THYROID RECOVERY AFTER CESSATION OF TREATMENT WITH THYROIDAL SUBSTANCES

By

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INTRODUCTION

Thyroid therapy has been employed in human subjects for about seventy years. More recently thyroid substances have been used extensively in lower animals for basic research and applications for improvement of productive processes. Despite a long history of application and research, little information is available on the duration of suppression of normal thyroid function after withdrawal of therapy or the extent to which normal function will be restored.

In studies reported to date radioiodine uptake and output have been used as indices of recovery of the functional state of the gland. Occasional mention is made of thyroid weights, thyroidal iodine, and histology as indices of thyroid function. Since no single measurement is sufficient for all conditions, a comparative study of the various parameters of thyroid function during suppression and recovery was planned.

Thyroxine, triiodothyronine, and thyroactive iodinated casein are the common thyroid substances used in therapy and research. No complete survey of the course of changes in thyroid function during and following different periods of suppression by these agents has been made.

Therefore, in this work various parameters of thyroid function were compared in rats treated for six weeks or six months by thyroxine, triiodothyronine, or iodinated casein and during the subsequent four week recovery period.

Since in practice thyroproteins are administered orally, this was the route of choice for these experiments, simulating as nearly as possible in rats the circumstances for routine use of thyroidal substances.

LITERATURE REVIEW

The suppressive effects of thyroxine, triiodothyronine, desiccated thyroid, and thyroactive iodinated protein have been applied by many workers in studies of the normal and thyrotoxic thyroid gland (Greer and Johnston, 1951; Dresner and Schneeberg, 1958; Greer, 1951; Morgans, et al., 1952; Starr and Liebhold-Schueck, 1953; Blaxter, 1952). These inhibitory effects are exerted through interruption of the thyroid-pituitary homeostatic mechanism (Miyai, et al., 1962; Perlmutter, et al., 1952; Oddie, et al., 1960). Exogenous thyroxine, triiodothyronine, and iodinated casein given at levels in excess of the thyroxine secretion rate block pituitary thyroprotein secretion (Bauman, et al., 1965; Pipes, et al., 1957; Premachandra and Turner, 1962). Few studies have been for the direct purpose of assessing thyroid recovery after suppression by thyroid hormones and other thyroproteins.

Purves (1964) has observed that although thyrotrophin secretion appears to resume promptly after short periods of suppression, there may be considerable delay after prolonged suppression by repeated injections of thyroxine. Greer (1951) found that radioiodine uptake of normal glands inhibited by ingestion of desiccated thyroid resumed normal function within four weeks after cessation of thyroid ingestion. Suppression of the thyroid of normal subjects by triiodothyronine administration for four weeks was followed by a gradual return of normal function in the third and fourth week after triiodothyronine was discontinued according to Rich (1958). All of the above observations were from human subjects, using radioiodine uptake to define thyroid function.

In discussing the difference in the suppressive action between potassium iodide and triiodothyronine, Spring (1964) states that the pituitary cannot be suppressed completely. He says that beyond a maximal dose of triiodothyronine, more will not suppress the pituitary further. Iodine can completely inhibit the organic binding of iodine in the thyroid gland provided the concentration of iodine in the gland is sufficiently high. Either directly or indirectly it can inhibit the pituitary.

The use of thyroproteins for increasing lactation in cattle has prompted research on resumption of thyrotrophin secretion following withdrawal of the inhibitory substance. Premachandra and Turner (1962) injected lactating cows daily with thyroxine 50 per cent above their thyroxine secretion rate for eleven to twelve weeks. Radioiodine uptake commenced twelve days after cessation of thyroxine treatment and was normal by three weeks. The thyroid and pituitary of one cow was blocked for twenty-nine weeks, yet recovery was the same as in those blocked for eleven to twelve weeks. Thyroprotein has also been fed for extended periods of time with no undesirable permanent effects on the pituitary or thyroid. In similar studies with a graded withdrawal of thyroxine, Bauman et al. (1965) observed that younger animals resumed thyroid and pituitary secretory function more rapidly after both partial and complete withdrawal of thyroxine than did older animals.

Yamada et al. (1961) used over 300 rats in assessing various parameters of thyroid function after either thyroxine administration or hypophysectomy. They found that the rate of release of thyroidal radioiodine was the most sensitive, the thyroidal radioiodine uptake the next, and the weight of the thyroid gland and histology were the least sensitive.

Working with dairy calves, Lodge et al. (1958) found questionable the information provided by radioiodine uptake in determining the thyroid status of these animals. They explained that "the iodine content of the thyroid gland is not indicative of the gland's activity but only shows its storage capacity. The same can be said of uptake of radioactive iodine in that it is a measure of the collecting ability of the gland and, owing to the storage of iodine, may not be indicative of the output of the gland."

A rebound phenomenon was observed by Yamada et al. (1961) in both radioiodine uptake and thyroid gland weight after cessation of thyroxine administration, but it was not observed in thyroidal iodine release. A rebound increase in radioiodine uptake has also been infrequently observed by Greer and Johnston (1951) during or after treatment of human subjects with thyroxine. Rich (1958) describes an intermediate phase of thyroidal rebound after four weeks of treatment with triiodothyronine during which the radioiodine uptake was greater than normal.

Protamone fed to growing chickens has consistently lowered their thyroid weights (Hill, 1953). Upon autopsy the thyroids of lambs given 0.5-1.0 g. daily of iodocasein for up to 126 days were found to be in a resting state (Winchester, 1953).

Few workers have reported exactly the lengths of suppression periods by thyroid substances. Human clinical studies probably vary a great deal in this respect. In some cases the I^{131} has been given for a few hours to 2 days following one large suppressive dose of thyroxine or triiodothyronine injected in either animals or humans. Desiccated thyroid was the therapeutic agent in many of the human cases and iodinated casein was fed to cows in lactation experiments. In basic research thyroproteins have been more often injected than administered orally.

PROCEDURE

The degree and rate of recovery of rat thyroids inhibited for different periods of time by the feeding of thyroxine (T_4), triiodothyronine (T_3), and iodinated casein (I.C.) were determined by thyroidal radioiodine uptake and output, total thyroidal iodine, thyroid weight and histology. Parallel experiments with thyroxine, triiodothyronine, and iodinated casein in the feed were run for six-week and six-month periods.

Albino female rats weighing from 100 to 150 grams ordered from Carworth Farms were used for all experiments. They were weighed, grouped with varying weights to a group, marked, and then fed normal feed SR-1 for two weeks before being started on the experiment. Room temperature was maintained at $25^{\circ}C \pm 1^{\circ}$ except for fluctuations as noted in Appendix A.

The feed (SR-1: see Appendix B for complete formula) was a finely ground meal composed of corn, soybean oil meal, vitamin supplements, and a special mineral salt Permix SR-1 (Appendix B) containing a known and constant amount of iodine. The Premix SR-1 was prepared in our laboratory and mixed with the other components at the Michigan State University Animal Husbandry department feed mixing plant.

Preliminary tests were made to determine the amounts of the three test materials necessary in the feed to inhibit and replace normal thyroxine secretion. A modification of the thyroxine substitution method of Reineke and Singh (1955) was used with increasing amounts of thyroxine and triiodothyronine being fed instead of injected. The per cent of injected dose of I^{131} was plotted on log scale against the μg of thyroxine (μg of triiodothyronine or mg of iodinated casein) fed per rat per day and the inhibiting dose determined from the change in the slope of the output curve. The calculations were based on the assumption that each rat ate an average of 10 grams of feed per day. Data from this preliminary experiment is presented under Results.

For each I^{131} output determination, intraperitoneal injections of 3 μc of carrier-free I^{131} were given each rat and *in vivo* thyroid and body counts (epigastric region) were taken under nembutal anesthesia (3 mg/100 g of body weight) every two days for six counts. Counts were taken by use of a scintillation counter with a 2" crystal connected to a radiation analyzer and a laboratory scaler. A count rate meter was connected in the circuit to help in determining the position of the rat for highest counts. A standard prepared at one-tenth of the injected dose was counted with each group. Counts were corrected for body and room background and I^{131} uptake as per cent of injected dose was calculated according to the formula:

$$\% \ I^{131} \text{ uptake} = \frac{\left(\text{Thyroid Count} - \text{Body background} + \frac{1}{2}\ \text{Room background}\right)}{\text{Standard count} \times 10} \times 100$$

For preparation of the test feed, the 1-thyroxine and triiodothyronine were dissolved in 0.1 N NaOH and made up to volume with distilled water. Calculated volumes of these solutions were mixed with 200 g of feed for ten minutes in a small mixer and then made up to 5000 g and mixed for several hours in a large mixer. Iodinated casein in powdered form was added to the feed and mixed thoroughly in the same way.

Four times the inhibiting doses of thyroxine and triiodothyronine and three times the inhibiting dose of iodinated casein were mixed with the feed for the experiment (Table 1). This insured maximum though not absolute inhibition of thyroid function. The rats continued normal weight gains and showed no symptoms of hyperthyroidism.

Two test groups were run for each of the three (T_4, T_3, and I.C.) six week experiments and for each of the three six month experiments. One of each pair of test groups was allowed a two-week recovery period after either six weeks or six months on a test diet and the other a four-week recovery period.

Three types of control groups were maintained: (1) Rats on test feed, given I^{131} and nembutal, and counted exactly like test groups; (2) Rats on normal feed, given I^{131} and nembutal, and counted each time test groups were

Table 1. Inhibiting doses of thyroxine, triiodothyronine, and iodinated casein mixed with feed.

	Inhibiting dose per 10 g feed per day	Increased inhibiting dose per 10 g feed per day	Amounts mixed per 5000 g feed
Thyroxine	3.00 μg (x 4)	12.00 μg	6.00 mg
Triiodo-thyronine	0.72 μg (x 4)	2.88 μg	1.44 mg
Iodinated casein	0.45 mg (x 3)	1.35 mg	675.00 mg

Table 2. Dosages of thyroxine, triiodothyronine, and iodinated casein calculated per 100 grams of body weight assuming 10 grams of feed consumed per day.

	Preliminary experiment	End of short term experiment	End of long term experiment
Thyroxine	2.50 μg	5.45 μg	5.20 μg
Triiodo-thyronine	0.63 μg	1.41 μg	1.21 μg
Iodinated casein	0.37 mg	0.67 mg	0.59 mg

Table 3. The dosages of thyroxine, triiodothyronine, and iodinated casein exprrssed as per cent of feed.

	Dosages expressed as per cent of feed	
	Inhibiting dose per 10 g feed per day	Increased inhibiting dose per 10 g feed per day
Thyroxine	0.0000300%	0.0001200%
Triiodo-thyronine	0.0000072	0.0000288
Iodinated casein	0.0045000	0.0135000

counted; (3) Rats on normal feed, given no I^{131} or nembutal and of course not counted. The experimental plan for each of the test groups and their controls is outlined in Fig. 1.

Each test and control group had at least 5 animals per group in the six-week experiment and six animals per group in the six-month experiment, except that normal controls (Group 3 above) had three animals per group in each case. Final group data were reduced in numbers in some cases because of animals lost due to sickness, anesthetic overdosage or samples lost in the analytical process. Numbers of rats remaining per group are indicated on the various graphs.

At the beginning of the experiment, thyroidal I^{131} output curves were plotted for 6 days (3 counts) and then continued for another 6 days (3 counts) after the animals were placed on the test feed. At the end of either the short term (ST) or long term (LT) test periods, the rats were returned to normal feed and thyroidal I^{131} output rates followed during the two-week recovery period for one test group and during the third and fourth week of recovery for the second test group in each of the three experiments. The rats were then killed and weights recorded.

Thyroids were removed, carefully trimmed, weighed on a Roller-Smith torsion balance, and fixed in Dietrich's solution. Later one lobe was prepared for histological sections (H & E stain) and the other analyzed for total

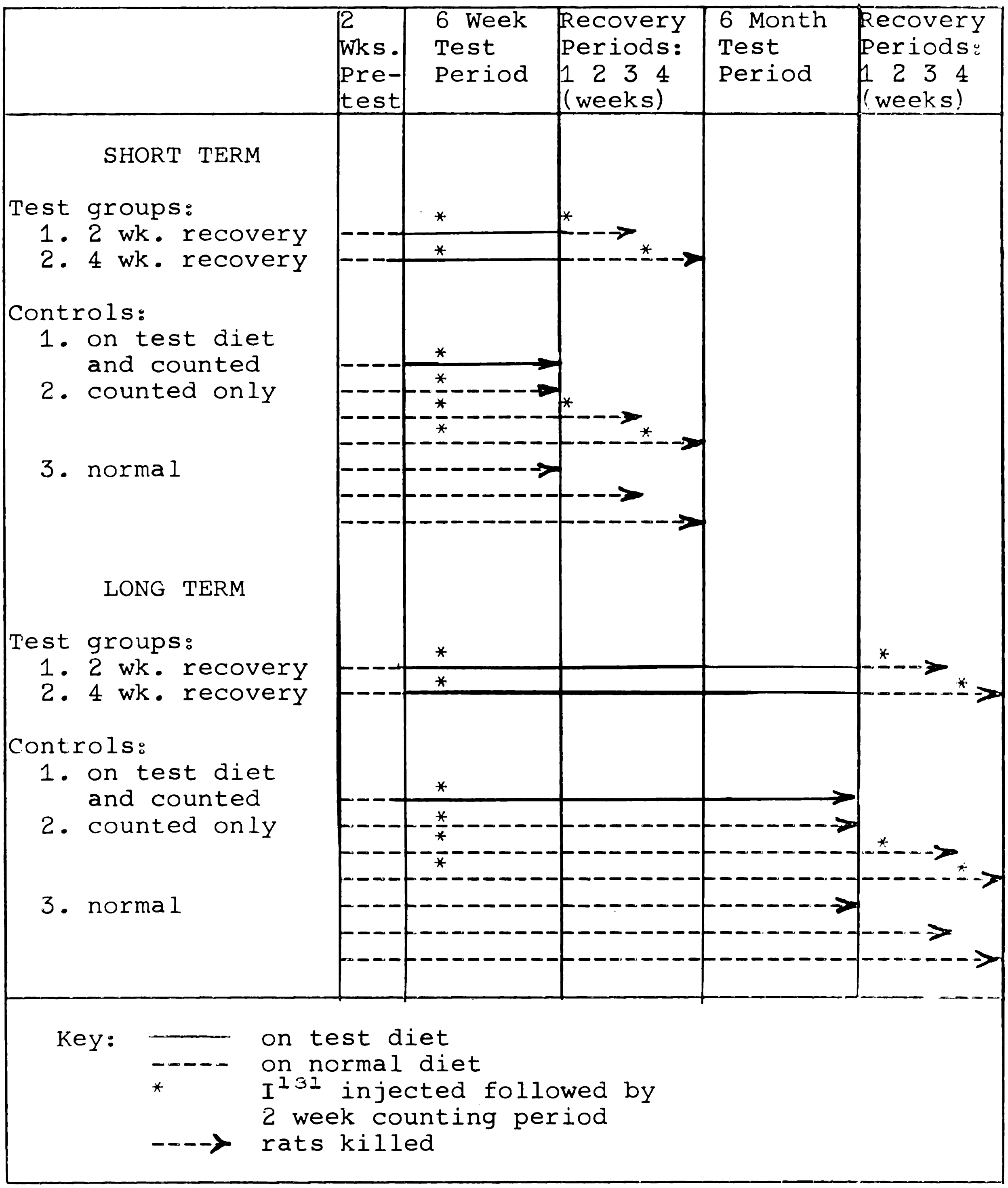


Fig. 1. Outline of the experimental plan for each of the three test groups: thyroxine, triiodothyronine, and iodinated casein.

thyroidal iodine using a modification of Barker's method for determination of PBI (Appendix C).

Control group 1 and one-third of control groups 2 and 3 were killed when test groups were changed from test feed to normal feed. This provided histological and thyroidal iodine data at the end of the inhibitory period to compare with recovery data. The remaining two-thirds of control groups 2 and 3 provided the same kind of data as well as thyroidal radioiodine output control data for the 2 and 4 week recovery groups.

Data collected and evaluated include:

1. I^{131} uptake at zero time, % of injected dose (U)
2. I^{131} output rate corrected for recycling iodine (K_4)
3. Biological half time in days ($T\frac{1}{2}$)
4. Thyroidal iodine, μg/100 g Body Weight (I)
5. Thyroid secretion rate (TSR)
6. Thyroid weights (mg/100 g Body Weight)
7. Thyroxine equivalents
8. Histology

These data were compared for:

1. Test animals and their controls
2. No recovery, 2-week and 4-week recovery groups
3. Long and short term experimental and control groups
4. Thyroxine, triiodothyronine, and iodinated casein test groups.

CALCULATIONS

Standard errors and tests of significance of difference (Students "t" test) were computed by standard statistical methods.

