

RESULTS

1. Determination of the dose-response to standard TSH

The dose-response curve was obtained by plotting the treatment response expressed as the logarithm of the proportional change in count against the logarithm of the dose in international unit (ImU) of TSH (Fig. 3 p.27). The three-hour blood count rates obtained are presented in Table 4,5 and 6 (pp.25-26). It appears very satisfactory that using standard TSH to determine the dose-response of the sensitivity ranges from 0.05 to 0.80 ImU giving the precision index of 0.173 and $p < 0.001$. The results could be compared favorably to any of those using McKenzie's method.

2. Determination of the level of TSH in sera

Once a standard curve is established, one may proceed with the assay of samples of sera of varying thyroid disorders. In this type of bioassay, it is imperative that each set of data be provided with its own individual dose-response curve; the value of serum TSH could then be read from the curve.

We then proceed with sera of:-

Normals in Table 7,8,9 and Fig. 4 (pp.28-30) for the representative of normal, and Table 10a (pp.31-34) which shows the abbreviated data of normal cases 1 to

Hypothyroids in Table 11,12,13 and Fig. 5 (pp.35-37) for the representative case, and Table 14a (pp.38-41) for hypothyroid cases 1 to 7.

Hyperthyroids in Table 15,16,17 and Fig. 6 (pp.42-44) for the representative of hyperthyroid, and Table 18a (pp.45-46) shows the data of cases 1 to 4.

Normal : Table 7-8-9 represent the application of assay design with the range of dose-response of 0.05 to 0.20 ImU from which curve and equation were drawn. The precision index (λ) was 0.188 and the treatment effects were highly significant ($p < 0.001$). The value of normal serum TSH of this case as read from the dose-response curve was 0.104 ImU/ml. This value appears to be within the limits of those reported by the previous workers (see Table 19 p.50). The value for the normal of this series range from 0.056 to 0.328 ImU/ml with the mean of 0.186 ± 0.096 ImU/ml as tabulated in Table 10b (p.34) and Table 3 (p.24).

Hypothyroid : Table 11-12-13 represent the application of assay design with range of dose-response of 0.05 to 0.40 ImU; curves and equations were drawn along the same line as those previously treated in the normal. The values of serum TSH for the hypothyroid cases range from 0.152 to 2.18 ImU/ml with the mean of 0.69 ± 0.678 ImU/ml as seen in Table 14b (p.41), and Table 3.

Hyperthyroid : The assay of TSH in hyperthyroid serum is shown in Table 15-16-17. Table 18a (pp.45-46) shows the range of dose-response of 0.092 to 0.378 ImU/ml with the mean of 0.268 ± 0.125 ImU/ml.

Table 3 - VALUES OF SERUM TSH IN VARIOUS CONDITIONS

Conditions	no. of cases	Mean (ImU/ml)	S.D.	N.B.
Normal	8	0.185	0.096	-
Hypothyroid	8	0.691	0.678	-
Hyperthyroid	5	0.268	0.125	-

On the following pages, are the results tabulated for entities of thyroid condition which are mentioned with the proceedings as stated above.



Table 4- APPLICATION OF ASSAY DESIGN TO THE DETERMINATION OF A STANDARD DOSE-RESPONSE CURVE OVER THE RANGE 0.05 TO 0.80 ImU

Mouse no.	Day 1			Day 2			log difference Day 1 - Day 2
	Treat-ment ImU TSH	3-hr count per 10.min	log 3-hr count rate	Treat-ment ImU TSH	3-hr count per 10 min	log 3-hr count rate	
Group 1							
1	0.05	1,212	3.08350	0.05	1,596	3.20303	-0.11953
2	0.05	1,160	3.06446	0.10	2,378	3.37601	-0.31175
3	0.05	1,019	3.00187	0.20	1,813	3.25840	-0.25023
4	0.05	798	2.90200	0.40	6,151	3.78895	-0.88695
5	0.05	904	2.95617	0.80	5,537	3.74327	-0.78710
Group 2							
1	0.10	667	2.82413	0.05	669	2.82543	-0.00130
2	0.10	1,458	3.16376	0.10	2,384	3.37731	-0.21355
3	0.10	1,304	3.11528	0.20	3,999	3.60195	-0.48667
4	0.10	1,093	3.03862	0.40	5,692	3.75526	-0.71664
5	0.10	1,317	3.11959	0.80	5,475	3.73838	-0.61879
Group 3							
1	0.20	1,805	3.25648	0.05	822	2.91487	0.34161
2	0.20	3,130	3.49554	0.10	3,003	3.47756	0.01798
3	0.20	1,724	3.23654	0.20	1,369	3.13640	0.10014
4	0.20	2,308	3.36324	0.40	4,313	3.63478	-0.27154
5	0.20	1,473	3.16820	0.80	4,947	3.69434	-0.52614
Group 4							
1	0.40	2,941	3.46850	0.05	965	2.89453	0.57397
2	0.40	1,891	3.27669	0.10	939	2.97267	0.30402
3	0.40	1,826	3.26150	0.20	830	2.91908	0.34242
4	0.40	1,735	3.23930	0.40	3,863	3.58692	-0.34762
5	0.40	2,519	3.40123	0.80	3,267	3.51415	-0.11292
Group 5							
1	0.80	10,250	4.01072	0.05	1,874	3.27370	0.73702
2	0.80	7,871	3.89603	0.10	1,395	3.14457	0.75146
3	0.80	12,018	4.07990	0.20	4,099	3.61268	0.46722
4	0.80	6,863	3.83651	0.40	6,919	3.84004	-0.00353
5	0.80	6,407	3.80665	0.80	4,224	3.62572	0.18093

Table 5 - ASSESSMENT OF TREATMENT EFFECTS
FROM THE DATA OBTAINED FROM TABLE 4

Treat- -ment ImU TSH	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	-0.11953	-0.00130	0.34161	0.57397	0.73702	1.53177
0.10	-0.31175	-0.21355	0.01798	0.30402	0.75146	0.54816
0.20	-0.25023	-0.48667	0.10014	0.34242	0.46722	0.17288
0.40	-0.88695	-0.71664	-0.27154	-0.34762	-0.00353	-2.22628
0.80	-0.78710	-0.61879	-0.52614	-0.11292	0.18093	-1.86402
Total	-2.35556	-2.03695	-0.33795	0.75987	2.13310	-1.83749

