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Biologically Available Iodine in Goitrogenic Diets (42050)

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Abstract. Eight different sources of low-iodine diet (LID) were tested in mice over 14 years. The available iodine in each diet was measured by isotopic equilibration. Commercially prepared Remington diets contained 6.8 to 69.3 ng available iodine/g, and the results were usually different from shipment to shipment. Some samples produced greatly enlarged thyroids. The Remington diets from two sources were occasionally low in iodine but produced little thyroid enlargement. Between 1977 and 1980 only one shipment of Remington diet was found to contain less than 10 ng available I/g, and it resulted in large goiters. Since 1980 other compositions of LID have been used, but they caused additional abnormalities during breeding or chronic feeding. A low-iodine wheat diet produced goiter in mice more readily than in rats. In the course of testing for unavailable forms of dietary iodine, it was found that only 34.2% of thyroxine iodine was available to the thyroid iodine pool of mice. It is concluded that unidentified nutritional deficiency or dietary contaminants can alter the goitrogenic response to restricted iodine intake. Furthermore, at least one natural form of potential dietary iodine is incompletely available to mice.

Studies in iodine metabolism frequently require a reproducible low-iodine diet (LID), and for many years this requirement was met by the commercially available Remington diet (1). In 1976 these diets were found to contain increased quantities of iodine and to be erratically goitrogenic. It became necessary to analyze each preparation of the diet to confirm the iodine content. Later, Okamura *et al.* (2) showed that one commercial Remington diet was low in iodine but poorly goitrogenic. The present report shows the biologically available iodine and goitrogenic properties of commercially available LID in the United States during the past 14 years. This study also shows that some potential forms of dietary iodine may not be available to the thyroid of mice.

Methods and Materials. Purina Laboratory Chow served as a basal diet. It was obtained from Ralston Purina Company, Checkerboard Square, St. Louis, Missouri. This diet is generally available, and it supports reasonable growth; but it contains a large excess of iodide.

Remington diet (1) contained yellow corn meal, 78%; wheat gluten, 18%; brewers yeast, 2%; $CaCO_3$, 1%; NaCl, 1%. It was purchased, usually in 100-lb lots, from the following sources: (a) General Biochemical Company which changed to Teklad in 1974; (b) Teklad Mills, Division of ARS/Sprague–Dawley, P.O. Box 4220, Madison, Wisconsin; (c) Nutritional Biochemical Corp., changed to ICN, approximately 1975; (d) ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, Ohio; and (e) U.S. Biochemical Corp., Box 22400, Cleveland, Ohio.

A soy flour diet was obtained from Theracon, Inc., Box 1493, Topeka, Kansas. It was supplied as Low Iodide Scan, a dog food, and it was composed of purified soy flour, 31.5%; corn starch, 15.8%; glucose, 15.8%; corn oil, 15.8%; cellulose, 13.1%; mineral mixture, 5.2%; vitamin mixture, 2.5%; choline chloride, 0.026%; DL methionine, 0.26%. The iodine content of this diet ranged from 9.0 to 36.0 ng/g from 1974 through 1981 and it supported good growth and breeding of the animals until 1979 when the source of soy flour was changed and hind limb paralysis occurred in many neonatal animals.

Low-iodine wheat was purchased as shredded wheat biscuits, prepared by Nabisco Brands, East Hanover, New Jersey. This cereal was purchased five times between 1982 and 1984 in cases of 12 boxes each. The composition was listed as protein, 8.47%; carbohydrate, 80.5%; fat, 4.2%; fiber, 2%. The available iodine content was measured

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in 10 different shipments, as described in this report, and was found to average 6.2 ng I/g (1 SEM ± 0.44 ng I/g). As a sole dietary source, wheat alone was not nutritionally adequate, but it contained less iodine than any other dietary material tested and was satisfactory for acute experiments.

Weanling male mice (CD-1) and rats (CD), from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, were depleted of iodine by feeding the soy flour diet with 1% potassium perchlorate in the drinking water for at least 10 days. When perchlorate was stopped, the animals were fed the ^{125}I-labeled diets to be studied.

