CHAPTER III

RESULTS

Total thyroxine levels in serum or plasma have long been recognized as an important indicator of thyroid status. Therefore, some important modifications of the sensitive radioimmunoassay of total serum thyroxine were performed to establish the optimum methods. The labelled compounds were found to be the most unstable reagents in RIA, so the $^{125}I\text{-}T_4$ using in this study was repurified by column chromatography in order to remove radioactive iodide and damaged compounds. The degree of interference depends on the proportion of binding proteins in each serum sample. In this investigation, the reaction medium has been formulated to minimize the interference by the combination of an appropriate buffer (barbital buffer, pH 8.6) and a TBG blocking agent (ANS). Thus, various concentrations of ANS were tested to exist the response standard curve as illustrated in Fig.3, page 28. The results indicated that optimum concentration of ANS at 600 ug per assay tube was chosen and used throughout of these experiments. Moreover, separating technique of the antibody-bound and free fractions in RIA is an essential part, and polyethylene glycol (PEG) is now very useful in thyroid hormone assays. For these reasons, the optimum concentrations of PEG in the incubation mixture were determined and

found that 15% of PEG in final volume was preferable, as shown in Fig.4, page 29. In addition, incubation time and temperature of the reaction mixture were made and compared, as shown in Fig.5, page 30. The results revealed that only one hour at 37°C of incubation was optimum conditions giving the best typical standard curve (see Fig.6, page 31.

The reproducibility of intra-and inter-assay variations in 15 replications of T_4 RIA has been evaluated using control standard T_4 diluted in T_4-free serum at low, medium and high concentrations. The coefficients of intra-assay variations were 7.3% at 1.25ugT_4/100 ml, 8.7% at 7.5 ugT_4/100 ml and 6.4% at 15ugT_4/100 ml, as summarized in Table 1, page 32. Greater coefficients of variations were obtained in inter-assay variations which illustrated in Table 2, page 32, 9.5% at 1.25 ugT_4/100 ml, 9.7% at 7.5 ugT_4/100 ml and 8.8% at 15 ugT_4/100 ml, respectively.

Cross-reactivity between T_4-antibody (from rabbit plasma) to MIT, DIT and L-T_3 (at physiological concentration) were determined to assess specificity of the assay. No cross-reaction occured with these analogue compounds but L-T_3 could cross react with this T_4-antibody when the concentrations of T_3 were increased, as shown in Fig. 7, page 33.

With the improvements, it became possible to finish each assay in one day that is suitable and useful for routine diagnosis. Therefore, serum total T_4 levels were measured in 219 euthyroid subjects and it was established that normal values lay within the

range 4-11 ugT_4/100 ml serum with the mean ± S.D. of 7.4 ± 1.6 ugT_4/100 ml. Good discrimination between 25 hypothyroids (2.2 ± 1.3 ugT_4/100 ml), 219 euthyroids (7.4 ± 1.6 ugT_4/100 ml) and 42 hyperthyroids (17.6 ± 4.2 ugT_4/100 ml) was obtained, as shown in Fig.8, page 34.

Moreover, the effects of acetaminophen, diazepam and nordiol on total T_4, T_3-uptake and free thyroxine index (FTI) in serum were analysed and compared to each control group. All results were expressed into the mean ± S.D.

Experiment No 1: The results showed that acetaminophen did not influence any of the thyroid function test studied between control and treated group because the value of total T_4 (7.7 ± 1.9 cf: 7.5 ± 1.8 ugT_4/100 ml), T_3-uptake test (104 ± 6.0 cf: 103 ± 7.4) and FTI (7.4 ± 1.9 cf: 7.4 ± 1.7%) were unaffected, as summarized in Table 3, page 35.

