CHAPTER III

RESULTS

Some preliminary tests were performed before starting the TSH receptor assay in order to establish the optimum methods. Therefore, the techniques of iodination, receptor purification of the labelled TSH, elution conditions, the dose-response curves and Scatchard analysis were evaluated.

Iodination of Bovine TSH

Highly purified bTSH was iodinated with Na ^{125}I by iodogen method. The iodination time was varied in order to give iodinated product with suitable specific activity for radioreceptor assay. The relation of iodination time to specific activity was illustrated in Table 1, page 29 These data also showed percent specific binding at various specific activities.

The lowest specific activity (43.2 - 71.3 μCi/μg or 1.6-2.6 Bq/pg) in Table 1 has shown to give the highest specific binding, so exposure time between bTSH, Na^{125}I and iodogen for 2 min was found to be satisfactory and was used throughout of this study.

Separation of pure ^{125}I-bTSH from damaged protein and free Na^{125}I after iodination was carried out using a Sephadex G-100-40 column. The fractions of damaged ^{125}I-bTSH were eluted first (peak I)

Table 1. Effect of iodination time on specific activity of ^{125}I-bTSH and on specific binding to human thyroid membranes.

Iodination time (minutes)	Specific acitvity		% Specific binding
	μCi/μg	Bq/pg	$\bar{X} \pm$ SD
2	43.2 - 71.3	1.6 - 2.6	35.5 ± 5.17
4	79.5 - 85.7	2.9 - 3.2	28.6 ± 4.59
6	90.7 - 98.5	3.4 - 3.6	21.7 ± 3.26
8	101.2 - 115.4	3.8 - 4.3	15.0 ± 1.93
10 - 15	120.0 - 170.0	4.4 - 6.3	10.0 ± 4.43

and the fractions of the second peak (peak II) were undamage ^{125}I-bTSH which was chosen for use in the TSH receptor assay. The fractions of the third peak were unreacted Na^{125}I, and the elution pattern was shown in Fig. 7, page 30.

