CHAPTER III

RESULTS

1. Cellulose acetate electrophoresis

HTg was shown a purity substance in Fig.5

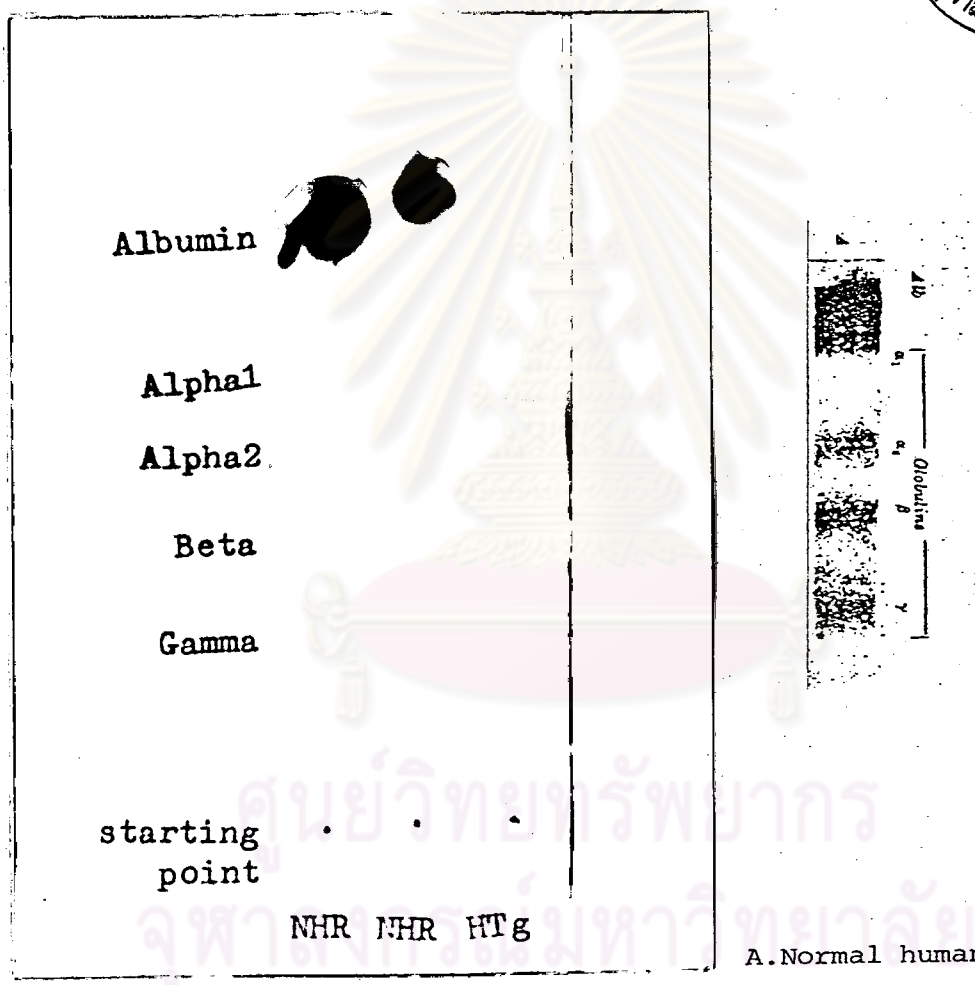

Fig.5 Cellulose acetate electrophoresis of HTg and NHR

Reference refers "Homogeneity of this HTg solution was established by cellulose acetate electrophoresis "Single band in the inter-alpha region." (3)

2. Immunodiffusion agar

A.Three rabbits can produce anti-HTg antiserum.

There are precipitin line in agar between HTg and Rabbit anti-HTg (Fig.6)

Fig. 6

Purified agar 1.5 % w/v

R_1, R_2, R_3 = Rabbit antiserum 1, 2 and 3 respectively.

