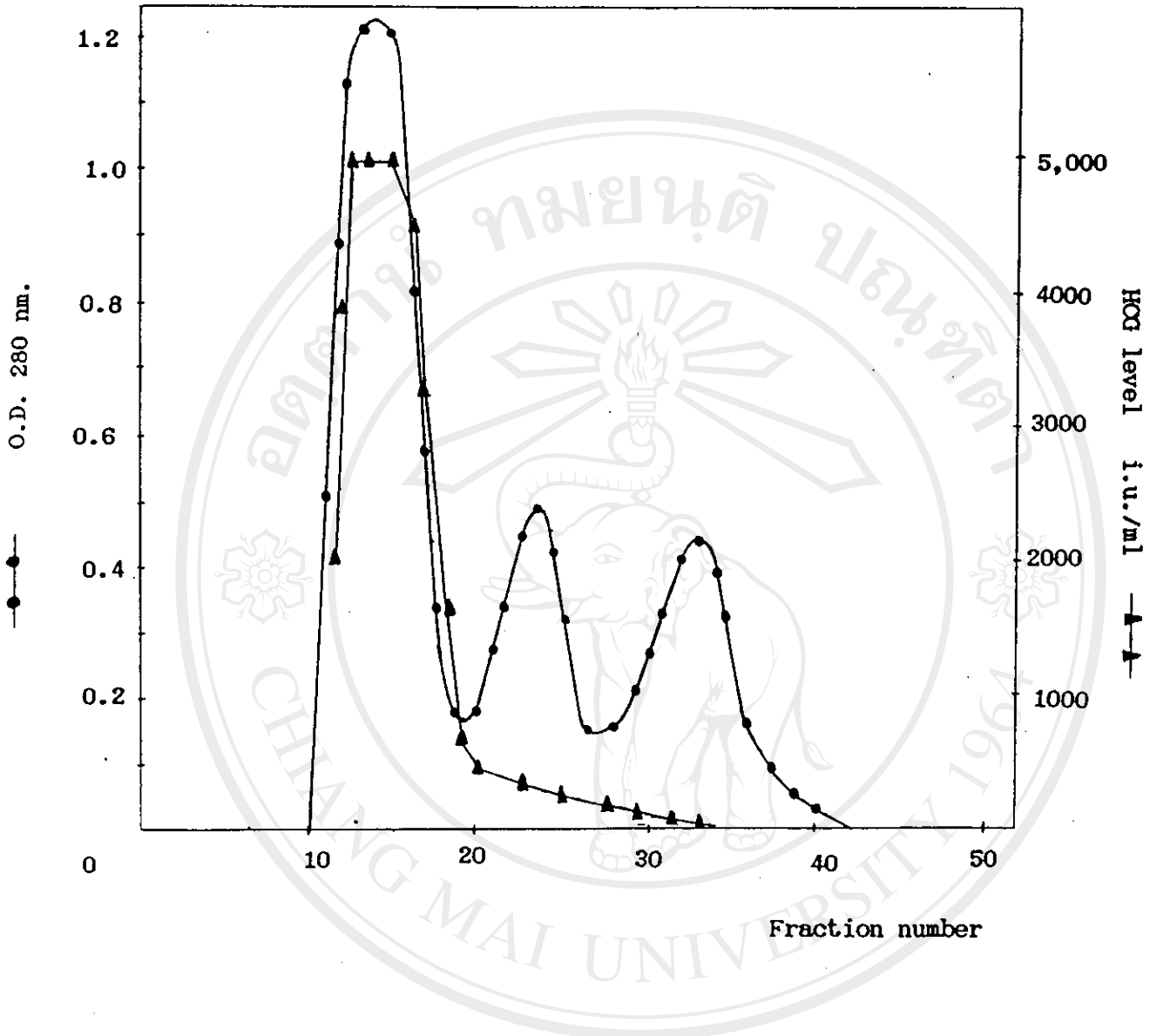

Figure III.2 Biogel P-60 Chromatography of Partially Purified HCG.

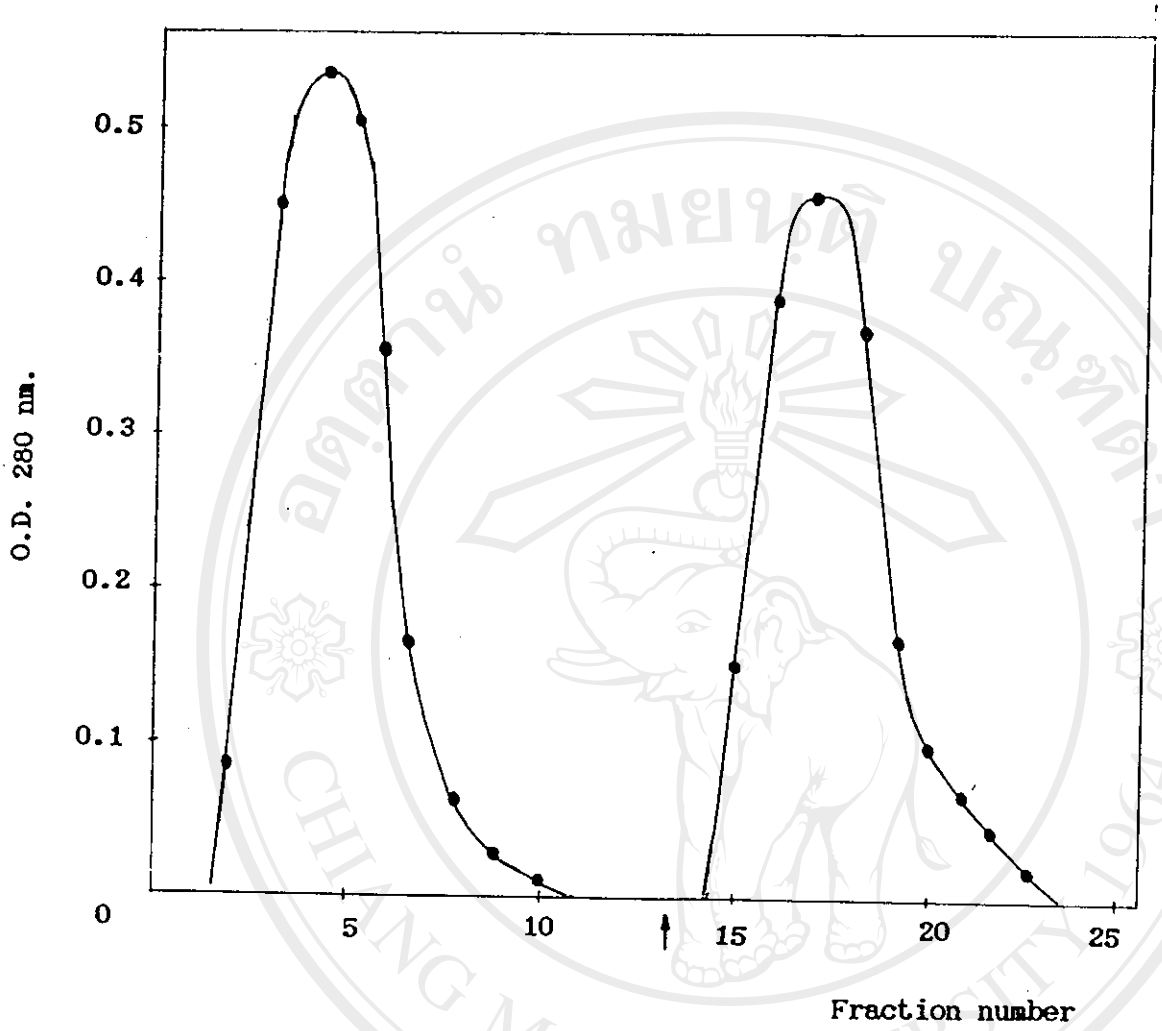

Figure III.3 8 M Urea - DEAE Sephadex A.25 Chromatography of Purified HCG.

↑ Application of 8 M Urea Containing 1 M NaCl and 0.2 M Glycine

Figure III.4 Native - Polyacrylamide Gel Electrophoresis for Isolation and Purification of HCG.

I : Partially purified HCG from DEAE Sephadex A.50.

II : Highly purified HCG from Biogel P-60.

III: Standard HCG.

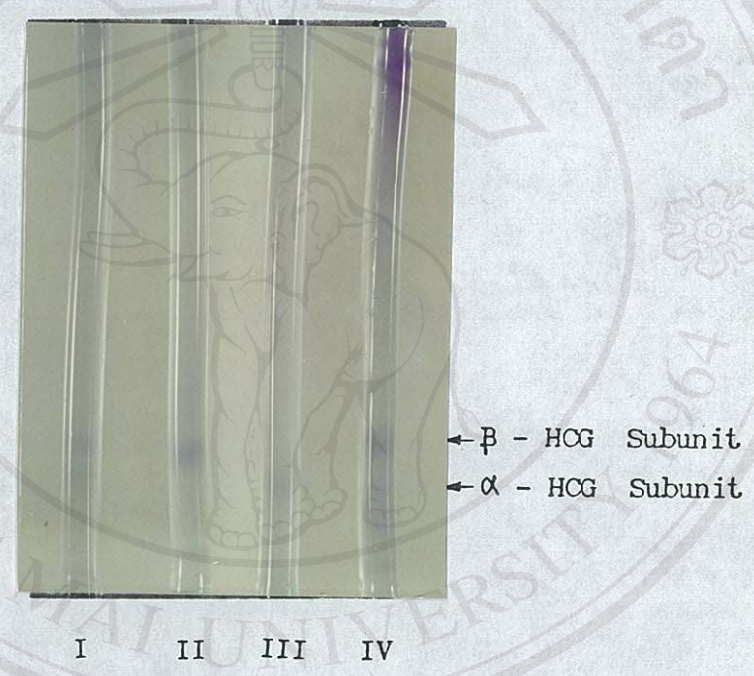

Figure III.5 SDS - Polyacrylamide Gel Electrophoresis for Isolation and Purification of β - HCG Subunit.

I : Highly purified HCG.

II : Second peak from 8 M urea - DEAE Sephadex A.25.

III: First peak from 8 M urea - DEAE Sephadex A.25.

IV : Standard HCG.