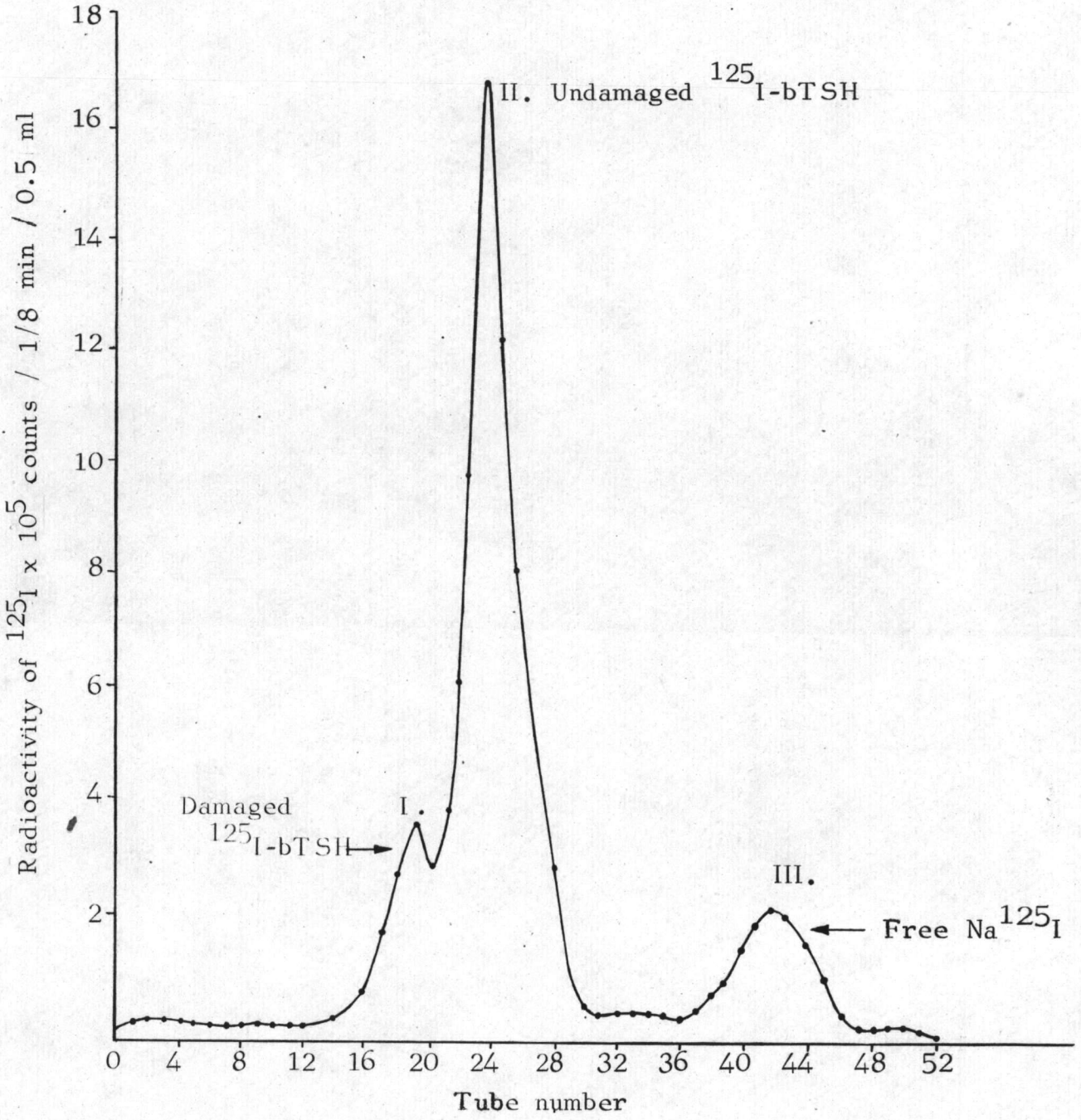

Fig. 7. Elution pattern of the reaction mixture after radioiodination (^{125}I) of bTSH from Sephadex G-100-40 column.

Receptor Purification of ^{125}I-bTSH.

^{125}I-bTSH was subjected to repurification by receptor adsorption. After incubation of ^{125}I-bTSH with thyroid membranes for 30 min at 37°C, the membrane pellet was centrifuged and rinsed (see page 25). The bound ^{125}I-receptor purified bTSH was then eluted from the membrane pellets by adding the elution buffer. The elution conditions were tested in different buffers, time and temperature, as summarized in Table 2, page 32 . From Table 2, the shorter incubation time for 30 min at 37°C in 2M NaCl, 1 g BSA/l, pH 5.0 was found to be suitable and better than the incubation for 1 h at 37°C since the former condition gave the least damaged labelled TSH. The eluant was then rechromatographed on Sephadex G-100-40 and a single narrow peak of ^{125}I-receptor purified bTSH was obtained, as indicated in Fig. 8., page 33. The top and the first descending fractions were pooled and stored at -20°C for further assay. Competition binding of ^{125}I-receptor purified bTSH and unlabelled TSH for normal thyroid membrane in different elution buffers was compared as presented in Fig. 9., page 34.

Table 2. Efficiency of elution (%) in different elution buffer, time and temperature.

No.	Elution buffers	% Efficiency of elution $\bar{X} \pm SD$		
		18 h, 4°C	1 h, 37°C	30 min, 37°C
1.	2M NaSCN, 1gBSA/1, pH 5.5	31.1 ± 2.4	57.0 ± 3.1	52.5 ± 8.4
2.	2M NaCl, 1gBSA/1, pH 5.0	30.6 ± 1.6	48.6 ± 6.6	43.8 ± 4.8
3.	200 mM NaCl, 10 mM Tris-Maleate, 1 gBSA/1 pH 5.0	28.5 ± 1.3	46.2 ± 6.8	43.6 ± 1.4

