The *in vitro* effect of thyrotropin (TSH) on the osmoregulation of surviving thyroid slices

In previous studies[1,2,3,4] of the *in vitro* effects of thyrotropin (TSH) on radioiodide turnover in surviving beef thyroid slices, accurate measurement of the weight of the tissue slice before and after incubation revealed that as little as $8 \cdot 10^{-4}$ U.S.P. units of TSH (1 μg of Armour TSH powder) in the incubation medium caused the final weight of the slice to be significantly higher than the final weight of the control incubated in the absence of TSH. Further study of this *in vitro* phenomenon might contribute to an understanding of the mechanisms by which TSH stimulates thyroid tissue and in addition might provide an empirical basis for the assay of TSH.

Thyroid slices, 0.4 mm thick, weighing 60 to 120 mg, were prepared in a Stadie-Riggs microtome from a single fresh beef thyroid. After rinsing and weighing they were incubated for 21 hours at 38° C under air in a shaking metabolic incubator of the Dubnoff type. The medium was Krebs-Ringer phosphate buffer[5] containing $10^{-3}M$ penicillin and $10^{-3}M$ propylthiouracil. Each incubation beaker contained 5 ml of medium per 100 mg of tissue. At the end of incubation the slices were removed, spread on hard filter paper for about 5 seconds, weighed, and placed in tared aluminum cups and dried to a constant weight in a vacuum oven at 50° C to obtain the dry weight. Both wet and dry weights were also obtained on unincubated slices for comparison.

When slices were removed and weighed at intervals, it was found that during the first 3 hours the tissue weights remained relatively constant. During the following 30 hours there was a slow decline in both the wet and dry weights until the control slices weighed less than they did initially. TSH in the incubation medium did not noticeably affect the weight change in the first 10 hours, but after 21 hours the slices usually weighed more than they did at the start of incubation, and they uniformly weighed significantly more than the control slices. For this reason all of the subsequent data were obtained after 21 hours incubation.

Because of the prolonged incubation period the QO_2 was determined before and after a 21 hour incubation. In 12 observations fresh tissue had an average QO_2 of 0.471 $\pm$ 0.07 (μl O_2/mg wet tissue/hour). After 21 hours incubation the QO_2 declined to 0.201 $\pm$ 0.09 (24 observations). However, the slices that had been incubated in TSH, $8 \cdot 10^{-4}$ U.S.P. units/ml, declined to 0.320 $\pm$ 0.11 (24 observations); *i.e.*, they were 59% above their controls.

Table I shows both wet and dry weight determinations in a typical experiment. All slices had lost dry weight at the end of incubation and also increased their percent water content. Because the dry weight was not affected by TSH only total weights are presented in the subsequent data. The two larger doses of TSH shown in Table I had a pronounced effect on the final weight of the slices ($p < 0.001$).

TABLE I

THE EFFECT OF TSH ON THYROID SLICE WEIGHT

(Thyroid slices incubated 21 hours in 5 ml of medium/100 mg of tissue. Data in mg/100 mg of initial tissue $\pm$ standard error; 7 slices/point.)

	Final wt (mg)/100 mg initial wt			*Change in wt (mg)*		
	Total	*Dry*	*Water*	*Total*	*Dry*	*Water*
Initial tissue	100	23	77	—	—	—
Final tissue						
Control	92 $\pm$ 3.7	17 $\pm$ 0.9	75 $\pm$ 3.5	—8	—6	—2
TSH, $8 \cdot 10^{-6}$ u./ml	96 $\pm$ 2.5	18 $\pm$ 1.8	78 $\pm$ 2.9	—4	—5	+1
TSH, $8 \cdot 10^{-5}$ u./ml	117 $\pm$ 6.1*	17 $\pm$ 1.9	100 $\pm$ 4.8*	+17*	—6	+23*
TSH, $8 \cdot 10^{-4}$ u./ml	118 $\pm$ 6.0*	16 $\pm$ 1.8	102 $\pm$ 5.4*	+18*	—7	+25*

* Denotes that the effect of TSH was statistically significant ($p < 0.001$).

