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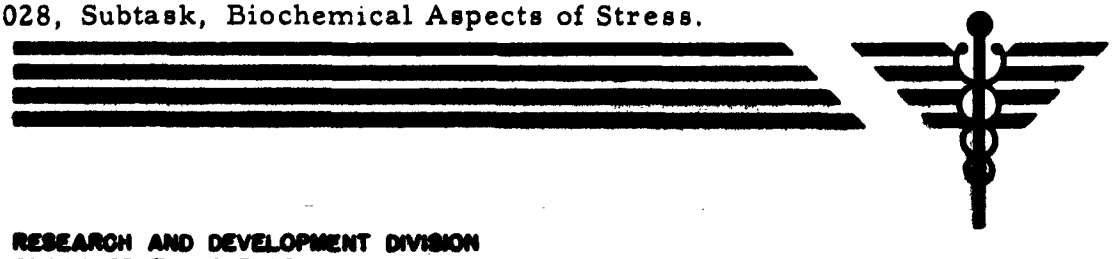
ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 133
12 January 1954

STUDIES ON THE ACTIVITY OF THE ISOLATED THYROID GLAND*

*Subtask under Environmental Physiology, AMRL Project No. 6-64-12-028, Subtask, Biochemical Aspects of Stress.



RESEARCH AND DEVELOPMENT DIVISION
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

Army Medical Research Lab. Project No. 6-64-12-028 Report No. 133

IN VITRO STUDIES OF THE ISOLATED THYROID GLAND

C.D. Eskelson, H.E. Firschein, A.L. Botkin and H. Jensen

12 pp & ii 12 January 1954
UNCLASSIFIED

Thyroxin has been found to exert in vivo an inhibitory effect on the thyroid by inhibiting the release of TSH from the hypophysis. The results of the in vitro studies on the thyroid, using TSH and I131 under various experimental conditions, indicate that the primary effect of TSH is to induce a release of hormone from the gland. Continuous stimulation of the thyroid by repeated TSH administration over a period of two to three days was found to result in an increased content of inorganic and organic radioiodine in the gland.

1. The thyroid gland. 2 Thyrotropin effects on thyroid function.
3. Thyroxin effects on thyroid function.

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STUDIES ON THE ACTIVITY OF THE ISOLATED THYROID GLAND*

by

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ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
12 January 1954

*Subtask under Environmental Physiology, AMRL Project No 6-64-12-028, Subtask, Biochemical Aspects of Stress.

Report No. 133
Project No. 6-64-12-028
Subtask AMRL S-11
MEDEA

12 January 1954

ABSTRACT

STUDIES ON THE ACTIVITY OF THE ISOLATED THYROID GLAND

OBJECT

To investigate the effect of thyroxin, of thiocyanate and of thyrotropic hormone (TSH) on the functional state of the isolated thyroid gland. Radioiodide (I^{131}) was employed to determine the functional activity of the thyroid gland.

RESULTS AND CONCLUSIONS

Thyroxin has been found to exert, in vivo, an inhibitory effect on the thyroid by inhibiting the release of TSH from the hypophysis.

Confirmatory evidence has been obtained that thiocyanate inhibits the uptake of iodide by the thyroid.

The results of the in vitro studies on the thyroid, using TSH and I^{131} under various experimental conditions, indicate that the primary effect of TSH is to induce a release of hormone from the gland. Continuous stimulation of the thyroid by repeated TSH administration over a period of two to three days was found to produce an increased content of inorganic and organic radioiodine in the gland.

The probable significance of these findings is discussed.

RECOMMENDATIONS

None.

Submitted by:

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STUDIES ON THE ACTIVITY OF THE ISOLATED THYROID GLAND

I. INTRODUCTION

Results of recent investigations of this laboratory on thyroid functions under certain conditions of experimental stress (1, 2, 3, 4, 5) have been obscured by the question of peripheral utilization and of excretion of released thyroid hormone.*

In the use of the radioiodine (I^{131}) level as an indication of the functional state of the thyroid in in vivo studies, the following must be considered:

A. The amount of I^{131} in the gland after I^{131} administration is dependent upon the rate of uptake and of the degree of conversion and release from the gland.

B. The organic I^{131} in the blood is dependent upon the rate of release of hormone from the gland and the rate of utilization and excretion.

