THYROID SECRETION RATE AS INFLUENCED BY METHOD OF MEASUREMENT, TYPE OF ANESTHESIA, AND ROUTE OF THYROXINE ADMINISTRATION

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ABSTRACT

THYROID SECRETION RATE AS INFLUENCED BY METHOD OF MEASUREMENT, TYPE OF ANESTHESIA, AND ROUTE OF THYROXINE ADMINISTRATION

By

Lawrence John Paulik

Three short acting experimental anesthetics (Brevital, Evipal and Metofane) were administered to determine if they significantly effected the thyroid secretion rate (TSR) of rats when compared with a control anesthetic (Nembutal) known not to effect the TSR of these animals. Two methods for measuring TSR were used, the substitution method and the direct output method. The Carworth Farms CFN strain of rat was employed in one substitution experiment and compared with the Sprague Dawley strain used in later substitution experiments.

Evipal was chosen as the anesthetic for use in a subsequent infusion experiment. In this infusion experiment, a comparison of the substitution method of once daily thyroxine injection versus a continuous infusion of thyroxine into unanesthetized, relatively unrestrained rats was performed using the weeks' chronic jugular cannulation procedure, harness, and rotary swivel mechanism. Group I rats were

cannulated, harnessed and received continuous thyroxine infusion. Group II (controls) were cannulated, harnessed and received continuous saline infusion with once daily thyroxine injection. Group III (controls) were unharnessed, noncannulated and received once daily thyroxine injection.

The results of the anesthesia experiments show that there were no significant differences in the thyroid secretion rates of the animals under the experimental anesthetics when compared to the control anesthetic within a given method and employing the same strain of rat. The three direct output values "U", "K_4", and "I" also show no significant differences between the three anesthetics Evipal, Metofane, and Nembutal. However, in the substitution method, the thyroid secretion rate of Sprague Dawley rats was significantly higher than the TSR obtained by the direct output method in the same animal strain. The Carworth Farms CFN rats also showed significantly higher TSR values when compared to the Sprague Dawley strain by the substitution method.

In the infusion experiment, Group I showed higher TSR values when compared to Groups II and III. These results indicate that the higher values of Group I were not due to any stress of the cannulation procedure or confinement in the harness, since these aspects would be reflected in Group II as well, but were due to the greater quantity of infused thyroxine needed to achieve the same inhibitory effects which occur in the once daily injection of thyroxine.

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DEDICATION

This thesis is affectionately dedicated to my wife, Barbara E. Paulik. Her devotion, understanding and artistic talents have made this work possible.

Lawrence J. Paulik

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INTRODUCTION

Before the introduction of isotopes into the research field, oxygen consumption, goiter prevention, and tadpole metamorphosis were some of the biological methods available to the thyroid physiologist in determining the functions of the thyroid gland. When isotopes became available, the thyroid physiologist was given the tools which enabled him to more precisely measure thyroid function.

Today, I^{131} is helping solve the complex mysteries of iodide uptake, hormone synthesis and release, sites of accumulation, rates of degradation, pathways for excretion and absorption, as well as cellular and subcellular mechanisms for hormone action.

The thyroid substitution method has found wide application in thyroid research. It is based on the classic negative feedback relationship which exists between the thyroid and the anterior pituitary gland. Thus, graded, daily injections of exogenous thyroxine over a period of time can effectively block the release of thyrotropin (TSH) from the pituitary and indicate the level of thyroid secretion rate (TSR).

Physiologically, the thyroid gland is in a dynamic relationship with the anterior pituitary gland. It is believed that small quantities of hormone are released, minute to minute as peripheral utilization of "free" thyroid hormone occurs. Perhaps circadian rhythms (Brody, 1945) could effectively regulate or alter the maximum and minimum quantities of TSH released during specific periods of the day. This would then be reflected in the quantity of thyroxine (T_4) released and utilized peripherally.

The question arose as to whether small quantities of thyroxine infused over a twenty-four hour period, would be comparable to a once daily injection which is absorbed into the circulation in a matter of a few hours. This is the basis for the following study.

LITERATURE REVIEW

It has been pointed out by Wiener (1948) that many physiologic processes are regulated by feedback control and he has suggested that much clarification and insight may be gained by the application of servo theory to such functions.

The dictionary defines a servo system as one in which the quantity of the output of an apparatus is "fed back" for the control of the system.

The pituitary-thyroid axis is an excellent example of a feedback (servo) mechanism. Hoskins (1949) discussed this thyroid-pituitary servo mechanism at length and suggested that in studying this relationship between the pituitary and thyroid, two questions arise: 1) the quantitative relationship between the blood thyroxine titer and thyrotropin discharge, and 2) the nature and control of the setting of the pituitary governor. In studying these and other questions concerning the thyroid and pituitary, researchers have utilized methods that take into account this servo mechanism.

Dempsey and Astwood (1943) introduced the goiter prevention method for estimating the daily rate of thyroid hormone secretion. In this method a goitrogen was given to

block thyroxine secretion from the thyroid while administering graded doses of thyroxine. The animals were killed and the thyroid glands were excised, weighed and compared with the thyroid glands of control animals. Mixner, Reineke and Turner (1944) studied the effects of thiouracil and thiourea on the thyroid gland of chicks. Reineke, Mixner and Turner (1945) studied metabolism and thyroid weight in the rat by the goiter prevention technique. Schultze and Turner (1945) applied the method in studying the thyroid secretion rate in small experimental animals and fowl, as did Hurst and Turner (1947). The method, however, has several drawbacks. It cannot be used on large animals because of the great expense in sacrificing them to determine thyroid weights. It also gives only an average thyroid secretion rate for a group of animals rather than having the capability of measuring each individual animal's TSR.

In 1959, Van Middlesworth, Jagiello and Vanderlaan showed that goiter prevention in propylthiouracil treated rats was only achieved through daily injections of sodium-1-thyroxine in amounts which elevated the plasma concentration of protein-bound iodine, two to three times normal. They felt that this clearly raised the possibility that the goiter-prevention method gives erroneously high values for the daily secretion rate of thyroxine by the thyroid gland.

Perry (1951) utilized the radioisotope, iodine^{131}, in determining the amount of thyroxine required to suppress

turnover of I^{131} by the thyroid gland. He showed that when rats are given I^{131} and different groups are then given graded doses of thyroxine, inhibition of thyroidal I^{131} output during a 48 hour period is proportional to the dosage of thyroxine administration.