Biological radioiodine half-time ($T_{\frac{1}{2}}$) and zero hour uptakes (U) were read from the graphs and used in the following calculations:

I^{131} output rate constant: $k = \frac{.693}{T_{\frac{1}{2}}}$ or $k = 2.302 \frac{(\log A_t - \log A_0)}{t}$

Fractional output daily: $k_4' = 1 - e^{-kT}$ (T = 1 day)

I^{131} daily output corrected for recirculating iodine: $k_4 = \frac{k_4'}{1-U}$ (U = uptake at zero time)

Output rate: $k_4 \times$ Thyroidal iodine content = hormonal iodine release

This value x 1.529 = the thyroxine equivalent.

Since the rat thyroid contains 6 parts thyroxine to 1 part triiodothyronine iodine (Pitt-Rivers and Rall, 1961) and T_3 is 4.85 times as potent per unit of iodine as T_4 in suppressing TSH release in the rat (Reineke, unpublished), factors correcting hormonal iodine release to the equivalent thyroxine potency are necessary. The potencies of the compounds can be assessed as follows (Bhatnager, 1963):

	T_4		T_3		Totals
Parts	6.00	+	1.00	=	7.00
Potency	x 1.00		x 4.85		
Activity	6.00	+	4.85	=	10.85

To adjust the T_4 equivalent in proportion to the potency of the mixture of T_4 and T_3 assumed to be released we use the factor $\frac{10.85}{7} = 1.55.$

Then

$$\text{Estimated daily thyroid secretion, } T_4 \text{ equivalent} = k_4 \times \text{total thyroid iodine } (\mu g) \times 1.529 \times 1.55$$

Comparisons of TSR values computed by this equation and by the thyroxine substitution method of Reineke and Singh (1955) show satisfactory agreement (Bhatnager, 1963).

RESULTS

I. The PRELIMINARY EXPERIMENT

The preliminary tests were run for the purpose of obtaining base figures for calculating dosages of thyroxine, triiodothyronine, and iodinated casein for the experimental diets. Results of feeding graded doses of each of the three test materials are plotted for individual rats in Fig. 2. The irregularity of the slopes of these curves is due to the writer's inexperience at that time in handling and positioning the rats for consistently high counts.

The inhibiting doses as indicated on the curves were judged to be 3.0 μg thyroxine, 0.72 μg triiodothyronine, and 0.45 mg iodinated casein.

According to the assay results, triiodothyronine has 4.17 times the potency of crystalline thyroxine. Since iodinated casein contains one percent thyroxine (as stated by the supplier), 0.45 mg of iodinated casein contains 4.5 μg thyroxine and the potency ratio of triiodothyronine to the thyroxine in iodinated casein is 6.25. In view of the variations inherent in this assay, there is not a real difference in the potency ratios according to these two comparisons. Both fall within the range reported by other investigators.

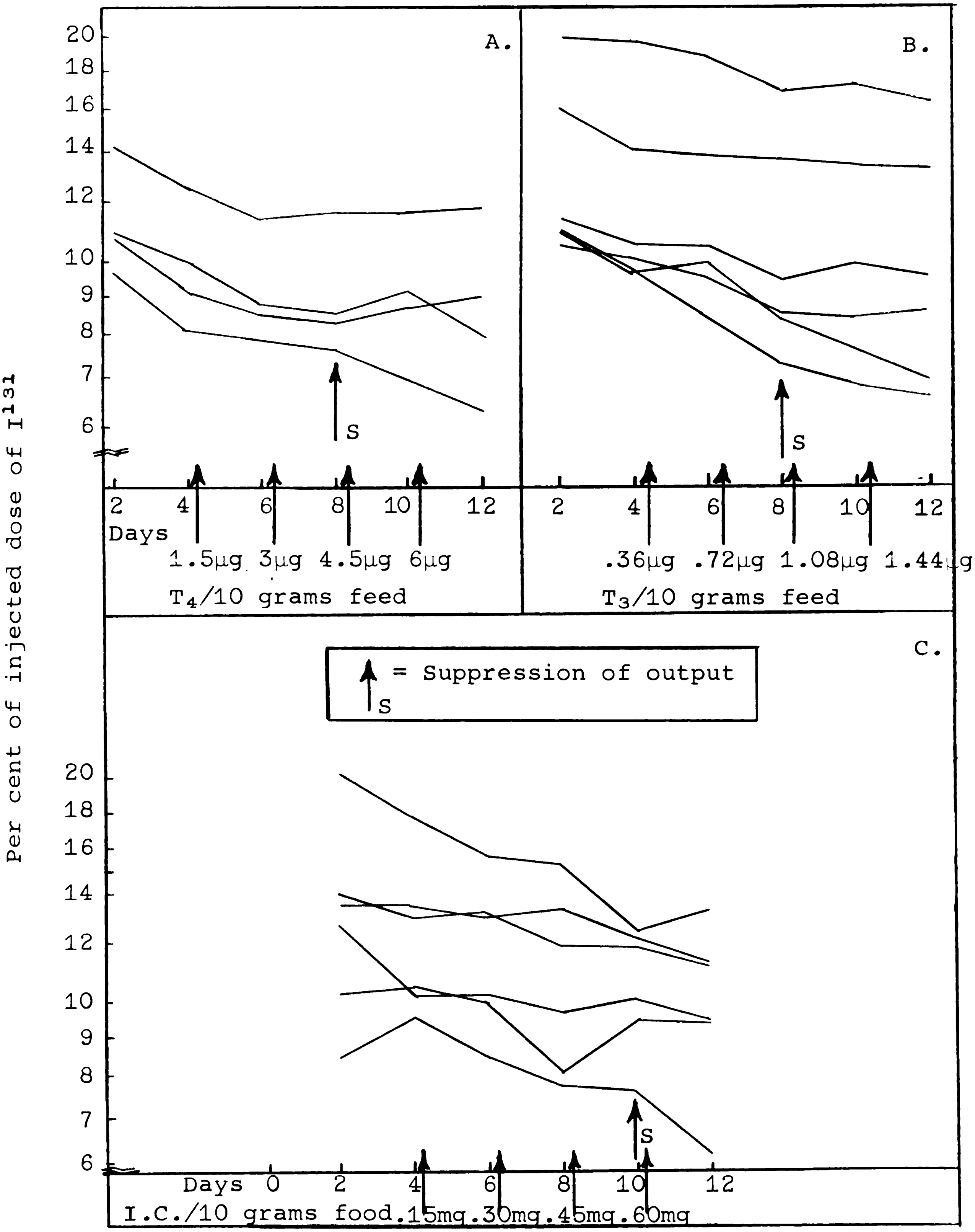


Fig. 2. Radioiodine output curves of rats fed increasing doses of either thyroxine, triiodothyronine, or iodinated casein.

Earlier comparisons of physiological potency have been made by parenteral administration of the test substance. In the present comparison all compounds were given orally. In as much as the potency ratios are within the same range, it can be concluded that all of the materials were absorbed to about the same extent.

II. THE SHORT AND LONG TERM SUPPRESSION EXPERIMENT

Radioiodine Uptake, Output, and Half-time Results

The mean thyroidal radioiodine output curves for each group before and following the period on the experimental diet are plotted for thyroxine in Fig. 3, for triiodothyronine in Fig. 4, and for iodinated casein in Fig. 5. The standard errors for the points plotted on all of the output curves are listed in Appendices D and E. The radioiodine uptakes (U), the radioiodine biological half-times ($T_{\frac{1}{2}}$), and the radioiodine output rates (corrected for recirculating iodine, k_4) were determined from the output curves and together with standard errors are listed in Appendices F through H.

<u>Normal pretest values</u>: Tables 4-9

At the start of the experiment, the normal thyroidal radioiodine output was determined for the test groups for six days and then inhibited by feeding thyroxine, triiodothyronine, or iodinated casein as shown by the flattening of the output slopes for the next six days (Figs. 3-5).

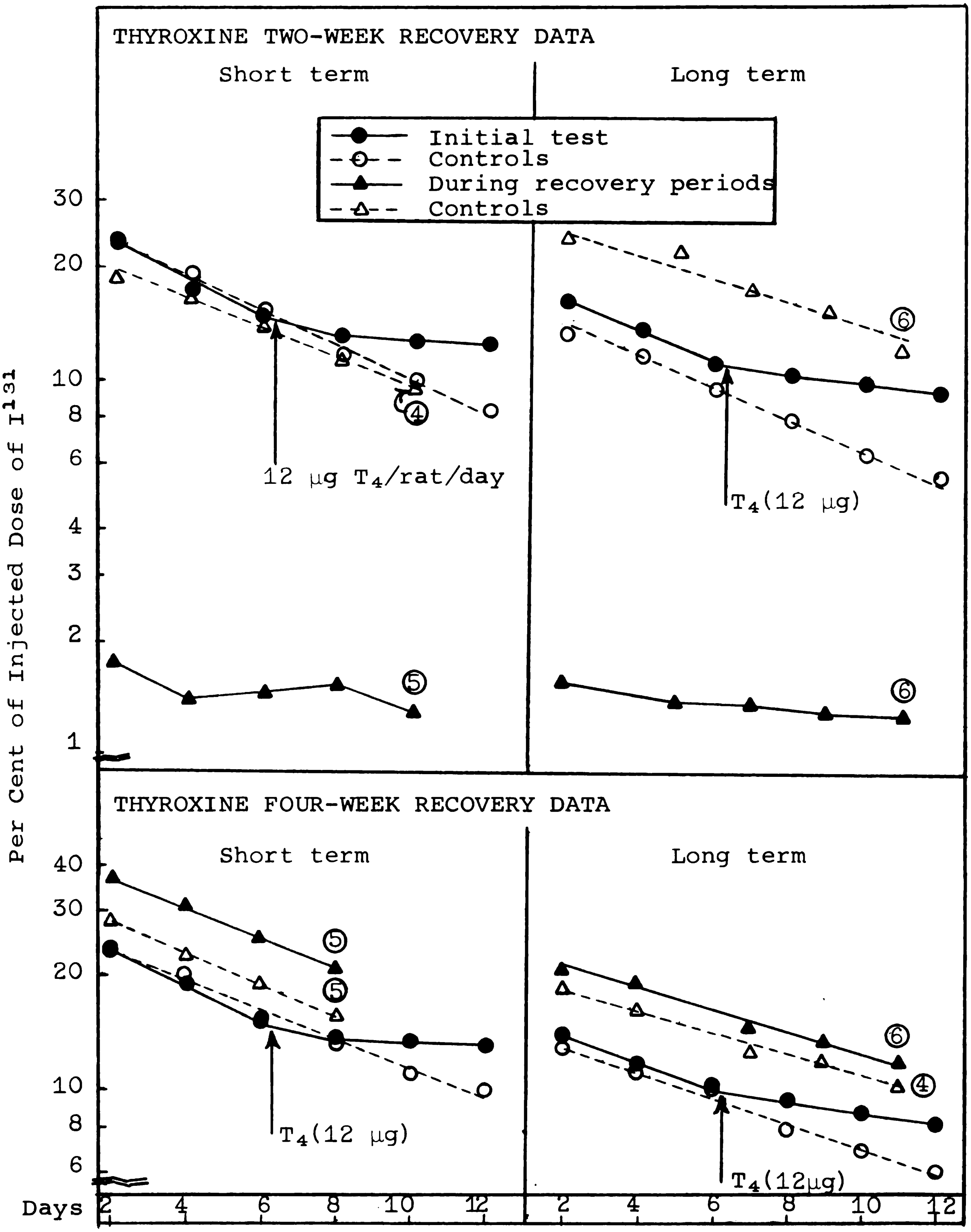


Fig. 3. Thyroidal radioiodine output curves for rats before, during, and after being fed thyroxine test diet for six weeks or six months and their controls.

O indicates number of rats in group.

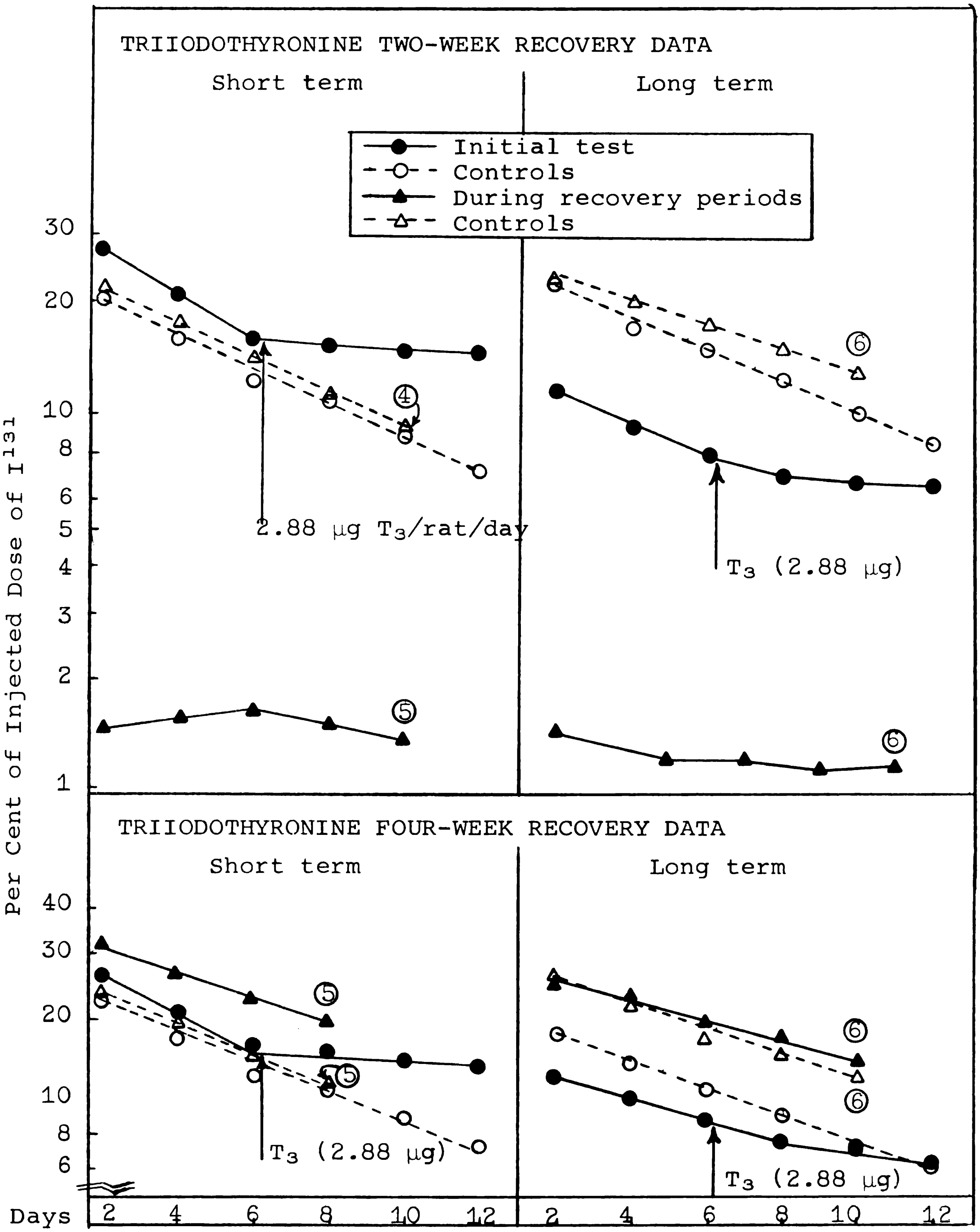


Fig. 4. Thyroidal radioiodine output curves for rats before, during, and after being fed triiodothyronine test diet for six weeks or six months and their controls.

O indicates number of rats in a group.

