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Some Quantitative Aspects of in Vitro Thyroid Diagnostic Tests

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1. Introduction

This communication presents data upon a number of aspects of *in vitro* analysis in the field of thyroid diagnosis. Results are examined quantitatively and the clinical value and limitations of the data are presented. All the information is derived from the use of Thyopac-3 and Thyopac-4, a T3 uptake test and a total serum thyroxine assay respectively. They differ from other methods in being based upon an equilibrium rather than a continuing reaction.

2. Thyopac-3

Maintenance of Scale

The nature of the equilibrium of this process is shown in figure 1. It can be seen that the reaction continues during the first ten minutes but at this point equilibrium is reached and the outcome is thereafter independent of time. Other methods of determining T3-uptake values involve sampling a continuing reaction and depend upon achieving identical rates of reaction in all tubes and upon sampling them all at the same time. With an equilibrium-based system, the result is independent of factors which might influence the rate of reaction, only the position of the final equilibrium is of importance. It follows that more reproducible and precise results can potentially be derived from such equilibrium systems.

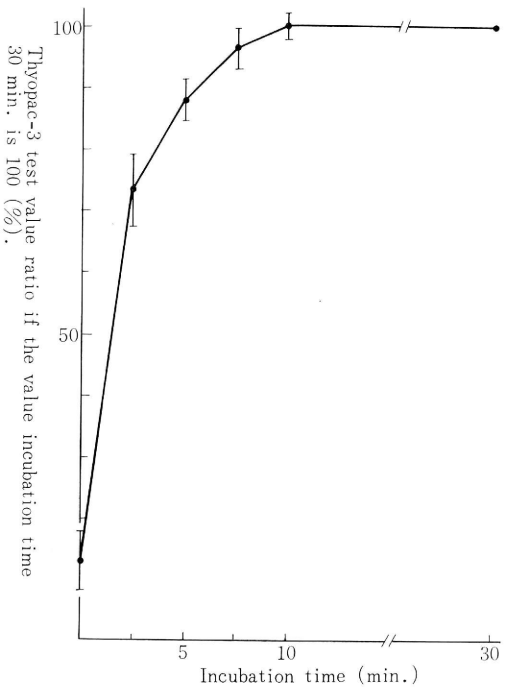


Fig. 1. Effect of incubation time upon the Thyopac-3 test value

In the long term management of thyroid disorders parameters such as T3 uptake are used to assess the patients' conditions over a period which may span several years. It is therefore vital that results obtained over such a period can be reliably inter-related.

Since T3 uptake is measured on an arbitrary scale, it is the responsibility of the manufacturer (whether commercial or private) to establish a unit and a scale and to maintain these unchanging over long periods.

This has been achieved by storing large numbers of freeze-dried aliquots of control sera at low temperatures. The sera were selected to span the working range of the assay as seen in figure 2.

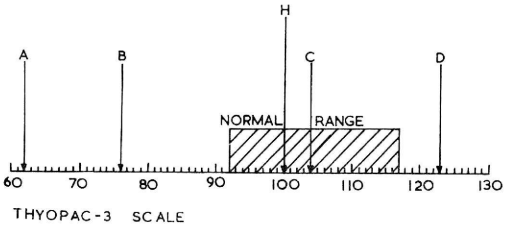


Fig. 2. Control Sera: A. B. C. D. H.

In order to test the stability of serum aliquots stored in this way, serum samples were obtained from a number of volunteers on different occasions over a period of some years. These were pooled and the T3 uptake value of each freshly prepared pool compared with that of a sample of the first pool obtained, and having been stored in the intervening period under exactly the same conditions as used for storing the control sera. The results of this examination are shown in Table 1.

Table 1. Mean Thyopac-3 values of a given group of volunteers when samples are collected over a period of years and referred to a stored standard serum.

Year	Mean Thyopac-3 value
1	107.2
2	107.0
3	107.0
4	107.7

Since the value is remarkably constant, it is reasonable to assume that the stored samples retained their T3 uptake value and that pooled serum from the group of volunteers had an identical T3 value each time it was collected.