Henneman, Griffin and Reineke (1952), and Henneman, Reineke and Griffin (1955), devised a method to determine the daily thyroid secretion rate in intact individual sheep. They injected I^{131} into the animals, allowed for uptake by the thyroid gland and measured the radioactivity of the thyroid region with a Geiger counter. L-thyroxine was then given to the animals in low concentration, the dosage being increased every three days. The counts were corrected for background and decay and the percentage of the previous corrected count was computed. As the quantity of l-thyroxine injected daily was increased, the percentage of the previous count increased, indicating that the thyroid gland itself was secreting less thyroxine. When the percent of previous count reached the 100% level, this represented the animal's daily thyroid secretion rate. Reineke and Singh (1955) confirmed and extended the method on the intact rat. They observed that in rats there appears to be a small iodine turnover even in those receiving thyroxine, equivalent to their thyroid secretion rate. In normal rats, output is approximately balanced by the uptake. If organic combination of iodine is blocked by thiouracil, output, even at

high thyroxine levels continues at an average rate of 4.2% per day. Thus, rats given thiouracil to prevent further thyroidal combination of I^{131} during treatment, yield values higher than those not receiving thiouracil.

The substitution method has become well established in studying thyroid function as related to other physiological and environmental factors. Amin, Chai and Reineke (1957) studied the thyroid activity in different strains of mice. An adaptation of the method has been used in determining the TSR of rainbow trout by Hoffert and Fromm (1959). In 1955, Lewis, Reineke and Lodge adapted the technique for thyroid secretion rate in dairy cattle. Lodge, Lewis and Reineke (1957) showed by use of the method that dairy calves were able to recycle iodine and that there were individual and breed differences in iodine assimulation. Flamboe and Reineke (1959) used the substitution method in studying dairy goats and the effects of age, pregnancy, lactation, and seasonal variations on thyroid function. Reineke, Travis and Kifer (1960) measured thyroid parameters in the mink. Recently, Reineke and Lorscheider (1967) found that the thyroid gland I^{131} output cannot be totally inhibited by thyroxine injections. In thyroids maximally blocked by T_4 injection, I^{131} is still released at the rate of 1.0 to 1.2 percent per day. They concluded that a more accurate endpoint for measuring the TSR is the 97.5% of previous count level rather than the 100% level previously used.

Nowhere in the literature to date has anyone continuously infused thyroxine for the purpose of measuring and comparing the TSR derived by infusion with that derived by the substitution method of once daily thyroxine injection, and indeed, this is why this research work was undertaken.

MATERIALS AND METHODS

General

The experiment was divided into two parts: The first dealt with finding a suitable anesthetic, capable of anesthetizing the animal for approximately twenty minutes, but not interfering with thyroid secretion rate. The second dealt with chronic infusion of thyroxine into the rat via jugular cannulation.

Anesthesia Experiment

Perry (1951) originally showed that sodium pentabarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.) does not cause any functional change in thyroid secretion rate. However, Nembutal anesthesia is of too long duration for use with rats restrained in the harness employed in later experiments. Therefore, an experiment was done to compare the thyroid secretion rate of rats immobilized with three short-acting anesthetics. Nembutal anesthesia was used as the control. The experimental anesthetics used were: 1) Methohexitol sodium (Brevital, Eli Lilly and Company, Indianapolis, Ind.), a short acting, intravenously administered anesthetic,

2) Hexobarbitol sodium (Evipal, Winthrop Laboratories, New York, N. Y.) given intraperitoneally, and 3) Methoxyflurane (Metofane, Pitman Moore, Indianapolis, Ind.) an inhalant. The dosages used for each anesthetic were:

Brevital--2% solution--0.1 ml/100 g. body wt. intravenously.

Evipal--6% solution--0.1 ml/100 gm. body wt. intraperitoneal.

Metofane--inhalant given to effect.

Nembutal--3% solution--0.1 ml/100 gm. body wt. intraperitoneal.

The animals used for the Brevital experiment were female rats from the Carworth Farms CFN strain weighing 180 to 200 grams and maintained on Wayne Lab. BLOX. All other experiments were performed on Sprague Dawley female rats weighing 180 to 200 grams and fed Zinn's Rat Feed. The laboratory changed from the CFN strain to the Sprague Dawley strain after experiencing difficulty in acclimating the CFN rats to a harness mechanism used later in the infusion experiment. Upon recommendation of the breeder, Zinn's Rat Feed was fed to the Sprague Dawley animals after a comparison between the two feeds showed both had similar nutritional and iodine contents. The rat room temperature was maintained at 76 $\pm$ 2° F and lights were on 14 hours daily. All animals had an adequate water supply.

The measurement of TSR in the Brevital experiment was done by the substitution method (Reineke and Singh, 1955). The anesthetic was administered via tail vein injection while the animal was contained in a cylindrical restraining device.

The Evipal and Metofane anesthetics were compared with sodium pentabarbital using the substitution method and the direct output method (Reineke and Lorscheider, 1967).

Counting Method

All animals received subcutaneous injections of 3 to 5 mci of carrier-free sodium iodide (I^{131}). Approximately 72 hours were allowed for I^{131} fixation by the thyroid and elimination of excess isotope by the kidneys and other routes.

In vivo radioactivity counts were taken with a 2-inch thallium-activated sodium iodide crystal connected to a radiation analyzer-scaler. The rat was positioned over the crystal so maximum counts over the thyroid region were obtained by observing a count rate meter. Two to three thyroid counts of one minute duration were then taken on the scaler. A one minute count of the epigastric region was taken to obtain the animal's body background count. Standards of 1/10th the injected dose were counted for one minute. The percent of injected dose over the thyroid region was then calculated from the equation:

$$\text{Percent of injected dose} = \left[\frac{\text{Thyroid count} - \left(\frac{\text{epigastric count}}{2} + \text{general background}\right)}{(\text{Standard count} - \text{general background}) \times 10}\right] \times 100$$

The log of percent injected dose was plotted against time.

The Substitution Method

In the substitution method, thyroid counts were taken on alternate days until an output slope of usually three points could be plotted. An alkaline saline solution of L-thyroxine (T_4), 0.5 μg./100 g. body wt., was injected into the animal after the third count for two successive days. A higher concentration of 1.0 μg. T_4/100 g. was injected following the fourth count again for the next two days. The concentration was increased after each count by 0.5 μg. T_4/100 g. until a leveling off or inhibition of the thyroid I^{131} output was achieved. Each count was expressed as percent of the previous count and plotted against T_4 concentration on linear graph paper. Inhibition was considered to occur at the 97.5% level as demonstrated by Reineke and Lorscheider (1967). The T_4 dosage corresponding to the 97.5% level then gave the concentration of exogenous thyroxine needed to block TSH release and, therefore, was considered to be equal to the animal's daily endogenous thyroid secretion rate.