Experiment No 2: The results indicated that only the levels of serum T_4 before and after diazepam therapy were significantly decreased (from 7.6 ± 2.1 to 6.7 ± 1.5 ugT_4/100 ml, $P<0.001$), respectively. No change was found on the values of T_3-uptake test (107 ± 7.6 cf: 108 ± 7.6) and of FTI (6.7 ± 1.6 cf: 6.6 ± 1.8%), as illustrated in Table 3, page 35.

Experiment No 3: Nordiol treated sera in 51 out-patients were analysed and the results revealed that two tested values were significantly increased in total serum T_4 (from 8.0 ± 2.0 to 9.2 ± 2.2 ugT_4/100 ml, $P<0.01$) and T_3-uptake test (from 108 ± 8.3 to 112 ±

7.1, $P < 0.005$), but the values of FTI (from 7.5 $\pm$ 2.0 to 8.2 $\pm$ 1.8%) were not significantly elevated from the control group ($P < 0.1$) as shown in Table 3, page 35.

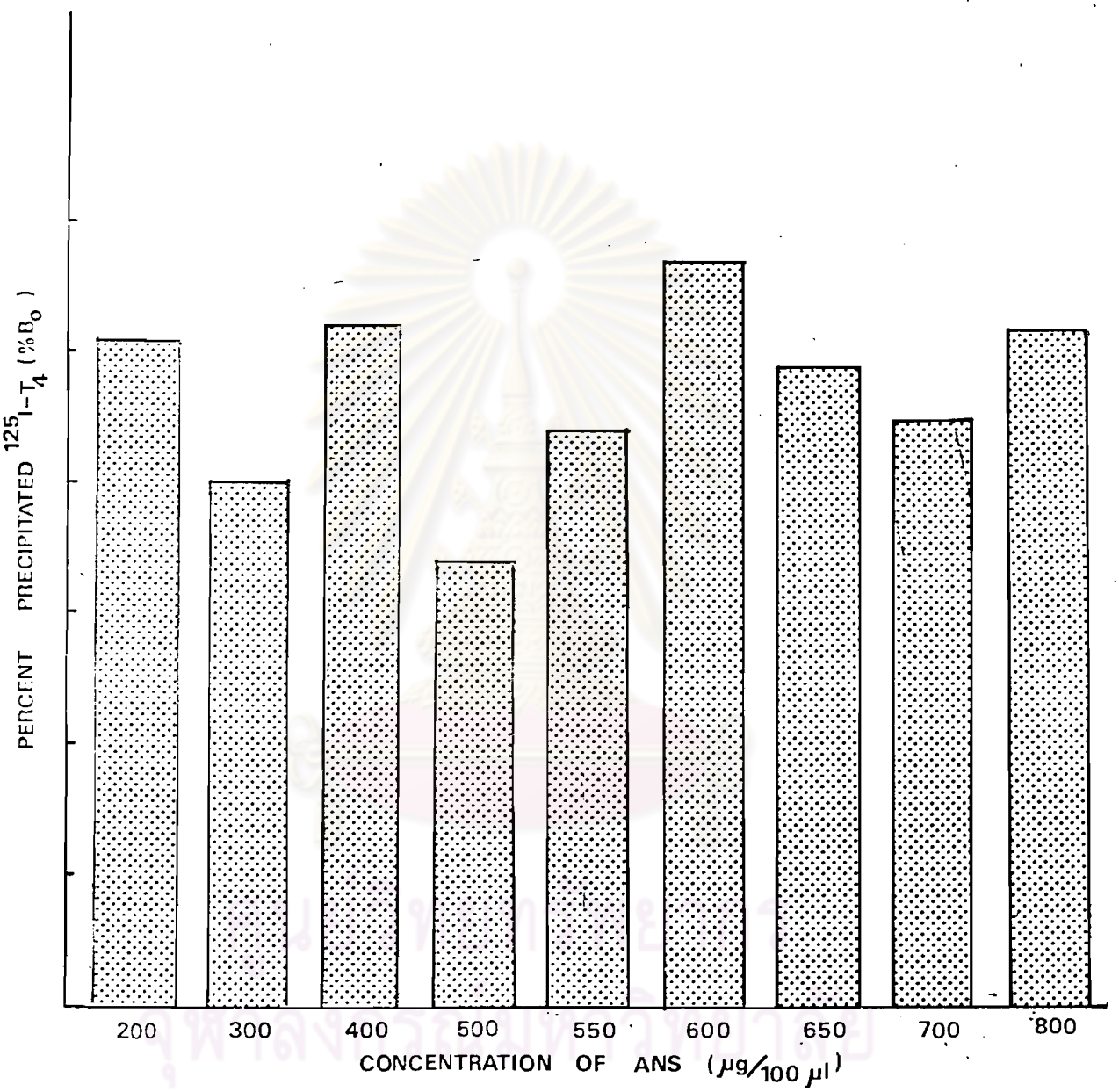

Fig.3. Percent radioactivity in bound fraction ($\%B_o$) using various concentrations of ANS.