Clinical usefulness

Generally, the two factors are important in determining the clinical usefulness of a measurement are firstly the reproducibility with which the measurement is made, and secondly the discrimination between different groups of patients which is obtained.

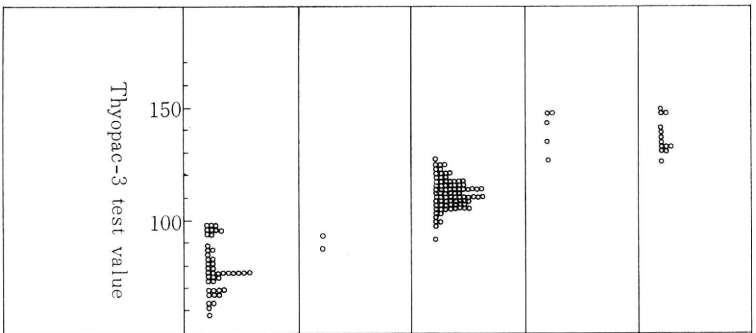
Table 2 shows replicate determinations of Thyopac-3 on a given serum and it can be seen that the coefficient of variation is rather less than 1%. Experience has shown that reproducibility of this order is not difficult to achieve.

Table 2. Replication of Thyopac-3 on one occasion.

100.4	102.4	100.6	101.3
101.8	102.5	102.8	100.7
101.4	101.8	103.5	101.3
102.8	101.1	103.2	102.4
100.4	100.3	102.8	102.3
101.7	101.7	102.1	102.5
102.3	103.2	102.3	101.1

Number of observations = 28
Mean value = 101.9
Standard deviation = 0.9

Discrimination according to thyroid status is illustrated in figures 3, 4 and 5, all of which data was obtained during clinical trials in Japan. Good discrimination of thyroid status is achieved in cases where TBG concentration is normal.



Subjects No. of subj.	Hyperthyroid 43	Subacute thyroiditis 2	Euthyroid 87	Pregnancy 5	Hypothyroid 13
Range	57.7~97.4	85.1~91.4	90.0~126.0	122.1~144.4	122.5~145.5
Mean + SD	80.0+11.0	88.3+4. 5	110.7+7.4	135.8+9.5	133.0+7.0

