

CHAPTER III

RESULTS

1. Standard curves of T₄ and T₃

Typical standard curve of thyroxine is shown in Fig. 2. The results of the assay of the T₄ standards were calculated as percentages of T₄-¹²⁵I bound to TBG ($\frac{\text{cpm of supernate}}{\text{total count}} \times 100$) and were plotted against doses of standard thyroxine. Ten aliquots of a single sample of serum T₄ were determined in the assay to find the variation of the method. The mean and the standard deviation of the ten determinations of a single sample were calculated and expressed in term of coefficient of variation.

$$\text{Coefficient of variation} = \frac{S.D.}{\bar{X}} \times 100.$$

Coefficient of variation of the serum T₄ method was found to be 11.4% which lay within very acceptable range for radioimmunoassay.

Standard curve of T₃ on the other hand was calculated as logit (see section 8 page 22) and was plotted against log dose of T₃ as shown in Fig. 3. The plot was a straight line. Ten determinations of a single sample were done to find coefficient of variation which was found to be 14.8% for T₃ method which was also well within acceptable range.

2. Normal ranges of serum T₄ and serum T₃

Blood from 57 normal subjects were drawn for serum T₄ determination. The range of normal was found to be from 3 to 11 μg%.

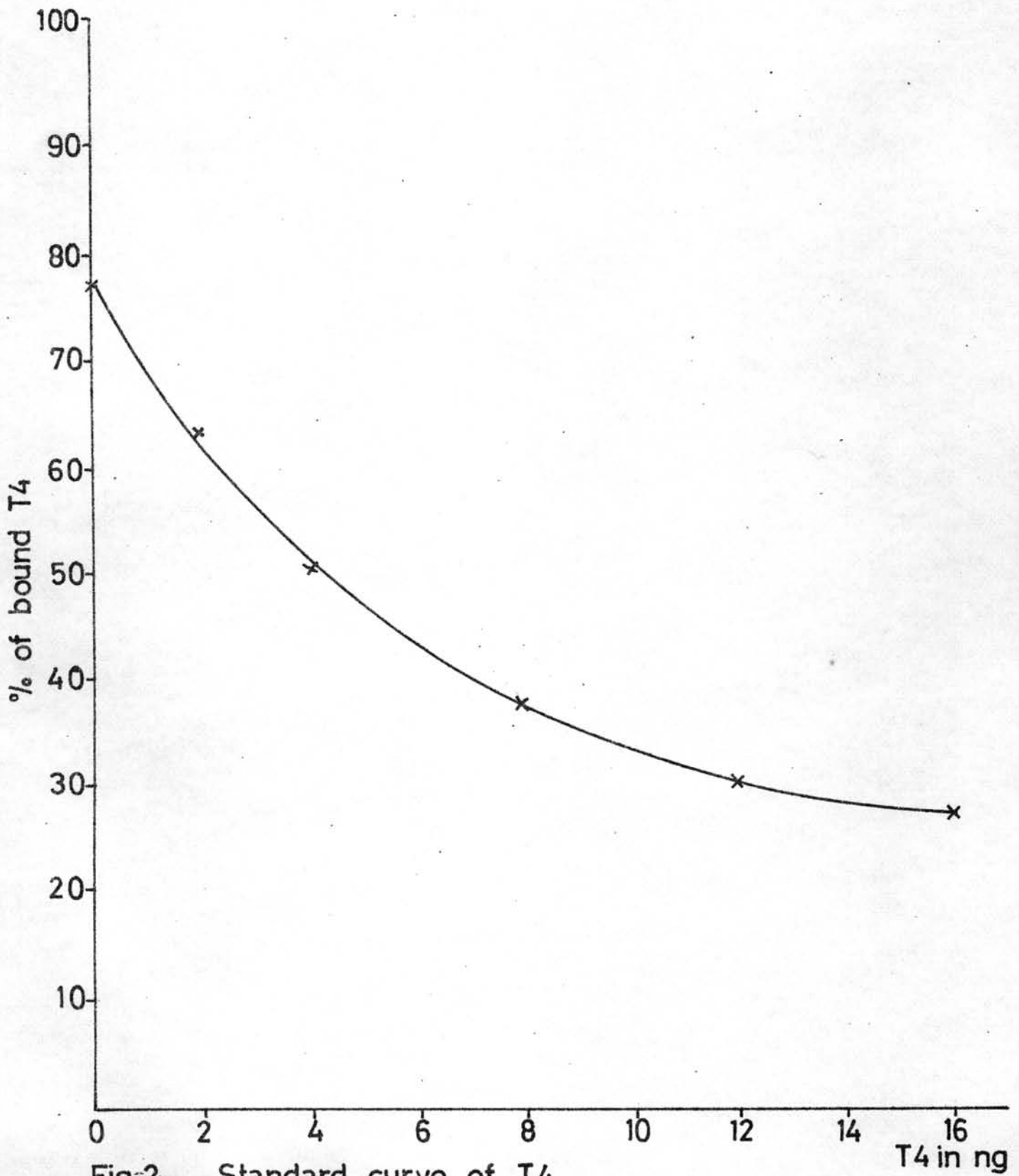


Fig:2 Standard curve of T4

T4 in ng

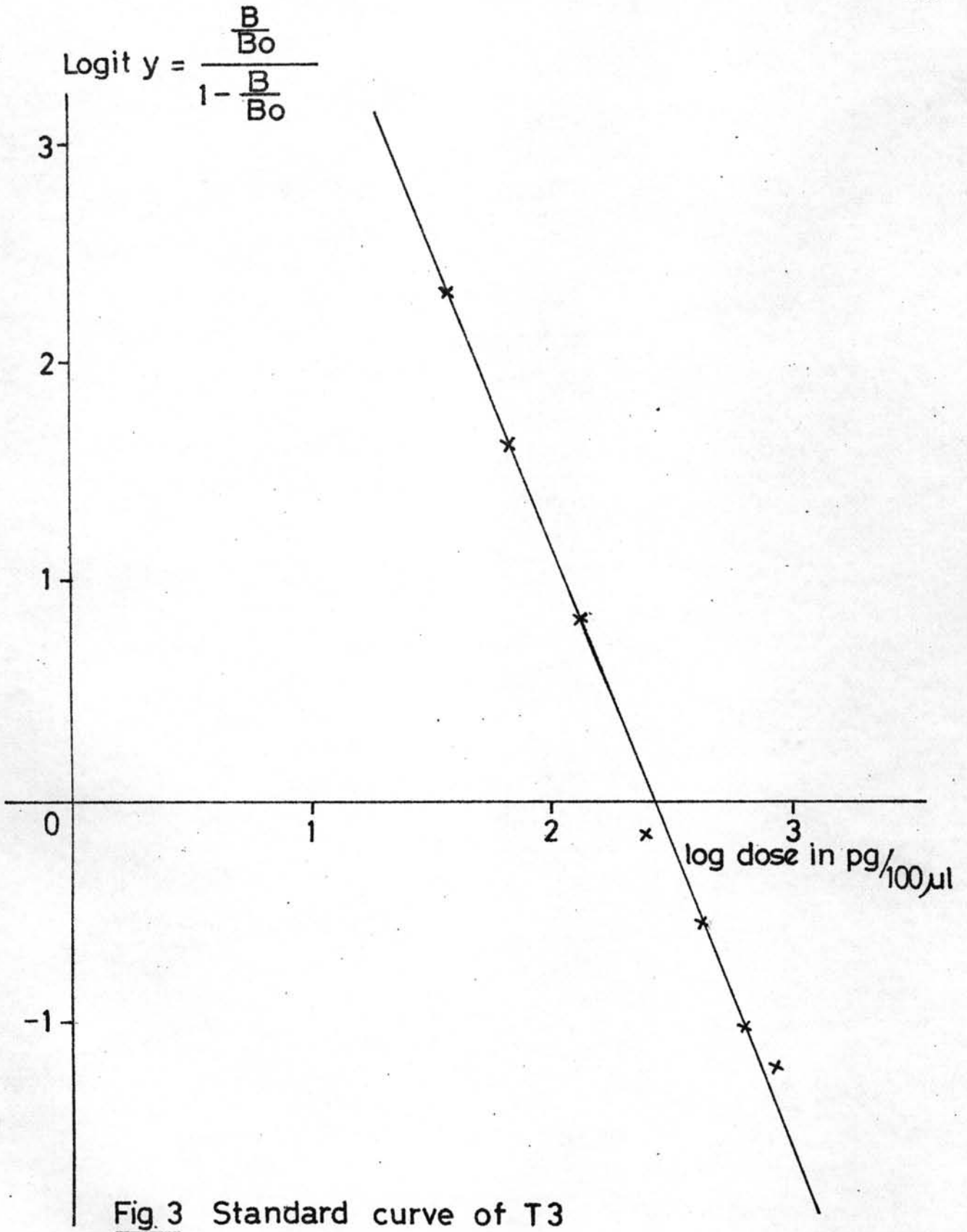


Fig. 3 Standard curve of T3

Table 1 Normal values of serum T₄ by competitive protein binding assay

sample no.	Serum T ₄ (ug/100 ml)	sample no.	Serum T ₄ (ug/100 ml)
1	3.90	30	7.65
2	3.30	31	9.30
3	6.90	32	4.05
4	5.60	33	8.10
5	10.20	34	5.30
6	8.85	35	9.30
7	4.35	36	4.35
8	7.35	37	6.60
9	7.40	38	6.00
10	7.50	39	9.15
11	7.35	40	10.20
12	5.70	41	3.30
13	6.30	42	4.05
14	11.10	43	11.85
15	5.10	44	10.50
16	7.05	45	6.60
17	3.30	46	4.50
18	8.85	47	3.60
19	9.00	48	10.10
20	6.00	49	7.35
21	8.55	50	5.70
22	3.60	51	6.00
23	7.65	52	7.65
24	9.75	53	7.65
25	3.30	54	11.10
26	5.10	55	6.60
27	6.30	56	7.05
28	11.25	57	10.20
29	4.80		
		mean	7.05
		range	3-11

