Labeled Iodinated Compounds in Tumors and Tissues of C3H Mice after the Injection of I\(^{131}\)-labeled Triiodothyronine*

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SUMMARY

Tumor-bearing C3H mice were injected with I\(^{131}\)-labeled triiodothyronine and sacrificed at 1, 4, and 8 hours later. The I\(^{131}\)-labeled compounds in mammary tumors, mammary gland, muscle, and plasma were separated by paper chromatography.

During the course of this work a previously unidentified contaminant, in the triiodothyronine solution, was identified as 3,3'-diiodothyronine on paper chromatographic evidence.

Triiodothyronine accounted for most of the organic I\(^{131}\) in the tissues and was accompanied by various amounts of iodide. In addition, a compound tentatively identified as 3,3'-diiodothyronine accounted for 10 per cent of the activity in plasma and tumors, and for 4–5 per cent in muscle and mammary gland.

In the previous study (1) the distribution of I\(^{131}\)-labeled triiodothyronine in C3H mice bearing mammary tumors was determined. Labeled compounds, precipitable with trichloroacetic acid, reached a concentration 2–4 times as large in tumors as in plasma. Preliminary chromatographic analysis indicated that triiodothyronine accounted for a major part of the activity. The following is a report on the paper chromatographic separation and identification of the radioactive compounds in tumors, mammary gland, muscle, and plasma after the intravenous injection of I\(^{131}\)-labeled triiodothyronine.

MATERIALS AND METHODS

Nine C3H mice with mammary tumors, both spontaneous and H\(_{2}\)712 tumors, or the latter alone, were given injections of 0.1 cc. saline containing the I\(^{131}\)-labeled L-3,5,3'-triiodothyronine. The details of these procedures were the same as in the previous study (1). Separate chromatographic analyses were carried out on tumors, plasma, and other tissues from each mouse. Quantitative results are reported on tissues from three representative mice.

For paper chromatographic analyses, thick water homogenates were prepared in glass homogenizers immersed in ice water. The homogenates were extracted with 95 per cent ethanol, and the extracts were concentrated under vacuum, at room temperature, and then kept at less than 0° C. while in storage.

Paper chromatographic analyses were generally made with 4:1 n-butanol:dioxane and/or tertiary amyl alcohol, both equilibrated with 2 N ammonia. For the identification of an unknown I\(^{131}\)-labeled contaminant in the triiodothyronine solution, additional paper chromatograms were made with tertiary amyl alcohol equilibrated with 6 N ammonia, and methanol: ammonium acetate solvents (5). Before the preparation of all solvents, the water phase was made 0.1 n in sodium thiosulfate to prevent any oxidation of iodide during chromatography. Ascending chromatography was employed, and development of the chromatograms was continued for 24 hours after the solvent front had reached the top of the paper. This resulted in good resolution in the thyroxine and triiodothyronine region, and allowed 3,3'-diiodothyronine to be distinguished from thyroxine as well as from tetraiodothyroacetic acid.

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On paper chromatograms, constant amounts of carriers of triiodothyronine, iodide, and sometimes thyroxine were applied. For the identification of the I\(^{131}\)-labeled contaminant in the triiodothyronine solution, carriers of monoiodothyronine, 3,3\(^{\prime}\)-diiodothyronine, 3,5\(^{\prime}\)-diiodothyronine, and the acetic and propionic analogs of thyroxine and triiodothyronine were employed. These compounds were obtained through the courtesy of Dr. R. L. Kroc, of the Warner and Chilcott Laboratories.

For the visualization of the active compounds on paper chromatograms, contact radioautograms were prepared with Kodak no-screen x-ray film. Single-dimensional chromatograms were, in addition, scanned for activity under a gas flow counter. The stable carriers of organic compounds were sprayed with ninhydrin or diazotized sulfanilic acid, and iodide was developed with palladium chloride (2). With the guidance of the radioautograms and the carriers, the paper chromatograms were cut into suitable pieces and assayed in a well counter.

By use of two-dimensional chromatography, the recovery of I\(^{131}\)-labeled triiodothyronine, after paper chromatographic separation, was determined. In the first dimension, with n-butanol: dioxane, triiodothyronine was separated from its contaminants. Then n-butanol:dioxane was used again for the second dimension and the distribution of the triiodothyronine activity determined.