Table 6 - ANALYSIS OF THE SET OF DATA PRESENTED IN TABLE 5

Analysis of Variance					
Source of variation	d.f.	S.S.	M.S.	Variance ratio (F)	p
Totals	24	5.13331	-	-	-
Replications	4	2.85285	0.71321	58.84570	p < 0.001
Treatments	4	2.08646	0.52161	43.03712	p < 0.001
Error	16	0.19400	0.01212	-	-

Standard deviation (s) = 0.11009, $\lambda = \frac{s}{b} = \frac{0.11009}{0.63553} = 0.17322$

Data from row totals	Estimation after removal of day variation ImU TSH	Estimation from regression ImU TSH
$5t_1 - 10d_1 = -1.53177$	$0.05 = t_1 = -0.37985$	$0.05 = t_1 = -0.38264$
$5t_2 - 10d_1 = -0.54816$	$0.10 = t_2 = -0.18313$	$0.10 = t_2 = -0.19132$
$5t_3 - 10d_1 = -0.17288$	$0.20 = t_3 = -0.10807$	$0.20 = t_3 = -0.00001$
$5t_4 - 10d_1 = 2.22628$	$0.40 = t_4 = 0.37175$	$0.40 = t_4 = 0.19131$
$5t_5 - 10d_1 = 1.86402$	$0.80 = t_5 = 0.29930$	$0.80 = t_5 = 0.38263$