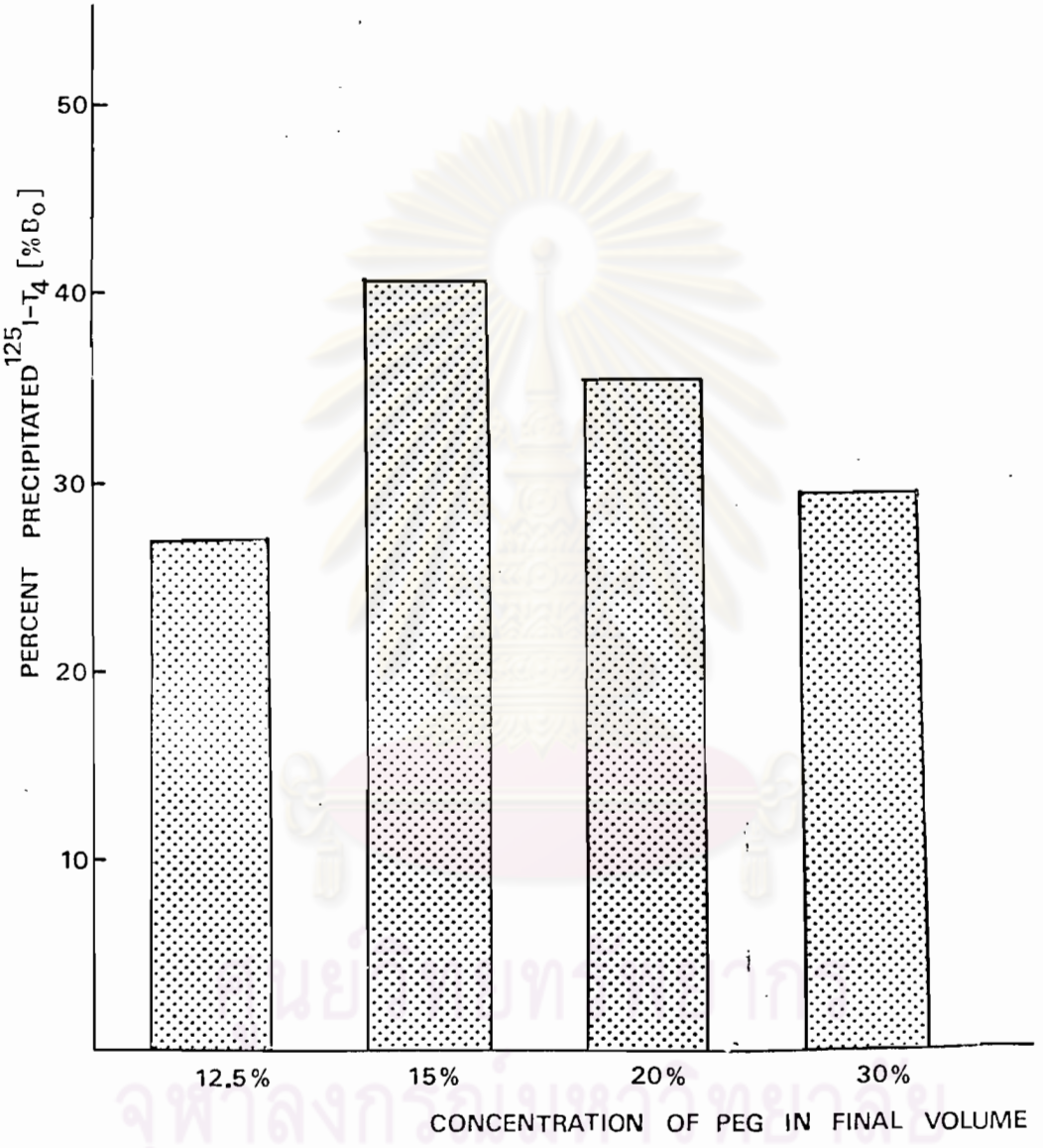

Fig. 4. Percent $^{125}I-T_4$ bound to T_4- antibody in various concentrations of PEG in final volume.

