

# THE INTRACELLULAR LOCALIZATION OF PITUITARY THYROTROPIC HORMONE

FRANCIS S. GREENSPAN, M.D., and JUDY R. HARGADINE

From the Department of Medicine, University of California School of Medicine, and the Radioactivity Research Center, University of California Medical Center, San Francisco

## ABSTRACT

The intracellular localization of a bovine anterior pituitary preparation of thyroid-stimulating hormone (TSH) was studied in guinea pigs and dogs. The preparation was administered intravascularly or applied directly to tissue sections. TSH was detected by an indirect technique utilizing bovine TSH antiserum and fluorescein-labeled anti-rabbit globulin; the presence of TSH in the tissue was indicated by fluorescence when the tissue was examined under the microscope with an ultraviolet light source. After either intravascular administration or direct application of the TSH preparation, striking fluorescence was found in the nuclei of the thyroid cells and to a lesser degree in the nuclei of retro-orbital fat tissue and kidney tubules in both species studied. A little fluorescence was also seen in spleen tissue. No fluorescence was noted in comparable tissues removed from control animals injected with bovine albumin or globulin or when the tissues were treated with the fluorescein-labeled globulin alone. Fluorescence was also noted in the nuclei of adrenal cells treated with unabsorbed antiserum, but this was greatly diminished when antiserum absorbed with crystalline ACTH was used. The positive reactions were all markedly decreased when the tissues were treated with antisera absorbed with the original TSH preparation. Fluorescence was noted in the cytoplasm of pituitary tissue from both treated and control animals, suggesting a cross-reaction between the bovine pituitary antisera and guinea pig or dog hypophysis. The indirect technique seems to be highly satisfactory for demonstration of the pituitary hormone within the cell. In addition, the demonstration of immunologically active anterior pituitary TSH bound to cell nuclei offers a clue to the site of action of this hormone.

## INTRODUCTION

The site of binding of anterior pituitary hormone within the target organ, although of critical importance in terms of hormone action, has not been established. Studies on the distribution of certain anterior pituitary hormones, for example, thyroid-stimulating hormone (TSH), adrenocorticotropin (ACTH), and somatotropin, have been conducted with hormone preparations labeled with radioactive sulfur, radioactive iodine, or fluorescein (1-4). The direct labeling of a hormone, however, has several serious drawbacks. Isotopic labeling

may lead to some inactivation of the hormone due to radiation damage or denaturation. The use of fluorescein for labeling presents a similar problem. If only small amounts of label are conjugated with the hormone, unphysiologically large doses of the hormone are needed before the concentration of the label is sufficient to permit its detection in tissues. Finally, when the label is detected in a tissue, one cannot be certain that it is still attached to the hormone.

Another method for the detection of proteins in

tissue is the use of fluorescein-labeled antibody (5). This technique has been used to determine the localization in tissue of bacterial antigens (6), viruses (7), and injected foreign protein (8). Marshall (9) and Cruickshank and Currie (10) used this method in an attempt to localize the site of synthesis of pituitary hormones, but their results were not conclusive. More recently, Leznoff *et al.* (11, 12) and McGarry *et al.* (13) have applied this technique to the study of the site of synthesis of human hypophyseal hormones.

A more sensitive method for the localization of proteins of low molecular weight is an indirect technique which utilizes fluorescein-labeled anti-rabbit globulin (14, 15). With this method, a protein localized in a tissue is reacted with a specific antiserum; the protein antiserum complex is then detected by the use of fluorescein-labeled anti-globulin. In the present investigation, this method was applied to the study of the intracellular localization of a pituitary hormone preparation of TSH in two species, the guinea pig and the dog.

