Demonstration of Iodide Transport Defect but Normal Iodide Organification in Nonfunctioning Nodules of Human Thyroid Glands

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ABSTRACT Benign and malignant nodules in human thyroid glands, which did not concentrate iodide in vivo, were also unable to accumulate iodide in vitro. The mean thyroid-to-medium hormone (T/M) in seven benign nodules was 0.8±0.2 compared with 7±2 in adjacent normal thyroid tissue. In four malignant thyroid nodules, the mean T/M was 0.5±0.1 compared with 11±4 in adjacent normal thyroid. Despite the inability of such nodules to concentrate iodide, iodide organification was present but was only one-half to one-third as active as in surrounding normal thyroid. Thyroid-stimulating hormone (TSH) increased iodide organification equally in both benign nodules and normal thyroid although it had no effect in three of the four malignant lesions. The reduction in organification is probably related to the absence of iodide transport, since incubation of normal thyroid slices with perchlorate caused similar diminution in iodide incorporation but no change in the response to TSH. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) accounted for most of the organic iodide in both the nodules and normal tissue. The MIT/DIT ratio was similar in normal and nodule tissue. The normal tissue contained much more inorganic iodide than the nodules, consistent with the absence of the iodide trap in the latter tissue. The thyroxine content of normal thyroid was 149±17 μg/g wet wt and 18±4 μg/g wet wt in the nodules. The transport defect in the nodules was not associated with any reduction in total, Na-K-, or Mg2+-activated ATPase activities or the concentration of ATP. Basal adenylate cyclase was higher in nodules than normal tissue. Although there was no difference between benign and malignant nodules, the response of adenylate cyclase to TSH was greater in the benign lesions.

These studies demonstrate that nonfunctioning thyroid nodules, both benign and malignant, have a specific defect in iodide transport that accounts for their failure to accumulate radioactive iodide in vivo. In benign nodules, iodide organification was increased by TSH while no such effect was found in three of four malignant lesions, suggesting additional biochemical defects in thyroid carcinomas.

INTRODUCTION

We previously reported that the failure of thyrotropin (TSH)1 to increase in vivo 131I uptake in benign nonfunctioning or “cold” thyroid nodules cannot be ascribed to defective binding of TSH or activation of the adenylate cyclase-cyclic AMP system (1). Basal- and TSH-responsive adenylate cyclase activities of such nodules were significantly greater than in the adjacent normal thyroid tissue. Although stimulation of iodide uptake and organification by TSH appears to be mediated by the adenylate cyclase-cyclic AMP system (2-4), the lack of iodide uptake and response to TSH in benign “cold” nodules does not reflect a generalized abnormality in the action of cyclic AMP. TSH increased colloid droplet formation and [1-14C]glucose oxidation in benign “cold” nodules as in adjacent normal thyroid (1). Although controversy exists as to whether augmented 32P

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1 Abbreviations used in this paper: DIT, diiodotyrosine; MIT, monoiodotyrosine; T, thyroxine; T/M ratio, thyroid-to-medium ratio; TSH, thyroid-stimulating hormone.
incorporation into phospholipids induced by TSH is
dependent on cyclic AMP (5), this parameter was
also more responsive to TSH in "cold" nodules.

Since this initial study compared effects of TSH on

"I uptake in "cold" nodules in vivo and other meta-
bolic responses to TSH in vitro, subsequent studies
were directed towards correlating the latter response
with iodine metabolism in vitro. Preliminary experi-
ments demonstrated defective iodide transport in "cold"
nodules in comparison with adjacent normal thyroid
tissue though organification of iodide was only moder-
ately decreased in this tissue compared with the

normal (6). DeGroot has also reported that "cold"
nodules were unable to concentrate iodide in vitro,
but could incorporate "I into protein (7).