Table 2 Normal values of serum T₃ by radioimmunoassay

Sample No.	Serum T ₃ (ng/100 ml)
1	70.84
2	194.98
3	147.91
4	81.28
5	186.21
6	199.53
7	190.55
8	181.17
9	162.18
10	199.53
11	201.10
12	144.54
13	151.36
14	137.78
15	162.18
16	123.03
17	162.18
18	144.54
19	138.04
20	181.97
mean	158.00
range	70-200

Blood from 20 normal subjects were drawn for serum T_3 determination. The normal range of serum T_3 was found to be 70-200 ng%.

3. Serum T_4 and serum T_3 determinations in the Graves' disease patients before therapy.

Serum T_4 in forty-eight Graves' disease patients (see page 11) before therapy was found to be 16.8 ± 5.3 $\mu\text{g}\%$ while serum T_3 was 485 ± 230 ng%. However six of the forty-eight patients with symptoms and signs of Graves' disease had normal serum T_4 level. All six patients had abnormal ^{131}I uptake and abnormally high serum T_3 . These six patients then fit the criteria for T_3 toxicosis. An incidence in this series is 12.5%.

4. Weekly serum T_4 and serum T_3 after the initiation of therapy

Twenty-three patients (see page 11) completed the weekly visits during the first month. The mean value of serum T_4 before therapy was 18.0 ± 5.6 $\mu\text{g}\%$. After one week of therapy the mean value of serum T_4 dropped to 10.3 ± 5.2 $\mu\text{g}\%$, which was already within the normal range and significantly different from the value before therapy ($p < 0.001$). The mean T_4 value dropped further in the second week to 7.6 ± 3.0 and in the third week to 6.0 ± 3.0 and stabilized at this level in the fourth week with the mean of 6.1 ± 2.9 (Fig. 4). The individual values of these patients are shown in Table 3.

Serum triiodothyronine before therapy of this group of 23 patients was 507 ± 239 ng%. After one week of therapy the mean T_3 dropped to 260 ± 182 ng% significantly different from the value before therapy ($p < 0.001$). Serum T_3 then gradually dropped further with each week of therapy to

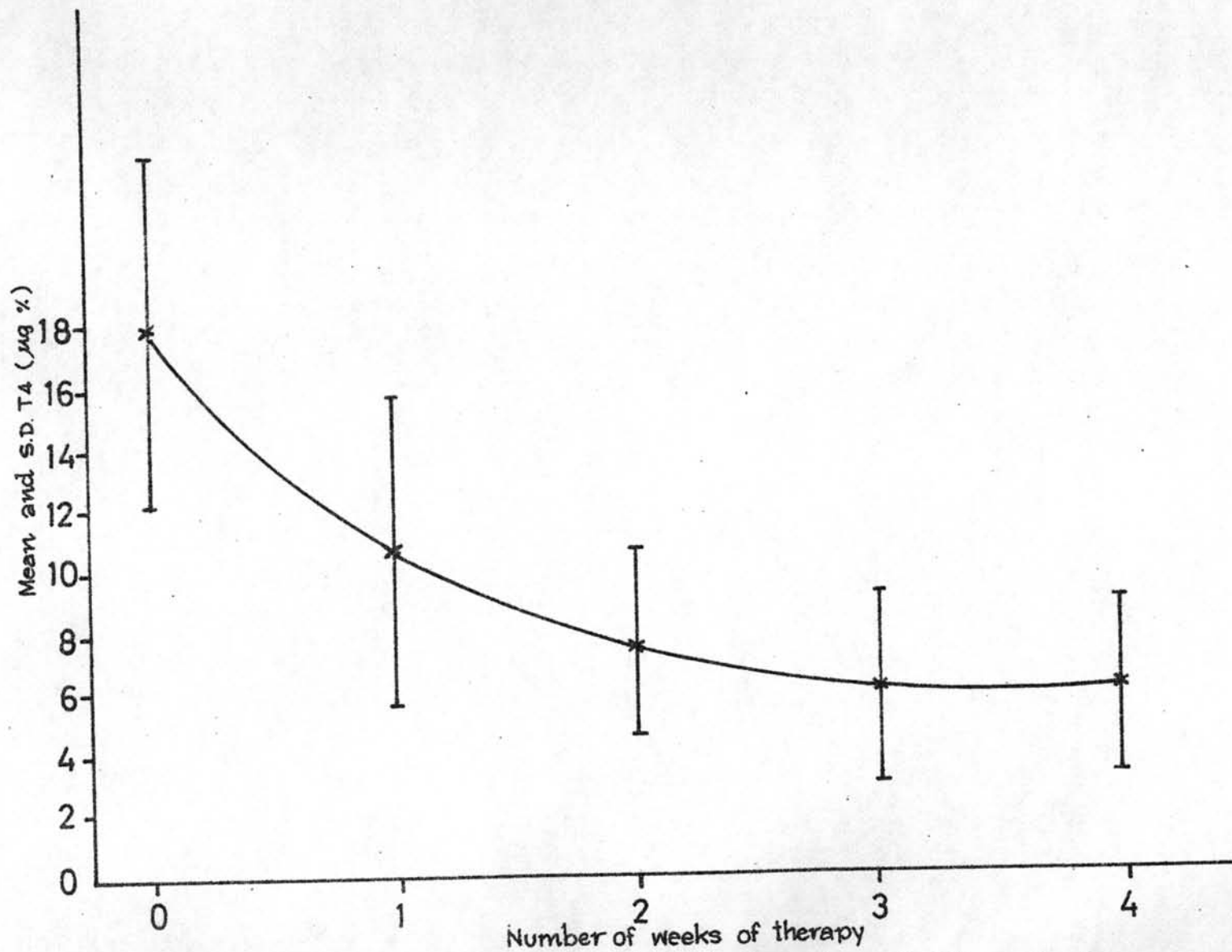


Fig.4 Serum T4 at weekly intervals before (week 0) and after initiation of therapy with methimazole