#### MATERIALS AND METHODS

**PREPARATIONS OF THYROTROPIN:** Five bovine TSH preparations were used interchangeably throughout the study. Preparations NIH-TSH-B1 (8 u.s.p. units per mg) and NIH-TSH-B2 (4 u.s.p. units per mg), a gift of the Endocrinology Study Section of the National Institutes of Health, and preparation 216-177-5 (1 u.s.p. unit per mg), kindly supplied by Armour Pharmaceutical Company, Chicago, were used in most of the studies. In addition, preparation 14-93A (8 u.s.p. units per mg) was kindly supplied by Dr. Peter Condliffe, and preparation R-157-2 (7 u.s.p. units per mg) was kindly supplied by Dr. Leo E. Reichert, Jr.

**PREPARATION OF TSH ANTISERA:** TSH antisera were prepared by immunization of female New Zealand albino rabbits with TSH as follows: Initially and after a 2 week interval, the animals received 4 units of TSH suspended in Freund's adjuvant,  $\frac{1}{3}$  given intraperitoneally and  $\frac{2}{3}$  injected subcutaneously. At 4 weeks, 4 units of TSH suspended in saline were administered similarly. At 6 weeks, blood samples were obtained, and the antibody titer was determined. The antibody titer was considered adequate if a 1:5 dilution of antiserum produced a 2 plus reaction against 2.5 milliunits TSH in the precipitin ring test. In addition, the activity of each antiserum was tested by the McKenzie mouse assay (16). The antiserum was considered adequate if 0.01 ml antiserum neutralized the biological activity of 1 milliunit TSH. If the titer was adequate, the animals were

bled by cardiac puncture; if not, they received a booster dose of 4 units of TSH subcutaneously and were bled 2 weeks later. The antiserum was absorbed with bovine albumin, bovine globulin, and desiccated thyroid powder (Armour Pharmaceutical Company, Chicago)<sup>1</sup> before being used. When tested in an Ouchterlony plate (17), 0.01 ml of absorbed antisera showed only a single precipitin line against 1.0 milliunit TSH and no precipitin lines against comparable quantities of bovine albumin, bovine globulin, or bovine pituitary growth hormone. For some studies, the antisera were also absorbed with synthetic ACTH.<sup>2</sup>

**PREPARATION AND LABELING OF ANTI-RABBIT GLOBULIN WITH FLUORESCIN:** Anti-rabbit globulin was prepared by immunization of guinea pigs with rabbit gamma globulin (Nutritional Biochemical Corporation, Cleveland). The animals received 0.2 mg of globulin every other week for five doses; the initial and final doses were given in saline solution, the other doses in Freund's adjuvant. Blood was obtained by cardiac puncture, and, if the titer of the antiserum was low, the animal received an additional dose of 0.2 mg of rabbit globulin in saline solution. The anti-rabbit globulin antiserum was absorbed with desiccated thyroid powder<sup>1</sup> prior to use.

The labeling was performed as follows: A mixture of 1 ml anti-rabbit globulin and 0.5 ml 0.05 M sodium carbonate buffer, pH 8.5, was shaken for approximately 3 minutes with 10 mg of Celite containing 10 per cent fluorescein-isothiocyanate (18) (California Corporation for Biochemical Research, Los Angeles). The mixture was centrifuged for 3 minutes, and the supernatant was layered onto a 20 × 1 cm column of Sephadex G-100 (Pharmacia, Uppsala, Sweden). The column was eluted with 0.05 M phosphate buffer, pH 6.8, and the labeled antiglobulin was found to be eluted between the 5th and 8th ml. This fraction was relatively free of albumin and completely free of unbound fluorescein.

**EXPERIMENTAL STUDIES:** Two animal species were used. In the first study, 2 normal male dogs, weighing 12 to 15 kg, were given 40 u.s.p. units bovine TSH intravenously. The animals were sacrificed by intravenous administration of pentobarbital 15 minutes later. Two control animals received no injections and were sacrificed similarly. Various tissues were removed and treated as described below. In later studies, healthy male guinea pigs, weighing

<sup>1</sup>Absorption of antisera with desiccated thyroid powder, 10 mg per ml, was found to diminish non-specific thyroid tissue fluorescence.

