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Alteration in Tissue and Serum Concentrations of TSH, Iodide, T_4 and T_3 Induced by Various Dietary Iodide Levels[1] (38990)

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(Introduced by D. M. Donaldson)

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In an earlier report from this laboratory (1) it was demonstrated that tissue concentrations of iodine containing compounds increased severalfold when rats were maintained on a diet supplying 3 μg iodide/day as opposed to a diet that supplied 1 μg iodide/day. The present study was undertaken to extend the original study to include additional increases in iodide intake in order to evaluate the effect of this increase on tissue, serum, and urine concentrations of iodine compounds. Such data would permit an estimate of the minimal daily iodide intake that would produce maximal tissue levels of thyroid hormones. In addition, we wish to compare Thyroxine (T_4) and Triiodothyronine (T_3) values obtained by isotopic equilibrium methods with the newer radioimmunoassay techniques.

Materials and methods. Animals and diets. Male Holtzman rats with an initial weight of 120–150 g were used. All animals were fed an iodine-deficient ration (General Biochemical, Chagrin Falls, Ohio) containing not more than 0.06 μg iodide/g for 21 days, after which time the animals were divided into three groups of 9–12 animals each and fed the iodine-deficient diet plus different quantities of stable iodide. For the first experiment animals were divided into groups and fed diets containing 0.59, 3.25, and 6.5 μg stable iodide per gram of feed. With a daily feed consumption of approximately 15 g of the powdered diet, the total iodide intake per rat in each group was 10, 50, and 100 μg/day. In the second experiment, an additional two levels of stable iodide were fed; 0.06 and 0.18 μg/g, which gave daily iodide intakes of 1 and 3 μg/day. These additional two groups approximated conditions of our previous experiments (1). All animals in both experiments were fed their respective ration for 60–75 days, at which time they were sacrificed by exsanguination under ether anesthesia (Experiment I) or decapitation (Experiment II). The latter method of sacrifice was used to minimize the effect of ether on serum thyrotropin (TSH) (2). Tissues and sera were collected and either assayed immediately for iodinated compounds (Experiment I) or frozen for analysis at a later date (Experiment II).

Experiments. Two experiments were performed. In Experiment I, we used an isotopic equilibrium technique that previously has been used in this laboratory (3) to quantitate iodine-containing compounds in tissues and serum of the rat. For this we added carrier-free sodium [^{125}I] to the diets in sufficient quantity to enable satisfactory radioactivity measurements in 1–2 g of tissue. Thus each diet would have a known specific activity and by the end of the 60–75 day period it was assumed that there would be nearly uniform labeling of the iodine pools in the animals; therefore, radioactivity measurements could be translated into absolute values. Sacrifice of animals, radioactivity determinations, chromatography of extracts of tissue homogenates, and quantitation of the iodocompounds were performed as previously described (1, 3).

For Experiment II the various diets were fed as described above and following collection of serum and removal of the thyroid and pituitary all samples were frozen for later analysis by radioimmunoassay methods (RIA).

Radioimmunoassay methods. Thyroxine (T_4) and triiodothyronine (T_3) content of thyroid tissue were determined following

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pronase digestion as specified by Tong *et al.* (4) and by radioimmunoassay methods described by Chopra (5) and Chopra *et al.* (6). Following digestion, the hydrolysate was diluted such that only 1/4000 of the total thyroid was assayed (25 μl). Preliminary experiments have shown that there is no significant binding of monoiodotyrosine (MIT) or diiodotyrosine (DIT) by the T_3 or T_4 antibody even under conditions of 1000-fold excess of iodotyrosine. No cross-reaction of the T_3 antibody with T_4 occurs so the presence of relatively high concentrations of T_4 in the assay system does not interfere with the T_3 assay. Serum T_4 and T_3 were assayed directly, without prior extraction, by the same procedure.

