# Inhibitory Effect of an Ultracentrifugal Fraction of Rat Renal Homogenate on TSH-Stimulated $^{131}I$ Uptake by Bovine Thyroid Slices[1] (35909)

L. O. LUTHERER, A. H. BURNS, M. J. FREGLY, AND F. P. FIELD[2]

*Department of Physiology, University of Florida, College of Medicine, Gainesville, Florida 32601*

The kidneys of rats contain a factor, extractable into saline, which depresses the uptake of $^{131}I$ both by the thyroid gland of recipient rats *in vivo* and by isolated porcine thyroid slices *in vitro* (1, 2). It is also present in ultracentrifugal fractions of rat renal homogenates but has not been found in similar fractions of either liver or skeletal muscle (1, 2).

The present studies were designed to determine whether thyroid depressing factor (TDF) of renal origin antagonized the effect of thyroid stimulating hormone (TSH) on $^{131}I$ uptake by bovine thyroid slices *in vitro*.

*Methods. Preparation of thyroid depressing factor.* A single preparation of renal factor was used for all the experiments described below. Kidneys were obtained from untreated rats of the Long Evans strain, cleaned of connective tissue and stored frozen in saline (0.15 *M*) until time of use. Twenty-five grams of kidney were homogenized for 1 min in 50.0 ml of saline in a Waring blender, and then the homogenate was centrifuged at 100*g* for 30 min. The resulting precipitate was washed twice by resuspension in 25.0 ml of saline and recentrifuged at 100*g* for 30 min. The precipitate was then discarded, and the original supernatant was combined with those from the 2 washes and centrifuged for 30 min at 35,000*g* in a Beckman Model L ultracentrifuge. The resulting pellet, after 2 washes in saline with recentrifugation at 35,000*g*, was resuspended in 50.0 ml of saline, and this final preparation was the one used in the experiments described below.

*Thyroid slice incubation.* The details of this procedure have been outlined previously (2). Bovine thyroid glands were used in these experiments, since we are unable to demonstrate an effect of bovine TSH (Thytropar, Armour Pharmaceutical Co.) on uptake of $^{131}I$ by porcine thyroid slices. The glands were obtained from freshly slaughtered steers at a nearby abattoir. About 45 min elapsed between removal of the glands and arrival at the laboratory. The glands were kept in iced saline during this time and all subsequent preparative work was done in a cold room maintained at 5°. Experiments usually started within 2.5 hr after removal of the glands. Slices were cut from cylindrical cores of thyroid tissue (1 cm in diam) using a modified Stadie-Riggs hand microtome and weighed on a torsion balance. Slice weights for the experiments ranged between 32.2 and 47.4 mg. The slices cut from each core were distributed randomly among groups (2). The incubation medium was Krebs-Ringer phosphate buffer containing 100 mg/100 ml of glucose and 0.2 $\mu$Ci of $^{131}I$/ml. No stable iodide was added to the buffer. The pH of the buffer was adjusted to 7.40 after the addition of test substances. Slices were incubated individually in 25 ml Erlenmeyer flasks containing 1.0 ml of buffer/10.0 mg slice weight. Incubations were carried out in air for 2 hr using a Dubnoff shaker (80 shakes/min), the water of which was maintained at 37°. After incubation, the slices were blotted on filter paper, placed in counting vials, and radioactivity was measured in a $4\pi$ scintillation detector. Accumulated $^{131}I$ was expressed as counts per minute per milligram slice weight (cpm/mg).

*Experimental design.* The first experiment was set up according to a 3 × 3 factorial

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[2] Present address: Department of Physiology, State University of New York, School of Medicine, Buffalo, New York.

**TABLE I. Effect of TSH and TDF, Separately and in Combination, on the Uptake of $^{131}I$ by Surviving Bovine Thyroid Slices.[a] [b]**

| | | TSH (mU/ml of incubation medium) | | |
|---|---|---|---|---|
| | | 0 | 20 | 100 |
| Thyroid depressing factor (ml/ml of incubation medium) | *0.000* | 1390 ± 232[c] | 1604 ± 78 | 2144 ± 179 |
| | *0.014* | 1356 ± 138 | 1284 ± 115 | 1362 ± 258 |
| | *0.030* | 906 ± 104 | 1101 ± 142 | 986 ± 73 |

[a] Five slices per group.
[b] $F$ (rows) = 9.56, <.01; $F$ (columns) = 9.16, <.01; $F$ (interaction) = 0.48, NS.
[c] Mean cpm/mg ± one standard error.

design, using 3 concentrations of renal factor (0.000, 0.007, and 0.030 ml/ml of buffer) and 3 concentrations (0, 20, and 100 mU/ml) of TSH (Thytropar, Armour Pharmaceutical Co.). Five slices were used in each group. The factorial analysis was carried out by means of the IBM 360/50 computer and University of Florida Computing Center program SPL 104.

