Defective Thyroglobulin Synthesis in an Experimental Rat Thyroid Tumor

LACK OF MEMBRANE-BOUND SIALYLTRANSFERASE ACTIVITY

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SUMMARY

The incorporation of carbohydrates has been studied in vitro in the experimental rat thyroid tumor 1-1C2. The uptake of N-[14C]acetylmannosamine, a precursor of sialic acid, is less than 5% of that in normal thyroid gland; no label is found in 19 S thyroglobulin or its precursors either in the soluble or in the solubilized proteins. N-[14C]Acetylgalactosamine is incorporated into thyroglobulin and may explain the defect in thyroglobulin synthesis. N-[14C]Mannose and [14C]fucose are incorporated into almost normal rate and [14C]fucose, at a subnormal rate; they are present mainly in the particle-bound proteins as ~18 S thyroglobulin and its precursors.

Measurement of sialyltransferase activity showed that, with desialylated thyroglobulin as acceptor, the 22,000-105,000 X g pellet of tumor contains ~3% of normal sialyltransferase activity, and the soluble fraction contains ~25% of normal activity. Desialylated fetuin and orosomucoid are generally better acceptors than desialylated thyroglobulin for tumor sialyltransferase.

The findings indicate that defective thyroglobulin release and absence of incorporation of sialic acid into thyroglobulin coexist in this tumor. The very low activity of thyroglobulin-specific sialyltransferase activity in the tumor particulate fraction accounts for the failure of sialic acid incorporation into thyroglobulin and may explain the defect in thyroglobulin release.

The transplantable rat thyroid tumor (1), Wollman line 1-1C2, shows very little formation of soluble thyroglobulin; most of the protein immonochemically and structurally related to thyroglobulin remains bound to cell membranes (2, 3). Since thyroglobulin is a glycoprotein (4) and since glycoproteins are generally extracellular products of secretion (5, 6) it was considered that a defect in carbohydrate incorporation into the oligosaccharide chains of thyroglobulin might be responsible for its incomplete maturation and release. A likely candidate for such a defect is N-acetylmuramid acid (sialic acid), a charged terminal residue of thyroglobulin oligosaccharides and known to be important in preventing tissue uptake of circulating glycoproteins (7).

We recently showed (8) that normal thyroid in vitro incorporates N-acetylmannosamine (ManNAc) into thyroglobulin-bound sialic acid, and incorporates N-acetylgalactosamine (GalNAc) into thyroglobulin-bound GalNAc and, to a lesser extent, into sialic acid. Similar studies with tumor 1-1C2, reported here showed no formation of thyroglobulin-bound sialic acid from these precursors, whereas all other carbohydrates normally present in thyroglobulin were incorporated into tumor thyroglobulin. Further investigation showed that the tumor is deficient in membrane-bound sialyltransferase activity.

MATERIALS AND METHODS

The tumor line 1-1C2 was kindly supplied by Dr. S. Wollman of the National Cancer Institute (1) and was carried in our laboratory as previously described (2, 3). Over the course of time the function of this tumor has changed significantly. Radioiodine uptake decreased from ~35% of the dose per g at 18 hours to ~1% per g (3), and the proportion of labeled iodoprotein in the soluble fraction from ~20% of total radioiodine (2) to ~8% (3). At present, 30 to 60% of the soluble fraction is 19 S thyroglobulin. From 10 to 78% of the particulate fraction can be solubilized, and 10 to 90% of the solubilized iodoprotein is 19 S thyroglobulin.