Table II shows experiments performed to evaluate the tissue specificity of the weight gain response to TSH. TSH did not alter the weight change measured in other beef tissues including heart muscle, liver, kidney and anterior hypophysis. Only thyroid slices responded to the TSH.

Table II also shows that the response of thyroid tissue to TSH was specific when compared with other pituitary proteins including ACTH and anterior pituitary powder known to be free of TSH (see acknowledgement). In addition, both Armour and Parke-Davis preparations of TSH

gave identical responses in spite of the fact that they may differ significantly in certain unidentified interfering materials[6].

TABLE II

THE SPECIFICITY OF THE WEIGHT GAIN RESPONSE TO TSH

(Same as Table I except data is the mean of 5 slices/point in Expt. #1, and 10 slices/point in Expt. #2; TSH, $5 \cdot 10^{-4}$ u./ml.)

Experimental conditions	*Final wt (mg)/100 mg initial wt*	
	Control	*TSH*
Expt. #1		
Thyroid	83 ± 8.5	117 ± 9.9*
Liver	80 ± 5.9	81 ± 3.8
Kidney	78 ± 6.2	80 ± 4.1
Ant. Pituitary	83 ± 3.1	81 ± 4.8
Heart muscle	68 ± 2.2	67 ± 1.4
Expt. #2		
Armour TSH	84 ± 6.2	113 ± 8.1*
Parke-Davis TSH	84 ± 6.2	113 ± 7.3*
Ant. Pituitary Po. T_{88}	84 ± 6.2	84 ± 6.9
ACTH	84 ± 6.2	79 ± 5.2

* Denotes that the effect of TSH was statistically significant ($p < 0.001$).

Further experiments studied the effect of various conditions and enzyme inhibitors on the response to TSH. Preliminary quick-freezing of the tissue on dry ice, incubation at 4° C, the addition of dinitrophenol (10^{-3} *M*), cyanide (10^{-3} *M*) or mercuric ion (10^{-5} *M*) abolished the effects of TSH. By contrast fluoride (10^{-3} *M*), thiocyanate (10^{-3} *M*), or iodide (in concentrations less than 10^{-2} *M*) did not interfere with the stimulation by TSH.

Preliminary experiments demonstrated that the final weight change was proportional to the total amount of TSH in the incubation medium when the TSH was varied from 10^{-5} to 10^{-4} units/ml of medium. This simple method may be the basis of a useful assay procedure for TSH.

The biological significance of the increased weight gain in the presence of TSH is not clear. However, the response appears to be dependent upon oxidative metabolism because it was blocked by cyanide or dinitrophenol. The response appears to be independent of thyroidal iodine uptake and organic binding since it was not altered by thiocyanate, propylthiouracil, nor by high concentrations of iodide in the medium. One hypothesis for the tissue weight change is that TSH preserves thyroid slice viability. Another hypothesis is that TSH stimulates proteolytic hydrolysis of large molecules (such as thyroglobulin) within the thyroid follicle resulting in increased colligative properties of the follicular colloid protein with increased water entry and weight gain. Experimental study of these possibilities will be presented elsewhere.

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[1] J. L. BAKKE AND NANCY LAWRENCE, (Abst.) *Am. J. Med.*, 17 (1954) 116.
[2] J. L. BAKKE AND NANCY LAWRENCE, (Abst.) *Clin. Research Proc.*, II (1954) 143.
[3] J. L. BAKKE AND NANCY LAWRENCE, (Abst.) *J. Clin. Endocrinol. and Metabolism*, 14 (1954) 771.
[4] J. L. BAKKE AND NANCY LAWRENCE, Submitted for publication in *Endocrinology* (1955).
[5] JOHN FIELD, *Methods in Med. Research*, 1 (1948) 229.
[6] W. TONG, personal communication.

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