# Thyroidal Particulate Protein in the Synthesis of Thyroid Hormones: Effect of Iodide on $^{131}I$ Distribution Among Iodoamino Acids in Iodine-Deficient Rat Thyroids (37471)

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Mayberry and Astwood (1) noticed in 1960 that injection of iodide caused a percentile increase in $^{131}I$-thyroxine in prelabeled rat thyroid gland. Subsequent studies by Mouriz, Morreale de Escobar and Escobar del Ray (2), Onaya and Halmi (3) and Inoue and Taurog (4, 5) have confirmed this observation. However, the mechanism of this iodide effect has remained to be elucidated. To find out which type of thyroid protein is responsible for this percentile increase in $^{131}I$-thyroxine, soluble protein, deoxycholate-soluble and deoxycholate-insoluble particulate proteins were separated after administration of iodide, and $^{131}I$ distribution among iodoamino acids in these fractions was investigated in the rat.

*Materials and Methods.* Male rats of the Holtzman strain (150–250 g) were used. Low iodine diet was the Remington-type test diet distributed by General Biochemicals, Chagrin Falls, OH (iodine content $<0.07$ $\mu$g/g). Distilled water was given *ad libitum*. In Expt 1, 35 animals were fed a low iodine diet for 10 days and were divided into 7 equal groups. Twenty microcuries of $^{131}I$ was injected into each animal, and they were killed 4, 8, 12, 16, 20 and 24 hr later. KI (200 $\mu$g) was injected into 5 experimental (8, 12, 16, 20 and 24 hr groups) 4 hr after administration of $^{131}I$ injection, while saline was injected into the control (24 hr) group. In the first part (A) of Expt 2, 4 animals were fed a low iodine diet for 10 wk. Neither $NaClO_4$ nor KI was administered, and the rats were killed 4 hr after 50 $\mu$Ci of $^{131}I$. In the second part (B) of Expt 2, 4 animals were also fed a low iodine diet for 10 wk. Two hundred microcurie of $^{131}I$ was injected intraperitoneally (ip) 10 min after administration of 5 mg $NaClO_4$ and the animals were killed 4 hr later. In the third part (C) of Expt 2, 5 animals were also fed a low iodine diet for 10 wk. Ten minutes after administration of 5 mg $NaClO_4$, 200 $\mu$Ci of $^{131}I$ was injected ip simultaneously with 200 $\mu$g KI. The animals were killed 4 hr after administration of 5 mg $NaClO_4$. The thyroids were excised free of connective tissues and homogenized in glass tissue grinders with 0.5 ml of 0.25 *M* sucrose containing 10 m*M* Tapazole. The separation of particulate and soluble fractions was accomplished by ultracentrifugation of homogenates at $10^5g$ for 1 hr. This centrifugation was repeated after resuspension of the pellet. The first supernatant was used to study soluble indoproteins. The second pellet was resuspended with 0.5 ml of 2.0 % deoxycholate solution in 0.25 *M* sucrose containing 10 m*M* Tapazole, and was centrifuged again at $10^5g$ for 1 hr. The supernatant obtained after this procedure was used to study deoxycholate-soluble particulate iodoprotein. The final pellet was resuspended, washed 3 times with 0.15 *M* NaCl, and used for the study of deoxycholate-insoluble particulate iodoprotein. Homogenates or homogenate fractions were hydrolyzed for 6 hr with streptomyces griseus protease (pronase) (5 mg/ml homogenate) at 38°. Portions of each hydrolysate were fractionated by ascending chromatography on Whatman No. 3 MM filter paper in a *n*-butanol–ethanol–0.5 *N* $NH_3$ (5:1:2) solvent system (BEA). Thyroxine ($T_4$), triiodothyronine ($T_3$), diiodotyrosine (DIT), monoiodotyrosine (MIT) and iodide were used as the carriers. The radioactivity on the chromatographic strips was located with a 4-pi, windowless, gas flow counter (Vanguard). The area of the

paper corresponding to each radioactive peak was cut out, and the radioactivity was measured in a well-type scintillation counter. Statistical significance of difference between fractions was done by means of the Student's $t$ test. A $p$ value $<0.05$ was considered statistically significant.