N-[14C]Acetylmannosamine, specific activity 400 mCi per mmole, and N-[13C]acetylgalactosamine, specific activity 1.0 Ci per mmole, were purchased from Tracer Lab, N-acetyl-d-[1-14C]glucosamine, specific activity 417 mCi per mmole, and L-[1-14C]fucose, specific activity 56.2 mCi per mmole, from New England Nuclear; d-[1-14C]mannose, specific activity 180 mCi per mmole; and d-[1-14C]galactose, specific activity 400 mCi per mmole, from International Chemical and Nuclear Corp. L-[1-14C]Leucine, specific activity 310 mCi per mmole, was from Schwarz-Mann.

activity 254 mCi per millie from New England Nuclear. The unlabeled carbohydrates were from Sigma except N-acetyl-D-galactose which was from Nutritional Biochemicals Corp. Aquasol was from New England Nuclear. Earle's solution was provided by the Media Unit of the National Institutes of Health. NCS solubilizer was from Amersham-Searle, Protosol from New England Nuclear. Digitonin was from Fisher Scientific Co. and was prepared as described previously (3); Triton X-100 was from Rohm and Haas; neuraminidase of Vibrio cholerae was from Schwarz-Mann; N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) was a gift of Dr. L. Van Lenten of this institute. The affinity column for neuraminidase (9) was provided by Dr. J. Hickman. N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)\(^1\) (10) was from Calbiochem.

**Incubation of Thyroid and Tumor**—Rats carrying tumors weighing 1.5 to 2 g were killed by guillotine and the tumors were removed, trimmed of connective tissue, and immersed in cold (4\(^\circ\)) 0.1 M Tris-HCl buffer, pH 7.4. The washed tumor was then cut into ~200 mg slices (\(\sim 5 \times 2 \times 3 \text{ mm}\)), immersed in 3 ml of Earle's solution which had been prepared for 20 min with \(\text{O}_2/\text{CO}_2 (95:5)\), and preincubated for 20 min at 37\(^\circ\) as described (8). (Because the tumor was very friable, thin slices could not be made uniformly and tended to disintegrate during incubation.) After the incubation, 50 \(\mu\)g of labeled carbohydrate, 25 \(\mu\)Ci of \([\text{PC}]\)lucine or 50 \(\mu\)Ci of \([\text{3H}]\)labeled, were added, and incubation at 37\(^\circ\) in a shaking water bath was continued for 1, 3, 6, and 12 hours. Each flask was gassed every 15 min with \(\text{O}_2/\text{CO}_2\). In some experiments the slices were directly homogenized (see below) in 3 ml of Earle's solution and the homogenate was incubated as above. In each experiment a parallel incubation of normal rat thyroid gland (pooled hemiblasts, 20 mg per flask) was performed as a control.

**Preparation of Soluble and Solubilized Proteins**—The homogenization and differential centrifugation were performed as previously described (3, 8). Briefly, after incubation the tumor slices were taken, washed with cold 0.1 M Tris-HCl, pH 7.4, and homogenized with 2 ml of 0.1 M Tris HCl, 0.25 M sucrose, pH 7.4, in a Potter-Elvehjem homogenizer with a Teflon pestle at 1,100 rpm, three strokes in ice. The homogenate was centrifuged at 105,000 \(\times g\) for 1 hour and the supernatant, \(S_{10}\), was dialyzed for 2 days at 4\(^\circ\) against Tris-HCl buffer, pH 7.4, and homogenized with 2 ml of 0.1 M Tris HCl, 0.25 M sucrose, pH 7.4, in a Potter-Elvehjem homogenizer with a Teflon pestle at 1,100 rpm, three strokes in ice. The homogenate was centrifuged at 105,000 \(\times g\) for 1 hour and the supernatant, \(S_{10}\), was dialyzed against Tris-HCl buffer for 2 days and the pellet was dissolved in 1 ml of NCS or Protosol. Aliquots of \(S_{10}\) and pellets were counted in a Nuclear Chicago Mark I liquid scintillation counter in 15 ml of Aquasol, shaken with 1 ml of distilled water.

Aliquots of the dialyzed soluble (\(S_1\)) and solubilized (\(S_2\)) proteins were precipitated with 95% ethanol or 10% trichloroacetic acid; the precipitate after washing was dissolved in 1 ml of distilled water added to 15 ml of Aquasol and counted in a scintillation counter (10).