Direct Output Method

The direct output method is based on the fractional daily output of the thyroid gland times the thyroxine equivalent of the total iodine released from the thyroid gland. The TSR is calculated as follows:

I^{131} turnover rate constant = $X = \frac{0.693}{T\frac{1}{2}}$ (1)

Fractional turnover = $K_4' = 1 - e^{-xt}$ ($t = 1$ day) where e is the base of the natural logarithms (2)

I^{131} actual turnover rate constant (corrected for the recycling of metabolized iodide to the thyroid) =

$$K_4 = \frac{K_4'}{1 - U}$$

where U = zero time uptake (3)

Hormonal iodine released = output rate K_4 x iodine content of thyroid (4)

T_4 activity, T_4 equivalent = K_4 x total thyroid iodine (mg.) x 1.53 x 1.52, the factor 1.52 is used to allow for the proportional contribution of triiodothyronine (T_3) presumed to be released from the thyroid. (5)

Thyroid Substitution by Intravenous Infusion

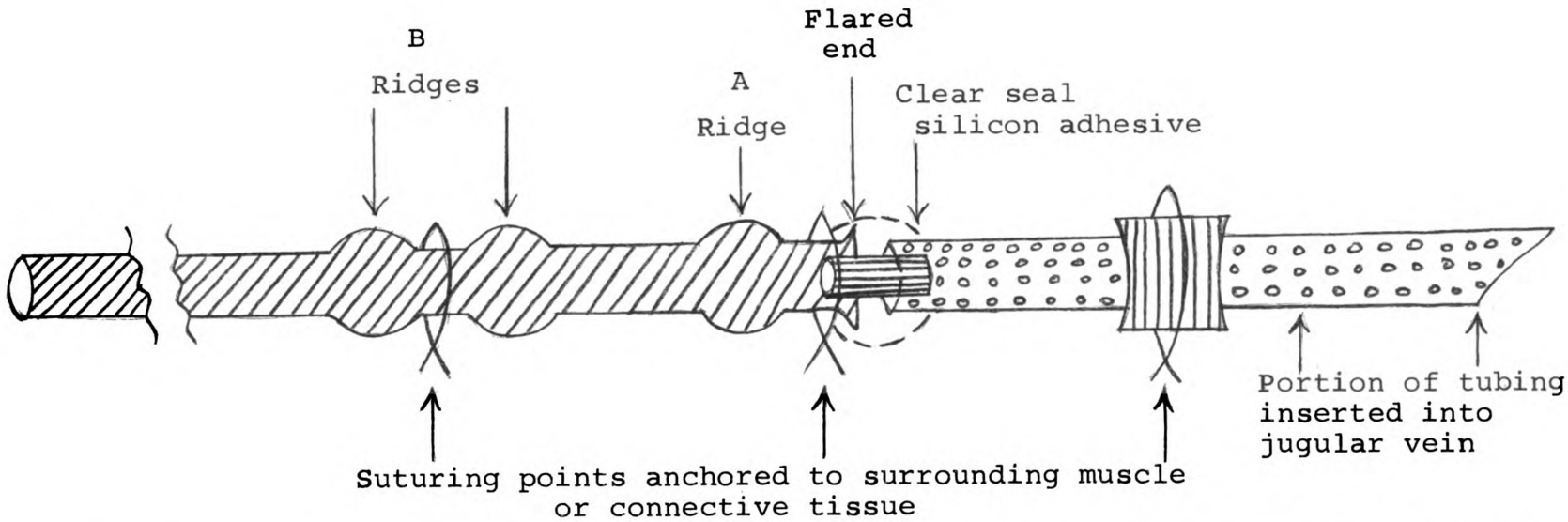
Cannula Construction

The cannulas used for infusion (Weeks and Davis, 1964) were constructed of 26 gauge needle tubing (Small Parts, Inc., Biscayne Annex, Miami, Fla.), silicone rubber tubing

and polyethylene as indicated in Figure I. The ridges were formed on the polyethylene tubing by passing a wire stylus through the lumen (Heatley and Weeks, 1964). Rotation of the tubing on the stylus wire in presence of a jet of hot air, together with linear compression formed the ridges. The flared pieces were made by holding the tubing near an open flame. A silicone adhesive (Clear Sealer--Dow Corning Corporation, Midland, Michigan) was used to give the connecting joint flexibility.

Cannula Implantation

The surgery was performed on 180 to 200 g. female rats, anesthetized with Metofane. The hair was shaved from the dorsal side of the neck region and over the right jugular area on the ventral side. An incision was made just to the left of the midline in the dorsal neck region. A transverse incision was also made over the jugular region. Approximately 5 mm. of the jugular vein was exposed by blunt dissection. A curved hemostat was inserted subcutaneously into the incision in the dorsal neck region and worked posteriorly around the right foreleg to the ventral skin incision. The cannula was then clamped (polyethylene side) with the hemostat and pulled back through the dorsal incision. A syringe containing 0.85% NaCl was attached to the polyethylene end of the cannula. The vein was punctured with a 23 gauge needle and the silicone rubber portion of the cannula was



Kay:

= P.E. 20 Length--170 mm.
A Ridge--2 mm. from flared end.
B Ridges--4 mm. from nonflared ends

= Silicone Rubber Tubing
Length--70 mm.

= Stainless Steel Needle Tubing
26 gauge, length 10 mm.
beveled at both ends

= P.E. 50 Length--3 mm
flared at both ends

Figure I. Cannula construction.

inserted into the vein. The suturing points were then attached to surrounding muscle tissue. The connective tissue on the ventral side was reunited over the cannula with absorbable sutures, and wound clips were used to close both incisions. The animal was allowed a week to recover from the operation before it was acclimated an additional week in the harness.

The Harness and Pump

The harness used in this method allows the rat to pivot freely because of a swivel mechanism (Lehigh Valley Electronics, Fogelsville, Pa.)(see Figures II through IV). When the rat is securely placed in the harness it cannot get at the cannula, yet is not restrained from moving freely around the cage.

The thyroxine solution was infused into the animal by using a Sage constant speed syringe pump (Model 234-7, Sage Instruments, Inc., White Plains, New York) modified to push four syringes simultaneously. One c.c. tuberculin syringes were used. The flow rates were varied by changing the gear ratio. A minimum flow rate of 0.23 ml/day up to a maximum flow rate of 0.57 ml/day could thus be achieved.