log prop'l
change in count

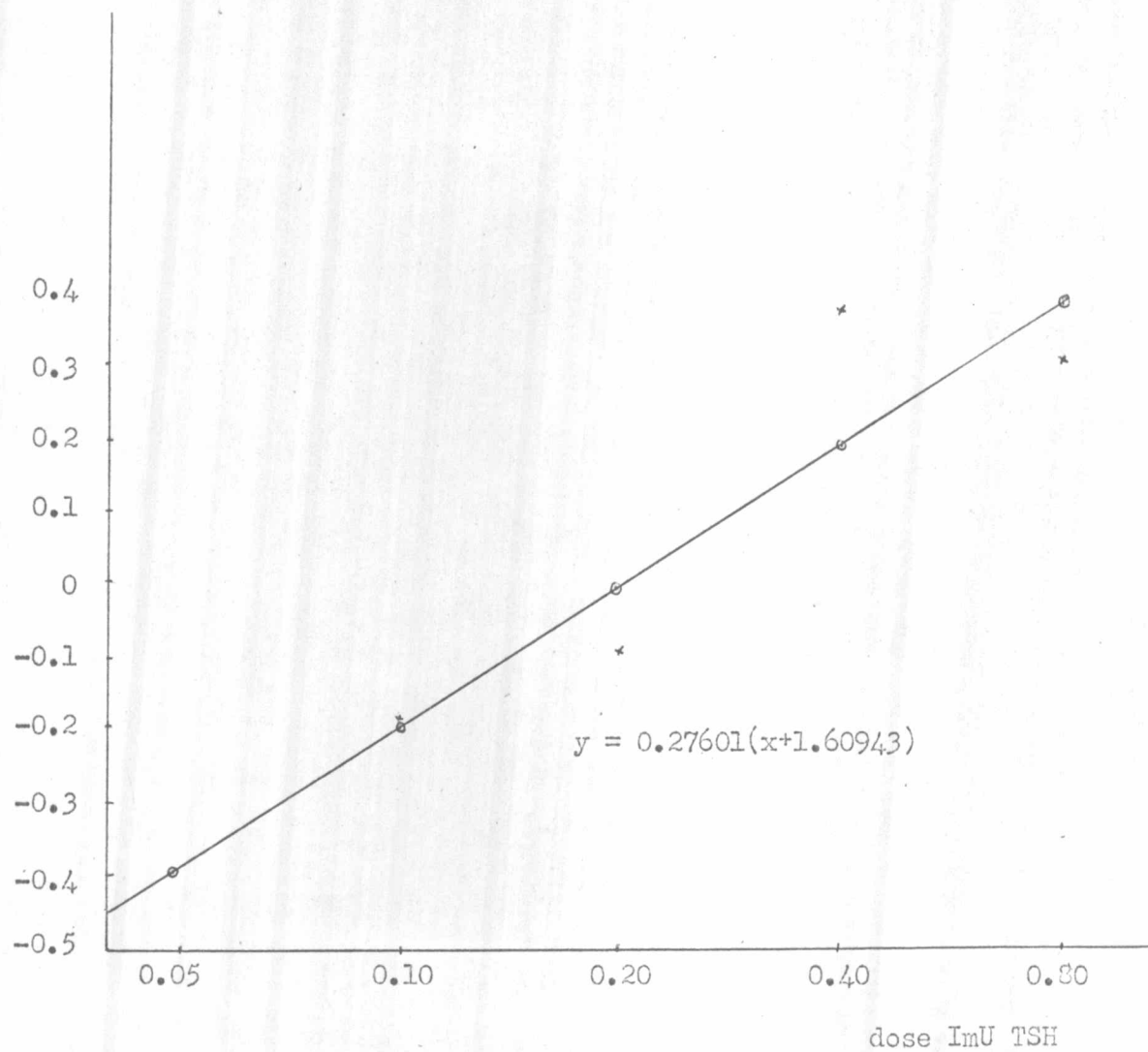


Figure 3 - Dose-response curve over the range 0.05-0.80 ImU standard TSH

Table 7 - APPLICATION OF ASSAY DESIGN TO THE DETERMINATION
OF TSH IN NORMAL SERUM*

Mouse no.	Day 1			Day 2			log Difference Day 1 - Day 2
	Treat- ment ImU TSH	3-hr count per 10 min	log 3-hr count rate	Treat- ment ImU TSH	3-hr count per 10 min	log 3-hr count rate	
Group 1							
1	0.05	511	2.70842	0.05	514	2.71096	-0.00254
2	0.05	739	2.86864	0.10	977	2.98989	-0.12125
3	0.05	1,026	3.01115	0.20	2,090	3.32015	-0.30900
4	0.05	357	2.55267	serum	296	2.47129	0.08138
5	0.05	926	2.96661	serum	784	2.89432	0.07229
Group 2							
1	0.10	1,543	3.18837	0.05	1,238	3.08272	0.10565
2	0.10	840	2.92428	0.10	942	2.97405	-0.04977
3	0.10	1,299	3.11361	0.20	1,381	3.13019	-0.01658
4	0.10	885	2.94694	serum	689	2.83822	0.10872
5	0.10	731	2.86392	serum	510	2.70757	0.15635
Group 3							
1	0.20	312	2.49415	0.05	134	2.12710	0.36705
2	0.20	691	2.83948	0.10	742	2.87040	-0.03092
3	0.20	891	2.94988	0.20	878	2.94349	0.00963
4	0.20	286	2.45637	serum	425	2.62839	0.17202
5	0.20	1,265	3.10209	serum	874	2.94151	0.16058
Group 4							
1	serum	1,547	3.18949	0.05	947	2.97635	0.21314
2	serum	837	2.92273	0.10	1,400	3.14613	-0.22340
3	serum	888	2.94841	0.20	2,420	3.38382	-0.43541
4	serum	427	2.72181	serum	665	2.82282	-0.10101
5	serum	438	2.64147	serum	613	2.78746	-0.14599
Group 5							
1	serum	568	2.75435	0.05	483	2.68395	0.07040
2	serum	489	2.68931	0.10	872	2.94052	-0.25121
3	serum	692	2.84011	0.20	1,062	3.02612	-0.18601
4	serum	835	2.92169	serum	657	2.81757	0.10412
5	serum	592	2.77232	serum	324	2.51055	0.26177

* 0.5 ml of serum is used in each intravenous injection.

Table 8 - ASSESSMENT OF TREATMENT EFFECTS
FROM THE DATA OBTAINED FROM TABLE 7

Treat- ment ImU TSH	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	-0.00254	0.10565	0.36705	0.21314	0.07040	0.75370
0.10	-0.12125	-0.04977	-0.03092	-0.22340	-0.25121	-0.67655
0.20	-0.30900	-0.01658	0.00639	-0.43541	-0.18601	-0.94061
serum*	0.07229	0.15635	0.16058	-0.14599	0.26177	0.50500
serum	0.08138	0.10872	0.17202	-0.10101	0.10412	0.36523
Total	-0.27912	0.30437	0.67512	-0.69267	-0.00093	0.00677

* Serum 0.5 ml done in duplicate.

Table 9 - ANALYSIS OF THE SET OF DATA PRESENTED IN TABLE 8

Analysis of Variance					
Source of variation	d.f.	S.S.	M.S.	Variance ratio (F)	p
Totals	24	0.86167	-	-	-
Replications	4	0.22122	0.05530	4.89813	<0.01
Treatments	4	0.45978	0.11494	10.18069	<0.001
Error	16	0.18067	0.01129	-	-

Standard deviation (s) = 0.10625, $\lambda = \frac{s}{b} = \frac{0.10625}{0.56268} = 0.189$

Data from row totals	Estimation after removal of day variation ImU TSH	Estimation from regression ImU TSH
$5t_1 - 10d_1 = -0.75370$	$0.05 = t_1 = -0.15047$	$0.05 = t_1 = -0.11155$
$5t_2 - 10d_1 = 0.67655$	$0.10 = t_2 = 0.13558$	$0.10 = t_2 = 0.05783$
$5t_3 - 10d_1 = 0.94061$	$0.20 = t_3 = 0.18839$	$0.20 = t_3 = 0.22722$
$5t_4 - 10d_1 = -0.50500$	serum = $t_4 = -0.10073$	
$5t_5 - 10d_1 = 0.36523$	serum = $t_5 = -0.07277$	