**Carbohydrate Analysis**—Hydrolysis was performed on labeled thyroglobulin isolated by sucrose gradient ultracentrifugation (3, 11). For determination of sialic acid, the protein was hydrolyzed with 0.1 M \(\text{H}_2\text{SO}_4\) for 1 hour at 80\(^\circ\) in a sealed tube, and neutralized with 0.1 M \(\text{Ba(OH)}_2\); the precipitate was removed and the supernatant was dried under vacuum. The dried material was dissolved in 0.2 ml of distilled water and applied on a strip of Whatman No. 3MM filter paper, 5 \(\times 47 \text{ cm}\). For determination of GlnNAc, thyroglobulin was hydrolyzed with 4 N HCl for 6 hours at 100\(^\circ\) in a sealed tube. The hydrolysate was then dried at 50\(^\circ\) under vacuum, redissolved in \(\text{H}_2\text{O}\), and passed through a small column of Dowex 50-X4 (H\(^+\) form) (12, 13), eluted with 2 N HCl, and the eluate dried at 50\(^\circ\) under vacuum. The material, dissolved in water, was then acetylated and analyzed by paper electrophoresis in borate buffer on a strip of Whatman No. 3MM paper (8). The neutral sugars were hydrolyzed from thyroglobulin with 2 N \(\text{H}_2\text{SO}_4\) for 6 hours at 100\(^\circ\) in a sealed tube (14). The hydrolysate was passed through a column of Dowex as above, coupled to a column of Dowex 1-X8, 200 to 400 mesh (formate form), and eluted as described (8). The effluent was concentrated under vacuum and then applied to Whatman paper strips as above.

Chromatography was performed by the descending method. For sialic acid, the system was isomyl acetate-acetic acid-water (3:3:1) for 8 hours (15). For neutral sugars, the system was 1-butanol-ethanol-water (10:1:2) for 12 hours. After chromatography the strips were dried, cut into 2-cm segments, and extracted for 24 hours with 1 ml of distilled water. Each extract and paper segment was placed in 15 ml of Aquasol for scintillation counting. Standard sugars were run on parallel chromatogram strips and were detected by the silver nitrate method (16).

**Assay of Thyroglobulin Catabolism**—This assay was performed using 19 S thyroglobulin from normal Fisher rats labeled by incubating normal thyroid hemiblasts with \(N'-\text{[3H]acetylglucosamine (specific activity }1.5 \times 10^{5} \text{ net cpm per mg of protein)}\) (17). Thyroid tumor or normal thyroid gland was homogenized gently (see above) in 0.1 M Tris-HCl, 0.25 M sucrose, 0.001 M EDTA, pH 7.4. The homogenate was centrifuged at 800 \(\times g\) for 10 min at 4\(^\circ\) and the supernatant was then centrifuged at 22,000 \(\times g\) for 10 min at 4\(^\circ\); the pellets were suspended in the same buffer and recentrifuged at 800 \(\times g\) and 22,000 \(\times g\) as before. The 800–22,000 \(\times g\) pellet was suspended in 0.1 M potassium acetate buffer, pH 5.6. The equivalent of 40 mg of protein, measured by the Lowry method (18), was incubated with 0.25 mg of labeled 19 S thyroglobulin for 1, 3, and 6 hours at 37\(^\circ\). After incubation the tubes were centrifuged at 30,000 \(\times g\) for 10 min at 4\(^\circ\) and the supernatant was precipitated with 1.4 M \(\text{NH}_4\text{SO}_4\), and then by 1.8 M \(\text{NH}_4\text{HSO}_4\). The 1.4 to 1.8 M \(\text{NH}_4\text{HSO}_4\) fraction, the 1.8 M supernatant fraction, and the 30,000 \(\times g\) pellet were counted for radioactivity.