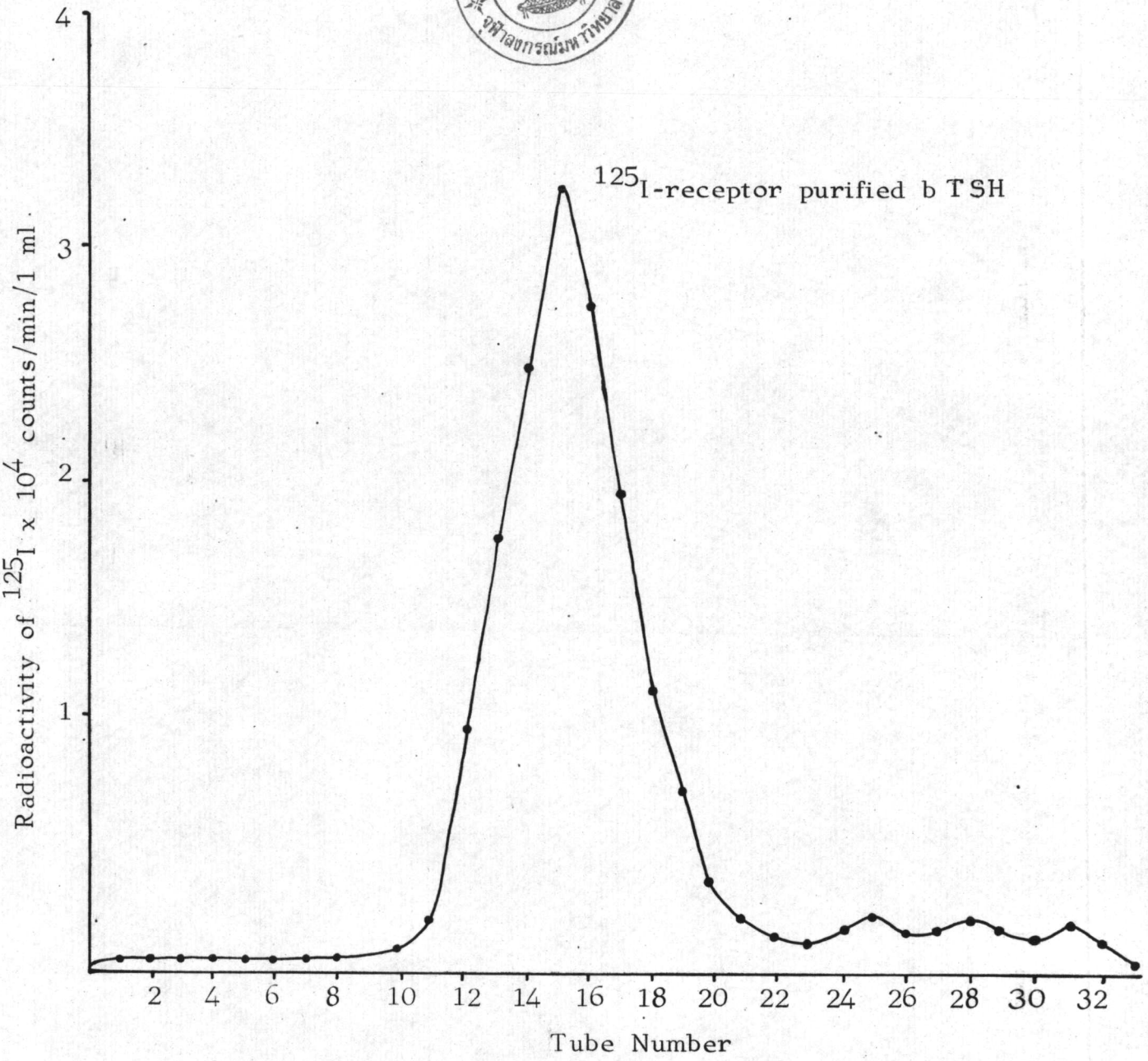

Fig. 8. Chromatography of receptor-purified ^{125}I-bTSH on a column of Sephadex G-100-40 (1x15 cm) in NaCl/Tris/BSA buffer, flow rate 8 ml/h, fraction volume 1 ml.