In order to circumvent the in vivo influence of possible peripheral utilization and of excretion of the thyroid hormone with regard to the physiological state of the thyroid, in vitro studies were undertaken on the effect of thyroxin, thiocyanate and thyrotropic hormone (TSH) on the functional activity of the isolated gland.

II. EXPERIMENTAL

Male rats of the Sprague-Dawley strain, weighing from 200 to 250 gms, were used throughout the experiment. Hypophysectomized rats and litter mates were purchased from Hormone Assay Laboratory, Inc., Chicago, Ill.

The animals were housed at 25°C ± 1°. The normal animals were fed Purina checkered dog chow and the hypophysectomized animals received in addition, oranges, carrots, ground horse meat and bread. Food, but not water, was withheld for 18 to 24 hours before sacrifice.

*Recently Gross and Pitt-Rivers (6) have presented evidence that the thyroid releases in addition to thyroxin, another active principle which was shown to be 3:5:3' - triiodothyronine.

I^{131}* was administered intraperitoneally 18 hours before sacrifice if not otherwise stated. Injections of thyroxin and TSH** were given at various time intervals before sacrifice. The animals were sacrificed by a sharp blow at the base of the skull and exsanguinated by thoracic incisions and heart puncture. The thyroids were removed quickly and with as little damage as possible. Approximately 3 minutes elapsed from the time of sacrifice to the start of the incubation.

The glands were incubated with constant agitation in glass stoppered flasks containing 2 ml modified Krebs buffer (7). The incubations were carried out at 37°C ± 1° for 4 hours unless otherwise stated.

After incubation the glands were removed from the incubation media, rinsed once in 0.9% saline, blotted on filter paper, then placed in 2 ml of 2 N NaOH and heated on a steam bath for 2 hours or until dissolved.

The incubation media (hereafter referred to as bath) and the gland hydrolysates (hereafter referred to as gland) each were diluted to 10 ml. Both solutions were analyzed for total and organic bound radioiodine employing the methods of Morton and Chaikoff (8). Further details of the experimental conditions are given in the various tables. A significant change in the radioiodine content of the gland was assumed to indicate a change in the functional state of the gland.

III. RESULTS AND INTERPRETATIONS

The experimental data may be classified into six groups:

Group 1: Effect of Thyroxin on Thyroid Function as Measured by Radioiodine Content.

Results on the distribution of I^{131}, in the gland and in the bath indicated that thyroxin administration induced a hypofunctional state

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* The radioactive iodine, I^{131}, used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotope Division, U. S. Atomic Energy Commission.

** The TSH used was obtained from two sources: A Parke, Davis preparation, containing 10 J. S. units per mg, and two Armour preparations, one containing 4 J. S. units per mg and the other, 5 J. S. units per mg. The authors are greatly appreciative of the generosity of Armour and Company, Chicago, Illinois, and of Parke, Davis and Company, Detroit, Michigan, for supplying these TSH preparations.

of the thyroid (Table 1). There was an apparent inhibition of both the in vivo (Table 1) and the in vitro (Table 2) uptake and conversion of I^{131}. These results are in general agreement with the findings of Purves and Griesbach (9) that oral administration of thyroid extract to rats decreased the TSH content of the pituitary to less than 5 per cent of control values, and are also in agreement with those of Greer (10) and of Perlmutter (11) who found that the uptake of I^{131} by the thyroid gland of normal persons was markedly inhibited by ingestion of dessicated thyroid.