**Assay of Glycosyltransferases**—The tumor, or thyroids from normal Fisher rats weighing 250 g, were taken and slices of tumor or pooled thyroid hemiblasts weighing 250 mg were gently homogenized in 2 ml of 0.1 M Tris-HCl, 0.25 M sucrose, pH 7.4, as described (8), then centrifuged for 10 min at 10,000 \(\times g\) at 4\(^\circ\). The supernatant was centrifuged at 22,000 \(\times g\) for 10 min at 4\(^\circ\) and the resulting supernatant was centrifuged at 105,000 \(\times g\) for 1 hour at 4\(^\circ\). The 105,000 \(\times g\) pellet was suspended with a hand-driven, all glass homogenizer in 0.1 M HEPES buffer, pH 6.5, then preincubated with 0.5 \(\mu\)Ci of CMP-[3H]sialic acid at 37\(^\circ\) with 0.1% Triton X-100. After 15 min of preincubation 0.5 or 1 mg of desialylated thyroglobulin (with less than 20% of its normal content of sialic acid) dissolved in 0.1 M Tris-HCl, pH 7.4, was added and incubated for 0.25, 0.5, 1, 3, and 6 hours. The number of theoretical acceptor sites was determined as the difference in the sialic acid content before and after treatment with neuraminidase (see below) and is expressed as nanomoles of sialic acid. The final pH of the incubation mixture was 6.6 in a final

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\(^1\) The abbreviation used is: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
volume of 0.4 ml. After incubation the mixture was chilled in ice and centrifuged at 105,000 × g for 1 hour at 4°C; the supernatant was dialyzed for 2 days at 4°C against several changes of 0.1 M Tris-HCl buffer, pH 7.4. An aliquot of 0.1 ml was precipitated with 10% trichloroacetic acid and the precipitate counted in a liquid scintillation counter as described (8); other aliquots were analyzed by density gradient centrifugation and by paper chromatography after hydrolysis (8).

To assay galactosyltransferase in the 105,000 × g pellet, incubation was carried out as described for the sialyltransferase assay except for the following differences: 0.1 ml of Tris-HCl buffer, pH 7.4, or 0.05 M acetate buffer, pH 5.6, at 37°C for 48 hours; the incubation mixture contained at the start 5 mg of thyroglobulin per 25 units of neuraminidase per ml (19). More enzyme was added every 12 hours so that at the end of incubation a total of 100 units of enzyme had been added. After incubation the mixture was centrifuged at 3000 × g for 10 min at 4°C; the supernatant was precipitated between 1.4 and 1.8 M ammonium sulfate, dissolved in the above Tris-HCl buffer, dialyzed, and residual neuraminidase was removed by affinity chromatography (9).

To prepare desialylated thyroglobulin, rat, bovine, and hog 19 S thyroglobulin, purified by ammonium sulfate fractionation, Sephadex G-200 chromatography, and sucrose gradient ultracentrifugation, were separately incubated in 0.1 M Tris-HCl, pH 7.4, or 0.05 M acetate buffer, pH 5.6, at 37°C for 48 hours; the incubation mixture contained at the start 5 mg of thyroglobulin per 25 units of neuraminidase per ml (19). More enzyme was added every 12 hours so that at the end of incubation a total of 100 units of enzyme had been added. After incubation the mixture was centrifuged at 3000 × g for 10 min at 4°C; the supernatant was precipitated between 1.4 and 1.8 M ammonium sulfate, dissolved in the above Tris-HCl buffer, dialyzed, and residual neuraminidase was removed by affinity chromatography (9). Desialylated thyroglobulin was eluted with 0.05 M acetate buffer, pH 5.6. At least 80% of sialic acid in thyroglobulin was released, as measured by the thiobarbituric acid assay (20).

