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ON THE EFFECT  
OF  
NEUROHYPOPHYSIAL HORMONE  
ON  
THYROID ACTIVITY

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## A B S T R A C T

### ON THE EFFECT OF NEUROHYPOPHYSIAL HORMONE ON THYROID ACTIVITY

As to the influence of neurohypophysial hormone on the thyroid activity, conflicting results have been reported according to investigators. The present experiments were carried out to elucidate the role of vasopressin in the thyrotropin discharge and thyroid secretion, measuring thyroïdal  $^{131}\text{I}$  uptake (T/S), plasma PB $^{131}\text{I}$  level, as well as histological examination of the thyroid gland, in rats.

Though a single dose of Pitressin failed to change the T/S in rats kept at 20°C, successive administrations of Pitressin tannate resulted in a suppression of the rise of T/S due to chronic cold exposure. These results well coincided with that in the histological examination.

Pitressin, but not synthetic lysine vasopressin, suppressed the PB $^{131}\text{I}$  level which was slightly elevated by minor surgical stress used. Pitressin and lysine vasopressin demonstrated suppressive effect in the same extent, if these preparations were given intraperitoneally. The rise of plasma PB $^{131}\text{I}$  level following exogenous thyrotropin was not affected by these neurohypophysial preparations. The administration of Pitressin in various doses and routes did never increase the plasma PB $^{131}\text{I}$  level in the rats under experimental conditions employed.

## INFLUENCE OF THE POSTERIOR PITUITARY HORMONE ON THE THYROIDAL IODIDE PUMP AND $^{131}\text{I}$ UPTAKE

### 1. Introduction

A number of attempts were made to elucidate the possible hypothalamic neurohumor in regulating the release of TSH by the anterior pituitary gland. The nature of this substance, however, remains unknown. It was reported that the corticotrophin releasing factor (CRF) is a peptide closely related to vasopressin (10). This posterior pituitary hormone was postulated by several investigators as a possible neurohumoral substance participating in TSH release (5,7). According to these researchers, the thyroidal  $^{131}\text{I}$  uptake, the conversion ratio of inorganic iodide to protein-bound-iodide (PBI) and the level of PBI were stimulated by the administration of vasopressin. On the other hand, entirely opposite evidences were presented by other investigators (4,9). We observed that vasopressin did not affect the thyroidal function of rats at a warm ambient temperature using oxygen consumption, PBI and histological examination as the indices of thyroidal activity, but significantly inhibited the increase in its activity during cold exposure (1). Skebelskaya (11) reported that the posterior pituitary hormone reduced the ability of the thyroid gland to accumulate  $^{131}\text{I}$ . These conflicting results may be due to the differences in the experimental conditions or the neurohypophyseal preparations used. To study further the conditions under which vasopressin may or may not affect the thyroidal function, we have decided to re-examine the previous work with thyroid-serum iodide ratio (T/S ratio),  $^{131}\text{I}$  uptake and histological examination to evaluate the thyroid activity.

### 2. Materials and Methods

Wister strain male rats were used throughout the experiment. The body weight of rats ranged from 130 to 340 g in all of the study. But the rats of as similar body weight as possible were employed in each series of experiments.

Pitressin (Park, Davis & Co.) was diluted with physiological saline, or polyethylenglycol (PEG) and Pitressin tannate (Park, Davis & Co.) with peanut oil.

The T/S ratio was determined by the method originally described by Vanderlaan and Greer (12). Two mg of methylthiouracil per 100 g body weight instead of propylthiouracil was subcutaneously injected and a tracer dose of carrier free  $^{131}\text{I}$  was given 45 minutes thereafter. The blood was withdrawn from the abdominal aorta under ether anesthesia 1 hour after  $^{131}\text{I}$  and a single lobe of thyroid was dissected out, cleaned off adhesive tissue and weighed on a torsion balance to the nearest 0.1 mg. The thyroid gland was then homogenized in 7 ml of 10 % trichloroacetic acid. The homogenate was centrifuged and 2 ml of the supernatant was transferred to a clean tube for radioassay by means of a well-type scintillation counter. The blood collected was allowed to clot, centrifuged and aliquot 0.5 ml of the serum placed in a test tube. The serum was added with 4.5 ml of 10 % trichloroacetic acid, mixed well and centrifuged. After the supernatant was transferred to another test tube, the precipitate was washed once with 5 ml of 10 % trichloroacetic acid and centrifuged. Two ml of the pooled supernatant was served for measurement of radioactivity.

Thyroidal uptake of  $^{131}\text{I}$  at 24 hour interval was measured by direct counting of the dissected thyroid.

The dissected thyroids for histological examination were fixed in formol-sublimate or Bouin's solution and stained with hematoxylin and eosin.

### 3. Results

#### a. Effect of a single dose of Pitressin on T/S ratio.

The mean of T/S ratio of nontreated rats was  $109 \pm 8.4$ .

Pitressin in a dose of 60 mU in 0.1 ml of physiological saline per 100 g body weight was given intraperitoneally 24 hours before the T/S determination. Pitressin was also given 90 minutes before the blood collection, e.g., it was injected subcutaneously 15 minutes after methylthiouracil and a tracer dose of  $^{131}\text{I}$  was administered 30 minutes after Pitressin. Physiological saline was given in the control animals in place of Pitressin. As shown in Table 1, no significant difference was observed in either case between control and Pitressin-treated rats.

**b. Effect of chronic administration of Pitressin tannate on T/S ratio.**

One hundred or 400 mU of Pitressin tannate suspended in 0.05 ml of peanut oil per 100 g body weight was subcutaneously injected into a rat once daily for 3 days. The rat was sacrificed 24 hours after the last injection. The control animals were treated with the vehicle only in the same fashion as those given Pitressin tannate. As illustrated in Fig. 1, the peanut oil used possessed a slight goitrogenic effect on the rats, resulting a significant increase in the T/S ratio. One hundred mU of Pitressin tannate per 100 g body weight of rat did not elevate the T/S further. Four hundred mU of Pitressin tannate, however, rather showed a suppressive effect on the T/S ratio, though without effect on the thyroid weight. There was statistically significant difference between the T/S ratios of the groups of rats treated with 100 mU and 400 mU of Pitressin tannate.

**c. Effect of Pitressin on the T/S ratio during cold exposure.**

Although Pitressin did not affect the T/S ratio of rats at the ordinary ambient temperature, 20 °C, it might modify the thyroidal activity in response to cold. Rats were transferred into a cold environment of 10 °C and the T/S was determined 7 days thereafter. During the rats were exposed to cold, they were injected subcutaneously with 40 mU of aqueous Pitressin dissolved in 0.05 ml polyethyleneglycol per 100 g of body weight twice daily, or the vehicle only. The results are shown in Fig. 2. The T/S ratio considerably increased in the polyethyleneglycol treated control rats after the cold exposure. The thyroid also increased in weight. However, in the rats injected with Pitressin the increase in T/S ratio by the cold was less in extent than that of the former, though no suppression on the increase in thyroid weight was observed by Pitressin.