Reagents for rat TSH radioimmunoassay were obtained from the NIAMDD Rat Pituitary Hormone Program, Bethesda, MD. A double antibody procedure was employed. Into 10 × 75 mm disposable culture tubes the following reagents were added in sequence: (a) 0.01 *M* phosphate–0.15 *M* NaCl buffer, pH 7.5 containing 2% normal rabbit serum. Buffer was adjusted to a final volume of 1 ml. (b) 100 μl 0.1 *M* EDTA. (c) 200 μl of test serum, (d) varying quantities of the reference TSH preparation (NIAMD-Rat TSH-RP-1) covering the range from 5 to 1000 μg/tube. Sufficient points were selected (usually 15) so the entire curve could be reconstructed graphically. (e) 100 μl of the rat TSH antibody (NIAMD-Anti-Rat TSH-S-1) in a final dilution of 1: 40,000 in buffer. (f) 100 μl of 125-I labeled TSH (NIAMD Rat TSH-I-1) in buffer (10,000 cm). After mixing, all tubes were incubated at room temperatures for 24 hr. (g) 75 μl of sheep antirabbit gamma globulin (second antibody) to precipitate all of the rabbit gamma globulin including the antibody-bound labeled TSH. An additional 24-hr incubation was carried out at 4° followed by separation of the bound and free hormone by centrifugation.

For pituitary TSH assay the pituitaries were homogenized in 1 ml of the phosphosaline buffer and diluted 1000×. Of this final dilution, 100 μl were assayed by the procedure described above.

Iodination of rat TSH was performed by the Chloramine-T method of Hunter and Greenwood (7). Specific activity was approximately 100 mCi/mg. Statistical comparison of sample means, using Tukey's *D* test were performed according to Snedecor (8).

Results. The effect of various dietary iodide levels on body, thyroid and pituitary weight is seen in Table I (data from Experiment II). Over the entire range of iodide levels no effect on body weight was observed. Thyroid weights expressed as milligrams per 100 g body wt were significantly higher in the 1 and 3 μg groups when compared to the 10, 50, and 100 μg groups. Pituitary weights were significantly higher only in the 1 μg group.

Concentrations of iodinated compounds in extra thyroidal tissues are presented in Table II (data from Experiment I). Total iodine increased directly with iodide intake, mainly as a result of increases in inorganic iodide since iodide concentration closely paralleled the total iodine values. There was generally no significant change in tissue T_4 or T_3 over the tenfold range of iodide intake that was examined. Exception to this was seen only with samples of liver in which both T_4 and T_3 plateaued at the 50 μg iodide intake level.

Urinary iodide excretion increased linearally with increases in iodide intake over the entire range (Table II).

It would appear from these data that peripheral tissue concentrations, in general, reach maximum levels when dietary iodide intake is no greater than 10 μg per day.

Thyroid and serum T_3 and T_4 concentrations are summarized in Table III. Data are presented from both Experiment I (isotopic

TABLE I. EFFECT OF IODIDE INTAKE ON THYROID, PITUITARY, AND BODY WEIGHT OF RATS

Iodide intake (μg/day)	Body weight (g)	Thyroid weight (mg%)	Pituitary weight (mg%)
1	333±30[a]	18.00±5.9	3.41±0.25
3	315±31	6.53±0.95	2.71±0.59
10	342±42	4.86±0.63	2.96±0.30
50	325±33	4.72±0.71	3.07±0.25
100	340±26	4.53±0.41	2.85±0.30

[a] All values are mean ± SD.

TABLE II. TISSUE AND URINE LEVELS OF IODINATED COMPOUNDS IN RATS FED VARIOUS DIETARY IODIDE LEVELS.