III.2. Production and Preparation of Rabbit Anti - β - HCG

Anti - β - HCG was produced in 10 New Zealand white rabbits and IgG preparation was done by ammonium sulfate precipitation (33% saturation) as described in Section II.5.2.a and II.5.2.B. Purification of rabbit anti - β - HCG was performed by affinity chromatography on HCG - Sepharose 4B and normal human serum protein - Sepharose 4 B column, respectively as described in Section II.5.2.c. Anti - β - HCG titer was detected by Ouchterlony's method and cross - reactive antibody was detected by immunoelectrophoresis as described in Section II.4.6 and II.4.7.

Total protein , total volume , anti - β - HCG titer , total titer, specific activity , purification fold and % yield of each step of anti - β - HCG preparation are summarized in Table III.2.

After two weeks of booster - injection, the immunized rabbit was bled. Rabbit serum (26 ml) contained 1:4 of anti - β - HCG titer determined by Ouchterlony's method as shown in Figure III.8. Total protein and specific activity were 1250 mg and 3.2 $titer^{-1}$/gm protein , respectively.

After ammonium sulfate precipitation, the rabbit IgG was redissolved and dialysed. The rabbit IgG (10 ml) contained 1:8 of anti - β - HCG titer as shown in Figure III.8. Total protein was 242 mg. The specific activity and purification fold were increased to 33.3 $titer^{-1}$/mg protein and 10 , respectively. The yield was 77% calculated

from total titer of anti - β - HCG content in total volume. The rabbit IgG was applied on HCG - Sepharose 4B column.

Elution profiles of HCG - Sepharose 4B chromatography of rabbit IgG are shown in Figure III.6. After acid elution with glycine - HCl pH 2.5 , fractions under the second peak were pooled , dialysed and lyophilized. The lyophilizate (2 ml) contained 7.5 mg of total protein and its titer was 1:8 detected by Ouchterlony's method as shown in Figure III.9. The specific activity and purification fold were increased to 1066.6 $titer^{-1}$/gm protein and 333 , respectively. This rabbit anti - β - HCG preparation showed no cross - reaction to normal human serum protein as shown in Figure III.10 The rabbit anti - β - HCG was applied on normal hunman serum protein - Sepharose 4B column to exclude undetectable cross - reactive antibody.

Elution profiles of normal human serum protein - Sepharose 4B chromatography are shown in Figure III.7. The protein containing fractions under the first peak were pooled , dialysed and lyophilized while glycine - HCl , pH 2.5 eluted protein containing under the second peak were discarded. The lyophilizate (1 ml) contained 7.2 mg of total protein and its titer was 1:8 detected by Ouchterlony's method as shown in Figure III.9 . The specific activity and purification fold were 1111 $titer^{-1}$/gm protein and 347, respectively. The yield was 15%. The adsorbed anti - β - HCG showed no cross - reaction to normal human serum protein detected by immunoelectrophoresis as shown in Figure III.10.

Table III.2 Production and Purification of Rabbit Anti - B - HCG.

Fractions	Total Protein	Total volume	Anti-B-HCG titer	Total Titer	Specific activity	Purification fold	Yield
	mg	ml			$titer^{-1}/g$ protein		%
Rabbit anti-serum	1250	26	1:4	104	3.2	1	100
Rabbit IgG	242	10	1:8	80	33.3	10	77
Rabbit anti-β-HCG	7.5	2	1:8	16	1066.6	333	15
Adsorbed anti-β-HCG	7.2	2	1:8	16	1111.1	347	15

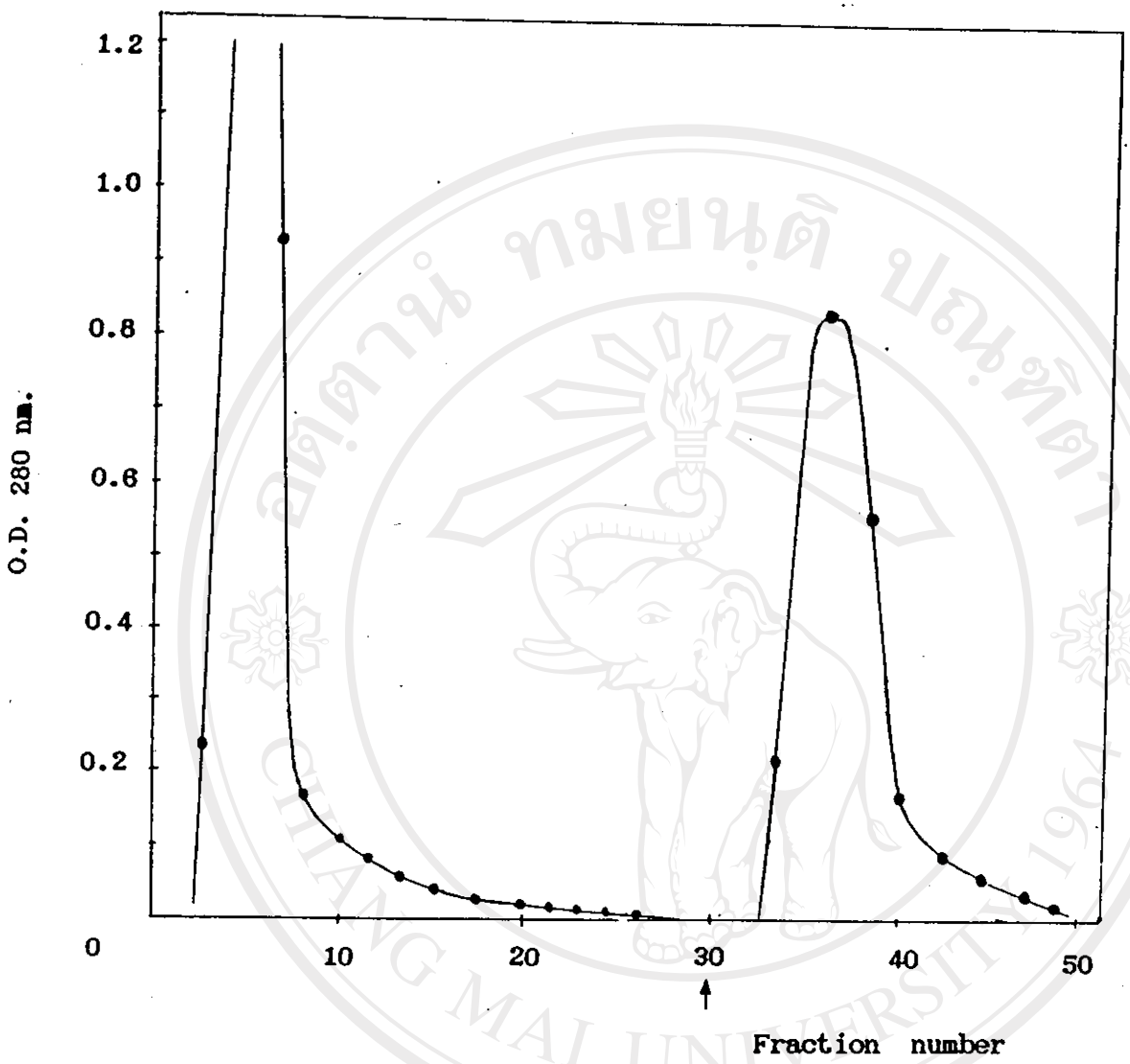

Figure III.6 HCG - Sepharose 4 B Chromatography of rabbit IgG.

↑ Application of Glycine - HCl, pH 2.5

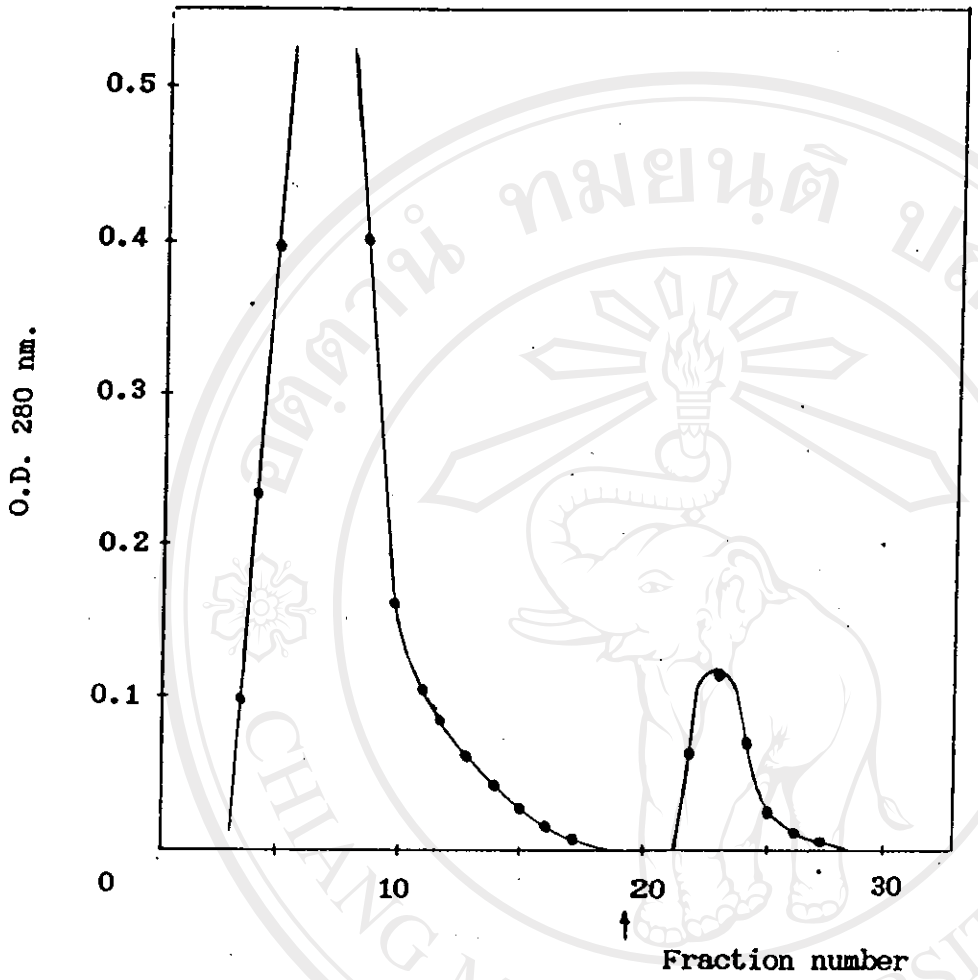

Figure III.7 Normal Human Serum Protein - Sepharose 4 B Chromatography of Rabbit Anti - β - HCG.