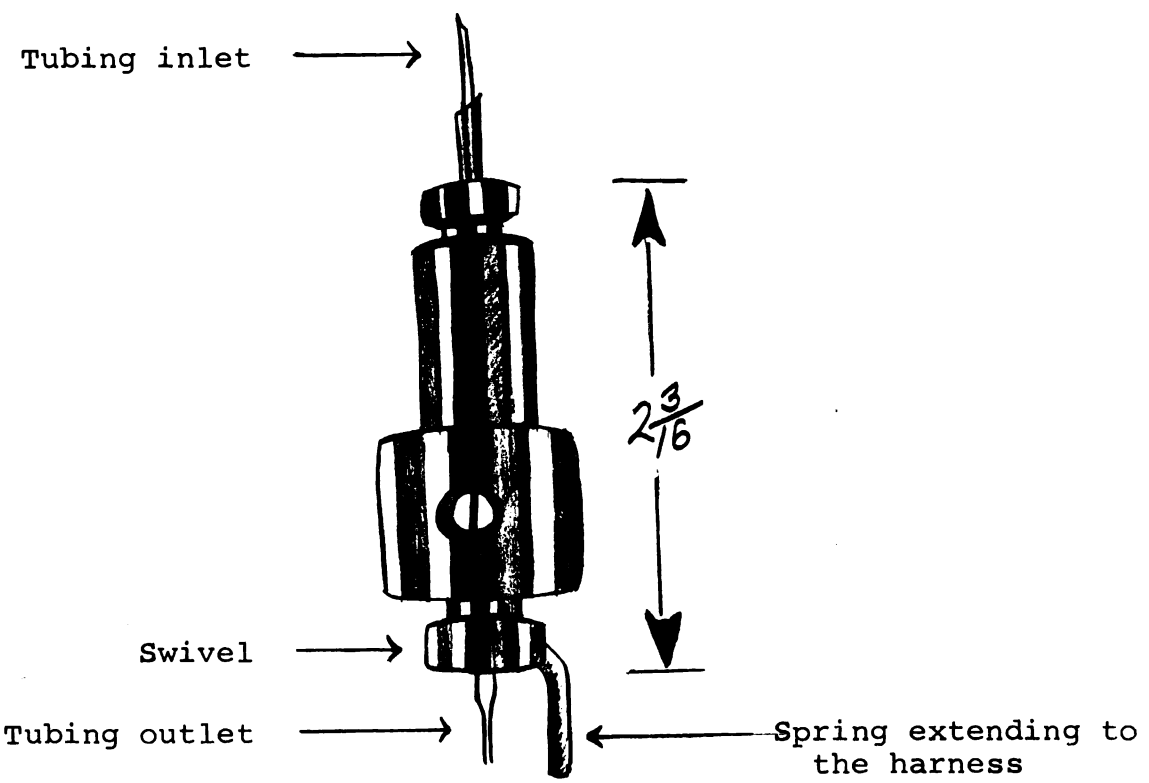


Figure II. Swivel mechanism.

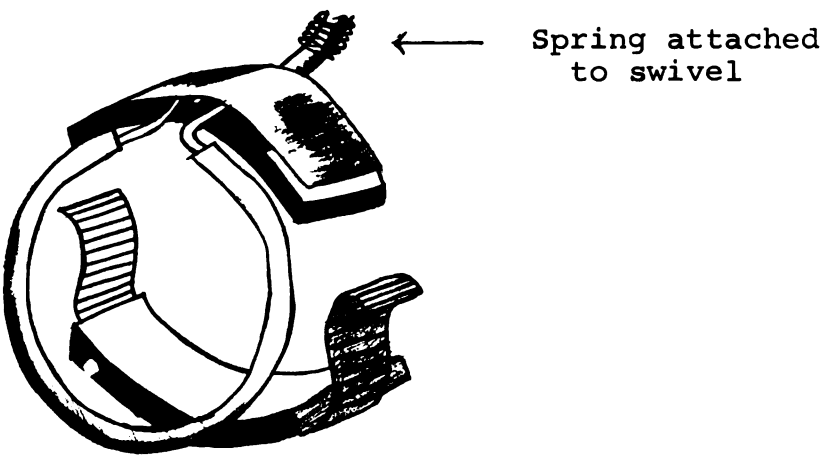


Figure III. Harness for holding the rat.

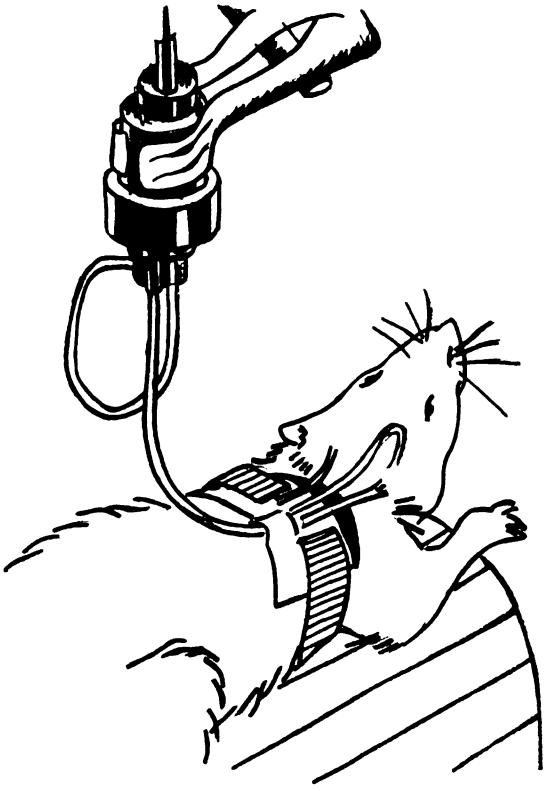


Figure IV. Entire assembly with rat attached to harness. Because the swivel turns freely, the rat can move at will without twisting the infusion tubing.

Once Daily Injection vs. Chronic Infusion of T_4

The experiment was performed on 3 groups of 4 rats each. The first group contained cannulated rats in harnesses, receiving thyroxine by infusion. The second group contained cannulated rats in harnesses, receiving 0.85% saline by infusion, but subcutaneous thyroxine injections. The third group contained non-cannulated, unharnessed rats receiving subcutaneous thyroxine injections.

Calculating the Amount of T_4 Infused per 100 Grams of Rat

First pump speed equals 0.23 ml/day

Thyroxine concentration equals 6 μg/ml

0.46 ml/48 hours of infusion x 6 μg/ml = 2.76 μg/48 hr. infusion

Average weight of rat between the 3rd and 4th count equals 209 g.

2.76 μg/2.09 g. = 1.32 μg T_4/100 g./48 hr.

0.66 μg T_4/100 g./24 hr. infusion

All new solutions were completely flushed through the polyethylene tubing from the pump to the junction point where the polyethylene tubing meets the cannula on the rat before a new infusion was begun.

Table 1. Comparing thyroxine concentration by injection and by infusion, assuming a 200 g. rat.

Thyroxine dosage and concentration by infusion expressed as micrograms.

T_4/100 g./24 hr.	Concentration T_4/ml	Days post I^{131}
0.7 μg.	6 μg.	9-11
1.05 μg.	6 μg.	12-14
1.80 μg.	6 μg.	15-17
2.28 μg.	8 μg.	18-20
2.70 μg.	9 μg.	21-23
3.30 μg.	11 μg.	24-26

Graded thyroxine dosage by injection.

μg. 100 g rat	Days post I^{131} injection
0.5 μg.	9-11
1.0 μg.	12-14
1.5 μg.	15-17
2.0 μg.	18-20
2.5 μg.	21-23
3.0 μg.	24-26

RESULTS AND DISCUSSION

Anesthesia is a necessity when taking thyroid radioactivity counts on small animals. However, the anesthetic must not alter thyroid secretion rate. Anesthesia Experiments I through III were performed to find a suitable anesthetic for the harnessed rats. The control anesthetic, Nembutal, was compared with experimental anesthetics by two different methods (substitution and direct output) for measurement of TSR.

