Further Studies on Effects of Thyroid-stimulating Hormone on Thyroid Nucleotide Biosynthesis*

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Previously it was reported that thyroid-stimulating hormone increased the incorporation of $^{14}$C-formate into acid-soluble and ribonucleic acid purines in calf thyroid slices (1). Evidence was presented relating the action of TSH to its known effect on stimulating the hexose monophosphate pathway (2), thereby increasing the supply of available ribose.

Further studies with other nucleotide precursors have been carried out to clarify the mechanism of action of TSH on nucleotide biosynthesis. The results of these experiments form the basis of this report.

EXPERIMENTAL PROCEDURE

Slices of calf thyroid were incubated in Krebs-Ringer phosphate at pH 7.4 in air as previously described (1). Incubation periods were 3-hours duration for $^{14}$C-formate, glycine-2-$^{14}$C, and adenine-8-$^{14}$C, and 1 hours for glucose-$U^{-14}$C and ribose-$^{14}$C, unless otherwise stated. The specific activities of the radioactive precursors are mentioned under "Results." Methods used for separation of nucleic acids and purines were as described (1).

Ribose from RNA-purine mononucleotides was obtained by the method of Marks and Feigelson (3). After liberation of the purine nucleotide ribose with 0.5 $\times$ H$_2$SO$\_4$ at 100$^\circ$ for 24 hours, purine and unhydrolyzed pyrimidine nucleotides were removed by adsorption on to Norit. Prior to paper chromatography of the ribose, ionic contaminants were removed by passing the acid hydrolysate through a mixed bed ion exchange resin (Amberlite CG-4B (Cl$^-$), 100 to 200 mesh, and nowex 50 (H$^+$), 200 to 400 mesh, 12% cross-linked). Ribose was estimated by the orcinol reaction (4).

Measurement of Specific Activity—Aliquots of the bases, nucleotides, and ribose were added to NE213 or NE220 (supplied by Nuclear Enterprises (G.B.) Ltd., Edinburgh, Scotland) liquid scintillators in glass counting vials and the radioactivity determined in a Packard Tri-Carb liquid scintillation counter. The results of these experiments form the basis of this report.

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RESULTS

TSH always increased the incorporation of $^{14}$C-formate, glycine-2-$^{14}$C, and adenine-8-$^{14}$C into RNA-adenine in calf thyroid (Table I).

Other pituitary hormones, ACTH and growth hormone gave no stimulation (Table II). A small effect was obtained with FSH which could be accounted for by its known contamination with TSH. When the concentration of TSH was reduced, no stimulation was observed. Addition of triiodothyronine (3 $\times$ 10$^{-7}$ M), hydrocortisone (6 $\times$ 10$^{-7}$ M), or stilbestrol (2.2 $\times$ 10$^{-8}$ M) to the medium caused no stimulation of formate incorporation into RNA.

Glucose, like TSH, increased the incorporation of formate, glycine, and adenine into RNA-adenine, and in the presence of TSH an additive effect was observed (Table III). Previously we noted that TSH was effective with amounts as small as 0.1 millimicron per ml (1). Similarly glucose is also effective at levels as low as 5 $\mu$moles per flask (Table IV).

Ribose caused marked stimulation of incorporation of $^{14}$C-formate into RNA-adenine, and in the presence of ribose, TSH and glucose had no additional effect (Table V). The action of graded amounts of ribose on RNA-adenine labeling is shown in Table VI. A response could be obtained when as little as 0.5 $\mu$mol of ribose was added to a flask.

With glucose-$U^{-14}$C as precursor, TSH caused an increase in the $^{14}$C-label in RNA-purine nucleotide ribose (Table VII). Glucose-$U^{-14}$C was used in preference to glucose labeled in a single carbon atom since it was available at very high specific activity (87 mC per mm) and allowed the use of an amount of glucose insufficient in itself to cause any stimulation of purine synthesis. Insulin, which increases glucose uptake by thyroid slices, had no effect on glucose-$U^{-14}$C incorporation. When ribose-$L^{-14}$C was used, TSH had no stimulating effect on the incorporation of the $^{14}$C-label into RNA-purine nucleotide ribose (Table VII).