↑ Application of Glycine - HCl, pH 2.5

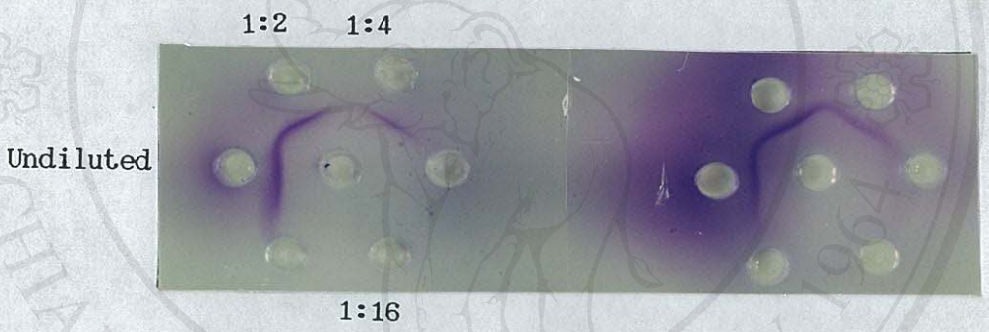

Figure III.8 Rabbit Anti - β - HCG Titer Detected by Ouchterlony's Method (I).

Left : Rabbit Anti - Serum

Right : Rabbit IgG

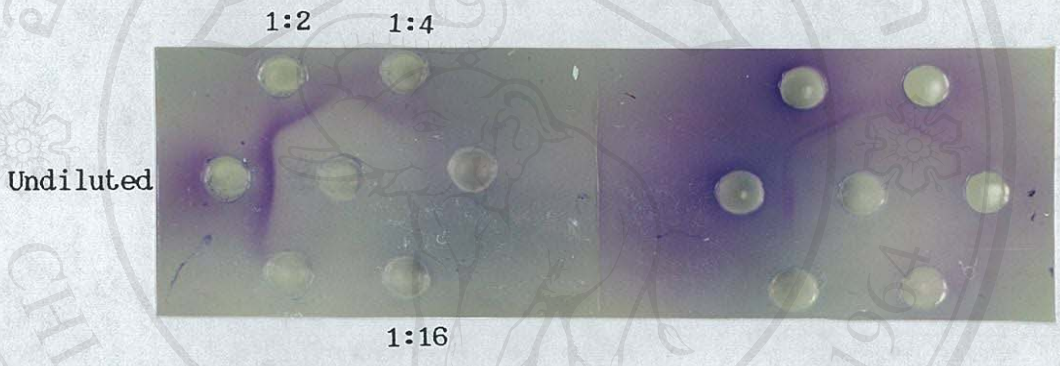

Figure III.9 Rabbit Anti - β - HCG Titer Detected by Ouchterlony's Method (II).

Left : Fraction from HCG - Sepharose 4 B chromatography.

Right : Fraction from normal human serum protein - Sepharose 4 B chromatography.

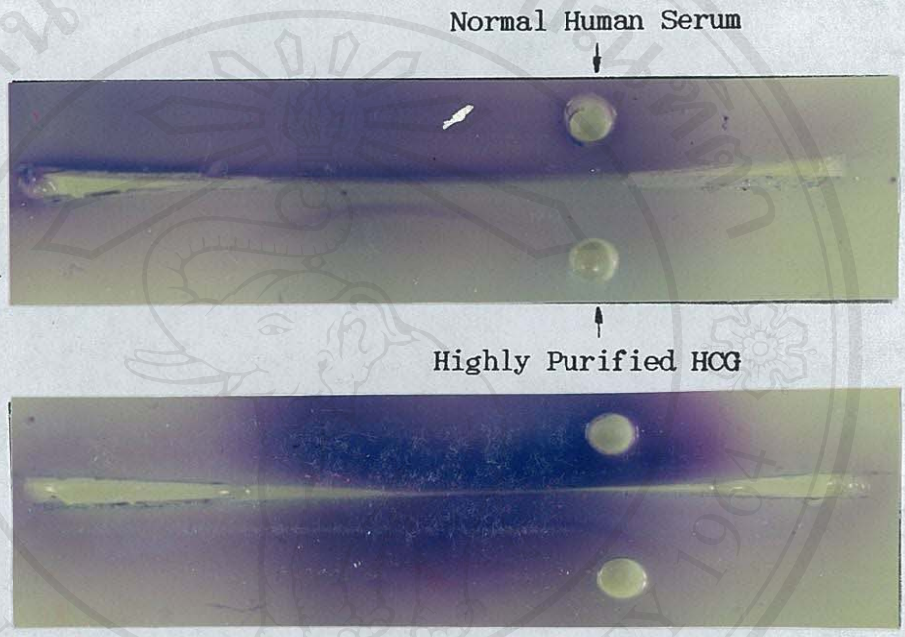

Figure III.10 Cross - Reaction Antibody Detected by Immunoelectro - phoresis.

Above : Fraction from HCG - Sepharose 4 B chromatography.

Below : Fraction from normal human serum protein - Sepharose 4 B chromatography.

III.3 Preparation and Isolation of Anti - β - HCG Horseradish Peroxidase Conjugate.

Anti - β - HCG - Horseradish peroxidase (HRP) conjugate was prepared by periodate oxidation method and separated by Sephadex G.150 chromatography as previously described in Section II.5.3.

Total protein , total HRP activity , R.Z. and % yield in the each step of preparation and isolation of anti - β - HCG - HRP conjugate are summarized in Table III.3.

Three mg of HRP (R.Z. = 2.0) contained 574 units of total HRP activity was done in the steps of amino groups blocking with FDNB , oxidation with sodium periodate , neutralization of excess peridate with glycerol and dialysis. The periodate oxidized HRP was coupled to 3.0 mg of rabbit anti - β - HCG and dialysed. After conjugation , the conjugate mixture contained 5.7 mg of total protein and 438 unit of total HRP activity while the R.Z. was decreased to 0.92. The yield was 76% as calculated from HRP activity. The conjugate mixture was applied on a Sephadex G.150 column.

Elution profiles of Sephadex G.150 chromatography are shown in Figure III.11. Protein containing fractions under the first peak were pooled and stored at -20 °C by adding an equal volume of glycerol. It totally contained 4.2 mg of protein and 156 units of HRP activity. An average R.Z. was decreased to 0.42 and the yield was 27%.

Table III.3 Preparation and Isolation of Anti - β - HCG - HRP Conjugate.

Fractions	Total protein	Total HRP activity	R.Z.	Yield
	mg.	unit*		%
Before conjugation				
Rabbit anti-B-HCG	3.0	-	-	-
HRP	3.0	574	2.0	100
After conjugation				
Conjugate mixture	5.7	438	0.91	76
Sephadex G.150				
Peak I	4.2	156	0.42	27

* Pyrogallol units of peroxidase activity

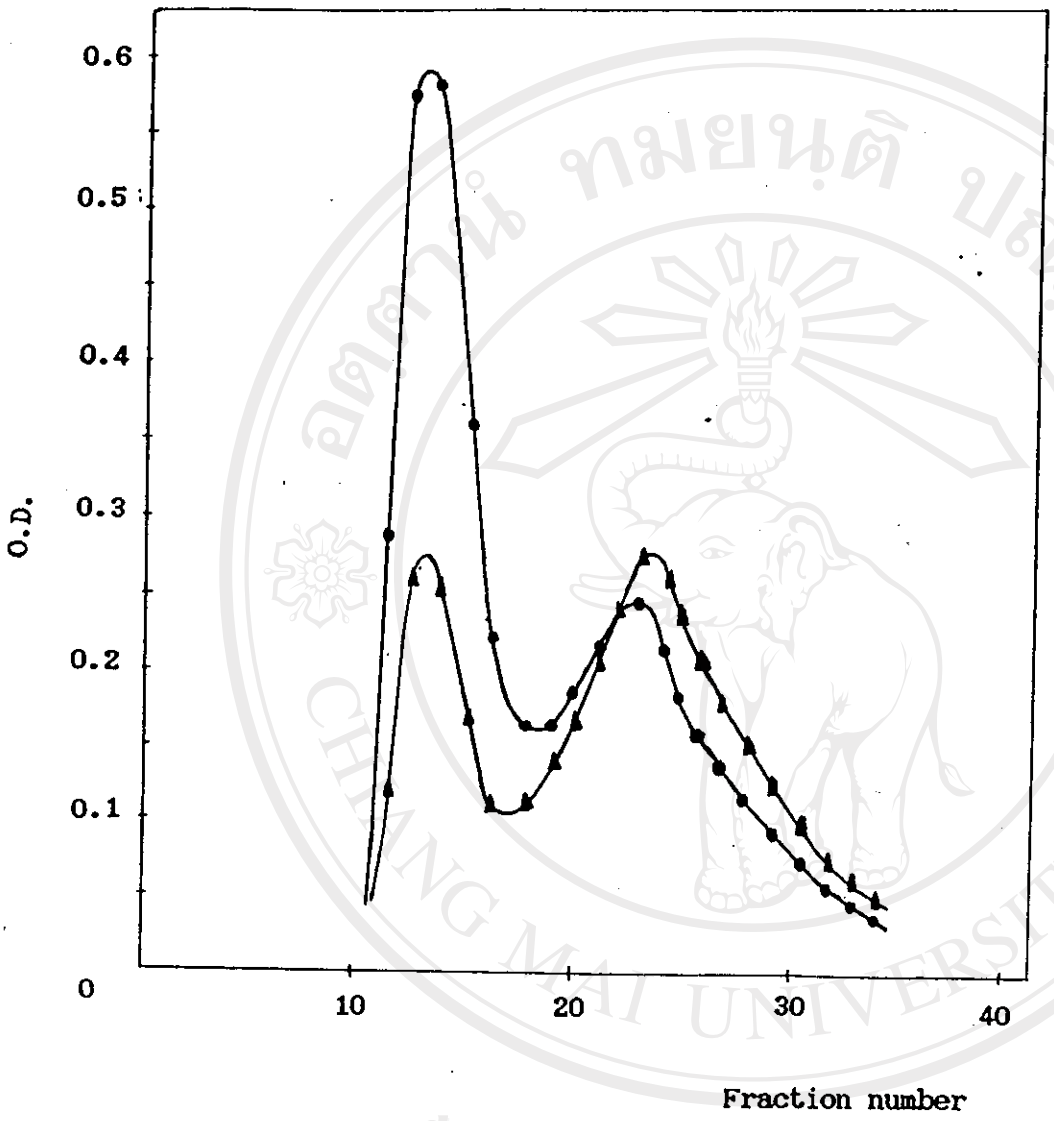

Figure III.11 Sephadex G.150 Chromatography of Anti - β - HCG - HRP Cojugate.