To prepare thyroglobulin from which galactose was removed, the desialylated rat thyroglobulin was dialyzed against 0.05 M citrate-phosphate buffer, pH 6.6, then incubated with β-galactosidase from D. pneumoniae (activity 2.5 μmoles per min per 100 μl) (21). Fifteen milligrams of desialylated rat thyroglobulin were incubated with 0.1 ml of β-galactosidase at 37°C for 24 hours in citrate phosphate buffer, pH 6.3. After incubation the mixture was passed through a DEAE-cellulose column (1.5 × 5 cm). Elution of free galactose was performed with 0.01 M ammonium acetate, pH 7.2. After a subsequent elution of β-galactosidase with 0.04 M phosphate buffer, pH 7.4, most of the thyroglobulin was eluted with 0.5 M NaCl, pH 6.9. The released galactose was measured with the galactose dehydrogenase assay (Boehringer Mannheim Corp.) ; this procedure showed that 60% of the galactose had been removed by the β-galactosidase.

Completely desialylated orosomucoid was a gift of Dr. G. Ashwell (22); fetuin was from Grand Island Biological Co. and was desialylated as described above for thyroglobulin. Its sialic acid content was <20% that of the starting preparation.

The protein content of the 105,000 × g pellet was determined by Lowry assay (18). The DNA content of the tissue was measured (kindly performed by J. Toal) with diphenylamine reagent with deoxyadenosine as standard (23). Sialic acid was determined by the thiobarbituric acid assay of Warren (on samples hydrolyzed in 0.1 M H2SO4 for 60 min at 80°C (20) and by the resorcinol reaction (24)). Immunoprecipitation with rabbit antiserum against rat 19 S thyroglobulin was performed as described previously (2).

RESULTS

Incorporation of Amino Acid and Iodine—Preliminary experiments with [14C]Leucine and [125I]—were carried out to see whether the tumor could synthesize thyroglobulin in the in vivo system employed and for comparison with the carbohydrate studies. Both [14C] and [125I] continued to accumulate over a 6-hour period, reaching 0.35% of the dose per g of tissue for [14C]leucine incubated with tumor slices, and 0.03% in homogenates; and for [125I] reaching 0.66% per g in tumor slices, and ~0.2% in homogenates.

The distribution of the label in soluble and solubilized proteins is presented in Table I as well as the immunoprecipitation results with anti-rat thyroglobulin serum. The data show that [14C]-leucine incorporation increases with time both in slices and homogenates. From the immunoprecipitation results it can be calculated that up to 9% of the total [14C] is immunologically related to thyroglobulin in S1 of slices and up to 18% in homogenates. From density gradient ultracentrifugation patterns it was seen that about 15% of the radioactivity in S1 was in an 18 S peak at 3 hours. In the solubilized protein (S2) after [14C]leucine, total [14C] accumulation was similar but the proportion in 18 S was about 2 times greater.

In the experiment with [125I] (Table I) the soluble proteins contained less radioactivity than in the case of [14C]leucine. However, a larger proportion was thyroglobulin-like, giving a value of 7% of total [125I] related to thyroglobulin in S1 of slices and 9% in homogenates. Density gradient ultracentrifugation showed that about 8% of S1 radioactivity was in the ~19 S zone at 1 hour. [125I] in the S2 fraction was low in homogenates, but in slices it increased to 39% of the total at 6 hours. Density gradient ultracentrifugation patterns showed ~30% of S2 radioactivity in the ~19 S zone at 1 hour.

These experiments showed that tumor slices and homogenates could form thyroglobulin from [14C]leucine and from [125I]—, and that most of the thyroglobulin-like protein, especially after [125I]—.
labeling, remained in the particulate fraction before digitonin treatment.

Incorporation of Carbohydrates—In Fig. 1 the uptake of labeled carbohydrates in tumor and normal thyroid is shown. Uptake of [3H]ManNAc in tumor was less than 5% that of the control in slices and about 8% in homogenate and showed no increase with time. The uptake of [3H]GlcNAc, although less than that of normal thyroid, showed a continuing increase with time up to 12 hours of incubation. [14C]Galactose uptake by tumor was no different from normal; for [14C]mannose and [14C]fucose the total uptake in the tumor was less than normal.