TABLE 1

THE EFFECT OF THYROXIN AND I^{131} INJECTION ON IN VITRO THYROID FUNCTION

	GLAND				BATH			
	TOTAL		ORGANIC		TOTAL		ORGANIC	
	EXPERIMENT I[1]							
	I^{131} & Thyroxin in vivo	I^{131} in vivo	I^{131} & Thyroxin in vivo	I^{131} in vivo	I^{131} & Thyroxin in vivo	I^{131} in vivo	I^{131} & Thyroxin in vivo	I^{131} in vivo
Mean[3]	10.55	24.62	8.23	17.88	14.84	28.18	6.62	12.19
S.D.	3.78	7.69	2.89	5.99	4.12	8.41	1.84	4.20
No of Animals	12	12	12	12	12	12	12	12
t	5.687**#		5.026**#		4.936**#		4.203**#	
	EXPERIMENT II-A[2]							
	Thyroxin & I^{131}	Control I^{131} 20 Hrs	Thyroxin & I^{131}	Control I^{131} 20 Hrs	Thyroxin & I^{131}	Control I^{131} 20 Hrs	Thyroxin & I^{131}	Control I^{131} 20 Hrs
Mean	1.64	5.03	1.32	3.88	1.93	4.62	0.51	1.50
S.D..	0.38	2.00	0.33	1.60	0.45	1.55	0.13	0.33
No. of Animals	6	6	6	6	6	6	6	6
t	4.079**#		3.820**#		4.092**#		6.908**	
	EXPERIMENT II-B[2]							
	Thyroxin & I^{131}	Control I^{131} 2 Hrs	Thyroxin & I^{131}	Control I^{131} 2 Hrs	Thyroxin & I^{131}	Control I^{131} 2 Hrs	Thyroxin & I^{131}	Control I^{131} 2 Hrs
Mean	0.62	1.86	0.59	1.80	0.73	1.82	0.50	1.09
S.D.	0.24	0.85	0.20	0.84	0.17	0.58	0.13	0.49
No. of Animals	6	6	6	6	6	6	6	6
t	3.437*#		3.443*#		4.413**#		2.875*#	

* Significant at the .05 level.
** Significant at the .01 level.
\# Modified t test due to rejection at .05 level that variances are equal.

1. 5 uc I^{131} and 0.5 mg l-thyroxin injected 18 hours before sacrifice. Gland incubated for 4 hours in Krebs buffer.
2. 0.5 mg l-thyroxin injected at 24, 12, and 2 hours before sacrifice In group A, 5 uc I^{131} given 20 hours and in group B, 2 hours before sacrifice. Gland incubated for 4 hours in Krebs buffer.
3. All means are expressed in thousands of counts per minute.

TABLE 2

THE EFFECT OF THYROXIN INJECTION ON THYROID I^{131} UPTAKE IN VITRO

	GLAND			
	TOTAL		ORGANIC	
	Thyroxin	Control	Thyroxin	Control
	EXPERIMENT I - A[1]			
Mean[3]	1.77	3.64	0.68	2.32
S.D.	0.46	1.09	0.37	0.95
No. of Animals	6	6	6	6
t	3.856**		3.952**	
	EXPERIMENT I - B[1]			
Mean	4.53	7.34	2.02	4.25
S.D.	0.43	0.97	0.22	1.05
No. of Animals	5	6	5	6
t	5.983**		5.089** #	
	EXPERIMENT II[2] -			
Mean	4.37	7.37	1.88	5.84
S.D.	2.08	3.61	1.26	4.16
No. of Animals	12	12	12	12
t	2.497*		3.159** #	

* Significant at the .05 level
** Significant at the .01 level
\# Modified t test due to rejection at .05 level that variances are equal.

1. 0.5 mg l-thyroxin administered 18 hrs. before sacrifice. Gland incubated for 4 hrs. in Krebs buffer containing 1 uc I^{131}.
2. 0.5 mg l-thyroxin injected at 24, 12 and 2 hrs. before sacrifice. Glands incubated for 4 hrs. in Krebs buffer containing 1 uc I^{131}.
3. All means are expressed in thousands of counts per minute.

No definite effect of thyroxin upon the uptake or release of I^{131} could be observed when the gland was incubated in vitro with I^{131} and thyroxin (data are omitted). Apparently thyroxin acts mainly upon the release-mechanism of TSH from the anterior pituitary rather than upon the thyroid itself. These results are in agreement with the generally accepted assumption that the rate of TSH release from the anterior pituitary is regulated predominantly by the level of circulating thyroid hormone.

Group 2: Effect of Thiocyanate on Thyroid Function as Measured by Radioiodine Content.

The data in Table 3 show that thiocyanate prevents the uptake of I^{131}, associated with a decreased content of organic I^{131} in the gland. These findings are in agreement with similar in vitro observations of Franklin, Chaikoff and Lerner (12) and with in vivo observations of Wyngaarden and his associates (13).

TABLE 3

EFFECT OF THIOCYANATE ON THE UPTAKE OF I^{131} BY THE THYROID GLAND IN VITRO

	GLAND			
	TOTAL		ORGANIC	
	KSCN	Control	KSCN	Control
	EXPERIMENT I			
Mean	1.58	4.19	0.051	0.363
S.D.	0.19	2.22	0.028	0.319
No. of Animals	6	6	6	6
t	2.863*#		2.387#	
	EXPERIMENT II			
Mean	2.26	10.37	1.39	6.12
S.D.	1.93	3.26	1.87	3.37
No. of Animals	6	6	6	6
t	5.236**		3.009*	

* Significant at the .05 level
** Significant at the .01 level
\# Modified t test due to rejection at .05 level that variances are equal.