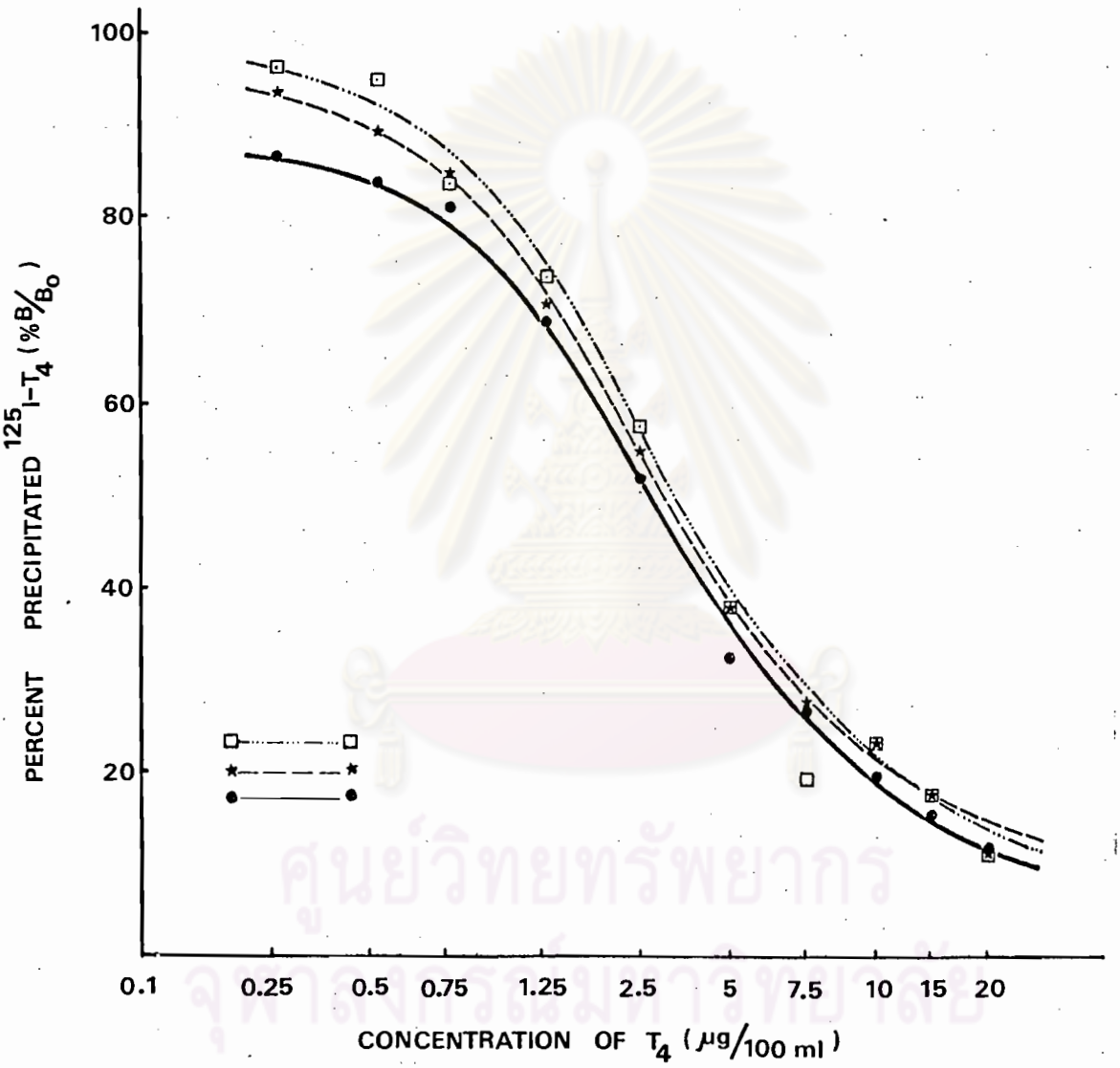

Fig. 5. Comparison of standard curves for T_4-RIA in different time and temperature. □—□ 2 hours at room temperature, ★—★ 2 hours at 37°C., ●—● 1 hour at 37°C.