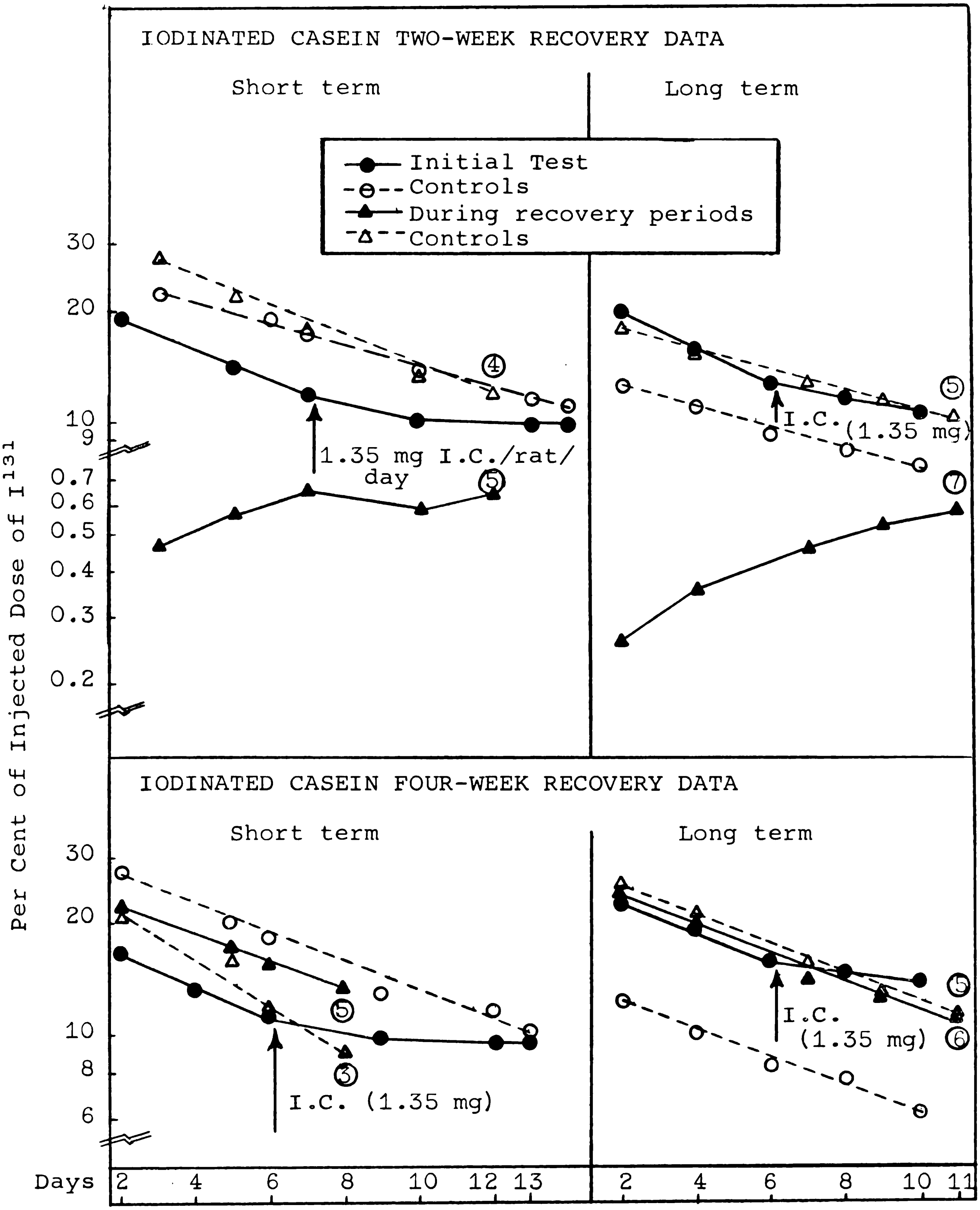


Fig. 5. Thyroidal radioiodine output curves for rats before, during, and after being fed iodinated casein test diet for six weeks or six months and their controls.

O indicates number of rats in group.

Controls counted at the same times showed normal output slopes for the whole twelve day period. The normal I^{131} uptakes at this time ranged from 13.6 per cent to 35.4 per cent of injected dose and maximum uptake had occurred by the time of the first count 48 hours after I^{131} injection. The normal I^{131} half-times ranged from 5.4 days to 10.5 days and the k_4 values ranged from 0.0747 to 0.1918 per day.

With few exceptions the short term (ST) and long term (LT) recovery groups showed similar results. Therefore, unless otherwise noted, they are considered together in reporting results.

Two-week recovery groups: Tables 4-9.

Maximal uptake of I^{131} by the two-week recovery groups was 2.9 per cent for T_4- and 2.8 per cent for T_3- inhibited glands and usually had occurred by the time of the first count, two days after removal from the test diets and injection of I^{131}. There was some variation of individuals within a group. The T_4 short term group had one animal with a delayed maximum uptake and the long term group had two such animals. In the T_3 recovery group at six weeks 3 out of 5 animals appeared not to reach maximum uptake until the sixth day after I^{131} injection. One animal in the six month T_3 recovery group also did not reach maximum uptake until the sixth day. Appendix I shows the thyroidal radioiodine output curves for the individual rats in the two-week recovery groups for both the six weeks and the six months periods.

Table 4. Significance of differences between thyroxine test and control group values for radioiodine output, uptake, and half-time for the six week experiment. The vertical bar (|) indicates the groups being compared.

THYROXINE: SIX-WEEK EXPERIMENT

A. Comparison between test and control groups.

<u>Before test diet</u>:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.1552	> 0.5	28.8	> 0.5	6.0	> 0.5
Controls	.1505		29.8		6.3	
4 wk. recov. gp.	.1593	> 0.1	30.2	0.1	6.2	> 0.5
Controls	.1280		29.2		7.3	
<u>After test diet</u>:						
2 wk. recov. gp.	.0386	< 0.01	1.96	< 0.01	23.4	> 0.05
Controls	.1187		23.9		7.5	
4 wk. recov. gp.	.1630	> 0.1	43.2	> 0.1	7.6	> 0.5
Controls	.1322		33.5		7.6	

B. Comparison between values before test diet and after test diet.

	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
<u>2 week recovery group</u>						
Before test diet	.1552	---	28.8	---	6.0	---
After test diet	.0386		1.96		23.4	
<u>2 week control group</u>						
Before test diet	.1505	---	29.6	---	6.3	---
After test diet	.1187		23.9		7.5	
<u>4 week recovery group</u>						
Before test diet	.1593	> 0.5	30.2	> 0.05	6.2	> 0.1
After test diet	.1630		43.2		7.6	
<u>4 week control group</u>						
Before test diet	.1280	> 0.5	29.2	> 0.5	7.3	> 0.5
After test diet	.1322		33.5		7.6	

Table 5. Significance of differences between thyroxine test and control group values for radioiodine uptake, output, and half-time for the six-month experiment. *(4) = number of rats included in average. The vertical bar (|) indicates the groups being compared.

THYROXINE: SIX-MONTH EXPERIMENT

A. Comparison between test and control groups.

Before test diet:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.1098	> 0.1	19.3	> 0.1	7.8	> 0.5
Controls	.1051		16.6		7.5	
4 wk. recov. gp.	.1165	> 0.1	16.7	> 0.5	9.6	> 0.5
Controls	.0951		16.2		8.8	
After test diet:						
2 wk. recov. gp.	.0284	< 0.01	1.98	< 0.01	25.9	< 0.01
Controls	.1030		28.2		9.4	
4 wk. recov. gp.	.0958	> 0.1	25.2	> 0.5	10.5	> 0.5
Controls	.0851		21.4		10.3	

B. Comparison between values before test diet and after test diet.

	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 week recovery group						
Before test diet	.1098	---	19.3	---	7.8	---
After test diet	.0284(4)*		1.98(4)*		25.9(4)*	
2 week control group						
Before test diet	.1051	---	16.6	---	7.5	---
After test diet	.1030		28.2		9.4	
4 week recovery group						
Before test diet	.1165	> 0.1	16.7	= 0.05	9.6	> 0.5
After test diet	.0958		25.2		10.5	
4 week control group						
Before test diet	.0951	> 0.1	16.2	> 0.1	8.8	> 0.5
After test diet	.0851		21.4		10.3	

Table 6. Significance of differences between triiodothyronine test and control group values for radioiodine output, uptake, and half-time for the six-week experiment. The vertical bar (|) indicates the groups being compared.

TRIIODOTHYRONINE: SIX-WEEK EXPERIMENT

A. Comparison between test and control groups.

Before test diet:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.1918	< 0.02	35.4	< 0.02	5.4	< 0.05
Controls	.1313		24.4		6.7	
4 wk. recov. gp.	.1668	< 0.02	32.7	> 0.1	5.9	< 0.02
Controls	.1166		26.3		7.9	
After test diet:						
2 wk. recov. gp.	-----	---	----	---	----	---
Controls	.1327		26.2		6.9	
4 wk. recov. gp.	.1315	> 0.5	37.6	> 0.1	8.2	< 0.01
Controls	.1335		28.5		7.0	

B. Comparison between values before test diet and after test diet.

2 week recovery group						
Before test diet	.1918	---	35.4	---	5.4	---
After test diet	-----		----		----	
2 week control group						
Before test diet	.1313	---	24.4	---	6.7	---
After test diet	.1327		26.2		6.9	
4 week recovery group						
Before test diet	.1668	> 0.05	32.7	> 0.1	5.9	< 0.01
After test diet	.1315		37.6		8.2	
4 week control group						
Before test diet	.1166	> 0.1	26.3	> 0.5	7.9	> 0.1
After test diet	.1335		28.5		7.0	

Table 7. Significance of differences between triiodothyronine test and control group values for radioiodine output, uptake, and half-time for the six-month experiment. *(4) = Number of rats included in average. The vertical bar (|) indicates the groups being compared.

TRIIODOTHYRONINE: SIX-MONTH EXPERIMENT

A. Comparison between test and control groups.

Before test diet:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.0953	> 0.5	13.6	> 0.02	9.4	> 0.5
Controls	.1075		23.2		8.5	
4 wk. recov. gp.	.0864	= 0.05	16.1	> 0.1	9.6	> 0.1
Controls	.1027		21.1		8.3	
After test diet:						
2 wk. recov. gp.	.0376(4)*	< 0.01	1.7	< 0.01	19.8	< 0.01
Controls	.1004		26.8		9.2	
4 wk. recov. gp.	.0825	> 0.05	27.4	> 0.5	12.2	< 0.05
Controls	.1057		29.8		9.1	

B. Comparison between values before test diet and after test diet.

	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 week recovery group						
Before test diet.	.0953	* ---	13.6	---	9.4	---
After test diet	.0376(4)		1.7		19.8	
2 week control group						
Before test diet	.1196	---	23.2	---	8.5	---
After test diet	.1004		26.8		9.2	
4 week recovery group						
Before test diet	.0864	> 0.5	16.1	< 0.01	9.6	> 0.1
After test diet	.0825		27.4		12.2	
4 week control group						
Before test diet	.1027	> 0.5	21.1	< 0.02	8.3	> 0.05
After test diet	.1057		29.8		9.1	

Table 8. Significance of differences between iodinated casein test and control group values for radioiodine output, uptake, and half-time for the six-week experiment. *(4) = Number of rats included in average. The vertical bar (|) indicates the groups being compared.

IODINATED CASEIN: SIX-WEEK EXPERIMENT

A. Comparison between test and control groups.

Before test diet:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.1193	< 0.02	23.2	> 0.5	7.4	< 0.01
Controls	.0861		24.4		10.5	
4 wk. recov. gp.	.1148	> 0.1	21.8	< 0.01	7.7	> 0.5
Controls	.1315		34.5		7.8	
After test diet:						
2 wk. recov. gp.	----	---	----	---	----	---
Controls	.1541		34.3(4)*		6.5	
4 wk. recov. gp.	.1094	< 0.01	26.6	> 0.5	8.6	< 0.02
Controls	.1605		23.6		5.4	

B. Comparison between values before test diet and after test diet.

	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 week recovery group						
Before test diet	.1193	---	23.2	---	7.4	---
After test diet	----		----		---	
2 week control group						
Before test diet	.0861	---	24.4	---	10.5	---
After test diet	.1541		34.3(4)*		6.5	
4 week recovery group						
Before test diet	.1148	> 0.5	21.8	> 0.1	7.7	> 0.5
After test diet	.1094		26.6		8.6	
4 week control group						
Before test diet	.1315	> 0.05	34.5	> 0.05	7.8	< 0.01
After test diet	.1605		23.6		5.4	

Table 9. Significance of differences between iodinated casein test and control group values for radioiodine output, uptake, and half-time for the six-month experiment. *(4) = Number of rats included in average. The vertical bar (|) indicates the groups being compared.

IODINATED CASEIN: SIX-MONTH EXPERIMENT

A. Comparison between test and control groups.

Before test diet:	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 wk. recov. gp.	.1274	< 0.05	23.9	> 0.02	7.8	< 0.05
Controls	.0747		14.3		8.9	
4 wk. recov. gp.	.1291	> 0.1	28.2	< 0.01	7.4	> 0.10
Controls	.0984		14.6		8.7	
After test diet:						
2 wk. recov. gp.	-----	---	----	---	----	---
Control	.0808		20.3		10.5	
4 wk. recov. gp.	.1105	> 0.1	27.2	> 0.5	8.6	> 0.10
Control	.1242		29.3(4)*		7.6	

B. Comparison between values before test diet and after test diet.

	K_4	P	U(%)	P	$T\frac{1}{2}$(days)	P
2 week recovery group						
Before test diet	.1274	---	23.9	---	7.8	---
After test diet	-----		----		---	
2 week control group						
Before test diet	.0747	---	14.3	---	8.9	---
After test diet	.0808		20.3		10.5	
4 week recovery group						
Before test diet	.1291	> 0.1	28.2	> 0.5	7.4	> 0.1
After test diet	.1105		27.2		8.6	
4 week control group						
Before test diet	.0984	> 0.1	14.6	< 0.01	8.7	> 0.5
After test diet	.1242		29.3(4)*		7.6	

For rat thyroids inhibited by iodinated casein, maximal I^{131} uptake was delayed until the sixth day for the short term test animals and until the eleventh day (and last count) for the long term two-week recovery group.

The highest uptake was always under 2.8 per cent of the injected dose for all two-week recovery groups compared to a range from 13.6 to 35.4 per cent of the injected dose for mean group values before being placed on the test diets.

Mean I^{131} half-times were much longer for the test groups than for the controls during the two-week recovery period. Because of the variation in time for reaching maximum uptake between individuals within groups, mean half-time could not be determined for T_3 and I.C. short term two-week recovery groups or for the long term I.C. two-week recovery group, and they could be determined for only part of the T_4 and T_3 long term two-week recovery groups.

Mean I^{131} half-times for the T_4 two-week recovery groups were 23.4 days for the short term experiment and 25.9 days (for 4 of the 6 rats) for the long term experiment (controls were 7.5 and 9.4 days respectively) compared to 6.0 days (ST) and 7.8 days (LT) before test diet (controls were 6.3 and 7.5 days respectively).

The T_3 long term two-week recovery half-time values were 19.8 days (for 4 of the 6 rats) and controls were 9.2 days compared to 9.4 days before test diet and 7.2 days for the controls during the initial counting period.

These obviously were the shortest of the extended half-times for any of the two-week recovery groups.

Since mean uptakes were not determined for T_3 short term and I.C. short and long term groups, the very low k_4 values could not be calculated for these groups. Mean k_4 values for T_4 two-week recovery groups were 0.0386 for the short term experiment and 0.0284 (for 4 of 6 rats) for the long term experiment (controls: 0.1187 and 0.1030 respectively) compared to 0.1552 (ST) and 0.1098 (LT) before test diet (controls: 0.1505 and 0.1051 respectively).

The mean T_3 long term two-week recovery group k_4 value was 0.0376 (for 4 of 6 rats) compared to 0.0953 before test diet while control values were 0.1004 compared to 0.1195 before the test diet period.

<u>Four-week recovery groups</u>: Tables 4-9

The slight increases in the k_4 values for the T_4 four-week recovery group following six weeks on test diet and their controls were not significantly different from pretest values. The k_4 values for the six month T_4 four-week recovery group and its controls were decreased from their pretest values but also not significantly.

The T_3 short term four-week recovery group mean k_4 value was decreased from 0.1668 before test diet to 0.1315 after test diet with a significant difference $P > 0.05$. There was no significant difference in the slight increase in control values between the first and second count.

There were no significant differences between first and second k_4 values for six month four-week recovery T_3 test or control groups.

Both short and long term I.C. four-week recovery groups had slight but nonsignificant decreases in the k_4 values after test diet at the same time controls had slight nonsignificant increases.

The mean I^{131} uptake for the short term four-week recovery T_4 test group increased from 30.2% to 43.2% at $P > 0.05$ after test diet. The long term T_4 test group increased from 16.7% to 25.2% at $P = 0.05$. Increases for controls were small (29.2% to 33.5% at $P > 0.5$ for short term and 16.2% to 21.4% at $P > 0.1$ for long term) and not significant.

Small increases in uptake were not significant for short term T_3 four-week recovery group (32.7% to 37.6% at $P > 0.1$) or their controls (26.3% to 28.5% at $P > 0.5$). Uptakes for the long term T_3 four-week recovery test and control groups were both increased significantly above pretest values (test group: 16.1% to 27.4% at $P < 0.01$ and control group: 21.1% to 29.8% at $P < 0.02$).

There were no significant changes in the I.C. short term (21.8% to 26.6% at $P > 0.1$) or long term (28.2% to 27.2% at $P > 0.5$) four-week recovery group mean uptakes. The short term controls were slightly lower (34.5% to 23.6% at $P > 0.05$) and long term controls were significantly higher (14.6% to 29.3% at $P < 0.01$).

Mean I^{131} half-times were not significantly different for T_4 short term (6.2 to 7.6 days at $P > 0.1$) or long term (9.6 to 10.5 days at $P > 0.5$) four-week recovery groups or for their controls (ST: 7.3 to 7.6 days at $P > 0.5$) (LT: 8.8 to 10.3 days at $P > 0.5$).

There was a significant increase in mean half-times for the short term T_3 four-week recovery group (5.9 to 8.2 days at $P < 0.01$) but not for the long term group (9.6 to 12.2 days at $P > 0.1$). Short term controls decreased slightly (7.9 to 7.0 days at $P > 0.1$) and long term controls increased slightly (8.3 to 9.1 days at $P > 0.05$).