log prop'l
change in count

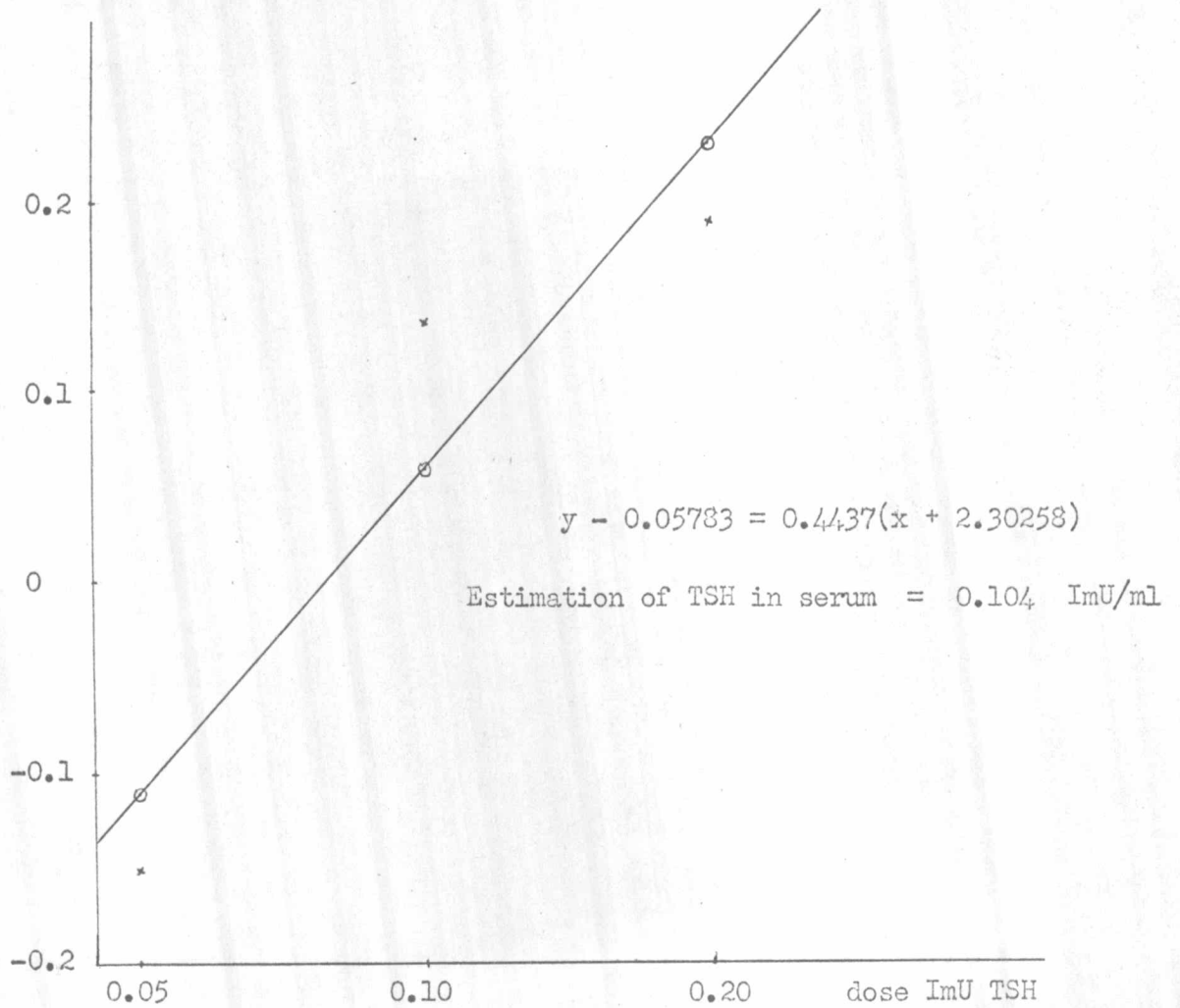


Figure 4 - Dose-response curve over the range 0.05-0.20 ImU standard TSH

Table 10a - ASSAY OF TSH IN NORMAL SERUM

No.1		Differences in log 3-hr count rates					Row Totals
Treat-ment	Group 1	Group 2	Group 3	Group 4	Group 5		
0.05	0.58940	0.56793	0.41777	0.25663	0.58802	2.41975	
0.10	-0.00236	0.37553	0.28587	0.06031	0.35406	1.07341	
0.20	-0.31089	0.24714	0.41281	-0.07026	0.37091	0.64971	
serum	0.27267	0.31876	0.52639	0.07045	0.03425	1.22252	
serum	0.15240	0.29186	0.43019	0.13843	0.25075	1.26363	
Total	0.70122	1.80122	2.07303	0.45556	1.59799	6.62902	

$$b = 0.58805 \quad s = 0.15962$$

$$\lambda = 0.27143$$

Estimation of TSH in serum = 0.334 ImU/ml, 0.322 ImU/ml

average = 0.328 ImU/ml

No.2		Differences in log 3-hr count rates					Row Totals
Treat-ment	Group 1	Group 2	Group 3	Group 4	Group 5		
0.05	0.15461	0.03974	0.49140	0.00491	0.45829	1.14895	
0.10	-0.04647	0.25675	-0.19588	0.25876	0.14437	0.41753	
0.20	-0.09306	-0.15125	-0.85236	0.20256	0.22032	-0.67379	
serum	0.11035	0.06600	-0.17930	0.08145	0.14284	0.22134	
serum	-0.38257	0.11515	0.34512	-0.08463	0.27855	0.27162	
Total	-0.25714	0.32639	-0.39102	0.46305	1.24437	1.38565	

$$b = 0.60550 \quad s = 0.27417$$

$$\lambda = 0.45279$$

Estimation of TSH in serum = 0.212 ImU/ml, 0.204 ImU/ml

average = 0.208 ImU/ml

No.3

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	-0.08582	0.11859	0.56934	0.18036	-0.53348	0.24899
0.10	0.03135	-0.23676	0.04026	-0.23869	-0.17010	-0.57394
0.20	-0.17771	0.22001	0.09251	-0.24686	-0.85161	-0.96366
serum	-0.07132	0.10848	0.16346	-0.47688	0.02431	-0.25195
serum	-0.02571	-0.06937	0.15189	-0.12217	-0.11993	-0.18529
Total	-0.32921	0.14095	1.01746	-0.90424	-1.65081	-1.72585

$$b = 0.40274 \quad s = 0.22766$$

$$\lambda = 0.56527$$

Estimation of TSH in serum = 0.082 ImU/ml, 0.076 ImU/ml

average = 0.079 ImU/ml



No.4

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.18258	0.11764	0.56307	0.27038	-0.13591	0.99776
0.10	-0.38633	0.02180	-0.07027	0.19087	0.00953	-0.23440
0.20	0.01702	-0.05392	0.52414	0.63086	0.03392	1.15202
0.40	-0.75637	0.50298	-0.49048	0.02592	-0.95237	-1.67068
serum	0.09661	-0.97275	0.24438	0.22160	0.01449	-0.39567
Total	-0.84649	-0.38425	0.77084	1.33963	-1.03034	-0.15061

$$b = 0.57458 \quad s = 0.39011$$

$$\lambda = 0.67894$$

Estimation of TSH in serum = 0.262 ImU/ml

| No.5 |

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.14907	-0.05428	-0.29676	0.29987	0.37955	0.47785
0.10	-0.24340	-0.01939	-0.10215	-0.00335	-0.07618	-0.44447
0.20	0.31954	-0.18595	-0.05278	0.17315	-0.30720	-0.05324
0.40	-0.52168	-0.02877	-0.35568	-0.11089	-0.26168	-1.27870
serum	0.62731	-0.11275	0.19503	0.00437	0.28889	1.00285
Total	0.33084	-0.40114	-0.61234	0.36315	0.02338	-0.29611

$$b = 0.32406 \quad s = 0.24093$$

$$\lambda = 0.74347$$

Estimation of TSH in serum = 0.056 ImU/ml

| No.6 |

Treat -ment	Differences in log 3-hr count rates				Row Totals
	Group 1	Group 2	Group 3	Group 4	
0.05	-0.13424	0.19575	0.59377	0.19813	0.85341
0.10	-0.14455	0.04598	0.30955	0.29515	0.50613
0.20	-0.00727	0.08514	-0.40993	-0.59235	-0.92441
serum	-0.40056	-0.25537	0.47462	0.13779	-0.04352
Total	-0.68662	0.07150	0.96801	0.03872	0.39161

$$b = 0.73813 \quad s = 0.30654$$

$$\lambda = 0.41529$$

Estimation of TSH in serum = 0.232 ImU/ml

No.7		Differences in log 3-hr count rates				Row Totals
Treat-ment	Group 1	Group 2	Group 3	Group 4		
0.05	0.52125	0.19960	-0.05208	0.23224	0.90101	
0.10	-0.09880	0.05596	-0.10094	0.21155	0.06777	
0.20	-0.20779	0.06765	-0.21038	0.11838	-0.23214	
serum	-0.05115	0.24816	-0.08174	0.06680	0.18207	
Total	0.16351	0.57137	-0.44514	0.62897	0.91871	