Pat = Patient serum

Staining by Ponceau S 0.2 % w/v in 7.5 % trichloroacetic acid

Tg = HTg

B. Rabbit anti-HTg has a specification to only HTg (Fig.7)

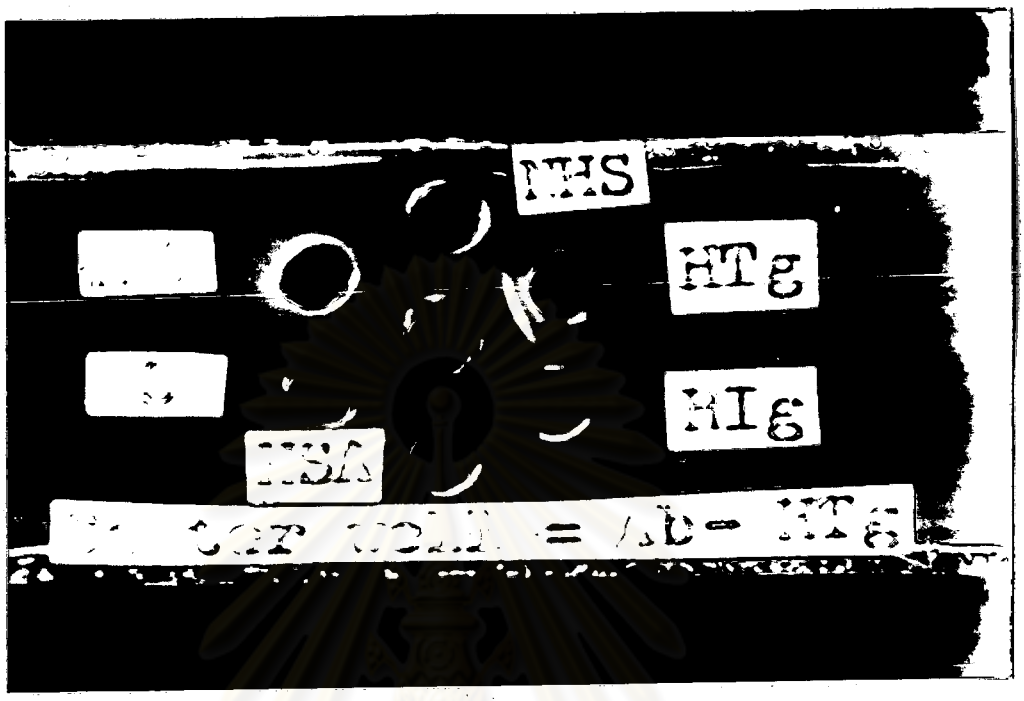

Fig. 7 Agarose 0.5 % w/v

Reference refers. Specificity.

A single immunodiffusion precipitation line (Fig.8) formed between the antiserum to HTg and HTg, while there was no reaction with either normal human serum or the most likely contaminants, albumin and γ-globulin, in pure form

(Ref : clinical chemistry, vol 24 (2) 1978, 269)

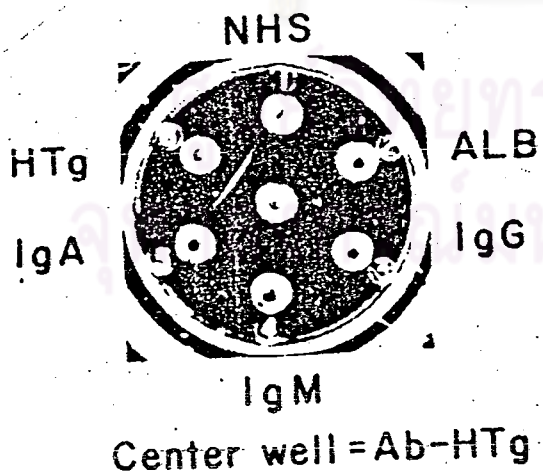

Fig. 8. Immunodiffusion analysis of rabbit antiserum to thyroglobulin (Ab-HTg)
NHS, normal human serum; *Alb*, albumin; *IgG*, immunoglobulin-G; *IgM*, immunoglobulin-M; *IgA*, immunoglobulin-A; and *HTg*, human thyroglobulin