The diets for analyses were prepared in a dry powdered form and mechanically mixed with an equal weight of distilled water containing carrier-free ^{125}I as sodium iodide; the final product contained 25 μCi (1.5 ng) ^{125}I/kg of dry diet. The thick, pasty material was transferred to paper or plastic cups, covered, and frozen. One cup, containing 2 or 3 days supply, was kept in a refrigerator at 5 to 6°C, and each day the animals were given distilled water *ad libitum* and fed the fresh, pasty diet in an open container. When different diets were tested simultaneously, carmine red, which contained no detectable iodine, was added at 50 mg/kg diet to minimize errors of identification. The animals were kept in clean, wire-bottom cages and, to avoid iodine contamination, they were fed distilled water and kept in a separate room from laboratory animals fed normal diets. The excreta were disposed of according to the regulations of the local radiation control office.

In vivo neck monitoring of ^{125}I was performed in some animals every 3 to 4 days to confirm the accumulation of iodine in the thyroid. After feeding the labeled diet for 18 to 60 days, the thyroids were removed from the mice and the ^{125}I in the thyroids was compared to the quantity of ^{125}I in 1 g of the labeled diet. The radiation counting standard was prepared by measuring a volume of ^{125}I from the same solution used to label the diet and equivalent to the ^{125}I in 1 g of the original labeled diet. The thyroid radioactivity as counts per minute (cpm) was divided by cpm in 1 g diet, measured the same day and with similar geometry, and the result was thereby corrected for radioactive decay. Usually, in the case of low-iodine diets, 4 to 10 mg thyroid glands contained the iodine from 5 to 10 g of diet.

The individual thyroid glands were digested in open vials (1.5 × 5 cm) by an excess (0.2 to 0.6 ml) of 28% iodine-free chloric acid (3) at 100 to 120°C for 30 min on a sand bath, taking care to avoid fuming. After the glands were digested, the solutions were made alkaline by the addition of 50 to 150 μl of 14 *N* sodium hydroxide and each solution was tested with pH paper. Radioiodine was not lost during chloric acid digestion or after pH was greater than 9.0.

The alkaline solutions were diluted with 1 to 5 ml water, and the probable iodine concentration was kept within the most accurate range of the chemical analytical procedure. Fifty to one hundred microliters of the alkaline solutions was analyzed for ^{125}I and compared to a radiation standard containing the ^{125}I of 1 g of diet. The total chemical iodine concentration was measured by the method of Benotti *et al.* (3). The method had a sensitivity limit of 2 to 3 ng/ml. The concentration of biologically available iodine in a diet was calculated as follows:

$$\frac{\text{ng I}}{\text{g diet}} = \frac{\text{cpm}}{\text{g diet}}\left[\frac{\text{ng I}}{\text{ml digested thyroid}} \div \frac{\text{cpm}}{\text{ml digested thyroid}}\right].$$

In some experiments iodide recovery tests were performed using Thericon Low-Iodine diet mixed with measured quantities of $Ca(IO_3)_2$, KIO_3, or iodinated compounds to be studied. As an example of poorly available dietary iodine, a small quantity of sodium thyroxine (T_4) pentahydrate was added to a basal low-iodine diet (35.6 μg of the T_4 salt/kg diet). The T_4 was dissolved in the same water solution used to add ^{125}I label.

Results. Isotopic equilibration was utilized to measure the available iodine intake of the test animals. The method depended upon initial depletion of the iodine pools within weanling mice followed by continuous ingestion of the ^{125}I-labeled diet for 18 to 60 days. The effectiveness of initial iodine depletion by feeding $KClO_4$ was proven by feeding 15 weanling mice 1% $KClO_4$ in their drinking

water and a low-iodine diet for 10 days. The thyroids were pooled in groups of five, digested, and analyzed for total iodine. The three groups of five glands each showed less than minimal detectable iodine (2 to 3 ng/ml) or less than 0.6 ng iodine per depleted thyroid.

When a diet was tested, the $KClO_4$ was discontinued after 10 days, and the iodine-labeled diet was fed continuously for 18 to 60 days. The thyroids were usually analyzed separately and usually contained 20 to 1000 ng iodine, labeled with ^{125}I.

