# Studies on Mono- and Diiodohistidine

## II. CONGENITAL GOITROUS HYPOTHYROIDISM WITH THYROGLOBULIN DEFECT AND IODOHISTIDINE-RICH IODOALBUMIN PRODUCTION

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ABSTRACT Butanol-insoluble iodinated compounds in the urine of patients with congenital goiters have been generally regarded as iodopeptides. Monoiodohistidine (MIH) and diiodohistidine (DIH) were identified from the urine of four patients with congenital goitrous hypothyroidism. From radioiodine studies, 40–70% of the urinary radioactivity was in the iodide-free fraction from which about 40% was identified as MIH and DIH by crystallizations to a constant specific activity.

Iodotyrosines were simultaneously identified in the urine. However the presence of an iodotyrosine-deiodinase activity was demonstrated in the two removed goiters with a normal $K_m$ for MIT. In vivo iodotyrosine deiodination was normal for hypothyroid subjects.

No thyroglobulin was identified in the thyroids from these patients. The major iodoprotein was iodoalbumin which, after in vivo labeling, contained 84–89% of the total soluble protein radioactivity. The thyroxine content of the goiter iodoalbumins and other iodoproteins was extremely low.

Iodohistidines were identified in comparable proportions in the iodoalbumin and in the other iodoproteins isolated from each goiter. The average iodohistidine content of these proteins as crystallizable MIH and DIH was in the individual cases 15 and 4% of the in vivo incorporated radioiodine. DIH was identified in all iodoprotein fractions. The mean DIH/MIH ratios from the individual cases were 1.16 and 0.35. The corresponding DIT/MIT ratios were 3.19 and 1.45, respectively.

The major consequence of this thyroglobulin defect is the iodination of inappropriate proteins (mainly albumin) resulting in low yields of thyroxine and high yields of iodohistidines. Iodohistidines from the goiter iodoproteins were not deiodinated and, at least for MIH, were quantitatively excreted in the urine of these patients. From the MIH iodoalbumin content and the MIH urinary excretion, goiter iodoalbumin turnover estimates were made and, although elevated, could not maintain a normal thyroxine secretion.

The urinary excretion of iodohistidines easily demonstrated by column chromatography is offered as a test for detecting this variety of congenital goiter.

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## INTRODUCTION

The discovery of "peptide-linked iodotyrosines and iodothyronines" in the blood of a congenital goitrous hypothyroid patient led to the description of the "syndrome of congenital goiter with butanol-insoluble serum iodine" (1, 2). The circulating iodo-compound was identified, at least in part, as iodoalbumin (3–5). An iodoalbumin has been identified in similar goiters (6), and thyroglobulin has been reported as reduced (3–5, 7) or apparently absent (2, 8–11). From similar patients, other butanol-insoluble compounds were detected either in thyroid (2, 4, 7) or serum (1, 4, 8) hydrolysates, and in urines (2, 3, 10, 11). These compounds, constituting the butanol-insoluble iodine (BII)[1] moiety of the urine, were not identified. In general, the assumption was made that they were iodopeptides and that their presence in urine indicated defective iodoprotein breakdown. In an effort to identify these compounds from the BII fraction

[1] *Abbreviations used in this paper:* BII, butanol-insoluble iodine; PBI, protein-bound iodine; MIH, 4-monoiodo-L-histidine; DIH, 2,4-diiodo-L-histidine; MIT, 3-monoiodo-L-tyrosine; DIT, 3,5-diiodo-L-tyrosine; $T_4$, 3,5,3′,5′-tetraiodo-L-thyronine; $T_3$, 3,5,3′-triiodo-L-thyronine; Tg, thyroglobulin; RISA, radioiodinated human serum albumin; Ta, thyralbumin.

of the urine of similar patients we have found that they were dialysable, non-TCA precipitable, cationic, and that unlike iodopeptides they did not liberate iodotyrosines after conventional acid hydrolysis. They were not detected in the urine of normal men after the intravenous injection of either 3,5,3′,5′-tetraiodo-L-thyronine ($T_4$), 3,5,3′-triiodo-L-thyronine ($T_3$), 3-monoiodo-L-tyrosine (MIT), or 3,5-diiodo-L-tyrosine (DIT). Moreover they were not unique to this variety of congenital goiter since they were detected in the urine of hyperthyroid and thyroid cancer patients treated by $^{131}I$. Free monoiodohistidine (MIH) and diiodohistidine (DIH) were identified in the BII moiety of the urine by the methods presented in the preceding paper (12). Therefore the aims of the present study have been: (*a*) to identify and quantify the iodohistidines excreted in the urine of four patients suffering from this variety of congenital goitrous hypothyroidism; (*b*) to isolate and identify the iodoproteins from these goiters, with particular consideration given to the thyroglobulin (Tg) content; (*c*) to determine the iodohistidine and the thyroxine content of these iodoproteins; (*d*) to detect the presence or absence of an iodotyrosine deiodinase activity.