METHODS

The criteria for selection and study of patients and
the preparation and incubation of tissue have been reported
(1). Iodide transport and organification were examined by
the method of Ahn and Rosenberg (3). For iodide trans-
port, thyroid slices were incubated initially for 1 h in
Krebs-Ringer phosphate buffer (pH 7.4) in an atmosphere
of air at 37°C in a Dubnoff metabolic shaker. Slices were
then incubated for 30 min in 4 ml of the same buffer con-
taining 1 mg/ml glucose, 0.5 mg/ml albumin, 1 μg/ml I–
as KI, 0.25 mg/ml Tapazol, and 0.05 μCi/ml "I. At the end of
the incubation, the tissue slice was rinsed in cold saline,
blotted, and digested in 1 ml of 2 N NaOH, and an aliquot
of the digest and of the medium was counted. In preliminary
experiments with normal thyroid tissue, the thyroid to
medium ratio (T/M) obtained at 30 min was similar to
that found at 120 min. However, since time curves were
not done with nodular tissue or with each normal thyroid
studied, the T/M ratios do not necessarily represent equilib-
rium values. The effect of perchlorate on iodide transport
in normal thyroid tissue was examined by inclusion of
1 × 10–8 M sodium perchlorate in the incubation buffer
during a 20 min incuba
tion preceding the 30 min incubation mentioned
above. Sodium perchlorate was also present during the latter
incubation. During the 20 min incubation, the buffer also
contained 1 mg/ml glucose and 0.5 mg/ml albumin. Organifi-
cation of iodide was studied as follows. Thyroid slices were
incubated for 1 h in Krebs-Ringer phosphate buffer
and then incubated for 30 min in 2 ml of the same buffer
containing 1 mg/ml glucose, 0.5 mg/ml albumin, 1 μg/ml I–
as KI, and 2.5 μCi/ml "I. At the end of the incubation,
the slices were homogenized in 3 ml of cold 10% trichlo-
roacetic acid (TCA). The precipitate was extracted twice
with 5 ml of 10% cold TCA and then dissolved in 1 ml of
2 N NaOH and counted.

The tissue uptake and distribution of iodine was also
measured using the methods of Ahn and Rosenberg (3)
except that the tissue was incubated for 90 min and
then homogenized in proximately 15 μCi of "I. The labeled components of
the thyroid were identified using methods previously described
(8). Briefly, the tissue slices were rinsed quickly in saline
and then homogenized in cold saline-Tris buffer (0.003 M
Tris, 0.11 M sodium NaCl), pH 8.5, containing 0.05 M
methylmercaptoimidazole as described by Inoue and Taurog
(9). Digestion was carried out with 1.75 mg Pronase for
18 h under anerobic conditions at 37°C as described by
these authors. Labeled "I thyroxine (T4) was added at
the start of the digestion to monitor recovery. After diges-
tion, 0.14 ml of methanol/concentrated NH4OH (1:1 vol/
vol) was added, the tubes were centrifuged, and the super-
nate was decanted. Residual "I and "I in the precipitate
were less than 10% of the total and were virtually com-
pletely extracted by a second wash of methanol/ammonia.
Sh–100 μl of this supernate was then chromatographed on
Whatman 3MM paper (3M Co., Photographic Products Div.,
St. Paul, Minn.) in two systems; tertiary amyl alcohol
(TAA)/hexane/2 normal NH4OH (5:1:6, vol/vol/vol) and
butanol(BAA)/2 N acetic acid (1:1, vol/vol). After dry-
ing, the strips were cut into 2-cm sections and counted in
an automatic gamma-well counter with suitable correction
for the overlap of "I counts in the "I spectrometer win-
don (about 10%). Identification of "I, was made by
location of "I in the TAA, system. The "I monochlor-
ate (MIT) and diiodotyrosine (MIT) were identi-
fied by reference to the migration of known unlabeled
compounds chromatographed periodically in the same system
(BAA) and identified by staining with diazotized sulfanilic
acid. Origin material on BAA chromatograms was less
than 5% in experiments using both normal slices and tissues
from nodules.