$$b = 0.47046 \quad s = 0.15962$$

$$\lambda = 0.32270$$

Estimation of TSH in serum = 0.216 ImU/ml

Table 10b - SUMMARY OF THE RESULTS OF NORMAL SERUM TSH

Eight normal cases showing serum TSH of:-

0.104 0.328 0.208 0.079

0.262 0.056 0.032 0.216

average = 0.185 ImU/ml

S.D. = 0.096 ImU/ml

Table 11 - APPLICATION OF ASSAY DESIGN TO THE DETERMINATION
OF TSH IN HYPOTHYROID SERUM

Mouse no.	Day 1			Day 2			log difference Day 1-Day 2
	Treat- ment ImU TSH	3-hr count per 10 min.	log 3-hr count rate	Treat- ment ImU TSH	3-hr count per 10 min.	log 3-hr count rate	
Group 1							
1	0.05	4,186	3.62180	0.05	2,225	3.34733	0.27447
2	0.05	1,552	3.19089	0.10	2,250	3.35218	-0.16129
3	0.05	3,358	3.52608	0.20	5,368	3.72981	-0.20373
4	0.05	2,161	3.33465	0.40	1,233	3.09096	0.24369
5	0.05	1,668	3.22220	serum	476	2.67761	0.54459
Group 2							
1	0.10	1,350	3.13033	0.05	1,130	3.05308	0.07725
2	0.10	1,909	3.28081	0.10	1,289	3.11025	0.17056
3	0.10	1,178	3.07115	0.20	6,061	3.78254	-0.71139
4	0.10	2,634	3.42062	0.40	2,119	3.32613	0.09449
5	0.10	901	2.95472	serum	383	2.58320	0.37152
Group 3							
1	0.20	3,417	3.53364	0.05	1,317	3.11966	0.41398
2	0.20	1,084	3.03503	0.10	248	2.39445	0.64058
3	0.20	1,248	3.09621	0.20	1,742	3.24105	-0.14484
4	0.20	4,856	3.68628	0.40	2,759	3.44075	0.24553
5	0.20	1,233	3.09096	serum	1,484	3.17143	-0.08047
Group 4							
1	0.40	2,218	3.34596	0.05	206	2.31387	1.03209
2	0.40	3,584	3.55437	0.10	589	2.77012	0.78425
3	0.40	2,579	3.41145	0.20	1,423	3.15320	0.25825
4	0.40	1,823	3.26079	0.40	1,228	3.08920	0.17159
5	0.40	6,487	3.81204	serum	967	2.98543	0.82661
Group 5							
1	serum	4,443	3.64777	0.05	1,877	3.27346	0.37431
2	serum	4,746	3.67633	0.10	1,758	3.24502	0.43131
3	serum	2,835	3.45255	0.20	3,951	3.59671	-0.14416
4	serum	2,449	3.38899	0.40	1,699	3.23003	0.15896
5	serum	8,170	3.91222	serum	4,960	3.69548	0.21674

Table 12 - ASSESSMENT OF TREATMENT EFFECTS
FROM THE DATA OBTAINED FROM TABLE 11

Treat- ment ImU TSH	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.27447	0.07725	0.41398	1.03209	0.37431	2.17210
0.10	-0.16129	0.17056	0.64058	0.78425	0.43131	1.86541
0.20	-0.20373	-0.71139	-0.14484	0.25825	-0.14416	-0.94587
0.40	0.24369	0.09449	0.24553	0.17159	0.15896	0.91426
serum	0.54459	0.37152	-0.08047	0.82661	0.21674	1.87899
Total	0.69773	0.00243	1.07478	3.07279	1.03716	5.88489

Table 13 - ANALYSIS OF THE SET OF DATA PRESENTED IN TABLE 12

Analysis of Variance					
Source of variation	d.f.	S.S.	M.S.	Variance ratio (F)	p
Totals	24	3.34979	-	-	-
Replications	4	1.04667	0.26166	4.20134	<0.025
Treatments	4	1.30651	0.32662	5.24438	<0.01
Error	16	0.99661	0.06228	-	-

$$\text{Standard deviation (s)} = 0.24955, \lambda = \frac{s}{b} = \frac{0.24955}{1.03572} = 0.24094$$

Data from row totals	Estimate after removal of day variation ImU TSH	Estimate from regression ImU TSH
$5t_1 - 10d_1 = -2.17210$	$0.05 = t_1 = -0.19902$	$0.05 = t_1 = -0.28249$
$5t_2 - 10d_1 = -1.86541$	$0.10 = t_2 = -0.13768$	$0.10 = t_2 = 0.02929$
$5t_3 - 10d_1 = 0.94587$	$0.20 = t_3 = 0.42457$	$0.20 = t_3 = 0.34107$
$5t_4 - 10d_1 = -0.91426$	$0.40 = t_4 = 0.05254$	$0.40 = t_4 = 0.65286$
$5t_5 - 10d_1 = -1.87899$	$\text{serum} = t_5 = -0.14040$	