O.D. at 280 nm. —●—●— O.D. at 403 nm. —▲—▲—

III.4. Optimization Study on ELISA Technique.

III.4.1 Optimal Dilution of Anti - β - HCG for Coating

Stock anti - β - HCG solution (0.5 mg/ml) was serially diluted to 1:125 , 1:250 , 1:500 and 1:1000 fold with 0.05 M sodium carbonate buffer, pH 9.5 containing 0.1% sodium azide.(w/v). One ml of the each diluted anti - β - HCG was incubated in 12 x 75 mm ethanol cleaned polystyrene tubes at room temperature for 12 hr. After coating , the tubes were washed with 0.01 M sodium carbonate buffer pH 9.5 and 1.0 ml of 1 % (w/v) bovine serum albumin in 0.05 M sodium carbonate buffer, pH 9.5 containing 0.1% sodium azide was added and incubated at room temperature for 4 hr. Excess bovine serum albumin was removed and washed the tubes with washing buffer. The solid phase anti - β - HCG coated polystyrene tubes were tested for suitable dilution of antibody for coating by the methods in section II.5.5. The incubation periods of the first and second reactions were maintained for 4 and 2 hr, respectively and 1:100 dilution of anti - β - HCG - HRP conjugate was constantly used.

Figure III.12 shows the effect of various dilution of anti - B - HCG on coating of the polystyrene tubes. The result from 1:125, 1:250 and 1:500 dilutions are slightly different while 1:1000 dilution shows the least binding to HCG. Due to the sensitivity and economy, 1:500 dilution of anti - β - HCG was more likely suitable and chosen for coating the tubes.

III.4.2 Optimal Dilution of Anti - β - HCG - HRP Conjugate for Test

The 1:500 diluted anti - β - HCG was used to coat polystyrene tubes. Procedure steps were performed as described in Section III.4.1. The dilution of anti - β - HCG - HRP conjugate were varied as a 1:50, 1:100, 1:200 and 1:400, respectively.

The result is illustrated in Figure III.13. It shows that the 1:200 dilution of the conjugate is suitable for enzyme immunoassay, because it increases sensitivity as nearly high as the other dilution (1:50 and 1:100) but with lower background as less as in the dilution of 1:400.

III.4.3 Effect of Incubation Periods of the First Reaction.

Incubation periods of the first reaction (1,2,4 and 6 hr.) were tested. The second reaction time had been fixed for 2 hr. The suitable dilution of coating anti - β - HCG (1:500) and the anti - β - HRP conjugate (1:200) were used in this experiment.

The result is illustrated in Figure III.14. Using low and medium concentrations of HCG (25 and 100 mi.u./ml), the saturation of solid phase anti - β - HCG occurred in 4 hours while high concentration of HCG (200 mi.u./ml) was not. The 4 hr incubation period was selected for the experiment. The omitted 6 hr. one, even gives a better linearity, but needs too time for the experiment.

III.4.4 Effect of Incubation Periods of the Second Reaction.

Different incubation periods (1,2,3 and 4 hr.) were tested for the second reaction with other fixed conditions previously described

(1:500 dilution of anti - β - HCG for coating, 1:200 dilution of the conjugate and 4 hr. incubation period for the first reaction).

The result is illustrated in Figure III.15. It was found that the 2 hr incubation period showed low background as well as 1 hr period but their O.D. values were too low. However, the 3 and 4 hr incubation periods exhibited higher background with high O.D. differences. The 2 hr incubation period of the second reaction was therefore suitably chosen for further use in ELISA technique.

III.4.5 Effect of Non-Specific Protein Added in Anti - β - HCG - HRP Conjugate.

Different concentrations of chicken serum (0, 6.2, 12.5 and 25%) containing in the working conjugate were tested with other fixed conditions previously described (1:500 dilution of anti - β - HCG for coating, 1:200 dilution of the conjugate and 4, 2 hr. incubation periods for the first and second reaction, respectively.)

The result is illustrated in Figure III.16. The conjugate containing without chicken serum showed high O.D. value of background. Chicken serum contained in the conjugate decreased the background, in the order of chicken serum concentrations. The 12.5 and 25% concentrations of chicken serum in the conjugate showed no significance of O.D. values of background while the 6.2% showed higher than the other two. The 12.5% concentration of chicken serum was chosen for use in the test as described in the text.(71)

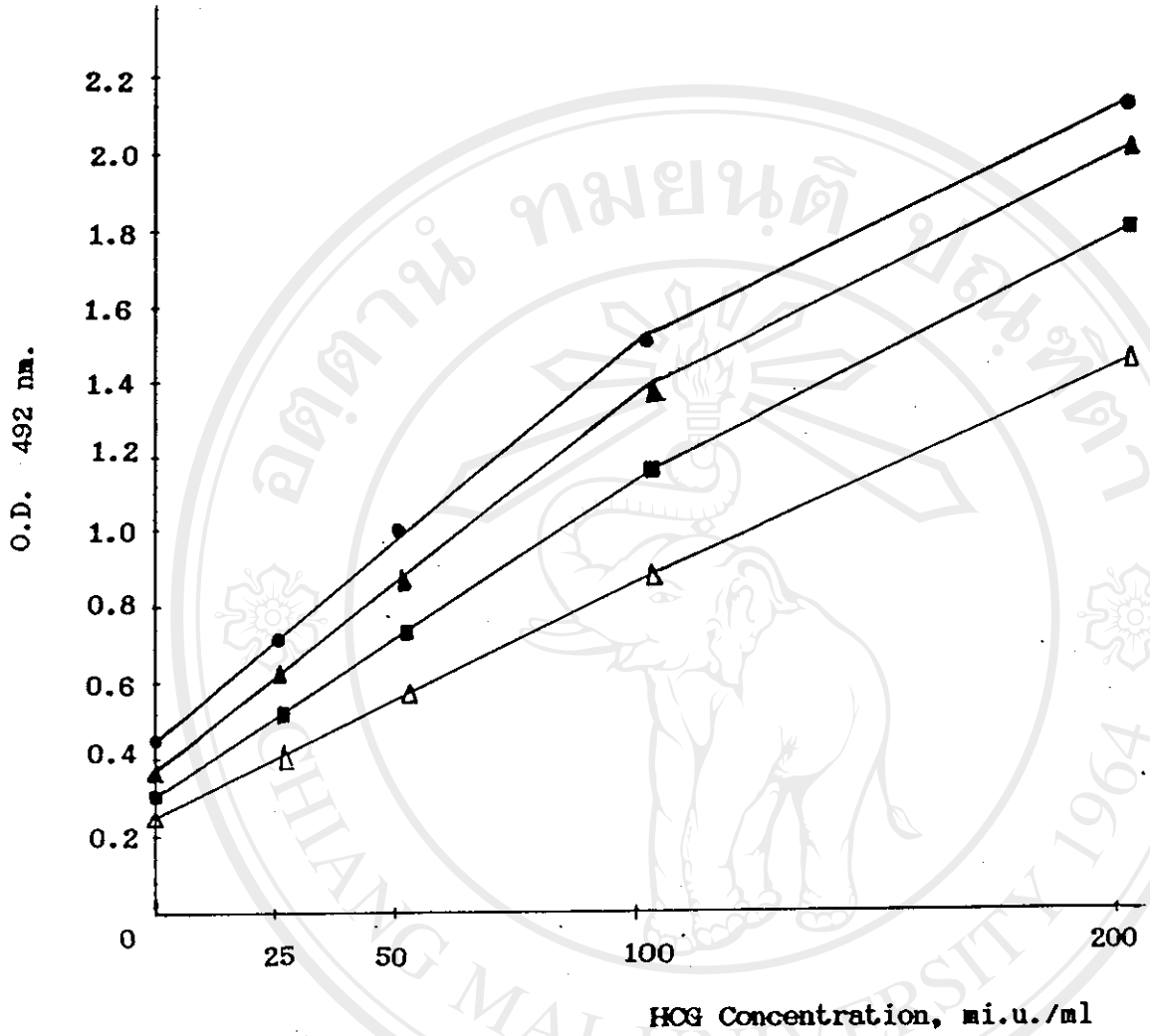

Figure III.12 Comparison of ELISA Technique Using Dilutions of Anti - β - HCG for Coating Polystyrene Tubes.

