METHODS

1. General procedure:

Male or female albino mice approximately 9 weeks of age and at a weight of 20-25 g were fed on commercial dog biscuit of low iodine content for at least 1 week before use. Multivitamins were added to drinking water (deionized water) to keep the mice healthy. Radioactive iodine ($Na^{125}I$) 5-10 uCi/mouse, was injected intraperitoneally. This quantity of radioactive iodine was found to produce maximal specific activity in the thyroid gland without causing radiation damage within the period of the assay.(32) It was assumed that radiation damage was assiciated with an increased rate of release from the animal 's thyroids, i.e. an increase in steepness of the release slope. Thus, it was determined that more than 2 uCi ^{125}I in the thyroid gland of the mouse might be assiciated with radiation damage. Since after a week on the low iodine diet, ^{125}I uptake average 20%, routinely 5-10 uCi ^{125}I was injected, thus achieving an average thyroid gland content of 1-2 uCi.

Endogenous secretion of thyrotropin was supressed by the subcutaneous injection of 10 ug L-thyroxine immediately after the radioiodide injection, and by the addition of L-triiodothyronine 0.5 ug/ml to the drinking water for the period of the assay.

The mice were used in the assay procedure 4 days later. They were numbered and distributed by random selection into 5 groups of 5 mice. These

groups were allotted the chosen treatments at random.

The standard solutions of TSH were freshly prepared immediately before use each day. International standards thyrotropin were dissolved in normal saline solution. The doses of standard TSH and test sample (0.5 ml) were injected into the tail vein dilated by heating the mice at 30°C for a few minutes.

Blood was obtained from the mice by cutting the tail vein with surgery blade number 24, and drawn into heparinized capillary tube. Sample of whole blood (0.1 ml) was hemolysed by transferring to 1 ml of distilled water. The radioactivity of the sample was measured by using an Automatic Gamma Well Counter System (Nuclear Chicago Model 4230 and 4233).

2. Modified assay design:

The design of the assay and the statistical analysis employed permits elimination of the factors of animal, day variation and the residual effect of the initial test doses, so that a pure estimate of the response to treat--ment is obtained. The assay design is expressed in two ways.

a) Determination of the dose response to standard TSH. The design used the mice on two successive days. On the first day, 25 mice were distributed at random into 5 groups which were allotted treatment of 0.05, 0.10, 0.20, 0.40, 0.80 ImU TSH. Blood samples were obtained at 0, 3, 7 and 24 hours. On the second day, the mice from each group were allotted the 5 treatments at random and the blood samples were again obtained at 0, 3, 7, and 24 hours.

b) Determination of the level of TSH in the serum of the patients. The same assay procedure followed that mentioned in a). Five groups of mice were treated with 0.05, 0.10, 0.20, 0.40 ImU TSH (in 0.5 ml normal saline solution) and 0.5 ml of serum sample (of normal, hypothyroid or hyperthyroid sera).

3. Calculation and statistical treatment

The response from this assay of TSH over the range of 0.05 to 0.80 ImU were expressed as the difference (Day 1 - Day 2) in the logarithmics trans--formation of the three-hour blood count rate. The individual values from the same treatment were then added altogether. This was called the row total. Finally, the log proportional change in count was calculated and plotted against various doses of standard TSH on semilogarithmic paper.

Theoretical considerations:[3]

Let m be the mean response

t_i be the deviation from the mean due to the i^{th} treatment

$i = 1, 2, \ldots\ldots\ldots, n \qquad t_i = 0$

a_j be the deviation from the mean due to the j^{th} animal

$j = 1, 2, \ldots\ldots\ldots, n \qquad a_j = 0$

d_k be the deviation from the mean due to the k^{th} day

$k = 1,2$ $\qquad d_k = 0$

$\sum_{i=1}^{n} r_i$ be the deviation from the mean due to the residual effect of the i^{th} treatment

$r_i = 0$ and this applied to Day 2 only

and y_{ijl} be the response to treatment i applied to animal j on Day 1.

Then $y_{ijl} = m + t_i + a_j + d_1 + e_{ijl}$

and for the second day with t_p applied to a_j

$$y_{pj2} = m + t_p + a_j + r_i + d_2 + e_{pj2}$$

where the errors e_{ijk} are normally an independently distributed $(0, \sigma^2)$, and the difference is

$$D_{ip} = y_{ijl} - y_{pj2} = t_i - t_p - r_i + d_1 - d_2 + e_{ip}$$

Summing over the n animals which had t_i on Day 1

$$\sum_{p=1}^{n} D_{ip} = n(t_i - r_i) + n(d_1 - d_2) + \sum e,$$

summing over the n animals which had t_p on Day 2

$$\sum_{p=1}^{n} D_{ip} = -nt_p + n(d_1 - d_2) + \sum e$$

$$= -nt_p + 2nd_1 + \sum e, \text{ since } d_1 = -d_2$$

whence may be obtained unbiased estimates of the treatment effects free of residual effects (see Table 1).