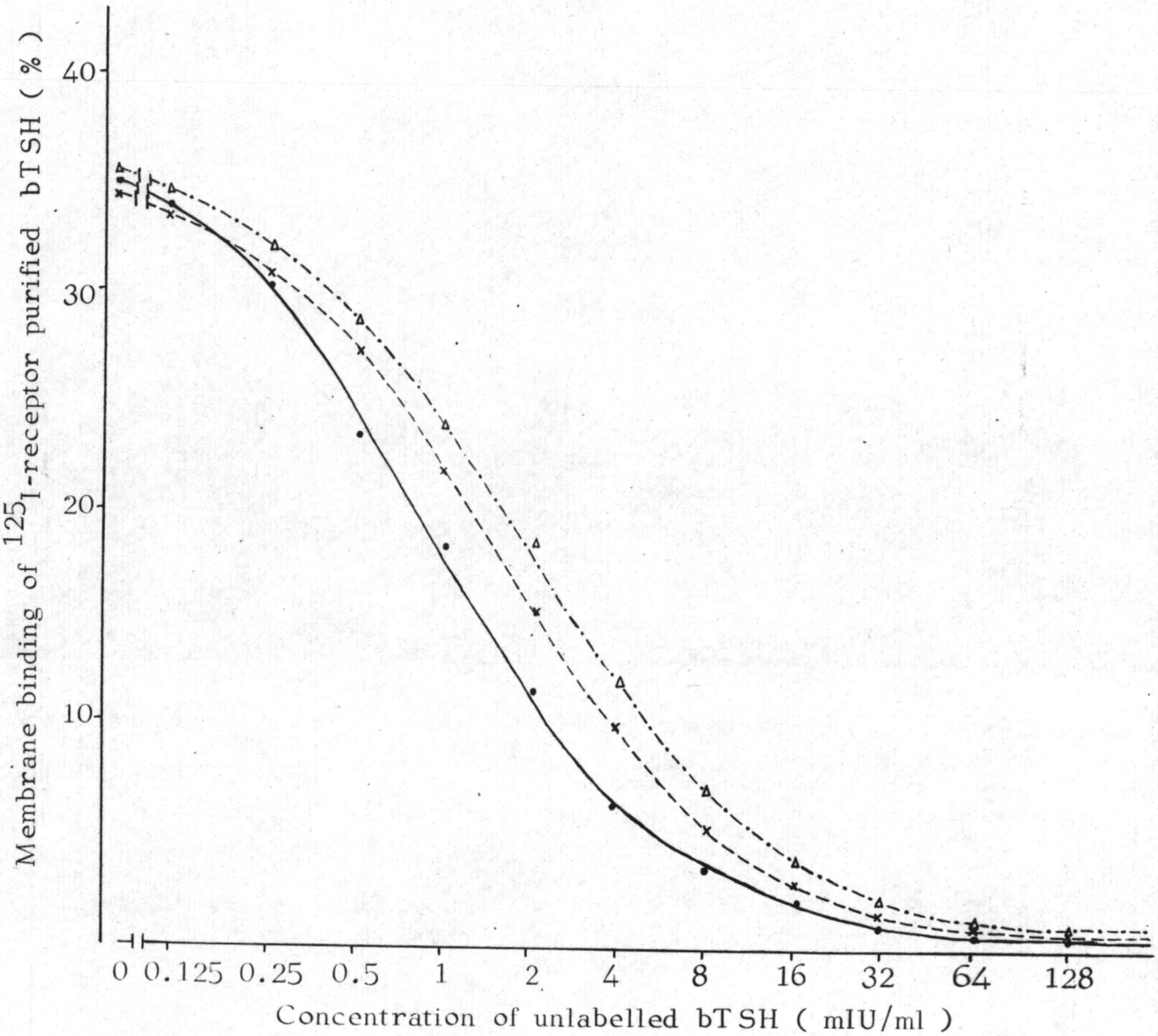

Fig. 9. Displacement curves of normal thyroid membrane eluted with different elution buffers (•—• 2 M NaCl, 1gBSA/l, pH 5.0, Δ-·-·-Δ 2 M NaSCN, 1gBSA/l, pH 5.5 and ×– – –× 200 mM NaCl, 10 mM Tris-Maleate, 1gBSA/l, pH 5.0).

Binding Properties of Normal Thyroid Tissues.

The maximum specific bindings (B_o) of ^{125}I-bTSH to TSH receptors of normal human thyroid tissue obtained at surgery were compared with tissues obtained at autopsy relation to the range of time after death, as shown in Table 3.

Table 3. Effectiveness of human thyroid tissues for specific binding of ^{125}I-bTSH.

Source of thyroid tissues	Membrane binding of ^{125}I-bTSH (%)
Surgery	31.4 - 42.8
Autopsy	
2 - 4 h	29.8 - 32.7
8 - 12 h	24.3 - 27.2
12 - 24 h	12.7 - 18.9
24 - 48 h	6.6 - 8.2
>48 h	2

Although data suggest that fresh human thyroid tissues are preferable in the radioreceptor assays, normal human thyroid tissues after 1 - 10 hours after death were used in this study due to difficulty in obtaining the fresh normal human thyroid tissues.

