Serum Triiodothyronine Concentration in the Iodine-deficient Rat

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ABSTRACT

A low iodine diet fed to rats resulted in decreased absolute amounts of circulating triiodothyronine and protein-bound iodine (PBI), but an increase in the triiodothyronine:protein-bound iodine ratio. Since baseline protein-bound iodine values were high, it is possible the “normal” diet fed was in fact a high iodine one. In this event, the fall in protein-bound iodine with the low iodine diet would be greater than that in thyroxine concentration. The relative increase in triiodothyronine compared to PBI concentration with the low iodine diet could result from preferential synthesis and secretion of triiodothyronine by the iodine-deficient thyroid; or could be due to accelerated removal of thyroxine due to increased peripheral utilization. Either change could keep body metabolism normal or nearly so over an extended period of iodine want.

An extensive literature documents the fact that the thyroid gland preferentially synthesizes and releases labelled triiodothyronine in the face of a lowered iodine intake compared to normal. However, there is little information concerning the effect of iodine deficiency on the circulating concentration of one of the two thyroid hormones, triiodothyronine. The subject has been recently reviewed (Greer et al., '68).

Determination of triiodothyronine in plasma has proven difficult in view of the low concentrations involved and the high concentrations of thyroxine which coexist. By the use of competitive protein-binding displacement as an endpoint, a practicable method was devised (Nauman et al., '67) and later adapted to resin sponges (Sterling et al., '69). However, both methods employ paper chromatography to separate the iodothyronines prior to quantification of the triiodothyronine. Recently, evidence has been elicited that paper chromatography deiodinates thyroxine to triiodothyronine, about 1% of the former being so converted (Fisher and Dussault, '71; Larsen, '71). In view of the large concentration of thyroxine relative to triiodothyronine in serum, the error in triiodothyronine estimation thus becomes large. Equally, although gas chromatography has been employed for serum triiodothyronine determination, the results have not been reassuring; from its introduction to the present time the method has given a wide variety of mean normal values. Most recently, radioimmunoassay has been introduced for triiodothyronine determination (Brown et al., '70) but still shows wide variation in normal values among laboratories (Gharib et al., '71; Mitsuma et al., '71; Lieblich and Utiger, '71; Chopra et al., '71). The present experiments were designed to study the effect of low iodine diet on serum triiodothyronine concentrations of the rat through use of a radioimmunoassay procedure.

MATERIALS AND METHODS

Charles River male rats weighing 200-250 gm at the start of the experiment were fed either Rockland “normal” diet or the Remington low iodine diet (General Biochemicals) for four to five weeks. The rats were then exsanguinated by cardiac puncture. Serum was frozen and an aliquot used for triiodothyronine assay.

Radioimmunoassay procedure. The radioimmunoassay method to determine triiodothyronine is as yet unpublished (Rubenstein et al., '71, '72). Rabbits were immunized with a triiodothyronine album
min conjugate produced by coupling the hormone to the protein by the use of carbodiimide (Goodfriend et al., '64). Serum proteins were denatured by the addition of 95% ethanol with about 92% recovery of triiodothyronine in the supernate. The supernate was dried, taken up in mildly alkaline buffer and reacted with the specific antiserum to triiodothyronine to which was added 125I-labelled triiodothyronine. After 48 hours incubation at 4°C to permit protein-binding displacement, anti-rabbit globulin serum was added to precipitate bound triiodothyronine. The amount of stable triiodothyronine originally present in the serum was estimated from a standard curve run contemporaneously. Serum protein-bound iodine concentrations were determined by a modification of the method of Zak et al. ("52).

OBSERVATIONS

In two experiments, serum from two or three rats had to be pooled to give sufficient serum for triiodothyronine assay. In one experiment, individual rat serums were tested. A decrease in PBI and triiodothyronine concentrations occurred in the iodine-deficient rats, but with a corresponding increase in triiodothyronine:PBI ratio (table 1). The range of values within each group is small and the direction of change is consistent.