C. Rabbit anti-HTg antiserum were shown concentration in the Fig.9

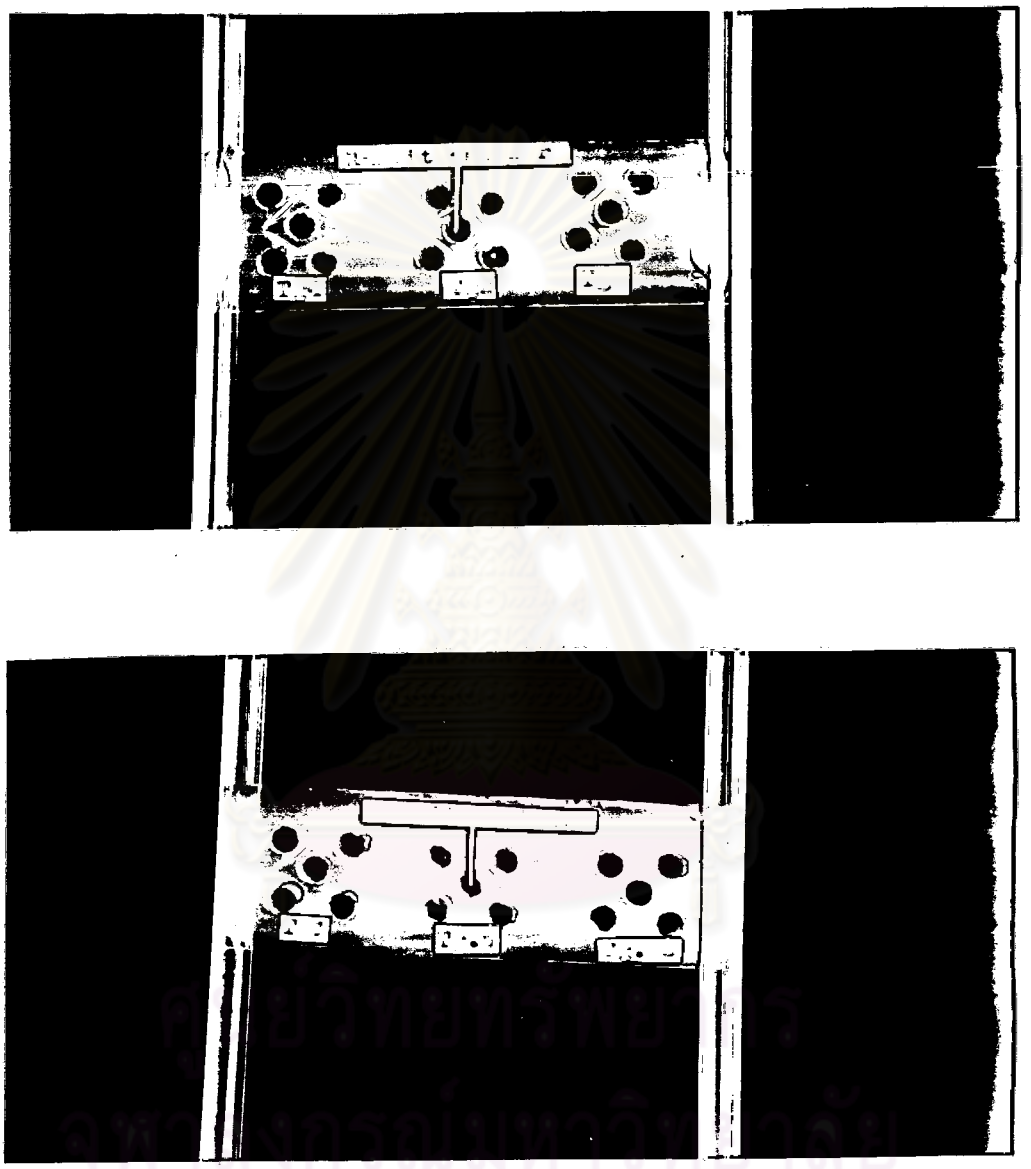

Fig 9 Agarose 0.5 % w/v

Tg 4 = HTg 4 µg/µl

Tg 2 = HTg 2 µg/µl

Tg 1 = HTg 1 µg/µl

Tg 0.5 = HTg 0.5 µg/µl

Tg 0.25 = HTg 0.25 µg/µl

3. Comparision the labelling of HTg with I^{125} when normal saline and veronal buffer were used as solvent to dissolve HTg. (Fig 10)

   A. LI, LII are the Labelling I,II and have reaction time for 15, 10 minutes respectively when normal saline was used as a solvent.

   B. L III, LIV are the Labelling III, IV and have reaction time for 8, 12 minutes respectively when veronal buffer was used as a solvent.

Table I Comparision the labelling of HIg with I^{125}

Labelling	% Bound	% Dissociation
L I	63.22	70.25
L II	62.13	64.52
L III	64.88	21.91
L IV	64.45	34.46