Radioreceptor Assays for TSH Receptors.

^{125}I-receptor purified bTSH was used in these radioreceptor assays. The displacement curves were performed by increasing doses of unlabelled bTSH, using the different thyroid membrane preparations. The different response curves from thyroid tissues of normal, toxic diffuse goiter, nodular goiter, follicular adenoma, papillary carcinoma and Hashimoto's thyroiditis were obtained and shown in Fig. 10, page **37**. The results of maximum specific binding at 0.0 concentration of unlabelled TSH (B_o) of each preparation were shown in Table 4.

Table 4. Maximum binding of ^{125}I-receptor purified bTSH to their receptors in various thyroid membrane preparations.

Thyroid membrane preparations	n	% Specific binding $\bar{X} \pm SD$	P values compared with normal
Normal	20	35.57 ± 5.17	
Toxic diffuse goiter	12	35.67 ± 5.26	$P > 0.5$
Nodular goiter	10	35.64 ± 4.23	$P > 0.5$
Follicular adenoma	10	33.88 ± 4.48	$P < 0.4$
Papillary carcinoma	2	14.80 ± 0.08	$P < 0.001$
Hashimoto's thyroiditis	3	11.52 ± 0.73	$P < 0.001$

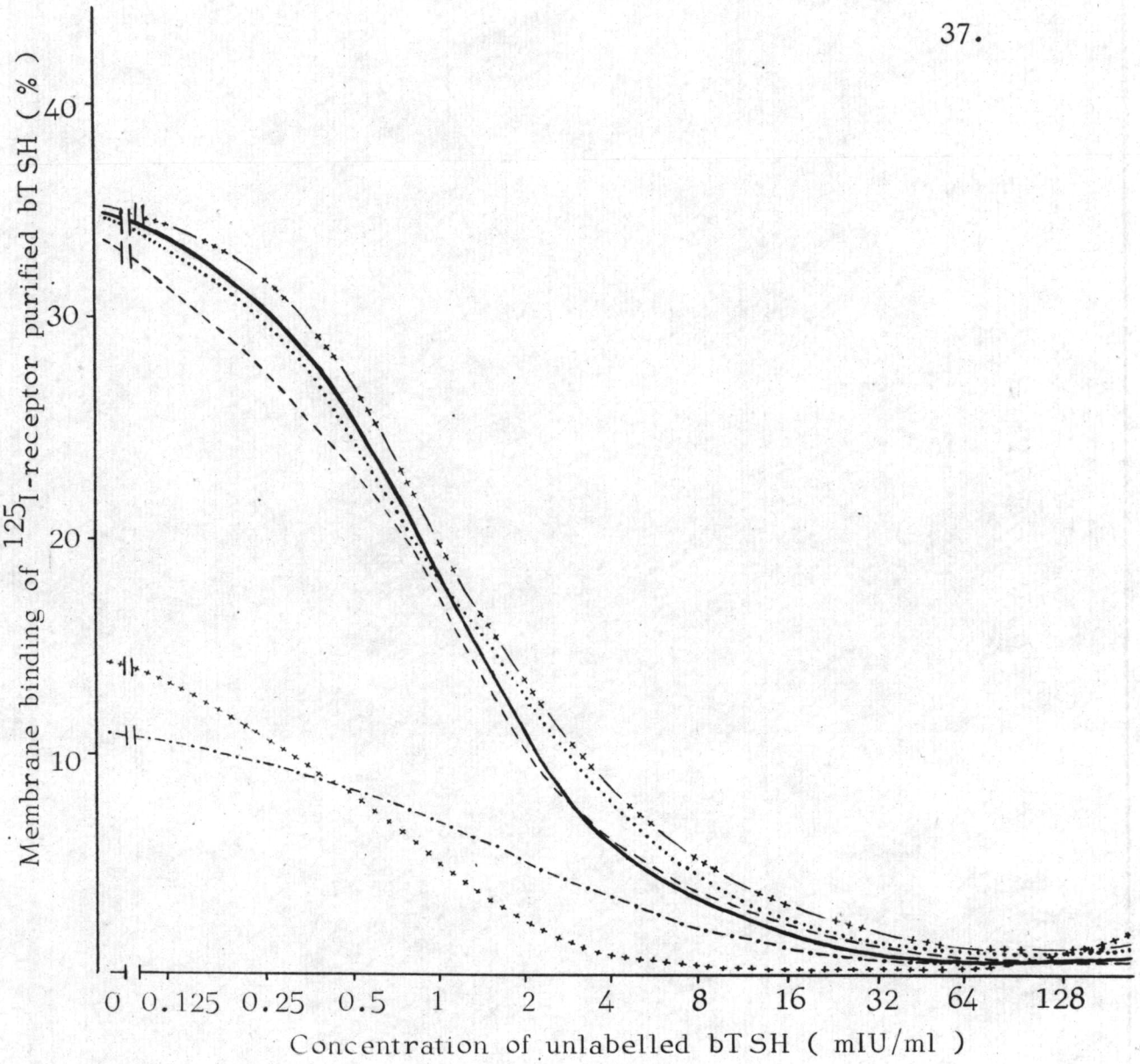

Fig. 10. The means of displacement curves of the different thyroid membrane preparations : ——normal, ... toxic diffuse goiter, —•••—•••—nodular goiter, -----follicular adenoma, +++++ papillary carcinoma and —•—•—Hashimoto's thyroiditis.

The percentages of zero binding of papillary carcinoma (14.80 ± 0.08%) and of Hashimoto's thyroiditis (11.52 ± 0.73%) were significantly ($P < 0.001$) lower than those of normal (35.57 ± 5.17%), toxic diffuse goiter (35.67 ± 5.26%), nodular goiter (35.64 ± 4.23%) and follicular adenoma (33.88 ± 4.48%).