1. Gland incubated for 4 hrs. in Krebs buffer containing 1 uc I^{131} and 0.3 mg KSCN
2. All means expressed in thousands of counts per minute.

The data in Table 4 illustrate that thiocyanate does not cause any significant release of I^{131} from the gland in vitro. Other investigators (14, 15) have reported that in rats, pre-treated with propylthiouracil, thiocyanate may cause the discharge of inorganic iodide previously accumulated by the thyriod gland. Apparently thiocyanate can cause the thyroid to expel inorganic iodide but not "iodine".

TABLE 4

EFFECT OF THIOCYANATE ON THE RELEASE OF I^{131} FROM THE THYROID GLAND

	GLAND			
	TOTAL		ORGANIC	
	KSCN	Control	KSCN	Control
	EXPERIMENT I			
Mean	19.15	17.55	14.49	16.40
S.D.	5.78	4.07	5.05	3.21
No. of Animals	6	6	6	6
t	0.554		0.780	
	EXPERIMENT II			
Mean	0.48	0.59	0.42	0.49
S.D.	0.16	0.08	0.11	0.10
No. of Animals	6	6	6	6
t	1.423		1.208	

1. 5 uc I^{131} injected 18 hours before sacrifice. Glands incubated for 4 hours in Krebs buffer containing 0.3 mg KSCN.
2. 5 uc I^{131} injected 15 minutes before sacrifice. Gland treated as in Experiment I.
3. All means expressed in thousands of counts per minute.

The mechanism of the action of thiocyanate has not yet been definitely established; apparently this agent inhibits the enzyme system responsible for the uptake of iodide. Raben (16) has suggested the possibility that large doses of thiocyanate could, in addition to the above effect, also directly affect organic synthesis.

Group 3: Administration of Both TSH and I^{131} in vivo.

The results of these experiments are given in Table 5. In Experiment 1, TSH and I^{131} were given 18 hours before sacrifice, followed by incubation of the gland in buffer for 4 hours. Under these conditions, TSH produced a lowering of total and organic I^{131} in the gland. This effect was also reflected in the difference of total and organic I^{131} in the bath between the TSH-treated and untreated animals.

Similar results were obtained on multiple TSH injections over a 24-hour period and I^{131} administration 24 hours prior to sacrifice (see Exp. II, Table 5).

In Experiment III, I^{131} was administered 1 hour before sacrifice of the animals which had received multiple injections of TSH ranging over a period of 26 to 62 hours. It is evident from the data presented in Table 5 that TSH administration under these conditions produced an increase in total and organic I^{131} in the gland as well as total I^{131} in the bath.

The results of Experiments II and III-Group A, in which 3 injections of TSH were given over a period of 24-26 hours, indicate that the time at which I^{131} was administered influenced the total and organic I^{131} content in the gland and also in the bath. In the case where I^{131} was given 24 hours before sacrifice (Exp. II), the administered TSH caused the gland to take up and release much of the accumulated I^{131}. On the other hand (Exp. III), when I^{131} was given 1 hour before sacrifice the gland could retain a greater percentage of the accumulated I^{131}. In the case where I^{131} was given 24 hours before sacrifice (Exp. II), the gland had access to the I^{131} throughout the TSH injections. The gland responded by increased release of hormonal iodine as well as increased uptake of inorganic iodine. At the last injection of TSH the gland had little if any available circulating inorganic I^{131} to take up, and thus one observes the release or lowering of I^{131} in the gland. In the case where I^{131} was given one hour before sacrifice (Exp. III), the gland had no I^{131} available until one hour after the last injection of TSH and, therefore, the only observable response was an uptake or increase in I^{131} content of the gland.