RESULTS

The data in Table I summarize the results for iodide
transport and organification in vitro in 11 patients
with nonfunctioning thyroid nodules. Seven patients had
benign lesions, and four had papillary carcinoma with
some follicular elements. Slices of normal thyroid
tissue from each patient concentrated iodide. In con-
trast, slices from nonfunctioning nodules, both benign
and malignant, did not concentrate iodide. Although one
of the nodules had a T/M ratio of 2, this was con-
siderably less than the adjacent normal thyroid
tissue. Extracellular space measurements were not made
in both tissues but expansion of this space cannot ex-
plain the differences in results between the control

Iodide Transport Defect in Nonfunctioning Thyroid Nodules

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**Table I**

**Comparison of Iodide Transport and Organification in "Cold" Thyroid Nodules and Adjacent Normal Thyroid**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Iodide transport</th>
<th>Iodide organification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T/M</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>TSH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ng I/mg DNA</td>
<td>ng I/mg DNA</td>
</tr>
<tr>
<td>Su</td>
<td>Follicular adenoma</td>
<td>6 0.8</td>
<td>26±1</td>
</tr>
<tr>
<td>Ha</td>
<td>Nodule in multinodular gland</td>
<td>10 0.7</td>
<td>60±5</td>
</tr>
<tr>
<td>Ju</td>
<td>Follicular adenoma</td>
<td>8 0.5</td>
<td>41±1</td>
</tr>
<tr>
<td>Ch</td>
<td>Embryonal adenoma</td>
<td>7 2.0</td>
<td>51±3</td>
</tr>
<tr>
<td>Mo</td>
<td>Follicular adenoma</td>
<td>15 0.5</td>
<td>166±12</td>
</tr>
<tr>
<td>An</td>
<td>Follicular adenoma</td>
<td>20 0.6</td>
<td>41±17</td>
</tr>
<tr>
<td>Om</td>
<td>Hurthle cell adenoma</td>
<td>2 0.7</td>
<td>15±2</td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td></td>
<td>7±2 0.8±0.2</td>
<td>57±19</td>
</tr>
</tbody>
</table>

The TSH concentration was 50 mU/ml. The values for iodide organification are the mean ± SEM of triplicate determinations. Tissues and the slices from the nodules. It is possible that differences in extracellular space could account for the minor differences between the T/M ratios in the various nodules.

Organification of iodide by slices of normal thyroid varied considerably. In all of the patients, 50 mU/ml TSH significantly augmented organification of iodide in normal thyroid tissue, and there was no apparent difference in organification between normal and nodular thyroid tissue.