Fig. 3. Distribution of the Thyopac-3 test values

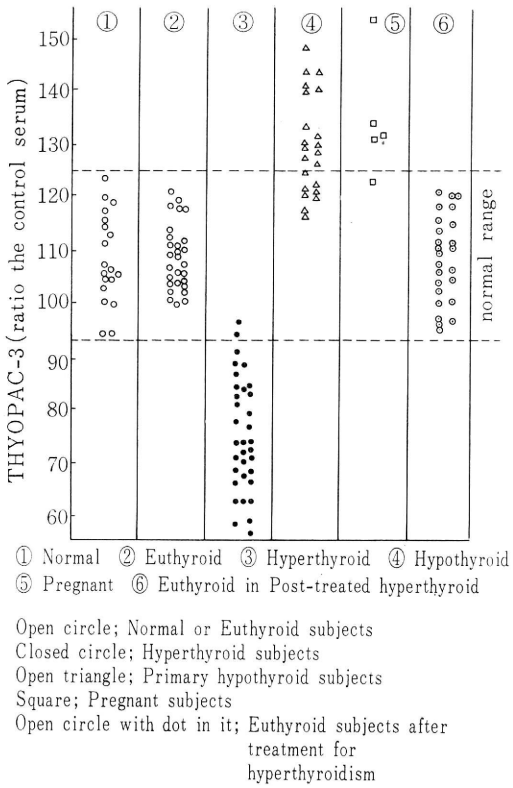


Fig. 4. Distribution of the values for Thyopac-3 test in various groups

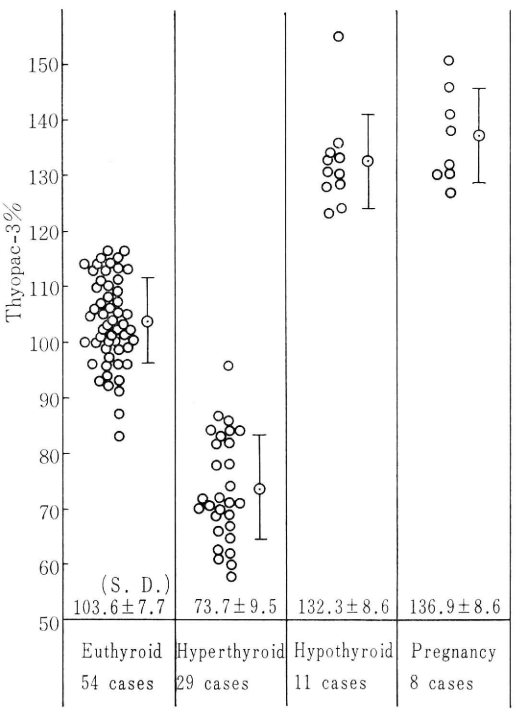


Fig. 5. Distribution of Thyopac-3 Values in various conditions

3. Thyopac-4

Figure 6 shows that the epuilibrium is established in 30 minutes and then remains constant at least up to 20 hours. As with Thyopac-3, this equilibrium is potentially well-suited to producing highly reproducible results.

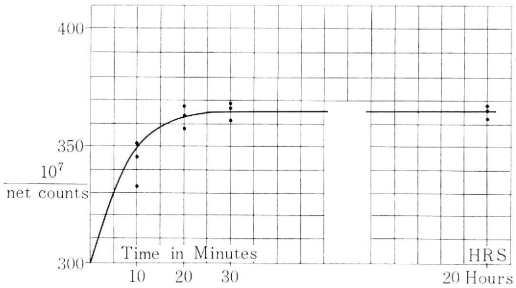


Fig. 6. Thyopac-4 Equilibration

Reproducibility

The within assay reprcducibility is illustrated in table 3 from which it can be seen that when the mean serum thyroxine concentration was 6.8 μg/100 mls, a standard deviation of 0.5 μg/ml was observed.

Table 3. Replication of Thyopac-4 on one occasion.

7.1	6.9	7.4	6.9
6.6	7.1	6.9	6.9
6.3	6.4	6.9	6.3
5.2	6.9	6.9	6.9
5.8	7.9	6.8	7.3
6.4	6.9	7.3	7.3
6.8	7.3	7.6	

Number of observations = 27
Mean value = 6.8 μg/100 ml
Standard deviations = 0.5 μg/100 ml

Table 4 shows the between assay reproducibility when the serum samples are stored at various temperatures between assays. When serum samples are stored at −20°C

Table 4. Reproducibility of Thyopac-4 values obtained on different occasions in one laboratory.

		RT	4°C	−20°C
5.10.71	Day 0	8.2	8.2	8.2
	1	8.3	8.9	7.5
	2	8.1	6.6	6.9
	3	9.4	7.3	7.7
	6	10.4	6.8	7.0
	7	12.4	7.6	7.4
	8	11.3	6.7	7.0
	9	12.5	7.5	6.4
	10	14.7	6.6	7.1
	13	14.3	7.2	6.4
	15	13.3	7.9	6.8
	17	14.0	7.8	7.3
			mean = 7.4	mean = 7.14
			S.D. = 0.71	S.D. = 0.52

over a period of seventeen days, the standard deviation was 0.52 μg, a value very similar to the within assay standard deviation. From this, it follows that the true between assay variance is extremely small. When serum samples are stored at +4°C the between assay standard deviation had increased to 0.71 μg showing that some additional variation had been introduced because of instability in the serum sample being measured. When the assay samples were stored at room temperature, a much bigger effect was seen. The apparent thyroxine concentration rose by a factor of almost two in seventeen days and even in three days a very significant error had been introduced.