Diets which contained very little iodine caused the mice to remain in positive iodine balance for more than 60 days (illustrated by the controls in Fig. 1). If chemical iodine contamination occurred during an experiment, it was shown by flattening of the thyroid accumulation curves. The thyroid specific activity ($^{125}I/^{127}I$, corrected for radioactive decay) was constant from the 18th day of positive iodine balance; this was shown by feeding 12 mice the very low-iodine wheat cereal, labeled with ^{125}I, and by measuring dietary iodine after 18 days and again after 51 days. The ^{125}I accumulation was continuously positive like that of the control mice in Fig. 1. Using the methods for thyroid and dietary analyses as described here, the iodine in two pooled samples of thyroids on the 18th day after beginning ^{125}I was 5.5 ng I/g of diet. Four separate thyroids on the 51st day showed 5.0 ± 0.4 ng I/g diet. Consequently, as iodine accumulated in the thyroid from this extremely low-iodine diet, radioactive iodine and nonradioactive iodine increased with constant specific activity.

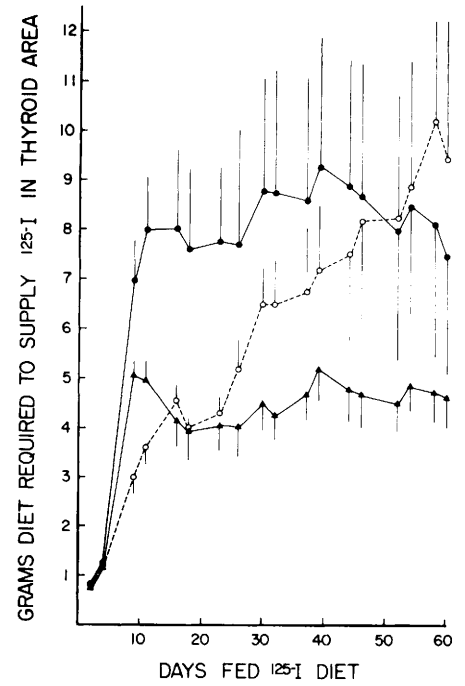

FIG. 1. Accumulation of ^{125}I in thyroid area of mice fed labeled low-iodine diets with and without added sources of iodine. Control basal diet (total iodine, 16.0 ng I/g), ○; basal diet plus 35.6 ng sodium thyroxine pentahydrate/g (total iodine, 35.9 ng I/g), ●; basal diet plus KI (total iodine, 39.6 ng I/g), ▲. The points show the means and SEM for five animals per group. Measurements were made by external gamma counting over the neck and compared to a standard, equivalent to the ^{125}I in 10-g diet.

To test the recovery of added iodine, increments of KIO_3, or $Ca(IO_3)_2$, were added to moderately low-iodine diets (Table I). Potassium iodate is a good primary standard for iodine and the iodate is rapidly converted to iodide *in vivo* (4). The measured recoveries of iodine from iodate were 104 to 109%; the recovery of added iodine from KI was too high by 3.6 ng/g diet or 118% of the added iodide. This discrepancy cannot be accounted for.

Some iodine compounds in a diet may appear in the total chemical analysis of a diet but may not be 100% biologically available as iodide. Thyroxine iodine is an example; it is incompletely absorbed, and traces of thyroxine may be present in most animal products. To test the availability of thyroxine iodine, 35.6 ng of sodium L-thyroxine pentahydrate, containing 19.9 ng iodine, was added to each gram of a labeled diet which initially contained 16.0 ± 0.4 ng iodine and 25 nCi of ^{125}I per gram. After the addition, there should have been 16.0 + 19.9 or 35.9 ng I/g diet. This diet was fed to six weanling mice previously deleted of iodine. Results (Table I, Fig. 1) were compared to other mice fed the same labeled basal diet with and without a similar dose (20 ng I/g diet) of added iodide as KI. Radioiodine was measured in their necks periodically, and they were killed 60 days after initiation of the labeled diet (Fig. 1). In this case, the

TABLE I. RECOVERY OF SMALL INCREMENTS OF IODINE IN DIET[a]

Iodine added to diet		No. mice	Initial iodine in diet[b] (ng I/g[c])	Total iodine in diet (ng I/g)	Measured total available iodine in diet[b] (ng I/g[c])	% of added iodine equilibrated with thyroid iodine (%)[d]
ng I/g	Source					
30.0	$Ca(IO_3)_2$	5	34.9 ± 1.9	64.9	66.8 ± 1.5	106
52.0	KIO_3	9	37.0 ± 0.8	89.0	91.0 ± 2.5	104
60.0	$Ca(IO_3)_2$	5	34.9 ± 1.9	94.9	98.6 ± 2.8	106
90.0	$Ca(IO_3)_2$	5	34.9 ± 1.9	124.9	130.7 ± 3.1	106
1000.	$Ca(IO_3)_2$	5	21.4 ± 2.0	1021.	1115. ± 5.4	109
19.9	Thyroxine	6	16.0 ± 0.4	35.9	22.8 ± 0.6	34.2
20.0	KI	7	16.0 ± 0.4	36.0	39.6 ± 1.2	118

[a] Thericon low-iodine diet.