**Table II**

**Distribution of Iodine Compounds Formed during In Vitro Incubation of Tissue from "Cold" Thyroid Nodules and Adjacent Normal Thyroid**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Condition</th>
<th>I- MIT</th>
<th>DIT MIT/DIT</th>
<th>I- MIT</th>
<th>DIT MIT/DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ng I/mg DNA</td>
<td></td>
<td>ng I/mg DNA</td>
<td></td>
</tr>
<tr>
<td>Ha</td>
<td>Adenoma</td>
<td>Control</td>
<td>1,220 48 28 1.7</td>
<td>156 30 6 5.0</td>
<td>151 48 10 4.8</td>
<td></td>
</tr>
<tr>
<td>Mo</td>
<td>Adenoma</td>
<td>Control</td>
<td>2,090 42 14 3.0</td>
<td>12 30 8 3.8</td>
<td>18 95 42 2.3</td>
<td></td>
</tr>
<tr>
<td>Sp</td>
<td>Adenoma</td>
<td>Control</td>
<td>1,530 69 23 3.0</td>
<td>146 37 11 3.4</td>
<td>138 38 10 3.8</td>
<td></td>
</tr>
<tr>
<td>An</td>
<td>Adenoma</td>
<td>Control</td>
<td>670 107 32 3.3</td>
<td>146 37 11 3.4</td>
<td>138 38 10 3.8</td>
<td></td>
</tr>
<tr>
<td>Le</td>
<td>Carcinoma</td>
<td>Control</td>
<td>994 49 20 2.5</td>
<td>276 16 5 3.2</td>
<td>56 8 3 2.7</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>Control</td>
<td></td>
<td>1,139 56 22 2.6</td>
<td>134 26 7 3.6</td>
<td>44 4 1 0.4</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>TSH</td>
<td></td>
<td>257 10 3 0.3</td>
<td>44 4 1 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>TSH</td>
<td></td>
<td>869 108 41 2.6</td>
<td>91 44 16 3.2</td>
<td>25 14 7 0.5</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>TSH</td>
<td></td>
<td>173 25 8 0.2</td>
<td>25 14 7 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH/Control, %</td>
<td></td>
<td></td>
<td>80 193 186</td>
<td>68 169 228</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values are the means of triplicate determinations. The TSH concentration was 50 mU/ml.
difference between patients with benign or malignant lesions. Cells of the nonfunctioning nodules, both benign and malignant, organified iodide even in the absence of an iodide-concentrating mechanism. TSH significantly stimulated organification of iodide in six of the seven benign lesions, and the percent increment induced by TSH was similar in normal and benign nodular tissue. In contrast to these results, TSH had no effect on organification of iodide in slices from three of the four carcinomas and only a small effect in the fourth carcinoma. To determine whether or not there were qualitative as well as quantitative differences in iodine organization between the nodules and normal tissues during the in vitro incubation, pronase digestion and chromatography of the [131I]labeled compounds were carried out separately in five patients (four with follicular adenomas and one with papillary carcinoma). Virtually all labeled iodine in these tissues was found in the form of iodide and MIT and DIT, though small quantities of labeled T4 were sometimes found in extracts of normal slices (Table I). Most of the radioactivity was in the form of iodide in both types of tissue. The mean iodide concentration in the five normal tissues represented 93.6% of the total 131I whereas in the five nodules it was 80.2% of tissue 131I. The marked difference in iodide content is further evidence of the defect in iodide transport characteristic of the "cold" thyroid nodules. During incubation with TSH, there was a small but statistically significant (P < 0.05) decrease in the content of iodide in the normal slices but no significant effect on iodide content in the tumor tissue. The mean quantities of MIT and DIT formed in the normal tissues were two- to threefold greater than in the nodules. The MIT/DIT ratio in the normals was not significantly different from that in the nodules under control conditions. Incubation with TSH resulted in a 86 and 93% increase in the incorporation of iodine into MIT and DIT in the normal tissue and a 68 and 128% increase in MIT and DIT formation in the nodular tissue. However, one adenoma (Sp) and the one carcinoma (Le) did not respond to TSH during in vitro stimulation. As in the studies using TCA precipitation to measure iodide organification (Table I), less iodide was organically bound in the tissue from the "cold" nodules. However the response to TSH was similar in the nodular and paranodular tissue in the two groups.

Since iodothyronine formation was difficult to detect in vitro, the T4 content of both the normal and nodular tissue was examined to determine whether iodothyronine synthesis was also decreased in vivo. The mean T4 content of the four samples of normal tissue was approximately eight times that of the "cold" nodules (Table III). This would suggest that despite the only moderate decrease in iodide organification demonstrated in vitro in these "cold" nodules, there was considerably less T4 formed in vivo.

To determine the effects of inhibition of iodide transport on organization in tissues from normal thyroid, comparative studies were done in the presence and absence of sodium perchlorate, a competitive inhibitor of iodide transport. Incubation with perchlorate markedly reduced the T/M ratio in comparison with control slices, although it did not reach a level of 1.0 as would have been anticipated (Table IV). The explanation for this is unknown but it is unlikely that there is organically bound iodine since even in the absence of methimazole, over 90% of the label is in the form of inorganic iodide. This reduction of iodide transport was associated with a decrease in basal iodide organification to 45% of the control level, but the response to TSH was unaltered. Thus, these results are qualitatively similar to the alterations in iodine metabolism that were observed in the nodules.