	Iodide intake (μg/day)		
	10	50	100
Brain			
Total I	3.3±0.9[b]	5.6±0.5	6.2±0.7
Iodide	0.6±0.4	1.9±0.6	3.5±0.7
T_4	2.5±0.3	3.6±1.0	2.7±1.9
T_3	1.5±0.5	1.6±0.5	1.3±0.3
Heart			
Total I	9.0±0.3	26.4±1.2	32.7±1.1
Iodide	3.2±1.1	18.6±3.7	25.5±5.5
T_4	6.3±2.0	9.1±2.0	7.4±2.3
T_3	1.6±0.8	2.1±1.1	1.5±0.9
Liver			
Total I	26.3±3.0	56.1±1.5	70.3±12.2
Iodide	5.6±2.1	20.8±10.7	36.8±14.6
T_4	25.6±3.9	40.0±16.6	37.2±11.5
T_3	4.6±1.1	8.8±3.1	8.8±2.8
Skeletal muscle			
Total I	3.71±0.40	11.86±1.07	14.8±0.3
Iodide	2.00±0.64	8.60±2.1	13.0±1.5
T_4	2.31±0.76	2.54±0.64	2.1±1.0
T_3	0.58±0.27	0.51±0.58	0.43±0.6
Kidney			
Total I	29.7±3.1	69.0±19.6	79.1±13.2
Iodide	6.0±3.2	33.2±9.0	43.6±18.8
T_4	22.8±3.9	35.2±4.7	28.9±9.6
T_3	9.1±2.7	14.5±9.0	9.9±4.1
Urine			
Iodide	4.1±1.3[a]	21.8±7.2	40.5±13

All values are mean ± SD.
[a] μg/24 hr
[b] ng/g tissue, wet weight.

equilibrium) and Experiment II (radioimmunoassay methods). Maximal thyroidal T_4 and T_3 were observed when iodide intake was 10–50 μg per day. The one exception to this was the observation that in Experiment II the thyroidal T_3 was maximal in the 3 μg group. Serum T_3 and T_4 in both experiments using the different analytical methods were maximal at an iodide intake no greater than 3 μg/day.

Table IV shows serum and pituitary concentrations of TSH. At 1 μg/day iodide intake, serum TSH was highest and pituitary TSH content lowest. When the iodide intake was increased to 3 μg/day, a sharp decrease in serum TSH and an increase in pituitary TSH was noted. As iodide intake increased to 10, 50, and 100 μg/day, there was no further change in either serum or pituitary TSH.

Discussion. Increases in dietary iodide within the range of 10–100 μg/day result in an almost parallel increase in total iodine in peripheral tissues, serum and urine. When the iodinated compounds were separated by chromatography it was observed that the component mainly responsible for the increase was inorganic iodide. These finds are in agreement with data reported by Nagataki *et al.* (9) in which they found a linear increase in plasma iodide in rats treated with progressively increasing doses of iodide for 10 days. With few exceptions, when hormone concentrations were calculated they were remarkably constant throughout the entire 10–100 μg/day range of iodide intake. It is logical to expect that within a given range of iodide intake there would be proportionate increases in hormone synthesis and secretion. The range over which this increase occurs is apparently quite narrow, since the data from previous work (1) and the data of the current study indicate an iodine intake of 1 μg/day results in extremely low hormone concentrations in tissues, whereas maximal tissue concentrations are generally reached at an iodide intake no greater than 10 μg per day. Simon (10) employed isotopic equilibrium methods and reported increases in rat plasma organic iodine when animals were maintained on 50 μg iodide/day as compared to values obtained from rats on 5 μg iodide/day. Our values for plasma organic iodine in the 50 μg group calculate out to be almost identical to those he reported, but our 3 μg group did not have plasma values different from our 50 μg group (Table III). The reason for the difference is not apparent.

If we examine the control values for tissue hormone concentrations of our previous work (1) and the values for the current 10 μg groups we see essentially no change. On this basis it may be that maximal concentrations are reached at even lower levels of iodide intake. The data of Experiment II where serum and pituitary TSH and serum T_4 and T_3 are measured would seem to bear this out; all values reached a plateau when iodide intake was 3 μg/day (Tables III and

TABLE III. COMPARISON OF SERUM AND THYROIDAL T_4 AND T_3 BY ISOTOPIC EQUILIBRIUM AND RADIOIMMUNOASSAY.