TABLE 5

THE EFFECT OF TSH AND I^{131} INJECTION ON IN VITRO THYROID FUNCTION

	GLAND				BATH			
	TOTAL		ORGANIC		TOTAL		ORGANIC	
	EXPERIMENT I-A[1]							
	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}
Mean	25.39	32.61	20.47	23.69	20.23	29.73	11.10	15.03
S.D.	5.25	8.46	4.74	9.05	3.46	5.36	2.13	3.53
No. of Animals	12	12	12	12	12	12	12	12
t	2.512*		1.092#		5.158**		3.294**	
	EXPERIMENT I-B[1]							
	TSH & I^{131}	Control & Albumin & I^{131}	TSH & I^{131}	Control & Albumin & I^{131}	TSH & I^{131}	Control & Albumin & I^{131}	TSH & I^{131}	Control & Albumin & I^{131}
Mean	20.50	31.02	15.34	25.26	17.60	27.97	9.39	15.64
S.D.	7.28	5.14	2.70	5.00	2.50	4.62	1.54	3.22
No. of Animals	6	5	6	5	6	5	6	5
t	2.708*		4.203**		4.757**		4.239**	
	EXPERIMENT II[2]							
	TSH & I^{131}	Control & Saline & I^{131}	TSH & I^{131}	Control & Saline & I^{131}	TSH & I^{131}	Control & Saline & I^{131}	TSH & I^{131}	Control & Saline & I^{131}
Mean	10.98	15.21	8.00	13.45	5.86	7.47	3.53	5.60
S.D.	4.73	3.13	2.03	2.73	1.89	2.64	1.38	2.10
No. of Animals	6	6	6	6	6	6	6	6
t	1.878		3.923**		1.212		2.013	
	EXPERIMENT III-A[3]							
	3 TSH & I^{131}	3 Saline & I^{131}	3 TSH & I^{131}	3 Saline & I^{131}	3 TSH & I^{131}	3 Saline & I^{131}	3 TSH & I^{131}	3 Saline & I^{131}
Mean[5]	8.74[4]	6.96	7.34	6.21	3.93	2.26	1.25	0.70
S.D.	1.99	0.55	1.50	0.36	1.06	0.57	0.36	0.25
No. of Animals	4	4	4	3	4	4	4	4
t	1.727		1.258		2.758*		2.190	
	EXPERIMENT III-B[3]							
	4 TSH & I^{131}	4 Saline & I^{131}	4 TSH & I^{131}	4 Saline & I^{131}	4 TSH & I^{131}	4 Saline & I^{131}	4 TSH & I^{131}	4 Saline & I^{131}
Mean	12.10[4]	5.96	9.21	5.30	5.46	3.06	1.58	1.04
S.D.	0.79	0.25	0.73	0.66	1.12	0.82	0.41	0.26
No. of Animals	4	4	4	4	4	4	3	4
t	14.813**		7.995**		3.449*		2.105	
	EXPERIMENT III-C[3]							
	5 TSH & I^{131}	5 Saline & I^{131}	5 TSH & I^{131}	5 Saline & I^{131}	5 TSH & I^{131}	5 Saline & I^{131}	5 TSH & I^{131}	5 Saline & I^{131}
Mean	6.34[4]	3.10	6.12	2.53	2.57	2.10	0.86	0.50
S.D.	0.84	0.50	0.90	0.46	1.51	0.60	0.47	0.15
No. of Animals	4	4	4	4	4	3	2	4
t	6.605**		7.078**		0.502		1.560	

(CONTINUED)

TABLE 5 CONTINUED

	GLAND TOTAL		GLAND ORGANIC		BATH TOTAL		BATH ORGANIC	
	EXPERIMENT III-D[3]							
	6 TSH & I^{131}	6 Saline & I^{131}	6 TSH & I^{131}	6 Saline & I^{131}	6 TSH & I^{131}	6 Saline & I^{131}	6 TSH & I^{131}	6 Saline & I^{131}
Mean	9.13[4]	5.26	7.68	4.60	3.44	2.24	1.17	0.90
S.D.	2.10	1.10	1.71	1.27	0.38	0.81	0.22	0.35
No. of Animals	4	4	4	4	4	4	4	4
t	3.271*		2.894*		2.678*		1.270	

* Significant at the .05 level.

** Significant at the .01 level.

\# Modified t test due to rejection at .05 level that variances are equal.

1. TSH and 5 uc I^{131} injected 18 hours before sacrifice. The group received 5 mg TSH (10 J.S. units/mg.), controls received 5 mg bovine albumin Gland incubated for 4 hours in Krebs buffer.

2. 5 uc I^{131} injected 24 hours before sacrifice, 1.25 mg TSH (10 J.S. units/mg) at 24, 12 and 2 hours before sacrifice. Controls received 5 uc I^{131} 24 hours, and saline 24, 12 and 2 hours before sacrifice. Glands incubated for 4 hours in Krebs buffer.