**TABLE III**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Normal</th>
<th>Nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg T4/g wet wt (mean ± SEM)</td>
<td></td>
</tr>
<tr>
<td>Ha</td>
<td>114±8</td>
<td>23±4</td>
</tr>
<tr>
<td>Sp</td>
<td>195±42</td>
<td>5±1</td>
</tr>
<tr>
<td>An</td>
<td>134±32</td>
<td>24±3</td>
</tr>
<tr>
<td>Le</td>
<td>153±9</td>
<td>20±9</td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td>149±17</td>
<td>18±4</td>
</tr>
</tbody>
</table>

Four to six specimens were used for each determination.

| TABLE IV |

<table>
<thead>
<tr>
<th>Patient</th>
<th>Perchlorate</th>
<th>Iodide transport, T/M</th>
<th>Iodide organification Basal</th>
<th>Iodide organification TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg I/mg DNA</td>
<td></td>
</tr>
<tr>
<td>Un</td>
<td></td>
<td>8.0</td>
<td>47±3</td>
<td>96±1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.0</td>
<td>25±3</td>
<td>42±1</td>
</tr>
<tr>
<td>Ap</td>
<td></td>
<td>4.0</td>
<td>58±6</td>
<td>115±6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.7</td>
<td>27±3</td>
<td>33±1</td>
</tr>
<tr>
<td>Le</td>
<td></td>
<td>12.0</td>
<td>267±9</td>
<td>484±70</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.3</td>
<td>111±4</td>
<td>220±34</td>
</tr>
<tr>
<td>Fe</td>
<td></td>
<td>18.0</td>
<td>38±3</td>
<td>103±5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3.5</td>
<td>14±5</td>
<td>41±3</td>
</tr>
<tr>
<td>Mean ±SEM Perchlorate, %</td>
<td>22±8</td>
<td>45±3</td>
<td>40±4</td>
<td></td>
</tr>
</tbody>
</table>

The iodide transport and organification were determined in triplicate. The concentration of perchlorate was 10 mM and TSH was 50 mU/ml.

Iodide Transport Defect in Nonfunctioning Thyroid Nodules
The ATPase trapping has been elucidated, the process is energy-dependent (15). A role for ouabain-sensitive Na⁺-K⁺-activated ATPase in this process has also been suggested (16), though a recent study did not find a stoichiometric relationship between these two processes (17). No consistent difference between ouabain-sensitive Na⁺-K⁺-activated ATPase activity was found in the nonfunctioning and normal thyroid tissue (Table V).

Although the biochemical steps involved in iodide trapping have not been elucidated, the process is energy-dependent (15). A role for ouabain-sensitive Na⁺-K⁺-activated ATPase in this process has also been suggested (16), though a recent study did not find a stoichiometric relationship between these two processes (17). No consistent difference between ouabain-sensitive Na⁺-K⁺-activated ATPase activity was found in the nonfunctioning and normal thyroid tissue (Table V).

The total and Mg⁺⁺-activated ATPase was actually significantly higher in the tissue from the nodules compared with normal. Furthermore, ATP concentrations were similar in the nodular and paranodular tissue.

As in the series of patients reported previously, basal adenylate cyclase activity was significantly higher in the benign "cold" nodules and was as responsive to TSH as that of the normal tissue (Table VI). There was no significant difference in basal adenylate cyclase activity of normal and abnormal thyroid tissue.

The ATPase assays were done in duplicate and those for ATP in triplicate.

### Table V

ATPase and ATP Concentration in "Cold" Thyroid Adenomas and Adjacent Normal Thyroid

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Total</th>
<th>Ouabain-sensitive Na⁺-K⁺-activated</th>
<th>Mg⁺⁺-activated</th>
<th>ATP concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ne</td>
<td>Follicular adenoma</td>
<td>2.9</td>
<td>0.6</td>
<td>1.0</td>
<td>52±4</td>
</tr>
<tr>
<td>Tu</td>
<td>Follicular adenoma</td>
<td>1.7</td>
<td>0.0</td>
<td>0.7</td>
<td>131±18</td>
</tr>
<tr>
<td>Ch</td>
<td>Embryonal adenoma</td>
<td>2.8</td>
<td>0.3</td>
<td>0.7</td>
<td>229±21</td>
</tr>
<tr>
<td>Mo</td>
<td>Follicular adenoma</td>
<td>5.1</td>
<td>1.3</td>
<td>1.0</td>
<td>390±15</td>
</tr>
<tr>
<td>Un</td>
<td>Follicular adenoma</td>
<td>3.5</td>
<td>1.0</td>
<td>0.6</td>
<td>58±3</td>
</tr>
</tbody>
</table>