It was found that 94 per cent had migrated as triiodothyronine, 1 per cent as iodide, 8 per cent as 3,3\(^{\prime}\)-diiodothyronine, 3,5\(^{\prime}\)-diiodothyronine, and the acetic and propionic analogs of thyroxine and triiodothyronine were employed. These compounds were obtained through the courtesy of Dr. R. L. Kroc, of the Warner and Chilcott Laboratories.

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It was found that 94 per cent had migrated as triiodothyronine, 1 per cent as iodide, 3 per cent with the front, 1 per cent was diffusely spread over the area between iodide and triiodothyronine, and 1 per cent over the rest of the paper. When tertiary amyl alcohol was used in the second dimension, the recovery of triiodothyronine was somewhat smaller.

RESULTS

In one experiment two mice bearing H2712J carcinoma were sacrificed at 1 and 8 hours after injection of 0.2 \(\mu\)g. of I\(^{131}\)-labeled triiodothyronine. Water homogenates of tumor and plasma were chromatographed in tertiary amyl alcohol with carriers of triiodothyronine, thyroxine, and iodide. The results showed that the major labeled compounds were triiodothyronine and iodide. In tumor tissue, at both 1 and 8 hours after injection, an additional unidentified compound which accounted for about 10 per cent of the activity was observed. It was located just in front of the thyroxine carrier. Control chromatograms of the injection solution demonstrated a similar compound accounting for 7.5 per cent of the injected activity. These unidentified compounds, with \(R_p\) values intermediate between thyroxine and triiodothyronine, were labeled U2. Such a compound had been observed earlier in triiodothyronine solutions, but due to lack of chromatographic resolution it was then thought to be thyroxine. The experiment was repeated with six mice sacrificed at 0.5, 1, and 4 hours. The presence of U2 in the H2712 tumor was confirmed, and it was also indicated in other tissues.

It was wondered if this compound could be of physiologic importance, and, therefore, identification was attempted. The U2 activity was eluted, with 0.5 N ammonia, from a single-dimensional chromatogram of the labeled triiodothyronine solution. When this solution was rechromatographed 60 per cent of the activity was recovered in the U2 area and the rest as iodide. Part of the same solution was added to 0.2 cc. of mouse plasma and precipitated with trichloroacetic acid, and 51 per cent of the activity remained with the plasma proteins. These evidences were taken to indicate that U2 was an organic compound. Some of the U2 solution was frozen and stored for 2 days, and then a new chromatogram was made. This time only 45 per cent of the activity was recovered in the U2 area. This demonstrated that under our conditions U2 was quite labile. When radioactive solutions of triiodothyronine, containing the contaminant U2, were chromatographed with carriers of a large number of thyroxine and triiodothyronine analogs, it was found that U2 corresponded exactly to 3,3\(^{\prime}\)-diiodothyronine. Figure 1 shows a radioautogram of a two-dimensional chromatogram. Similar correspondence was obtained when the solvent was tertiary amyl alcohol: 6 N ammonia or methanol: ammonium acetate.

Next, it was investigated whether the unidentified compound in the tumor tissue also was 3,3\(^{\prime}\)-diiodothyronine. One mouse, bearing simultaneously H2712 and spontaneous mammary tumors, was given 6 \(\mu\)g. of labeled triiodothyronine and sacrificed 4 hours later. Blood was taken with a chilled syringe and the tissues cooled on ice immediately after excision. Single-dimensional chromatograms were prepared at once of water homogenates. On the following day, single-dimensional chromatograms of ethanol extracts, and two-dimensional chromatograms of both homogenates and extracts, were made. Carriers of 3,3\(^{\prime}\)-diiodothyronine, triiodothyronine, and iodide were used. Chart 1 shows scans of the single-dimensional chromatograms of the homogenates. The major peak corresponds, as before, to triiodo-
thyronine, and the smaller peaks to iodide and U2. The latter corresponds well with the 3,3'-diiodothyronine carrier. One other unidentified compound, between origin and iodide, can be seen in plasma. Table 1 gives the quantitative data obtained from these chromatograms and from that of muscle, which were made simultaneously. In some of the one-dimensional chromatograms prepared from ethanol extracts, and in all two-dimensional chromatograms, very little activity was found in the U2 area. Since the iodide fraction had increased by comparison with the first chromatograms, it was concluded that U2 had become deiodinated during the storage. Consequently, visible spots of U2 were not obtained in the two-dimensional radioautograms. A radioautogram of a typical two-dimensional chromatogram is shown in Figure 2. The two-dimensional chromatograms confirmed the identification of labeled iodide and triiodothyronine in these tissues.