The distribution of all the carbohydrates studied in the soluble and solubilized fractions and their trichloroacetic acid and immunoprecipitates are shown in Table II. Ethanol precipitation gave results similar to trichloroacetic acid and are not reported in the table. There is in general an increase in the soluble proteins with time except for galactose in which the soluble fraction is maximal at 1 hour. The soluble fraction, however, never exceeded 36% of the total and the solubilized fraction always exceeded the soluble. The trichloroacetic acid precipitate and the immunoprecipitate ranged from 35 to 67% of the S₁ and S₂ fractions for all carbohydrates except ManNAc; in the latter, <15% was precipitable with trichloroacetic acid and none with antithyroglobulin serum.

The soluble and solubilized proteins were further analyzed by density gradient ultracentrifugation as shown in Figs. 2 and 3. All carbohydrates were incorporated into thyroglobulin except [3H]ManNAc; in more than 30 separate analyses, glucose incubated with [3H]ManNAc never showed a peak of labeled thyroglobulin or its 19 S precursor. It is to be noted that all carbohydrates showed a greater peak of 19 S in the solubilized than in the soluble proteins at all times studied.

To identify the labeled products (8, 25-27) the soluble and solubilized proteins were precipitated between 1.4 and 1.8 in ammonium sulfate, hydrolyzed with H₂SO₄ or HCl, and chromatographed.

### Table II

<table>
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<tr>
<th>Precursor</th>
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<th>Distribution of nondialyzable radioactivity²</th>
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<tr>
<td></td>
<td>hrs</td>
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<td>ManNAc</td>
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<td></td>
<td>3</td>
<td>22 ± 12</td>
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<tr>
<td></td>
<td>6</td>
<td>27 ± 11</td>
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<td>6</td>
<td>36</td>
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<td>GlcNAc</td>
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<tr>
<td></td>
<td>3</td>
<td>24 ± 12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>28 ± 10</td>
</tr>
</tbody>
</table>

* The values are the means of nine experiments ± S.D. for [3H]GlcNAc and [3H]ManNAc and of two experiments for the remaining carbohydrates. The values for trichloroacetic acid precipitate and immunoprecipitate are the mean of two experiments.

* TCA, trichloroacetic acid.

* Radioactivity precipitated by rabbit antiserum against rat 19 S thyroglobulin plus sheep antiserum against rabbit IgG.

![Fig. 1](http://www.jbc.org/) * In vitro uptake of labeled carbohydrates in normal thyroid (N) and in tumor (T). Values are the means of six experiments in slices incubated with GlcNAc, or three experiments in the other cases.
control experiment was performed: $^{131}$I-labeled thyroglobulin was desialized with *V. cholerae* neuraminidase, then homogenized with a tumor slice or with normal thyroid hemolobes, and subjected to differential centrifugation as described under "Materials and Methods." For normal thyroid, 7% of the desialized $^{131}$I-thyroglobulin remained with the pellet, and <20% for the tumor; this radioactivity was completely released with vigorous homogenization and detergent both for normal thyroid and for tumor.

**Glycosyltransferase Activity**—The 105,000 × g pellet from normal thyroid of Fisher rats showed sialyltransferase activity that was dependent on time, on the amount of enzyme and on the amount of substrate (Fig. 5). A similar pellet from the tumor revealed little or no sialyltransferase activity (Fig. 5). The largest quantity of tumor represented in Fig. 5B was 250 mg. A further test was performed using the 105,000 × g pellet obtained from 2 g of tumor. Under these conditions, sialyltransferase activity amounting to 3% of that in normal thyroid could be shown.

The sucrose gradient ultracentrifugation pattern of the reaction product with the pellet from normal thyroid is shown in Fig. 6; it can be seen that CMP-$^{14}$C-sialic acid was transferred mainly into 19 S thyroglobulin. Labeled 19 S thyroglobulin was also seen when the pellet from 2 g of tumor was used. Treatment of the labeled thyroglobulin with neuraminidase removed all the $^{14}$C from thyroglobulin, as determined by sucrose gradient ultracentrifugation.