The results of a study of the between-laboratories variance is shown in table 5. In this evaluation, nine hospital laboratories and three different technicians at The Radiochemical Centre all examined samples of a serum on several different occasions. It can be seen that there was good agreement over the mean

Table 5. Reproducibility of Thyopac-4 on different occasions and in different laboratories

Centre Ref.	No. of assays	Mean value	S.D. of results
TRC (1)	10	12.4 μgT4	0.31 μgT4
TRC (2)	14	11.8	0.54
TRC (3)	54	12.35	0.95
1	10	12.3	0.87
2	4	12.0	0.35
3	21	12.7	0.34
4	5	11.8	1.10
5	5	12.7	0.29
6	30	11.3	0.75
7	18	12.0	1.24
8	11	11.9	0.53
9	12	12.6	0.54
Mean	—	12.15	0.43

value of 12.15 μg/100 mls and that most laboratories had a between-assay standard deviation which agreed well with that shown in table 4, but laboratories 4 and 7 did not perform quite so well as the others. It is apparent that there is small but significant loss of precision in comparing results carried out in different laboratories as compared to results obtained in a single laboratory.

Clinical usefulness

The correlation of total serum thyroxine concentration with thyroid status is illustrated in figure 7 which shows the results of analysis of sera from one thousand unselected thyroid clinic patients. It can be seen that there is an excellent cut off between normal and pathological groups when the TBG concentration is normal. Euthyroid patients who are pregnant or receiving oestrogens have thyroxine concentrations which spread over the normal and hyperthyroid interface.

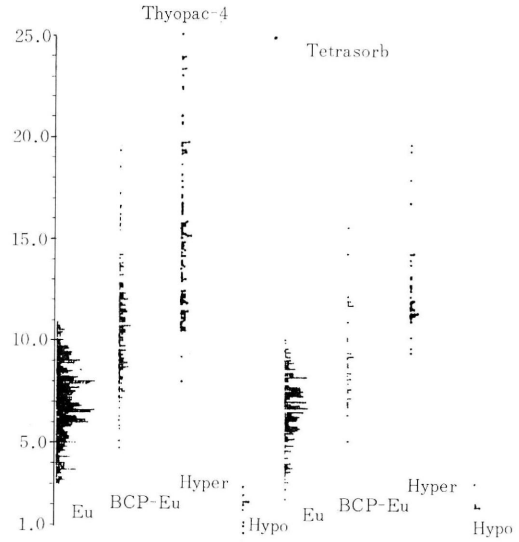


Fig. 7.

4. Free Thyopac Index

It is now well known that the thyroid status correlates well with the concentration of free thyroxine and that this relates well to the concentration of total thyroxine where the concentration of thyroid binding proteins is normal. Variations in the concentration of thyroid binding proteins can be associated with abnormal total thyroxine concentrations where thyroid function is normal. Well known and frequent examples are pregnancy and subjects taking oral contraceptives. A well-established method of obtaining a measure of the concentration of free thyroxine was described by Clark and Horn (1965) and is summarized below:—

$$FT_4 + UTBP \rightleftharpoons TBP\text{-}T_4$$

$$K = \frac{[FT_4]\,[UTBP]}{[TBP\text{-}T_4]}$$

$$[FT_4] = \frac{K[TBP\text{-}T_4]}{[UTBP]}$$

$[FT_4]$ = free thyroxine concentration

$[UTBP]$ = the concentration of unoccupied binding sites to thyroid binding proteins

$[TBP\text{-}T_4]$ = the concentration of thyroxine bound to thyroid binding proteins.

Clark and Horn used a T3 uptake test as a measure of $[UTBP]$ and PBI as a measure of $[TBP\text{-}T_4]$.