log prop'l
change in count

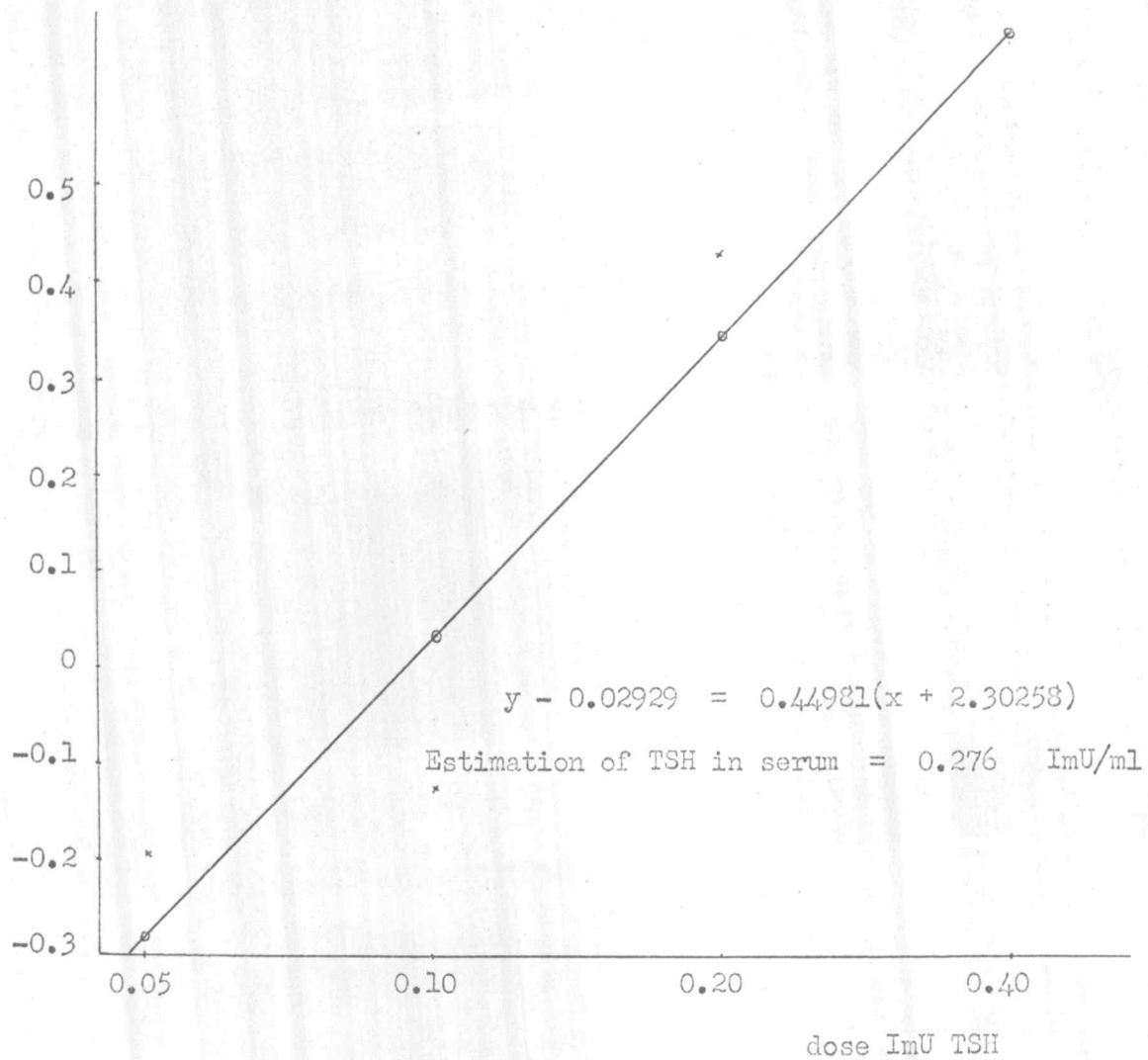


Figure 5 - Dose-response curve over the range 0.05-0.40 ImU standard TSH

Table 14a - ASSAY OF TSH IN HYPOTHYROID SERUM

No.1						
Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.13408	0.13767	0.49103	0.29237	0.12068	1.17583
0.10	0.06249	0.27238	0.33478	0.43790	-0.03547	1.07208
0.20	-0.03641	0.23516	0.26909	0.63436	0.06989	1.17209
0.40	-0.03474	-0.48233	-0.27775	0.04908	-0.63706	-1.38280
serum*	-0.49614	0.16157	0.48397	0.32107	0.03343	0.50390
Total	-0.37072	0.32445	1.30112	1.73478	-0.44853	2.54110

* serum 0.25 ml + normal saline solution 0.25 ml

$$b = 0.50332 \quad s = 0.20032$$

$$\lambda = 0.39799$$

Estimation of TSH in serum = 0.568 ImU/ml

No.2						
Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.40455	0.15272	0.06338	0.52809	0.82595	1.97469
0.10	0.44446	0.15704	0.25102	0.36993	0.66749	1.88994
0.20	0.35910	0.07055	-0.00994	0.23664	0.61553	1.27188
0.40	0.10775	-0.07996	0.07655	0.20254	-0.07245	0.23443
serum*	0.24274	-0.08496	0.37708	-0.08111	0.42679	0.88054
Total	1.55860	0.21539	0.75809	1.25609	2.46331	6.25148

* serum 0.125 ml + normal saline solution 0.375 ml

$$b = 0.38798 \quad s = 0.17603$$

$$\lambda = 0.45370$$

Estimation of TSH in serum = 1.47 ImU/ml

No.3

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.38780	0.51068	-0.17246	0.47657	0.11354	1.31613
0.10	0.54373	0.11083	0.00165	0.06145	0.30060	1.01826
0.20	0.31574	0.05670	0.15733	0.25423	0.41466	1.19866
0.40	0.04287	0.39209	0.20178	0.20035	0.44107	1.27816
serum	-0.15804	0.01751	-0.20003	0.59301	0.66027	0.91272
Total	1.13210	1.08781	-0.01173	1.58561	1.93014	5.72393

$$b = 0.19820 \quad s = 0.24572$$

$$\lambda = 1.23975$$

Estimation of TSH in serum = 0.256 ImU/ml

No.4

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.39660	-0.15873	0.01532	-0.26243	0.10803	0.09879
0.10	-0.78608	0.14680	0.13404	0.19205	0.06702	-0.24617
0.20	-0.12617	0.37452	0.01871	0.22670	0.43090	0.92466
0.40	-0.10730	0.29557	0.09180	-0.01702	0.34867	0.61172
serum	-0.05797	-0.16010	-0.09103	-0.02822	0.01217	-0.32515
Total	-0.68092	0.49806	0.16884	0.11108	0.96679	1.06385

$$b = 0.22887 \quad s = 0.25612$$

$$\lambda = 1.11906$$

Estimation of TSH in serum = 0.468 ImU/ml

No.5		Differences in log 3-hr count rates					Row Totals
Treat	-ment	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05		0.27028	0.04883	0.33920	0.11287	0.21925	0.99043
0.10		0.21256	0.31910	0.25768	0.28670	-0.09681	0.97923
0.20		0.24861	0.44918	0.13425	0.24859	0.03243	1.11306
0.40		0.25623	0.48333	-0.09918	0.03787	-0.47955	0.19870
serum		0.08283	-0.01360	0.01936	-0.00502	0.04365	0.12722
Total		1.07051	1.28684	0.65131	0.68101	-0.28103	3.40864

$$b = 0.18733 \quad s = 0.17930$$

$$\lambda = 0.95713$$

Estimation of TSH in serum = 2.18 InU/ml

No.6		Differences in log 3-hr count rates					Row Totals
Treat	-ment	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05		-0.04315	0.09731	0.42436	0.38849	0.24281	1.10982
0.10		0.28342	0.34962	-0.52936	0.20125	0.19527	0.50020
0.20		-0.24303	-0.27022	-0.24554	0.12942	0.05464	-0.57473
0.40		-0.51704	-0.22559	-0.12166	0.00849	-0.09082	-0.94662
serum*		-0.39121	-0.18578	-0.22944	0.08548	-0.07669	-0.79764
Total		-0.91101	-0.23466	-0.70164	0.81313	0.32521	-0.70897