Mean ±SEM: 3.2±0.6 7.0±1.9 0.6±0.2 0.9±0.3 0.9±0.1 1.8±0.4 154±71 204±72

### Table VI

Adenylate Cyclase Activity in Homogenates

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Normal</th>
<th>TSH</th>
<th>Normal</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ne</td>
<td>Follicular adenoma</td>
<td>0.16±0.02</td>
<td>0.3±0.03</td>
<td>0.5±0.02</td>
<td>3.2±0.03</td>
</tr>
<tr>
<td>Tu</td>
<td>Follicular adenoma</td>
<td>0.19±0.01</td>
<td>0.4±0.02</td>
<td>0.5±0.01</td>
<td>1.8±0.04</td>
</tr>
<tr>
<td>Ch</td>
<td>Embryonal adenoma</td>
<td>0.6±0.02</td>
<td>1.3±0.02</td>
<td>1.0±0.02</td>
<td>2.4±0.01</td>
</tr>
<tr>
<td>Mo</td>
<td>Follicular adenoma</td>
<td>0.4±0.03</td>
<td>1.5±0.03</td>
<td>0.9±0.03</td>
<td>4.8±0.04</td>
</tr>
<tr>
<td>Un</td>
<td>Follicular adenoma</td>
<td>0.6±0.04</td>
<td>1.7±0.08</td>
<td>0.3±0.08</td>
<td>1.1±0.03</td>
</tr>
<tr>
<td>An</td>
<td>Follicular adenoma</td>
<td>0.2±0.01</td>
<td>0.9±0.04</td>
<td>0.17±0.01</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>Fl</td>
<td>Follicular adenoma</td>
<td>0.5±0.05</td>
<td>1.1±0.05</td>
<td>0.7±0.08</td>
<td>2.4±0.02</td>
</tr>
<tr>
<td>Am</td>
<td>Follicular adenoma</td>
<td>0.2±0.03</td>
<td>0.6±0.04</td>
<td>0.5±0.11</td>
<td>2.0±0.03</td>
</tr>
<tr>
<td>Om</td>
<td>Hürthle cell adenoma</td>
<td>0.15±0.01</td>
<td>0.3±0.02</td>
<td>0.3±0.01</td>
<td>1.3±0.03</td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td></td>
<td>0.3±0.06</td>
<td>0.8±0.2</td>
<td>0.6±0.1</td>
<td>2.3±0.4</td>
</tr>
</tbody>
</table>

The values are the mean ±SEM of triplicate determinations. TSH concentration was 1 mU/ml. The mean DNA content of normal thyroid and tumor was 3.4±0.4 µg/mg wet wt (range 1.4–6.5) and 4.2±0.6 µg/mg wet wt (range 1.7–8.6), respectively.

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activity between the benign and malignant nodules. In 2 of the 4 malignant nodules, TSH stimulated adenylate cyclase activity less than 50% whereas such stimulation exceeded 75% in all 10 of the benign nodules. Although the mean data suggests that the adenylate cyclase activity in the malignant nodules was as responsive to TSH as in the benign lesions, the value is distorted by the results obtained in patient Maf.