Evidence is presented here for the first time that: (*a*) MIH and DIH make up a large part of the BII fraction in the urine of this variety of congenital goiter patients; (*b*) the iodohistidine content of the iodoalbumin and of the minor unidentified iodoproteins produced by these goiters is similar, is higher than that of normal Tg, and is comparable with that of normal thyralbumin (Ta); (*c*) the true [$^{127}I$] thyroxine content of the iodoalbumin produced by these congenital goiters is extremely low; (*d*) estimation of the iodoalbumin turnover in the congenital goiters affords a biochemical and metabolic understanding of this disease.

## METHODS

*Patients.* Four patients with congenital goiters and a large urinary BII fraction were studied. Their main clinical and biological findings are summarized in Table I. The first three cases had not been previously treated. Case 4 had been on an unknown replacement medication from age 4–6. Treatment was discontinued because of restlessness, and she received no further treatment during the 6 yr before her admission. When first examined she was clearly a hypothyroid cretin as were cases 1 and 2. In contrast, case 3 had none of the clinical features of hypothyroidism but had been known to have goiter since birth. His low $T_4$-iodine and high thyroid-stimulating hormone (TSH) level were typical of hypothyroidism. Two cases were submitted to thyroid surgery after in vivo labeling of their goiters by oral doses of 300 $\mu$Ci of $^{125}I$ 7 days (case 1) and 6 days (case 3) and 100 $\mu$Ci of $^{131}I$ 3 h before their thyroidectomies.

*Iodoprotein isolation.* Goiter tissues from patients 1 and 3 weighed 77 and 592 g, respectively. They were divided into parts and those displaying the highest $^{125}I$ and $^{131}I$ radioactivities per gram were saved. From patient 1 a 3.1 g fragment, and from patient 3 a 5.2 g fragment were minced in 4 vol of 0.9% NaCl and kept 24 h at 4°C. The extracts were then centrifuged at 4°C for 1 h in a Spinco model L2 (rotor 50, Spinco Div., Beckman Instruments, Inc., Palo Alto, Calif.) ultracentrifuge at 105,000 *g*.

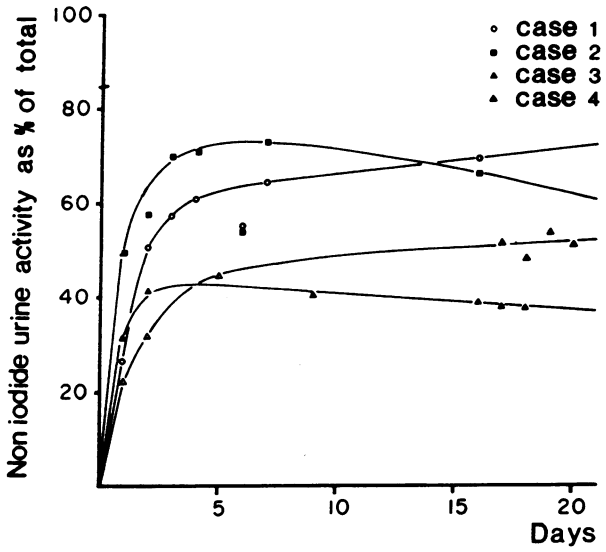


FIGURE 1 Iodide-free labeled fraction of the 24-h urines after radioiodine uptake by the four congenital goiters. This fraction, obtained by Dowex 1 column chromatography, and containing iodohistidines, reached a plateau near the 5th day.

The 105,000 *g* supernate, in a volume of 20 ml, was filtered at 4°C over a 4 × 100 cm column of Sepharose 6B-50 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden), prepared and eluted with 0.9% NaCl. Each 5 ml fraction was counted (Autogamma, Packard Instrument Co., Downers Grove. Ill.), and the OD at 280 nm and at 410 nm were obtained with a Beckman DU Spectrophotometer.

In the soluble protein fractions and in the twice washed sediment, the $^{127}I$ concentration (16) the protein content (17) and the protein iodination levels were determined.

*Protein identification.* This was done by Sephadex G-200 filtration (12); 5–20% sucrose gradient ultracentrifugation (18); electrophoresis in agar (19) polyacrylamide gel (20); immunoprecipitin reaction against rabbit antihuman-albumin serum (Miles Laboratories, Inc., Kankakee, Ill.) by a modification (Fig. 5) of a described method (21); immunodiffusion in agar against antihuman-Tg precipitating sera (22). In order to increase the sensitivity of the immunoidentification of the iodoproteins, portions of normal Tg and of the soluble protein fractions were *in vitro* $^{125}I$ iodinated (23), sp act: 10–40 $\mu$Ci/mg. Immunoelectrophoreses in agar (24) were then made.

The general methods applied to the isolation, the separation and the identification of the iodohistidines from the Pronase hydrolysates of the iodoproteins have been described in the preceding paper (12). Briefly, the isolation was made by Dowex 1 chromatography; a partial purification was obtained by butanol partition; the separation of MIH and DIH from each other was obtained by Sephadex G-10 chromatography, and the identification relied upon crystallizations to a constant specific activity.