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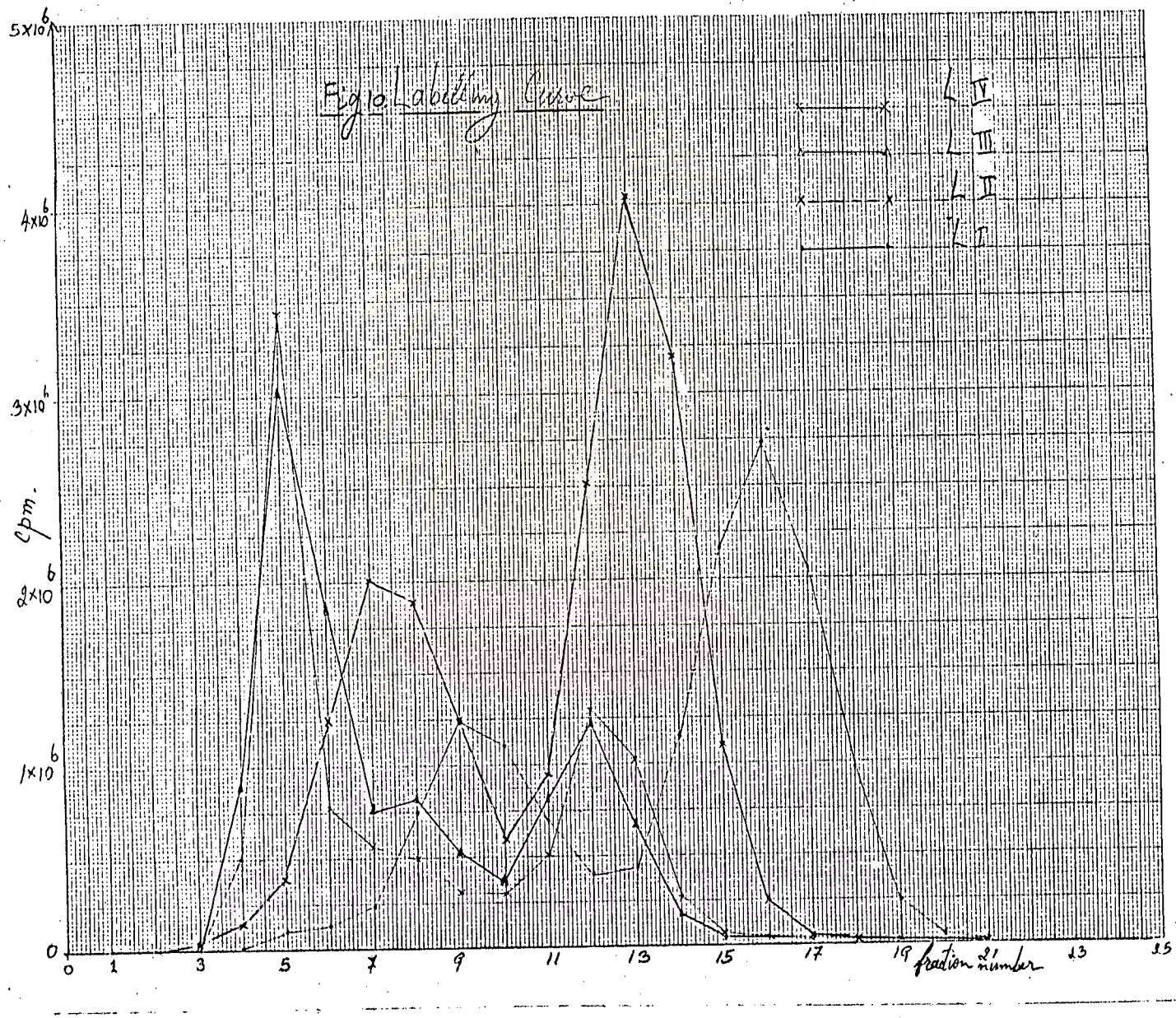

Fig 10. Labelling Curve

From table 1 and experiments concept that.

1. Veronal buffer is a better solvent than normal saline
   a. Percentage of dissociation is lower
   b. The peak is sharp (Fig. 10)
   c. $HTgI^{125}$ is more stable
2. Both veronal buffer and normal saline have the same reaction, percentage of dissociation is higher when reaction time is longer, and percentages of bound are not difference value.

4. Titre of antisera (Rabbit anti-HTg)

Titre was assessed by measuring the binding of $HTgI^{125}$ by serial dilution of antisera. Table 2 and Fig. 11 illustrates the binding of $HTgI^{125}$ to antisera obtained in rabbit 2.

Table 2

Comparision of % Bound $HTgI^{125}$ to antisera dilution.

Antisera dilution	% Bound (Rabbit 2)
1 : 1,000	74.3
1 : 5,000	67.8
1 : 10,000	58.0
1 : 20,000	40.5
1 : 30,000	36.0
1 : 50,000	10.5
1 : 100,000	2.0
1 : 150,000	1.9

The antisera dilution 1 : 20,000 was suitable for experiment.