The I.C. four-week recovery groups showed no significant change in I^{131} half-times after six weeks (7.7 to 8.6 days at $P > 0.5$) or six months (7.4 to 8.6 days at $P > 0.1$) on test diet. Short term controls showed a significant decrease in half-time (7.8 to 5.4 days at $P < 0.01$) and long term controls showed no significant decrease (8.7 to 7.6 days at $P > 0.5$).

Thyroidal Iodine

Mean thyroidal iodine in the short term thyroxine-inhibited group was not appreciably increased after two weeks on a normal diet over the thyroidal iodine of those allowed no recovery period. By the end of the fourth week of recovery, mean thyroidal iodine had increased to 4.77 μg/100 g B.W. compared to 2.00 μg I/100 g B.W. ($P < 0.05$) for the no recovery group and was not significantly different from values for either counted or normal controls. Mean thyroidal iodine in the long term T_4 two-week recovery group was increased over that of the no recovery group (6.04 μg I/100 g B.W. compared to 3.80 μg I/100 g B.W. in the no recovery group at $P < 0.05$) and over that of the counted control (4.90 μg I/100 g B.W. at $P > 0.1$). (Figure 6 and Tables 10-11.)

The mean thyroidal iodine values of the two-week recovery groups for both short and long term T_3 test groups were significantly increased over the no recovery groups (ST: 4.42 μg I/100 g B.W. for the test group and 2.92 μg I/100 g B.W. for the control group at $P < 0.05$; and LT: 5.44 μg I/100 g B.W. for the test group and 2.02 μg I/100 g B.W. for the control group at $P < 0.01$) and were close to the values for the counted controls (Figure 7 and Tables 12-13).

The mean thyroidal iodine values for all groups (no recovery, two-week recovery, and four-week recovery) that had been on I.C. test diet were elevated above values for counted controls, but only the long term no recovery group was significantly higher than its controls (8.6 μg I/100 g B.W. for the test group and 5.25 μg I/100 g B.W. for the control group at $P < 0.01$). (Figure 8 and Tables 14-15.)

This same long term I.C. no recovery group also had a significantly higher thyroidal iodine content than the

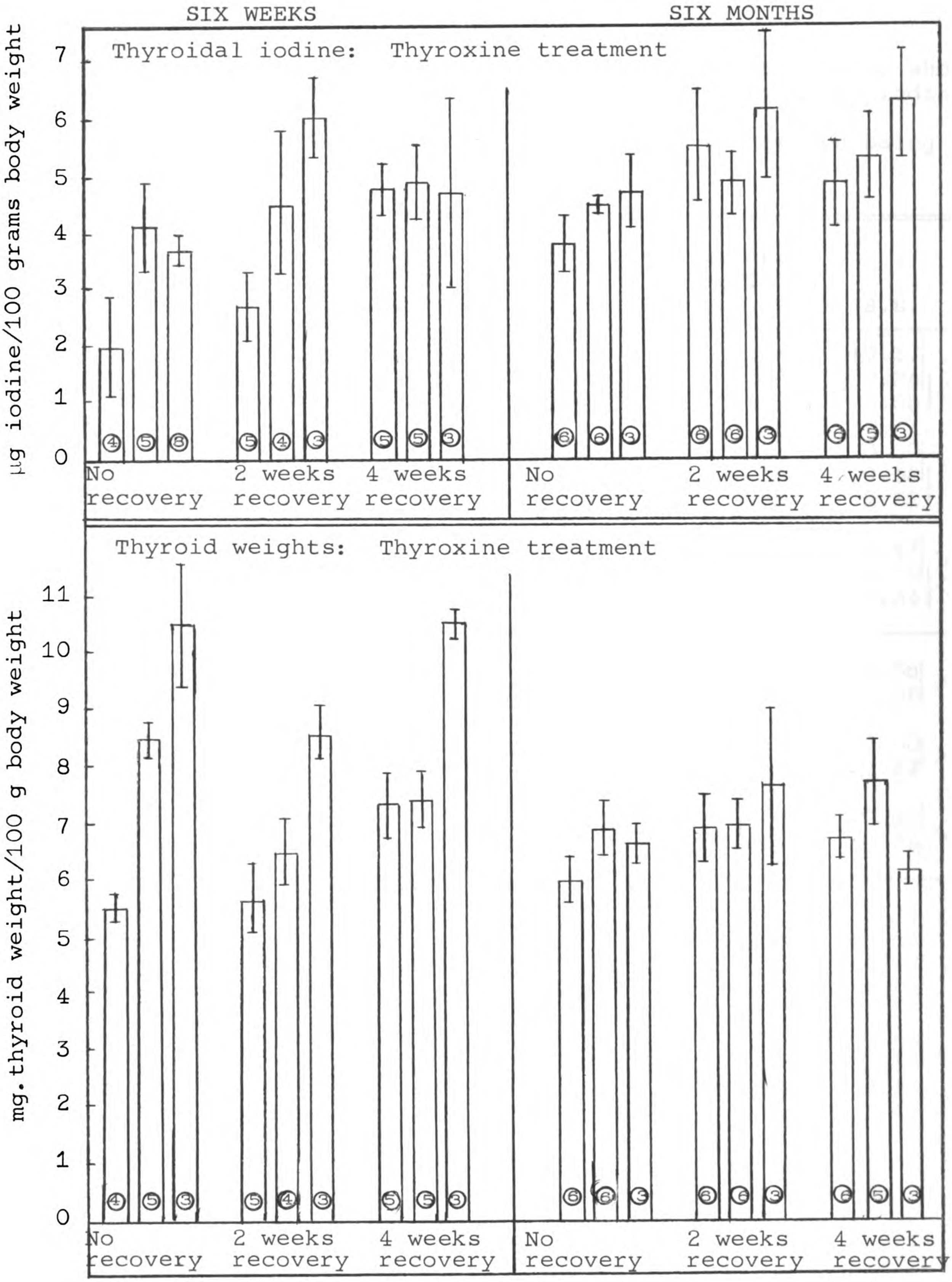


Fig. 6. Thyroidal iodine and thyroid weights for thyroxine test and control groups. The first bar in each group of three is the test group, the second is the counted control group, and the third is the normal control group. O = the number of rats in each group.

Table 10. Significance of difference between thyroxine short-term test and control groups for thyroidal iodine and weight.
The vertical bar (|) indicates the groups being compared.

THYROXINE: SHORT TERM Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	4.14	±0.74	>0.10	8.46	±0.37	<0.01
No recovery	2.00	0.93	>0.5	5.59	0.24	<0.01
Normal controls	3.61	0.34		10.56	1.05	
Counted controls	4.43	1.21	>0.1	6.45	0.60	>0.1
Two-week recovery	2.71	0.58	<0.01	5.63	0.58	<0.01
Normal controls	6.07	0.72		8.58	0.52	
Counted controls	4.84	0.61	>0.5	7.41	0.47	>0.5
Tour-wk. recovery	4.77	0.41	>0.5	7.35	0.56	<0.01
Normal controls	4.67	1.73		10.57	0.34	
Two-week recovery	2.71	0.58	<0.02	5.63	0.58	>0.05
Four-wk. recovery	4.77	0.41		7.35	0.56	
Two-wk. count.con.	4.43	1.21	>0.5	6.45	0.60	>0.1
Four-wk.count.con.	4.84	0.61		7.41	0.47	
Two-wk. nor. con.	6.07	0.72	>0.1	8.58	0.52	<0.02
Four-wk. nor. con.	4.67	1.73		10.57	0.34	
No recovery	2.00	0.93	>0.5	5.59	0.24	>0.5
Two-wk. -recovery	2.71	0.58		5.63	0.58	
No rec.count.con.	4.14	0.74	>0.5	8.56	0.37	<0.05
Two-wk.count.con.	4.43	1.21		6.45	0.60	
No recovery	2.00	0.93	<0.05	5.59	0.24	<0.05
Four-wk. recovery	4.77	0.41		7.45	0.56	
No rec. count. con.	4.14	0.74	>0.1	8.56	0.37	>0.1
Four-wk.count.con.	4.84	0.61		7.41	0.47	

Table 11. Significance of difference between thyroxine long term test and control groups for thyroidal iodine and weight.
The vertical bar (|) indicates the groups being compared.

THYRCXINE: LONG TERM

Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	4.54	±0.13	=0.1	6.89	±0.53	>0.1
No recovery	3.80	0.43	>0.1	6.02	0.40	>0.1
Normal controls	4.74	0.61		6.64	0.37	
Counted controls	4.90	0.47	>0.5	6.94	0.42	>0.5
Two-week recovery	5.55	1.03	>0.5	6.90	0.65	>0.5
Normal controls	6.19	1.30		7.64	1.44	
Counted controls	5.32	0.83	>0.5	7.67	0.72	>0.1
Four-wk. recovery	4.90	0.72	>0.1	6.68	0.44	>0.5
Normal controls	6.30	0.92		6.17	0.22	
Two-week recovery	5.55	1.03	>0.5	6.90	0.65	>0.5
Four-wk. recovery	4.90	0.72		6.68	0.44	
Two-wk. count.con.	4.90	0.47	>0.5	6.94	0.42	>0.1
Four-wk.count.con.	5.32	0.83		7.67	0.72	
Two-wk. nor. con.	6.07	0.72	>0.5	7.64	1.44	>0.5
Four-wk.nor. con.	4.67	1.73		6.17	0.22	
No recovery	3.80	0.43	>0.5	6.02	0.40	>0.1
Two-wk. recovery	5.55	1.03		6.90	0.65	
No rec. count. con.	5.54	0.13	>0.1	6.89	0.53	>0.5
Two-wk. count. con.	4.90	0.47		6.94	0.42	
No recovery	3.80	0.43	>0.5	6.02	0.40	>0.1
Four-wk. recov.	4.90	0.72		6.68	0.44	
No rec. count. con.	4.54	0.13	>0.1	6.89	0.53	>0.1
Four-wk. count. con.	5.32	0.83		7.67	0.72	

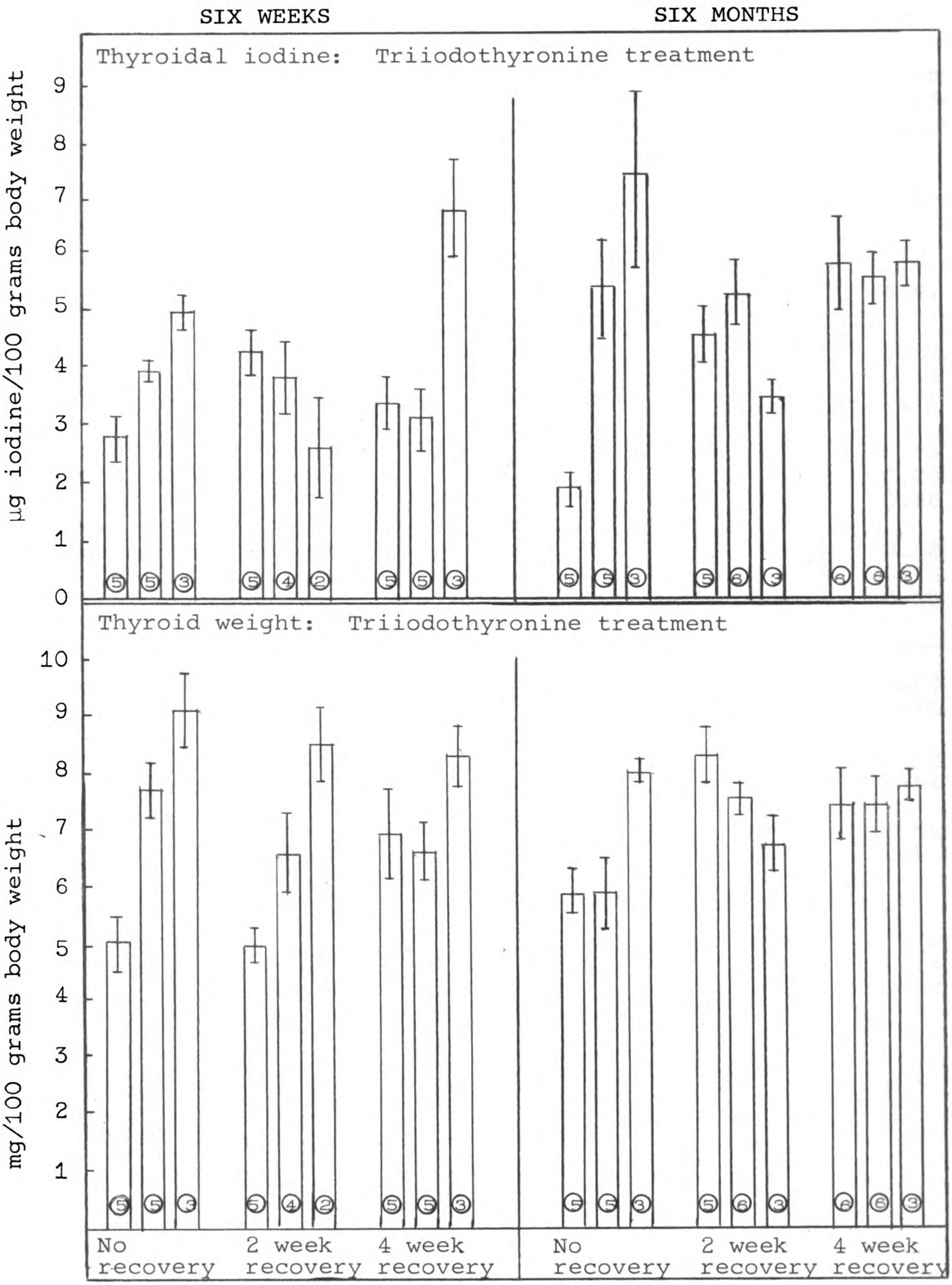


Fig. 7. Thyroidal iodine and thyroid weights for triiodothyronine test and control groups. The first bar in each group of three is the test group, the second is the counted control group, and the third is the normal control group. O = the number of rats in each group.

Table 12. Significance of difference between triiodothyronine short term test and control groups for thyroidal iodine and weight. The vertical bar (|) indicates the groups being compared.

TRIIODOTHYRONINE: Group	SHORT TERM Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	4.07	±0.11	< 0.02	7.69	±0.52	< 0.01
No recovery	2.92	0.37	< 0.01	5.02	0.48	< 0.01
Normal controls	5.11	0.28		9.11	0.71	
Counted controls	3.95	0.64	> 0.1	6.59	0.69	> 0.05
Two-week recovery	4.42	0.41	> 0.1	4.98	0.29	< 0.01
Normal controls	2.73	0.86		8.48	0.66	
Counted controls	3.25	0.57	> 0.5	6.60	0.52	> 0.5
Four-wk. recovery	3.51	0.48	< 0.02	6.97	0.75	> 0.1
Normal controls	6.82	0.86		8.25	0.55	
Two-week recovery	4.42	0.41	> 0.1	4.98	0.29	< 0.05
Four-wk. recovery	3.51	0.48		6.97	0.75	
Two-wk. count.con.	3.95	0.64	< 0.01	6.59	0.69	> 0.5
Four-wk. count.con.	3.25	0.57		6.60	0.52	
Two-wk. nor. con.	2.73	0.86	< 0.02	8.48	0.66	> 0.5
Four-wk. nor. con.	6.82	0.86		8.25	0.55	
No recovery	2.92	0.37	< 0.05	5.02	0.48	> 0.5
Two-wk. recovery	4.42	0.41		4.98	0.29	
No rec. count. con.	4.07	0.11	> 0.5	7.69	0.52	> 0.1
Two-wk. count. con.	3.95	0.64		6.59	0.69	
No recovery	2.92	0.37	> 0.1	5.02	0.48	> 0.05
Four-wk. recovery	3.51	0.48		6.97	0.75	
No rec. count. con.	4.07	0.11	> 0.1	7.69	0.52	> 0.1
Four-wk. count. con.	3.25	0.57		6.60	0.52	

Table 13. Significance of difference between triiodothyronine long term test and control groups for thyroidal iodine and weight. The vertical bar (|) indicates the groups being compared.