1 : 125 —●—●— 1 : 250 —▲—▲—

1 : 500 —■—■— 1 : 1,000 —△—△—

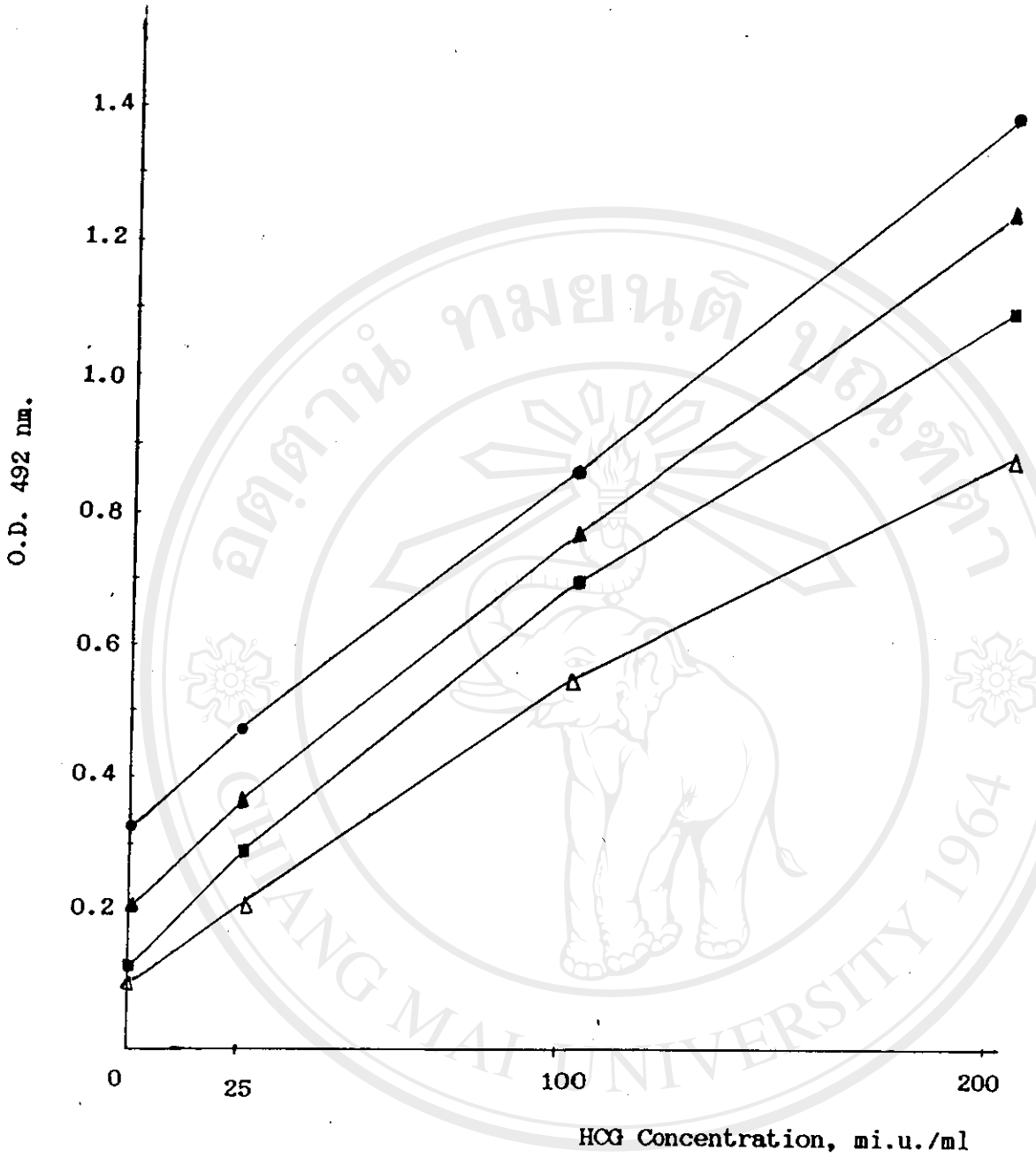

Figure III.13 Comparison of Dilutions of Anti - β - HCG - HRP Conjugate Used in ELISA Technique.

1 :50 —●—●— 1 : 100 —▲—▲—

1 : 200 —■—■— 1 : 400 —△—△—

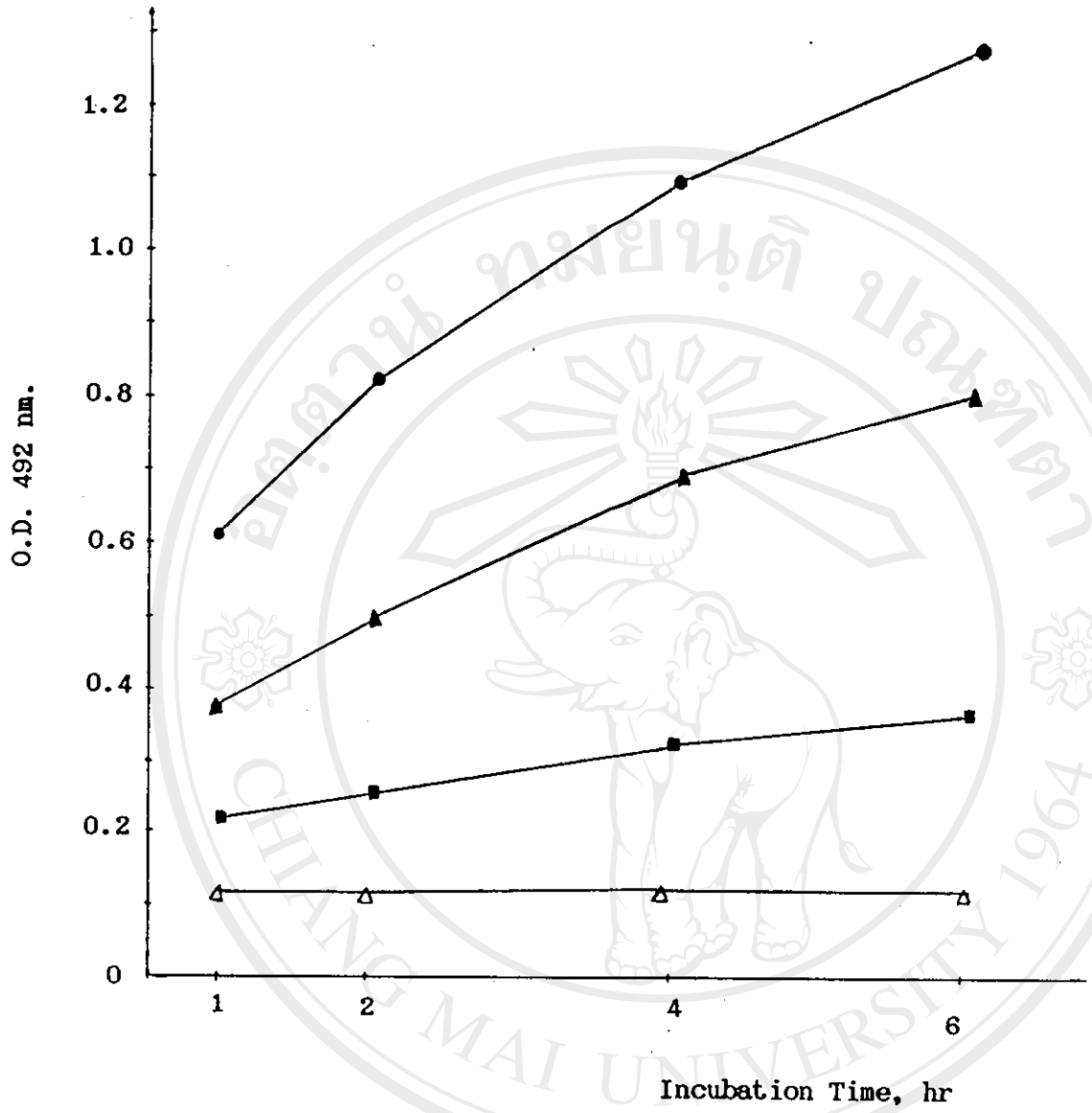

Figure III.14 Comparison of Incubation Periods for the First Reaction of Solid Phase Anti - β - HCG with HCG in ELISA Technique.

200 mi.u./ml —●—●— 100 mi.u./ml —▲—▲—

25 mi.u/ml —■—■— 0 mi.u./ml —△—△—

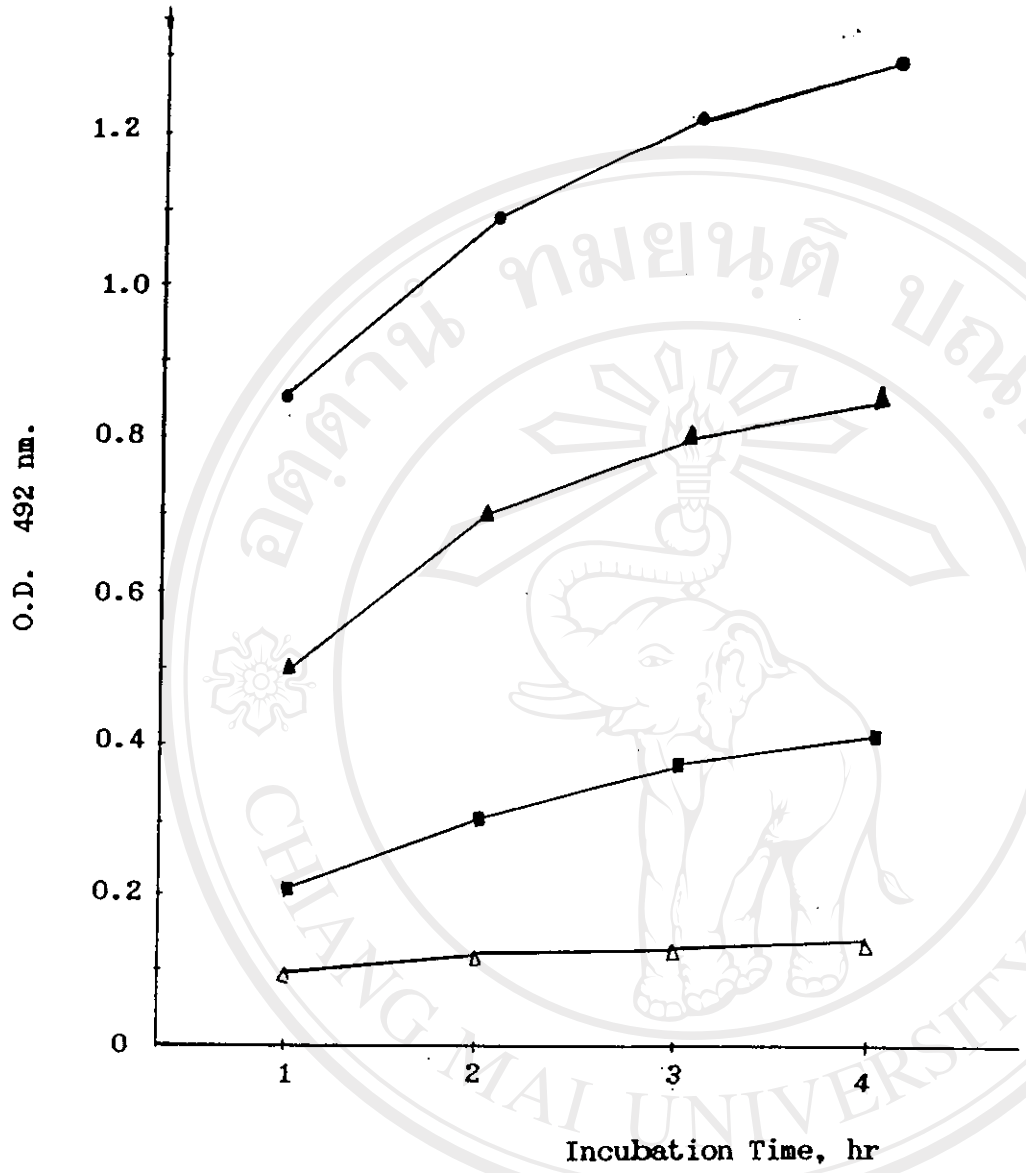

Figure III.15 Comparison of Incubation Periods for the Second Reaction of Solid Phase Bound HCG and Anti - β - HCG - HRP Conjugate in ELISA Technique.