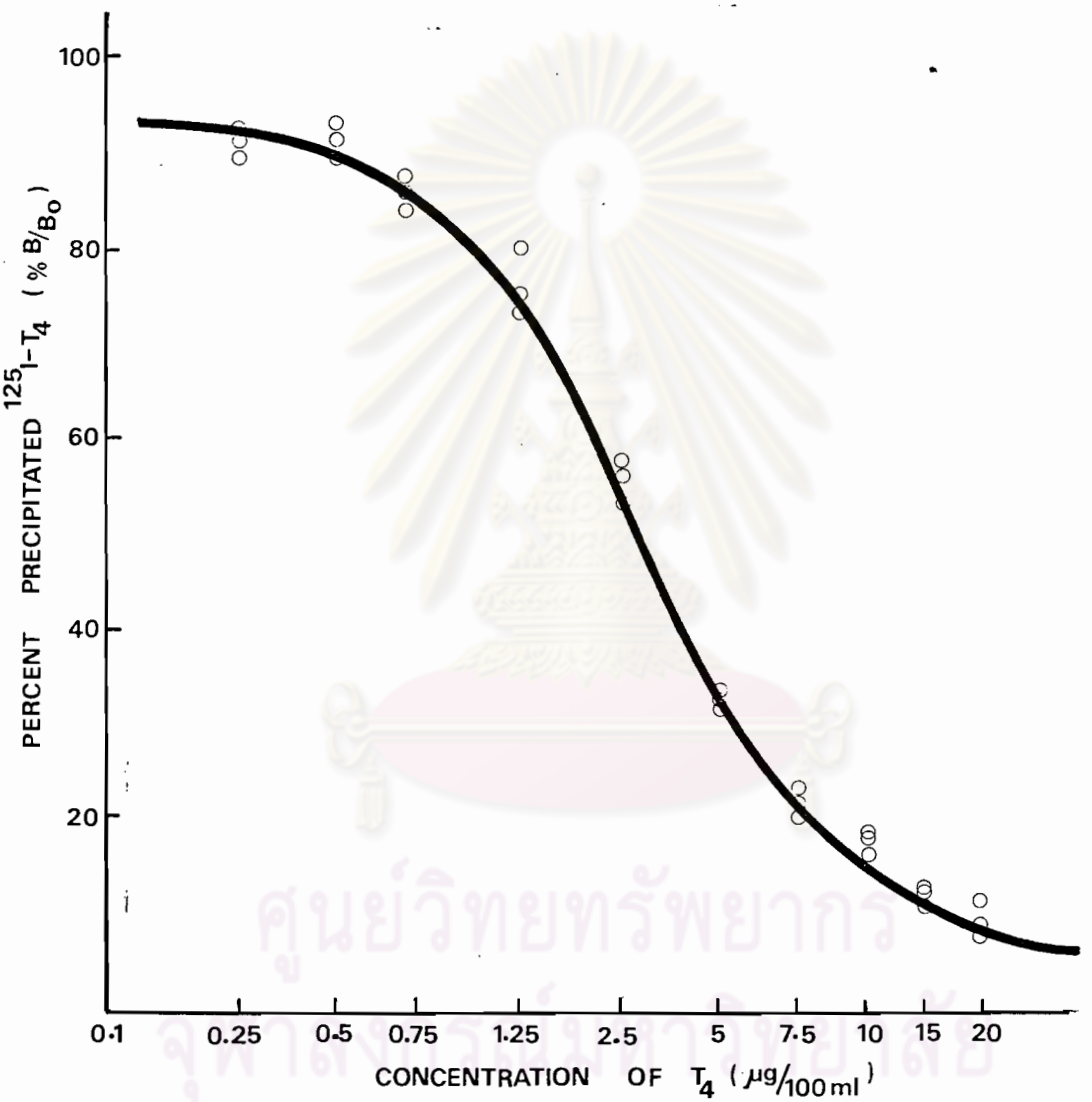

Fig.6. Typical standard curve of T_4 RIA using 600 μg ANS/tube and 15 % PEG in final volume.

Table 1. Coefficient of variation [%] of intra- assay with 15 replications by T_4- RIA.

T_4 CONCENTRATIONS	$\bar{X} \pm$ S.D.	% C.V.
Low [1.25]	1.3 ± 0.1	7.3
Medium [7.5]	7.4 ± 0.6	8.7
High [15]	14.9 ± 0.9	6.4

Table 2. Coefficient of variation [%] of inter-assay with 15 replications by T_4- RIA.

T_4 CONCENTRATIONS	X ± S.D.	% C.V.
Low [1.25]	1.2 ± 0.1	9.5
Medium [7.5]	7.4 ± 0.5	9.7
High [15]	15.1 ± 1.3	8.7