*Results. Expt 1. Time study of the effect of iodide on the proportion of $^{131}I$ iodinated amino acids in the prelabeled thyroid gland.* In the 2 control groups (4 and 24 hr groups) without KI injection, thyroidal $^{131}I$ uptake at 4 hr was 40% and it decreased gradually to 30% at 24 hr. Distribution of radioiodine among the various iodinated amino acids was analyzed in the control group at 24 hr. Iodide was 4%; MIT, 41%; DIT, 32%; $T_4$, 18%; $T_3$, 5% of the total thyroidal radioactivity.

Administration of 200 $\mu$g KI at 4 hr did not significantly depress 24 hr thyroidal radioiodine uptake. However, administration of KI produced a marked alteration in the distribution of radioiodine among the various iodinated amino acids. As shown in Fig. 1, the proportion of $^{131}I$-iodothyronines in thyroid digest increased progressively with time after 200 $\mu$g KI administration ($T_4$, 38.6%; $T_3$, 9.2% at 24 hr), associated with the marked percentile decrease in $^{131}I$-iodotyrosines.

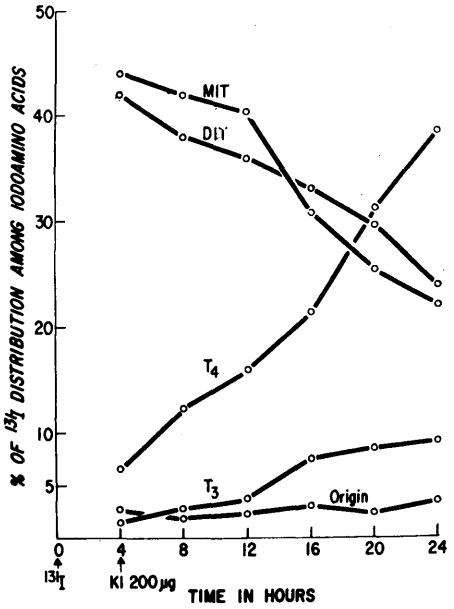


FIG. 1. Time study of the effect of iodide on the proportion of $^{131}I$-iodoamino acids in the prelabeled thyroids. Thyroid lobes of 5 animals were pooled for each group. KI (200 $\mu$g) was injected 4 hr after $^{131}I$ administration. MIT = monoiodotyrosine, DIT = diiodotyrosine, $T_4$ = thyroxine, $T_3$ = triiodothyronine.

*Expt 2. Proportion of $^{131}I$-iodoamino acids in soluble protein and particulate proteins (deoxycholate-soluble and -insoluble) in iodine-deficient rat thyroid.* In the first part of this experiment (A), the relative distribution of $^{131}I$ among 3 fractions (soluble, deoxycholate-soluble and deoxycholate-insoluble fractions) was determined by counting the radioactivity in each fraction (Table 1A). As expected, more than 92% of radioactivity was found in the soluble fraction, and 3.3 to 4.5% of total thyroidal radioactivity was found either in the deoxycholate-soluble or deoxycholate-insoluble fractions. The proportional distribution of $^{131}I$ among iodoamino acids was also studied after digestion of 3

TABLE I. $^{131}I$ Distribution in Fractions (% of Total cpm in Thyroid).[a]

| Group | No. of animals | $^{131}I$ Distribution (%) in fractions | | |
|---|---|---|---|---|
| | | Soluble | DOC-soluble particulate | DOC-insoluble particulate |
| A | 4 | 92.2 ± 0.1[b] | 3.3 ± 0.3 | 4.5 ± 0.2 |
| B | 4 | 92.4 ± 0.3 | 2.8 ± 0.2 | 4.8 ± 0.3 |
| C | 5 | 89.7 ± 0.6[c] | 5.4 ± 0.6[c] | 4.9 ± 0.3 |

[a] The experimental designs for A, B and C groups were indicated in Expt 2 of the text. DOC = deoxycholate.