DISCUSSION

The sharp drop in serum protein-bound iodine reported to follow feeding of a low iodine diet (Goldman et al., '66) was duplicated in our experiments. There was also a decrease in serum total triiodothyronine concentration. However, the triiodothyronine:PBI ratio became significantly increased, as a result of the smaller decrement in the triiodothyronine concentration compared to that of the protein-bound iodine. It should be noted that the protein-bound iodine concentrations found in the present experiments are unusually high for the rat and suggest that the normal diet was indeed a high iodine one (Rosenberg et al., '64). With iodine excess, iodide binds to albumin and the iodide-albumin complex precipitates as protein-bound iodine. Thus thyroxine concentrations are not accurately reflected under these conditions.

Turnover of iodine within the normal thyroid appears to be extremely slow (Loweinstein and Wollman, '67). However, there is a rapid component to the turnover, related to thyroglobulin deposition in, and removal from, the alveoli. This has given rise to the "last-come, first-served" hy-

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>No. of rats</th>
<th>Triiodothyronine (T3)</th>
<th>Protein-bound iodine (PBI)</th>
<th>T3:PBI ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Pool</td>
<td>8</td>
<td>0.058 ± 0.039</td>
<td>18.6 ± 4.6</td>
<td>28.3</td>
</tr>
<tr>
<td>Low iodine</td>
<td>5</td>
<td>0.057 ± 0.019</td>
<td>9.2 ± 2.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Normal Single</td>
<td>4</td>
<td>0.053 ± 0.019</td>
<td>1.2 ± 0.2</td>
<td>28.3</td>
</tr>
<tr>
<td>Low iodine Single</td>
<td>5</td>
<td>0.056 ± 0.019</td>
<td>9.2 ± 2.8</td>
<td>6.2</td>
</tr>
</tbody>
</table>

1 Mean of three pools of serum from three, three, and two rats.
2 Serum volume insufficient for triiodothyronine determination in two of the five.

2 Material of high specific activity was obtained through the courtesy of Dr. John J. Coupal, Abbott Laboratories, North Chicago, Illinois.
pothesis (Schneider, '63). In rats main-
tained for four to five weeks on low iodine
diet, the thyroid contains extremely low
absolute amounts of both triiodothyronine
and thyroxine as determined by gas chro-
matography (Volpert, '71). If the gland
of rats fed a low iodine diet synthesizes
triiodothyronine preferentially over thy-
roxine and secretes both as soon as formed,
then the gland would release into the circula-
tion, the triiodothyronine:thyroxine ratio
would become increased over normal. This is suggested
by the triiodothyronine:protein-bound io-
dine ratios of the present experiments.8

Alternatively, it is possible that the pro-
duction rates of triiodothyronine and thy-
roxine though decreased remain normal
relative to each other, but that the concen-
tration of circulating thyroxine is reduced to a greater extent than that of triiodo-
thyronine, possibly because utilization of thyroxine becomes increased peripherally as a consequence of the low iodine diet. This would resemble the increased thyrox-
line metabolism by the liver after admin-
istration of phenobarbital (Oppenheimer,
'68) or phenylhydantoin (Larsen, '70).

With respect to iodine deficiency in man,
few data are available concerning the ef-
effect of a low iodine diet on the serum con-
centration of triiodothyronine. However, it has been claimed in man that the absolute plasma triiodothyronine concentration be-
comes increased (Mitsuma et al., '71). The other scattered studies reporting in-
creased serum triiodothyronine concentra-
tions in euthyroid individuals were made in patients with sporadic non toxic nodular
goiter and nontoxic diffuse goiter (Werner
et al., '60; Radichevich and Werner, '68;
Shimaoka, '63), and presumably are not
related to the events in iodine deficiency.

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8This conclusion has been confirmed in a small
group of rats: normal mean T4 concentration 2.8 ±
0.53 (S.D.) μg/dl; low iodine 1.5 ± 0.08 μg/dl.