<sup>2</sup>Synthetic ACTH No. 30, 920 BA, E 7235 (100 IU per mg), a 24 amino acid preparation, was obtained from CIBA Pharmaceutical Company, Summit, New Jersey. One ml antiserum was absorbed with 1 mg ACTH.

about 350 gm, were pretreated with L-thyroxine, 100  $\mu$ g intraperitoneally daily for 4 days to suppress endogenous TSH secretion. Four of the animals were given 0.5 U.S.P. units bovine TSH intracardially and were sacrificed under barbital anesthesia 15 minutes later. Three groups of 2 control animals each were used: one group received 125  $\mu$ g of bovine globulin intracardially and the second 125  $\mu$ g of bovine albumin intracardially; the third group was given no injections. Healthy animals are required for such studies since we found uptake of non-specific protein when sick or fasted guinea pigs were used.

For additional studies on the uptake of TSH by thyroid tissue slices *in vitro*, thyroid glands were obtained from 40 dogs which had not been subjected to major stress<sup>3</sup> and from 15 thyroxine-treated guinea pigs.

**TECHNIQUE FOR DETECTION OF TSH IN TISSUE:** Tissues were frozen in dry ice and cut into slices 7  $\mu$  thick with a Pierce-Slee cryostat microtome. The presence of TSH in tissue was ascertained as follows: Tissues were covered with 0.1 ml TSH antiserum diluted 1:2 with 0.5 M phosphate buffer, pH 6.8, and allowed to stand at room temperature for 40 minutes. The slide was washed for 10 minutes in phosphate buffer, then covered with 0.1 ml of a 1:6 dilution of the fluorescein-labeled anti-rabbit globulin for 40 minutes, washed for 20 minutes in phosphate buffer, and counterstained with a 0.05 per cent solution of Evans Blue for approximately 15 seconds. Control sections were prepared in a similar fashion except that the TSH antiserum was omitted. All sections were mounted with glycerin and coverslips and examined with a Carl Zeiss microscope with ultraviolet light source using exciter filters BG 12 and UG 2 and barrier filters BG 23 and RG 1.

The *in vitro* uptake of TSH by various tissues was studied in a similar manner except that 0.1 ml TSH solution, 4 milliunits per ml in phosphate buffer, was applied to the tissue section and allowed to stand at room temperature for 40 minutes. The slide was then washed with phosphate buffer and treated with antisera as described.

## RESULTS

### *The Distribution of TSH after Intravascular Administration*

The distribution of TSH 15 minutes after intracardiac injection in the guinea pig is shown in Table I. A plus mark indicates the presence of

<sup>3</sup>These tissues were made available through the courtesy of the Departments of Experimental Surgery, Neurosurgery, and Physiology, University of California School of Medicine.

bright apple-green fluorescence in a frozen tissue section stained by the indirect technique and examined under an ultraviolet light source.

Photomicrographs of thyroid tissue from treated and control animals stained by the indirect method and with hematoxylin and eosin are compared in Fig. 1. Striking fluorescence was present in thyroid sections from the TSH-treated animals but not in comparable sections from the animals in the three control groups. The fluorescence appeared to be concentrated in the nuclei of the thyroid cells.

Retro-orbital tissue in the guinea pig consists mostly of large globules of fat surrounded by stroma and cell nuclei. Brilliant fluorescence was seen in the nuclei of some of the fat cells from the TSH-treated animals, but not in comparable tissue from the control animals (Fig. 2). Fluorescence was also noted in the nuclei of renal tubular cells (Fig. 3) and to a lesser degree in the spleen (Fig. 4) of TSH-treated animals. No fluorescence was seen in similar tissues from the control animals.