Table 3

Weekly serum T_4 , serum T_3 and T_4/T_3 ratio of 23 patients before (week 0) and after initiation of therapy with methimazole

No.	Subjects	T_4, T_3 of T_4/T_3 *	Week of Therapy				
			0	1	2	3	4
1	A.K.	T_4	23.40	21.80	9.50	8.20	6.10
		T_3	630.96	389.05	120.23	181.97	120.23
		T_4/T_3	37.09	56.03	95.00	45.06	50.73
2	S.L.	T_4	9.20	6.30	7.20	3.80	1.80
		T_3	275.42	104.71	134.90	208.93	117.49
		T_4/T_3	33.40	60.17	53.40	18.19	15.32
3	V.P.	T_4	24.00	16.20	10.80	5.50	8.20
		T_3	794.33	616.60	162.18	123.03	208.93
		T_4/T_3	30.21	26.27	66.59	44.70	39.25
4	K.H.	T_4	13.70	8.80	5.90	3.50	4.80
		T_3	588.84	79.43	83.18	27.54	54.95
		T_4/T_3	23.27	110.79	70.93	127.09	87.35
5	N.L.	T_4	11.60	7.70	5.40	0.60	1.50
		T_3	436.52	162.18	169.82	154.88	87.10
		T_4/T_3	26.57	57.00	47.48	3.87	17.22
6	P.S.	T_4	24.00	7.20	9.60	10.90	9.40
		T_3	476.74	263.03	181.97	114.82	144.54
		T_4/T_3	50.34	27.37	52.76	94.93	65.03

* T_4 is in $\mu\text{g}/100 \text{ ml}$

T_3 is in $\text{ng}/100 \text{ ml}$

Table 3 (Cont.)

No.	Subjects	T_4, T_3 or T_4/T_3	Week of Therapy				
			0	1	2	3	4
7	C.V.	T_4	24.00	13.70	12.60	6.90	8.40
		T_3	363.08	173.78	109.65	169.82	173.78
		T_4/T_3	66.10	78.84	114.91	40.63	48.34
8	V.Y.	T_4	24.00	10.30	9.60	6.20	9.00
		T_3	354.81	213.80	199.53	229.09	218.78
		T_4/T_3	67.64	48.18	48.11	27.04	41.14
9	S.P.	T_4	11.00	6.90	3.20	4.50	1.50
		T_3	416.87	131.83	67.61	46.77	112.20
		T_4/T_3	26.39	52.24	47.33	96.21	13.37
10	P.B.	T_4	17.25	6.50	5.30	5.10	4.40
		T_3	426.58	87.10	123.03	79.43	120.23
		T_4/T_3	40.44	74.63	43.08	64.13	36.60
11	P.B.	T_4	12.90	3.60	6.20	3.00	7.20
		T_3	297.49	151.36	63.10	41.69	223.87
		T_4/T_3	43.36	57.05	40.96	11.94	32.16
12	P.S.	T_4	14.70	7.20	7.65	12.75	6.45
		T_3	416.87	128.82	123.03	144.54	114.82
		T_4/T_3	35.26	55.89	62.17	121.76	56.17
13	M.I.	T_4	12.40	3.75	1.05	2.10	2.60
		T_3	346.74	57.54	38.81	72.44	53.70
		T_4/T_3	35.76	65.17	27.05	28.99	48.41
14	T.N.	T_4	19.20	10.80	10.90	10.40	7.65
		T_3	575.44	162.18	223.87	144.54	181.97
		T_4/T_3	33.37	78.24	48.69	71.95	42.04

Table 3 (Cont.)

No.	Subjects	T_4, T_3 or T_4/T_3	Week of Therapy				
			0	1	2	3	4
15	P.B.	T_4	21.70	10.40	10.13	9.52	9.30
		T_3	912.34	363.08	275.42	223.87	134.90
		T_4/T_3	23.78	28.64	36.78	42.54	60.04
16	S.T.	T_4	19.50	15.00	12.45	7.95	8.02
		T_3	602.56	588.84	446.68	660.69	151.36
		T_4/T_3	32.36	25.47	27.87	12.03	52.99
17	P.Y.	T_4	6.45	4.80	4.20	2.10	1.50
		T_3	218.78	141.25	251.19	123.03	69.18
		T_4/T_3	29.48	33.98	16.72	17.07	21.68
18	T.N.	T_4	24.00	8.85	7.05	3.15	6.90
		T_3	313.23	257.04	398.11	331.13	100.00
		T_4/T_3	76.62	33.26	17.70	5.12	69.00
19	K.S.	T_4	20.30	10.50	7.80	5.70	4.35
		T_3	446.68	243.47	281.84	54.95	134.90
		T_4/T_3	45.45	42.97	27.67	103.73	32.25
20	M.I.	T_4	24.00	10.88	10.30	7.10	7.00
		T_3	478.13	245.47	165.96	162.18	302.00
		T_4/T_3	50.20	44.32	62.06	43.77	23.18
21	B.Y.	T_4	15.10	9.45	4.50	7.00	5.70
		T_3	346.74	213.80	75.86	263.08	158.49
		T_4/T_3	43.55	44.20	59.32	26.60	35.96
22	A.B.	T_4	17.60	24.00	7.00	4.40	7.35
		T_3	660.69	645.65	457.09	190.55	199.53
		T_4/T_3	26.64	37.17	15.31	23.09	36.84

Table 3 (Cont.)

No. Subjects	T_4, T_3 * or T_4/T_3	Week of Therapy				
		0	1	2	3	4
23 N.M.	T_4	24.00	12.70	5.30	7.35	11.40
	T_3	1288.25	575.44	501.18	371.54	275.42
	T_4/T_3	18.63	22.07	10.57	20.59	41.39
Mean \pm S.D.	T_4	18.0 \pm 5.6	10.3 \pm 5.2	7.6 \pm 3.0	6.0 \pm 3.0	6.1 \pm 2.9
	T_3	507 \pm 239	260 \pm 182	202 \pm 134	179 \pm 137	150 \pm 65
	T_4/T_3	39 \pm 15	50 \pm 21	47 \pm 25	47 \pm 37	42 \pm 18

* T_4 is in $\mu\text{g}/100 \text{ ml}$

T_3 is in $\text{ng}/100 \text{ ml}$

202±134 in the second week, 179±137 in the third week and 150±65 in the fourth week (Fig. 5). The individual values of these patients are shown in Table 3. Figure 6 illustrates the combined scattogram of serum T_4 and T_3 before and weekly after therapy.

The values of T_4/T_3 ratio were calculated for each patient visit and are shown in Table 3. The mean value of T_4/T_3 ratio before therapy was 39±15. This value increased to 50±21 in the first week, 47±25 in the second week, 47±37 in the third week and 42±18 in the fourth week. However there was no significant difference between each of these results. Figure 7 shows the mean of T_4/T_3 ratio before and weekly after therapy.