### Table VI: Response to graded amounts of ribose on RNA-adenine labeling

<table>
<thead>
<tr>
<th>Ribose (mM)</th>
<th>Incorporation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>100</td>
</tr>
<tr>
<td>0.005</td>
<td>120</td>
</tr>
<tr>
<td>0.010</td>
<td>150</td>
</tr>
<tr>
<td>0.020</td>
<td>200</td>
</tr>
<tr>
<td>0.050</td>
<td>250</td>
</tr>
</tbody>
</table>

### Table VII: Incorporation of $^{14}$C-formate into RNA-adenine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incorporation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>TSH</td>
<td>120</td>
</tr>
<tr>
<td>Glucose</td>
<td>150</td>
</tr>
<tr>
<td>TSH + Glucose</td>
<td>200</td>
</tr>
</tbody>
</table>

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* This work was supported by funds from Grant R.6-136(2) of the British Empire Cancer Campaign and by the Wellcome Trust.
† Wellcome Senior Research Fellow in Clinical Science and Honorary Lecturer in Medicine.

The abbreviations used are: TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; and PP-ribose-P, 5-phosphoribosyl 1-pyrophosphate.

Glucose-$U^{-14}$C refers to the uniformly or randomly labeled compound.
**TABLE I**

Effect of TSH on incorporation of $^{14}$C-formate, adenine-$8^{14}$C, and glycine-$2^{14}$C into RNA-adenine in calf thyroid

One unit of TSH, 2.5 $\mu$moles (25 $\mu$C) of $^{14}$C-formate, 9.5 $\mu$moles (25 $\mu$C) of glycine-$2^{14}$C, and 1.52 $\mu$moles (2.6 $\mu$C) of adenine-$8^{14}$C were added to the appropriate flasks.

<table>
<thead>
<tr>
<th>Precursor and drug</th>
<th>Relative specific activity of RNA-adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-Formate</td>
<td>Control: 741</td>
</tr>
<tr>
<td></td>
<td>TSH: 1,622</td>
</tr>
<tr>
<td>Adenine-$8^{14}$C</td>
<td>Control: 12,826</td>
</tr>
<tr>
<td></td>
<td>TSH: 17,840</td>
</tr>
<tr>
<td>Glycine-$2^{14}$C</td>
<td>Control: 103</td>
</tr>
<tr>
<td></td>
<td>TSH: 208</td>
</tr>
</tbody>
</table>

**TABLE II**

Effect of graded doses of glucose on incorporation of $^{14}$C-formate into RNA-adenine and RNA-guanine in calf thyroid

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5 $\mu$oles</td>
<td>547</td>
</tr>
<tr>
<td>Glucose</td>
<td>25 $\mu$oles</td>
<td>1099</td>
</tr>
<tr>
<td>Glucose</td>
<td>125 $\mu$oles</td>
<td>970</td>
</tr>
</tbody>
</table>

**TABLE III**

Effect of various pituitary hormones on incorporation of $^{14}$C-formate into RNA-adenine in calf thyroid

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Relative specific activity of RNA-adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH*</td>
<td>1 unit</td>
<td>863</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone†</td>
<td>1 unit</td>
<td>412</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH‡</td>
<td>3 units</td>
<td>454</td>
</tr>
<tr>
<td>FSH</td>
<td>1 mg</td>
<td>539</td>
</tr>
<tr>
<td>FSH</td>
<td>0.1 mg</td>
<td>512</td>
</tr>
<tr>
<td>FSH</td>
<td>0.01 mg</td>
<td>468</td>
</tr>
</tbody>
</table>

† Growth hormone, NIH-GH-D0 Bovine (1 U.S.P. unit per mg) and follicle-stimulating hormone, NIH-FSH-S1 ovine (2.6 times the Armour standard No. 264-151-X) were gifts of the Endocrinology Study Section of the National Institutes of Health.
‡ ACTH (Armour, bovine, DG.05.02).

**TABLE IV**

Effect of graded amounts of ribose on incorporation of $^{14}$C-formate into RNA-adenine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Relative specific activity of RNA-adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose and TSH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE V**

Effect of ribose, glucose, and TSH on incorporation of $^{14}$C-formate into RNA-adenine

Ribose, 25 $\mu$moles, glucose, 25 $\mu$moles, and TSH, 1 unit, were added to the appropriate flasks.

**TABLE VI**

Effect of graded amounts of ribose on incorporation of $^{14}$C-formate into RNA-adenine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Relative specific activity of RNA-adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose and TSH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE VII**

Effect of TSH on incorporation of glucose-$U^{14}$C and ribose-$1^{14}$C into RNA-purine nucleotide ribose

Either 0.03 $\mu$mole (2.5 $\mu$C) of glucose-$U^{14}$C or 0.66 $\mu$mole (2.5 $\mu$C) of ribose-$1^{14}$C were used as precursors. One unit of TSH was added to the appropriate flasks.