**FIG. 1.**—Radioautogram of a two-dimensional chromatogram of an $^{131}$I labeled triiodothyronine solution. Solvents were n-butanol:dioxane (BDA) and tertiary amyl alcohol (TAA), both equilibrated with 2 N ammonia. Carriers are outlined with ink. $T_1$ = triiodothyronine; $T_2$ = 3,3'-diiodothyronine; $T_4$ = thyroxine; $I^-$ = iodide; $O$ = origin.

**FIG. 2.**—Radioautogram of a two-dimensional chromatogram from an ethanol extract of the spontaneous tumor. Solvents were n-butanol:dioxane (BDA) and tertiary amyl alcohol (TAA), both equilibrated with 2 N ammonia. Carriers are outlined with ink. $T_1$ = triiodothyronine; $T_2$ = 3,3'-diiodothyronine; $T_4$ = thyroxine; $I^-$ = iodide; $O$ = origin.

**CHART 1.**—Scans of chromatographic analyses of plasma, $H_2$71$^9$ tumor, spontaneous tumor, and mammary gland. $O$ = origin; $F$ = front; $T_1$ = triiodothyronine; $T_2$ = 3,3'-diiodothyronine; $I^-$ = iodide.
DISCUSSION

The chromatographic analyses showed that the major I\(^{131}\)-labeled compound in all tissues at 1, 4, and 8 hours after injection was triiodothyronine accompanied by smaller amounts of iodide. In addition, another compound tentatively identified as 3,3'-diiodothyronine was observed. A contaminant in the labeled triiodothyronine solution was also identified as 3,3'-diiodothyronine, based on correspondence in four chromatographic solvent systems.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Per cent of I(^{131}) on Paper Chromatograms in Areas Identified by Carriers as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iodide</td>
</tr>
<tr>
<td>Plasma</td>
<td>18.4</td>
</tr>
<tr>
<td>Spontaneous tumor</td>
<td>10.2</td>
</tr>
<tr>
<td>H2712 tumor</td>
<td>4.9</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>4.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.6</td>
</tr>
<tr>
<td>Injection solution</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* T\(_2\) = 3,3'-diiodothyronine.
† T\(_3\) = 3,5,3'-triiodothyronine.

On trichloroacetic acid precipitation at least 85 per cent of 3,3'-diiodothyronine was precipitated. Therefore, the organic I\(^{131}\), obtained with trichloroacetic acid fractionation in the previous study, probably included most of the 3,3'-diiodothyronine that was present. Here it was found that diiodothyronine accounted for from 4 per cent of the I\(^{131}\) in muscle up to about 10 per cent in tumor and plasma.

The 3,3'-diiodothyronine found in the tissues could either have been a metabolite of triiodothyronine or have come from the injected quantity. Roche et al. believe that the formation of 3,3'-diiodothyronine is one pathway for the metabolism of triiodothyronine. These authors observed diiodothyronine in kidney and muscle of rats after the injection of triiodothyronine (3). In a report of the distribution of I\(^{131}\)-labeled diiodothyronine in rats, it was shown to be rapidly metabolized and to reach concentrations in tissues that were much smaller than that in plasma (4). In our work, the concentrations in tumors were about equal to that in plasma, and, therefore, it seems possible that it was formed by deiodination of triiodothyronine. Whether the 3,3'-diiodothyronine reached the tissues from the injection solution or was formed by deiodination of triiodothyronine, the relatively large concentration in mammary tumors is interesting and may be significant, especially since it also has been shown by Roche et al. that 3,3'-diiodothyronine has thyroid hormone activity (6) and is normally present in plasma (5).

REFERENCES

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