**d. Histological findings.**

The histological parameters indicating the thyroidal activity was mainly based on the height of follicular epithelium and the extent of the colloid retention. Since the histological appearance was not

uniform throughout one gland, the activity of the gland was judged by inspecting the serial sections and it was arbitrarily divided into 6 stages. The stage 0 represents the thyroid of the lowest activity as seen in the hypophysectomized rats. Stage 5 is of the maximum activation as seen in the goitrogen treated rats. The activity stages falling between the stage 0 to 5 were divided in 4, i.e., Stages 1, 2, 3 and 4.

The histological picture of the thyroid of rat adapted in a warm ambient temperature of 20 °C showed a moderate activity. Nine of these rats' thyroids examined had many inactive large follicles located in the peripheral region of the gland containing the squamous epithelium and the hard and dense colloid. In the center part of the gland, there were groups of the follicles smaller than 50  $\mu$  in diameter with cuboidal epithelium of 5 - 8  $\mu$  in height and dense colloid. In five out of 9 thyroids, some lobules contained follicles with the cuboidal epithelium and resorption vacuoles in the faintly stained colloid (Stage 2). Other 4 thyroid glands contained a larger number of these active follicles (Stage 3) (Table 3).

The thyroid glands of rats exposed to cold environment of 10 °C for 1 week were also histologically examined. They showed the activities of various stages. Table 3 shows the thyroid activity of rats used for the control animals in the experiment for Pitressin effect. Two out of 10 thyroid glands examined showed the activity of Stage 2, and 4 glands Stage 3. But other 4 had an appearance of increased activity indicated by the presence of centrally located small follicles with tall columnar epithelium 10 - 12  $\mu$  high and the faintly stained colloid containing many vacuole - like spaces. In these cases some of the peripheral large follicles also showed hyperactivity (Stage 4). In general histological appearance, the secretory activity of the thyroids of rats exposed to cold seems to be increased although considerable difference was seen from gland to gland (Table 3).

On the other hand, the glands of rats given Pitressin during the period of cold exposure remained in Stages 2 and 3, i.e., in a moderate active stage, and no hyperactive gland of Stage 4 was found

among these rats. The suppression on the increased thyroid activity due to cold was seen with both Pitressin and Pitressin tartrate. The histological picture of these glands did not depart from that of the 20 °C adapted rat's thyroid with the activity of Stages 2 and 3 (Table 3).

e. Effect of Pitressin on the  $^{131}\text{I}$  uptake by the rat's thyroid.

Skebelskaya reported that the thyroïdal  $^{131}\text{I}$  uptake at a 3-hour-interval was markedly suppressed by the posterior pituitary hormone (11). We have reinvestigated the effect of Pitressin on the thyroïdal  $^{131}\text{I}$  uptake in the identical fashion as Skebelskaya's experiment, except we performed the experiment at a room temperature of approximately 20 °C, while he made his study in a considerably cold environment ranging from 4.5 to 6.7 °C. Pitressin in a dose of 100 mU or 25 mU per rat was subcutaneously injected and a tracer dose of carrier free  $^{131}\text{I}$  was given immediately after the Pitressin injection. As shown in Table 2, neither 100 mU nor 25 mU of Pitressin exerted any influence on the thyroïdal  $^{131}\text{I}$  uptake at a 3-hour-interval.

#### 4. Discussion

The effect of Pitressin in a various doses on the Rat's thyroid activity was investigated using T/S ratio, thyroïdal  $^{131}\text{I}$  uptake and histological examination as the parameters. Although several investigators reported a stimulative effect of the posterior pituitary hormone, we failed to observe this effect but rather inhibitory action in some cases.

Bottari reported that Pitressin administered intraperitoneally into a rabbit causes immediate rise of TSH level in the peripheral blood (2).

If it is the case in rats, an increased TSH secretion following the intraperitoneal injection of Pitressin may be reflected by an increased T/S ratio. The effect of exogenous TSH on the T/S ratio in rats was reported to be the most strikingly manifested 24 hours after TSH (6,12). The T/S ratio in rats, however, did not depart from the control value 24 hours after 100 mU of Pitressin per 100 g body weight.

Acute effect of subcutaneous Pitressin was also investigated, but no effect on the T/S ratio was demonstrated.

Long acting Pitressin tannate in a dose of 100 mU per 100 g body weight given once daily for 3 days also failed to produce any demonstrable effect on the T/S and the thyroid weight, but a larger dose, 400 mU per 100 g body weight, seemed to suppress the thyroidal activity. Four hundred mU of Pitressin may be an enormously large amount for a rat and might result in the pituitary adrenal activation, which is likely to exert a suppression on the thyroid function (3,8,13).

It is interesting to see that 40 mU of Pitressin per 100 g body weight has possessed a suppressive effect on the rise of T/S ratio due to the cold exposure, though this suppression is moderate in extent. This result coincided well with the histological findings. Our previous work demonstrated an inhibitory effect of Pitressin on the increase in thyroid activity during the cold exposure using FBI, oxygen consumption and histological examination as the indices of the thyroidal function (1). The present study using T/S ratio and histological examination of the thyroid gland has reconfirmed the previous work.

It may be certain that Pitressin in a dose smaller than 100 mU per 100 g given subcutaneously or intraperitoneally does not show any demonstrable effect on the thyroid function of rat at an ordinary room temperature. But, when the thyroid is stimulated by cold, exogenous Pitressin suppresses the response.

## References

- 1) Arimura, A., Y. Takagi and T. Ueno, *Jap. J. Physiol.*, 6 : 284, 1956
- 2) Bottari, P.M., *Ciba Foundation Colloquia in Endocrinology*, 11 : 52, 1957
- 3) Brown-Grant, K., G.W. Harris & S. Reichlin, *J. Physiol.*, 126 : 41, 1954
- 4) Crosson, J., J. Falch and S. Reichlin, *Endocrinology*, 66 : 777, 1960
- 5) Garcia, J., G.W. Harris and W.J. Schindler, *J. Physiol.*, 162 : 64, 1962
- 6) Halmi, N.S., B.N. Spirtos, E.M. Bogdanove & H.J. Lipner, *Endocrinology*, 52 : 19, 1953
- 7) Maeda, H., *Folia Endocr. Jap.*, 37 : 364, 1961
- 8) Money, W.L., L. Kirshner, L. Krains, P. Merrill & Rawson, R.W., *J. Clin. Endocrinol.*, 10 : 1282, 1950
- 9) Rose, S., J. Nelson, T.R. Bradley, *Ann. New York Acad. Sc.*, 86 : 647, 1960
- 10) Schally, A.V., R.N. Anderson, H.S. Lipscomb, J.M. Long & R. Guillemin, *Nature*, 188 : 1192, 1960
- 11) Skebelskaya, Y.B., *Problemy Endokrinologii: Gormonterapii*, 4 : 32, 1961
- 12) Vanderlaan, W.P. & M.A. Greer, *Endocrinology*, 47 : 35, 1950
- 13) Williams, R.H., H. Jaffe & C. Kemp, *Amer. J. Physiol.*, 159 : 291, 1949

Table 1

Effect of Single Injection of Aqueous Pitressin  
on Thyroidal Iodide Pump

Treatment	No. of Rats	Body Weight (g) mean $\pm$ S.E.	Thyroid Weight (mg) mean $\pm$ S.E.	T/S mean $\pm$ S.E.	P vs Control
Control	11	309 $\pm$ 9.5	18.4 $\pm$ 1.71	109 $\pm$ 8.4	
24 hr. after Pit. 60 mU/100g. i.p.	6	238 $\pm$ 6.5	17.2 $\pm$ 0.93	124 $\pm$ 10.9	N.S.
Saline 15 mins. after MTU	5	234 $\pm$ 5.1	19.0 $\pm$ 1.71	139 $\pm$ 14.8	
Pit. 60 mU/100g 15 mins. after MTU	6	220 $\pm$ 8.8	15.8 $\pm$ 0.93	129 $\pm$ 17.1	N.S.