The 105,000 × g supernatant was also tested by incubation with CMP-$^{14}$C-sialic acid and desialylated thyroglobulin. Soluble sialyltransferase activity was detected in normal thyroid gland and also in tumor as shown in Figs. 7 and 8. The soluble enzyme activity in normal thyroid was much less than that in the pellet on the basis of total protein content. In the case of the tumor, the soluble enzyme activity was slightly lower than normal when calculated on the basis of protein content of the soluble fraction. It was much lower than normal, however, when calculated on the basis of DNA content of the tissue from which the soluble fraction was prepared, since the DNA content of tumor was about 4 times that of normal thyroid.

To test the specificity of sialyltransferase in normal thyroid and tumor, desialylated fetuin and desialylated orosomucoid were incubated as above with the 105,000 × g pellet and the 105,000 × g supernatant. As shown in Table III, these proteins were acceptors for the sialyltransferase activity for the particulate and soluble fractions of normal thyroid gland and tumor. Desialylated fetuin was a comparable or poorer acceptor than desialylated thyroglobulin in tumor, both in supernatant and pellet fractions. Desialylated orosomucoid was a poorer acceptor than desialylated thyroglobulin in all fractions except the tumor pellet. The tumor pellet is deficient especially in the sialyltransferase activity specific for desialylated thyroglobulin.

![Fig. 2. Density gradient ultracentrifugation pattern of the soluble (S1) and solubilized (S2) proteins after incubation of tumor slices with [H]ManNAc.](http://www.jbc.org/)

The identification of the labeled product after digestion with 0.1 N H$_2$SO$_4$ at 80° for 1 hour and chromatography showed that in all of the cases studied, the only labeled product was sialic acid.

Since this tumor incorporates $^{14}$C-galactose into thyroglobulin at a normal rate, the membrane-bound galactosyltransferase was presumed to be present in the tumor. As shown in Table IV, the tumor pellet showed galactosyltransferase activity that was only slightly less than that found in the control using desialylated-degalactosylated thyroglobulin as acceptor. When desialylated-degalactosylated fetuin was used as exogenous acceptor the galactosyltransferase activity of the tumor was about 5% of that using thyroglobulin. The product of the incubation of desialyl-
FIG. 3. Density gradient ultracentrifugation pattern of the soluble (S₁) and solubilized (S₂) protein after incubation of tumor slices with labeled carbohydrates. The conditions are as in Fig. 2.

ated-degalactosylated thyroglobulin and UDP-[14C]galactose when examined by sucrose gradient centrifugation showed a protein with a sedimentation coefficient of 18.4 Sₐ, of which 96% was precipitated with an anti-rat thyroglobulin antiserum.

DISCUSSION

In the experimental rat thyroid tumor 1-1C2 it is evident that there is no detectable incorporation of N-[3H]acetylmannosamine either into thyroglobulin or its precursors in soluble or solubilized...
be partly explained by the failure of partial conversion of this label into sialic acid in tumor compared to normal gland (8). Since \(^{14}C\)galactose is present mainly in membrane-bound thyroglobulin while \(^{3}H\)ManNAc is not incorporated at all, and since the latter sugar was shown to be incorporated only in soluble thyroglobulin in normal gland (8) it appears that sialic acid may play a role in the release and solubilization of thyroglobulin.

The lack of incorporation of \(^{14}C\)ManNAc into thyroglobulin could result from defective transformation of this sugar to CMP-sialic acid (29), a lack of sialyltransferase (30, 31), lack of formation of an intermediate glycolipid as recently described for other transferases (32) or from the presence of an inhibitor of any of the previous steps. From the data presented here, it is evident that the rat tumor shows a defect in membrane-bound sialyltransferase activity.