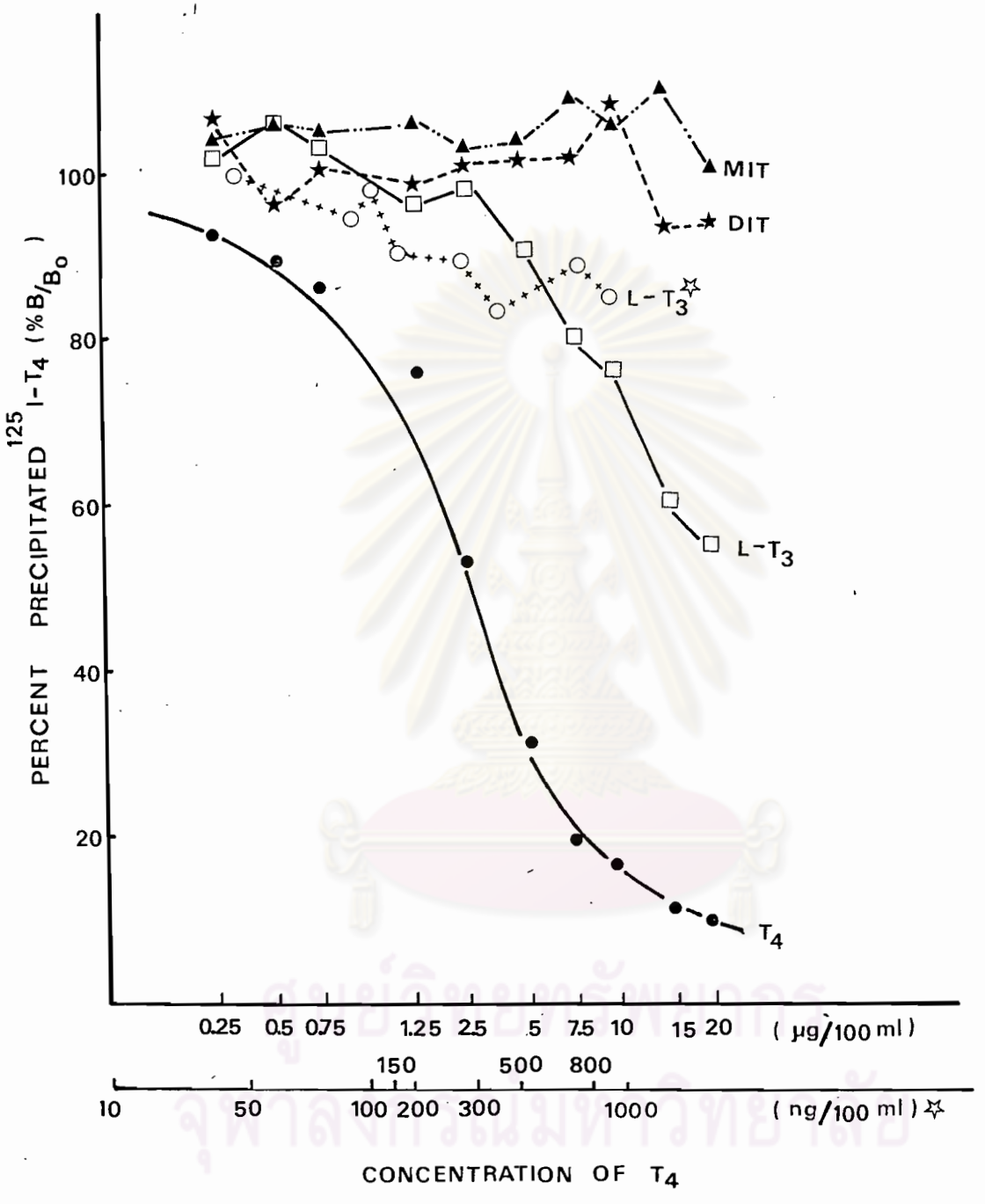

Fig. 7. Cross-reactivity of T_4-antibody with MIT, DIT and L-T_3

[☆ at biological concentration]