5. Side Effects of Methimazole

Of the 51 subjects (see page 11) treated with methimazole five developed skin rashes, an incidence of 9.8%. These skin rashes surprisingly developed in the third and fourth weeks after initiation of therapy (one in the third week and four in the fourth week). There were no changes of complete blood count and liver function determinations in any of the patients during the course of therapy.

Very significant finding during the course of follow up was the incidence of hypothyroxinemia. Of the forty-six patients (see page 11) that had been followed for eight months serum T_4 was found to be below normal (<3 ug%) in 33 patients (72%) at various times after the start of medication (Fig. 13). Two patients started to have serum T_4 below normal after only 2 weeks of therapy and the incidence increased with each follow up visit with the peak incidence in the fourth month after

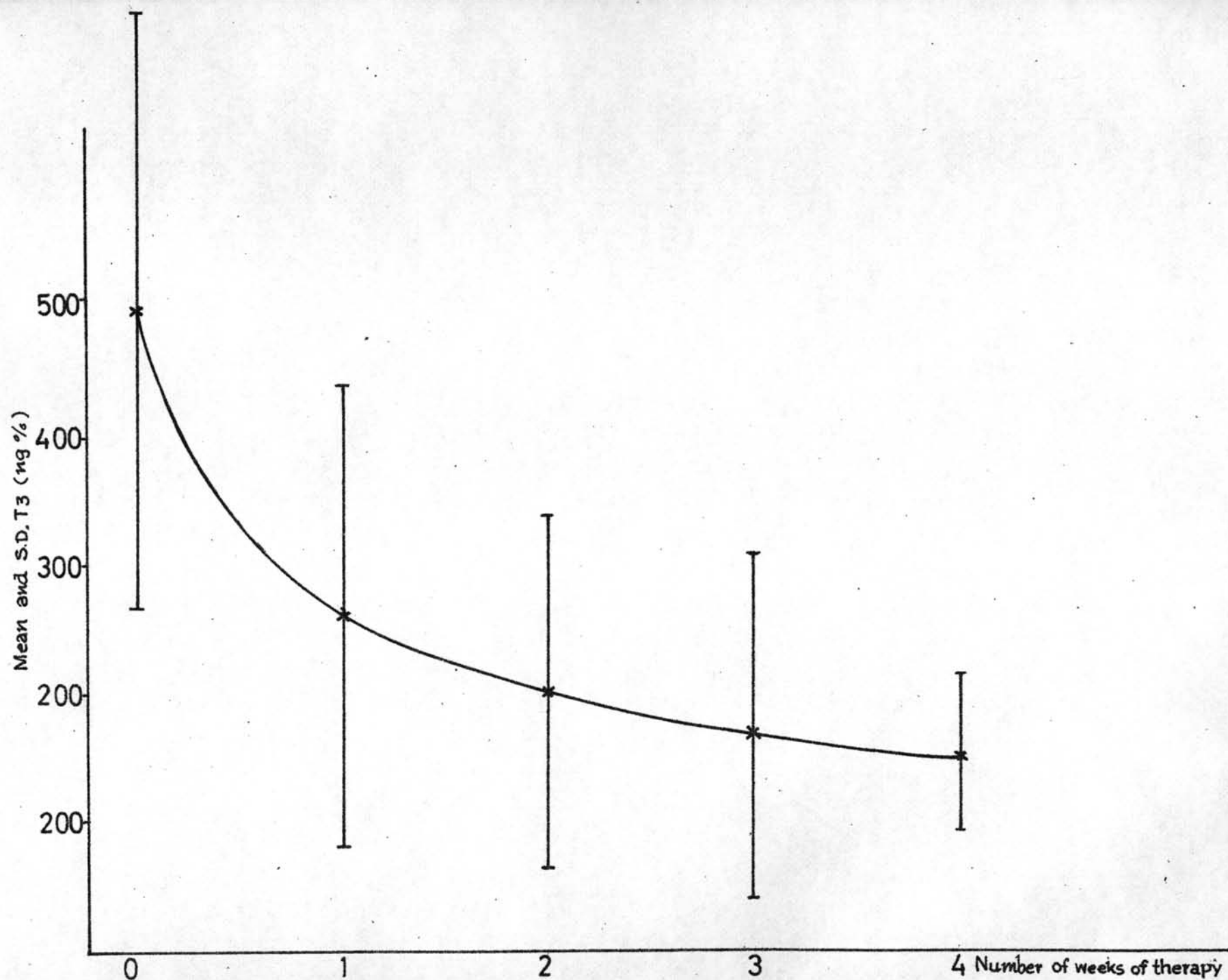


Fig.5 Serum T3 at weekly intervals before (week 0) and after initiation of therapy with methimazole

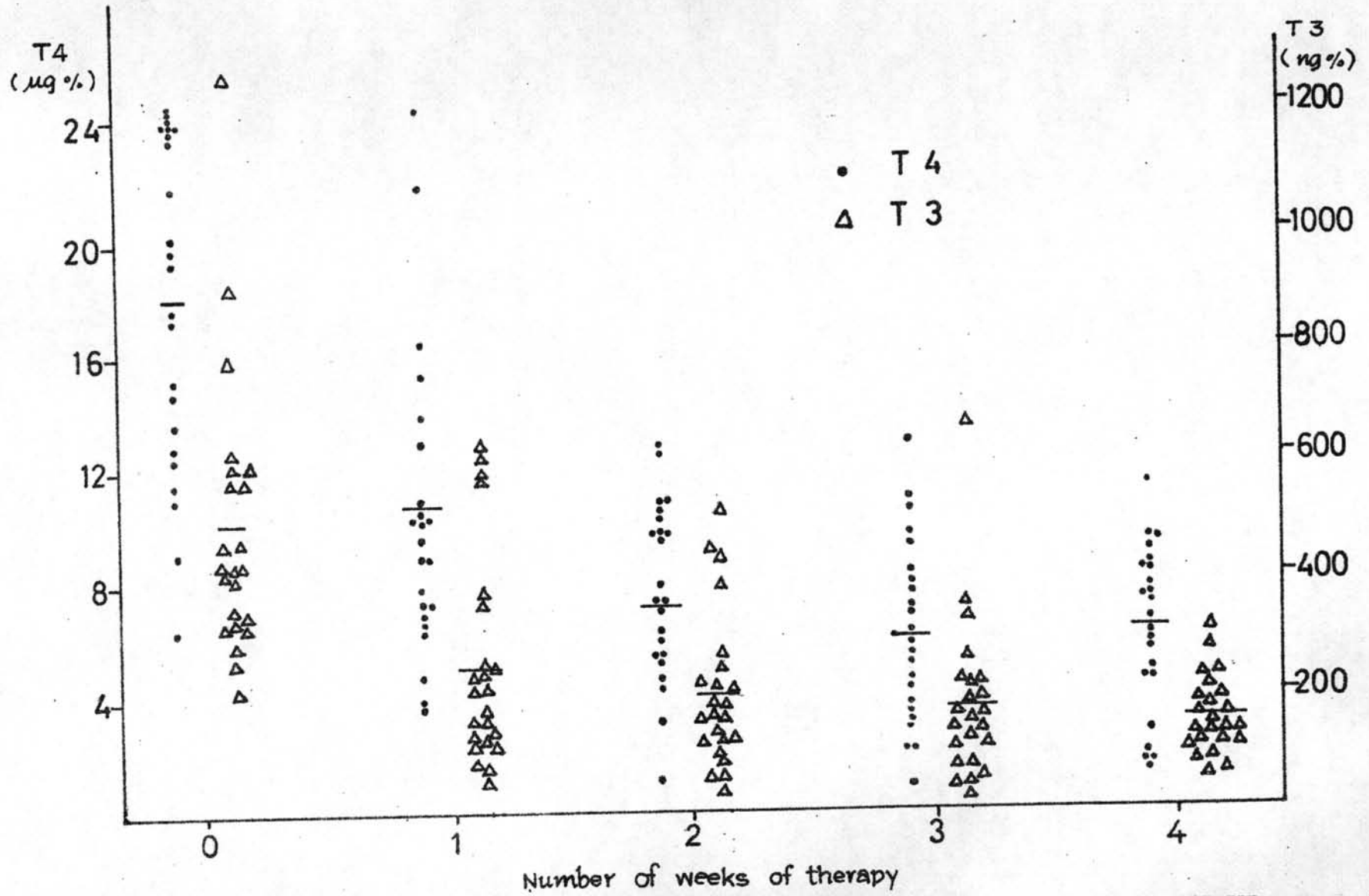


Fig. 6 Combined scattogram of weekly serum T4 and serum T3 before (week 0) and after initiation of therapy with methimazole