TRIIODOTHYRONINE: Group	LONG TERM Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	5.58	±0.77	< 0.01	5.85	±0.62	> 0.5
No recovery	2.02	0.25	< 0.01	5.88	0.45	< 0.01
Normal controls	7.59	1.56		8.04	0.21	
Counted controls	5.44	0.52	> 0.1	7.54	0.25	> 0.1
Two-week recovery	4.74	0.55	> 0.1	8.25	0.52	> 0.05
Normal controls	3.63	0.30		6.68	0.50	
Counted controls	5.69	0.45	> 0.5	7.41	0.51	> 0.5
Four-wk. recovery	5.87	0.81	> 0.5	7.41	0.63	> 0.5
Normal control	5.96	0.44		7.74	0.27	
Two-week recovery	4.74	0.55	> 0.1	8.25	0.52	> 0.1
Four-wk. recovery	5.87	0.81		7.41	0.63	
Two-wk. count. con.	4.74	0.52	> 0.5	7.54	0.25	> 0.5
Four-wk. count. con.	5.69	0.45		7.41	0.63	
Two-wk. nor. con.	3.63	0.30	< 0.01	6.68	0.50	> 0.1
Four-wk. nor. con.	5.96	0.44		7.74	0.27	
No recovery	2.02	0.25	< 0.01	5.88	0.45	< 0.01
Two-wk. recovery	4.74	0.55		8.25	0.52	
No rec. count. con.	5.58	0.77	> 0.5	5.85	0.62	< 0.05
Two-wk. count. con.	5.44	0.52		7.54	0.25	
No recovery	2.02	0.25	< 0.01	5.88	0.45	> 0.05
Four-wk. recovery	5.87	0.81		7.41	0.63	
No rec. count. con.	5.58	0.77	> 0.5	5.85	0.62	> 0.05
Four-wk. count. con.	5.69	0.45		7.41	0.51	

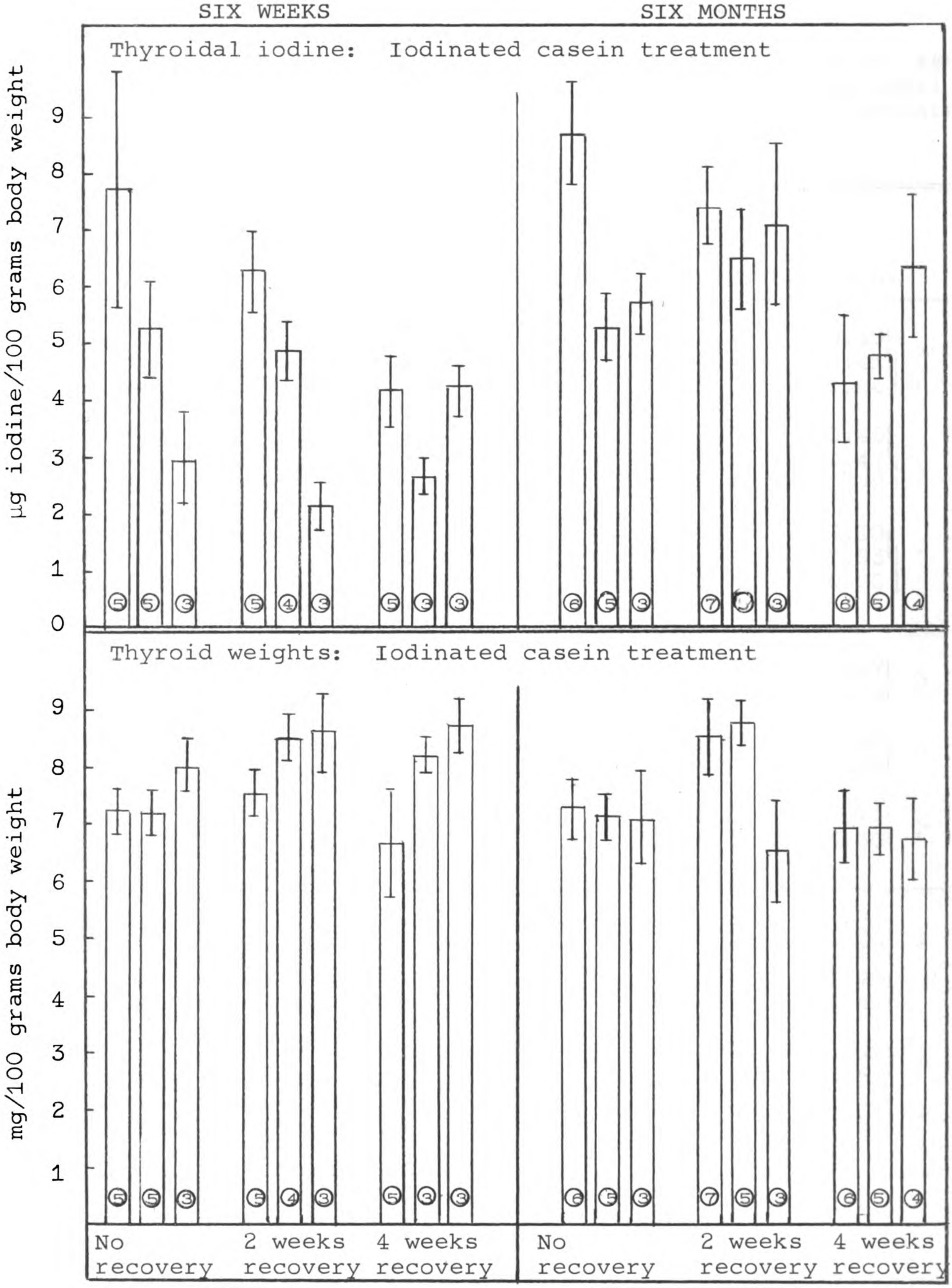


Fig. 8. Thyroidal iodine and thyroid weights for iodinated casein test and control groups. The first bar of the group of three is the test group, the second is the counted control group, and the third is the normal control group. O = the number of rats in each group.

Table 14. Significance of difference between iodinated casein short term test and control groups for thyroidal iodine and weight. The vertical bar (|) indicates the groups being compared.

IODINATED CASEIN: SHORT TERM

Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	5.27	±0.84	> 0.5	5.21	±0.40	> 0.5
No recovery	7.68	2.17	> 0.1	7.23	0.41	> 0.1
Normal controls	2.98	0.84		8.01	0.44	
Counted controls	4.90	0.54	> 0.1	8.52	0.40	> 0.1
Two-wk. recovery	6.34	0.72	< 0.01	7.50	0.47	> 0.1
Normal controls	2.17	0.45		8.61	0.72	
Counted controls	2.66	0.32	> 0.05	8.18	0.30	> 0.1
Four-wk. recovery	4.22	0.65	> 0.5	6.67	0.93	> 0.1
Normal controls	4.30	0.43		8.70	0.50	
Two-week recovery	6.34	0.72	> 0.05	7.50	0.47	> 0.1
Four-week recovery	4.22	0.65		6.67	0.93	
Two-wk. count. con.	4.90	0.54	< 0.02	8.52	0.40	> 0.5
Four-wk.count. con.	2.66	0.32		8.18	0.30	
Two-wk. nor. con.	2.17	0.45	< 0.02	8.61	0.72	> 0.5
Four-wk. nor. con.	4.30	0.43		8.70	0.50	
No recovery	7.68	2.17	> 0.5	7.23	0.41	> 0.5
Two-week recovery	6.34	0.72		7.50	0.47	
No rec. count.con.	5.27	0.84	> 0.5	7.21	0.40	> 0.05
Two-wk. count.con.	4.90	0.54		8.52	0.40	
No recovery	7.68	2.17	> 0.1	7.23	0.41	> 0.5
Four-wk. recovery	4.22	0.65		6.67	0.93	
No rec. count.con.	5.27	0.84	< 0.05	7.21	0.40	> 0.1
Four-wk.count.con.	2.66	0.32		8.18	0.30	

Table 15. Significance of difference between iodinated casein long term test and control groups for thyroidal iodine and weight. The vertical bar (|) indicates the groups being compared.

IODINATED CASEIN: LONG TERM						
Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
Counted controls	5.25	±0.58	< 0.01	7.17	±0.39	> 0.5
No recovery	8.68	0.93	< 0.05	7.31	0.47	> 0.5
Normal controls	5.78	0.53		7.10	0.84	
Counted controls	6.56	0.85	> 0.1	8.81	0.43	> 0.5
Two-week recovery	7.40	0.69	> 0.5	8.50	0.65	> 0.1
Normal controls	7.07	1.43		6.57	0.85	
Counted controls	4.83	0.40	> 0.1	6.97	0.36	> 0.5
Four-week recovery	4.27	1.04	> 0.5	6.93	0.62	> 0.5
Normal controls	6.42	1.25		6.73	0.72	
Two-week recovery	7.40	0.69	> 0.5	8.50	0.65	> 0.1
Four-week recovery	7.27	1.04		6.93	0.62	
Two-wk. count.con.	6.56	0.85	> 0.1	8.81	0.43	> 0.05
Four-wk.count.con.	4.83	0.40		6.97	0.36	
Two-wk. nor. con.	7.07	1.43	> 0.5	6.57	0.85	> 0.5
Four-wk. nor. con.	6.42	1.25		6.73	0.72	
No recovery	8.68	0.93	> 0.1	7.31	0.47	> 0.1
Two-week recovery	7.40	0.69		8.50	0.65	
No rec. count.con.	5.25	0.58	> 0.1	7.17	0.39	> 0.05
Two-wk. count.con.	6.56	0.85		8.81	0.43	
No recovery	8.68	0.93	> 0.1	7.31	0.47	> 0.5
Four-wk. recovery	7.27	1.04		6.93	0.62	
No rec. count.con.	5.25	0.58	> 0.5	7.17	0.39	> 0.5
Four-wk. count.con.	4.83	0.40		6.97	0.36	

corresponding T_4 and T_3 long term no recovery groups while counted control groups were not significantly different (Table 17). Short term I.C. two-week recovery group mean thyroidal iodine was also significantly increased over the parallel T_4 and T_3 groups (Table 16). The long term I.C. two-week recovery group was significantly increased over the corresponding T_3 group. It was also higher than the long term T_4 two-week recovery group but because of a large standard error for the T_4 group, the increase was not significant (Table 17).

Thyroid Weights

The mean thyroid weights of the short term T_4 and T_3 no recovery groups were significantly lower than their controls. The T_4 no recovery group had a mean weight of 5.59 mg/100 g B.W. compared to 8.46 mg/100 g B.W. for the control group at $P < 0.01$. The T_3 no recovery group had a mean weight of 5.02 mg/100 g B.W. compared to 7.69 mg/100 g B.W. for the control group at $P < 0.01$.

None of the mean thyroid weights from the two- and four-week recovery groups were significantly different from the mean thyroid weights of their control groups. There were some significant differences between the recovery groups and the no recovery groups.

In the T_4 test groups, the mean weight of the four-week recovery group was significantly increased above that

Table 16. Comparison of mean thyroidal iodine and weight between corresponding thyroxine, triiodothyronine, and iodinated casein short term test and control groups. The vertical bar (|) indicates the groups being compared.

SHORT TERM TEST AND CONTROL GROUPS						
Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
T_4 no recov. test	2.00	±0.93	>0.1	5.59	±0.24	>0.1
T_3 no recov. test	2.92	0.37		5.02	0.48	
T_4 no recov. cont.	4.14	0.74	>0.5	8.46	0.37	>0.1
T_3 no recov. cont.	4.07	0.11		7.69	0.52	
T_4 no recov. test	2.00	0.93	>0.05	5.59	0.24	<0.01
I.C. recov. test	7.68	2.17		7.23	0.41	
T_4 no recov. cont.	4.14	0.74	>0.1	8.46	0.37	<0.05
I.C. recov. cont.	5.27	0.84		7.21	0.40	
T_3 no recov. test	2.92	0.37	>0.1	5.02	0.48	<0.01
I.C. recov. test	7.68	2.17		7.23	0.41	
T_3 no recov. cont.	4.07	0.11	>0.1	7.69	0.52	>0.1
I.C. recov. cont.	5.27	0.84		7.21	0.40	
T_4 2-wk. recov. test	2.71	0.58	<0.05	5.63	0.58	>0.1
T_3 2-wk. recov. test	4.42	0.41		4.98	0.29	
T_4 2-wk. recov. cont.	4.43	1.21	>0.5	6.45	0.60	>0.5
T_3 2-wk. recov. cont.	3.95	0.64		6.59	0.69	
T_4 2-wk. recov. test	2.71	0.58	<0.01	5.63	0.58	<0.05
I.C.2-wk. recov.test	6.34	0.72		7.50	0.47	
T_4 2-wk. recov. cont.	4.43	1.21	>0.5	6.45	0.60	<0.05
I.E.2-wk. recov.cont.	4.90	0.54		8.52	0.40	
T_3 2-wk. recov. test	4.42	0.41	>0.05	4.98	0.29	<0.01
I.C.2-wk. recov.test	6.34	0.72		7.50	0.47	
T_3 2-wk. recov. cont.	3.95	0.64	>0.1	6.59	0.69	<0.05
I.C.2-wk. recov.cont.	4.90	0.54		8.52	0.40	
T_4 4-wk. recov. test	4.77	0.41	>0.05	7.35	0.56	>0.5
T_3 4-wk. recov. test	3.51	0.48		6.97	0.75	
T_4 4-wk. recov. cont.	4.84	0.61	>0.05	7.41	0.47	>0.1
T_3 4-wk. recov. cont.	3.25	0.57		6.60	0.52	
T_4 4-wk. recov. test	4.77	0.41	>0.5	7.35	0.56	>0.5
I.C.4-wk.recov. test	4.22	0.65		6.67	0.93	
T_4 4-wk. recov. cont.	4.84	0.61	<0.02	7.41	0.47	>0.1
I.C.4-wk. recov.cont.	2.66	0.32		8.18	0.30	
T_3 4-wk. recov. test	3.51	0.48	>0.1	6.97	0.75	>0.5
I.C.4-wk. recov.test	4.22	0.65		6.67	0.93	
T_3 4-wk. recov. cont.	3.25	0.57	>0.1	6.60	0.52	>0.05
I.C.4-wk. recov.cont.	2.66	0.32		8.18	0.30	

Table 17. Comparison of mean thyroidal iodine and weight between corresponding thyroxine, triiodothyronine, and iodinated casein long term test and control groups. The vertical bar (|) indicates the groups being compared.

	LONG TERM TEST AND CONTROL GROUPS					
Group	Iodine µg/100 g body wt.	S.E.	P	Weight mg/100 g body wt.	S.E.	P
T_4 no recov. test	3.80	±0.43	<0.01	6.02	±0.40	>0.5
T_3 no recov. test	2.02	0.25		5.88	0.45	
T_4 no recov. cont.	4.54	0.13	>0.1	6.89	0.53	>0.1
T_3 no recov. cont.	5.58	0.77		5.85	0.62	
T_4 no recov. test	3.80	0.43	<0.01	6.02	0.40	>0.05
I.C. no recov. test	8.68	0.93		7.31	0.47	
T_4 no recov. cont.	4.54	0.13	>0.1	6.89	0.53	>0.5
I.C. no recov. cont.	5.25	0.58		7.17	0.39	
T_3 no recov. test	2.02	0.25	<0.01	5.88	0.45	>0.05
I.C. no recov. test	8.68	0.93		7.31	0.47	
T_3 no recov. cont.	5.58	0.77	>0.5	5.85	0.62	>0.1
I.C. no recov. cont.	5.25	0.58		7.17	0.39	
T_4 2-wk. recov. test	5.55	1.03	>0.5	6.90	0.65	>0.1
T_3 2-wk. recov. test	4.74	0.55		8.25	0.52	
T_4 2-wk. recov. cont.	4.90	0.47	>0.5	6.94	0.42	>0.1
T_3 2-wk. recov. cont.	5.44	0.52		7.54	0.25	
T_4 2-wk. recov. test	5.55	1.03	>0.1	6.90	0.65	>0.1
I.C. 2-wk. recov.test	7.40	0.69		8.50	0.65	
T_4 2-wk. recov. cont.	4.90	0.47	>0.1	6.94	0.42	<0.02
I.C.2-wk. recov.cont.	6.56	0.85		8.81	0.43	
T_3 2-wk. recov. test	4.74	0.55	<0.02	8.25	0.52	>0.5
I.C.2-wk. recov.test	7.40	0.69		8.50	0.65	
T_3 2-wk. recov. cont.	5.44	0.52	>0.1	7.54	0.25	<0.05
I.C.2-wk.recov. cont.	6.56	0.85		8.81	0.43	
T_4 4-wk. recov. test	4.90	0.72	>0.1	6.68	0.44	>0.1
T_3 4-wk. recov. test	5.87	0.81		7.41	0.63	
T_4 4-wk. recov. cont.	5.32	0.83	>0.5	7.67	0.72	>0.5
T_3 4-wk. recov. cont.	5.69	0.45		7.41	0.51	
T_4 4-wk. recov. test	4.90	0.72	>0.05	6.68	0.44	>0.5
I.C. 4-wk. recov.test	7.27	1.04		6.93	0.62	
T_4 4-wk. recov. cont.	5.32	0.83	>0.5	7.67	0.72	>0.1
I.C.4-wk.recov. cont.	4.83	0.40		6.97	0.36	
T_3 4-wk. recov. test	5.87	0.81	>0.1	7.41	0.63	>0.5
I.C. 4-wk.recov.test	7.27	1.04		6.93	0.62	
T_3 4-wk. recov.cont.	5.69	0.45	>0.1	7.41	0.51	>0.5
I.C. 4-wk.recov.cont.	4.83	0.40		6.97	0.36	

of the no recovery group in the short term experiment (7.45 mg/100 g B.W. for the four-week recovery group and 5.59 mg/100 g B.W. for the no recovery group at $P < 0.05$). (Figure 6 and Tables 10-11.)