Table 2

Effect of Pitressin on the Thyroidal  $^{131}\text{I}$  Uptake  
at 3 Hours Interval

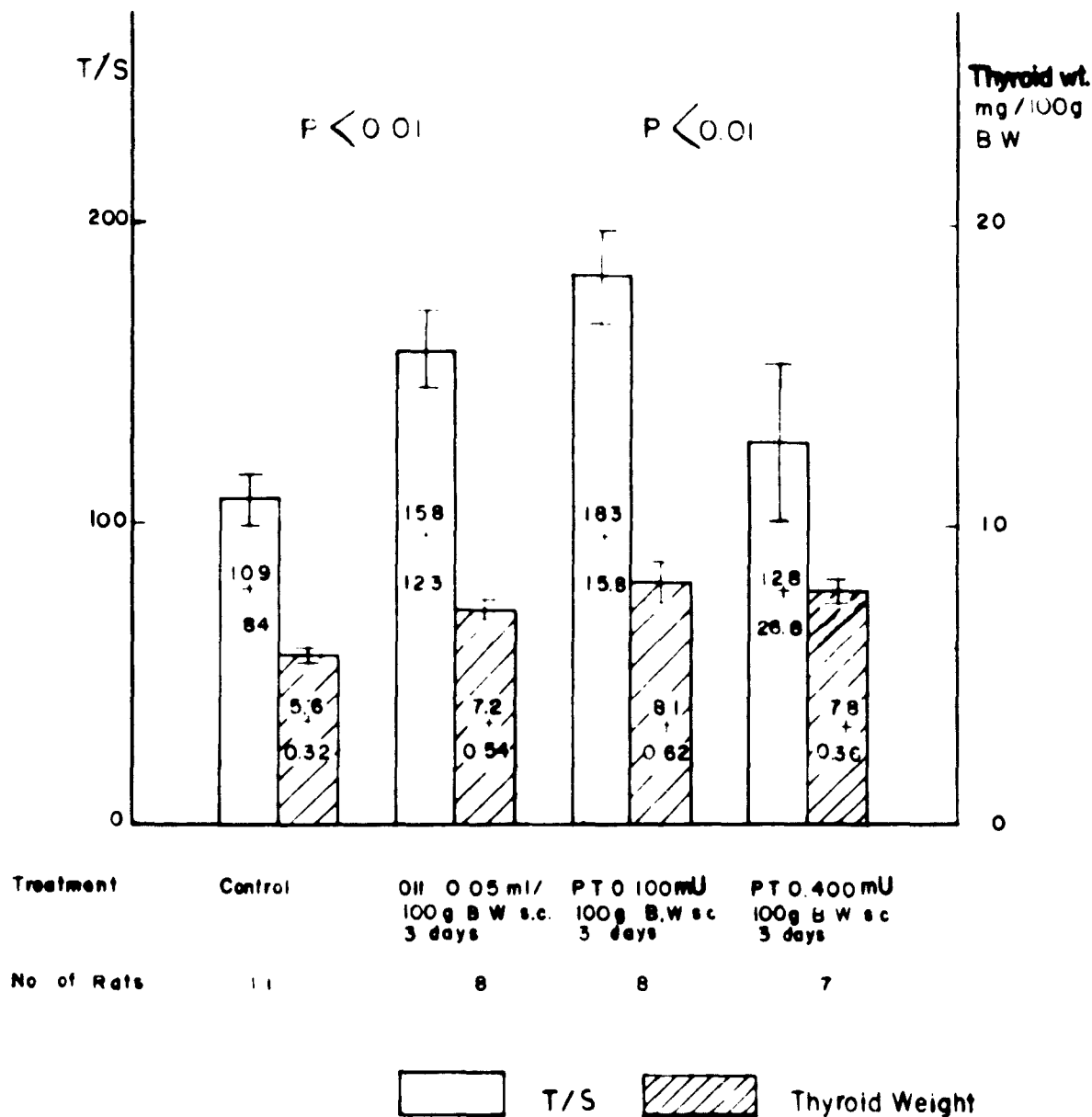
Procedure	No. of Rats	Body Weight (g) mean $\pm$ S.E.	Thyroid Weight (mg) mean $\pm$ S.E.	Thyroidal $^{131}\text{I}$ Uptake (%) mean $\pm$ S.E.	P vs Control
Control	7	154 $\pm$ 4.4	13.6 $\pm$ 1.2	14.1 $\pm$ 1.7	
Pitressin 100 mU/Rat s.c.	6	152 $\pm$ 4.8	13.8 $\pm$ 1.5	15.2 $\pm$ 2.2	N.S.
Pitressin 25 mU/Rat s.c.	6	148 $\pm$ 5.5	12.8 $\pm$ 0.7	15.0 $\pm$ 2.8	N.S.