3. Each animal received multiple injections of 1.25 mg TSH (10 J.S. units/mg) at intervals of 12 hours, the last injection being given 2 hours before sacrifice; 5 uc I^{131} given 1 hour before sacrifice. Controls received saline instead of TSH and also 5 uc I^{131} 1 hour before sacrifice. Glands incubated for 2 hours in Krebs buffer.

4. Weight of gland increased, compared with controls receiving 0.9% saline.

5. All means are expressed in thousands of counts per minute.

Group 4: Administration of TSH in vivo and Incubation of the Gland in vitro with I^{131}.

As can be seen from Table 6, the glands from normal animals which had received a single injection of TSH 42 hours prior to sacrifice took up less I^{131}. This was associated with an indicated decreased content of organic I^{131}, as compared with glands from untreated animals. The increased blood level of thyroid hormone, caused by TSH, may have produced a hypofunctional state of the thyroid similar to that caused by thyroxin administration.

Administration of TSH to hypophysectomized animals increased thyroid functional activity as shown by an increased total amount of I^{131} in the gland as compared with glands from the untreated hypophysectomized animals (Exp. II, Table 6). In this case TSH administration apparently caused a shift from the hypofunctional state of the thyroid of hypophysectomized animals towards the functional state of a normal gland.

TABLE 6
EFFECT OF IN VIVO TSH ON IN VITRO I^{131} THYROID UPTAKE

	GLAND			
	TOTAL		ORGANIC	
	TSH	Control & Saline	TSH	Control & Saline
	EXPERIMENT I[1]			
Mean[2]	4.44	6.89	2.61	4.92
S.D.	1.60	2.50	1.10	2.60
No. of Animals	5	6	5	6
t	1.888		1.842	
	EXPERIMENT II[1] (Hypophysectomized Animals)			
Mean	5.09	1.84	1.42	0.67
S.D.	2.29	0.56	1.06	0.48
No. of Animals	10	8	10	8
t	4.327**#		1.848	

** Significant at the .01 level.
# Modified t test due to rejection at .05 level that variances are equal.
1. Normal Animals injected with 20 J.S. units of TSH hypophysectomized animals with 50 J.S. units of TSH or saline 42 hours before sacrifice. Glands incubated 4 hours in Krebs buffer containing 1 uc I^{131}.
2. All means are expressed in thousands of counts per minute.

Group 5: Administration of I^{131} in vivo and Incubation of Gland in vitro with TSH.

In this experiment, the animals were injected with I^{131} and upon sacrifice the thyroids were incubated in buffer with TSH or albumin. As can be seen from the data of Table 7 (Exp. 1), there was an apparent reduction in total the I^{131} content of those glands incubated with TSH. There also appeared to be some decrease in the organic I^{131} content; however, the significance of this reduction was questionable. Similar observations were made with thyroids from hypophysectomized rats. However, it is obvious that the glands from the hypophysectomized animals were in a less functional state than the gland from normal animals. Again it can been seen that TSH caused an apparent release of I^{131} from the gland.

TABLE 7
IN VITRO EFFECT OF TSH ON THYROID I^{131} RELEASE

	GLAND			
	TOTAL		ORGANIC	
	Normal & TSH	Normal Control	Normal & TSH	Normal Control
	EXPERIMENT I[1]			
Mean[2]	24.66	30.50	22.35	28.44
S.D.	7.55	8.60	6.59	7.95
No. of Animals	12	12	12	12
t	1.767		2.044	
	EXPERIMENT II (Hypophysectomized Animals)			
Mean	0.071	0.206	0.040	0.135
S.D.	0.041	0.059	0.017	0.035
No. of Animals	3	2	3	2
t	Not performed		Not performed	

1. Animals were injected with 5 uc I^{131} 18 hours before sacrifice. Glands incubated for 4 hours in Krebs buffer containing either TSH (24 J.S units or 5 mg of bovine albumin (control).
2. All means are expressed in thousands of counts per minute.

Group 6: Incubation of Gland *in vitro* with both TSH and I^{131}.

Results of incubation of the thyroid (from normal and hypophysectomized animals) with TSH and I^{131} simultaneously indicate that TSH produced a lowering of total I^{131} with an apparent simultaneous release of organic I^{131} (Table 8).