In the Thyopac system free thyroxine can be evaluated by a simple ratio of Thyopac-3 and Thyopac-4:—

$$\text{Free Thyopac Index} = \frac{\text{Thyopac-3}}{\text{Thyopac-4}}$$

The combination of these two measurements to produce a measure of the concentration of free thyroxine improves the discrimination between normal and abnormal thyroid function in all subjects and addition-

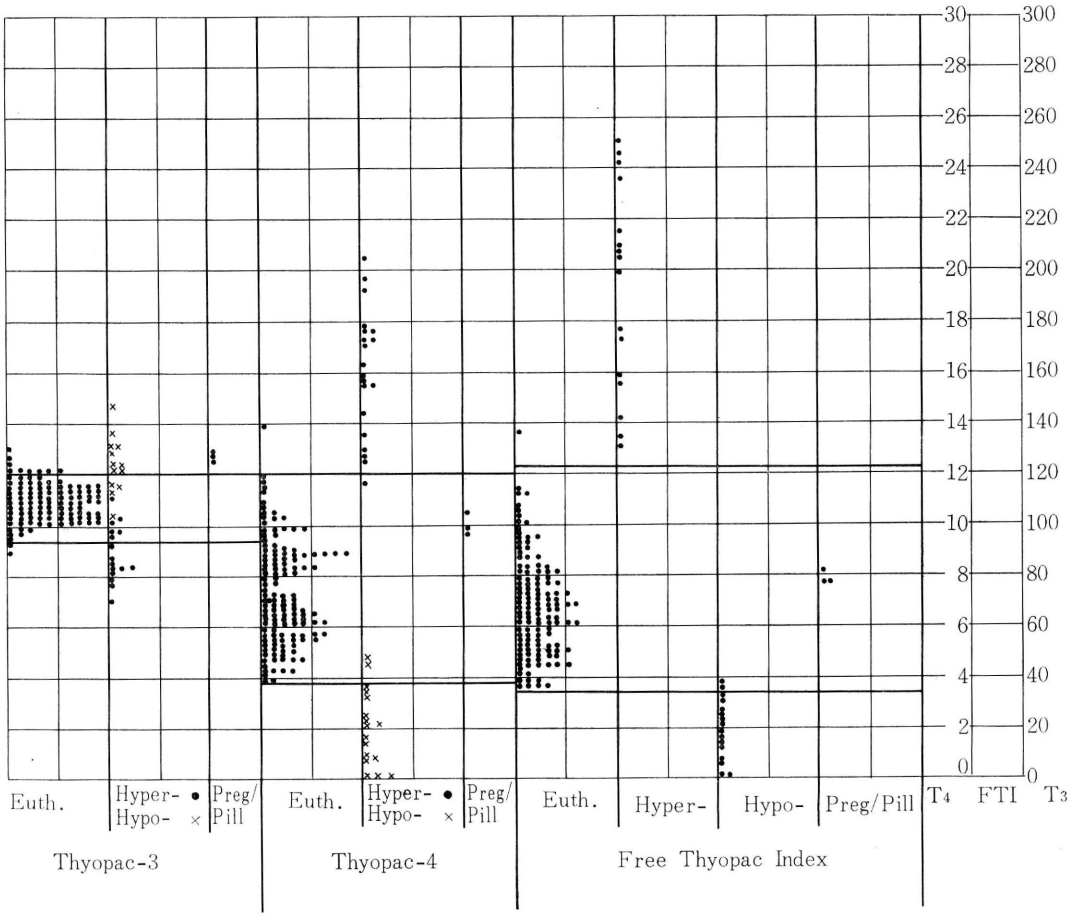


Fig. 8.

ally brings data from euthyroid subjects having abnormal TBG concentrations into the normal range, permitting useful measurements to be made on pregnant and oral contraceptive-taking subjects. This is illustrated in figure 8.