The Scatchard analyses of assay data were performed for assessing binding capacity and binding affinity for normal thyroid tissues and for different types of thyroid diseases were illustrated in Fig. 11, page 39. The binding capacities and binding affinities in various thyroid membrane preparations were summarized in Table 5, page 40.

The number of high affinity binding sites (binding capacity) for toxic diffuse goiter (2.33 ± 0.55 pmol/g equiv.), nodular goiter (2.33 ± 0.26 pmol/g equiv.) and Hashimoto's thyroiditis (2.13 ± 0.26 pmole/g equiv.) were similar to the binding capacity obtained with normal (2.17 ± 0.30 pmol/g equiv.). Some of these results revealed that the binding capacities of follicular adenoma (1.76 ± 0.31 pmol/g equiv.) and of papillary carcinoma (0.57 ± 0.00 pmol/g equiv.) were found of be significantly lower ($P < 0.005$ and $P < 0.001$, respectively) than those of the normal. With regard to binding affinity (K_a), these were no significant difference between normal ($1.73 \pm 0.47 \times 10^9\ M^{-1}$), toxic diffuse goiter ($1.59 \pm 0.42 \times 10^9\ M^{-1}$), nodular goiter ($1.59 \pm 0.30 \times 10^9\ M^{-1}$), follicular adenoma ($2.4 \pm 0.28 \times 10^9\ M^{-1}$) and papillary carcinoma ($2.14 \pm 0.00 \times 10^9\ M^{-1}$) but the lowest binding affinity was noted in three patiens of Hashimoto's thyroiditis ($0.41 \pm 0.00 \times 10^9\ M^{-1}$, $P < 0.001$)