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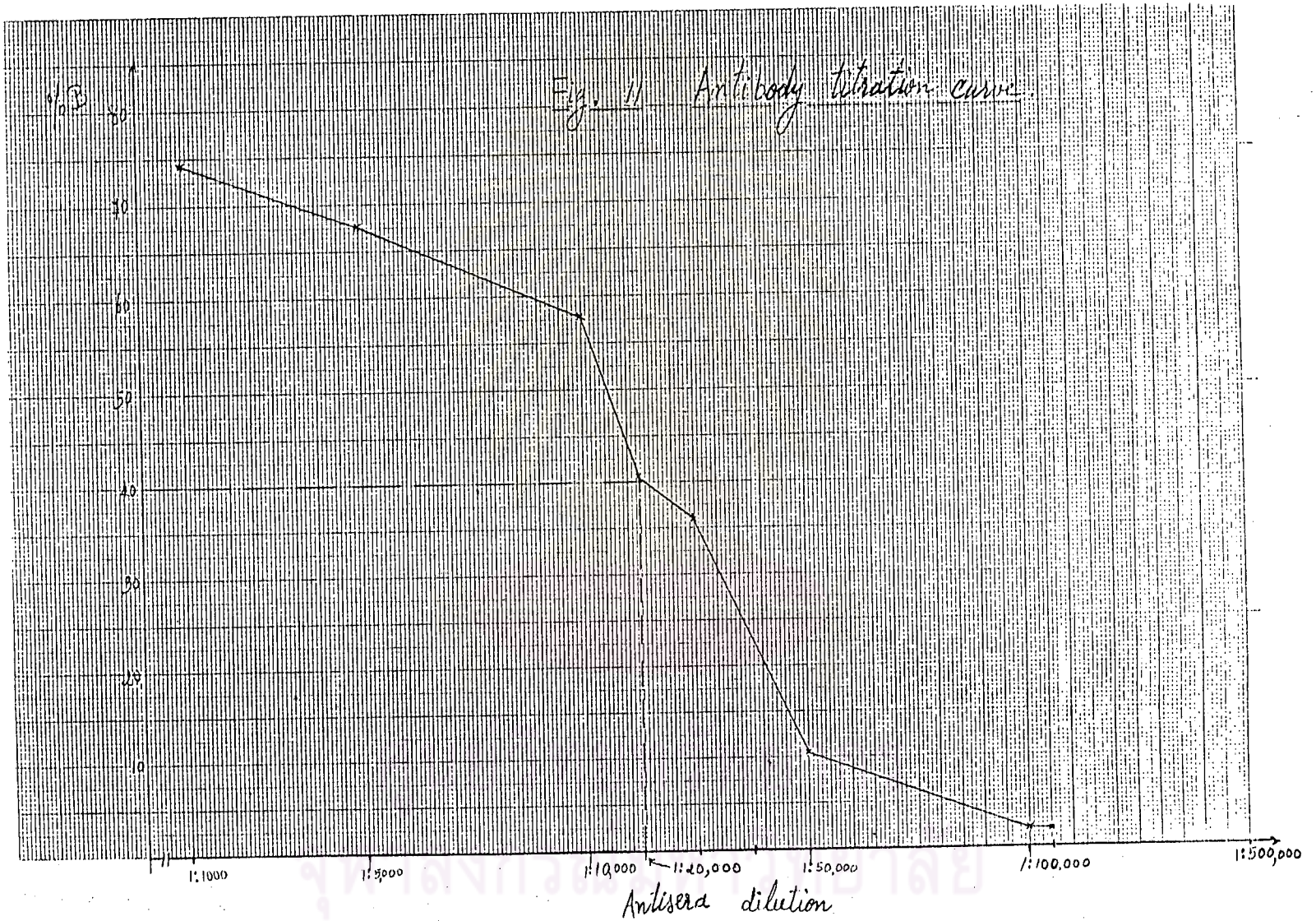

Fig. 11 Antibody titration curve.

5. Scatchard Plot

Estimation of binding capacity, q, and equilibrium constant, K, are obtained from a Scatchard Plot. Table 3 shows the data processing and Fig 12. shows the Scatchard Plot in which : -

K = Equilibrium Constant = Slope of curve

q = number of binding site by extrapolate the curve to the abcissa

Table 3.