DISCUSSION

The above data indicate that tissue slices obtained from “cold” thyroid nodules do not concentrate iodide in vitro. However, despite the defect in iodide transport, the organification of iodine and, in a smaller series, the rate of synthesis of MIT and DIT were only moderately slower in the nodules than in the surrounding normal tissue. The moderate reduction in the quantity of iodine organified in these tissues probably reflects decreased uptake of iodide from the media during in vitro incubation. Inhibition of transport in normal human thyroid with perchlorate decreased the basal rate of organification to 45% of control, but the process was still stimulated normally by TSH. Similar observations in isolated thyroid cells have been reported by Tong (18). However, since these nodules are “cold” by in vivo radioiodine scan, the amounts of iodide organified in vivo must be considerably less than in the surrounding normal tissue. The low T4 content of these nodules also suggests an in vivo defect in organification. The apparent discrepancy between the in vivo and in vitro observations is explained if one postulates that in vivo iodide transport is rate limiting for the synthesis of thyroid hormone whereas in vitro it is not. Evidence for this is seen in the high inorganic iodide content of the normal thyroid tissues incubated for 90 min. This component accounts for over 90% of the radioactivity present in such tissues. Thus, an inability to concentrate iodide appears to be the primary defect in iodine metabolism in the “cold” nodules.

Whereas these “cold” nodules and those reported by DeGroot (7) have all shown decreased iodide transport, both benign and malignant tumors have been described in which the ability to concentrate iodide appeared to be preserved although the organification of radioiodine by these tumors could not be demonstrated (19–21). Thus, since it would appear to be generally true that “cold” thyroid nodules are “cold” because they cannot concentrate radioiodine, transport ability may be preserved and the capacity for organification lost in some thyroid tumors.

Although it has generally been well accepted that thyroid carcinomas are less efficient than normal thyroid in concentrating and organifying iodide (22), there was no consistent difference between organifica-
tion in benign or malignant tissue. However, even though the number of cases is small, there was a striking difference in the response of organification to TSH in these two tissues. TSH augmented organification in slices from normal thyroid and benign “cold” nodules to about the same extent. In contrast, TSH did not stimulate organification in three of the four carcinomas in which it was studied, and the response in the fourth was small. A decrease in responsiveness to TSH was also suggested by the results obtained measuring adenylate cyclase activity. Although such data could be explained by decreased binding of TSH to the cells of some thyroid carcinomas, other possibilities exist.

The cause of the defective iodide transport in “cold” nodules is unknown. Since the biochemical basis for concentration of iodide in normal thyroid tissue has not been elucidated, characterization of the defect in the “cold” nodules is difficult. The role of an ouabain-sensitive, Na+-K+-activated ATPase in trapping is controversial (16, 17). Although inhibition of this enzyme by ouabain inhibited transport, the studies of Brunberg and Halmi failed to demonstrate a stoichiometric relationship between enzyme activity and iodide transport in vivo (17). Even if an ouabain-sensitive Na+-K+-activated ATPase is intimately associated with iodide accumulation, its activity was certainly not diminished in nodule tissue. Since the assays were done on whole homogenates, they do not provide any information concerning enzyme activities in different compartments of the cell. In general, ATPase is a membrane-associated enzyme (23). Since the fraction of total or ouabain-sensitive, cation-activated ATPase contained in the plasma membrane fraction is unknown, it is possible that changes in this fraction would be missed when the whole homogenate is assayed.

Iodide transport is energy-dependent (15), and the defect in the nodules could reflect an abnormality in production or coupling of energy for this process. An abnormality in energy production seems most unlikely since ATP concentrations in the nodules were not reduced (Table V). Furthermore, 35P incorporation into phospholipids (24) and colloid droplet formation (25), both energy-requiring processes, was not diminished in slices from the “cold” nodules (1). Although the concentrating defect could reflect a block in the utilization of ATP for this process, it is also possible that the membrane carrier for iodide transport is deficient. The chemical nature of this proposed carrier is not known, although involvement of phospholipids has been suggested (26). Deletion of such a substance in neoplastic tissue would not be unique since malignant cells have sometimes been characterized by absence of enzymes (27).
Pochin has emphasized that the metabolic defects, which have been described in thyroid carcinomas, are similar to the defects that have been observed in patients with congenital goiter (22). The present results would extend this concept to include benign thyroid tumors as well. It is hoped that continued study of such "nonfunctioning" tissues will provide further insights into the biochemical processes involved in normal thyroid tissue.

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