[b] Mean ± SE of the mean.

[c] Differences between the mean values of group C and the other groups are statistically significant, $p < 0.025$.

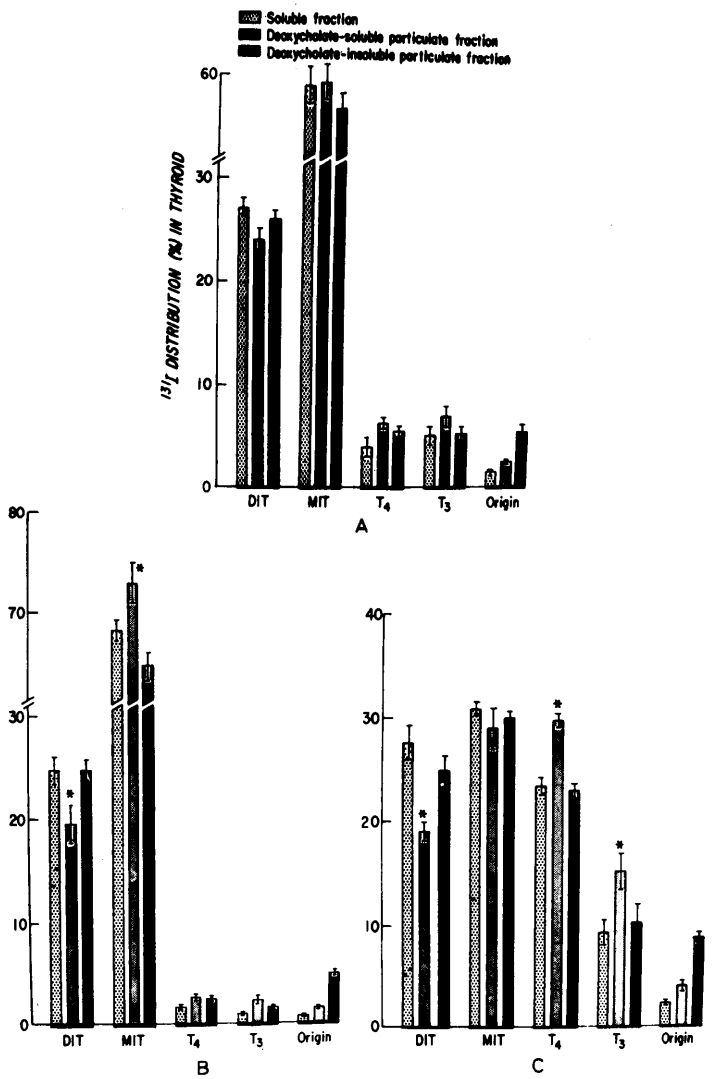


FIG. 2. Effect of $NaClO_4$, $NaClO_4$ + KI on the distribution of radioiodine among iodinated amino acids in soluble, deoxycholate-soluble and deoxycholate-insoluble particulate proteins, (A): Control (the first part of Expt 2), (B): $NaClO_4$ (the second part of Expt. 2), (C): $NaClO_4$ + KI (the third part of Expt 2). Abbreviations were the same as in Fig. 1. The experimental designs were indicated in the text. * Significantly different ($p < 0.01$) as compared to soluble and deoxycholate-insoluble fractions.

fractions. As shown in Fig. 2A, distribution of radioactivity among iodoamino acids were the same in 3 fractions.