Table 3

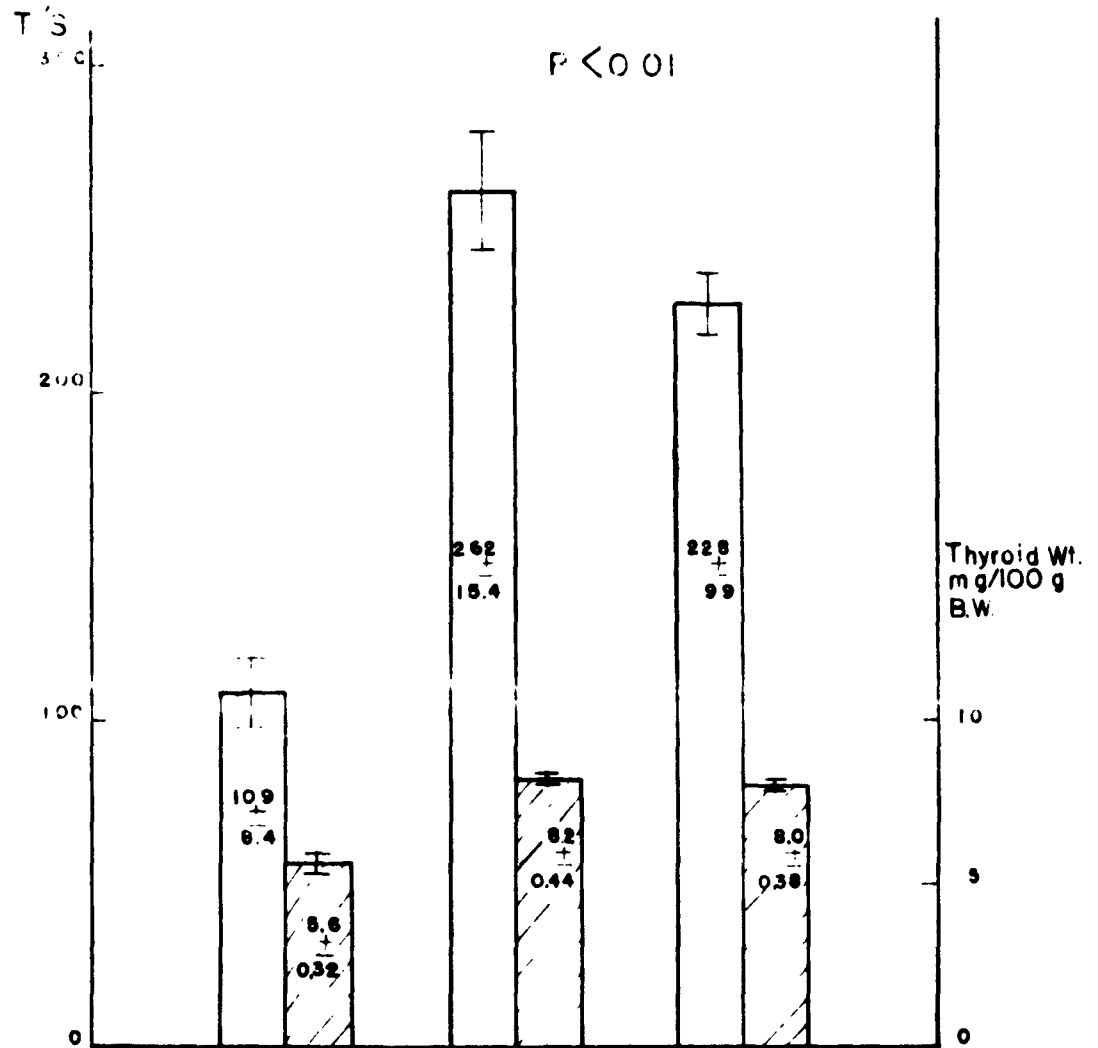
Histological Appearance  
of the Thyroid Gland

Group	No. of Rats Examined	Stages of Activity					
		0	1	2	3	4	5
20 °C Normal	9	-	-	5	4	-	-
29 °C Normal	6	-	2	4	"	-	-
10 °C Oil Cont.	6	-	-	1	3	2	-
Pit. Tann.	5	-	-	4	1	-	-
PEG Cont.	4	-	-	1	1	2	-
Pit.	5	-	-	2	3	-	-

# Effect of Chronic Administration of Pitressin Tannate in Oil on Thyroidal Iodide Pump in Rats



# Influence of Cold Exposure on Thyroidal Iodide Pump in Rats



Treatment

Control

10°C for 1 week  
PEG 0.05 ml/100g  
s.c. twice daily

10°C for 1 week  
Pit. 40mU/100g  
s.c. twice daily

No. of Rats

11

9

10



T/S



Thyroid Weight

PLASMA  $PB^{131}I$  LEVEL IN RATS AND  
THE INFLUENCE OF THE NEUROHYPOPHYSIAL HORMONE  
DURING THE SLIGHT SURGICAL STRESS

1. Introduction

Exogenous neurohypophysial preparations, either natural or synthetic, has been reported to increase  $PB^{131}I$  in rabbits (8) and mice (12) whose endogenous TSH secretion was blocked by administration of thyroxine. During the course of investigation dealing with the effect of neurohypophysial hormone on  $PB^{131}I$  in rats, we have found that a minor surgical manipulation for blood collection seemed to stimulate the thyroid activity, though slight in extent. Further, the neurohypophysial hormone in a variety of doses failed to increase  $PB^{131}I$  in the peripheral blood of rat, but rather suppressed it during the stress. The present study was made in an attempt to elucidate the mechanism of the phenomena mentioned above.

2. Materials and Method

Wistar strain male rats weighing 150 to 300 g were used throughout the experiment. They were kept at 20 °C for at least 3 weeks prior to the experiment and fed Oriental rat's biscuit.

The rats were injected with a tracer dose of carrier free  $^{131}I$ , usually 10 to 15  $\mu$ c per rat, and the experiment was performed 40 hours thereafter. The rats were usually used twice for experiment, but at least a period of 2 weeks was allowed to elapse after the first experiment.

Blood collection was made under ether anesthesia from the external jugular vein by making a small cutaneous incision in the clavicular region. Ether was inhaled to the rat only at the time of blood withdrawal. Approximately 0.3 ml of blood was collected each time into an oxalated centrifuge tube and the plasma separated by centrifugation. One tenth ml of plasma was placed in a small test tube containing 1 ml of 10 % trichloroacetic acid (TCA). The plasma protein precipitated by TCA was washed once with 1 ml of 10 % TCA and its radioactivity counted by a well type scintillation counter.

Plasma protein content was determined by refractometry. Hypophysectomy was carried out through the external auditory canal. Adrenalectomy and adrenomedullectomy were made through dorsal approach.

The neurohypophysial preparations used were Pitressin (Parke, Davis & Co.) and Synthetic Lysine Vasopressin (Sandoz). TSH used was Ambinon (Organon). Other hormone preparations employed were Cortisone acetate (Nippon Merck Banyu Co., Ltd.) and Cortrophine (Organon). L-thyroxin was supplied by Takeda Pharmaceutical Co. Phenoxybenzamine hydrochloride and guanithiadine used for adrenolytic agents were Dibenzylamine (Smith, Kline & French Labs.) and Ismelin (Chiba), respectively.

### 3. Results

a. Level of  $PB^{131}I$  in the peripheral blood during the repeated blood collections. When the blood collections were made at 24-hour interval,  $PB^{131}I$  fell exponentially with time as illustrated in Fig. 1. The mean of  $PB^{131}I$  level of 11 rats 24 hours after 0 time was 85 % of that at 0 time. If the blood collections were made at 2, 4, 6 and 24 hours after 0 time, the  $PB^{131}I$  for the first 24 hour period was maintained approximately at the same level as that at 0 time or rather tended to elevate above the initial level (Table 1).

If the rat was hypophysectomized 24 hours after the  $^{131}I$  injection, i.e., 24 hours before 0 time, the plasma  $PB^{131}I$  concentration during the period of blood collections needs the presence of the normally functioning thyroid gland under the pituitary control, and in some cases the gland seems to secrete more hormone than sufficient to maintain the initial level resulting higher  $PB^{131}I$  value than the initial concentration (Table 1). The pattern of the plasma  $PB^{131}I$  during the period of repeated blood collections was not modified by methylthiouracil (Table 2), 4 mg/100 g body weight, given subcutaneously 45 minutes before 0 time, excluding the possibility of involvement of organic conversion of iodide by the thyroid gland in the process of maintenance of  $PB^{131}I$  level.