[b] $\frac{\text{ng I}}{\text{g diet}} = \frac{\text{cpm}}{\text{g diet}} \left[\frac{\text{ng I}}{\text{ml digested thyroid}} \div \frac{\text{cpm}}{\text{ml digested thyroid}} \right]$.

[c] Mean ± 1 SEM; $n = 5$ in each case except where indicated.

[d] {[(Measured total available iodine) − (initial diet iodine)] ÷ (iodine added to the diet)} × 100.

radioactive diet was fed for 60 days to ensure isotopic equilibrium between dietary iodine and the iodide derived from breakdown of administered T_4. Figure 1 shows a rapid accumulation of radioiodine in the neck of the animals fed T_4 for 10 days. There was little further change the following 50 days. On the 60th day the mean (±I SEM) iodine in the thyroid of mice fed T_4 was 171 ± 8.0 ng, which was similar to the thyroid iodine in mice fed KI (179 ± 9.8 ng), but the radioactivity in the thyroids of the animals fed T_4 was greater (Fig. 1). The difference suggests that the available iodine had a higher specific activity in the mice fed T_4. The total iodine concentration in the diet supplemented with KI was very close to that in the diet with T_4 (36 ng/g vs 35.9 ng/g), but the thyroid retention of radioiodide was so different between the two groups (Fig. 1) that it was concluded that a large fraction of the T_4 iodine was not available as iodide in the thyroid of mice fed T_4. The data on T_4 could be accounted for if only 34.2% of thyroxine iodine was available to the thyroid as iodide in the mice and 65.8% did not enter the thyroidal iodide pool.

After feeding either T_4 or KI, the accumulation of approximately 150 to 200 ng of iodine in the thyroids reduced the rate of accumulation of thyroid iodine compared to controls. In the case of T_4 feeding, the endogenous TSH was probably inhibited by the dietary T_4 in addition to increased thyroid hormone from added iodine. The 36 ng I/g diet fed to mice receiving KI was evidently enough iodide to increase thyroid hormone formation and partially inhibit the thyroid accumulation of iodine.

Figure 2 shows the available dietary iodine in different low-iodide diets. The iodine concentrations are compared to thyroid sizes in rats fed LID obtained from different sources during 1970 through 1982. In general, the thyroid sizes were inversely related to the iodine content if the diets contained less than 20 ng I/g. However, there were exceptions where diets contained 13 to 20 ng I/g and were only weakly goitrogenic. Examples of Remington diets which were low in iodine but weakly goitrogenic were found during 1971, 1974, 1975, and 1979. Since 1976 no Remington diet has been found to contain less than 8.7 ng I/g. We have found the least iodine in the wheat cereal; the mean and standard error of 10 analyses of shredded wheat were 6.2 ± 1.4 ng I/g cereal, and the thyroids of mice fed this cereal for 40 to 60 days averaged 112 ± 16 mg/100 g body wt. Weight gain was subnormal, but the mice survived up to 6 months when only wheat and water were fed, with or without added iodine.

Rats were studied less extensively than mice, but rats survived and reproduced when fed only the wheat diet and distilled water.

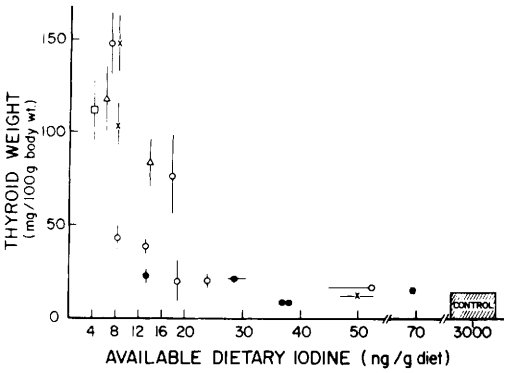

FIG. 2. Thyroid weights in mice fed different low-iodine diets for 40 to 60 days (see Table II). The low-iodine (Remington) diets were obtained from the following sources: Teklad, ●; General Biochemical Corp. (changed to Teklad in 1974), △; ICN or Nutritional Biochemical Corp., ○; U.S. Biochemical Corp., X. Whole wheat cereal was from Nabisco, □. Controls were fed different shipments of Purina Laboratory Chow. Points show the means and standard errors of the means. The number of animals tested for each point are shown in Table II. Nearly all the standard errors of the iodine determinations were smaller than the diameters of the points.