Sections from the pituitary glands of all the animals, whether TSH-treated, control-injected, or uninjected, showed fluorescence in many cells. The fluorescence was not confined to the nuclei (Fig. 5). The presence of fluorescence in pituitary tissue treated with TSH antiserum and fluorescein-labeled antiglobulin, but not in tissue treated with the labeled globulin alone, suggests that a cross-reaction between guinea pig pituitary TSH and bovine TSH antiserum had occurred.

No fluorescence was observed in any of the other tissues studied, with the exception of the adrenal gland. When adrenal tissue was treated with antisera which had been absorbed only with albumin, globulin, and thyroid powder, fluorescence was found in the nuclei of adrenal cells in the TSH-injected animal; it was not present in similarly treated adrenal tissue from control animals. Further absorption of the antiserum with synthetic ACTH partially eliminated the fluorescence in the adrenal cells (Fig. 6), but did not diminish the fluorescent reaction in the thyroid, retro-orbital, renal, and pituitary tissues. Absorption of the antiserum with the original TSH used for immunization markedly decreased the fluorescent reaction in all tissues. This finding suggests that the original TSH used to prepare the antisera may have contained a small amount of ACTH.

Almost identical results were obtained after intravenous injection of TSH in the dog (Table I). Striking fluorescence was noted in the thyroid,

TABLE I  
Occurrence of Tissue Fluorescence with Indirect Staining after Intravascular Administration of Bovine TSH

Tissue	Guinea pig				Dog	
	TSH	Albumin	Globulin	Uninjected	TSH	Uninjected
	(5 U.S.P. units i.c.)	(125 $\mu$ g i.c.)	(125 $\mu$ g i.c.)		(40 U.S.P. units i.v.)	
Thyroid	+ (n)	—	—	—	+ (n)	—
Retro-orbital fat	+ (n)	—	—	—	+ (n)	—
Kidney	+ (n)	—	—	—	+ (n)	—
Spleen	$\pm$	—	—	—	$\pm$	—
Hypophysis	+ (c)	+ (c)	+ (c)	+ (c)	+ (c)	+ (c)
Testes	—	—	—	—	—	—
Adrenal	*	—	—	—	—	—
Brain	—	—	—	—	—	—
Hypothalamus	—	—	—	—	—	—
Liver	—	—	—	—	—	—
Muscle	—	—	—	—	—	—
Skin	—	—	—	—	—	—
Foot pad fat	—	—	—	—	—	—
Intestine	—	—	—	—	—	—
Aorta	—	—	—	—	—	—
Lung	—	—	—	—	—	—

+ (n) indicates that fluorescence was concentrated in the nuclei of the cells; + (c) indicates that fluorescence was not limited to the nuclei but was also found in the cytoplasm of the cells; — indicates that no fluorescence was observed; and  $\pm$  indicates slight tissue fluorescence.

\* Fluorescence was noted on the nuclei of the adrenal cells when unabsorbed antiserum was used in the indirect staining technique. It was markedly diminished when the antiserum was absorbed with synthetic ACTH.

retro-orbital fat, and renal tissue of the TSH-injected animals, but not in similar tissues from the control animals. The fluorescence appeared to be concentrated in the nuclei. A small amount of fluorescence was noted in the spleen of the TSH-treated animals but not in that of the control animals. In both TSH-treated and control dogs the cytoplasm of many pituitary cells showed a fluorescent reaction. No fluorescence was found in any of the other tissues studied.