The presence in the tumor of a soluble sialyltransferase capable of adding CMP-sialic acid to thyroglobulin was unexpected. It is possible that the thyroid contains soluble glycosyltransferases

proteins. \(^{14}C\)Galactose, on the other hand, is incorporated into tumor thyroglobulin at a normal rate suggesting that the formation of the oligosaccharide unit proceeds at an almost normal rate up to this penultimate sugar (28). The finding that \(N\) acetylglucosamine is incorporated at a lower rate than in normal could...
The conditions are as in Fig. 2.

Table III
Incorporation of CMP-[14C]sialic acid into various acceptor proteins

The incubation was carried out in 0.1 M HEPES buffer, pH 6.5, for the 105,000 × g pellet and 0.1 M Tris-HCl, pH 7.4, for the soluble protein in a final volume of 0.4 ml. The mixtures contained 4 μl of Triton X-100, 33 nmoles of theoretical acceptor sites (see text), 5 nmoles of CMP-[14C]sialic acid, 2.2 mg of protein for the soluble fraction, and 1.6 mg of protein for the pellet. The results are expressed as picomoles of [14C]sialic acid incorporated per mg of pellet protein and are means of two experiments for fetuin and orosomucoid.

<table>
<thead>
<tr>
<th>Time</th>
<th>Desialylated thyroglobulin</th>
<th>Desialylated fetuin</th>
<th>Desialylated orosomucoid</th>
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<tr>
<td>hrs</td>
<td>Thyroid 1-IC2 tumor</td>
<td>Thyroid 1-IC2 tumor</td>
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<td>1</td>
<td>4.2 0.2 1.8 0.9 0.6 0.7</td>
<td>3.0 3.0 1.4 1.4 1.4 1.4</td>
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<tr>
<td>3</td>
<td>6.8 0.3 1.7 1.0 0.8 0.8</td>
<td>3.0 3.0 1.4 1.4 1.4 1.4</td>
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<tr>
<td>105,000 × g pellet</td>
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</tr>
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<td>1.2 0.3 1.2 0.5 0.3 0.2</td>
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<tr>
<td>3</td>
<td>1.8 0.5 1.9 0.9 0.4 0.3</td>
<td>3.0 3.0 1.4 1.4 1.4 1.4</td>
<td></td>
</tr>
</tbody>
</table>

which have a different physiological role than the membrane-bound enzymes and which are not involved in thyroglobulin synthesis. This is consistent with the fact that glycosyltransferases are considered specific even though they show some activity with different acceptors as has been reported here and by others (33, 34).

Glycosyltransferases have been previously described in normal calf and sheep thyroid; most of the enzyme activity has been shown in the 78,000 × g pellet from calf (33, 35) or in a "Golgi"-rich fraction from sheep thyroid (26, 37). Glycosyltransferase activity in the soluble fraction has been described only for sialyltransferase and galactosyltransferase but no detailed data have been reported (33, 35, 38).

The significance of the finding of some incorporation of label into soluble thyroglobin with precursors other than [3H]ManNAc is not clear. A possible explanation could be that membrane-bound thyroglobulin is partially solubilized during the homogenization and fractionation procedures. The relatively smaller amount of [3H]-labeled thyroglobulin compared to [14C]- and [3H]-labeled thyroglobulin could be explained if only the more mature molecules can be labeled with iodine, perhaps because of their location in the cell. However, a defect in the iodinating system itself has not been excluded.

Decreased sialyltransferase activity necessary for the synthesis of gangliosides of the cell membranes has been recently described in a virus-transformed cell of hamsters (30) and in a mouse cell (31). The finding of defective membrane-bound sialyltransferase activity in the thyroid tumor studied here is the first evidence of a specific defect in the oligosaccharide chain synthesis of thyroglobulin. A search for similar defects in other thyroid diseases in which there is an increase in the "particulate" iodoproteins is warranted.

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REFERENCES
Defective Thyroglobulin Synthesis in an Experimental Rat Thyroid Tumor: LACK OF MEMBRANE-BOUND SIALYLTRANSFERASE ACTIVITY

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