Table 2 shows the results of the first experiment. Nembutal was compared with Brevital (intravenous injection) by the substitution method. The average thyroid secretion rate of the rats under Brevital anesthesia was 1.94 ± 0.159 μg.* of thyroxine per 100 g. of rat with a range of 1.13 μg. to 3.10 μg./100 g. Nembutal gave an average TSR of 2.14 ± 0.220 μg./100 g. T_4 with a range of 1.30 to 3.38 μg./100 g. The student "t" test (Goldstein, 1964) was performed on the data and showed the difference to be non-significant ($P > 0.10$).

In Anesthesia Experiment II (Table 3), Nembutal was compared with Evipal (subcutaneous injection) and Metofane (veterinary inhalant) by the substitution method. The average

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*Standard error of the mean.

Table 2. Anesthesia Experiment I utilizing the substitution method to measure thyroid secretion rate per 100 g. Nembutal used as the control and compared with methohexitol sodium (Brevital).

Brevital		Nembutal	
Rat No.	TSR/100 g./day	Rat No.	TSR/100 g./day
1	3.100	1	3.375
2	2.413	2	3.125
3	2.150	3	3.050
4	2.125	4	2.163
5	1.950	5	2.100
6	1.875	6	2.050
7	1.850	7	1.850
8	1.825	8	1.575
9	1.475	9	1.525
10	1.450	10	1.425
11	1.125	11	1.300
Average TSR/100 g./day* 1.94 ± 0.159 (11)		Average TSR/100 g./day 2.14 ± 0.220 (11)	

*Mean ± SE (N)

Table 3. Anesthesia Experiment II utilizing the substitution method to measure thyroid secretion rate per 100 g. Nembutal was used as the control and compared with hexobarbitol sodium (Evipal) and methoxyflurane (Metofane).

Rat Number	Evipal TSR/100 g./day	Metofane TSR/100 g.	Nembutal TSR/100 g./day
1	2.800	2.900	2.125
2	1.950	1.850	1.875
3	1.875	1.700	1.875
4	1.800	1.600	1.825
5	1.775	1.525	1.675
6	1.575	1.350	1.675
7	1.550	1.225	1.625
8	1.550	1.100	1.500
9	1.475	1.000	1.325
10	1.225		
Average TSR/100 g.*	1.76 ± 0.134(10)	1.58 ± 0.189(9)	1.72 ± 0.078(9)

*Mean ± S.E. (N).

TSR for Evipal, Metofane, and Nembutal was 1.76 ± 0.134 μg./100 g., 1.58 ± 0.189 μg./100 g., and 1.72 ± 0.078 μg./100 g., respectively. Evipal showed a range of 1.23 μg. to 2.80 μg./100 g. Metofane showed 1.00 μg. to 2.90 μg. T_4/100 g. and Nembutal gave a range of 1.33 μg. to 2.13 μg. T_4/100 g. The student "t" test showed a non-significant difference ($P > 0.10$) between the three groups.

In experiment I, female rats from the Carworth Farms CFN strain were used whereas all other experiments were performed on Sprague Dawley female rats. Inasmuch as there were no differences between groups within either strain, a single mean was calculated for each strain of rat. The mean TSR's of the CFN and Sprague Dawley strains, respectively, were 2.04 ± 0.134 μg./100 g., and 1.69 ± 0.080 μg./100 g. This is a significant difference $P < 0.05$ level.

In experiment III (Table 4), Nembutal was again compared with Evipal and Metofane using the direct output method. Brevital was not further tested because it would not have been possible to give intravenous injections to harnessed rats via tail vein. Table 4 gives an average TSR for Evipal of 1.15 ± 0.096 μg. T_4 100 g. rat with a range of 0.636 μg. to 1.47 μg. T_4/100 g. Metofane showed a TSR average of 1.16 ± 0.129 μg./100 g. with a range of 0.775 μg. to 1.72 μg T_4/100 g. The Nembutal control averaged 1.31 ± 0.121 μg./100 g. with a range of 0.850 μg. to 1.76 μg. T_4/100 g. The student "t" test showed a non-significant difference $P > 0.10$ for the three groups. In Table 5, the values of "U", the percent

Table 4. Anesthesia Experiment III utilizing the direct output method to measure thyroid secretion rate per 100 g. Nembutal was used as the control and compared with hexobarbitol sodium (Evipal) and methoxyflurane (Metofane).

Rat Number	Evipal TSR/100g./day	Metofane TSR/100g./day	Nembutal TSR/100g./day
1	1.470	1.723	1.757
2	1.383	1.378	1.414
3	1.315	1.357	1.390
4	1.271	1.064	1.278
5	1.266	0.976	1.196
6	1.262	0.839	0.850
7	0.877	0.775	
8	0.841		
9	0.636		
Average TSR/100g.*	1.15 ± 0.096(9)	1.16 ± 0.129(7)	1.31 ± 0.121(6)

*Mean ± S.E. (N)

Table 5. Anesthesia Experiment III. Direct output method. Comparing the three values U, K_4, and I for the anesthetics Evipal, Metofane and Nembutal.

Evipal			Metofane			Nembutal		
U	K_4	I(mg)	U	K_4	I(mg)	U	K_4	I(mg)
17.00	0.1248	11.475	13.97	0.1234	12.588	18.10	0.0694	23.038
12.31	0.1153	12.869	7.15	0.0843	17.894	6.15	0.0540	27.300
10.60	0.0911	14.500	13.57	0.0889	13.950	10.88	0.1192	11.013
8.49	0.0825	16.856	10.16	0.1646	5.300	7.01	0.0949	14.450
10.07	0.0982	11.013	8.13	0.0634	15.844	19.75	0.1009	9.413
6.52	0.0704	20.625	5.24	0.0932	11.469	7.48	0.0456	19.600
8.73	0.1511	7.600	10.46	0.0474	14.025			
7.50	0.0916	11.038						
11.38	0.0956	5.850						
Average values*								
10.29 ± 1.041 (9)	0.1023 ± .008 (9)	12.425 ± 1.503 (9)	9.81 ±1.223 (7)	0.0950 ±0.015 (7)	13.010 ± 1.510 (7)	11.56 ± 2.428 (6)	0.0807 ±0.011 (6)	17.469 ± 2.875 (6)

*Mean ± S.E. (N)

of thyroidal I^{131} uptake extrapolated to zero time, "K_4", the fractional I^{131} output rate per day corrected for recycling of metabolized I^{131}, and "I", the total iodine present in the thyroid gland were compared for each of the three anesthetics (Evipal, Metofane, and Nembutal) used in the direct output experiment. All three parameters, "U", "K_4", and "I" gave non-significant differences between groups using the student "t" test ($P > 0.10$). The average "U" values for Evipal, Metofane, and Nembutal were 10.29 ± 1.041, 9.81 ± 1.223, and 11.56 ± 2.428 respectively. This shows that the experimental anesthetics did not alter or radically change the uptake of I^{131} by the thyroid gland when compared to the control anesthetic. The "K_4" values were 0.1023 ± 0.008 for Evipal, 0.0950 ± 0.015 for Metofane, and 0.08007 ± 0.011 for Nembutal. Here again, the experimental anesthetic values show non-significant differences from the control anesthetic indicating no gross alterations in the animals' fractional output or release of I^{131} from the thyroid gland. The values for total iodine ("I") present in the thyroid gland were 12.425 ± 1.503 µg. for Evipal, 13.010 ± 1.510 µg. for Metofane, and 17.469 ± 2.875 µg. for Nembutal. The non-significance of the experimental values when compared to the control, show no adverse changes in the iodine content of the thyroid gland. All of these values indicate no physiological change in the normal function of the thyroid gland due to repeated use of these experimental anesthetics.