* serum 0.25 ml + normal saline solution 0.25 ml

$$b = 0.14830 \quad s = 0.20791$$

$$\lambda = 1.40195$$

Estimation of TSH in serum = 1.176 InU/ml

No.7

Treatment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	1.71147	0.85223	0.20180	0.03553	0.49128	3.29231
0.10	-0.18483	0.16642	-0.26699	0.35608	0.14543	0.21611
0.20	-0.24580	0.09209	0.59260	-0.11925	-0.67343	-0.35379
0.40	0.03527	0.08516	-0.36560	0.70605	-0.01389	0.44699
serum	-0.00254	0.30478	0.77295	0.48677	0.23179	1.79375
Total	1.31357	1.50068	0.93476	1.46518	0.18118	5.39537

$$b = 1.21106 \quad s = 0.47446$$

$$\lambda = 0.39177$$

$$\text{Estimation of TSH in serum} = 0.152 \text{ ImU/ml}$$



Table 14b - SUMMARY OF THE RESULTS OF TSH IN HYPOTHYROID SERUM

Eight hypothyroid cases showing serum TSH of:-

0.568 1.470 0.256 0.468

2.180 1.176 0.276 0.152

average = 0.691 ImU/ml

S.D. = 0.678 ImU/ml

Table 15 - APPLICATION OF ASSAY DESIGN TO THE DETERMINATION
OF TSH IN HYPERTHYROID SERUM

Mouse no.	Day 1			Day 2			log difference Day 1-Day 2
	Treat- ment ImU TSH	3-hr count per 10 min.	log 3-hr count rate	Treat- ment ImU TSH	3-hr count per 10 min.	log 3-hr count rate	
Group 1							
1	0.05	1,623	3.21032	0.05	857	2.93298	0.27734
2	0.05	1,103	3.04258	0.10	2,959	3.47114	-0.42856
3	0.05	625	2.79588	0.20	2,838	3.45301	-0.65713
4	0.05	652	2.81425	0.40	1,341	3.12743	-0.31318
5	0.05	523	2.71850	serum	1,004	3.00173	-0.28323
Group 2							
1	0.10	1,159	3.06408	0.05	798	2.90200	0.16208
2	0.10	666	2.82347	0.10	794	2.89982	-0.07635
3	0.10	716	2.85491	0.20	1,375	3.13830	-0.28339
4	0.10	1,098	3.04060	0.40	1,936	3.28691	-0.24631
5	0.10	1,521	3.18213	serum	1,944	3.28870	-0.10657
Group 3							
1	0.20	1,404	3.14737	0.05	736	2.86688	0.28049
2	0.20	1,029	3.01242	0.10	717	2.85552	0.15690
3	0.20	1,336	3.12581	0.20	994	2.99739	0.12842
4	0.20	887	2.94792	0.40	704	2.84757	0.10035
5	0.20	1,336	3.12581	serum	797	2.90146	0.22435
Group 4							
1	0.40	442	2.64542	0.05	274	2.43775	0.20767
2	0.40	3,427	3.53491	0.10	1,643	3.21564	0.31927
3	0.40	2,026	3.30664	0.20	1,138	3.05614	0.25050
4	0.40	2,615	3.41747	0.40	2,297	3.36116	0.05631
5	0.40	6,833	3.83461	serum	1,804	3.25624	0.57837
Group 5							
1	serum	358	2.55388	0.05	256	2.40824	0.14564
2	serum	997	2.99870	0.10	961	2.98272	0.01598
3	serum	211	2.32428	0.20	518	2.71433	-0.39005
4	serum	445	2.64836	0.40	1,008	3.00346	-0.35510
5	serum	322	2.50786	serum	580	2.76343	-0.25557

Table 16 - ASSESSMENT OF TREATMENT EFFECTS
FROM THE DATA OBTAINED FROM TABLE 15

Treatment ImU TSH	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.27734	0.16208	0.28049	0.20767	0.14564	1.07322
0.10	-0.42856	-0.07635	0.15690	0.31927	0.01598	-0.01276
0.20	-0.65713	-0.28339	0.12842	0.25050	-0.39005	-0.95165
0.40	-0.31318	-0.24631	0.10035	0.05631	-0.35510	-0.75793
serum	-0.28323	-0.10657	0.22435	0.57837	-0.25557	0.15735
Total	-1.40476	-0.55054	0.89051	1.41212	-0.83910	-0.49177

Table 17 - ANALYSIS OF THE SET OF DATA PRESENTED IN TABLE 16

Analysis of Variance					
Source of variation	d.f.	S.S.	M.S.	Variance ratio (F)	p
Totals	24	2.13603	-	-	-
Replications	4	1.14385	0.28596	9.72653	< 0.001
Treatments	4	0.52168	0.13042	4.43605	< 0.025
Error	16	0.47050	0.02940	-	-

Standard deviation (s) = 0.17146, $\lambda = \frac{s}{b} = \frac{0.17146}{0.67267} = 0.25489$

Data from row totals	Estimate after removal of day variation ImU TSH	Estimate from regression ImU TSH
$5t_1 - 10d_1 = -1.07322$	$0.05 = t_1 = -0.23431$	$0.05 = t_1 = -0.22941$
$5t_2 - 10d_1 = 0.01276$	$0.10 = t_2 = -0.01711$	$0.10 = t_2 = -0.02692$
$5t_3 - 10d_1 = 0.95165$	$0.20 = t_3 = 0.17066$	$0.20 = t_3 = 0.17557$
$5t_4 - 10d_1 = 0.75793$	$0.40 = t_4 = 0.13191$	$0.40 = t_4 = 0.37807$
$5t_5 - 10d_1 = -0.15735$	serum = $t_5 = -0.05114$	