Data Processing

Standard HTg (P) (ng/100µl)	B	B-NS	F = T-B	$[B] = B/T = \frac{(B-NS)}{T}$	(P+P*) (ng)	$[B](P+P^*)$ ng/100 (µl)	B/F
Bo	10,642	8,830	11,141	0.4053	1.529	0.6197	0.7926
1.0	9.850	8,038	11,933	0.3690	2.529	0.9332	0.6736
5.0	7,854	6,042	13,929	0.2773	6.529	1.8105	0.4338
10.0	6,440	4,628	15,343	0.2125	11.529	2.4499	0.3016
25.0	5,809	3,997	15,974	0.1835	26.529	4.8681	0.2502
50.0	5,053	3,241	16,730	0.1488	51.529	7.6675	0.1937
100.0	4,363	2,551	17,420	0.1171	101.529	11.8890	0.1464

T = 21783

NS = 1812

P* = 1.529 ng/100 µl ($HTgI^{125}$)

[B] = B/T = Fraction Bound

$[B](P+P^*)$ = Concentration of bound

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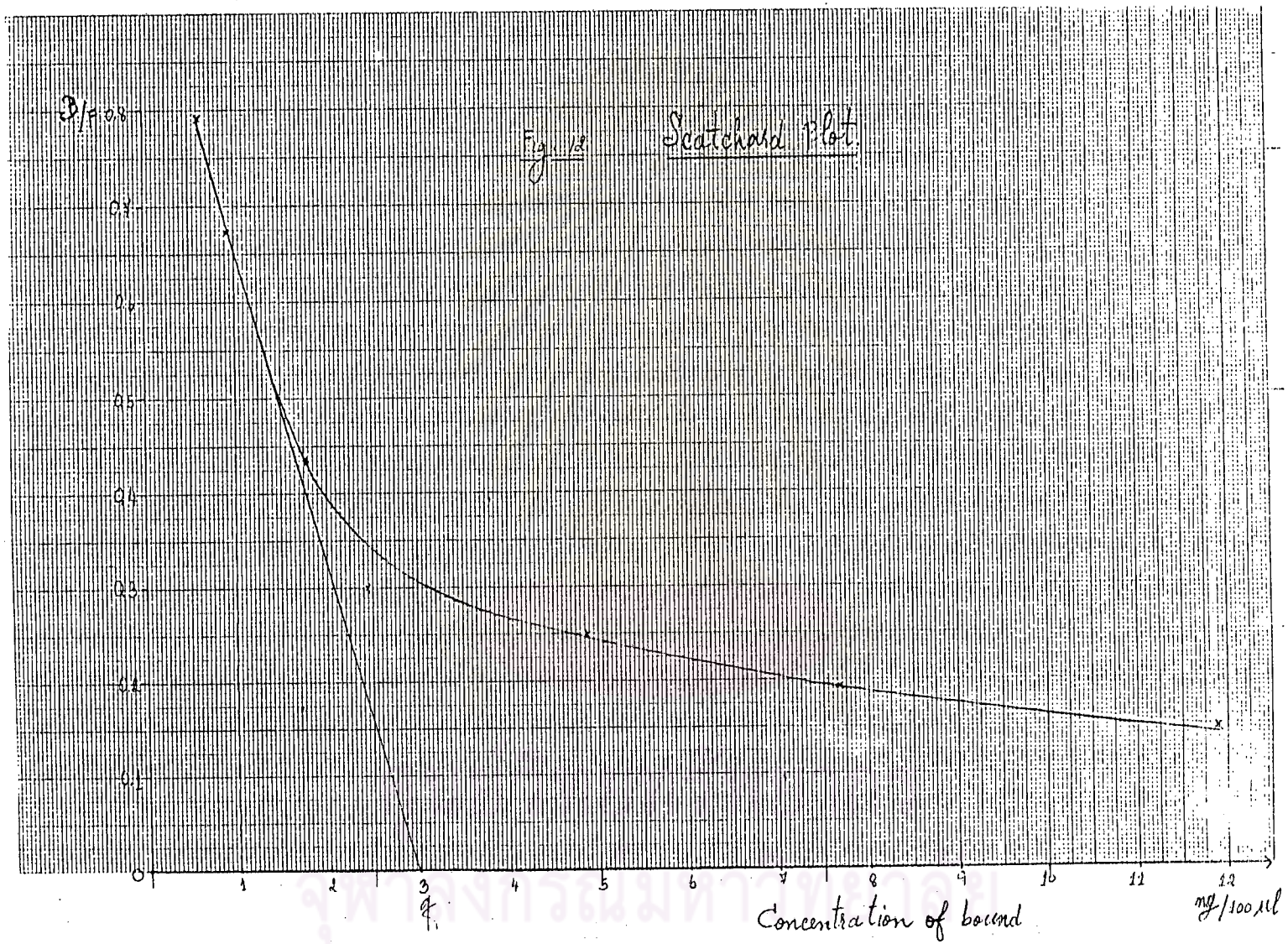