TABLE I

*Clinical and Biological data from the*

| Case | Sex | Clinical data | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Age when first examined | Height | | Weight | | Bone age‡ | Cretinism | Intelligence quotient | Goiter estimated weight |
| | | *yr* | *cm* | *SD** | *kg* | *SD** | *yr* | | | *g* |
| 1 | F | 12 | 97 | −7 | 18.5 | −3.3 | 3.5 | + | 25 | 70 |
| 2¶ | M | 6 | 92 | −5 | 14.8 | −2.5 | 2.5 | + | 38 | 50 |
| 3 | M | 22 | 171 | 0 | 55 | −1.2 | (adult) | 0 | 80 | 600 |
| 4 | F | 12 | 126 | −4.5 | 29 | −1.7 | 13 | + | 58 | 60 |

* According to French standards of reference (13).
‡ From Greulich and Pyle's radiographic atlas of skeletal development.
§ Normalized reference value 30±3.5% (14).
‖ Normal range 1.6–12 μU/ml (15).
¶ Brother of case 1.
** Under suboptimal therapy with desiccated thyroid 30 mg/day.

The thyroxine content from the goiter iodoprotein hydrolysates was determined by competition analysis (25). The 60% vol/vol acetic acid Dowex 1 eluate of the Pronase hydrolysates was dried in a rotating vacuum evaporator. This fraction was dissolved in methanol-ammonia (1% vol/vol). The $T_4$ assays (minimum sensitivity: 0.5 ng of $T_4$) were considered as valid only when the experimental values from four dilutions were on a straight line extrapolating to zero.

Urines were analyzed for their iodohistidine content after a 4 N HCl acid hydrolysis at 60°C, for ½ h (12).

*Iodotyrosine deiodinase activity.* Thin slices weighing 40–100 mg were incubated in 2.5 ml of 0.15 M Tris-HCl buffer (pH 7.4), in the presence of $10^{-5}$ M labeled MIT and without the addition of NADPH. After incubating 30 min at 37°C, the $I^-$ produced was separated from MIT by Dowex 50 chromatography.

The enzyme was further characterized by determining from goiter homogenates the $K_m$ for MIT as previously described (12).

The iodotyrosine deiodinase peripheral activity was determined in vivo from labeled MIT or DIT in the conditions described (12). The intravenous-injected tracers-sp act about 1 mCi/mg, 5–10 μCi dose—were previously sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.).

*Ancillary methods.* [$^{131}$I] Thyroid uptakes, and perchlorate discharge tests were routine techniques. The plasma hormonal iodine (and other $^{127}$I determinations) were measured with an AutoAnalyzer Technicon method (Technicon Instruments, Inc., Tarrytown, N. Y.) (16); human TSH (H-TSH) by radioimmunoassay (15); BII from plasma by a modification of a described method (9).

## RESULTS

*Identification of MIH and DIH in the urine of congenital goitrous patients.* The iodide-free fraction of 24-h urine samples has been obtained by Dowex 1 chromatography. This fraction increased with time up to a maximum of 40-71% of the 24 h urine radioactivity on the 5th day (Fig. 1). The size of this fraction was comparable from $^{127}$I and radioiodine determinations (Table II). The iodocompounds constituting this fraction were not butanol-extractable and could be obtained by butanol partition with similar results. The activity prepared in either way was resolved into two peaks by Sephadex G-10 chromatography (Fig. 2). When differently labeled MIH and/or DIH were added as internal indicators before the Sephadex G-10 chromatography, the first peak was superimposed with MIH and the second with DIH. The final identification was obtained by crystallizations to constant specific activity. The crystallizable MIH and DIH fractions obtained from the urines of three of the four patients are presented in Table II. In every urine sample analyzed, DIH was identified. The total crystallizable iodohistidine content was between 22 and 70% (mean 42%) of the iodide-free fraction of the urine radioactivity.

*Iodotyrosine deiodinase activity.* This was demonstrated in vitro in the two removed goiters and in vivo in all four cases. The MIT deiodination rate of goiter slices at MIT concentrations near the $V_{max}$ were estimated from the data obtained after 30 min of incubation. They were found to be: 6 and 2 nmol $I^-$/min per mg of wet tissue from cases 1 and 3 respectively (normal control value 3.75±0.9 SE nmol $I^-$/min per mg). Deiodination kinetics obtained with goiter tissue homogenates yielded a $K_m$ for MIT of: 1.27 and 0.82 μM from cases 1 and 3, respectively. These results do not differ significantly from the normal human control value: 0.60 μM±0.28 SD (n = 12). Table III summarizes the in vivo iodotyrosine deiodination studies in all four patients. Six of the peripheral deiodination tests were obtained when the patients were still untreated and three differ from control values (12). However, these results are within the normal range for hypothyroid subjects (26), and the peripheral deiodination returned

*Congenital Goitrous Hypothyroid Cases*

| Biological data | | | | | Radioiodine uptake | | | |
|---|---|---|---|---|---|---|---|---|
| Serum cholesterol | PBI | Iodine from $T_4$ | $T_3$ resin test§ | H-TSH‖ | 6 h dose admin. | 24 h dose admin. | Thyroid half retention time | Cl $O_4$ discharge test |
| *mg/100 ml* | *μg/100 ml* | *μg/100 ml* | | *μU/ml* | % | % | *days* | |
| 375 | 2.0 | 1.8 | 22.0 | 40** | 77 | 81 | 6.1 | negative |
| 235 | 2.0 | | 17.3 | 48** | 81 | 74 | 5.0 | negative |
| — | 0.4 | 0.7 | 27.3 | 100 | 66 | 61 | 15.0 | negative |
| 258 | 0.9 | | 21.1 | — | 78 | 82 | 8.0 | negative |

to normal after case 3 was on replacement therapy. This decrease of the peripheral iodotyrosine deiodinase activity together with the high iodoprotein turnover rate might explain the detection of some MIT and DIT in the urines from all four patients.