200 mi.u./ml —●—●— 100 mi.u./ml —▲—▲—

25 mi.u/ml —■—■— 0 mi.u./ml —△—△—

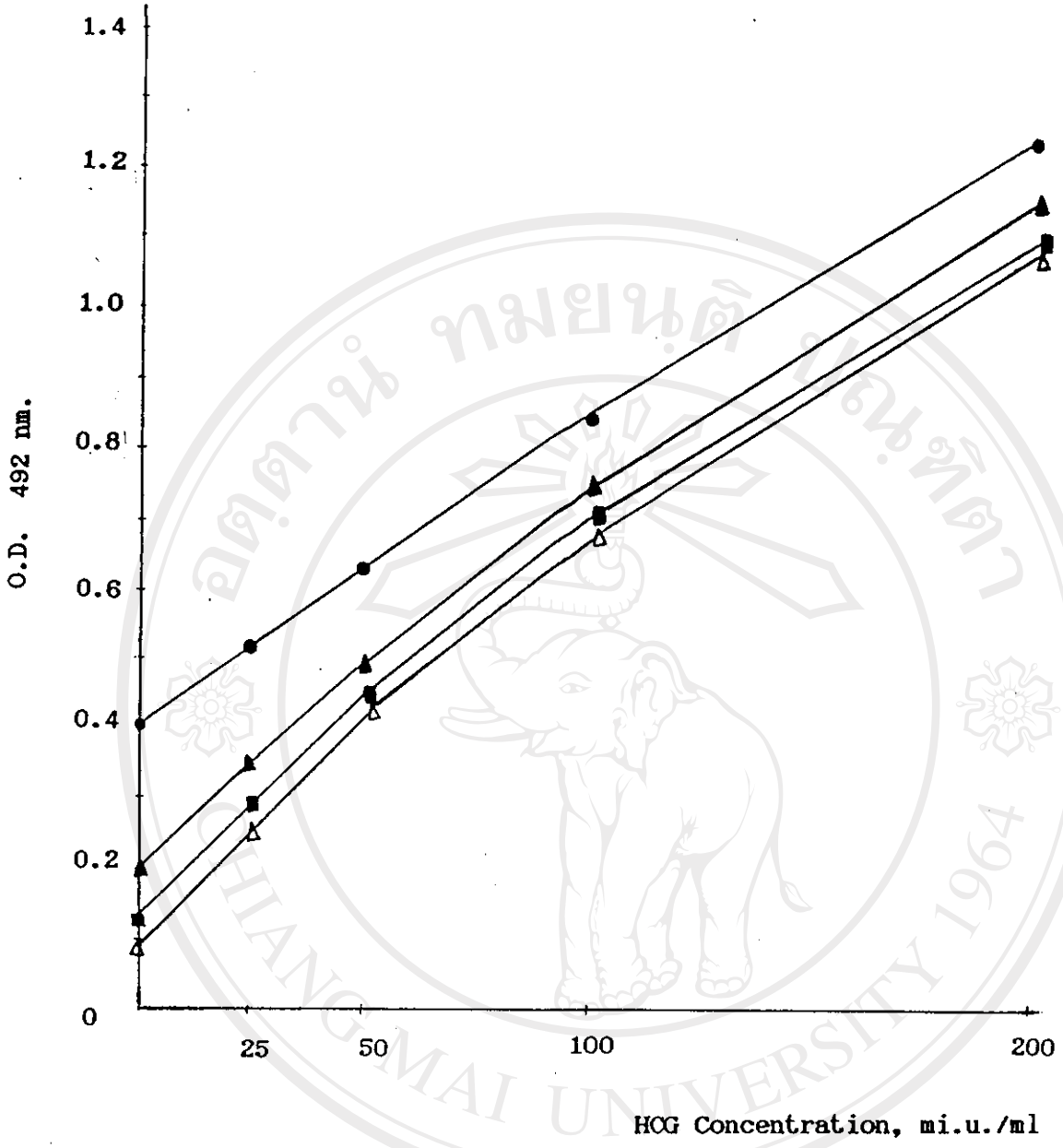

Figure III.16 Effect of Different Concentrations of Chicken Serum Added into the Conjugate.

0 % —●—●— 6.2 % —▲—▲—

12.5 % —■—■— 25.0 % —△—△—

Polystyrene tubes coated with 1:500 diluted anti - β - HCG

↓ R.T., 12 hr.

↓ Washed

1% Bovine serum albumin

↓ R.T., 4 hr.

↓ Washed 3 times

Standard or serum samples and diluent buffer

↓ 37 °C, 4 hr.

↓ Washed 3 times

1:200 of Anti - β - HCG - HRP Conjugate

↓ 37 °C, 2 hr.

↓ Washed 3 times

Subtrate - chromogen solution

↓ R.T., 30 min

↓ Dark condition

1.0 M Sulfuric acid

↓

O.D. 492 nm

Chart III.1 Flow Diagram of the Procedure of Local-Made ELISA for HCG.

III.5 Sensitivity of the Test.

The sensitivity of local - made ELISA was determined by O.D. reading from different concentrations of standard HCG. The least HCG concentration which gave O.D. value higher than mean + 2 S.D. level of that obtained from the blank (zero concentration of HCG in normal human serum) was recognized as the sensitivity of the test (72). Ten blank controls were examined and mean (+ 2 S.D.) O.D. was 0.12 + 0.02. The sensitivity was found to be about 4 mi.u./ml as calculated from the calibration curve shown in Figure III.17.

III.6 Effect of Serum Dilution

High levels over 200 mi.u./ml of HCG in native serum samples could not be directly measured by ELISA , they had to be appropriately diluted. In this study , a serum sample containing 315 mi.u./ml HCG was diluted to 1:2 , 1:4 , 1:16and 1:32 with normal human serum and brought for the ELISA test.

The results are presented in Table III.4. It is shown that from the confident interval of calibration curve , no significant difference between the expected values and the estimated ones. However , at higher dilution , (i.e. 1:32) the recovery was highly fluctuated.

III.7 Cross - Reaction Effect of LH and FSH

A series of standard LH (100 , 50 , 25 and 12.5 mi.u./ml) and FSH (75 , 37.5 , 18.75 and 9.37 mi.u/ml) dissolved in normal human serum were prepared and tested for cross - reaction by local - made B - HCG

ELISA as the previously described.

The results are illustrated in Figure III.18. Cross - reaction was calculated from $\frac{\text{assay concentration}}{\text{actual concentration}} \times 100\ \%$ (50)

LH and FSH showed 24 and 15% cross - reaction at the concentration of 100 and 75 mi.u/ml , respectively. However , at the concentrations of LH < 12.5 mi.u./ml and FSH < 9.3 mi.u./ml , the cross - reaction was nil

III.8 The Precision Test

The precision study both within assay and between assay was carried out by using low , medium and high serum levels. Repeated determinations (N=10) for each sample were performed.

The results are illustrated in Table III.5. The coefficients of variation of within assay were 6.5 , 5.8 and 8.1 % and between assay were 11.1 , 7.6 and 7.3 % in the respective order of low , medium and high serum levels. The precision of all levels of serum control both within assay and between assay were therefore acceptable. In general , the accepted variation of any assay should not be far over 10%.

III.9 The Accuracy Test

Serum samples obtained from 30 patients with choriocarcinoma , hydatidiform mole , ectopic pregnancy and normal pregnancy , and each 20 of normal healthy men and women were tested both by commercial β - HCG ELISA (Roche) and local - made β - HCG ELISA.

The results are illustrated in Figure III.19. Serum HCG values

measured by commercial β - HCG ELISA were given as X and those measured by local - made ELISA were given as Y. The correlation coefficient (r) was 0.945. The linear - regression equation was Y = 1.01X + 3.34. This high correlation coefficient indicates that serum HCG values measured by both commercial β - HCG ELISA and local - made ELISA were gave similar results.

However , mean serum HCG levels of normal healthy women (9.5 ± 5.4 mi.u./ml) was significantly higher than that of normal healthy men (5.0 ± 2.1 mi.u./ml) at $p < 0.01$ (X^2 test) determined by local - made , ELISA. While , by commercial β - HCG ELISA determination , mean serum HCG levels both in normal healthy women (<1.7 mi.u./ml) and men (<1.7 m i.u./ml) were not significantly different. Since cross - reaction effect of LH and FSH influenced in the determination HCG by local - made ELISA , the elevation of serum HCG levels in women may be due to the presence of serum LH and FSH which always higher than in men. The frequent distribution of serum HCG levels in normal healthy women and men are shown in Figure III.20.

Serum HCG levels measured by both commercial β - HCG (Roche) and local - made ELISA in each group of patients are presented in Table III. 6.

III.9 Stability of Reagents of Local - Made ELISA.

Reagents of the developed β - HCG ELISA i.e. solid phase anti - β - HCG, working conjugate and substrate buffer stored at 4° C all the

time were tested for their stability at 1, 2, 3 and 4 weeks after preparations.

The results are illustrated in Figure III.21. After 2 weeks stored at 4°C, the dose - curve showed significantly decreased compared to that of storage at 1 and 2 weeks, respectively. When the solid phase anti - β - HCG was replaced with a new one, the dose - response curve was retained.