The thyroid secretion rate obtained by the substitution method in Sprague Dawley rats was consistently higher than the TSR obtained by the direct output method in the same strain of rat. Since there were no significant differences between either method due to the anesthetics used, the values for each method were combined. The overall average TSR's were 1.69 ± 0.080 μg./100 g. by the substitution method and 1.20 ± 0.064 μg./100 g. by the direct output method. The difference was highly significant by the student "t" test ($P < 0.01$). Singh and Reineke (1968), measuring TSR in the chicken, obtained values by the direct output method that were one-half as large as those obtained using other methods for measuring TSR.

Evipal was chosen as the anesthesia for the infusion experiments. It is short acting, has minimal side effects, and, as the tables indicate, does not effect the TSR.

Thyroid secretion rates of rats receiving thyroxine by continuous infusion are compared in Table 6 with two control groups in which the T_4 was injected once daily. Group I had a mean TSR of 2.84 ± 0.175 μg. T_4/100 g. which was significantly higher than either the mean TSR value of 2.12 ± 0.248 g. T_4/100 g. Group II ($P < 0.05$) or the mean TSR value of 1.86 ± 0.147 μg. T_4/100 g. for Group III ($P < 0.01$). Group I ranged from 1.70 μg. to 3.43 μg. T_4/100 g. Groups II and III had a range of 1.40 μg. to 3.95 μg. T_4/100 g. and 1.18 μg. to 3.28 μg. T_4/100 g., respectively. The student "t" test gave a non-significant difference ($P < 0.10$) between

Table 6. Infusion experiment utilizing the substitution method.

Rat Number	Group I: Continuous T_4 infusion. µg.T_4/100g./day TSR/100g.	Group II: Continuous 0.85% NaCl infusion with daily T_4 injection. µg.T_4/100g./day	Group III: Daily T_4 injection. µg.T_4/100g./day
1	3.425	3.950	3.280
2	3.420	3.730	2.730
3	3.400	2.550	2.630
4	3.375	1.975	2.130
5	3.350	1.875	2.075
6	3.250	1.850	1.825
7	3.200	1.750	1.800
8	3.100	1.675	1.800
9	3.025	1.650	1.675
10	2.625	1.625	1.560
11	2.125	1.400	1.500
12	1.975	1.400	1.475
13	1.825		1.450
14	1.700		1.300
15			1.300
16			1.175
Average TSR/100 g.*	2.84 ± 0.175(14)	2.12 ± 0.248(12)	1.86 ± 0.147(16)

*Mean ± S.E. (N)

Groups II and III. This shows that the higher TSR values of Group I are not due to any stress of the cannulation procedure or confinement in the harness since these aspects would be reflected in Group II as well.

It required higher infusion concentrations at the maximum pump speed to achieve the inhibition of the thyroid in the experimental animals. Group I contained fourteen animals of which eleven had TSR values above 1.98 μg. T_4/100 g. and nine had values of 3.03 μg. T_4/100 g. of larger. Group II contained twelve animals, nine of which had TSR values of 1.98 μg. T_4/100 g. or less. Group III had sixteen animals, eleven of them having TSR values of 1.83 μg. T_4/100 g. or less.

If Groups II and III are taken collectively and compared with the Evipal animals of Anesthesia Experiment II, they show a mean TSR of 1.97 μg. T_4/100 g. The Evipal anesthesia animals have a mean TSR of 1.76 μg. T_4/100 g. (Table 3) which compares well with these controls.

In order to explain the significant differences between Group I and the control Groups II and III, we must take into account the possible effects of extrathyroidal sites upon the negative feedback relationship between the thyroid and pituitary.

Thyroxine is carried in rat plasma bound chiefly to the alpha 1 and alpha 2 globulins, the so-called thyroxine binding globulins (TBG) (Larson and Albright, 1955). TBG binds a limited amount of thyroxine and this is exchangeable with

free thyroxine added to the plasma. When thyroxine in excess of the quantity that the TBG can bind, is added to blood serum containing radioactive thyroxine, some of the radioactive thyroxine on the TBG is displaced and becomes bound to the albumin fraction of the serum. Triiodothyronine has been shown to have a lower affinity than thyroxine for TBG. With the highest doses of thyroxine, the albumin appears to be saturated, some of the thyroxine becoming bound to the other protein fractions of the serum.

The thyroid secretion rate of rats used in these experiments ranged approximately from 1.0 to 3.5 μg. of T_4/100 g./day.

Myant, (1957), in work on the biliary clearance rate of thyroxine and the binding of thyroxine by the serum proteins, found that the biliary clearance rate of endogenous thyroid hormone averages about 2 ml plasma/hour under physiological conditions. Let us assume that a 200 g. rat's plasma volume is approximately 4% of its body weight (Best and Taylor, 1950) or equal to 8 ml, and that its TSR is 1.5 μg. T_4/100 g./day or 3.0 μg. T_4 total output/day (0.125 μg.T_4/hr.). If the liver clears 2 ml plasma/hr., that amount of plasma should contain 0.032 μg. of the 0.125 μg. T_4 secreted by the thyroid per hour. It appears, therefore, that about 26% of the thyroxine secreted hourly by the thyroid is removed by the liver and (possibly) excreted in the bile (see Table 7).

In the present experiment, at the maximum pump speed and maximum concentration of T_4, approximately 0.13 μg.T_4/hr.

Table 7. Calculations for the determination of the percent of T_4 cleared from plasma, hourly by the liver.

If we assume a steady state thyroid secretion rate of 1.5 μg./100 g./day the thyroid secretion rate of a 200 g. rat equals:

TSR = 1.5 μg. T_4/100 g./day

= 3.0 μg. T_4 total/day/200 g.

= 0.125 μg. T_4/hr./200 g.

The plasma volume (PV) of a 200 g. rat is approximately equal to 4% of its body weight.

PV = 8 ml.

μg. T_4/ml./hr. of plasma equals

$$\frac{0.225 \text{ μg. } T_4/\text{hr./200 g.}}{8 \text{ ml. plasma/200 g.}} = 0.016 \text{ μg./ml./hr.}$$

If the liver clears 2 ml. plasma per hour (based on the work of Myant, 1957)

μg. T_4 cleared/hr. by liver equals

2 ml/hr. x 0.016 μg. T_4/ml. plasma/hr = 0.032

T_4 cleared hourly by liver equals

$$\left(\frac{0.125 \text{ μg. } T_4/\text{hr.}}{0.032 \text{ μg. } T_4 \text{ cleared/hr. by liver}}\right)\left(100\right) = 26\%.$$

were infused. In the individual experiments this either approached the average hourly endogenous T_4 release or exceeded it.