In the second experiment the effects of several concentrations of TSH (0, 15, 30, 60, and 100 mU/ml) were tested separately and in the presence of a single concentration of renal factor (0.030 ml/ml of buffer). Either 5 or 6 slices were used in each group. Results were evaluated statistically by means of an analysis of variance (3).

In the third experiment the effects of several concentrations of renal factor (0.000, 0.004, 0.008, 0.015, 0.030, and 0.060 ml/ml of buffer) were tested against a single concentration of TSH (100 mU/ml). An additional group to which only saline (0.15 *M*) was added was run as a control. Six slices were used in each of the seven groups. Results were evaluated statistically by an analy-

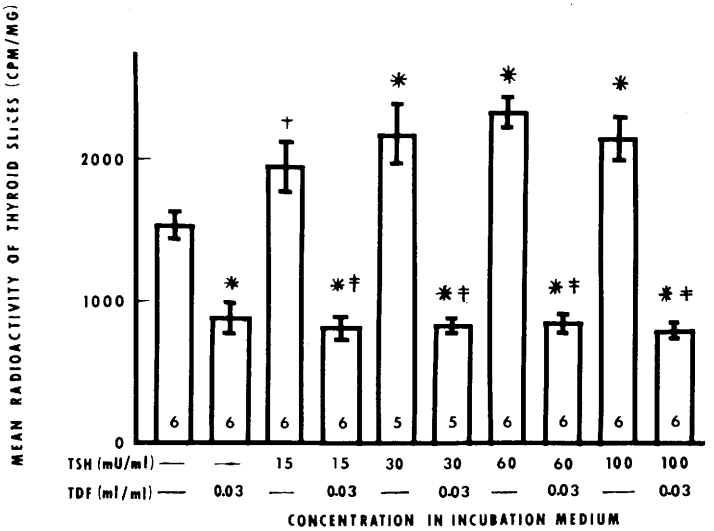


FIG. 1. Total $^{131}I$ (cpm/mg) accumulated by bovine thyroid slices during a 2 hr incubation in buffer, in buffer containing renal factor (0.03 ml/ml), in buffer containing TSH and renal factor in combination. The number of slices used in each group is given at the base of each bar. One standard error is set off at each mean. Significant differences from the untreated group are represented by symbols above the bars: (*), $p<.01$; and (†), $p<.05$. Significant difference ($p<.01$ from the TSH-treated slices is represented by (‡).

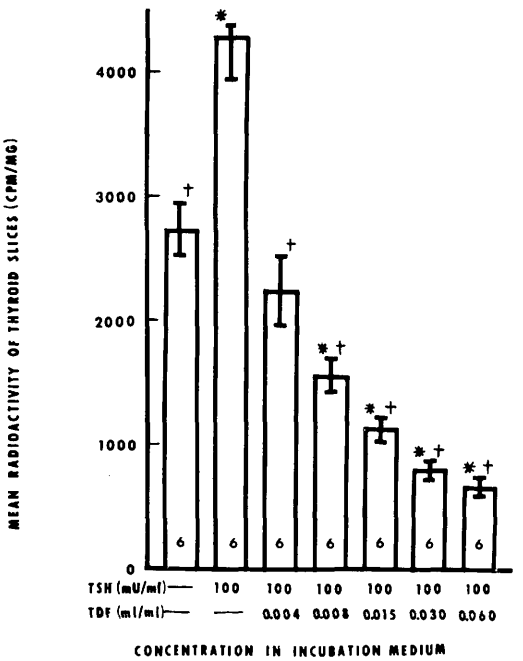


FIG. 2. Total $^{131}I$ (cpm/mg) accumulated by bovine thyroid slices during a 2 hr incubation in buffer, in buffer containing TSH (100 mU/ml), and in buffer containing TSH plus various concentrations of renal factor (0.004 to 0.060 ml of TDF/ml). The number of slices used in each group is given at the base of each bar. One standard error is set off at each mean. Significant differences from the untreated group are represented by symbols above the bars: (*) significantly different from the untreated group ($p<.01$); (†) significantly different from the group treated with TSH alone ($p<.01$).

sis of variance (3).

*Results.* The results of the first experiment are presented in Table I. The factorial analysis demonstrated that both TSH and renal factor had significant ($p<.01$) effects on the uptake of $^{131}I$. TSH produced an increase in the uptake whereas renal factor produced a depression. The analysis also showed that there was no statistical interaction between TSH and renal factor. In all groups having additions of both TSH and renal factor, the renal factor completely blocked the expression of any stimulatory effect of TSH.