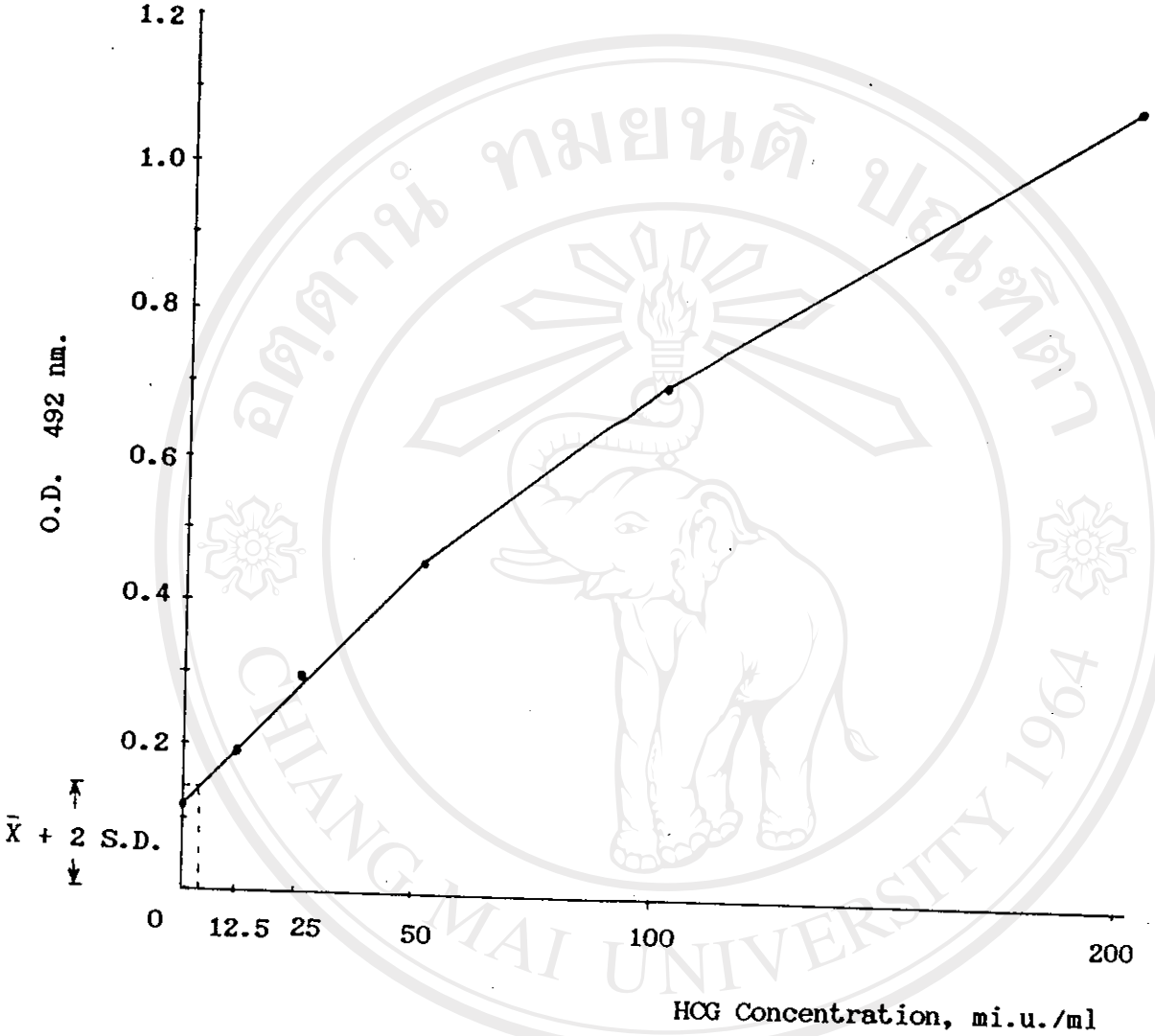

Figure III.17 Calibration Curve and Sensitivity of Local - Made β - HCG ELISA.

Table III.5 Precision Test of Local-Made ELISA Technique for β - HCG Determination.

Assay	HCG Concentration		C.V.
	mean (mi.u./ml)	S.D.	%
Within assay			
Low	16.9	1.1	6.5
Medium	67.2	3.9	5.8
High	161.4	13.2	8.1
Between assay			
Low	15.8	1.8	11.1
Medium	60.6	4.6	7.6
High	180.0	13.1	7.3

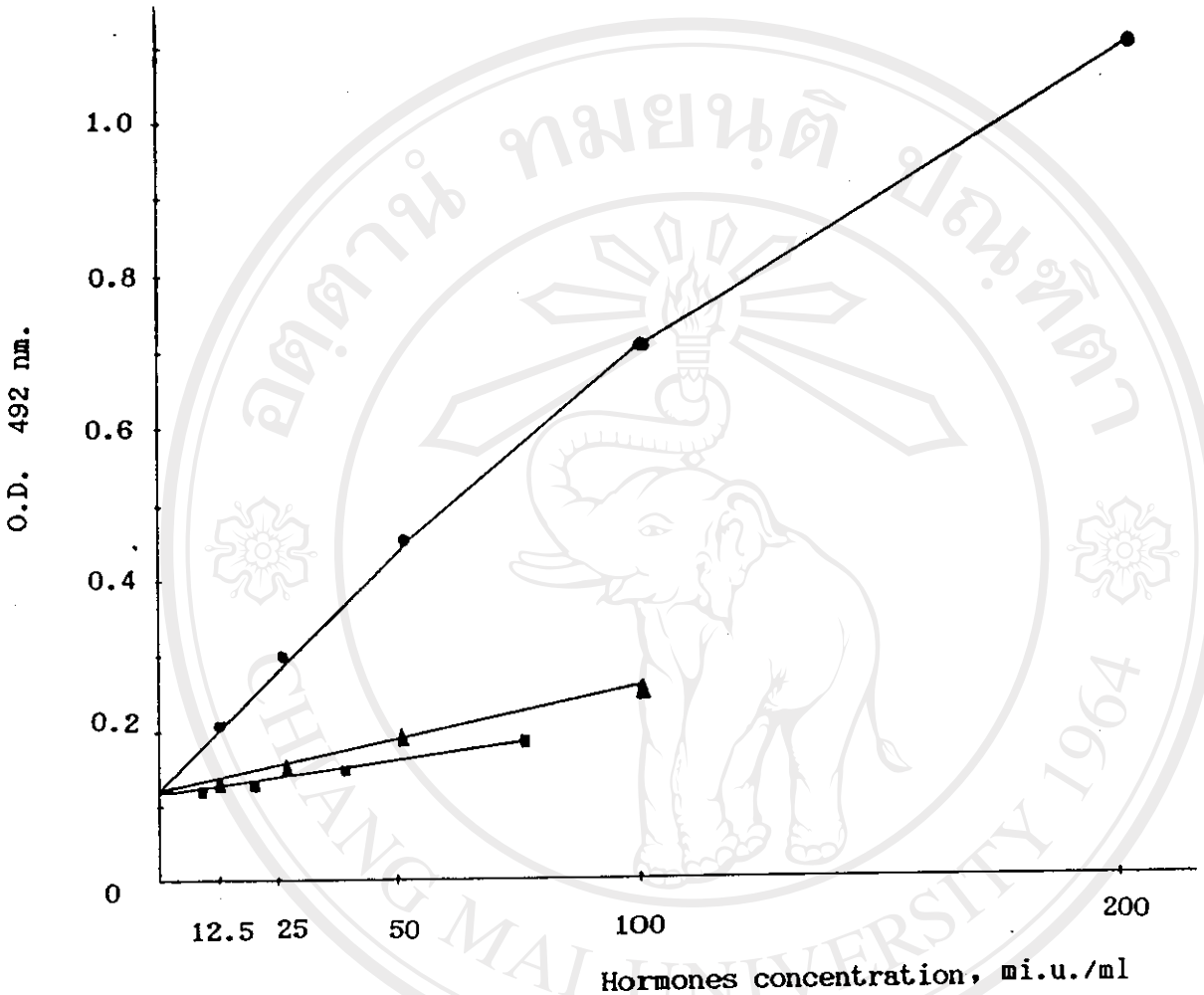

Figure III.18 Cross - Reaction Effect of LH and FSH on HCG Determined by Local - Made ELISA Technique.

HCG —●—●— LH —▲—▲—

FSH —■—■—

Table III.4 Effect of Serum Dilution in ELISA Technique for β - HCG Determination

Serum Dilution	Expected	Measured	Recovery
	mi.u./ml	mi.u./ml	%
Undiluted	-	315	100
1:2	157.5	160	101.6
1:4	78.7	80	101.7
1:8	39.3	42.5	108.1
1:16	19.6	20	102.0
1:32	9.8	11	111.1