log prop'l
change in count

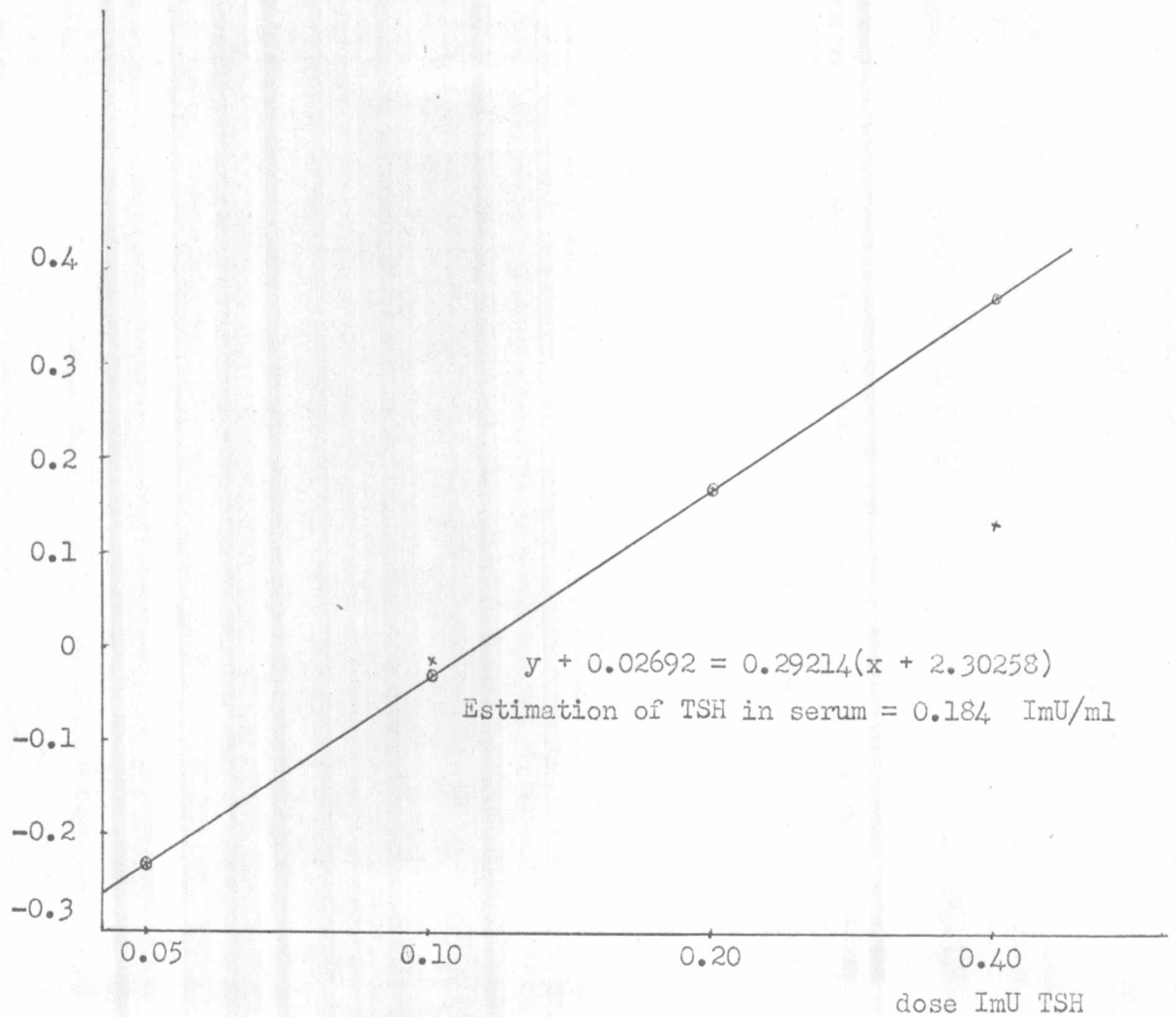


Figure 6 - Dose-response curve over the range 0.05-0.40 ImU standard TSH

Table 18a - ASSAY OF TSH IN HYPERTHYROID SERUM

No.1						
Treat-ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.47571	0.69438	0.26584	0.32745	0.50231	2.26569
0.10	0.34052	0.39026	0.34280	0.77001	0.17576	2.01935
0.20	0.47341	0.01680	0.22465	0.15179	-0.18735	0.67930
0.40	0.03469	0.30301	0.27797	0.00682	-0.23485	0.38764
serum	0.41342	0.22456	0.31423	0.35247	-0.22218	1.08250
Total	1.73775	1.62901	1.42549	1.60854	0.03369	6.43448

$$b = 0.46332 \quad s = 0.19310$$

$$\lambda = 0.41677$$

$$\text{Estimation of TSH in serum} = 0.364 \text{ ImU/ml}$$

No.2						
Treat-ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	-0.04613	-0.17362	-0.07485	0.30878	-0.18814	-0.17396
0.10	-0.15631	-0.10062	0.07188	0.21457	-0.15373	-0.12421
0.20	-0.31602	-0.09680	-0.05838	0.20284	-0.17756	-0.44592
0.40	-0.27957	-0.19845	-0.14656	0.05590	-0.20150	-0.77018
serum	-0.29794	-0.14326	-0.04116	0.02495	0.11159	-0.34582
Total	-1.09597	-0.71275	-0.24907	0.80704	-0.60934	-1.86009

$$b = 0.21457 \quad s = 0.09939$$

$$\lambda = 0.463$$

$$\text{Estimation of TSH in serum} = 0.322 \text{ ImU/ml}$$

No.3

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.27734	0.16208	0.28049	0.20767	-0.40516	0.52242
0.10	-0.42856	-0.07635	0.15690	0.31927	-0.15469	-0.18343
0.20	-0.65713	-0.28339	0.12842	0.25050	-0.54174	-1.10334
0.40	-0.31318	-0.24631	0.10035	0.05631	-0.13116	-0.53399
serum	0.15989	0.25671	0.35086	0.11974	0.58909	1.47629
Total	-0.96164	-0.18726	1.01702	0.95349	-0.64366	0.17795

$$b = 0.54002 \quad s = 0.23964$$

$$\lambda = 0.44376$$

$$\text{Estimation of TSH in serum} = 0.092 \text{ ImU/ml}$$

No.4

Treat -ment	Differences in log 3-hr count rates					Row Totals
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	0.03838	-0.03870	-0.15398	0.16557	0.44225	0.45352
0.10	-0.05081	-0.10447	-0.34504	0.39794	-0.24712	-0.34950
0.20	-0.41710	-0.24551	1.47716	0.02870	-0.06075	0.78250
0.40	-0.27939	-0.63129	-1.58173	0.06131	-0.05607	-2.48717
serum	-0.38086	-0.33632	-0.14506	-0.26717	-0.09168	-1.22109
Total	-1.08978	-1.35629	-0.74865	0.38635	-0.01337	-2.82174

$$b = 0.65957 \quad s = 0.51321$$

$$\lambda = 0.77809$$

$$\text{Estimation of TSH in serum} = 0.378 \text{ ImU/ml}$$

Table 18b - SUMMARY OF THE RESULTS OF TSH IN HYPERTHYROID SERUM

Five hyperthyroid cases showing serum TSH of:-

case	ImU/ml
1	0.184
2	0.364
3	0.322
4	0.092
5	0.378
average	= 0.268 ImU/ml
S.D.	= 0.125 ImU/ml