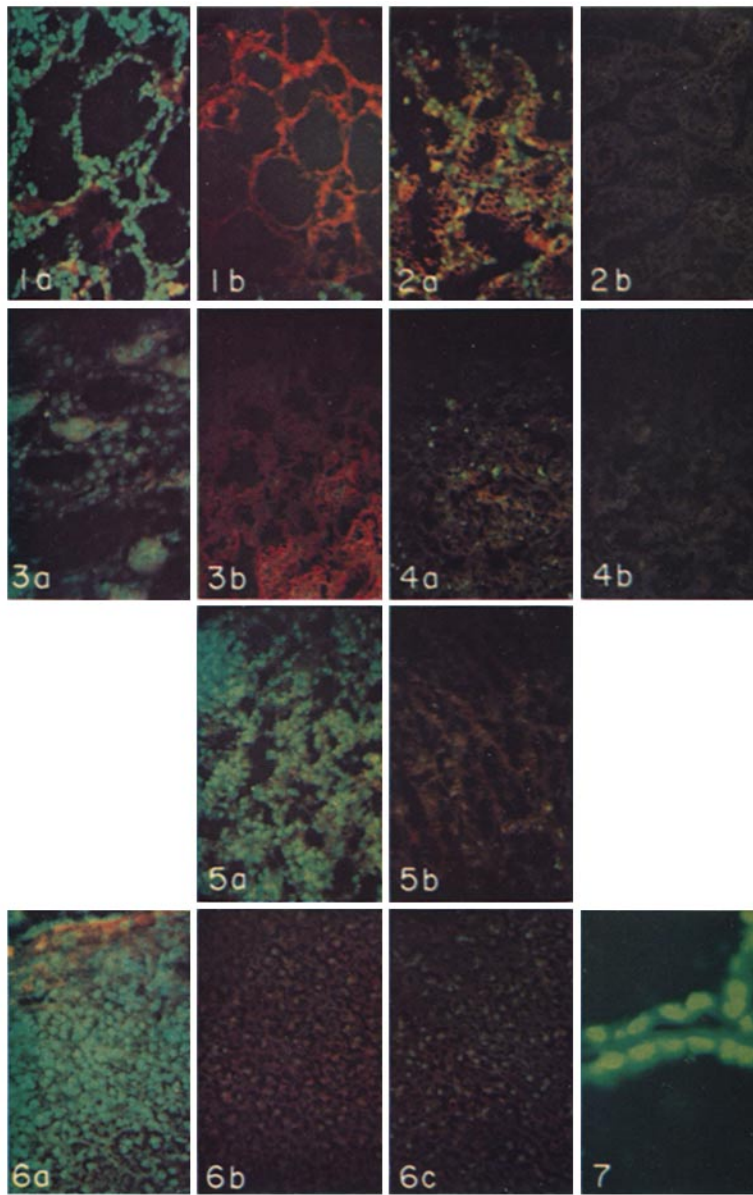
#### *Detection of TSH in Thyroid and Other Tissues in Vitro*

Various tissues were removed from thyroxine-treated guinea pigs and examined for fluorescence after application of TSH solution directly on the tissue sections, followed by fluorescent staining by the indirect technique. Control sections were prepared with TSH solution and fluorescein-labeled anti-rabbit globulin, but without TSH antiserum. The results are presented in Table II. Brilliant fluorescence was seen in the nuclei of thyroid,

retro-orbital fat, and renal tissue. A small amount of fluorescence was noted in the spleen. No fluorescence was observed in any of the other tissues studied, with the exception of adrenal tissue. As in the *in vivo* study, fluorescence was present in the nuclei of adrenal tissue treated with unabsorbed antisera but was markedly diminished in the sections treated with antiserum absorbed with crystalline ACTH. No fluorescence was noted in any of the control sections.

Similar results were obtained in studies of tissues removed from dogs (Table II). Nuclear fluorescence was found in thyroid, retro-orbital fat, and kidney tissues treated with TSH but not in the control sections. Slight fluorescence was noted in the spleen. There was no uptake of TSH by the liver sections. When examined under a high power objective, the fluorescence in the thyroid tissue of the dog was clearly concentrated in the cell nuclei (Fig. 7).

To evaluate the specificity of the reaction, canine thyroid slices were incubated with other antigens.



Frozen sections of various tissues, stained with anti-TSH antiserum and fluorescein-labeled anti-rabbit globulin and photographed under ultraviolet light. Except where noted,  $\times 307$ .