Treatment with TSH produced a significant ($p<.05$ to $p<.001$) increase in the $^{131}I$ uptake in the second experiment (Fig. 1). The increase appeared to be concentration dependent through 60 mU/ml (significantly greater than the response at 15 mU/ml, $p<.05$). Treatment with 100 mU produced a stimulation ($p<.01$) but it was no greater than that achieved with 60 mU. Treatment with 0.03 ml of TDF/ml of incubation medium produced a depression in uptake of 43% below the value for the untreated control group ($p<.01$), and the absolute value for the uptake of $^{131}I$ in the presence of TDF was not altered by the addition of any of the concentrations of TSH.

The results of the third experiment are presented in Fig. 2. Treatment with TSH produced an increase in $^{131}I$ uptake of 58% ($p<.001$). The addition of the lowest concentration of TDF (0.004 ml/ml) completely blocked any stimulation by TSH; but, in the presence of TSH, the lowest concentration of TDF was insufficient to produce any significant depression in uptake below the level of the untreated group. The addition of progressively higher concentrations of TDF led to a progressively greater depression in uptake which was, in all cases, significantly less than that of the untreated group ($p<.01$). With the highest concentration of TDF, the uptake was only 25% that of the untreated group in spite of the presence of the TSH.

*Discussion.* These studies demonstrate that a factor, present in crude extracts of rat kidneys, has the capacity to inhibit partially the active uptake of iodide by thyroid tissue *in vitro*. Previous studies demonstrated this inhibition *in vivo* in rats and *in vitro* in thyroid tissue of porcine origin (1, 2). The present studies extend this observation to bovine thyroid tissue. The results of the first experiment showed that TDF not only reduced the $^{131}I$ uptake of non-TSH stimulated thyroid slices, but it also blocked the stimulation observed with the addition of TSH. There was, however, no evidence of a significant interaction between TDF and TSH. This was confirmed in the other two experiments. In the presence of TDF, a maximally stimulatory concentration of TSH failed to produce any increase in uptake over the level obtained when TDF alone was present. In the presence of the lowest concentration of TDF used, addition of 100 mU of TSH/ml

of buffer failed to induce any stimulation in uptake of $^{131}I$ by bovine thyroid slices.

The exact mechanism by which TDF blocks iodide uptake by thyroid tissue both *in vitro* and *in vivo* cannot be ascertained from these or earlier studies. However, the results suggest that TDF interferes with some aspect of the concentrating mechanism for iodide. Since there is no significant interaction between TSH and TDF, it seems unlikely that there is a direct inhibition of TSH by TDF. It seems more likely that TDF inhibits some aspect of iodide transport on which the manifestation of a TSH effect is dependent.

Earlier *in vivo* studies from this laboratory showed that the target organ for renal extract is the thyroid gland since the organ to body weight ratios of heart, kidneys, adrenals, and ovaries of recipient rats were unaffected by chronic treatment (1). In addition, the kidney appears to be a unique source of thyroid depressing factor since extracts of skeletal muscle and liver, similarly prepared, failed to influence either thyroid weight ratio or uptake of $^{131}I$ in the same way as renal extract. Further, neither the crude renal extract nor ultracentrifugal fractions thereof apparently had any general toxic effect on recipients in that food intake and increase in body weight were unchanged during the 6 days of treatment (1).

The chemical characteristics of the TDF isolated from rat kidneys are currently being pursued. Studies from this laboratory suggest that it is neither renin nor associated with renin (1). It does not have measurable vasopressor activity, is found in greatest potency in the renal medulla and is not found in the renal fraction precipitated at 103,000*g* for 24 hr. This fraction contains potent vasopressor activity which is thought to be due to the presence of renin (4). In addition, angiotensin failed to mimic the effect of renal factor on $^{131}I$ uptake in rats (1). It is also unlikely that renal factor is prostaglandins $E_1$, $E_2$, $B_1$, or $F_{1\alpha}$ since these are reported either to increase or to have no effect on thyroid activity (5, 6). Characterization of the renal factor will require further studies.

*Summary.* The kidneys of rats contain a factor, extractable in normal saline, which depresses the uptake of $^{131}I$ by slices of bovine thyroid gland *in vitro*. The thyroid depressing factor (TDF) is present in ultracentrifugal fractions of kidney (35,000*g* for 0.5 hour) and inhibits the stimulatory effect of TSH on $^{131}I$ uptake. Thus, a graded increase in concentration of renal factor in the incubation medium resulted in a graded depression in $^{131}I$ uptake by bovine thyroid slices stimulated to take up iodide by TSH (100 mU/ml medium). An experiment designed statistically to reveal interaction between TSH and TDF failed to demonstrate significant interaction. The exact mechanism by which TDF blocks the stimulatory effect of TSH on iodide uptake cannot be ascertained from these studies but the results suggest that TDF inhibits some aspect of iodide transport and thereby prevents TSH from manifesting its typical effect.

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