The Mapping Approach

It was seen above that free thyroxine index gives more accurate information than either the T3 uptake or the total thyroxine from which it was calculated. Even so, there is still a loss of information in simply computing a free thyroxine index. A more complete clinical picture can be obtained by use of a two dimensional plot of T3 uptake and total serum thyroxine. By this approach an even better correlation with thyroid status (independent of TBG concentration) is obtained additionally results for any thyroid category are subdivided according to TBG concentration.

It was seen above that

$$\text{Free Thyopac Index} = \frac{\text{Thyopac-4}}{\text{Thyopac-3}}$$

Then, if a graph is drawn with Thyopac-3 on the horizontal axis and Thyopac-4 on the vertical axis, any line passing through the origin will join points have the same Free Thyopac Index; a scale of Free Thyopac Index can be drawn as a quadrant (see figure 9).

It follows that we would expect points arising from sera obtained from patients with

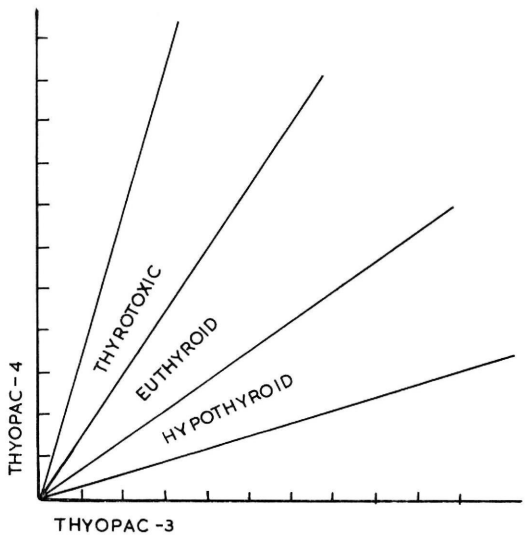


Fig. 10.

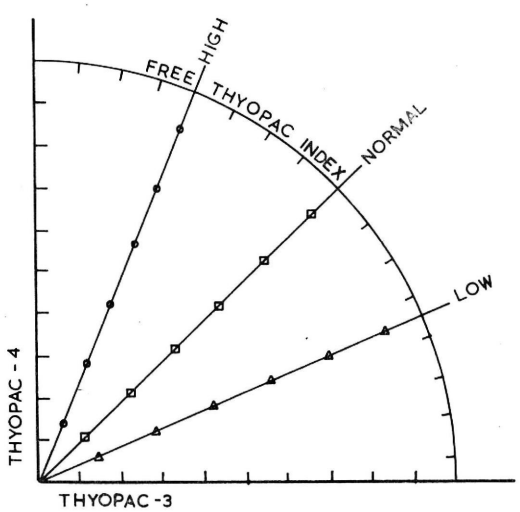


Fig. 9.

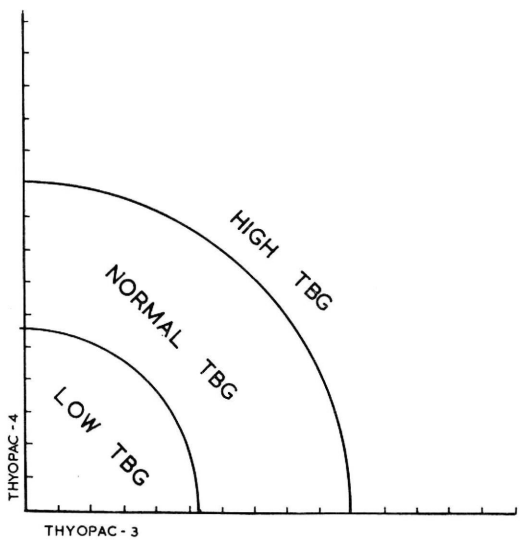


Fig. 11.