**FIGURE 1** Thyroid gland: (a) from a TSH-injected guinea pig, (b) from a globulin-injected guinea pig. The bright green areas in (a) indicate fluorescence, which appears to be concentrated in the nuclei of thyroid cells.

**FIGURE 2** Retro-orbital tissue: (a) from a TSH-treated guinea pig, (b) from a globulin-injected guinea pig. The bright green areas in (a) indicate fluorescence, which appears to be concentrated in the nuclei of the fat cells. The round black areas represent intracellular fat globules.

**FIGURE 3** Kidney tissue: (a) from a TSH-treated guinea pig, (b) from a globulin-injected guinea pig. The bright green fluorescence in (a) appears to be concentrated in the nuclei of the renal tubular cells.

**FIGURE 4** Spleen tissue: (a) from a TSH-treated guinea pig, (b) from a globulin-injected guinea pig.

Only scattered areas of fluorescence are seen in (a) and these are difficult to localize.

**FIGURE 5** Pituitary tissue: (a) from a globulin-injected guinea pig, (b) from the same animal but stained with fluorescein-labeled anti-rabbit globulin alone. Note the marked fluorescence in (a), absent in (b). Fluorescence appears throughout the cytoplasm of many cells.

**FIGURE 6** Adrenal tissue: (a) from a TSH-treated guinea pig, (b) from a globulin-injected guinea pig, (c) from the TSH-treated guinea pig but stained with antiserum that was absorbed with ACTH. Note the striking fluorescence in (a), absent in (b). This fluorescence was markedly diminished in the adrenal when antisera absorbed with ACTH were employed in the indirect technique of staining (c). See text for details.

**FIGURE 7** Thyroid tissue from a TSH-treated dog. Note that the fluorescence is clearly concentrated in the nuclei of the thyroid cells. The fluorescence in this section is yellow due to lack of counterstain and photography under an oil immersion objective.  $\times 1205$ .

TABLE II  
Occurrence of Tissue Fluorescence after Direct Application of Bovine TSH to Frozen Tissue Sections Followed by Indirect Staining Technique\*

Tissue	Guinea pig		Dog	
	TSH	Control	TSH	Control
Thyroid	+ (n)	-	+ (n)	-
Retro-orbital fat	+ (n)	-	+ (n)	-
Kidney	+ (n)	-	+ (n)	-
Spleen	±	-	±	-
Testes	-	-	-	-
Adrenal	‡	-	-	-
Brain	-	-	-	-
Hypothalamus	-	-	-	-
Liver	-	-	-	-
Muscle	-	-	-	-
Skin	-	-	-	-
Foot pad fat	-	-	-	-
Intestine	-	-	-	-

+ (n) indicates that fluorescence was concentrated in the nuclei of the cells; ± indicates slight tissue fluorescence; - indicates no tissue fluorescence.

\* TSH was applied to frozen sections of normal animal tissues and stained by the indirect technique. Control tissues were treated with TSH and stained only with fluorescein-labeled anti-rabbit globulin.

‡ Fluorescence was noted on the nuclei of adrenal cells when antiserum absorbed with highly purified TSH was used in the indirect staining technique but not when antiserum absorbed with synthetic ACTH was used.

Bovine albumin, bovine growth hormone, and human growth hormone, when applied on the thyroid slice with either their respective antisera or TSH antiserum did not induce nuclear fluorescence.

We have interpreted the presence of fluorescence in the tissues after application of TSH and indirect staining to indicate the presence of TSH within the cell. The finding of fluorescence in adrenal tissue suggested the possibility that ACTH was being detected in a similar manner. To clarify the relationship between TSH and ACTH in the reaction, the TSH antiserum was absorbed with highly purified TSH<sup>4</sup> or with a synthetic ACTH preparation and used in the indirect technique to detect the uptake of crude TSH or synthetic ACTH by adrenal or thyroid tissue. As shown in Table III, absorption of the TSH antiserum with purified TSH completely eliminated the fluorescent reaction in thyroid or kidney tissue, but had no effect on the fluorescence in adrenal tissue. Conversely, use of the ACTH-absorbed antiserum in the indirect staining technique almost completely eliminated the fluorescence of the adrenal tissue, but had no effect on the fluorescent reaction in the thyroid. These reactions provide strong evidence for the specificity of the uptake of TSH by the thyroid cell nucleus and also of ACTH by the adrenal cell nucleus.