In Myant's (1957) observations, he found that thyroxine causes a marked and immediate rise in the biliary clearance rate. If thyroxine is cleared at a higher rate when bound to albumin than when bound to TBG, the observed clearance rate of thyroxine should rise as the proportion bound to albumin increases. This would explain why the clearance rate rises with increasing doses of carrier and why the difference in the clearance rate of thyroxine, according to whether it is bound by strong bonds to TBG or by weaker bonds to albumin, might depend on the ease with which the liver can detach T_4 molecules from their binding sites.

Before the onset of infusion, the experimental rats have a normal amount of endogenous thyroxine bound to plasma proteins. However, as the experiment progresses, the steady hourly infusion of exogenous thyroxine approaches or exceeds the hourly glandular release and must tend toward the saturation of the TBG or increase the amount of T_4 bound to the albumin fraction of the plasma proteins. Indeed, Freinkel, Ingbar and Dowling (1957) have shown that in mixtures of plasma proteins, thyroxine can be displaced from TBG onto the albumin by the addition of increasing amounts of T_4.

If the liver removes the plasma T_4 more easily when it is bound to the serum proteins (albumin) by weak bonds, we can then conclude that as the plasma T_4 concentration rises,

the binding sites with strong affinity for T_4 are filled and thyroxine becomes attached to sites with weaker affinity.

Gross and Leblond (1949) and other workers have shown a rapid uptake of I^{131} labeled thyroxine by the liver. They found that even in physiological doses of several micrograms of radiothyroxine injected into the rat, the plasma contained only about 2% of the administered dose two hours after injection. As mentioned before, the liver metabolizes T_3 and T_4 and the principle route of excretion occurs via the biliary route.

Albert and Keating (1952) have observed that the liver concentrates T_4 and they proposed that T_4 underwent an enterohepatic circulation.

The possibility may exist that the liver not only metabolizes thyroxine, but also may store it. Singh and Reineke (1968) observed in experiments done on chickens with radiothyroxine, that blood samples obtained by heart puncture contained more radioactivity than earlier samples taken via the brachial vein. They proposed that the amount of radioactive substance released to the blood during the sampling of relatively large quantities of blood may indicate that the liver or other extrathyroidal tissue plays a role in regulating the circulating thyroid hormone level. It is interesting to note here that the chicken has no TBG in the blood (Tata and Shellabarger, 1959).

Recently, Gorman et al. (1966) isolated and perfused livers of rats at 1 to 20 hr. intervals following

administration of the thyroid hormones. He observed that unchanged T_4 was released to the perfusing blood until an equilibrium was reached. If we assume that the rat's plasma proteins contain normal levels of bound T_4, a once daily injection of increasing T_4 concentration will enter the blood rapidly and tend toward the saturation of the strong TBG bonds and overlap onto the weaker albumin sites. The liver (or other extrathyroidal tissue) could then rapidly remove this thyroxine and either store it or release it via an entero-hepatic route, thereby permitting its absorption into the blood at a later period (see Figure V). This exogenously derived thyroxine would then exert (for longer periods) its effects on the pituitary and cause a functional endogenous block of T_4 from the thyroid.

In the slow constant infusion method, the saturation of the plasma sites would occur at a much slower rate than by injection. It would then take a longer period or higher concentration to saturate the plasma proteins. The liver could keep up with the removal of T_4 possibly storing less and conjugating more for its removal in the feces. It would then take a longer period for the endogenous T_4 blockage in the thyroid to occur (see Figure VI).

There may exist not only a negative feedback mechanism regulating thyroxine release from the thyroid, but also some extrathyroidal mechanism that controls and conserves the hormone that is already present in the circulation.

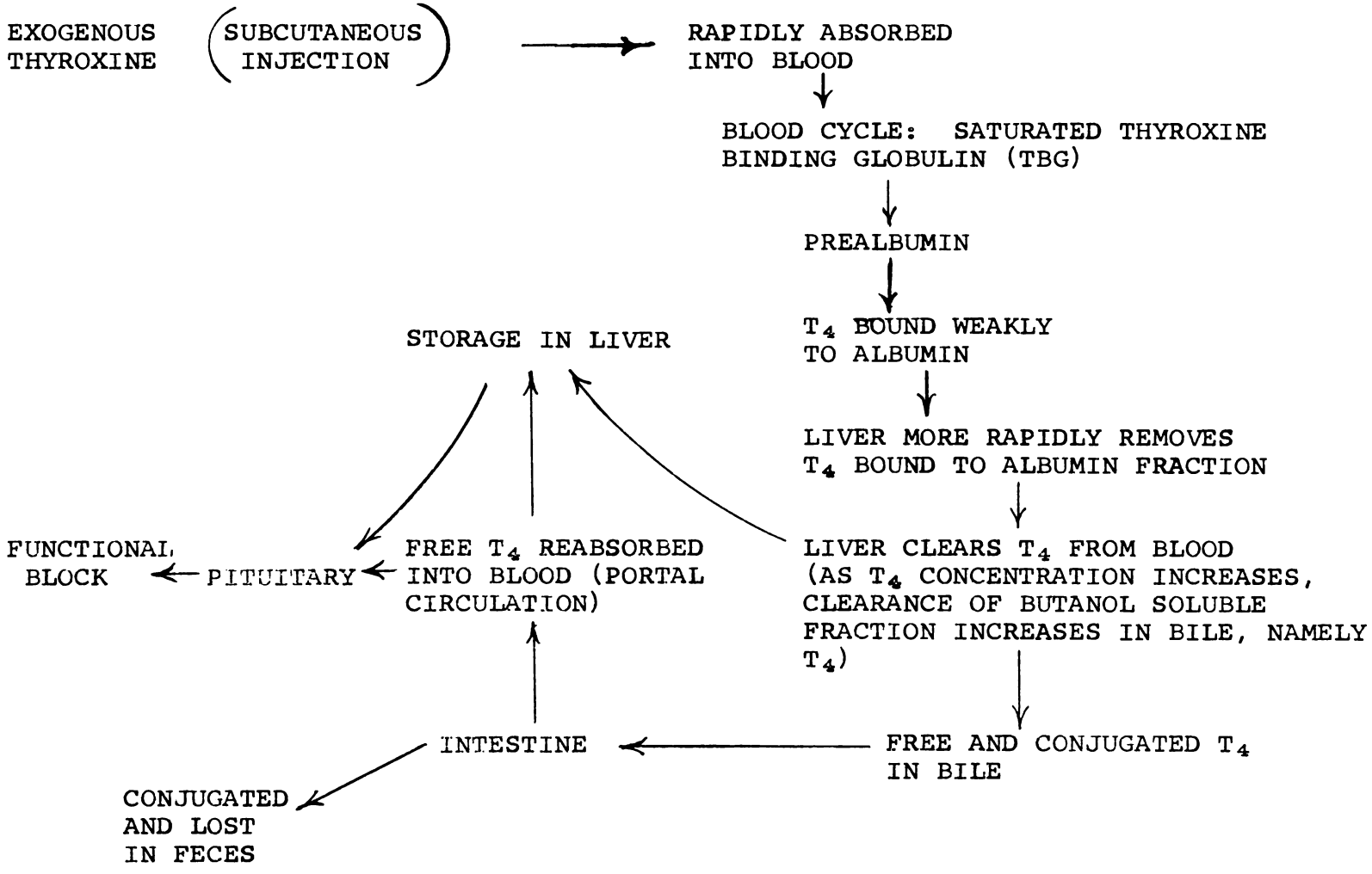

Figure V. Possible pathways of exogenous thyroxine following injection.