**b. Sympathetic influence on the plasma  $PB^{131}I$  level during the repeated blood collections.** Since the procedure for the blood withdrawal is stressful stimulus to the rat, it may be followed by stimulation of the sympathetic nervous system. Therefore, the effect of adrenolytic agents on the plasma  $PB^{131}I$  was investigated first. The rats were injected intravenously with 1 mg per 100 g of Dibenzylamine 18 hours before 0 time and the blood was taken at 2, 4, 6 and 24 hour intervals. As shown in Table 2, the pattern of plasma  $PB^{131}I$  level was not modified by Dibenzylamine.

The effect of Ismelin, 2 mg per 100 g, given intraperitoneally 24 hours before the experiment was also examined. The means of 4 and 24 hours  $PB^{131}I$  values in the Ismelin treated rats were lower than those of control, but no significant difference was observed in the 2 and 6 hour values (Table 2).

Observations were made in the rats in which adrenal medullectomy was performed 2 weeks prior to the experiment. Shamly operated rats at the same time were served as the control animals. As shown in Table 2, the  $PB^{131}I$  level at each time interval was not different between the medullectomized and the control rats.

Epinephrine in a dose of 24  $\mu$ g per 100 g body weight was injected into a group of rats at 0 time, but this procedure did not change the plasma  $PB^{131}I$  pattern during the experimental condition employed (Table 2).

These results suggest that the adrenal medulla and other sympathetic nervous system may not affect the plasma  $PB^{131}I$  level during the minor surgical stresses, or, if any, the sympathetic system other than adrenal medulla might play some role favoring the maintenance of  $PB^{131}I$  concentration.

**c. Effect of vasopressin preparations on the plasma  $PB^{131}I$  level in rats.** Plasma  $PB^{131}I$  level was reported to rise by administration of vasopressin preparations in rabbits (8) and mice (12). On the contrary to these reports, the plasma  $PB^{131}I$  level fell as rapidly with time as seen in hypophysectomized rats when 100 mU of Pitressin per 100 g body weight was subcutaneously or intraperitoneally

injected into rats at 0 time (Table 2). A subcutaneous injection of lysine vasopressin in a dose of 100 mU per 100 g body weight at 0 time, however, did not suppress the  $PB^{131}I$  level under the same experimental condition. In order to investigate whether this inhibition by Pitressin is mediated through the suppression on the pituitary TSH secretion or not, 50 mU per 100 g body weight of TSH was given into the rats intravenously at 0 time with or without Pitressin or synthetic lysine vasopressin. As shown in Fig. 2, Pitressin 100 mU per 100 g body weight seemed to suppress the rise of  $PB^{131}I$  due to exogenous TSH, though statistically not significant, while lysine vasopressin had no effect.

Although this result can not completely exclude the suppressive action of Pitressin on the pituitary gland to secrete TSH under the experimental condition used, it indicates that exogenous Pitressin might inhibit the TSH action to elevate the plasma  $PB^{131}I$  level. However, this inhibition might be exerted in the thyroid gland or resulted from Pitressin's peripheral influences, i.e., an increased excretion or degradation, or increase in the distribution space of  $PB^{131}I$ . In order to clarify these possible mechanisms, the rats given carrier free  $^{131}I$  were hypophysectomized 24 hours thereafter and the effect of Pitressin was investigated 24 hours after the operation. There was no difference in the plasma  $PB^{131}I$  levels at 2, 4, 6 and 24 hour intervals between the hypophysectomized control and Pitressin injected animals (Fig. 2). This indirect method may make it unlikely for the extrathyroidal influence of Pitressin to account for the suppression of  $PB^{131}I$  level.

The plasma protein concentration was also measured on the samples obtained at 2, 4, 6 hour intervals. In the control group of rat subcutaneously injected physiological saline, 0.1 ml per 100 g body weight, the plasma protein concentration was lower in 2, 4, and 6 hours' samples than the initial one indicating hemodilution by repeated blood collections. On the other hand, a subcutaneous injection of Pitressin in a dose of 100 mU per 100 g body weight

led to rather an increase in its concentration at 2 hour time (Fig. 3). These facts infer that Pitressin did not cause hemodilation in the rats under the experimental condition used resulting in a fall of plasma  $PB^{131}I$  concentration.

These results may suggest that exogenous Pitressin inhibited the release of  $PB^{131}I$  from the thyroid gland into the general circulation, though its suppressive influence on TSH secretion from the pituitary gland could not be entirely ruled out.

d. The effect of intravenous infusion of Pitressin and synthetic lysine vasopressin. The findings on the stimulatory effect of vasopressin preparations on the circulating thyroid hormone level made by other investigators (8,12) were carried out by injecting the neurohypophysial hormone into animals intravenously. To observe whether an intravenous infusion of Pitressin or synthetic lysine vasopressin results in a rise of the plasma  $PB^{131}I$  in rats, these preparations in a dose of 100 mU per 100 g body weight were infused into the external jugular vein over 1 minute period at 0 time. This procedure, however, failed to induce an elevation of plasma  $PB^{131}I$  during the period of observation, but was again followed by the suppression not only in Pitressin but lysine vasopressin treated animals (Table 2).

e. Effect of ACTH. Since vasopressin in the dose used in these experiments provokes ACTH secretion from the anterior pituitary gland which may induce thyroidal suppression (6), the suppressive effect of Pitressin on the plasma  $PB^{131}I$  level might be mediated by ACTH release. In an attempt to clarify this question, 4 mU of ACTH (Organon) per rat was injected into the jugular vein of rat at 0 time. ACTH injection, however, did not suppress the plasma  $PB^{131}I$  during the period of observation (Table 2).

f. Effect of exogenous L-thyroxine.  $PB^{131}I$  levels were followed up in the L-thyroxine treated rats. Twenty-four and 48 hours after the rats were given carrier free  $^{131}I$ , they were injected subcutaneously with 50  $\mu$ g of L-thyroxine. Zero time was set up 72

hours after  $^{131}\text{I}$  injection and blood collections were made at shorter intervals in this experiment, i.e., at 30 mins., 1, 2, 4, and 6 hour time. Maintenance of plasma  $\text{PB}^{131}\text{I}$  level at rather higher-than-the-initial value was observed in these rats, indicating no inhibitory effect of exogenous L-thyroxine on the plasma  $\text{PB}^{131}\text{I}$  concentration under the experimental condition used (Table 3). The dose of L-thyroxine was thought to be sufficient enough to suppress the endogenous TSH secretion in the resting states, which was indicated by as low cpm of the plasma of these rat at 0 time as that of hypophysectomized ones. If Pitressin, in a dose of 1 U. per rat, was given intravenously in these thyroxine treated rats, a suppression of the plasma  $\text{PB}^{131}\text{I}$  levels was seen (Table 3).

#### 4. Discussion

Many controversial reports have appeared dealing with the influence of stress on the thyroid activity in man and animals. The stress seems to induce the thyroidal activation in some cases and not in another. No explanation, however, on the factors governing the stimulation or inhibition has yet been made. In the control experiments reported in the present paper, the plasma  $\text{PB}^{131}\text{I}$  levels during the repeated blood collections were considerably higher than those of the initial values in some rats. In spite of a constant condition in the animal room, there seemed to be also a systemic difference in the plasma  $\text{PB}^{131}\text{I}$  pattern between different groups of rats.