<sup>4</sup> TSH preparation F1-51B2 (34 u.s.p. units per mg) was kindly supplied by Dr. Peter Condliffe. One ml of antiserum was absorbed with 1 u.s.p. unit TSH.

TABLE III  
Effect of Absorption of TSH Antiserum with Highly Purified TSH or Synthetic ACTH on Hormone Uptake of Guinea Pig Tissue Slices

Preparation applied to tissue	TSH antiserum	Tissue reaction		
		Thyroid	Adrenal	Kidney
Bovine TSH*	Absorbed with purified TSH ‡	-	+	-
Synthetic ACTH §	Absorbed with purified TSH ‡	-	+	-
Bovine TSH*	Absorbed with synthetic ACTH	+	-	-
Synthetic ACTH §	Absorbed with synthetic ACTH	-	±	-

+ indicates fluorescence in cell nuclei after application of indirect staining technique; - indicates no fluorescence in tissue after indirect staining; ± indicates slight tissue fluorescence.

\* NIH-B2 TSH, 4 u.s.p. units per mg.

‡ TSH preparation F1-51B2, 34 u.s.p. units per mg.

§ 24 amino acid ACTH.

### Detection of TSH in Serum

Sera obtained from the experimental dogs 15 minutes after intravenous injection of 40 U.S.P. units TSH contained approximately 26 milliumits TSH per ml, as determined by the McKenzie method (16). Canine thyroid slices treated with this sera and stained by the indirect technique showed strongly positive nuclear fluorescence, whereas no such reaction was obtained in slices treated with normal dog serum. This finding suggests that thyroid tissue was able to take up the TSH even in the presence of large quantities of serum protein.

### DISCUSSION

One major problem in evaluating the significance of these findings lies in the lack of purity of the pituitary preparations used for formation of the antisera and for the distribution and tissue-uptake studies. Although these preparations were relatively rich in TSH, they were certainly contaminated by other pituitary gland proteins. The original preparations evidently contained some ACTH, for we found that the fluorescence of the nuclei of adrenal cells was markedly diminished when the tissue was treated with antiserum absorbed with synthetic ACTH. The synthetic ACTH certainly contained no other pituitary gland protein, and the absorption of the antiserum with ACTH did not modify the reaction in other tissues such as thyroid, retro-orbital fat, and kidney. Conversely, absorption of the antiserum with the original TSH preparation markedly reduced the reaction in all these tissues, suggesting that the pituitary preparation was, in fact, bound to the nucleus of the cell and was reacting in the indirect fluorescent staining technique. Bovine albumin, bovine globulin, and bovine growth hormone did not induce nuclear fluorescence in the control tissues, so it is not likely that we were dealing with these fractions as contaminants. Other possible contaminants of pituitary TSH preparations are follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. Apparently very little biologically active FSH was present in the NIH-TSH-B2 preparation used in many of these experiments.<sup>5</sup> Werner (19) and Selenkow *et al.*

<sup>5</sup> The levels of contamination of NIH-TSH-B2 with other pituitary hormones, as reported in the specifications sheet for this preparation distributed by the Endocrinology Study Section of the National Insti-

(20), however, have demonstrated antibodies to LH and to prolactin in antisera prepared from various TSH preparations. We were unable to detect any uptake of the injected TSH in testicular tissue. More significantly, when the TSH antiserum was absorbed with highly purified TSH (presumably free of LH and prolactin) (21), we were no longer able to detect fluorescence on thyroid cell nuclei. This finding suggests that TSH, rather than another pituitary hormone or a protein contaminant, was being taken up by the thyroid cell.