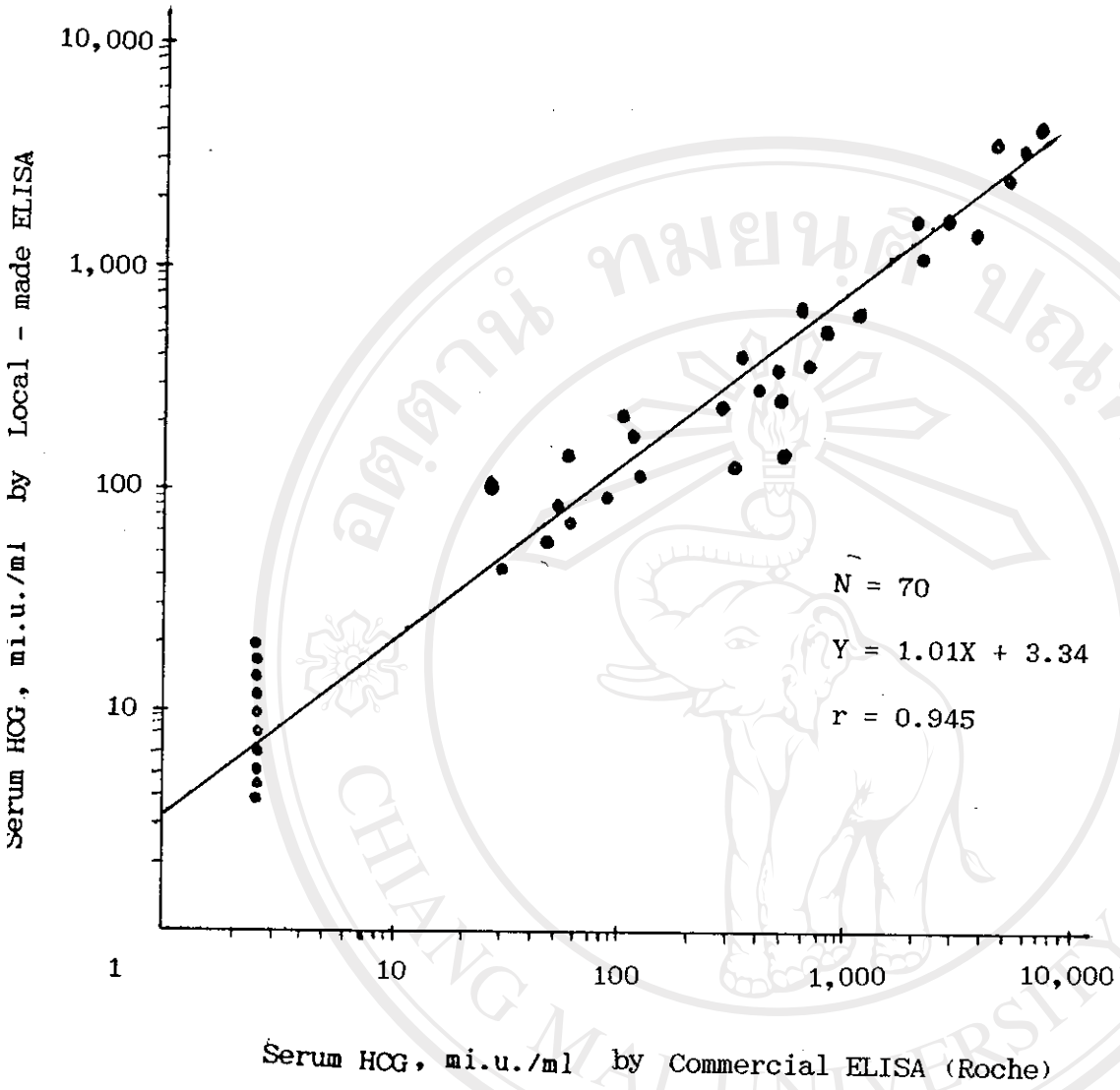

Figure III.19 Correlation Curve of Commercial β - HCG ELISA (Roche) and Local - made ELISA.

Table III. Range and Mean Serum HCG Levels of Each Group of Patients Measured Both Commercial β -HCG ELISA (Roche and Local - Made β - HCG ELISA)

Group of patients	N.	Serum HCG levels (mi.u./ml)			
		Commercial β-HCG ELISA		Local-made β-HCG ELISA	
		Range	Mean	Range	Mean
Normal healthy men	20	-	<1.7	<4 - 10	5
Normal healthy women		-	<1.7	<4 - 20	9.5
Hydatidiform mole	16	19-2400	518	45-2780	541
Choriocarcinoma	6	72-370	146	80-400	371
Ectopic pregnancy	5	172-800	296	119-560	274
Normal pregnancy	3	16-1700	789	37-1660	787

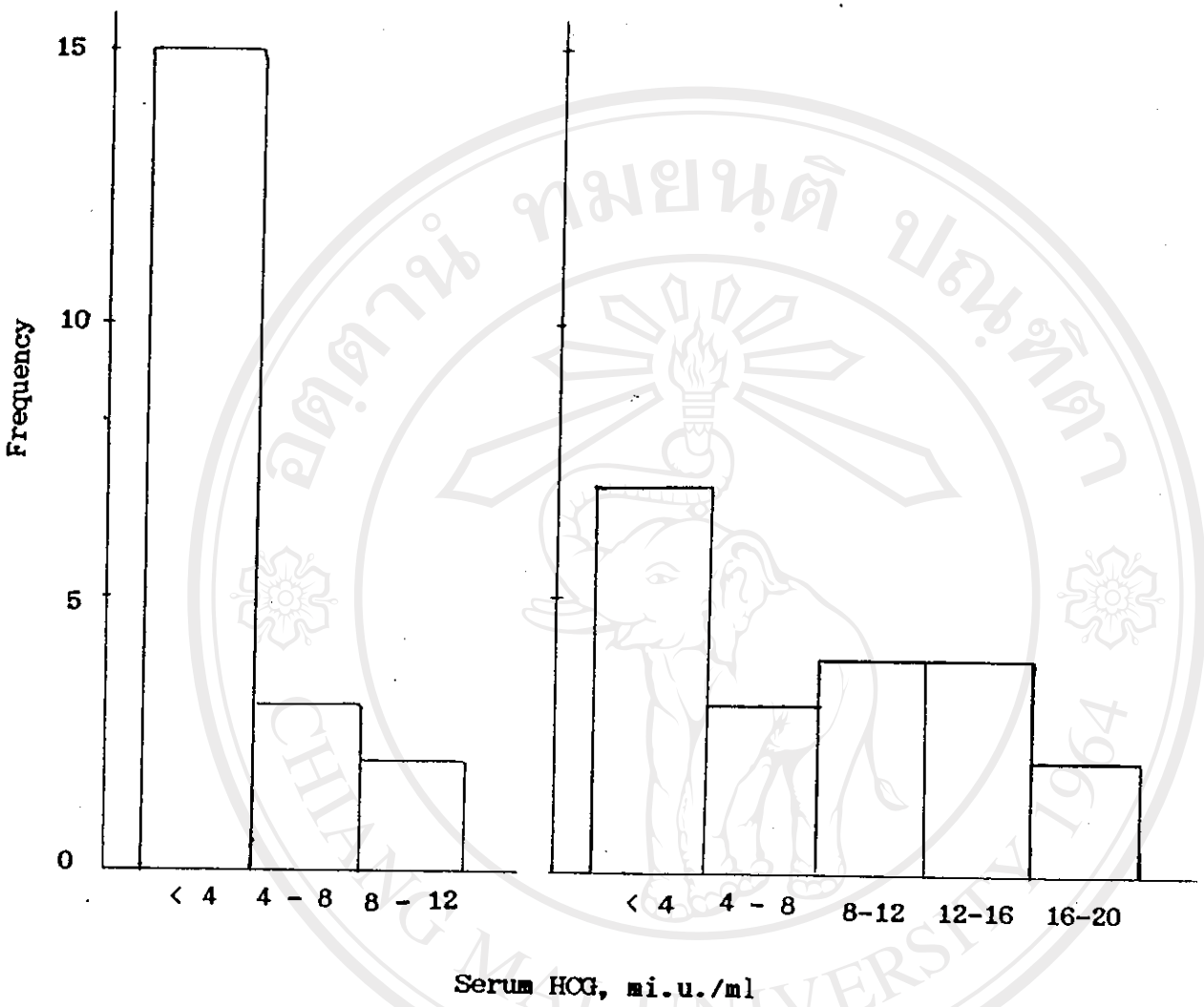

Figure III.20 Frequent Distribution of Serum HCG Levels in Normal Healthy Men (Left) and Women (Right) Measured by Local - Made ELISA.

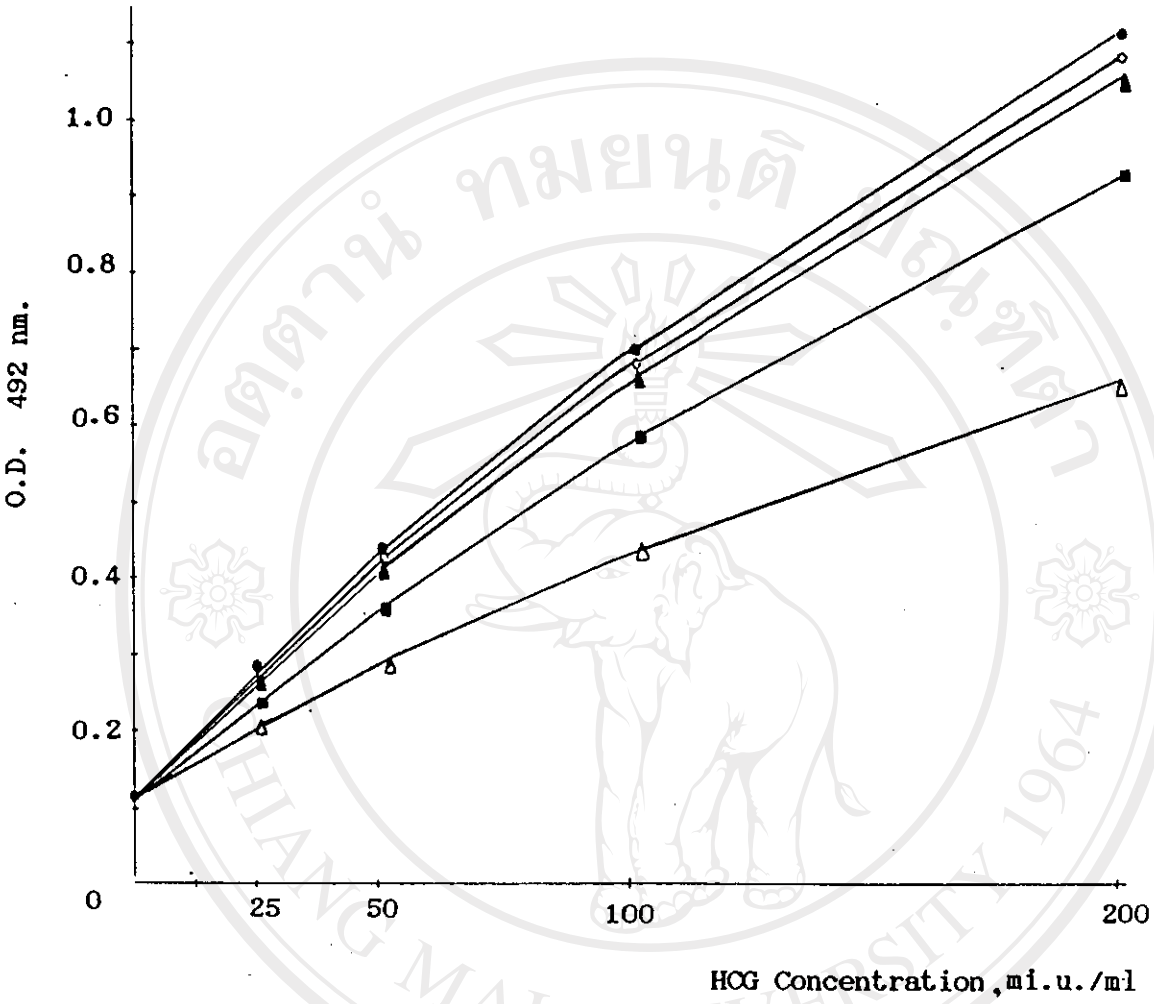

Figure III.21 Stability of Reagents of Local - Made ELISA.

Week After Preparations.

1 Week —●—●— 2 Weeks —▲—▲—

3 Weeks —■—■— 4 Weeks —△—△—

Replaced 4th Week Solid Phase Anti - β - HCG

With New Preparation. —○—○—