The plasma  $\text{PB}^{131}\text{I}$  levels roughly exponentially fell with time, if the blood collections were made at 24 hour interval. Since the first blood collection is already a stressful stimulus to the rats, the decay of plasma  $\text{PB}^{131}\text{I}$  concentration might not be the same as that in the non-treated rats. However, the repeated stimuli for collection of the samples at 2, 4, 6 and 24 hours caused a maintained  $\text{PB}^{131}\text{I}$  level for the period of experiment which departed from the pattern of  $\text{PB}^{131}\text{I}$  level in the 24-hour interval blood collections. Hemodilution during the repeated surgical procedures excluded the possibility that the maintained or higher  $\text{PB}^{131}\text{I}$  values might be due to hemococentration. Failure of the maintenance of plasma

$PB^{131}I$  under an identical experimental condition in the hypophysectomized rats suggested the necessity of the presence of the normally functioning thyroid for the maintenance of  $PB^{131}I$ . These facts may indicate that the minor surgical procedure under ether anesthesia with blood withdrawal caused a thyroidal activation, if the plasma  $PB^{131}I$  level reflects the thyroidal function. Or, if the stress caused both thyroidal activation and inhibition simultaneously, the former must have been induced more predominantly than the latter.

There was no significant difference in the  $PB^{131}I$  pattern between the first and second used groups of rats. But that found in the third used rats clearly demonstrated a considerable thyroid activation comparing with the former groups (Table 1). Although a systemic difference in the thyroidal response to the stress between the groups can not be ruled out, it might be also possible to postulate the adaptation of the rats to the stresses resulting in an escape from inhibition of the thyroid by the repeated stress (6) with the predominant manifestation of its stimulatory influence.

Many investigations on the effect of the sympathetic nervous system and catecholamines on the thyroid function were made, and again controversial findings reported (4,10,13,14). In this experiment reported here, they were found not to produce a critical influence on the maintenance of plasma  $PB^{131}I$  concentration under the experimental condition used, though guanethidine administration resulted in a slight inhibition on the  $PB^{131}I$  level.

Although the activation of pituitary-adrenal axis has been reported to suppress the thyroid function (7,10,11), it did not seem to play an important role in the mechanism of the  $PB^{131}I$  maintenance during the minor surgical stimuli. The adrenalectomized rats off cortisone failed to maintain the  $PB^{131}I$  level during the repeated blood collection, however, at present it is difficult to conclude on whether it was result of the lack of adrenocortical steroid or the secondarily induced change in the body of rat.

It is very interesting to note no demonstrable effect of exogenous L-thyroxine on the  $PB^{131}I$  pattern during the repeated blood collections. The initial  $PB^{131}I$  level in the L-thyroxine treated rats as low as hypophysectomized ones indicates the thyroidal suppression in the resting status. The thyroxine treated rats demonstrated the same  $PB^{131}I$  pattern during the experiment as the control animals, while the hypophysectomized rats failed to maintain the  $PB^{131}I$  level. Three possibilities to explain these phenomena may be considered: (1) There was no TSH in the hypophysectomized rats, while the TSH content in the pituitary gland of L-thyroxine treated rats markedly increased (10). The stress provoked the release of pituitary TSH breaking through the barrier made by exogenous thyroxine in the dose used resulting the thyroidal activation. (2) The inhibitory effect of exogenous L-thyroxine on the pituitary TSH release was no use for the release provoked by the stress used. (3) The failure of maintenance of plasma  $PB^{131}I$  in the hypophysectomized rats was not directly related to the lack of endogenous TSH, but resulted from secondarily induced change in the body of rat following hypophysectomy.

The possibilities in (1) and (2), however, need another hypothesis that the stress provoked TSH release. This hypothesis is true if the stress is cold stimulus (5), but no available data has been obtained with regards to other stressful stimuli. As far as hypophysectomy is concerned, the operation was performed only 24 hours before 0 time. The gland showed an excellent response to exogenous TSH. The thyroid may not be considered to be extremely hypoactive in comparison with that of L-thyroxine treated rats.

Another interesting finding is the inhibitory effect of Pitressin on the plasma  $PB^{131}I$  level. Pitressin did not induce hemodilution to account for the fall of  $PB^{131}I$ . Other extrathyroidal action of Pitressin may also be excluded from the probable mechanism of the plasma  $PB^{131}I$  suppression (see Results). The tendency of this hormone to suppress the rise of  $PB^{131}I$  produced by exogenous TSH might indicate its direct inhibition of the thyroid activity. The inhibition might be more markedly demonstrated if a smaller dose of TSH was employed to avoid masking the inhibitory effect of Pitressin on the TSH action, if any, by the overdosage of exogenous TSH.

On the other hand, synthetic lysine vasopressin subcutaneously injected in the same dose as Pitressin showed no effect on the plasma  $PB^{131}I$  levels. The rise of  $PB^{131}I$  following TSH administration in the hypophysectomized rats was not affected by it either. However, the intravenous injection of lysine vasopressin well suppressed the  $PB^{131}I$  levels. This may indicate that the biological activity of Pitressin to suppress the plasma  $PB^{131}I$  level is more potent than synthetic lysine vasopressin even on the same IU doses,

Although no definite information on the action site of vasopressin to suppress the plasma  $PB^{131}I$  level is available, the following possibilities to account for the mechanism may be considered at present: (1) The direct effect of exogenous vasopressin on the thyroidal activity through its vasoconstrictive effect or other mechanism. (2) Inhibition of the stress-induced TSH release from the pituitary gland by vasopressin. Since the stress of ether inhalation causes an increased blood flow both in the anterior pituitary gland and the thyroid gland (9), it is likely that the increase in blood flow of these glands results in an increased output of the hormones. Vasopressin, a vasoconstrictive agent, might possibly block the vasodilatation in these glands. However, there are evidences not favoring the postulation. Vasoconstrictive effect of exogenous vasopressin in rats does not last long, because its biological half life is very short. The suppression of the  $PB^{131}I$  due to vasopressin is evident at least for 6 hours. Unless a temporal irreversible vasoconstriction is induced by exogenous vasopressin, it may be difficult to fully account for the mechanism of  $PB^{131}I$  suppression by its vasoconstrictive effect. Further, the pituitary portal vessels were found not to be affected by exogenous Pitressin in rats (1).

The possibility that Pitressin resulted in an inhibition of the pituitary TSH secretion through the activation of the pituitary adrenal axis was ruled out in the experiment.

Another effort to elucidate whether the surgical stress provokes the pituitary TSH release and whether vasopressin inhibits it or not is the project of study to be undertaken in the future.