*Separation and identification of the protein fractions from the 105,000 g supernates.* The Sepharose pattern of filtration was comparable from both goiters and five peaks were partially separated in case 3 (Fig. 3).

Peak 1 eluted in the fractions 45–65, was better individualized in case 3 than in case 1. In control experiments normal Tg was recovered here.

Peak 2 was high both in protein content and in radioactivity. Peak 2 radioactivity was, in case 1, 84% of the

TABLE II

*Iodohistidines in the Large Iodide-Free Fraction of the Urines from Hypothyroid Congenital Goitrous Patients*

| | | From radioiodine determinations | | | | | From $^{127}I$ determinations | |
|---|---|---|---|---|---|---|---|---|
| | | | Crystallizable iodohistidines | | | | | |
| | Urine sample | Iodide-free fraction* Total activity | MIH | DIH | MIH and DIH | MIH and DIH | $^{127}I$ in urine sample | $^{127}I$ in the iodide free fraction‡ |
| | | | Urine radioactivity | | | Fraction 1 | | |
| | | % | % | % | % | % | *μg/day*§ | *μg/day* |
| Case 1 | 6–24 h | 38 | 11.91 | 2.75 | 14.65 | 38 | — | — |
| | 5–6 day | 52 | — | — | — | | 41.5 | 17.9 |
| Case 2 | 6–24 h | 32 | 16.2 | 6.4 | 22.6 | 70 | — | — |
| | 24–48 h | 53 | — | — | — | | 55.5 | 14.7 |
| | 2–3 day | 63 | 9.2 | 4.75 | 13.95 | 22 | — | — |
| | 5–6 day | 71 | — | — | — | | 72.0 | 19.2 |
| Case 3 | 6–24 h | 32 | 5.6 | — | — | | 55.0 | 27.0 |
| | 24–48 h | 42 | 14.6 | 1.15 | 15.75 | 37 | 75.5 | 39.6 |
| Case 4 | 24–48 h | 32 | ‖ | ‖ | | | | |

* The labeled iodide-free fractions were Dowex 1 fraction 1 from 4 N HCl hydrolyzed urines.

‡ For $^{127}I$ determinations the iodide-free fractions were Dowex 1 (acetate form) fraction I from 4 N acetic acid acidified urines. This method analogous to the former avoids the HCl photometric blanks.

§ Normal range in Paris 40–80 μg/day.

‖ MIH and DIH identified by Sephadex G-10 chromatography only (Fig. 2).

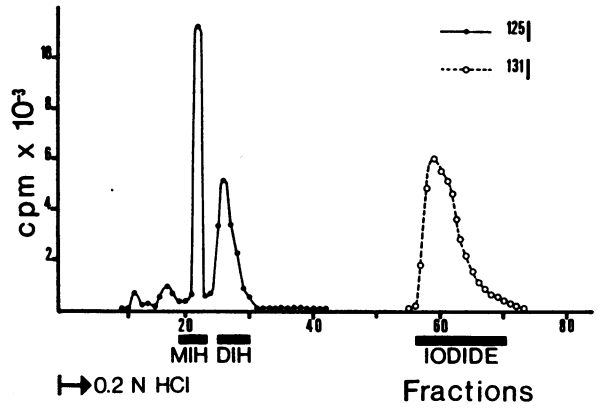


FIGURE 2 Sephadex G-10 chromatography of the [$^{125}$I] iodide-free fraction of urine from case 4. MIH and DIH separation from each other, and from [$^{131}$I] iodide added as indicator, was obtained.

total 105,000 *g* supernatant protein radioactivity and 89% in case 3.

Peak 3, included in the unaffected steadily descending branch of peak 2 radioactivity, was individualized by a pink color of the corresponding tubes. The strong absorbancy at 410 nm gave evidence for the hemoglobin nature of this peak.

Peak 4 was characterized by the contrast between a strong 280 nm absorbancy and the absence of a corresponding peak of radioactivity. The 260 greater than 280 nm absorbancy suggested that peak 4 was due to the presence of nucleoproteins.

Peak 5 was characterized by a rise in the radioactivities with a $^{131}I/^{125}I$ ratio higher than in any other peak, and no corresponding 280 nm absorbancy. The radioactivity from peak 5 was identified by routine chromatography as free iodide mixed with free MIT and DIT; no free iodohistidines were detected.