**TABLE VIII**

Effect of TSH on incorporation of $^{14}$C-formate into RNA-adenine produced by TSH reflects the

**DISCUSSION**

It has previously been shown that the increased incorporation of $^{14}$C-formate into RNA-adenine produced by TSH reflects the
Increased formation of adenine nucleotides (1). Because of the occurrence of exchange reactions between free formate and carbon in the second position of the purine ring of inosinic acid (7, 8), estimation of the rate of purine synthesis of the adenosine is best made with glycine-2-14C (8). Stimulation of glycine incorporation by TSH and glucose confirms an increase in purine synthesis in vivo (1).

The specificity of the action of TSH on thyroid purine synthesis is indicated by the lack of action of other pituitary hormones, ACTH, growth hormone, and FSH on this process.

All the actions of TSH on nucleotide biosynthesis could be reproduced by glucose, and an additive effect was obtained when the two compounds were present together. Evidence was presented from previous studies that TSH and glucose share a common mechanism of action, that of increasing the supply of ribose for purine biosynthesis (1).

Field et al. (2) have shown that TSH stimulates thyroid glucose oxidation via the hexose monophosphate pathway and Hiatt and Lareau (9) showed this to be the principal source of nucleic acid ribose in man in vivo and in human blood cells in vitro, although in other tissues such as rat liver the nonoxidative sequences were more important (3). The relative contributions of other sources of ribose-5-phosphate to nucleic acid ribose, e.g., purine and pyrimidine nucleosides, free ribose, and other pentoses remain to be elucidated. Harrington (10) and Thomson, Ricceri, and Peretta (11) have postulated that in the Ehrlich ascites cell glucose stimulates purine synthesis by providing a source of ribose, and hence of PP-ribose-P. In the carcinoma cell direct evidence for this theory was lacking since ribose and ribose-5-phosphate were ineffective even when as much as 100 μmoles were added per flask. Therefore, it was suggested that these cells lacked ribokinase and were impermeable to phosphorylated sugars. Addition of ribose to calf thyroid slices caused a marked increase in purine synthesis. Other workers have also found that ribose-5-phosphate can be utilized by intact cells and slices from normal and tumor tissue (12). It must be assumed that, unlike the Ehrlich ascites cell, calf thyroid contains ribokinase and so is able to utilize added ribose. If TSH and glucose both act by providing ribose, their failure to cause any increase in purine synthesis in calf thyroid in the presence of ribose would be explained.

The action of TSH and glucose on incorporation of adenine-8-14C into RNA-adenine could also be accounted for by their provision of PP-ribose-P which reacts with free bases to form nucleotides under the influence of nucleotide pyrophosphorylase (13).

Most studies of purine nucleotide biosynthesis have been concerned with the incorporation of 32P and various radioactive precursors of the purine ring. Itzhaki (14) has shown that the 14C-label from glucose-U-14C is readily incorporated into the ribose of RNA-purine nucleotides in rat thymus, spleen, liver, and the Ehrlich ascites cell. Similarly, in our studies, 14C from glucose-U-14C is rapidly introduced into the ribose of RNA-mononucleotides. TSH by increasing glucose oxidation via the hexose monophosphate pathway could increase the specific activity of the ribose-5-phosphate pool, of PP-ribose-P, and hence, of purine nucleotide ribose. Further studies are being carried out to study the effect of TSH on the incorporation of the 14C-label from glucose-6-14C and glucose-1-14C into nucleotide ribose.

Two alternative modes of action of TSH on glucose-U-14C incorporation need to be excluded before this theory can be accepted. Firstly, TSH is known to increase the uptake of glucose by thyroid slices (2) and a similar effect is obtained with insulin. If TSH acted solely by increasing the uptake of labeled glucose by the slices, its effect on ribose labeling should be reproduced by insulin. It has been shown that insulin has no such action and this agrees with Field's finding (2) that insulin was without effect on thyroid glucose oxidation to CO2.

Secondly, if TSH increased the availability of ATP, more of the labeled ribose from glucose-U-14C could be converted to PP-ribose-P and then to purine nucleotide ribose. Henderson and Le Page (11) suggested that this was the mechanism by which glucose stimulated nucleotide biosynthesis in the Ehrlich ascites cell. To test this hypothesis thyroid slices were incubated with ribose 14C and the RNA purine nucleotide ribose obtained. Unlabeled ribose has already been shown to stimulate purine synthesis, and with the labeled ribose considerable incorporation was demonstrated into nucleotide ribose. TSH, however, had no stimulating effect on incorporation of the 14C-label into nucleotide ribose supporting the view that the hormone acts on the conversion of glucose to ribose and not on the incorporation of ribose into nucleotides by provision of ATP or by other mechanisms. The slight reduction of nucleotide-ribose labeling in the presence of TSH could have resulted from dilution of the labeled ribose with additional ribose derived from TSH-stimulated glucose oxidation.