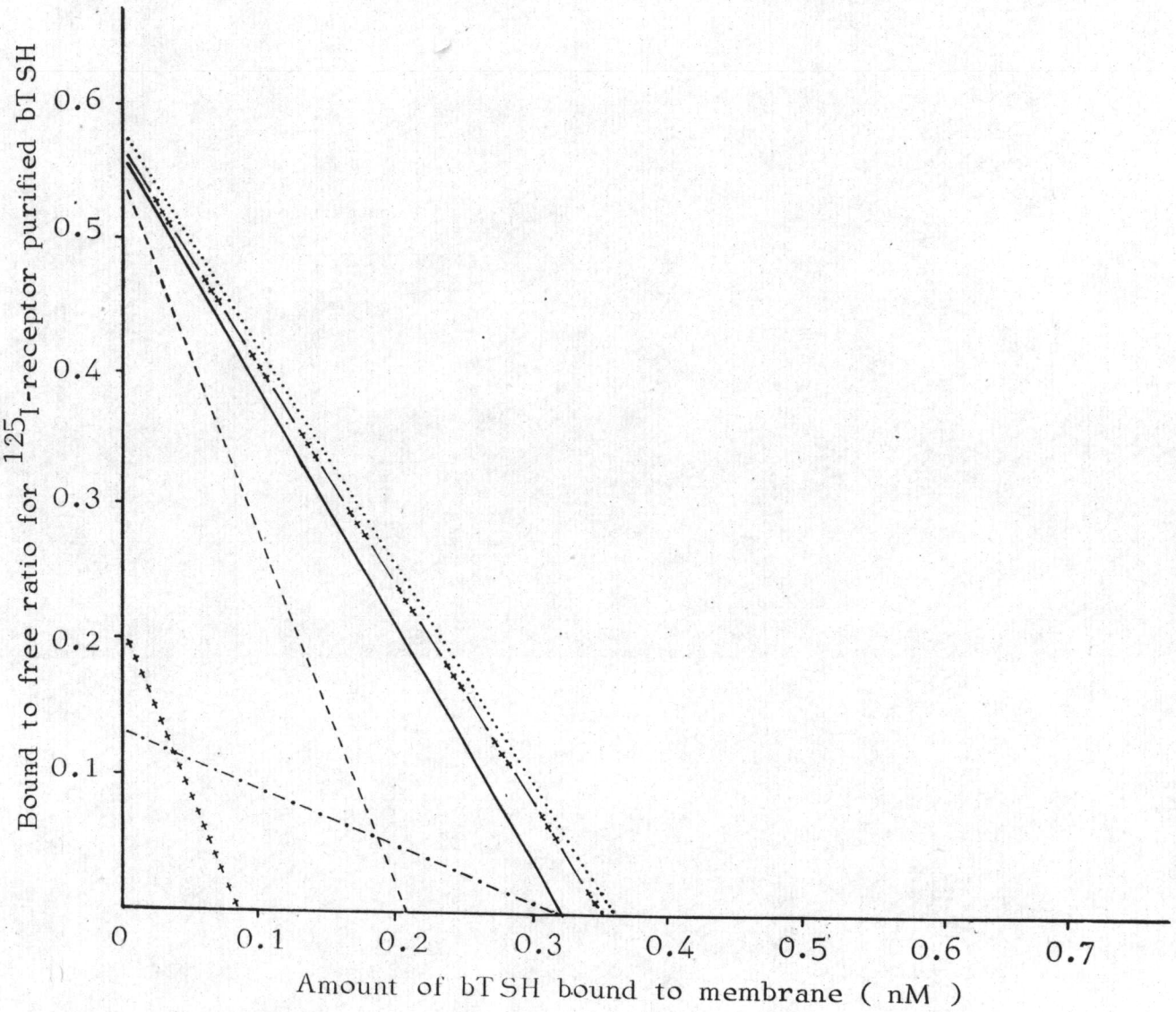

Fig. 11. Scatchard plot of bTSH binding to normal human thyroid membrane (——), human thyroid membrane of toxic diffuse goiter (·····), of nodular goiter (–+++–), of follicular adenoma (-----), of papillary carcinoma (+++++) and Hashimoto's thyroiditis (–·–·–).

Table 5. Binding capacities and binding affinities of human thyroid membranes for bovine TSH.

Thyroid membrane preparation	N	Binding capacity pmol/g equiv. $\bar{X} \pm SD$	P values compared with normal	Binding affinity $(K_a) \times 10^9 M^{-1}$ $\bar{X} \pm SD$	P values compared with normal
Normal	20	2.17 ± 0.30		1.73 ± 0.47	
Toxic diffuse goiter	12	2.33 ± 0.55	$P < 0.4$	1.59 ± 0.42	$P < 0.5$
Nodular goiter	10	2.33 ± 0.26	$P < 0.2$	1.59 ± 0.30	$P < 0.5$
Follicular adenoma	10	1.76 ± 0.31	$P < 0.005$	2.04 ± 0.28	$P < 0.1$
Papillary carcinoma	2	0.57 ± 0.00	$P < 0.001$	2.14 ± 0.00	$P < 0.4$
Hashimoto's thyroiditis	3	2.13 ± 0.26	$P > 0.5$	0.41 ± 0.00	$P < 0.001$