Fig. 12 Scatchard Plot

K = Equilibrium Constanst = Slope of curve

$= \frac{0.65}{3.0-1.0} = 0.325$ 100 µl/ng

$= 0.0325$ ml/ng

$= 0.0325 \times 10^{-3} \times 10^{9}$ L/gm

$= 0.0325 \times 10^{6} \times 660{,}000$ L/mole

$= 2.145 \times 10^{10}$ L/mole

q = Number of binding site

$= 3.0$ ng/100 µl

$= 3.0 \times 10 \times 10^{-9} \times 10^{3}$ gm/L

$= \frac{3.0 \times 10^{-5}}{660{,}000}$ mole/L

$= 4.545 \times 10^{-11}$ mole/L

6. Standard curve of HTg

From Fig 13. I,II & III was standard curve of HTg in experiment I, II and III respectively. The concentration of thyroglobulin in serum samples was calculated by reading the percent of radioactivity bound from a standard curve.

7. HTg levels in serum

Serum of euthyroid and thyroid diseases were assayed with HTg antiserum, the results were shown that.

A. Determinations of HTg concentration in 112 serum samples.

The range of the detectable samples from 2 to 1,000 ng/ml Above the level of 1,000 ng/ml, 1: 5 or 1 : 10 dilution method were required.