In the T_3 test groups, the four-week recovery group was significantly increased in mean weight over the two-week recovery group in the short term experiment (6.97 mg/100 g B.W. for the four-week group and 4.98 mg/100 g B.W. for the two-week group at $P < 0.05$) and the two-week recovery group (8.25 mg/100 g B.W.) was significantly increased over the no recovery group (5.88 mg/100 g B.W.) at $P < 0.01$ in the long term experiment (Figure 7 and Tables 12-13).

There were no significant differences between any I.C. test group or control groups (Figure 8 and Tables 14-15).

The mean weight of the no recovery and two-week recovery short term I.C. groups were significantly higher than their corresponding T_4 and T_3 groups compared to no significant difference between corresponding control groups (Table 17).

The thyroid glands of the long term no recovery group fed triiodothyronine were large and pale in appearance and were soft and friable in texture. No other glands in any group had this distinctive gross appearance.

Total Iodine Output and Thyroxine Equivalents (TSR)

Total iodine output and thyroid hormone secretion rates expressed as thyroxine equivalents (TSR) are presented in

Table 18. Total iodine output values for all short and long term two-week recovery groups were significantly less than their controls ($\leq$ 0.17 μg I/da/100 g B.W. compared to $\geq$ 0.49 μg I/da/100 g B.W. for control groups at $P < 0.05$).

By the fourth week of recovery, the total iodine output values of test groups were slightly but not significantly higher than their controls except for the long term T_3 four-week recovery group which was still significantly lower than the control group (0.45 μg I/da/100 g B.W. compared to 0.62 μg I/da/100 g B.W. for the controls at $P < 0.05$).

The thyroxine equivalents (TSR) ranged from less than 0.25 μg T_4 eq./da/100 g B.W. to 0.46 μg T_4 eq./da/100 g B.W. for three of the six two-week recovery groups that had uptakes high enough so that output could be calculated. This compared to a range of 1.00-1.78 μg T_4 eq./da/100 g B.W. for the controls.

Four-week recovery group values ranged from 1.06-1.90 μg T_4 eq./da/100 g B.W. compared to 0.95-1.53 μg T_4 eq./da/100 g B.W. for the control groups.

HISTOLOGY

Findings are recorded in Table 19. Fig. 9 shows photomicrographs of typical examples of recovering thyroid glands and controls. All no recovery groups had glands filled with very large follicles containing solid masses of colloid and lined with squamous epithelium. Occasionally a follicle appeared to be partially depleted of colloid but it did not

Table 18. Significance of difference between total iodine output of thyroxine, triiodothyronine, and iodinated casein test and control groups and the thyroxine equivalents (TSR) for each test and control group. The vertical bar (|) indicates the groups being compared.

GROUP	Total Iodine Output µg/da/100 g B.W.	S.E.	P	T_4 equiv. (µg/100 g B.W.) (TSR)
T_4 SIX-WEEK EXPERIMENT				
2-wk. recov. test gp.	0.11	±0.03	< 0.05	0.25
Control	0.50	0.15		1.20
4-wk. recov. test gp.	0.80	0.13	> 0.1	1.90
Control	0.65	0.09		1.53
T_4 SIX-MONTH EXPERIMENT				
2-wk. recov. test gp.	0.16	0.03	< 0.01	0.43
Control	0.49	0.04		1.15
4-wk. test gp.	0.46	0.06	> 0.5	1.09
Control	0.45	0.09		1.09
T_3 SIX-WEEK EXPERIMENT				
2-wk. recov. test gp.	----	----	----	----
Control	0.52	0.09		1.00
4-wk. recov. test gp.	0.46	0.07	> 0.5	1.10
Control	0.40	0.07		0.95
T_3 SIX-MONTH EXPERIMENT				
2-wk. recov. test gp.	0.18	0.05	< 0.01	0.42
Control	0.55	0.05		1.31
4-wk. recov. test gp.	0.45	0.05	< 0.05	1.06
Control	0.62	0.05		1.49
I.C. SIX-WEEK EXPERIMENT				
2-wk. recov. test gp.	----	----	----	----
Control	0.75	0.01		1.78
4-wk. recov. test gp.	0.45	0.06	> 0.5	1.06
Control	0.42	0.12		0.99
I.C. SIX-MONTH EXPERIMENT				
2-wk. recov. test gp.	----	----	----	----
Control	0.52	0.05		1.22
4-wk. recov. test gp.	0.70	0.10	> 0.1	1.86
Control	0.60	0.07		1.43

Table 19. Histological evaluation of functional state of rat thyroid glands after 2 and 4 week recovery periods following 6 weeks and 6 months on feed containing thyroxine, triiodothyronine, or iodinated casein.

	SIX WEEKS ON EXPERIMENTAL DIET			SIX MONTHS ON EXPERIMENTAL DIET		
	T_4	T_3	I.C.	T_4	T_3	I.C.
No recovery	-	-	-	-	-	-
Counted controls	+	+	+	+	+	+
Normal controls	+	+	+	+	+	+
2 week recovery	+ -	+ -	+ -	+ -	+ -	+ -
Counted controls	+	+	+	+	+	+
Normal controls	+	+	+	+	+	+
4 week recovery	+ -	+ -	+ -	+ -	+	+ -
Counted controls	+	+	+	+	+	+
Normal controls	+	+	+	+	+	+

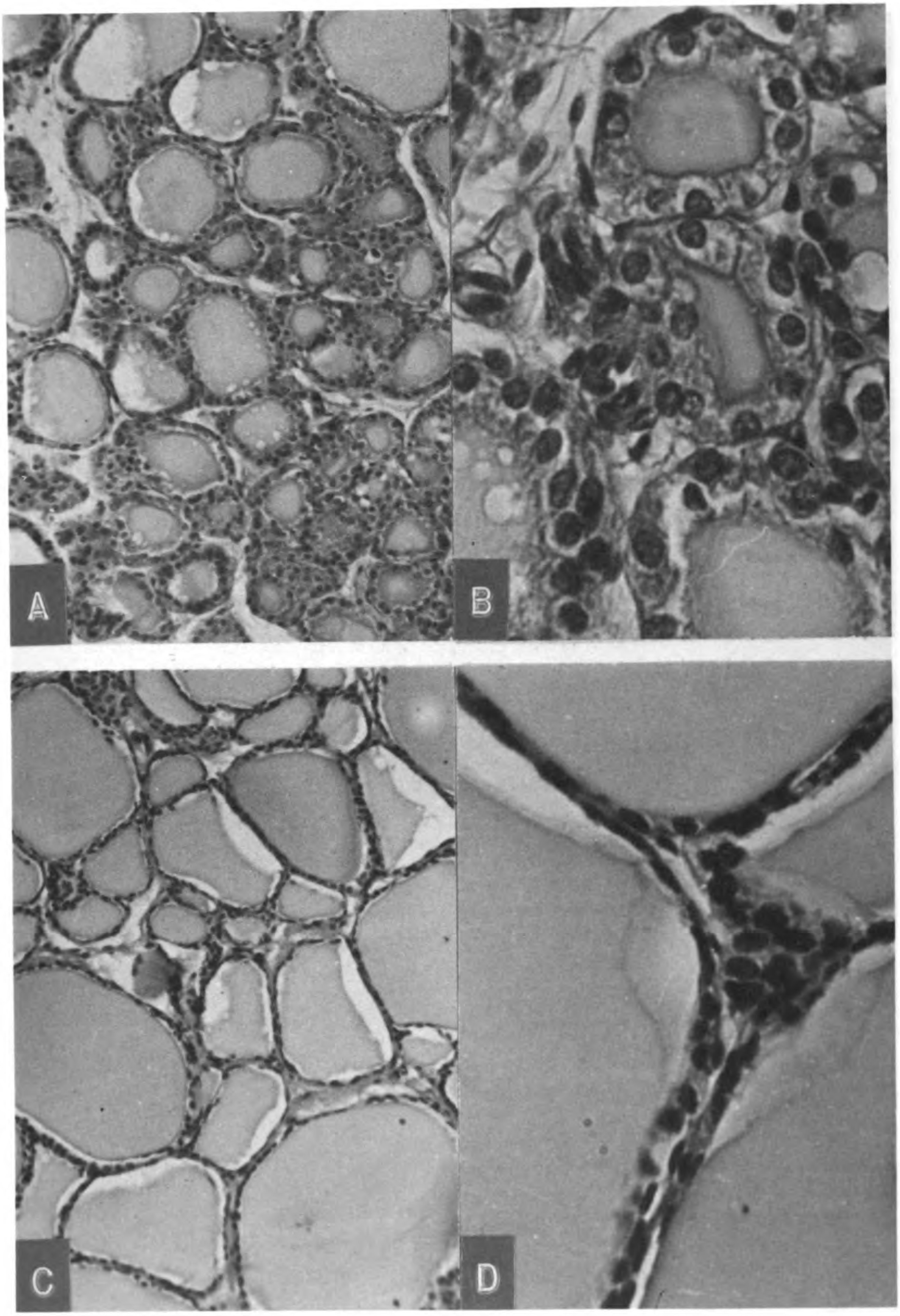
KEY: - Inactive gland
1. Squamous epithelium.
2. All follicles large and filled with colloid.
3. Little or no vacuolization in colloid.

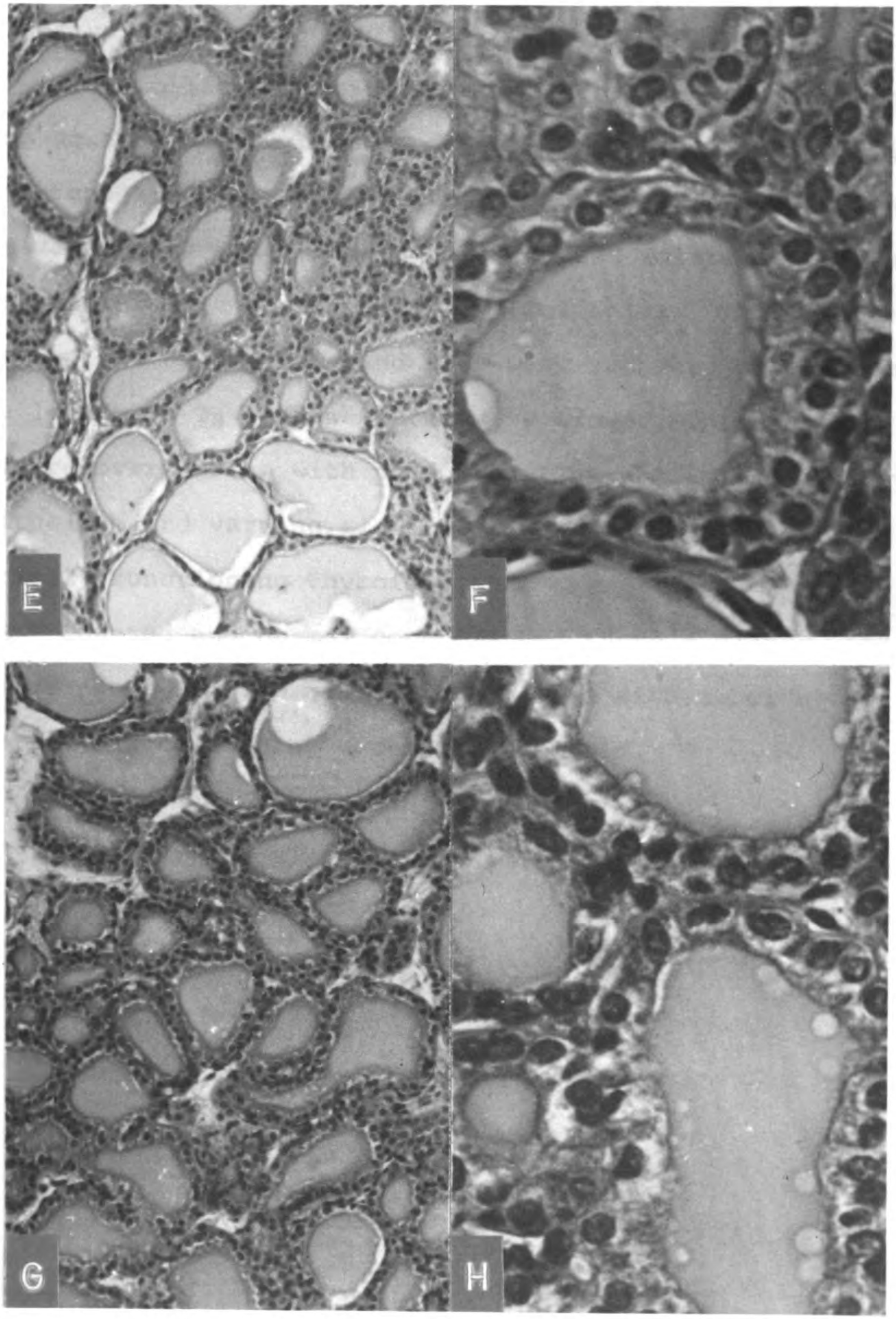
± Recovering gland
1. Range from squamous to cuboidal to columnar epithelium.
2. Follicles large and full.
3. Much enzymatic action as shown by vacuolated colloid.

\+ Active gland
1. Cuboidal epithelium; a few squamous and high cuboidal.
2. Some large follicles at periphery; otherwise most follicles are medium and small.
3. Some vacuolization of colloid.

Fig. 9. Photomicrographs of typical examples of thyroid glands in the suppressed, recovering, and normal states. The "+" or "-" symbols indicate the evaluation system followed in Table 18.

Key:
A. + Active gland: normal control (X198)
B. + Active gland: normal control (X818)
C. - Inactive gland (X198)
D. - Inactive gland (X818)
E. ± Recovering gland (X198)
F. ± Recovering gland (X818)
G. + Active gland: after four weeks of recovery (triiodothyronine) (X198)
H. + Active gland: after four weeks of recovery (triiodothyronine) (X818)


A
B
C
D


E
F
G
H

have the vacuolated appearance typical of colloid in a normally active gland.

The follicular epithelium in recovering glands ranged from squamous through low and high cuboidal to columnar in different follicles within the same gland. The colloid had the vacuolated appearance seen in active follicles and was one-half or more depleted in some follicles. This picture was typical of all two- and four-week recovery groups except the long term T_3 four-week recovery group. This was the only recovery group with predominantly cuboidal folliclar epithelium and varying sized follicles indicative of a normally functioning thyroid gland.

Counted and normal controls had thyroid glands with a normal range in size of follicles lined with cuboidal epithelium and containing vacuolated colloid.

DISCUSSION

Many of the differences between test and control groups are not statistically significant because of large standard errors due to one very high or very low value in the group and to small numbers of rats in some groups. The following may account for some of these variations:

More than one-half of all the animals used in this experiment showed varying degrees of lymphocyte infiltration and fibrosis upon histological examination of the thyroid. Since these changes were noted in normal controls receiving no radioiodine or nembutal as well as in counted controls, they cannot be attributed solely to the possible degenerative effects of these two materials. The rats suffered periodically from a respiratory virus that was evidenced by sore eyes and wheezing, especially when they were subjected to stresses such as temperature variations and anesthesia. When room temperatures fluctuated during counting periods, the combination of stresses resulted in some scattering of points on the output curves and caused the death of some rats, resulting in smaller groups. Temperature changes are noted in Appendix A.

The question arises as to whether these occasional individual differences in the various parameters of thyroid function exhibited by these rats in this experiment are inherent in the rat, due to the viral disease or environmental conditions, or some combination of these. High temperatures depress the thyroid function of rats (Dempsey and Astwood, 1943) and this depression was observed several times when the room temperature rose during counting periods.

In spite of these problems there were definite indications of differences in the recovery of the thyroid gland after inhibition by the three different thyroid inhibiting substances and in the reliability of the various parameters of thyroid function in assessing the functional state of the gland.

All comparisons have been made between test animals and counted controls. Thyroidal iodine and thyroid weights for normal controls have been included on the graphs (Fig. 6-8), but because group numbers were small (2 or 3) and these controls were not exposed to the stresses inherent in the radioiodine counting procedure, it was felt that valid comparisons could not be justified.

Throughout this paper, the terms inhibition and suppression have been used synonamously to mean depression of thyroid function by thyroidal substances given in excess of the thyroxine secretion rate.

The Inhibitory Doses

The inhibitory doses of thyroidal substances observed in the preliminary experiment (2.5 μg T_4/100 g B.W., 0.63 μg

T_3/100 g B.W., and 0.37 mg I.C./100 g B.W.) reflect the increased dosages necessary because of incomplete absorption from the gut compared to the lower dosages when administered by injection. Purves (1964) has reported the continuous suppression dose rates to be 1.5 μg/100 g/da for thyroxine and 0.3 μg/100 g/da for triiodothyronine at 25° C for both. Von Euler and Swartling (1960) found that 2.85 μg thyroxine per day at 28° C was necessary for depression of the gland. Thyroidectomized rats required 3 μg thyroxine per day according to Leblond and Eartly (1952).

Because of variations in the amount of feed consumed and problems inherent in mixing small amounts of the inhibiting substances in large lots (100 g) of feed, the amounts of thyroxine and triiodothyronine were increased by four and of iodinated casein by three times the inhibiting dose. There still remained a small iodine turnover also noted previously by Reineke and Singh (1955) in glands inhibited by thyroxine equal to the thyroxine secretion rate. Purves (1964) too observed that a certain low level of synthetic and secretory activity remained as an intrinsic property of normal thyroid tissue.