TABLE 8

EFFECT OF TSH IN VITRO ON IN VITRO THYROID I^{131} UPTAKE AND RELEASE

	GLAND				BATH			
	TOTAL		ORGANIC		TOTAL		ORGANIC	
	EXPERIMENT I[1]							
	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}	TSH & I^{131}	Control & I^{131}
Mean[2]	4.66	14.89	1.07	4.84	83.4	74.7	2.40	1.48
S.D.	0.92	4.20	0.53	4.25	8.1	9.7	0.22	0.22
No. of Animals	6	6	12	12	12	12	12	12
t	5.829**#		3.052*#		2.377*		10.312**	
	EXPERIMENT II[1] (Hypophysectomized Animals)							
Mean[2]	1.46	1.94	0.12	0.70				
S.D.	0.23	0.37	0.01	0.25				
No. of Animals	3	2	3	2				
t	Not performed		Not performed					

* Significant at the .05 level.

** Significant at the .01 level.

\# Modified t test due to rejection at .05 level that variances are equal.

1. Thyroids incubated for 4 hours in Krebs buffer containing either TSH (10 J.S. units) or bovine albumin (controls).

2. All means are expressed in thousands of counts per minute.

IV. GENERAL DISCUSSION

The distribution of radioiodine in the gland and bath was subject to considerable variation in the different experiments and not all differences of means were statistically significant.

The overall results of the in vitro studies on the normal thyroid, treated with TSH and I^{131} under various experimental conditions, seem to indicate that the primary effect of TSH is to induce a release of hormone from the thyroid. Histological studies of the gland seemed to bear out this assumption since TSH appeared to cause in vitro a breakdown of colloid in the lumen of the follicle and transport of the products through the cell. These observations are in agreement with the in vivo findings of DeRobertis (17) that the proteolytic activity within the thyroid follicles increases soon after the administration of TSH. According to the author, thyroid hormone is liberated from the thyroglobulin complex, present in the colloid of the follicle, under the influence of an enzyme and is then transported through the follicular cells to the blood stream.

In vitro studies on thyroids from hypophysectomized animals gave in general results similar to those obtained with normal glands. As was expected, the glands from the hypophysectomized animals were in a less functional state than normal glands. According to Randall, Lorenz and Albert (18) iodide collection, conversion to organic iodine, and discharge of hormone can occur in the absence of the pituitary but at a much reduced rate. Apparently, collection and secretion are affected to about the same extent. This lowered function can be overcome, at least in part, by TSH administration.

The finding that TSH in vitro produced a release of organic iodine is in agreement with in vivo observations. Using I^{131}, Wolff (19) and Botkin and Jensen (3) demonstrated, in rats, a marked release of organic I^{131} from the thyroid two to four hours after an intraperitoneal injection of TSH, reaching a maximum after about 8 hours. In young chicks, Rawson and his callaborators (20) observed an increased release of thyroid hormone within 6 hours with a maximum 24 hours after a subcutaneous injection of TSH. The uptake of I^{131} apparently increases upon continuous stimulation of the thyroid by TSH, as illustrated by the results of Experiment 3 in Group 3 (Table 5). These findings are in agreement with similar observations of Keating, Rawson, Peacock and Evans (21) and of Vanderlaan and Greer (22).

Rawson (20) has suggested that the primary effect of TSH is to liberate hormone from the thyroid, while other responses of the gland to TSH might actually be secondary to the release of hormone. Studies by Morton, Perlman and Chaikoff (23) on the iodine partition within the gland indicate that the loss of a hormone-like fraction occurs faster than that of the diiodotyrosine or inorganic iodine. It is quite

possible that there exists in the gland a dynamic equilibrium between inorganic iodine, intermediate organic iodine and hormone iodine. Release of hormone then produces a shift of this equilibrium towards new formation of hormone iodine.

It would appear that the thyrotropic hormone has at least two main effects on the thyroid. These are a) the liberation of thyroid hormone stored in the gland, which in turn may cause an increased turnover rate of thyroid hormone, and b) an increase in the hormone manufacturing capacity, bringing with it the ability of the gland to take up greater amounts of iodide and to store larger amounts of hormone (continuous stimulation of the gland, see results of Exp. 3 in Group 3).

V. SUMMARY

Thyroxine has been found to exert, in vivo, an inhibitory effect on the thyroid by inhibiting the release of TSH from the hypophysis.

Confirmatory evidence has been obtained that thiocyanate inhibits the uptake of iodide by the thyroid.

The results of the in vitro studies on the thyroid, using TSH and I^{131} under various experimental conditions, indicate that the primary effect of TSH is to induce a release of hormone from the gland. Continuous stimulation of the thyroid by repeated TSH administration over a period of two to three days was found to produce an increased content of inorganic and organic radioiodine in the gland.