*Sepharose peak 2 iodoprotein identification.* (*a*) When filtered over a Sephadex G-200 column with added [$^{131}$I] radioiodinated human serum albumin (RISA), this fraction gave a single peak of radioactivity in which [$^{125}$I] and [$^{131}$I] RISA were superimposed and in constant ratios. (*b*) The sedimentation coefficient of the protein was 3–8S. (*c*) Disc polyacrylamide gel electrophoresis is shown in Fig. 4. A large albumin disc was the predominant protein. Other unidentified protein fractions of minor importance were separated from albumin. (*d*) Tg-precipitating sera from two Hashimoto patients did not precipitate peak 2 iodoproteins by immunoelectrophoresis. (*e*) The immunoprecipitin reactions from cases 1 (Fig. 5) and 3 gave identical results. Peak 2 iodoproteins were precipitated by rabbit antihuman-albumin serum. When the radioactivities recovered in the immunoprecipitates were compared with the total radioactivities of the individual samples, 65–80% of the radioactivity was precipitated near the equivalence, and 100% when the antibody was in large excess. Thus peak 2 was largely if not entirely iodoalbumin.

*Sepharose peak 1 iodoproteins, and attempts to identify Tg.* Purification of peak 1 iodoproteins was first obtained by a Sephadex G-200 filtration. Only the proteins excluded from the gel and separated from contaminant peak 2 proteins were further analyzed. (*a*) Electrophoresis in agar, in barbital buffer (pH 8.2) of the purified proteins from peak 1, revealed the presence of several components. If electrophoretic mobility (EM) of albumin is normalized to 1, the two major protein components from peak 1 had an EM of 1 and 0.85. Only traces of proteins were detected having the

TABLE III
*Peripheral Deiodinating Activity in the Four Congenital Goiter Cases*

| Cases | Thyroid status | Protocol | Urine collections 0–2 h | 2–6 h | 6–24 h | 0–4 h | 4–24 h | Total 0–24 h |
|---|---|---|---|---|---|---|---|---|
| Case 1 | Hypothyroid | MIT—i.v. | 0.58 | 0.65 | 0.75 | — | — | 1.93 |
| | | DIT—i.v. | 2.60 | 1.76 | 0.47 | — | — | 4.89 |
| Case 2 | Hypothyroid | MIT—i.v. | 0.99 | 0.49 | 0.23 | — | — | 1.71 |
| | | DIT—i.v. | 6.50 | 1.48 | 0.29 | — | — | 8.27 |
| Case 3 | Hypothyroid | MIT orally | — | — | — | 10.75 | 0.32 | 11.07 |
| | Euthyroid (treated)* | MIT orally | — | — | — | 0.95 | 0.75 | 1.70 |
| Case 4 | Hypothyroid | DIT orally | — | — | — | 9.55 | 0.86 | 10.41 |

Results in percent of given iodotyrosines recovered as such in the urine fractions.
* Under replacement therapy by D-L $T_4$ 250 μg/day.

0.6 EM of normal human Tg; (*b*) Disc polyacrylamide gel electrophoresis from case 1 is shown in Fig. 3. No albumin was detected. The two major proteins were not identified; only two small discs near the origin were compatible with Tg; (*c*) Sucrose gradient ultracentrifugation of these proteins disclosed the presence of two peaks, the major peak, 12 to 20S, being compatible with Tg; (*d*) Immunodiffusion on Ouchterlony plates demonstrated the precipitation of human Tg by antithyroid sera from two Hashimoto patients and from a rabbit, a goat and a monkey, but no precipitation with peak 1 iodoproteins from case 3; (*e*) Immunodiffusion was sensitized in two ways: the IgG, contained in the previously mentioned sera, were specifically precipitated by an anti-IgG reagent; in vitro labeled proteins from cases 1 and 3 (peak 1) were added. No radioactive precipitates were obtained; (*f*) Immunoelectrophoresis was made from in vitro labeled normal human Tg and from cases 1 and 3 (peak 1) labeled proteins, against the same antisera. No precipitation was observed from peak 1 proteins with the exception of a doubtful reaction from the monkey antithyroid serum. Thus peak 1 proteins were not Tg but were not otherwise identified.

*Iodoamino acid content of the iodoproteins prepared from the goiters.* Three protein fractions from both goiters, namely: (*a*) The Sepharose peak 2 iodoalbumin; (*b*) The Sepharose peak 1 heavy proteins, differing from Tg; (*c*) The 105,000 *g* washed sediment, was hydrolyzed with Pronase.

The Dowex 1 purification of the Sepharose peak 2 (iodoalbumin) hydrolysate from case 1 is illustrated in Fig. 6. The main result was the extraordinarily high "crude iodohistidine fraction:" 31.4% of the total $^{125}I$ activity of the hydrolysate (corresponding fraction obtained from normal Tg: 1.94±0.54 SE [12]).

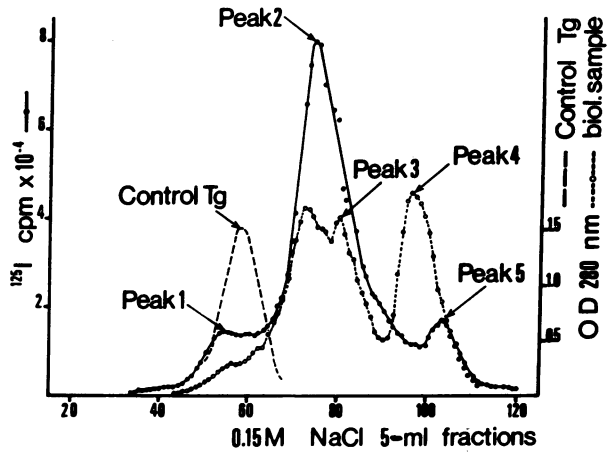


FIGURE 3 Sepharose gel filtration. Comparison of the 105,000 *g* supernate from case 3 goiter extract with normal human Tg.