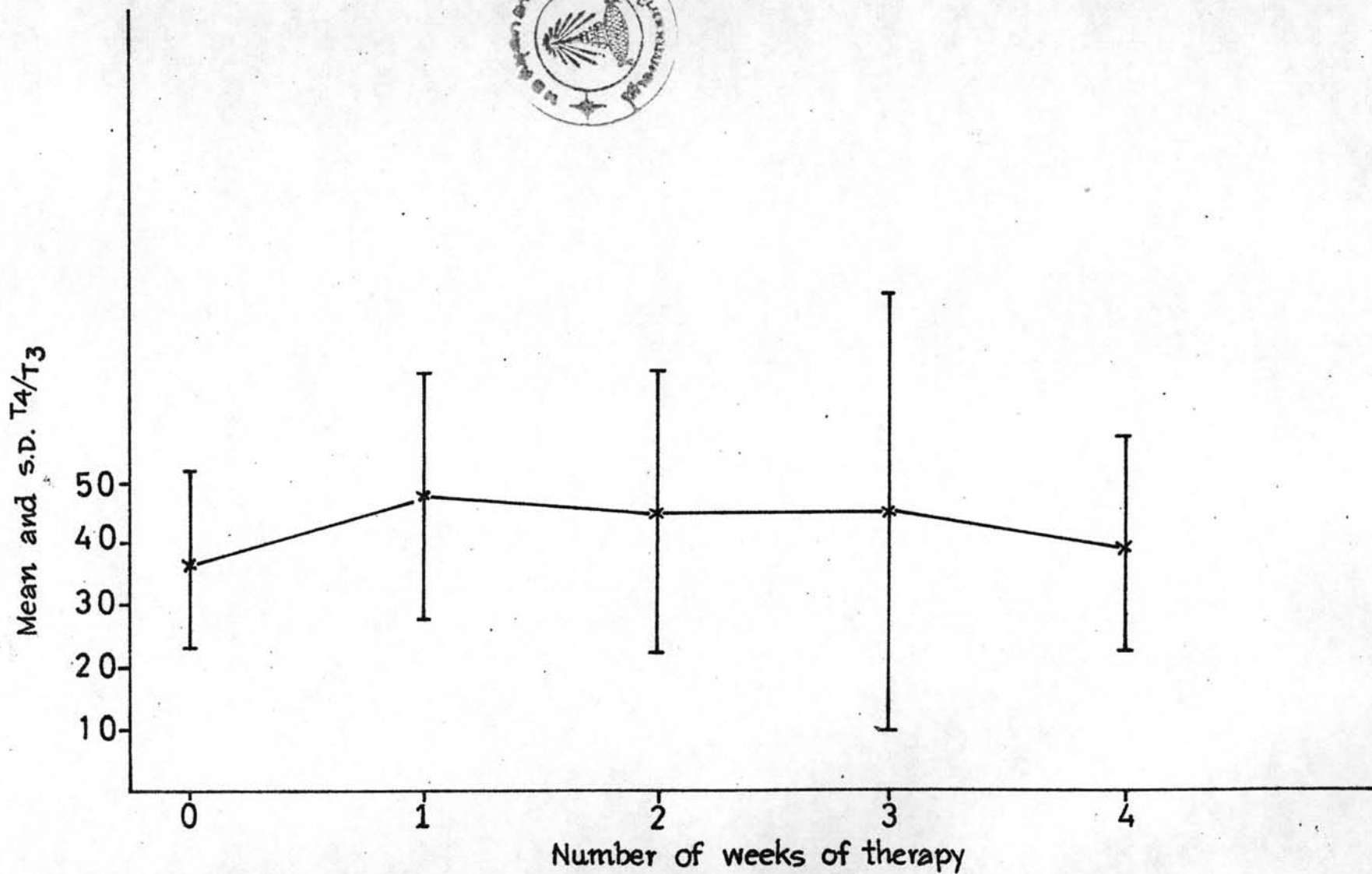


Fig.7 Weekly T_4/T_3 ratio before (week 0) and after initiation of therapy with methimazole

Table 4 Period of development of signs and symptoms of hypothyroidism in relation to period of discovery of hypothyroxinemia

Subjects	Time when T ₄ below Normal (3 µg%)	Time when Subject Developed Signs and Symptoms of Hypothyroidism	Lag Period (months)	Signs and Symptoms of Hypothyroidism
1 S.B.	week 3	month 6	5	Cold intolerance coarse skin
2 C.B.	week 4	month 4	3	Dry hair, dry skin
3 L.P.	month 2	month 8	6	Fatigue
4 H.L.	month 4	month 6	2	Tight skin
5 V.Y.	month 4	month 8	4	Lid edema, tight skin, positive hypothyroid reflex
6 Y.S.	month 4	month 6	2	Thyroid enlarge- ment
7 K.C.	month 4	month 4	0	Anasarca, lid edema
8 M.I.	week 2	month 4	3	Dry skin

initiation of therapy. Incidence of new cases of hypothyroxinemia plateaued off in the six and eight months (Fig. 10). On analysis of serum T_3 however it was found that only 11 out of 29 patients with low serum T_4 had serum T_3 below normal range (Fig. 13).

Out of the 33 patients, with low serum T_4 only 8 patients (24.2%) developed signs and symptoms of hypothyroidism. These signs and symptoms of hypothyroidism lagged behind development of hypothyroxinemia by several months (Table 4). Of the eight patients with signs and symptoms of hypothyroidism only three had both serum T_4 and serum T_3 below normal.

The T_4/T_3 ratios were plotted against T_4 and T_3 values in the hyperthyroid state and compared with the hypothyroid state; these are shown in Fig. 8, 9, 11 and 12. When correlation coefficients were calculated for each of the curve; it was found that in hyperthyroid state T_4/T_3 ratio correlated significantly with T_3 ($p < 0.001$) but not with T_4 (Fig. 8 and 9).

In hypothyroid state T_4/T_3 ratio correlated significantly with both T_4 ($p < 0.001$) and T_3 ($p < 0.01$). However T_4/T_3 ratio markedly decreased with a mean of 24 ± 15 , almost half of the value before therapy (39 ± 15).