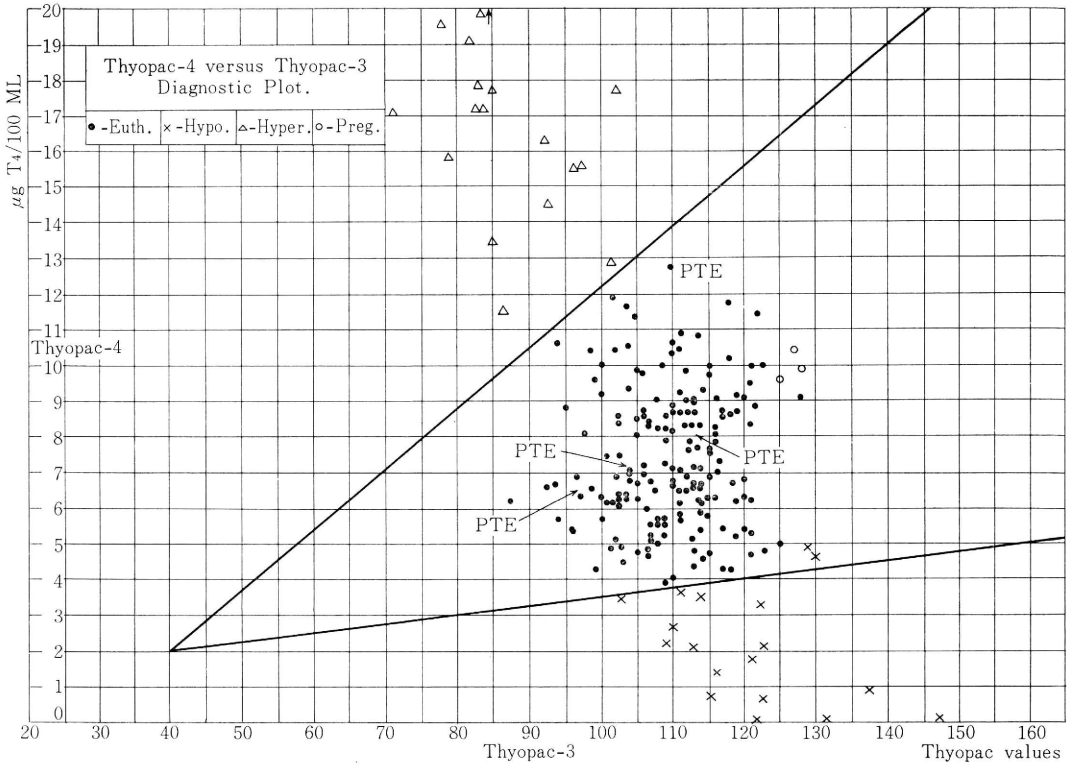


Fig. 12. PTE-Post Thyroidectomy

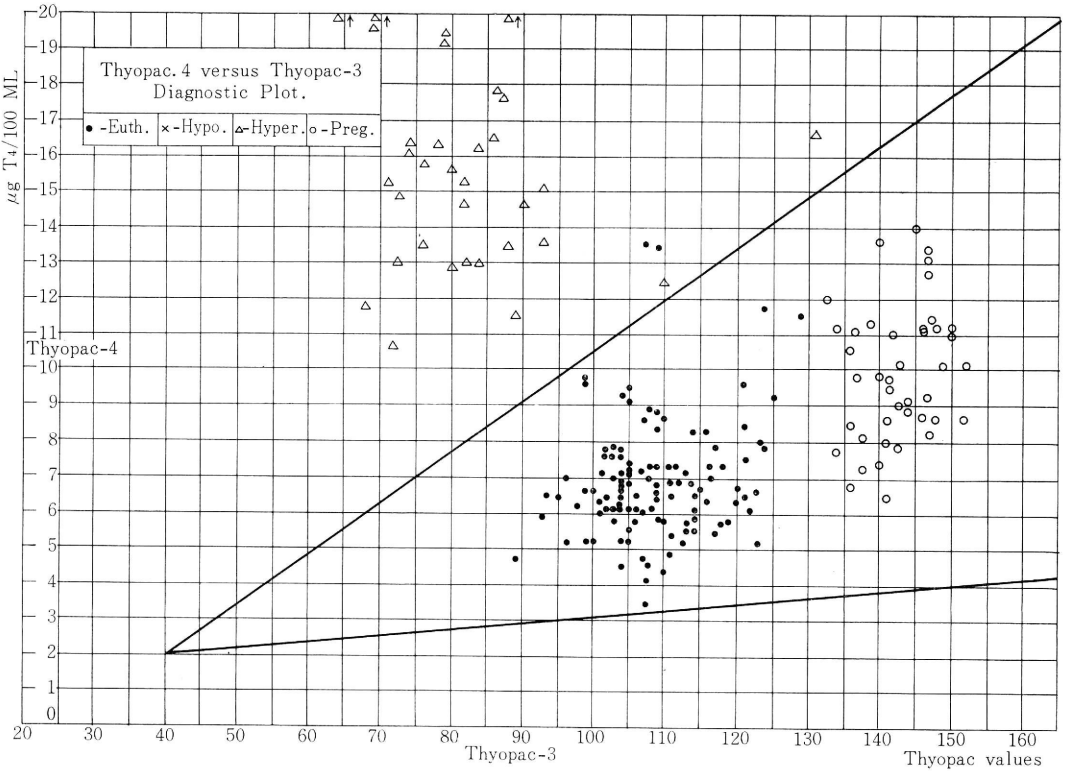


Fig. 13.

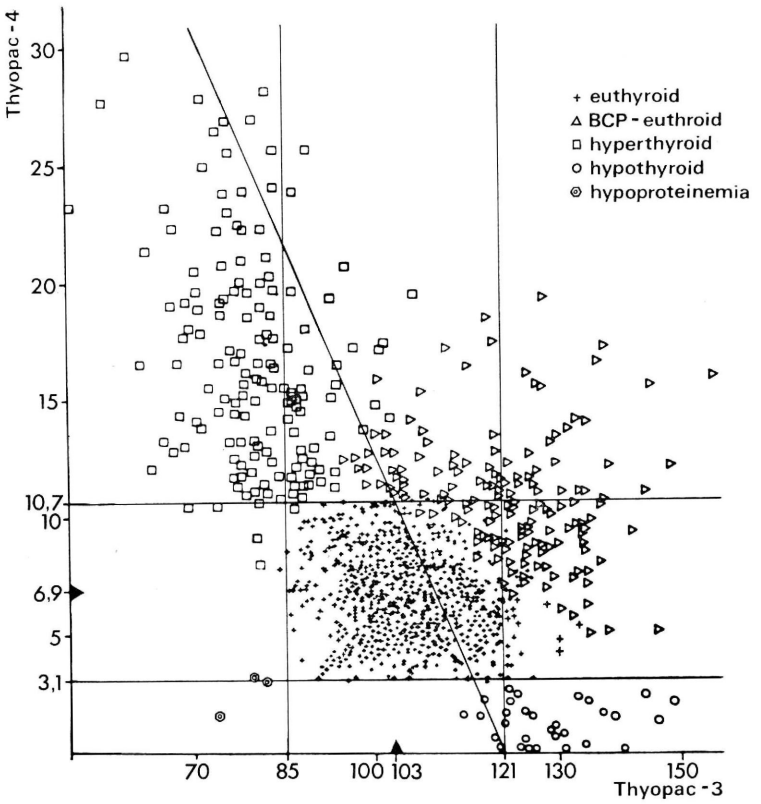


Fig. 14.

differing thyroid function to be distributed as shown in figure 10; this grouping being based upon the concentration of free thyroxine ought to be independent of the concentration of TBG. Discrimination according to TBG concentration for any particular thyroid category is predictable and is shown in figure 11.

The results of practical application of this technipue is shown in figures 12, 13 and 14.

In all cases it can be seen that there is a virtually complete separation according to thyroid status regardless of TBG concentration. Additionally, in the cases where TBG concentration was abnormal, the point was in the area of the graph predicted on theoretical grounds.

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