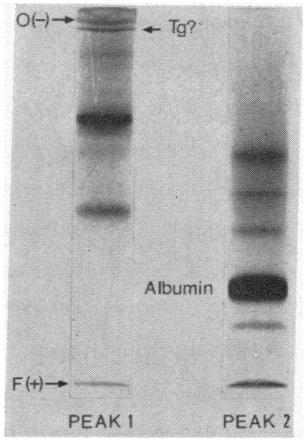


FIGURE 4 Polyacrylamide gel electrophoresis. Comparison of the proteins from case 1 goiter extract separated by Sepharose gel filtration into peaks 1 and 2.

The Sephadex G-10 chromatography was also remarkable disclosing a DIH peak higher than that of MIH. This finding was confirmed by the crystallization results.

The crystallizable MIH and DIH content of each iodoprotein and their $^{127}I$ iodination levels are presented in Table IV. The iodohistidine content of the three protein fractions from each goiter was comparable but differed from case to case. The mean total crystallizable iodohistidines from the protein fractions purified from cases 1 and 3 goiters were of the order of 15 and 4%

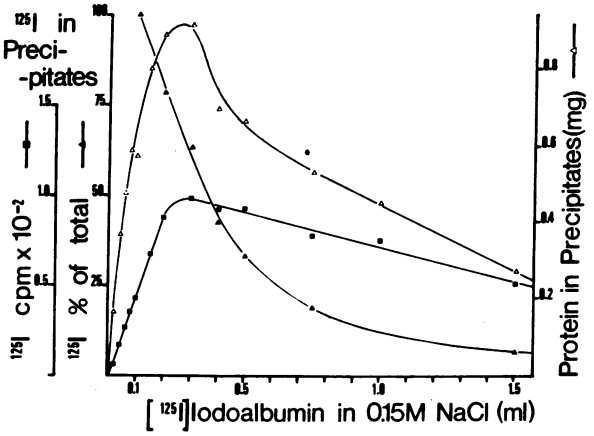


FIGURE 5 Identification by precipitin reaction of Sepharose peak 2 proteins from case 3. Increasing volumes of the in vivo-labeled protein solution, 1 mg/ml in 0.15 M NaCl, were allowed to react with a 0.2 ml constant volume of rabbit antihuman-albumin serum at 4°C, 48 h. From the precipitates, twice washed with 0.15 M NaCl were obtained: the protein content, the radioactivities, and the precipitable radioactivities in percent of total radioactivity recovered in the immunoprecipitates.

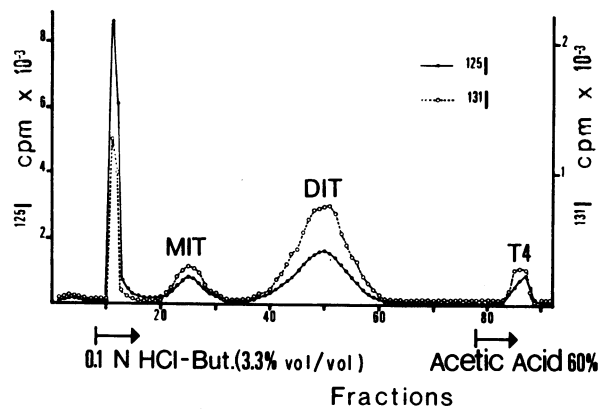


FIGURE 6 Dowex 1 column chromatography of case 1 iodoalbumin Pronase hydrolysate. The predominance of peak 1, containing iodohistidines, is shown. In this fraction, the $^{125}I/^{131}I$ ratio, of lately to newly incorporated radioiodine was two to three times higher than in the other iodoamino acid fractions.

respectively of the $^{125}I$ iodoprotein radioactivities. The mean DIH/MIH ratio from case 1 was 1.16±0.07 SE (n = 6) and from case 3 was 0.35 (n = 2). From case 1, the 3 h newly incorporated $^{131}I$ in iodohistidines was already in a proportion as high as two-thirds of the 7 day incorporated $^{125}I$.

The mean DIT/MIT ratio from case 1 was 3.19 ±0.43 SE (n = 6), and 1.45±0.21 SE (n = 5) from case 3. There was no apparent correlation between the high DIT/MIT ratio or DIH/MIH ratio and the calculated iodination levels of the iodoproteins.

The thyroxine content of the separated protein fractions, presented in Table IV, was obtained by the specific competition analysis. The average $T_4$ iodine was about 12 μg/g of protein from case 1 and 3.6 μg/g of protein from case 3. The very low $T_4$ content of the iodoproteins is better demonstrated by the percent of the protein iodine identified as $T_4$ iodine than by the conventional content per gram of protein if other uniodinated proteins are present as contaminants. $T_4$ iodine amounted to 1.2% of the $^{127}I$ content of the predominant peak 2 iodoalbumin from case 1, and 3.6% from case 3.