In the second part of this experiment (B), intrathyroidal distribution of $^{131}I$ was studied under the influence of $NaClO_4$. As shown in Table 1B, more than 92% of total thyroidal radioactivity was again found in the soluble fraction. The radioactivity in the deoxycholate-soluble fraction was somewhat less than that of Table 1A, although the difference was not statistically significant. Effect of $NaClO_4$ on the distribution of $^{131}I$ among iodoamino acids in 3 fractions was shown in Fig. 2B. It was apparent that MIT increased significantly in 3 fractions after $NaClO_4$, and that DIT and iodothyronines decreased significantly in the deoxycholate-soluble

particulate fraction after $NaClO_4$ administration.

In the third part of this experiment (C), effect of excess iodide on intrathyroidal distribution of radioiodine was studied under the influence of $NaClO_4$. As shown in Table 1C, the radioactivity of soluble fraction decreased significantly, but the radioactivity in the deoxycholate-soluble fraction increased significantly. No alteration of radioactivity was found in the deoxycholate-insoluble fraction, however. As compared to Fig. 2A and B, a significant decrease of MIT was found in all 3 fractions after KI + $NaClO_4$ administration (Fig. 2C). Furthermore, an increase of $T_4$ and $T_3$ was found after administration of KI + $NaClO_4$. This increase was the greatest in the deoxycholate-soluble particulate fraction.

*Discussion.* Iodide is not only the element essential for the synthesis of thyroid hormones, but also the agent involved in modifying thyroid activity. For instance, excess iodide blocks the synthesis (6) and release (3, 7–11) of thyroid hormone in patients with Graves' disease and in experimental animals with overactive thyroid. Furthermore, *in vitro* studies have shown that some amount of iodide promotes the synthesis of thyroid hormone (12–15). In agreement with these *in vitro* studies, our present study clearly indicated that some amount of iodide stimulated coupling of iodotyrosines to iodothyronines in the rat thyroid gland. Thus the mode of action of iodide on thyroid proteins is of considerable interest. DeGroot and Carvalho (16) have reported an *in vitro* experiment in which hydrolysis of the deoxycholate-soluble iodoprotein yielded labeled MIT and DIT, but suggested that this kind of iodination in the deoxycholate-soluble iodoprotein does not represent the normal pathway of thyroid hormonogenesis. In contrast, our *in vivo* experiment clearly indicated that the deoxycholate-soluble and -insoluble fractions contained $T_4$ and $T_3$ as the soluble protein did. Although the absolute amounts of iodothyronines formed should be less in the particulate fractions than in the soluble protein because of a low iodine uptake, our data suggested that thyroidal particulate fraction did actually participate in the synthesis of thyroid hormones at least under our experimental conditions. This hypothesis was further supported by the finding that thyroxine concentration increased significantly after iodide administration in all 3 fractions (soluble, deoxycholate-soluble and deoxycholate-insoluble). Also, it was shown that $NaClO_4$ depressed iodothyronine concentrations in all 3 fractions. Thus the data accumulated indicated that thyroidal particulate proteins (deoxycholate-soluble and -insoluble fractions) are actually involved in the synthesis of thyroid hormones when thyroidal iodine is depleted. However, since iodine depletion may produce a drastic alteration of thyroidal protein (5), our data on iodine depleted thyroid are not necessarily applicable to the physiology of the thyroid in iodine-sufficient animals. Further experiments are required to establish the concept that thyroidal particulate proteins are involved in the synthesis of thyroid hormones in normal animals.

*Summary.* The effect of iodide on $^{131}I$ distribution among iodinated amino acids was studied after separation of thyroid soluble protein, deoxycholate-soluble and deoxycholate-insoluble particulate proteins in rats with iodine depleted and prelabeled thyroid gland. Although iodide caused an increase in labeled thyroxine in all 3 fractions, the effect was the most marked in the deoxycholate-soluble particulate protein and associated with a significant percentile decrease in $^{131}I$-DIT and $^{131}I$-MIT. It is suggested that thyroidal particulate proteins are also involved in the synthesis of thyroid hormones, at least in rats with iodine depleted thyroids.

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