## References

- 1) Arimura, A. and C.N.H. Long, *Jap. J. Physiol.*, 12 : 429, 1962
- 2) Ackerman, N.B. & W.L. Arons, *Endocrinology*, 71 : 7, 1962
- 3) Bakke, J.L. & N. Lawrence, *The Endocrine Soc., 44th Meet. Program 2*, 1962
- 4) Batkin, A.L. & J.T. Tew, *Proc. Soc. exp. Biol. Med.*, 79 : 376, 1952
- 5) Bottari, P.M., In *Ciba Foundation Colloquia on Endocrinology*, Little, Brown & Co., Boston, 11 : 52, 1957
- 6) Brown-Grant, K., G.W. Harris & S. Reichlin, *J. Physiol.*, 126 : 29, 1954
- 7) *ibid.* 126 : 41, 1954
- 8) Gracia, J., G.W. Harris and W.J. Schindler, *J. Physiol.*, 162 : 64, 1962
- 9) Goldman, H., *The Endocrine Soc. 44th Meeting Program 48*, 1962
- 10) Harris, G.W., *Neural Control of the Pituitary Gland*, Edward Arnold Ltd., London, 132, 1955
- 11) Myant, N.B., *J. Physiol.*, 120 : 288, 1953
- 12) Noguchi, A., E. Yamaguchi S. Sato & H. Kurihara, *Hormone to Rinsyo*, 10 : 939, 1962
- 13) Seffer, L.J., J.L. Gabilove & J.W. Gailer, *Proc. Soc. exp. Biol. Med.*, 71 : 117, 1949
- 14) Sonderberg, U., *Physiol. Rev.*, 39 : 777, 1959

Table 1-1

Control First Used Rats

No.	B.W. (g)	PB <sup>131</sup> I %				
		0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
1	190	100	78*	87	64	149*
2	185	100	95	99	120	103
3	220	100	144*	139*	139	158*
4	210	100	111	103	95	103
5	180	100	103	114	109	144
6	170	100	113	159*	124	123
7	225	100	101	105	115	75
8	270	100	115	74	83	75
9	260	100	128	109	110	84
10	220	100	97	83	67	73
11	210	100	94	80	81	63
12	160	100	99	91	75	70
13	235	100	89	75	103	86
14	300	100	115	92	109	118
15	250	100	90	91	115	106
16	185	100	120	115	135	98
17	260	100	85	75	90	65
<b>M<sub>t</sub>SE</b>		100	104 $\pm$ 4.0	99 $\pm$ 3.8	102 $\pm$ 5.9	100 $\pm$ 7.1

Table 1-2

## Control Second Used Rats

No.	B.W. (g)	PB <sup>131</sup> I %				
		0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
1	325	100	93	86	89	100
2	310	100	123	106	96	105
3	210	100	126	128	112	106
4	270	100	101	101	97	109
5	265	100	108	98	85	109
6	225	100	87	103	84	99
7	245	100	118	109	100	96
8	210	100	122	115	92	108
9	270	100	124	99	97	89
10	230	100	106	86	111	90
11	290	100	101	97	91	92
12	270	100	134	128	113	81
13	260	100	121	-	90	59
14	200	100	92	90	69	90
<b>M.S.E.</b>		100	111±3.8	103±3.8	95±3.2	94±3.5

Table 1-3

Changes in Plasma  $\text{PB}^{131}\text{I}$  Level in the 5rd Used Rats

No.	B.W. (g)	$\text{PB}^{131}\text{I}$ %				
		0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
1	230	100	103	101	104	96
2	310	100	140	159	121	103
3	310	100	162	165	151	134
4	250	100	158	178	176	195
5	290	100	108	112	100	80
<u>M+S.E.</u>		100	134 $\pm$ 12.4	143 $\pm$ 15.3	130 $\pm$ 14.8	122 $\pm$ 20.4

Table 2

Changes in Plasma  $PB^{131}I$  during the Repeated Blood Collections under Various Experimental Conditions

Procedure	No. of Rats	0 hr.	2 hr.	4 hr.	6 hr.	24 hr.
Control 1st used	17	100	104±4.0	99±3.8	102±5.9	100±7.1
Control 2nd used	14	100	111±3.8	103±3.8	95±3.2	94±3.5
Hypophysectomy 24 hrs. before 0 hr., 1st used	10	100	**90±3.2	**83±4.4	*78±2.8	*56±3.7
Methylthiouracil 4mg/100g 45 min. before 0 hr., 1st used	8	100	103±3.7	102±4.1	98±3.5	94±7.1
Dibenzylamine, 1mg/100g 18 hrs. before 0 hr., 2nd used	10	100	101±2.8	93±2.7	92±4.9	98±4.6
Ismelin, 2mg/100g 24 hrs. before 0 hr., 2nd used	6	100	98±2.1	*84±1.6	92±9.2	*70±7.0
Pitressin 100mU/100g s.c. at 0 hr.	12	100	*88±1.0	*78±1.7	*72±2.9	**80±3.6
Pitressin 100mU/100g i.p. at 0 hr. 1st used	6	100	92±2.9	**83±4.3	*73±5.3	**73±4.2
Synthetic lysine vasopressin 100mU/100g s.c. at 0 hr., 1st used	14	100	107±4.6	99±3.9	93±3.5	**80±5.5
Pitressin 100mU/100g i.v. at 0 hr. over 1 min. 2nd used	4	100	86	75	70	62
Synthetic lysine vasopressin 100mU/100g i.v. at 0 hr. over 1 min. 2nd used	7	100	**97±4.4	**87±3.6	**80±4.7	*64±5.0
ACTH 5 mU/rat i.p. at 0 hr. 2nd used	6	100	102±1.9	97±3.7	96±3.1	*77±3.0
Bil. Adrenalectomy, on cortisone 2.0mg per rat, 1st used	7	100	99±3.2	92±6.3	83±4.7	**72±5.6
Bil. Adrenalectomy, off cortisone, 1st used	6	100	*79±2.4	*72±3.3	*66±5.7	79±6.6
Control, 1st used Sham operated	7	100	102±3.1	91±5.4	91±3.4	77±2.7
Adrenomegaly 3 wks. before 0 hr., 1st used	8	100	*90±3.4	87±3.8	87±5.1	76±5.6
Control, saline at 0 hr. in place of Epinephrine	6	100	106±7.0	94±8.7	83±7.0	73±4.7
Epinephrine, 200µg/100g at 0 hr.	6	100	98±3.6	85±3.5	80±2.2	79±5.7

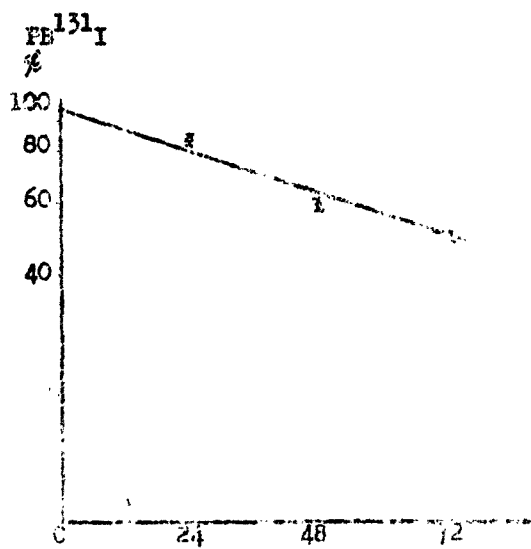
\* P < 0.01      \*\*P < 0.05

Table 3

Plasma PB<sup>131</sup>I Level in the L-Thyroxine Treated Rat

Procedure	No. of Rats	PB <sup>131</sup> I %					
		0 hr.	0.5 hr.	1 hr.	2 hr.	4 hr.	6 hr.
Control							
Saline 0.5cc i.v. over 2 min. at 0 time	5	100	125±6.9	130±4.3	114±6.6	111±8.3	115±4.2
Pitressin							
1 U/0.5cc i.v. over 2 min. at 0 time	5	100	85±8.2	94±8.3	91±6.4	91±9.9	83