*Iodoalbumin turnover in the thyroid glands.* This was estimated from simplified kinetics. They were derived from the findings that MIH is almost quantitatively recovered in urine after intravenous injection (12). In addition the assumption was made that urinary MIH was a specific metabolite of iodoalbumin, disregarding the partial conversion of DIH to MIH and the proteolysis of other minor thyroidal iodoproteins. In case 1 it can be calculated from Table II that about 5 μg of MIH iodine was excreted per day. MIH iodine in iodoalbumin amounts to 51 μg/g (Table IV). Therefore the iodoalbumin turnover in case 1 can be estimated to 100 mg/day and the $T_4$-iodine secretion to 1 μg/day. Similar calculations indicate an iodoalbumin turnover in case 3 of 7 g/day with a $T_4$-iodine secretion of 12 μg/day. Thus an over-stimulation of the thyroid gland may explain the latent or compensated hypothyroidism in case 3. Hypothyroidism is not always present in similar patients. Euthyroid and hypo-

TABLE IV

*Iodine Determinations in the Protein Fractions Obtained from the Two Surgically Removed Congenital Goiters*

| 105,000 g protein fractions from 0.15 M NaCl tissue extracts | Patient case no§ | $^{127}I$ Protein iodination level | $^{127}I$ Iodine from $T_4$‖ | $^{131}I$* DIT/MIT ratio | $^{131}I$* Crude iodohistidine fraction¶ | $^{131}I$* MIH** | $^{131}I$* DIH** | $^{125}I$‡ DIT/MIT ratio | $^{125}I$‡ Crude iodohistidine fraction¶ | $^{125}I$‡ MIH** | $^{125}I$‡ DIH** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | μg/g | μg/g | | % protein radioactivity | | | | % protein rad.oactivity | | |
| Sepharose peak 2 | 1 | 822 | 10.1 | 4.98 | 15.9 | 4.2 | 5.55 | 3.54 | 31.4 | 6.2 | 7.55 |
| (iodoalbumin) | 3 | 47 | 1.7 | 1.12 | 5.45 | | | 2.18 | 8.75 | 2.9 | 1.17 |
| Sepharose peak 1 | 1 | 273 | 15.0 | 2.94 | 21.2 | 5.17 | 6.65 | 3.13 | 31.4 | 8.2 | 10.1 |
| (differing from Tg) | 3 | 238 | 5.4 | | | | | 1.43 | 7.9 | | |
| 105,000 g sediment‡‡ | 1 | 4750 | 11.0 | 1.81 | 17.7 | 4.35 | 4.2 | 2.75 | 24.6 | 6.9 | 6.45 |
| | 3 | 6000 | | 0.96 | 9.8 | | | 1.54 | 11.9 | 2.9 | 0.87 |

* Given orally 3 h before operation.
‡ Given orally 7 days (case 1) and 6 days (case 3) before operation.
§ See Table I.
‖ Obtained by competition analysis from the 60% acetic acid Dowex 1 eluate of Pronase hydrolysate (Fig. 7).
¶ Fraction 1 from Dowex 1 chromatography of Pronase hydrolysate.
** MIH and DIH purified from the crude iodohistidine fraction by successive crystallizations to constant specific activity.
‡‡ Twice washed with 0.15 M NaCl. Sediment from case 1 was the 700–105,000 g insoluble protein fraction. Sediment from case 3 was the total 105,000 g insoluble protein fraction.

thyroid cases in the same kindred have been reported (3, 7).

*Fractionation of the labeled compounds from the serum.* About 100 μCi of $^{131}I$ were intravenously injected to cases 1, 3, and 4, and the serum radioactive concentrations (SRC) determined. The SRC decreased in 6 h to a low minimum of between 0.17 and 0.52% of the given radioactivity per liter (D/L). Then the SRC were found approximately constant over a period of 3 days. The BII fraction of serum samples obtained during this period was found to be 50, 71, and 42% of the SRC in cases 1, 3, and 4, respectively. In addition 32 and 38% of the BII from cases 3 and 4, respectively were found TCA precipitable, but the low SRC were not compatible with the identification of this iodoprotein.

## DISCUSSION

Mono- and diiodohistidine have been identified in the quantitatively large BII moiety of the urine from four patients with congenital goitrous hypothyroidism. Urinary iodohistidines were metabolites of thyroidal iodoproteins. Tg was absent, and the predominant protein identified in the two removed goiters was an iodoalbumin. This protein was found to be rich in iodohistidines as well as were the other unidentified protein fractions from the goiters. In contrast, their thyroxine contents were very low. Iodoalbumin turnover estimates were found to be very high but insufficient to maintain a normal thyroxine production.

The identification and the classification of congenital goitrous hypothyroidism has been mainly due to a penetrating analysis of the clinical and biological syndromes by Stanbury (27). Five main varieties have been described in relation to the defective step of the thyroid hormonogenesis namely: the active transport of iodide, the organification of iodine, the iodotyrosine deiodination, the iodotyrosyl "coupling," and protein synthesis or breakdown.

This last variety or iodoprotein defect was first defined by the presence of BII compounds in the serum (1, 2). The stable BII fraction of the serum may be high enough to raise the protein-bound iodine (PBI) above normal values (28, 29). In other reports, only the labeled BII moiety of the serum was elevated (2) and shown to contain peptide-linked iodotyrosines and iodothyronines (1).