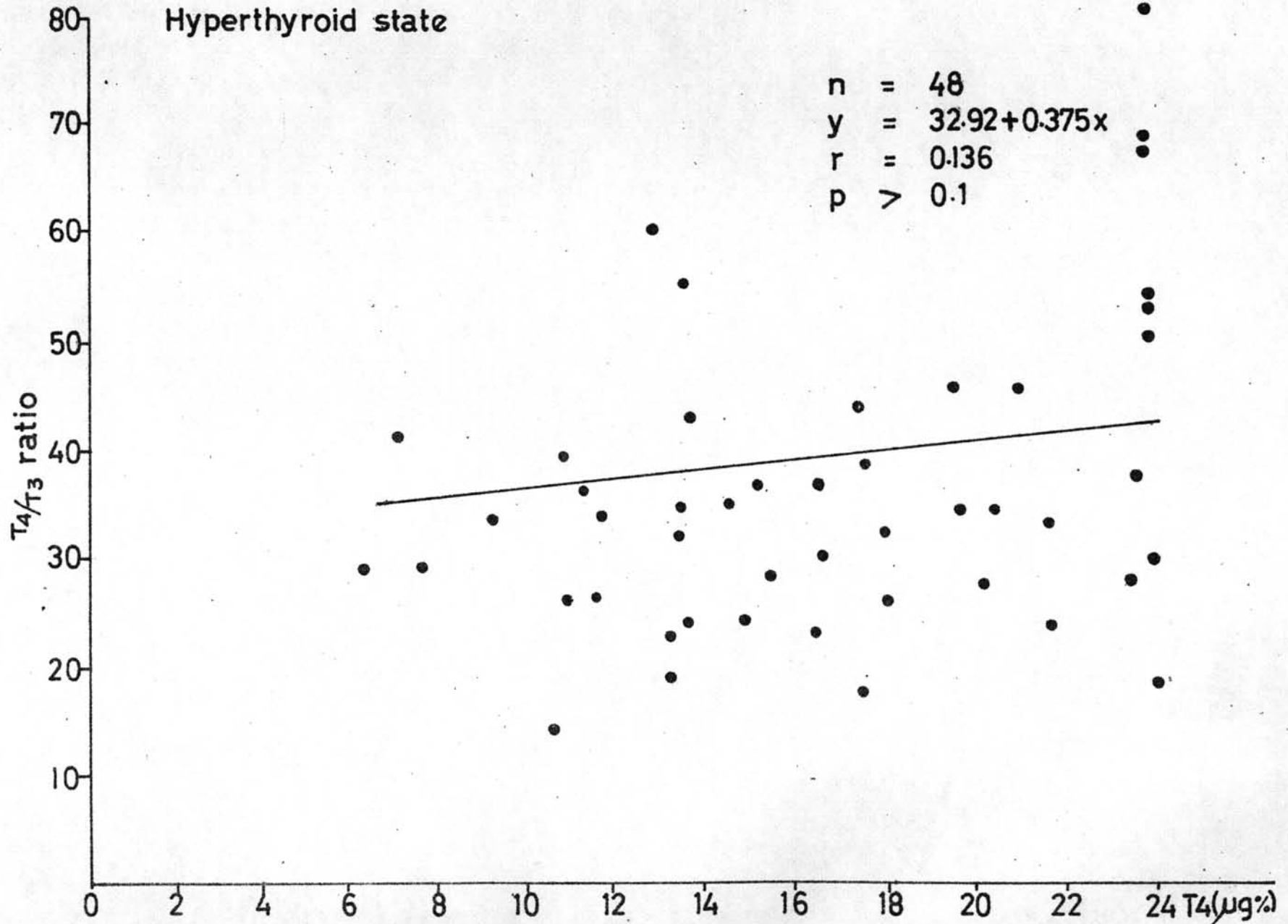
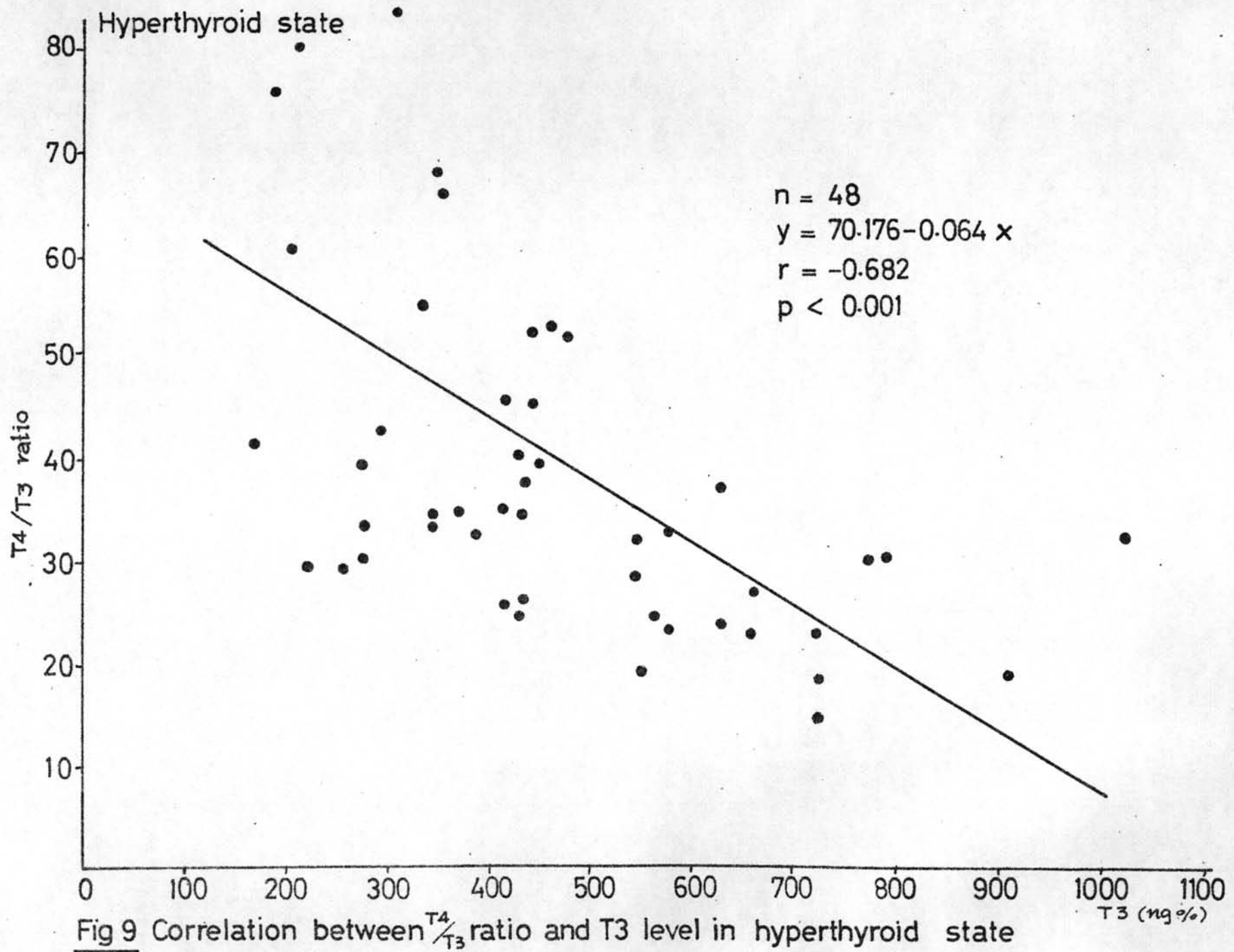