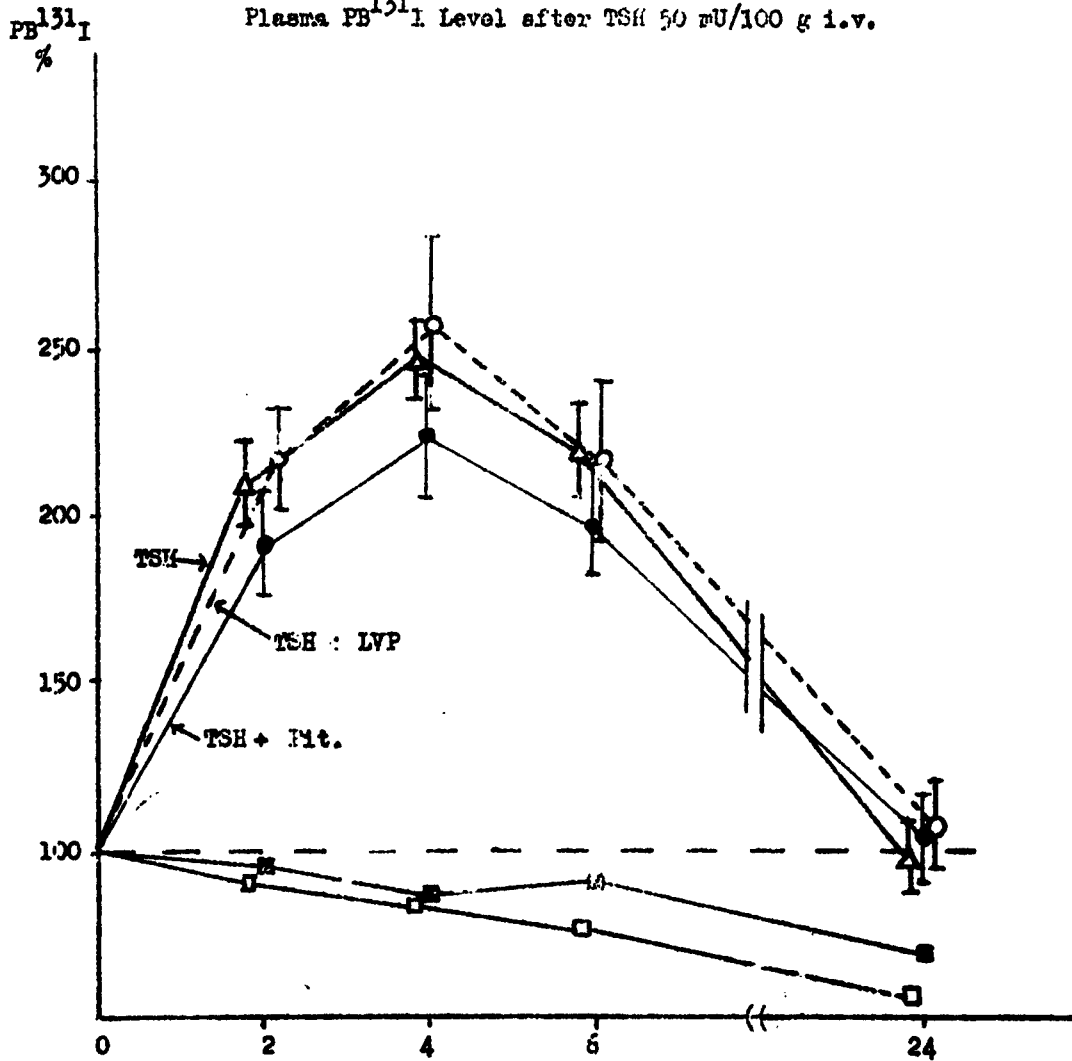
Fig. 1  
Change in PB<sup>131</sup>I at 24 hr. Interval



Physical decay of <sup>131</sup>I was corrected.

Fig. 2

Plasma  $PB^{131}I$  Level after TSH 50 mU/100 g i.v.



Pit.: Pitressin 100 mU/100 g s.c. at 0 time.

LVP: Synthetic lysine vasopressin 100 mU/100 g s.c. at 0 time.

TSH was injected into the external jugular vein at 0 time.

Δ—Δ TSH

□ Hypophysectomy

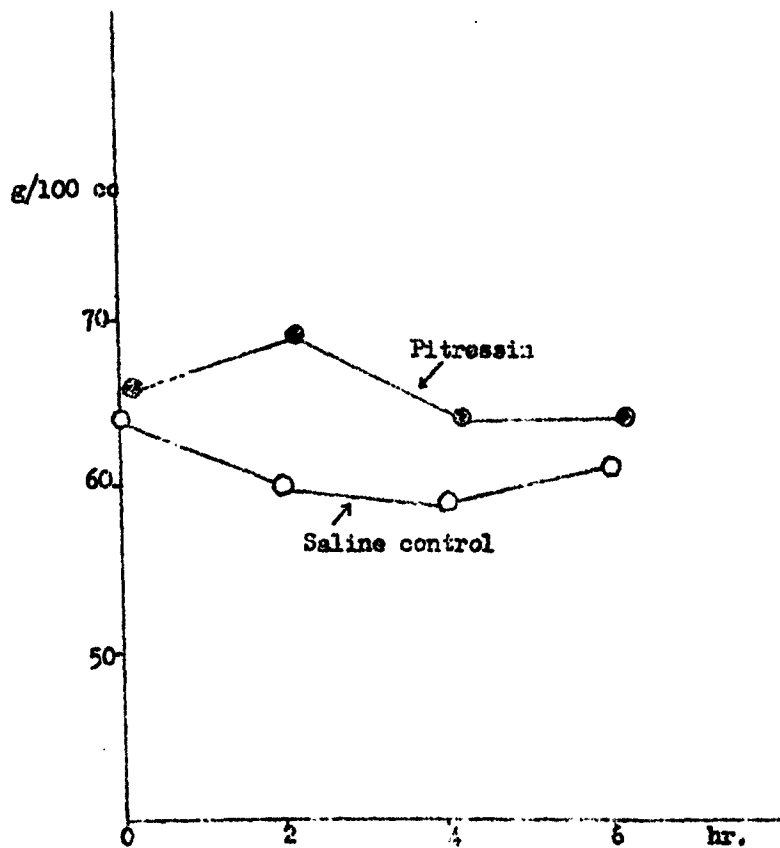
○—○ TSH + Pit.

■ Hypophysectomy + Pit.

○---○ TSH + LVP

Fig. 3

Plasma Protein Content  
During the Repeated Blood Collections



Pitressin 100 mU/100 g s.c. at 0 time.  
Saline was given in the control animals at 0 time.