Our findings are not in complete agreement with those of others who have studied the distribution of TSH after intravenous administration in the rat. Mancini *et al.* (4), using TSH labeled with acid rhodamine B, found fluorescence in thyroid, kidney, spleen, and retro-orbital fat. In the thyroid tissue, the fluorescence was present in the basement membrane of the follicles and in small granules in the basal portions of scattered follicular cells. The TSH conjugate appeared in the basement membrane 5 minutes after injection and was completely gone in 30 minutes. Our studies were made at only one time interval after injection of TSH, namely 15 minutes. TSH was detected by direct application of fluorescein-labeled anti-rabbit globulin to excised tissue slices, and fluorescence was noted in the nuclei of thyroid cells. The discrepancy between our results and those of Mancini *et al.* may be owing in part to differences in the time interval after injection of TSH, or to species difference, or more likely to the difference in the method used for detecting TSH. It is possible that if studies were made at earlier time intervals, for example, 5 minutes after injection, TSH would be found at the cell membrane. However, since Mancini *et al.* did not find fluorescence in the nucleus at any time, we think it more likely that after intravenous injection of labeled TSH the fluorescent label breaks off as the protein enters the cell. The use of the fluorescein-labeled anti-rabbit globulin and the indirect technique on excised tissue slices allows us to detect immunologically active protein within the cell. Sonenberg *et al.* (1), using a pituitary TSH preparation labeled with radio-

tutes of Health, are as follows: luteinizing hormone, mean potency 0.003 times NIH-LH-S1 per mg; follicle-stimulating hormone, less than 0.012 times NIH-FSH-S1 per mg; ACTH, less than 0.13 milli-unit ACTH per mg; growth hormone, less than 0.002 times U.S.P. growth hormone per mg.

active sulfur, found a high concentration of S<sup>35</sup> in the liver. This too may have resulted from breaking off of the radioactive label and its concentration by the liver. Levey and Solomon (22), by direct biological assay of homogenates of tissue, found biologically active TSH in the kidney but not in the liver of their animals, as did Asch and Aron (23). Our results confirm their observations. Mack *et al.* (24), after applying fluorescein-labeled TSH antisera directly to beef thyroid slices, found localization of the fluorescence in the lumen and at the apex of the follicular cell. These data are not directly comparable with ours, however, since the technique and the species were different. We found considerable non-specific tissue fluorescence when fluorescein-labeled bovine TSH antiserum was applied to beef thyroid slices. In our hands, the indirect technique proved more satisfactory. In addition, the use of guinea pig thyroid tissue obviated the possibility of reactions between bovine TSH antisera and bovine tissue antigens. We were able to confirm the observation of Mancini and associates (4) regarding the uptake of TSH by retro-orbital fat tissue. Freinkel (25) and Jungas and Ball (26) have shown that TSH stimulates the metabolism of adipose tissue. It is possible that the metabolic stimulation reflects a direct effect of TSH on the nuclei of certain adipose tissue cells.

A number of recent reports have presented evidence suggesting that many hormones exert their effect by acting directly on nuclear DNA, resulting in increased synthesis of "messenger" RNA. Some of these observations have been briefly reviewed by Ferguson (27), who pointed out that estrogens, androgens, thyroxine, growth hormone, and possibly ACTH may act in this fashion. In regard to TSH, Hall reported that one of the major effects of TSH is increased incorporation of purine into nuclear ribonucleic acid (28). Using the McKenzie assay, we have shown that actinomycin D blocks the action of TSH on the release of I<sup>131</sup>-labeled compounds from the thyroid (29). This finding, coupled with the observation that pituitary TSH can actually be detected on the nucleus of the thyroid cell after intravascular or direct application, suggests that one of the actions of this trophic hormone may be directly on the nucleus of the thyroid cell.

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