Fig. 8 Correlation between T_4/T_3 ratio and T_4 level in hyperthyroid state



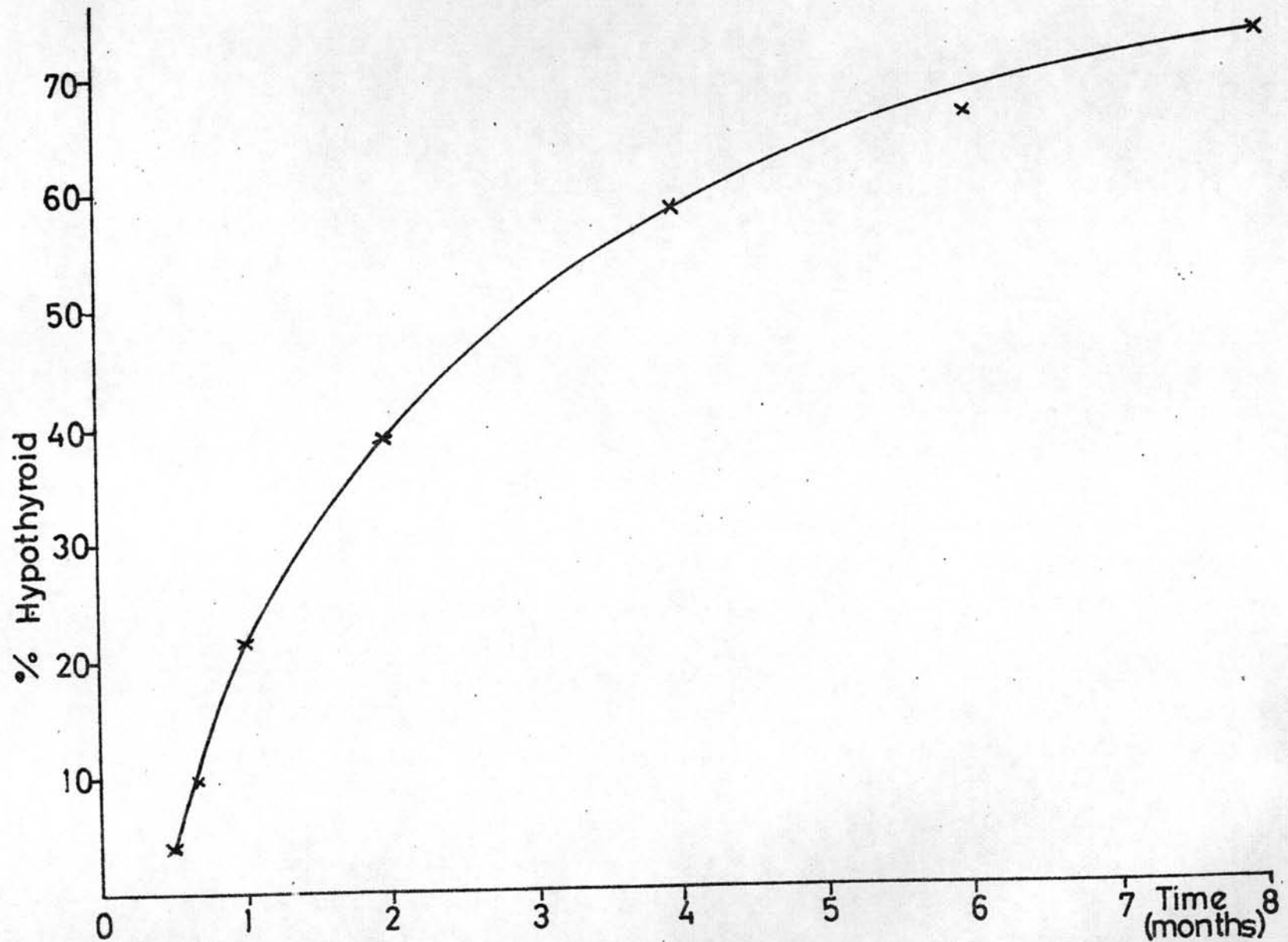


Fig.10 Accumulated percentage of incidence of hypothyroxinemia during the eight month follow up period