In the four patients studied here, the PBI were low, and the PBI-$T_4$ differences were not significant. A serum BII fraction was found in three cases. The latter analysis was not done in case 2 (brother of case 1). From the serum data only it is questionable whether the cases reported here could be included in the iodoprotein defect.

Investigations of the thyroid itself give more conclusive evidence for the identification of this Tg defect. Firstly, the high radioiodide uptake, the negative perchlorate discharge test, the detection of an iodotyrosine deiodinase activity eliminate other syndromes except for the rare occurrence of associated defects (3, 10). More important is the identification in the goiter extracts of an iodoalbumin (6). This iodoalbumin has also been identified from the serum of the patients (3–5). From the poorly iodinated iodoalbumin, low peptide-linked levels of hormone were already reported (8, 11). However the detection of iodoalbumin is not specific for this type of goiter. Iodoalbumin has been found in normal animal and human thyroid glands (30, 31) and in a large variety of thyroid diseases (32).

The iodoprotein defect is better defined by the contrast between the presence of iodoalbumin and the absence of Tg. The possibility that the disease was a Tg defect has already been suggested (6). False identification of the heavy iodoproteins collected in the Sepharose-peak 1 was avoided. Similar proteins were detected in congenital goiters from sheep (33) and cattle (34).

In some reported cases, however, Tg was found only to be reduced (3–5, 7). This might be due to incomplete protein identification, to incomplete forms of the disease or to the presence of a different disease. Evidence for the complete absence of Tg in our cases is strongly supported by the presented methods. Tg radioimmunoassay (35) with increased specificity and sensitivity might have demonstrated the presence of Tg traces. Tg defect means that the normal function of Tg as the support of normal hormonogenesis is completely missing. Tg structure has been shown to facilitate the thyroxine formation (36). In addition Tg defect suggests a genetic origin. Examples of this congenital defect in siblings were reported (2, 3, 9, 37). Two of the reported four patients (cases 1 and 2) are siblings. A brother of case 3 suffers from the same disease.

The main findings of this study came from a completely different approach to the Tg defect. BII compounds, other than the circulating iodoalbumin, were often mentioned in this variety of congenital goiter as unidentified and puzzling substances. They were found after hydrolysis of the serum (1) or the thyroidal iodoproteins (7) as "resistant to digestion" material, remaining at the origin of the chromatograms (2) or insoluble in acid butanol (9). They were detected in the urine of patients (8, 11) and presumed to be short-chain polypeptides (2), suggesting a iodoprotein breakdown defect. In the urine of the patients studied, a large part of these BII compounds was identified as iodohistidines. Evidence is presented in the preceding paper (12) that MIH and DIH are normally biosynthetized

in thyroidal proteins, that Ta is richer in iodohistidines than Tg, and that iodohistidines, unaffected by iodotyrosine deiodinase, are excreted in urine. The high iodohistidine content of the iodoalbumin produced by the congenital goiters studied is only comparable with that of normal Ta. Iodohistidine levels of in vitro-iodinated human serum albumin were found comparable with that of in vivo-iodinated albumin in preliminary experiments. The unidentified Sepharose-peak 1 heavy iodoproteins resembled iodoalbumin by their high iodohistidine and low $T_4$ content. This is one more reason to differentiate this protein from Tg.

The recovery in the urine of large amounts of iodohistidines is directly related to a high iodoalbumin turnover in the goiters. Since iodohistidines have physicochemical properties which easily differentiate them from other iodinated molecules (12) it is suggested that simple methods such as ion-exchange chromatographies of the urine should be performed in every congenital goiter case, in an attempt to detect the Tg defect. The detection of iodohistidines in the urine rather than that of BII compounds in the serum may appear in the future to be a more specific and more constant parameter of the iodination of inappropriate proteins in the absence of Tg.

The question arises as to whether the loss of iodohistidine iodine in the urine could explain the hypothyroidism of the Tg defect patients. This loss is comparable with that of iodotyrosine iodine in the iodotyrosine-deiodinase defect (26). However the high DIT/MIT ratio (3) as well as the observed high DIH/MIH ratio in case 1 are strong arguments against the iodine-deficiency hypothesis. In contrast with the iodotyrosine-deiodinase defect (38) attempts to improve the thyroid hormonogenesis by supplementing the diet of the patients with iodine were unsuccessful. 10 mg of iodine per day for 8 mo to 5 yr before the operation—given to case 1, and 2 mg of iodine per day for 2 mo to case 4 did not enhance the thyroid hormone secretion. Therefore iodine deficiency seems to play only an auxiliary role (if any) in this disease.

It can be concluded that the fundamental metabolic trouble in this disease, despite individual minor differences, is the genetic absence of Tg. The consequences are (*a*) the iodination of inappropriate proteins, mainly albumin, (*b*) a very low yield of thyroxine biosynthesis, (*c*) the formation of a goiter by a TSH oversecretion and a rapid thyroxine-poor iodoalbumin turnover, (*d*) a high production of iodohistidines by the proteolysis of the iodohistidine-rich iodoalbumin, (*e*) iodohistidines, not being deiodinated, are excreted in urine. The identification of iodohistidines in the urine may prove to be helpful in the detection of the Tg defect.



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