	Assay method	Iodide intake (μg/day) 1	3	10	50	100
Thyroid[a]						
T_4	I.E.	(1.7 ± 0.9)*	(134 ± 37)	251 ± 46	331 ± 47	346 ± 65
	RIA	1.6 ± 2.5	240 ± 57	345 ± 84	442 ± 134	350 ± 83
T_3	I.E.	(0.98 ± 0.4)	(21.4 ± 5.6)	26.7 ± 8	31.7 ± 8	32.1 ± 9
	RIA	0.34 ± 0.31	36.0 ± 8.1	34.8 ± 6.8	35.0 ± 12	30.1 ± 8
Serum						
T_4[b]	I.E.	(1.8 ± 0.5)	(307 ± 8)	3.9 ± 1.2	4.5 ± 1.6	3.2 ± 1.9
	RIA	1.6 ± 0.8	5.8 ± 1.1	6.2 ± 0.8	5.8 ± 2.1	6.7 ± 1.5
T_3[c]	I.E.	(100 ± 52)	(43 ± 41)	99 ± 66	51 ± 143	Not detectable
	RIA	68 ± 20	102 ± 15	92 ± 21	93 ± 39	96 ± 26

* All values are mean ± SD.
[a] ng/mg tissue, wet weight.
[b] μg/100 ml.
[c] ng/100 ml.
( ) data previously reported (1).

TABLE IV. SERUM AND PITUITARY TSH IN RATS MAINTAINED ON VARIOUS DIETARY IODIDE LEVELS.

Iodide intake (μg/day)	Serum[a]	Pituitary[b]
1	2924 ± 2272*	88 ± 52
3	374 ± 189	144 ± 36
10	245 ± 169	133 ± 103
50	247 ± 182	106 ± 39
100	329 ± 148	139 ± 52

* All values are mean ± SD.
[a] ng RTSH-RP-1/ml.
[b] ng RTSH-RP-1/mg.

IV). Data from the work of Lamas and Escobar (11) show plateau values for serum PBI at an iodide intake of 10 μg/day. No data were available for iodide intakes between 1 and 10 μg/day.

Exceptions to the generalization that we get a stabilized thyroid hormone content in the body at an iodide intake of somewhere near 3–10 μg/day might be with liver (Table II) and thyroid (Table III), where stable levels are not reached until iodide intake is 50 μg/day. It must be recognized that liver has functions quite different from those of body tissues in general and its additional role in the enterohepatic circulation of thyroid hormones may provide a partial explanation. With respect to the thyroid, Lamas and Escobar (11) obtained results similar to ours through a range of iodide intake up to 30 μg/day. This higher plateau level may not be physiologically significant, i.e., the increase in gland hormone content with an increase in iodide intake may not be accompanied by a parallel increase in secretion rate at high iodide intakes Experiments are underway to examine this relationship in more detail.

From Table III we see there is generally good agreement between the isotopic equilibrium and RIA methods of estimating hormone concentrations. Serum T_4 values, however, were consistently higher with the RIA method. If there is significant artifactual deiodination of T_4 during chromatography as demonstrated by Taurog (12) then this could provide the answer. We have no way of evaluating the current data to test this possibility.

Summary. Isotopic equilibrium and radioimmunoassay methods were used to evaluate the effects of increases in iodide intake on tissue and serum concentrations of thyroid hormones. Within the range of iodide levels used total iodine in peripheral tissues and serum increase directly with iodide intake but this change is mainly due to an increase

in inorganic iodide. It is concluded that increases in tissue thyroid hormone concentrations occur within a relatively narrow range of iodide intake and maximal concentration occurs at an iodide intake of 3–10 μg/day.

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