The amounts of thyroxine, triiodothyronine, and iodinated casein/100 g body weight that the rats were getting based on their weights at the end of the six-week and six-month test periods are listed in Table 2. These calculations are based on the assumption that each rat ate 10 grams of feed per day, though, in fact, as they grew older they ate less feed per day. Dosages expressed as per cent of feed are listed in Table 3. The figures in these two tables confirm that dosages of the three suppressive agents were within physiological limits.

The Inhibited Gland

Parameters of thyroid function involving the use of radioiodine were not determined at the end of the suppression period (i.e. on the "no recovery" groups). Only thyroidal iodine, thyroid weights, and histology were used to assess the state of the glands of these groups.

<u>The thyroxine no recovery group</u>:

The total thyroidal iodine concentrations tended to be lower than controls in the thyroxine no recovery groups, but these decreases were not significant because of the variation between individuals (Tables 10 and 11). The short term no recovery group had a mean thyroidal iodine content significantly less than the four-week recovery group but this was not so for the long term groups. The thyroidal iodine content of the long term T_4 no recovery group appears to be comparatively high.

Although mean thyroid weight appears to be significantly reduced in the short term thyroxine no recovery group compared to controls, the differences in mean thyroid weights between control groups raised in the same cage (and killed within two weeks of each other) discount the significance of the weight difference between the test and control group in this instance (Tables 10-11). Mean thyroid weights of the long term no recovery group are no different from the thyroid weights of the controls.

The histological sections of thyroid glands from these no recovery animals show the typical picture of the gland in the resting state and can easily be distinguished from sections made of recovering and normal glands.

The triiodothyronine no recovery group:

The thyroidal iodine concentrations were significantly lower than in controls for the triiodothyronine short and long term groups (Tables 12 and 13). The short term triiodothyronine group was not significantly different from the short term thyroxine group, but the long term triiodothyronine group was significantly lower than the corresponding thyroxine group (Tables 16 and 17).

The mean thyroid weight of the short term triiodothyronine group was significantly lower than the control group while the long term test group was almost the same as the control group (Tables 12 and 12). However, the mean thyroid weight of this long term control group was significantly lower than the two-week control group which was raised in the same cage and killed only two weeks later. Thus, as in the case of the thyroxine controls, the variation in thyroid weights of individuals reduces the reliability of these data in evaluating thyroid function.

Histological sections of the triiodothyronine-treated animals also reveal the gland to be in a resting state when allowed no recovery period.

<u>The iodinated casein no recovery group</u>:

The total thyroidal iodine concentration of the short term iodinated casein group was higher than the control group but was not significantly so because of a high standard error for the test group and variation between control groups as in the case of thyroxine mentioned previously. On the other hand, the value for the long term group was significantly higher than for the controls and the controls in this case did not show the variation observed in controls discussed previously (Tables 14 and 15).

The iodinated casein groups had iodine concentrations two to three times higher than the total thyroidal iodine concentrations for the thyroxine and triiodothyronine groups (Tables 16 and 17). The increased thyroidal iodine concentration in these rats can be accounted for by the high iodine content of the iodinated casein and the fact that the concentrating mechanism for iodine is not blocked.

The thyroid weights of the iodinated casein no recovery groups were not significantly different from the controls and the controls again did not show a wide variation in thyroid weights.

The histological sections showed the gland to be in the resting state the same as sections from the thyroxine- and triiodothyronine-treated glands.

The variation observed between control animals precludes the use of thyroidal iodine concentration as a test of thyroid function in individual rats but it may serve as an indicator of decreased thyroid function in thyroxine- and triiodothyronine-treated rats when large numbers of animals are used.

The flattening of the output curve at the start of the experiment together with thyroid histology provides substantial evidence that these glands were inhibited. If a low level of activity had continued throughout the suppression period, it could not be determined from these data.

The Recovering Gland at Two Weeks

During the two-week recovery period, radioiodine uptake and output were very low for all groups. The delayed uptake and low levels of iodine collection may be the result of low levels of TSH after prolonged periods of suppression. According to Purves (1964), this delay in resumption of thyrotrophin secretion may be the result of regressive changes in the thyrotrophs under conditions of continuous suppression.

<u>The thyroxine-treated two-week recovery group</u>:

The two-week recovery picture was essentially the same for both the short and long term thyroxine test groups (Fig. 3). Output rates were depressed to approximately one-fourth of the original output rates (Tables 4 and 5). Per cent radioiodine uptakes were reduced to about seven and ten per cent of their original levels and half-times were three and four times longer than before the thyroxine treatment for the short and long term groups (Tables 4 and 5).

Total thyroidal iodine (Tables 10 and 11) remained low at the end of the two-week recovery period in the short term group but the high standard error in the control group clouds the significance of this value. In the long term experiment the thyroidal iodine was higher than controls but there was a high standard error in the test group.

Thyroid weights were not significantly different from control groups (Tables 10 and 11).

Histologically these thyroxine-treated two-week recovery glands would have to be considered as at least partially functioning. According to Cowdry (1960), increased activity in a thyroid follicle is characterized by a decrease in the amount of colloid and an hypertrophy of the epithelium. Only a few follicles in some of the histological sections from these glands that have been recovering for two weeks showed this complete picture. There was an increase in vacuolization and a decrease in the amount of colloid compared to the no recovery groups, but follicles were still enlarged and only a very few in all of the slides showed hypertrophy of the epithelium. Many follicles were still inactive as judged by the squamous epithelium. The entire gland could be considered only as recovering with perhaps occasional follicles showing increased activity.

<u>The triiodothyronine two-week recovery group</u>:

There was more variation in the delay of resumption of I^{131} uptake between individuals in the short term triiodothyronine two-week recovery group than in the long term group (Appendix I). The radioiodine uptake for the short term group was reduced to five per cent of the initial uptake and half-time was two and one-half times longer than originally. Because the mean initial uptake in the long term group was low, the per cent recovery of the initial uptake (13%) was high even though the actual mean uptake value was within one per cent of the short term T_3 value and both short and long term T_4 values. Output rate for the long term T_3 group was one-fourth of the initial output rate and half-time was twice as long as before treatment and two weeks of recovery (Tables 6 and 7).

Total thyroidal iodine values were slightly higher than controls for the short term and slightly lower for the long term groups (Tables 12 and 13). The short term triiodothyronine thyroidal iodine value was almost twice as high as the short term thyroxine value after two weeks of recovery. There was no significant difference between the long term group values for T_4 and T_3, but the long term thyroxine group had a large standard error.

Thyroid weights tended to be low in the short term triiodothyronine group and high in the long term group compared to controls and were within the weight range of the corresponding thyroxine groups (Tables 12 and 13).

Histologically there was no difference in the triiodothyronine-treated glands at two weeks of recovery compared to the thyroxine-treated glands.

Radioiodine half-times were shorter and output rates therefore higher for the triiodothyronine-treated two-week recovery groups than for the thyroxine-treated groups. This indication of an earlier recovery from the suppressed state is not unexpected since triiodothyronine is metabolized faster than thyroxine.

Total thyroidal iodine data tend to confirm this earlier recovery for triiodothyronine-treated rats in the short term experiment, but these data are not consistent when comparing thyroxine- and triiodothyronine-treated rats in the long term experiment. In this case, thyroidal iodine is higher for T_4-treated rats at two weeks of recovery than for the T_3-treated rats.

<u>The iodinated casein two-week recovery group</u>:

The delayed and low level of radioiodine collection by iodinated casein suppressed rats was more pronounced than for either thyroxine or triiodothyronine suppressed animals (Appendix I). The high iodine content of iodinated casein has increased the extrathyroidal as well as the intrathyroidal iodine concentration far above the normal so that per cent of uptake is reduced below one per cent of the injected dose. The output curves appeared to be beginning to level off by the end of the two-week counting period for two or three animals in each of the short and long term two-week recovery groups, but half-times and therefore output rates could not be determined.

The resumption of output may be delayed because of the inhibitory effects of the high iodine concentration on the reaction leading to the iodination of the phenolic group of tyrosine and to the conversion of DIT to thyroxine (Wolff and Chaikoff, 1948). This inhibitory action has been reported by Wolff et al. (1949) as being temporary in nature lasting only 26 hours after an inhibiting dose of iodide. Probably, in this experiment, the combination of the suppressive actions of the thyroprotein and the iodine in iodinated casein on both the pituitary and the thyroid, together with the extended periods of suppression, have contributed to the delay in resumption of thyroid function.

Both the short and the long term iodinated casein groups had total thyroidal iodine concentrations two to three times higher than the thyroxine and triiodothyronine groups (Tables 16 and 17).

As in the groups mentioned previously, thyroid weights were not significantly different from those of control groups (Tables 14 and 15).

Histologically, the iodinated casein-treated groups were no different from the thyroxine- and triiodothyronine-treated groups.

Short term compared to long term suppression:

The radioiodine output curves for individual rats (Appendix I) in the two-week recovery groups show an increased variation between individual rats following the long term suppression period as compared to the short term suppression period.

The Recovering Gland at Four Weeks

The thyroxine-treated four-week recovery group:

By the end of the four-week recovery period for the thyroxine-treated test groups, the radioiodine output rates and half-times were not significantly different from control values or pretest values for either the short or long term groups (Tables 4 and 5). Radioiodine uptake was increased over pretest values for both the test and control groups. This increase was significant for the long term group but not for the short term group because of a large standard error for the short term test group. This tendency to a rebound in uptake confirms observations made by Yamada *et al*. (1961) and Greer and Johnston (1951) and is more pronounced in this experiment after long term suppression by thyroxine.

The rebound in thyroid weight also observed by Yamada *et al*. (1961) was not confirmed in this experiment. Thyroid weights as well as total thyroidal iodine values had returned to the normal range by the end of the four-week recovery period (Tables 10 and 11). Total iodine output values and thyroxine equivalent values (TSR) were also within the range of the controls by this time.

Histologically, these four-week recovery glands appeared to be in the same state of recovery as described for the two-week recovery glands with some follicles still enlarged and lined with squamous epithelium.

The delay in thyrotrophin secretion after prolonged suppression which was postulated by Purves (1964) was not observed in these rats even after six months of suppression.

The triiodothyronine-treated four-week recovery group:

Evaluating the radioiodine data for the triiodothyronine test and control groups is difficult because of the significant differences getween pretest values for the test and control groups. In addition, output and uptake values for the short term test group were higher than those for the control group while half-time values were lower for the test group and higher for the control group. The opposite was true for the long term experiment. Output and uptake values were low for the test group and high for the control group while the half-time value was high for the test group and low for the control group. Because of these differences, valid comparisons cannot be made between test and control groups. Radioiodine output rates decreased from pretest values for test groups and increased for control groups in both the short and long term experiments but changes were not significant (Tables 6 and 7). The longer half-times observed were significant only for the short term group. Radioiodine uptake was increased in both test groups. This increase was significant for the long term group but uptake values for the control group also increased significantly (Table 7).

Total thyroidal iodine and thyroid weight values were very close to values for the counted controls (Tables 11 and 12).

The total iodine output value for the short term group was not significantly different from the control value, but it was still significantly low for the long term group compared to its control. However, the long term value was almost the same as the short term group value; the long term control value was high compared to the short term control group value (Table 18).

Thyroid sections from the short term triiodothyronine four-week recovery animals showed the same recovering picture seen in the thyroxine test groups and in the two-week recovery triiodothyronine test groups. Thyroid sections from the long term four-week triiodothyronine recovery group could not be distinguished from sections of normal glands.

As mentioned previously, an earlier recovery for the triiodothyronine-treated groups is to be expected. This earlier recovery was not apparent histologically until the fourth week of recovery and then only in the long term group. The output rates and uptakes were lower and the half-times longer for the triiodothyronine four-week recovery groups compared to the thyroxine four-week recovery groups, but the total iodine output values and the thyroxine equivalents (TSR) were very nearly the same by the end of the four-week recovery period.

Whether or not the increase in radioiodine uptake could be the rebound phenomenon described by Rich (1958) following treatment with triiodothyronine is difficult to say because of the increases observed also in the controls.

The iodinated casein-treated four-week recovery group:

By the end of the four-week recovery period, the radioiodine output, uptake, and half-time values were not significantly different from the pretest values for both the short and long term recovery groups (Tables 8 and 9). By this time, the total thyroidal iodine and thyroid weight values were within the range of the control values (Tables 14 and 15) and of the values for thyroxine and triiodothyronine after four weeks of recovery (Tables 16 and 17).

The total iodine output value tends to be high for the long term group as it was also in the short term thyroxine four-week recovery group, but the control value was also high in each case.

The histological picture for both the short and long term iodinated casein four-week recovery groups again was that of a recovering gland.

Sensitivity of Parameters of Thyroid Function

The sensitivity for the various parameters of thyroid function following suppression by thyroxine, triiodothyronine, or iodinated casein is (in decreasing order) (1) the rate of release of thyroidal radioiodine, (2) thyroidal uptake of radioiodine, (3) total thyroidal iodine, (4) thyroid weight, and (5) thyroid histology.

This is in partial agreement with work reported by Yamada et al. (1961) for suppression by thyroxine in which he too found the output rate to be the most sensitive and thyroidal iodine, thyroid weight, and histology to be the least sensitive parameters for assessing thyroid function.

Thyroidal iodine was a good indicator of decreased function for glands suppressed by thyroxine or triiodothyronine and allowed no recovery period, but not for those thyroids suppressed by iodinated casein.

The histological picture is well defined for glands suppressed continuously by thyroxine, triiodothyronine, or iodinated casein but not for determining the degree of recovery of function after suppression.

SUMMARY AND CONCLUSIONS

The thyroid gland of the rat recovers normal function by the third or fourth week following up to six months suppression by oral administration of thyroxine, triiodothyronine, or iodinated casein as determined by radioiodine output, radioiodine uptake, radioiodine biological half-time, total thyroidal iodine, thyroid weight, and thyroid histology.

Thyroidal radioiodine output was the most sensitive parameter for evaluating thyroid function followed in sensitivity by radioiodine uptake and half-time, total thyroidal iodine, thyroid weight, and histology.

Low thyroidal iodine concentrations were indicative of glands suppressed by thyroxine or triiodothyronine and allowed no recovery time, but there was considerable variation between individuals within a group. Thyroidal iodine concentration was increased above normal in glands suppressed by iodinated casein.

Thyroid weights also varied within groups and changes in thyroid weights were not consistent for control groups.

Histological examination easily identified glands suppressed and allowed no recovery period.

APPENDIX A

Record of room temperatures during counting periods

	First count °F	Second count °F
Thyroxine		
ST 2-wk. recov.	81	78-70-75
ST 4-wk. recov.	81	
LT 2-wk. recov.		78-80
LT 4-wk. recov.		78-80 before counting
Triiodothyronine		
ST 2-wk. recov.	81	78-70-75
ST 4-wk. recov.	81	
LT 2-wk. recov.		78-80
LT 4-wk. recov.		78-80 before counting
Iodinated casein		
ST 2-wk. recov.	74-79	82-85-78
ST 4-wk. recov.		
LT 2-wk. recov.		
LT 4-wk. recov.		

Record of room temperatures that fluctuated from $75^{\circ} \pm 1^{\circ}$ F. during counting periods.

APPENDIX B

RAT FEED MIXTURE SR-1

Add 0.5 lb. of special mineral salt Premix SR-1 per each 100 lbs. of basic feed mix and mix thoroughly.

A. Basic feed mix:

Ingredient	Amount/100 lbs. of mix
1. Shelled yellow corn ground through 1/8" screen	68.9 lbs.
2. Soybean oil meal (50% protein)	28.0 lbs.
3. Dicalcium phosphate	1.8 lbs.
4. Limestone	0.6 lb.
5. Dawes & Forbes Vitamin B Supplement	0.1 lb.
6. Dawes & Forbes Vitamin B_{12} Supplement	0.2 lb.
7. Standard Brands 9F yeast 9000 i.u. (Vit. D_2/g)	5.0 grams
8. Pfizers Vitamin A Supplement (10,000 i.u. Vit. A/g)	15.0 grams

B. Mineral Salt Premix SR-1:

Element	% Element	Compound	grams/10 lbs.
1. Iodine	0.010	KI-10% CA stearate (Pfizer)	0.653
2. Zinc	0.800	$ZnSO_4 \cdot 7H_2O$ (Baker)	160.574
3. Manganese	0.542	$MnSO_4 \cdot H_2O$ (Baker)	73.12
4. Iron	0.270	$FeSO_4 \cdot 7H_2O$ (Baker)	60.782
5. Copper	0.054	$CuSO_4$ (anhydrous Baker)	6.169
6. Sodium chloride (plain)		(Morton's)	4234.72

APPENDIX C

IODINE DETERMINATION

In this modification of Barker's method (1948) for determination of protein-bound iodine, the dry ashing technique with Na_2CO_3 has been substituted for the distillation step in determining total thyroidal iodine (Reineke, unpublished).