Table 1-MODEL OF EXPERIMENTAL DESIGN

The response of a mouse on Day 1 to treatment (i) is compounded of the following factors:

$$m + a_j + t_i + d_1 + e_{ij1}$$

The response of the same mouse on Day 2 to treatment (p) is compounded of the following factors:

$$m + a_j + t_p + r_i + d_2 + e_{pj2}$$

Thus, if the difference of a mouse's response on Day 1 and Day 2 is denoted by D_{ip}, then:

$$D_{ip} = t_i - t_p - r_i + 2d_1 + e_{ip}$$

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	D_{11}	D_{21}	D_{31}	D_{41}	D_{51}	$-5t_1 + 10d_1$
0.10	D_{12}	D_{22}	D_{32}	D_{42}	D_{52}	$-5t_2 + 10d_1$
0.20	D_{13}	D_{23}	D_{33}	D_{43}	D_{53}	$-5t_3 + 10d_1$
0.40	D_{14}	D_{24}	D_{34}	D_{44}	D_{54}	$-5t_4 + 10d_1$
0.80	D_{15}	D_{25}	D_{35}	D_{45}	D_{55}	$-5t_5 + 10d_1$
	$5t_1-5r_1$ $+10d_1$	$5t_2-5r_2$ $+10d_1$	$5t_3-5r_3$ $+10d_1$	$5t_4-5r_4$ $+10d_1$	$5t_5-5r_4$ $+10d_1$	$50d_1$

Hence, after removal of the day effect, unbiased estimates of the treatment effects are obtained from the row totals. Row Totals = $-nt + 2nd$

Analyses of variance were carried out on the differences in log 3-hr count rates.

Treat-ment ImU TSH	Differences in log 3-hr count rates					Total
	Group 1	Group 2	Group 3	Group 4	Group 5	
0.05	D_{11}	D_{12}	D_{13}	D_{14}	D_{15}	$D_{1\cdot}$
0.10	D_{21}	D_{22}	D_{23}	D_{24}	D_{25}	$D_{2\cdot}$
0.20	D_{31}	D_{32}	D_{33}	D_{34}	D_{35}	$D_{3\cdot}$
0.40	D_{41}	D_{42}	D_{43}	D_{44}	D_{45}	$D_{4\cdot}$
0.80	D_{51}	D_{52}	D_{53}	D_{54}	D_{55}	$D_{5\cdot}$
Total	$D_{\cdot 1}$	$D_{\cdot 2}$	$D_{\cdot 3}$	$D_{\cdot 4}$	$D_{\cdot 5}$	$D_{\cdot\cdot}$

Table 2 - ANALYSIS OF THE SET OF DATA PRESENTED IN TABLE 1

Analysis of variance (ANOV)

S.V.	d.f.	S.S.	M.S.	F	P
Totals	rt-1	$D^2_{ij}-D^2_{\cdot\cdot}/rt$	-	-	-
Replications (residuals)	r-1	$D^2_{\cdot j}/t-D^2_{\cdot\cdot}/rt$	R	R/E	
Treatments	t-1	$D^2_{i\cdot}/r-D^2_{\cdot\cdot}/rt$	T	T/E	
Error	(r-1)(t-1)	Subtraction	E	-	

Data from row totals	Estimate after removal of day variation ImU TSH	Estimate from regression ImU TSH
$-5t_1 + 10d_1 = D_1.$	$0.05 = t_1$	$0.05 = t_1$
$-5t_2 + 10d_1 = D_2.$	$0.10 = t_2$	$0.10 = t_2$
$-5t_3 + 10d_1 = D_3.$	$0.20 = t_3$	$0.20 = t_3$
$-5t_4 + 10d_1 = D_4.$	$0.40 = t_4$	$0.40 = t_4$
$-5t_5 + 10d_1 = D_5.$	$0.80 = t_5$	$0.80 = t_5$

S.V. = Source of Variation

d.f. = degree of freedom

S.S. = Sum of Square

M.S. = Mean Square

D_{ij} = Observation

i = treatment

t = number of treatment

j = replication

r = number of replication

Let $D^2../rt = C$

Calculation of S.S.

Totals $= \sum D^2_{ij} - C$

$= (D^2_{11} + D^2_{12} + D^2_{13} + D^2_{14} + D^2_{15} + D^2_{21} + \ldots\ldots D^2_{55}) - C$

Residuals $= \sum D^2_{.j}/t - C$

$$= \frac{D_{\cdot 1}^2 + D_{\cdot 2}^2 + D_{\cdot 3}^2 + D_{\cdot 4}^2 + D_{\cdot 5}^2}{t} - C$$

Treatments $= \frac{\sum D_{i\cdot}^2}{r} - C$

$$= \frac{D_{1\cdot}^2 + D_{2\cdot}^2 + D_{3\cdot}^2 + D_{4\cdot}^2 + D_{5\cdot}^2}{r} - C$$

Error = Total S.S. - (Residual S.S. + Treatment S.S.)

Calculation of M.S.

Residual (R) $= \frac{\sum D_{\cdot j}^2/t - C}{r-1}$

Treatment (T) $= \frac{\sum D_{i\cdot}^2/r - C}{t-1}$

Error (E) $= \frac{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})}{(r-1)(t-1)}$

Calculation of F

Residual = R/E

$$\frac{\sum D_{\cdot j}^2/t - C}{r-1} \cdot \frac{(r-1)(t-1)}{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})}$$

$$= \frac{(\sum D_{\cdot j}^2/t - C)(t-1)}{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})}$$

Treatment = T/E

$$= \frac{\sum D_i^2./r - C}{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})} \cdot \frac{(r-1)(t-1)}{}$$

$$= \frac{(\sum D_i^2./r - C)(r-1)}{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})}$$

Calculation of s (Standard Deviation)

$$s^2 = \text{Error M.S.}$$

$$s = \sqrt{\frac{\text{Total S.S.} - (\text{Residual S.S.} + \text{Treatment S.S.})}{(r-1)(t-1)}}$$

Calculation of Index of precision (λ)

$$\text{Index of precision} = \frac{s}{b}$$

b = slope in term of log dose interval

s = standard deviation