The probable significance of these findings is discussed.

VI. RECOMMENDATIONS

None.

VII. BIBLIOGRAPHY

1. Schachner, H. G., Z. S. Gierlach, and A. T. Krebs. The response of the thyroid gland to a low environmental temperature as studied with radioiodine. MDFRL Report 6-64-12-02-(6) June 1949.

2. Hamolsky, M. W., Z. S. Gierlach, and H. Jensen. Uptake and conversion of radioactive iodine (I^{131}) by thyroid gland in vivo and in vitro in tourniquet shock in rats. Am. J. Physiol. 164: 35, 1951.

3. Botkin, A. L., and H. Jensen. The effect of epinephrine and TSH on thyroid function in rats. Endocrinology 50: 68, 1952.

4. Botkin, A. L., and J. T. Tew. Thyroid and adrenal response to varying dose levels of epinephrine. Proc. Soc. Exper. Biol. & Med. 79: 378, 1952.

5. Botkin, A. L., E. H. Praytor, M. E. Austing, and H. F. Jensen. Thyroid response to total body x-irradiation. Endocrinology 50: 550, 1952.

6. Gross, J., and R. Pitt-Rivers. 3:5:3' - Triiodothyronine. I. Isolation from thyroid gland and synthesis. Biochem. J. 53: 645, 1953.

7. Krebs, H. A. Body size and tissue respiration. Biochimica et Biophysica Acta 4: 249, 1950.

8. Morton, M. E., and I. L. Chaikoff. The formation in vitro of thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator. J. Biol. Chem. 147: 1, 1943.

9. Purvis, H. D., and W. E. Griesbach. The effect of thyroid administration on the thyrotropic activity of the rat pituitary. Endocrinology 39: 274, 1946.

10. Greer, M. A. The effect on endogenous thyroid activity of feeding desiccated thyroid to normal human subjects. New England J. Med. 244: 385, 1951.

11. Perlmutter, M. Clinical discussion - The use and abuse of thyroid hormone therapy. Metabolism 2: 81, 1953.

12. Franklin, A. L., I. L. Chaikoff, and S. R. Lerner. The influence of goitrogenic substances on the conversion in vitro of inorganic iodide to thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator. J. Biol. Chem. 153: 151, 1944.

13. Wyngaarden, J. B., J. B. Stanbury, and B. Rapp. The effects of iodide, perchlorate, thiocyanate and nitrate administration upon the iodide concentrating mechanism of the rat thyroid. Endocrinology 52: 568, 1953.

14. Vanderlaan, J. E., and W. P. Vanderlaan. The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate. Endocrinology. 40: 403, 1947.

15. Stanley, M. M. and E. B. Astwood. The accumulation of radioactive iodide by the thyroid gland in normal and thyrotoxic subjects and the effect of thiocyanate on its discharge. Endocrinology 42: 107, 1948.

16. Raben, M. S. The paradoxical effects of thiocyanate and of thyrotropin on the organic binding of iodine by the thyroid in the presence of large amounts of iodide. Endocrinology 45: 296, 1949.

17. DeRobertis, E. Proteolytic enzyme activity of colloid extracted from single follicles of the rat thyroid. Anatomical Record 80: 219, 1941.

18. Randall, R., N. Lorenz, and A. Albert. The effect of hypophysectomy on the biological decay of thyroidal radioiodine. Endocrinology 48: 339, 1951.

19. Wolf, J. Some factors that influence the release of iodine from the thyroid gland. Endocrinology 48: 284, 1951.

20. Rawson, R. W. Physiological reactions of the thyroid-stimulating hormone. Ann. New York Acad. Sci. 50: 491, 1949.

21. Keating, Jr., F. Raymond, R. W. Rawson, W. Peacock, and R. D. Evans. The collection and loss of radioactive iodine compared with the anatomic changes induced in the thyroid of the chick by the injection of thyrotropic hormone. Endocrinology 36: 137: 1945.

22. Vanderlaan, W. P., and M. A. Greer. Some effects of the hypophysis on iodine metabolism by the thyroid gland of the rat. Endocrinology 47: 36, 1950.

23. Morton, M. E., I. Perlman, and I. L. Chaikoff. Radioactive iodine as an indicator of the metabolism of iodine. J. Biol. Chem. 140: 603, 1941.