I. Iodine determination

Place 1 ml of 4 N Na_2CO_3 into a Pyrex test tube.
Add weighed ½ of thyroid to tube. Place in drying oven overnight at 90-95° C.
Dried residue is then incinerated in the muffled furnace for 2-2½ hours at 600°C.

II. Dissolving iodine from ash.

After incineration, add:
2.0 ml 2 N HCl
2.0 ml 7 N H_2SO_4
21.0 ml glass distilled water
After making up to a volume of 25.0 ml, mix well with a glass stirring rod and centrifuge 20 min.

III. Colorimetry

Pipette 1 ml of the digest into colorimeter tubes in duplicate. Add 4.0 ml of glass distilled water to bring up to a volume of 5 ml.
Add:
0.5 ml arsenious acid solution from a blowout pipette. Place in a water bath for 15 min. at 27°C.
Then add:
0.5 ml ceric ammonium sulfate at 1 minute intervals. Let stand in bath exactly 15 minutes from time of first addition.
After 15 min. add 0.5 ml of bruccine and read in the Coleman Universal Spectrophotometer at a wavelength of 480 millimicrons.

APPENDIX D

GROUP	Count #1		Count #2		Count #3		Count #4		Count #5		Count #6	
	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.
T_4 SHORT TERM												
2-wk. test	23.64	±3.18	16.92	±2.49	14.84	±2.20	13.14	±1.93	12.66	±1.90	12.44	±1.76
Control	22.90	4.17	19.22	1.85	14.95	1.75	11.40	1.37	9.85	1.34	8.40	1.24
4-wk. test	23.62	2.60	18.84	1.87	15.12	1.27	13.60	1.15	13.54	1.04	13.04	1.01
Control	23.60	1.82	20.42	1.44	15.76	1.36	12.96	1.31	10.96	1.29	9.94	1.09
T_4 LONG TERM												
2-wk. test	16.1	2.57	13.45	2.07	11.0	1.63	10.33	1.51	9.80	1.69	9.18	1.52
Control	13.33	1.02	11.58	0.95	9.48	0.64	7.75	0.63	6.25	0.53	5.50	0.50
4-wk. test	14.1	0.94	11.7	1.05	10.30	0.96	9.35	1.05	8.70	0.99	8.18	0.92
Control	12.83	1.14	11.08	1.07	10.02	1.49	7.86	1.14	6.94	1.02	6.04	0.95
T_3 SHORT TERM												
2-wk. test	27.38	2.10	20.78	1.20	16.22	1.34	15.28	1.25	14.76	1.29	14.36	1.10
Control	20.26	1.82	15.94	1.28	12.22	1.04	10.74	0.88	8.70	0.70	7.12	0.59
4-wk. test	25.28	2.70	20.52	2.94	16.30	2.53	16.24	2.38	15.28	2.11	14.60	2.26
Control	22.30	1.01	17.38	0.49	13.76	0.71	12.82	0.46	10.82	0.63	9.46	0.35
T_3 LONG TERM												
2-wk. test	11.47	2.38	9.27	1.09	7.83	1.29	6.85	1.17	6.57	1.10	6.47	1.10
Control	22.88	3.70	16.68	2.90	14.48	2.90	12.33	2.70	9.87	2.30	8.25	2.01
4-wk. test	13.72	1.98	12.02	1.79	10.50	1.79	9.18	1.79	8.68	1.57	8.08	1.50
Control	17.95	1.64	14.85	1.65	12.62	1.27	10.72	1.24	8.88	0.91	7.82	0.93
I.C. SHORT TERM												
2-wk. test	18.90	1.26	14.00	0.73	11.90	0.65	10.20	0.58	9.82	0.56	9.74	0.62
Control	22.22	2.91	18.75	2.59	16.67	2.66	13.50	2.13	11.47	2.01	11.15	1.88
4-wk. test	16.52	1.32	13.06	1.21	11.26	0.95	9.80	0.97	9.62	0.97	9.54	1.01
Control	27.06	2.46	19.93	2.30	18.23	1.87	12.96	1.53	11.60	1.67	10.00	1.72
I.C. LONG TERM												
2-wk. test	19.27	2.57	15.46	1.36	12.69	1.06	11.43	0.72	10.48	0.70	---	---
Control	12.24	1.19	10.78	0.89	9.18	0.81	8.30	0.61	7.46	0.55	---	---
4-wk. test	22.12	1.20	19.47	1.22	15.40	1.37	14.67	1.35	13.67	1.24	---	---
Control	12.30	0.78	10.04	0.86	8.08	0.83	7.50	0.89	6.07	0.93	---	---

Counts expressed as per cent of injected dose of I^{131} (%*) and standard errors for each counted group for the initial two-week counting period (plotted in Fig. 2-4).

APPENDIX E

	Count #1		Count #2		Count #3		Count #4		Count #5		Count #6	
	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.	%*	S.E.
T_4 SHORT TERM												
2-wk. test	1.78	±0.150	1.42	±0.188	1.48	±0.154	1.54	±0.174	1.30	±0.106	---	---
Control	18.55	3.66	16.61	2.82	13.88	2.56	11.60	2.4	9.63	2.30	---	---
4-wk. test	35.92	4.3	31.46	3.92	24.02	2.59	21.18	2.41	---	---	---	---
Control	28.06	2.67	22.92	2.6	19.04	1.88	15.80	1.75	---	---	---	---
T_4 LONG TERM												
2-wk. test	1.56	0.32	1.40	0.28	1.39	0.257	1.31	0.24	1.29	0.22	---	---
Control	23.67	3.12	21.29	2.98	17.35	2.18	15.18	1.41	11.93	0.89	---	---
4-wk. test	20.91	2.61	19.07	2.44	14.81	1.78	13.49	1.67	11.89	1.49	---	---
Control	18.46	2.69	16.52	2.37	12.63	2.18	12.28	2.16	10.36	2.19	---	---
T_3 SHORT TERM												
2-wk. test	1.49	0.18	1.61	0.138	1.67	0.221	1.53	0.043	1.38	0.049	---	---
Control	21.55	3.62	17.30	2.6	14.28	1.91	11.21	1.44	9.43	1.12	---	---
4-wk. test	31.34	3.13	26.90	4.24	22.48	3.02	19.66	2.91	---	---	---	---
Control	23.20	2.42	19.28	2.13	15.56	1.58	13.04	1.74	---	---	---	---
T_3 LONG TERM												
2-wk. test	1.45	0.31	1.22	0.239	1.23	0.204	1.15	0.197	1.19	0.23	---	---
Control	22.65	2.84	19.83	3.07	17.37	2.60	14.57	2.32	12.97	2.75	---	---
4-wk. test	24.26	2.64	22.28	2.66	19.01	2.59	17.84	2.26	15.26	2.32	---	---
Control	25.51	1.81	21.87	1.67	17.36	1.44	16.02	1.43	13.92	1.20	---	---
I.C. SHORT TERM												
2-wk. test	0.47	0.068	0.57	0.056	0.65	0.050	0.58	0.019	0.64	0.077	---	---
Control	27.80	2.68	21.25	2.22	17.05	1.42	13.35	1.85	11.95	1.57	---	---
4-wk. test	22.34	2.97	17.18	2.19	15.64	1.38	13.64	1.89	---	---	---	---
Control	21.08	4.92	15.85	3.44	11.93	2.74	9.03	2.36	---	---	---	---
I.C. LONG TERM												
2-wk. test	0.268	0.056	0.353	0.051	0.456	0.050	0.519	0.055	0.565	0.049	---	---
Control	17.86	1.03	15.37	1.03	12.32	0.94	11.39	0.81	10.29	0.70	---	---
4-wk. test	23.05	2.09	19.91	1.85	14.25	1.68	12.67	1.44	11.06	1.26	---	---
Control	24.45	2.20	20.67	1.76	15.19	1.19	12.93	1.10	11.54	1.14	---	---

Counts expressed as per cent of injected dose of I^{131} (%*) and standard errors for each counted group for the final two-week counting period (plotted in Fig. 2-4).

APPENDIX F

	SIX-WEEK EXPERIMENT				SIX-MONTH EXPERIMENT			
	Before Test Diet		After Test Diet		Before Test Diet		After Test Diet	
	K_4	S.E.	K_4	S.E.	K_4	S.E.	K_4	S.E.
Thyroxine								
2 weeks recovery							(4)	
Test	.1552	±.0081	.0386	±.0078	.1098	±.0100	.0284	±.0041
Control	.1505	.0126	.1187	.0076	.1051	.0037	.1030	.0118
4 weeks recovery								
Test	.1593	.0229	.1630	.0148	.1165	.0140	.0958	.0103
Control	.1280	.0020	.1322	.0081	.0951	.0050	.0851	.0058
Triiodothyronine								
2 weeks recovery							(4)	
Test	.1918	.0163	-----	-----	.0953	.0168	.0376	.0058
Control	.1313	.0081	.1327	.0150	.1075	.0107	.1004	.0055
4 weeks recovery								
Test	.1668	.0125	.1315	.0112	.0864	.0063	.0825	.0093
Control	.1166	.0087	.1335	.0050	.1027	.0037	.1057	.0048
Iodinated Casein								
2 weeks recovery								
Test	.1193	.0087	-----	-----	.1274	.0195	-----	-----
Control	.0861	.0055	.1541	.0132	.0747	.0045	.0808	.0032
4 weeks recovery								
Test	.1148	.0127	.1094	.0118	.1291	.0091	.1105	.0089
Control	.1315	.0116	.1605	.0058	.0984	.0145	.1242	.0071

Radioiodine output rates corrected for recirculating iodine (K_4) and standard errors (S.E.) for thyroxine, triiodothyronine, and iodinated casein, short and long term, two- and four-week recovery groups and controls.

APPENDIX G

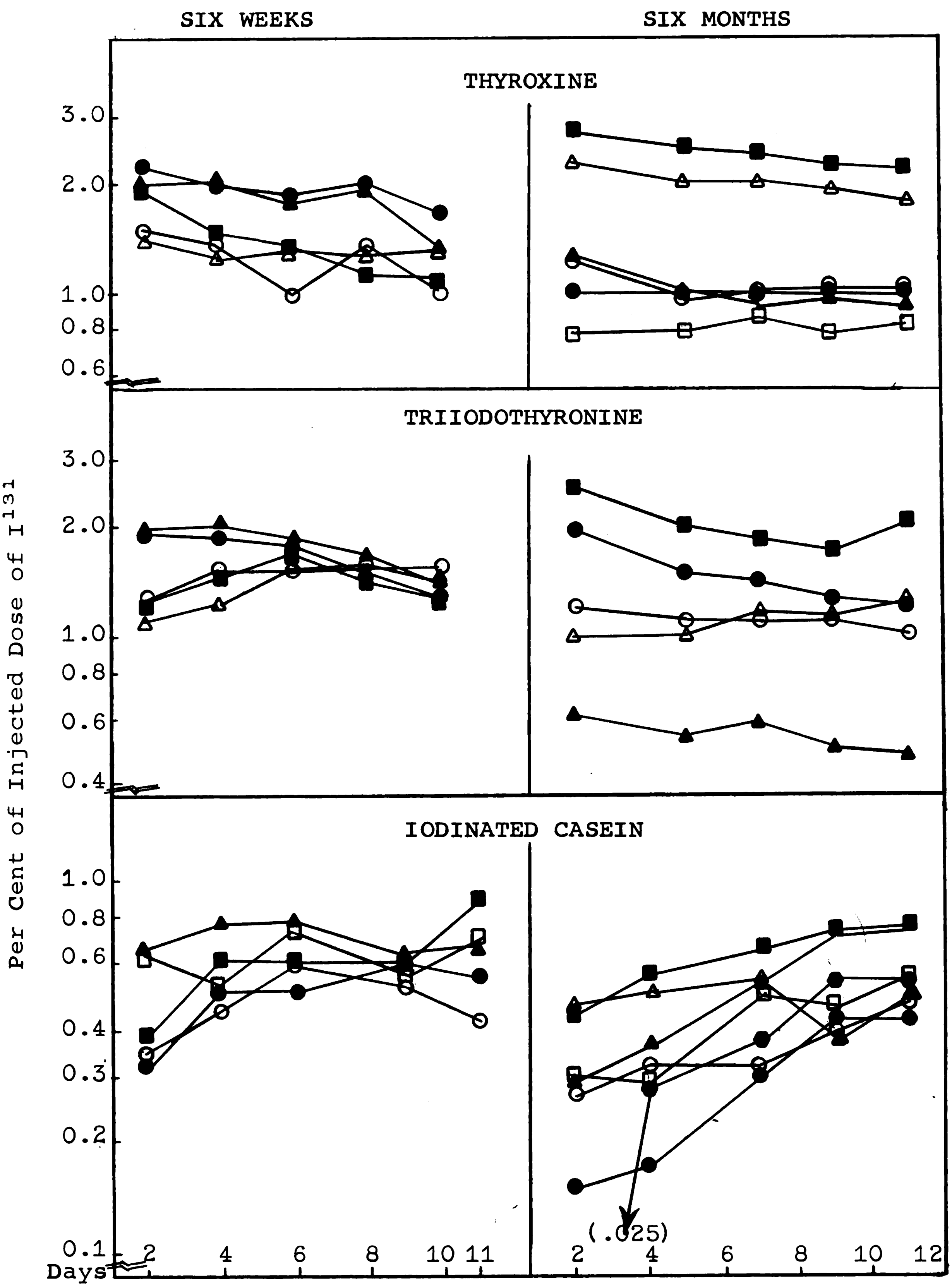
	SIX-WEEK EXPERIMENT				SIX-MONTH EXPERIMENT			
	Before Test Diet		After Test Diet		Before Test Diet		After Test Diet	
	U	S.E.	U	S.E.	U	S.E	U	S.E.
Thyroxine								
2 weeks recovery								
Test	28.8	±3.8	1.96	±0.19	19.3	±3.08	1.98	±0.39
Control	29.6	2.9	23.9	3.8	16.6	1.1	28.2	3.7
4 weeks recovery								
Test	30.2	3.9	43.2	4.9	16.7	1.2	25.2	3.5
Control	29.2	1.7	33.5	3.1	16.2	1.7	21.4	3.4
Triiodothyronine								
2 weeks recovery								
Test	35.4	2.5	----	---	13.6	3.0	1.7	0.44
Control	24.4	2.0	26.2	4.2	23.2	1.97	26.8	3.5
4 weeks recovery								
Test	32.7	4.4	37.6	5.1	16.1	2.1	27.4	2.8
Control	26.3	1.4	28.5	3.1	21.1	2.0	29.8	2.3
Iodinated Casein								
2 weeks recovery								
Test	23.2	1.5	----	---	23.9	3.3	----	---
Control	24.4	3.7	34.3	3.0	14.3	1.4	20.3	1.1
4 weeks recovery								
Test	21.8	1.9	26.6	3.6	28.2	1.3	27.2	2.4
Control	34.5	2.5	23.6	4.2	14.6	0.72	29.3	2.4

Radioiodine uptake as per cent of injected dose (U) and standard errors (S.E.) for thyroxine, triiodothyronine, and iodinated casein short and long term two- and four-week recovery groups and controls.

APPENDIX H

	SIX-WEEK EXPERIMENT				SIX-MONTH EXPERIMENT			
	Before Test Diet		After Test Diet		Before Test Diet		After Test Diet	
	$T\frac{1}{2}$	S.E.	$T\frac{1}{2}$	S.E.	$T\frac{1}{2}$	S.E.	$T\frac{1}{2}$	S.E.
Thyroxine								
2 weeks recovery								
Test	6.0	±0.3	23.4	±7.2	7.8	±0.7	25.9	±3.8
Control	6.3	0.4	7.5	0.8	7.5	0.3	9.4	0.5
4 weeks recovery								
Test	6.2	0.5	7.6	0.5	9.6	2.0	10.5	1.1
Control	7.3	0.3	7.6	0.3	8.8	0.9	10.3	1.1
Triiodothyronine								
2 weeks recovery								
Test	5.4	0.4	---	---	9.4	1.6	19.8	2.9
Control	6.7	0.3	6.9	0.4	8.5	0.8	9.2	0.6
4 weeks recovery								
Test	5.9	0.3	8.2	0.2	9.6	1.0	12.2	1.2
Control	7.9	0.6	7.0	0.1	8.3	0.3	9.1	0.3
Iodinated Casein								
2 weeks recovery								
Test	7.4	0.5	---	---	7.8	1.1	----	---
Control	10.5	0.7	6.5	0.4	8.9	0.5	10.5	0.004
4 weeks recovery								
Test	7.7	0.8	8.6	0.01	7.4	0.7	8.6	0.01
Control	7.8	0.4	5.4	0.01	8.7	1.3	7.6	0.01

Radioiodine biological half-time ($T\frac{1}{2}$) expressed in days and standard errors (S.E.) for thyroxine, triiodothyronine, and iodinated casein short and long term two- and four-week recovery groups and controls.



Thyroid output curves for individual rats during two-week recovery period following six weeks and six months on T_4, T_3, or I.C.

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