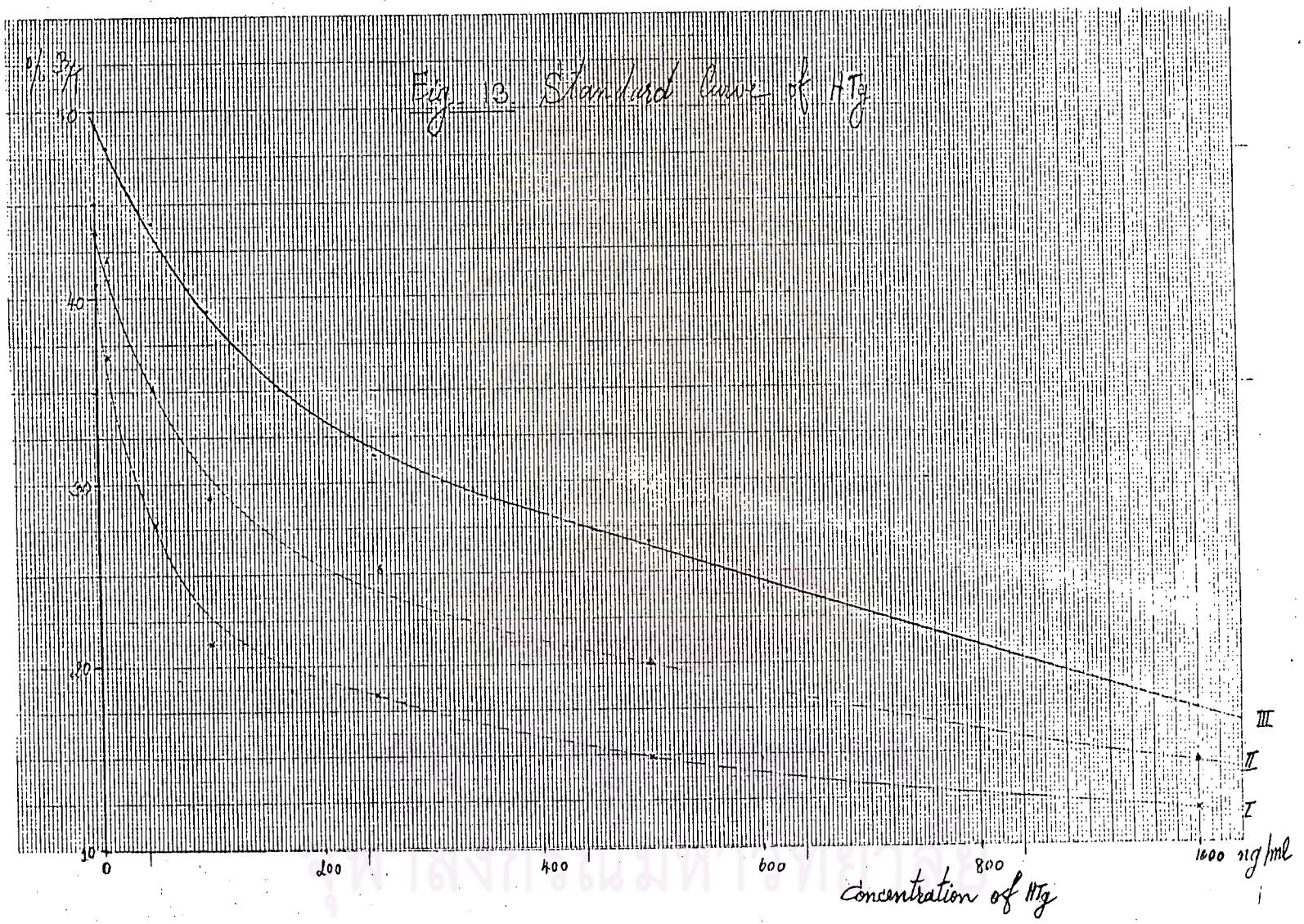

Fig. 13 Standard Curve of Hg

B. Normal test sera. Determinations on samples from 27 euthyroid. The mean of the detectable samples was 57.9 ± 27.5 ng/ml. a range from 7 to 104 ng/ml.

C. Chronic thyroiditis.

1. Determinations of HTg concentration in 14 autoanti-HTg titre 1: 640 samples, showed that range was from 120 to 5,000 ng/ml.

2. Determinations of HTg concentration in 19 autoanti-HTg titre 1 : 640 samples. The range was from 140 to 1,175 ng/ml.

D. Hashimoto's thyroiditis

Table 4

Data processing.

patients	HTg	T_3	T_4	TSH	Autoanti-HTg titre
1.	524	93	2.5	22.4	1 : 1280
2.	5,000	116	8.3	-	1 : 1280
3.	840	60	3.0	>100	1 : 1280
4.	>1,000	88	5.1	0	neg
5.	444	107	4.6	0	neg
6.	464	63	2.5	71.6	neg
7.	1,800	69	2.3	6.0	1 : 640
8.	> 500	151	7.7	-	neg
9.	224	136	6.0	4.3	1 : 1280
10.*	176	89	2.0	2.3	neg
11.*	254	<50	2.3	0.4	1 : 80
12.*	273	83	3.4	0.8	1 : 640
13.*	334	96	4.4	0.3	1 : 1280
14.**	148	76	9.2	-	neg
15.**	212	89	8.9	7.5	neg
16.**	452	130	6.1	6.2	neg

A range of HTg from 148 to 5,000 ng/ml.

The last 7 patients were four patients of the same Siblings(*) and three patients of the same Siblings (**) have had no current history of autoimmune thyroid disease, but owing to the level of HTg intensive follow - up should be implemented.

T_3 normal range 80 - 160 ng %

T_4 normal range 4.5 - 11.0 ng %

TSH normal range 2.5 - 12.0 ng/ml

E. Subacute thyroiditis (De Quervain)

TABLE 5

Data processing

patients.	HTg	T_3	T_4	TSH	Autoanti-HTg.
1	364	154	9.0	-	neg.
2	798	145	5.6	0	neg.
3	>1,000	144	7.8	-	neg.
4	>1,000	116	6.8	-	1 : 60

All 4 patients showed thyroid cell destruction with a substantial amount of released HTg. Follow-up should also be advised for HTg estimation at every 3 months interval during the course of disease which may run up to 1 - 2 years with self remissions.

F. Simple goitre

TABLE 6

Data processing

Patients	HTg	T_3	T_4	TSH	Autoanti-HTg
1	25	131	9.2	-	neg.
2	42	151	7.0	5.6	1 : 80
3	58	311	7.1	-	neg.
4	67	151	8.6	-	neg.
5	75	105	7.3	-	neg.
6	80	111	6.2	0	neg.
7	201	215	14.4	-	neg.
8	334	128	7.5	-	neg.
9	>500	-	4.7	4.5	neg.

G. Thyroid cancer.

One female thyroid cancer (metastasis to bone) has HTg concentration 6,640 ng/ml.

7. Recovery experiment

When 10.0 ng/ml of HTg was added to normal sera, percent recovery was almost complete. (Table 7)

TABLE 7

HTg recovery experiment

Experiment	Serum HTg concentration			% recovery.
	Initial	Add	Final	
1	42	10	56	107.7
2	36	10	44	95.7
3	88	10	99	101.0

8. Storage of antibodies obtained

Each antiserum was storage 200, 400 µl per ampule, lyophilized and store at $-70^{o}C$.