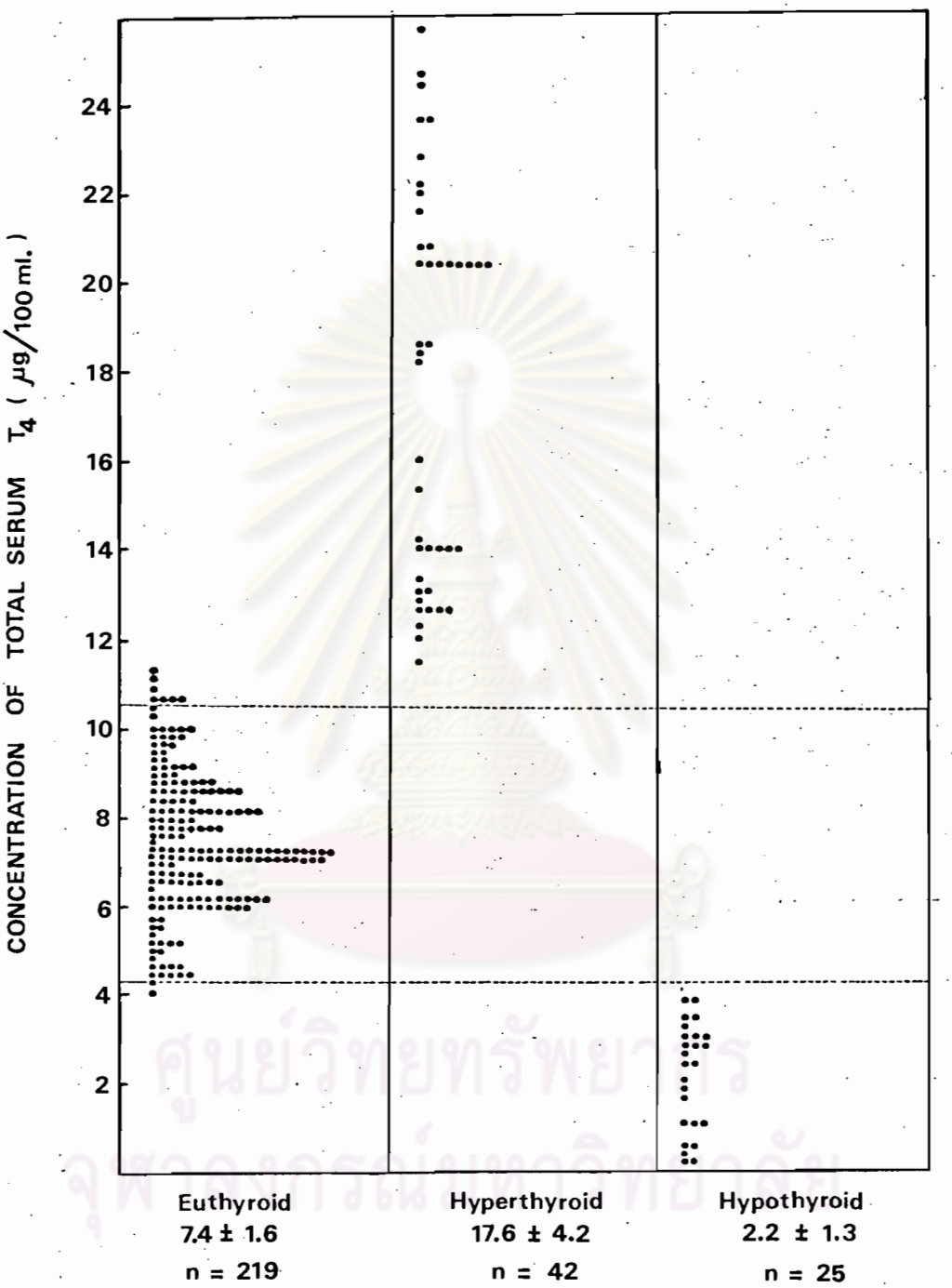

Fig. 8. Scatter diagram of total serum T_4 RIA in various thyroid conditions.

Table 3. The effects of Acetaminophen, Diazepam and Nordiol on total serum T_4, T_3- uptake and FTI between control and treatment groups.

		$\bar{X} \pm$ S.D.		
DRUGS	SUBJECTS	TOTAL SERUM T_4 ($\mu g/100$ ml.)	T_3- UPTAKE	%FTI.
ACETAMINOPHEN (Paracetamal)	Control	7.7 ± 1.9	104 ± 6.0	7.4 ± 1.9
		$P^{\star} < 0.4$	$P^{\star} < 0.5$	$P^{\star} < 0.9$
	Treatment (n = 61)	7.5 ± 1.8	103 ± 7.4	7.4 ± 1.7
DIAZEPAM (Valium)	Control	7.6 ± 2.1	107 ± 7.6	6.7 ± 1.6
		$P^{\star} < 0.001$	$P^{\star} < 0.6$	$P^{\star} < 0.4$
	Treatment (n = 52)	6.7 ± 1.5	108 ± 7.6	6.6 ± 1.8
NORDIOL (Nordiol 21 ED)	Control	8.0 ± 2.0	108 ± 8.3	7.5 ± 2.1
		$P^{\star} < 0.01$	$P^{\star} < 0.005$	$P^{\star} < 0.1$
	Treatment (n = 51)	9.2 ± 2.2	112 ± 7.1	8.2 ± 1.8

$N^{\star}$ pair t-test George W. Snedecor, Statistical Methods, 5th ed. (Iowa, The Iowa State University Press, Ames, 1966), p 60 - 62.