Further evidence that TSH does not cause an in crease in the energy available to the slices is seen in the failure of other substrates such as acetate, known to be oxidized in thyroid slices, to have any action on purine biosynthesis.

Field et al. have suggested that the primary action of TSH on the thyroid is to stimulate glucose oxidation via the hexose monophosphate pathway (2). This view has recently been disputed by Freinkel (15) because TSH effects can be demonstrated in unstimulated systems despite the paucity of intrathyroidal glycogen. Amounts of glycogen as low as 0.01% of the wet weight of thyroid tissue could still yield enough ribose by TSH-stimulated glucose oxidation to account for the increased purine synthesis observed in the present experiments. If stimulation of the hexose monophosphate pathway is the fundamental action of TSH on the thyroid, it should be possible to relate the diverse effects of TSH to its action on glucose metabolism. Some progress has already been made in this

### Table VIII

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Relative specific activity of RNA-adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>393</td>
</tr>
<tr>
<td>Acetate</td>
<td>20 μmoles</td>
<td>387</td>
</tr>
<tr>
<td>Succinate</td>
<td>20 μmoles</td>
<td>422</td>
</tr>
<tr>
<td>Aspartate</td>
<td>20 μmoles</td>
<td>338</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>401</td>
</tr>
<tr>
<td>Albumin-palmitate</td>
<td>5 μmoles</td>
<td>340</td>
</tr>
<tr>
<td>TSH</td>
<td>1 unit</td>
<td>607</td>
</tr>
<tr>
<td>TSH and albumin-palmitate</td>
<td>1 unit and 5 μmoles</td>
<td>698</td>
</tr>
</tbody>
</table>
correlation in thyroid tissue. Schussler and Ingbar (16) have
demonstrated that the incorporation of iodine into iodotyrosines is
dependent on the generation of NADPH by the hexose monophosphate pathway. The NADPH produced is subsequently
oxidized by a flavin enzyme which in turn reduces oxygen to
form hydrogen peroxide. The latter oxidizes iodide in a reac-
tion catalyzed by a thyroid peroxidase. A further effect of
TSH on intrathyroidal iodine metabolism, stimulation of de-
iodination of iodotyrosines, can be related to an increase in
NADPH, since deiodination of these compounds is catalyzed by
NADPH-linked thyroid microsomal deiodinase (17). The
specific action of TSH on phospholipid synthesis in the thyroid
demonstrated by Morton and Schwartz (18) may also be medi-
ated by production of NADPH necessary for lipid synthesis.
Thus, stimulation of the hexose monophosphate pathway by
TSH is a mechanism which can explain at least four apparently
different effects of the hormone on the thyroid, namely nucleotide
formation and deiodination of iodotyrosines, and phospholipid synthesis and
degradation, and phospholipid synthesis by providing NADPH.

Further studies may allow other effects of TSH to be related to
its action on glucose oxidation.

Any theory of TSH action must account for the specificity of
the hormone, since apart from the epididymal fat pad of the
rat (21) its actions are impressed entirely on the metabolic
processes of the thyroid gland. One might speculate that TSH
could increase the activity of an NAD-kinase isoenzyme present
in greatest amount in the thyroid, either by some direct action
or indirectly by stimulation of nuclear-RNA formation leading to
increased synthesis of the enzyme. Such a theory is open to
experimental verification and studies are at present under way
to assess the effects of TSH on NAD kinase activity in the
thyroid. In addition the action of inhibitors of protein synthesis
on the TSH effect is being examined.

SUMMARY

Thyroid-stimulating hormone (TSH) increased the incorpora-
tion of 14C-formate, glycine-2-14C, and adenine-8-14C into ribo-
nucleic acid purines in calf thyroid slices in vitro. The specificity of
the effect was confirmed by the lack of action of other pituitary
hormones. Glucose and TSH had similar and additive effects
on nucleotide biosynthesis. Further evidence is presented that
glucose and TSH both act by increasing the available ribose and
hence of 5-phosphoribosyl 1-pyrophosphate. Stimulation of
purine synthesis by ribose was direct support for this mechanism
of action. TSH increased the incorporation of the 14C-label of
glucose-U-14C into RNA-purine nucleotide ribose but had no
effect on the incorporation of ribose-14C. This also supports the
view that TSH acts by converting glucose to ribose via the
hexose monophosphate pathway. Four apparently diverse
actions of TSH on the thyroid, viz. nucleotide synthesis, forma-
tion and deiodination of iodotyrosines, and phospholipid synthesis
can now be related to its action on glucose oxidation via the
hexose monophosphate pathway.

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