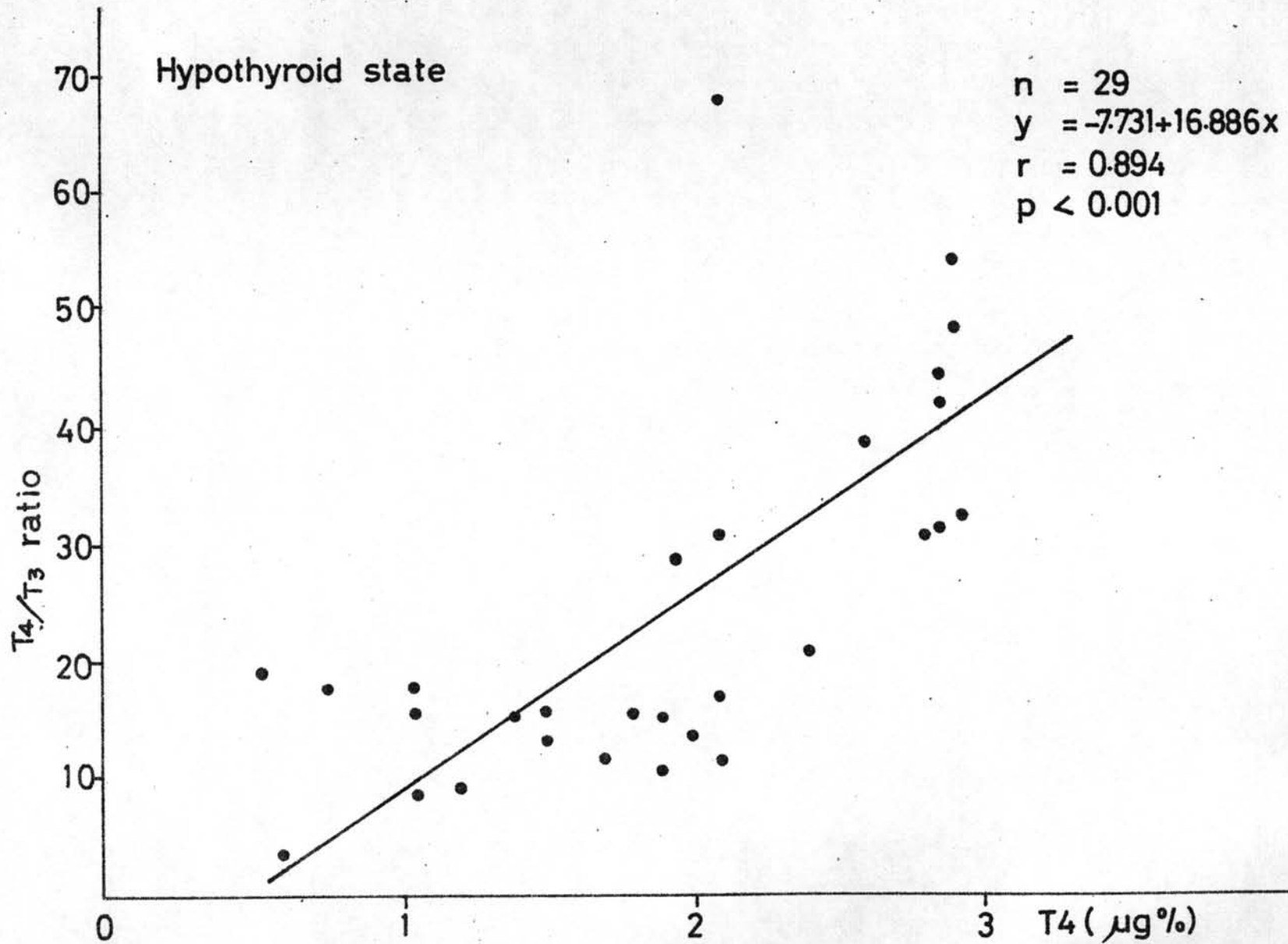


Fig. 11 Correlation between T_4/T_3 ratio and T_4 level in hypothyroid state

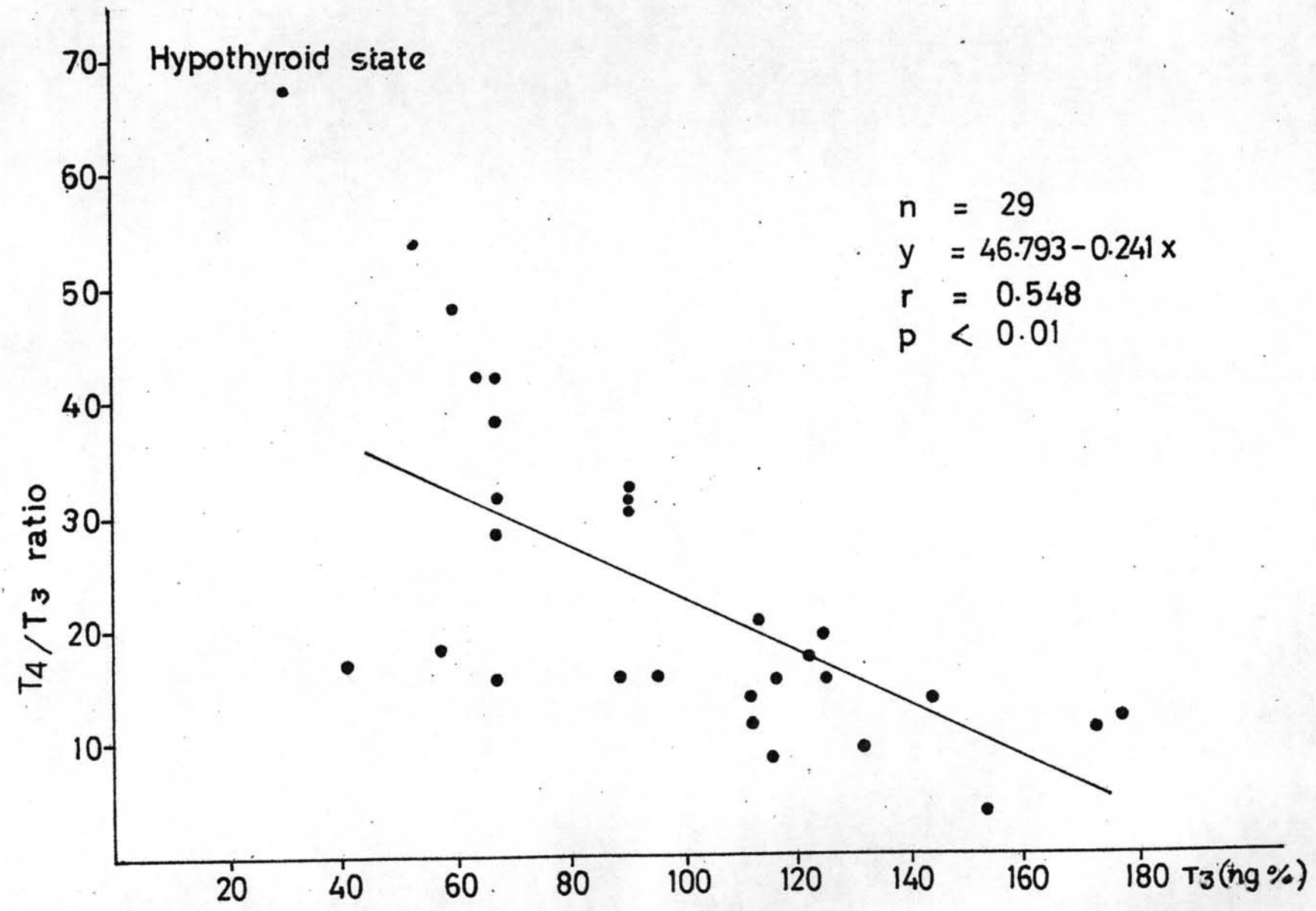


Fig 12 Correlation between T_4/T_3 ratio and T_3 level in hypothyroid state

Fig.13 Serum T₄, Serum T₃, T₄/T₃ ratio and period of the beginning of hypothyroxinemia in 33 patients

second week	third week	fourth week	second month	fourth month	sixth month	eighth month
1 M.J. T ₄ = 1.05 T ₃ = 57.54 T ₄ /T ₃ = 18.24	1 S.B. T ₄ = 290 T ₃ = 53.70 T ₄ /T ₃ = 54.00	1 C.B. T ₄ = 1.50 T ₃ = 95.40 T ₄ /T ₃ = 15.70	1 S.T. T ₄ = 1.95 T ₃ = 67.61 T ₄ /T ₃ = 28.84	1 H.L. T ₄ = 1.90 T ₃ = 173.78 T ₄ /T ₃ = 10.93	1 P.R. T ₄ = 2.10 T ₃ = 30.90 T ₄ /T ₃ = 67.96	1 P.P. T ₄ = 2.10 T ₃ = 177.83 T ₄ /T ₃ = 11.81
2 T.S. T ₄ = 2.85 T ₃ = 67.61 T ₄ /T ₃ = 42.15	2 N.L. T ₄ = 0.60 T ₃ = 154.88 T ₄ /T ₃ = 3.87	2 V.P. T ₄ = 1.40 T ₃ = 89.13 T ₄ /T ₃ = 15.70	2 L.P. T ₄ = 2.10 T ₃ = 67.61 T ₄ /T ₃ = 31.06	2 B.B. T ₄ = 1.20 T ₃ = 131.83 T ₄ /T ₃ = 9.10	2 V.P. T ₄ = 2.85 T ₃ = 64.57 T ₄ /T ₃ = 44.14	2 P.S. T ₄ = 2.85 T ₃ = 91.30 T ₄ /T ₃ = 31.25
	3 P.Y. T ₄ = 2.10 T ₃ = 123.03 T ₄ /T ₃ = 17.07	3 S.P. T ₄ = 1.50 T ₃ = 112.20 T ₄ /T ₃ = 13.36	3 S.P. T ₄ = 2.40 T ₃ = 114.82 T ₄ /T ₃ = 20.90	3 L.M. T ₄ = 0.75 T ₃ = 41.86 T ₄ /T ₃ = 17.91	3 S.P. T ₄ = 2.93 T ₃ = 91.20 T ₄ /T ₃ = 32.13	
		4 N.V. T ₄ = 2.90 T ₃ = 60.26 T ₄ /T ₃ = 48.12	4 P.B. T ₄ = 2.60 T ₃ = 67.61 T ₄ /T ₃ = 38.46	4 V.Y. T ₄ = 1.05 T ₃ = 117.49 T ₄ /T ₃ = 8.93	4 A.S. T ₄ = 2.80	
		5 N.Y. T ₄ = 2.00 T ₃ = 144.54 T ₄ /T ₃ = 13.84	5 N.V. T ₄ = 1.05 T ₃ = 67.61 T ₄ /T ₃ = 15.53	5 Y.S. T ₄ = 2.80 T ₃ = 91.20 T ₄ /T ₃ = 30.70		
		6 S.L. T ₄ = 1.80 T ₃ = 117.49 T ₄ /T ₃ = 15.32	6 V.P. T ₄ = 1.70 T ₃ = 131.83 T ₄ /T ₃ = 12.29	6 K.C. T ₄ = 1.60 T ₃ = 125.89 T ₄ /T ₃ = 15.09		
			7 L.B. T ₄ = 0.53 T ₃ = 125.89 T ₄ /T ₃ = 12.21	7 M.I. T ₄ = 2.70		

T₄ is in $\mu\text{g} \%$

T₃ is in $\text{ng} \%$

8 P.B.

T₄ = 1.80

9 Y.C.

T₄ = 0.00