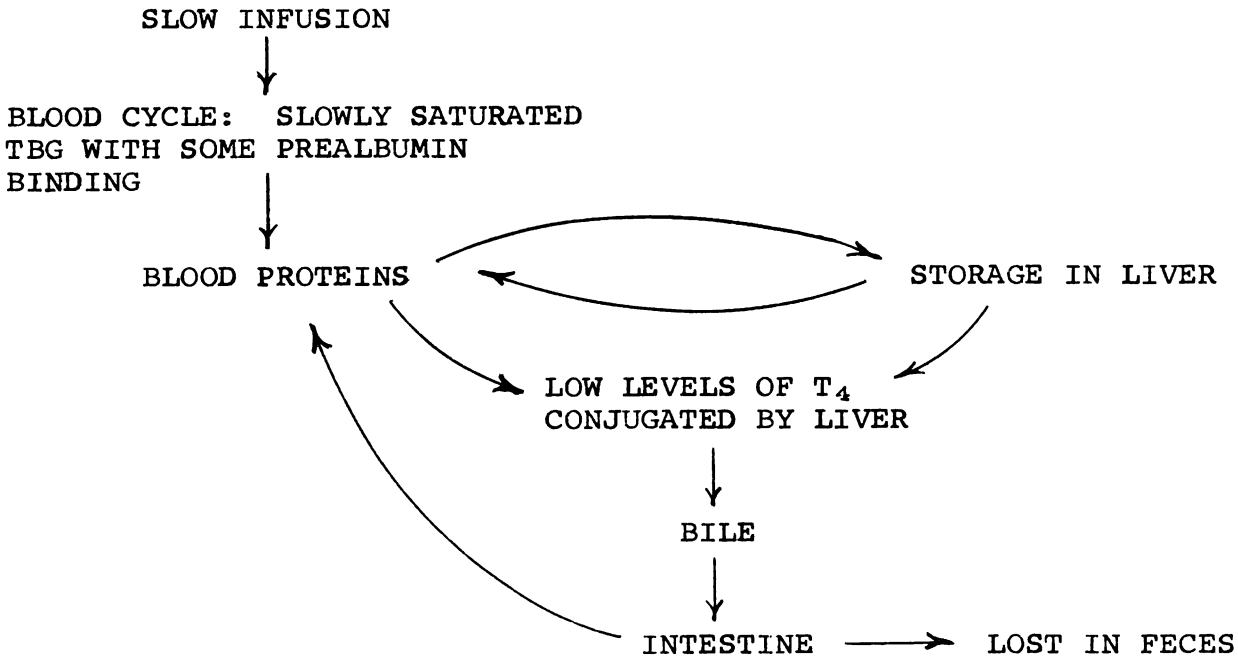

Figure VI. Possible pathways of exogenous thyroxine infusion.

SUMMARY OF RESULTS

Three short acting experimental anesthetics (Brevital, Evipal, and Metofane) were administered to determine if they significantly effected the thyroid secretion rate of rats when compared with a control anesthetic (Nembutal) known not to effect the TSR of these animals. The experimental anesthetics were compared to the control anesthetic by two separate methods for measuring the thyroid secretion rate; namely, the direct output method and the subsitution method. The Carworth Farms CFN strain of rat was employed in one substitution experiment and compared with the Sprague Dawley strain used in a later substitution experiment.

Evipal was chosen as the best anesthetic for use in the subsequent infusion experiment. In this infusion experiment, the substitution method employing once daily thyroxine injections was compared to a continuous infusion of thyroxine into unanesthetized, relatively unrestrained laboratory rats. The weeks' chronic jugular cannulation procedure, harness, and rotary swivel mechanism was employed to achieve the experimental results. Two sets of control animals were used. One group was cannulated, harnessed, and received continuous

saline infusion with once daily thyroxine injection. The other control group was unharnessed, non-cannulated and received once daily thyroxine injections. The experimental group of rats were cannulated, harnessed, and received continuous thyroxine infusion.

The two sets of controls were compared to each other to determine if any stress due to the Weeks' procedure was present and also compared to the experimental rats to observe any significant difference between the continuous infusion of thyroxine versus the once daily thyroxine injection.

CONCLUSIONS

1) The results of the anesthesia experiments I, II, and III show that there is no significant difference in the thyroid secretion rate of the animals under the experimental anesthetics (Evipal, Metofane and Brevital) when compared to the control anesthetic (Nembutal) within a given method and employing the same strain of rat.

2) The thyroid secretion rate obtained by the substitution method in Sprague Dawley rats was significantly higher than the thyroid secretion rate obtained by the direct output method in the same strain of rat. The overall average thyroid secretion rates were 1.69 $\pm$ 0.080 μg. T_4/100 g./day by the substitution method and 1.20 $\pm$ 0.064 μg. T_4/100 g./day by the direct output method. The difference was highly significant ($P > 0.01$).

3) The three direct output parameters "U", "K_4" and "I" showed non-significant differences ($P > 0.10$) for the three anesthetics, Evipal, Metofane, and Nembutal by the student "t" test.

4) The Carworth Farms CFN strain of rat gave a mean thyroid secretion rate of 2.04 $\pm$ 0.134 μg. T_4/100 g./day for the substitution method. The Sprague Dawley rats gave

a mean TSR of 1.69 ± 0.80 μg. T_4/100 g./day for the same method. A significant difference ($P > 0.05$) in the thyroid secretion rate exists between these two strains.

5) In the infusion experiment Group II and Group III controls had mean TSR values of 2.12 ± 0.248 μg. T_4/100 g./day and 1.86 ± 0.147 μg. T_4/100 g./day, respectively. The student "t" test gave a non-significant difference ($P < 0.10$) between the two control groups. The Group I experimental animals had a mean TSR of 2.84 ± 0.175 μg. T_4/100 g./day which was significantly higher than either the mean TSR value for Group II ($P < 0.05$) or the mean TSR value for Group III ($P < 0.01$). The results therefore showed that the higher TSR values of Group I were not due to any stress of the cannulation procedure or confinement in the harness, since these aspects would be reflected in Group II as well, but were due to the significantly greater quantity of infused thyroxine needed to achieve the same inhibitory effects which occur in the once daily injection of thyroxine.

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