Body and thyroid growth of rats was more impaired than in the case of mice. Sixty days after weaning to the wheat diet, 16 male rats weighed 111 ± 2 g each (31% of normal) and their thyroids weighed 16 ± 0.8 mg, or less than the thyroids of normal rats of the same age.

Discussion. This report describes erratic goitrogenesis from commercially available Remington diets during the past 14 years. These observations extend and confirm the studies of Okamura *et al.* (2) which showed that one commercial preparation of Remington diet produced small goiters when the iodine content of the diet was only 13 ng I/g diet. They also showed that the same Remington diet with or without added iodine caused abnormally elevated concentrations of serum T_4 and T_3. In further studies, they (5) showed that unidentified nutritional deficiency in that Remington diet reduced the uptake of serum T_3 by the tissues of rats without changing oxygen consumption. This phenomenon is still unexplained. The limited

TABLE II. BIOLOGICALLY AVAILABLE IODINE AND GOITROGENESIS OF COMMERCIAL REMINGTON DIETS FED 40–60 DAYS

Year	Manufacturer[a]	No. animals	Diet I (ng/g)[b]	Thyroid (mg/100 g body wt)[b]
1970	GBC	5[d]	14.0 ± 1.7	83.8 ± 13.8
1971	NBC	3[d]	8.6[c]	42.7 ± 7.9
1972	NBC	5	53.0 ± 8.9	18.4 ± 2.4
1973	GBC	3	6.8 ± 1.1	119.4 ± 19.7[e]
1974	TEK	5	12.3[c]	22.7 ± 3.2*
	TEK	8	38.0 ± 0.85	11.8 ± 0.49
1975	ICN(NBC)	3	24.0 ± 0.58	20.8 ± 2.8
	ICN(NBC)	6	7.8 ± 0.77	147.8 ± 17.5
	ICN(NBC)	3	12.6 ± 0.70	36.2 ± 2.9*
	TEK	8	37.7 ± 0.84	11.8 ± 0.49
1976	USB	6	8.7 ± 0.32	105.3 ± 12.5
	TEK	4	29.0 ± 4.0	21.0 ± 1.2*
1977	USB	4	8.7 ± 0.20	144.8 ± 15.3
1978	ICN	3	17.5 ± 1.2	77.7 ± 22.9
	USB	6	50.0 ± 3.8	13.3 ± 0.57
	TEK	3	69.3 ± 0.29	15.5 ± 1.7
1979	ICN	5	18.7 ± 0.53	21.6 ± 4.2*

[a] USB = US Biochemical Corp., Box 22400, Cleveland, Ohio. GBC = General Biochemical Co., changed to Teklad, approximately 1975. ICN = ICN Nutritional Biochemicals, 26201 Miles Rd., Cleveland, Ohio. NBC = Nutritional Biochemicals Corp., after 1975 name changed to ICN. TEK = Teklad Test Diets, Madison, Wisc. Controls—fed Purina Lab. Chow from Ralston Purina Co., Checkerboard Square, St. Louis, Mo.

[b] Mean ± SE.

[c] Pooled samples.

[d] Rats; all others are mice.

[e] Underline marks largest goiters.

* Thyroid relatively small with diet of low-iodine content.

observations presented here show that rats fed a wheat diet may be more susceptible than mice to the lack of nutritional growth factors.

The present study presents a method for measurement of the biologically available iodine to which experimental animals are exposed during nutritional studies. The method involved initial depletion of iodine pools in weanling mice, followed by continuous ingestion of ^{125}I-labeled diets for 18 to 60 days. Finally, the thyroids of the mice were removed and analyzed for chemical and radioactive iodine; the specific activity of thyroid iodine was measured and it was considered equal to the specific activity of the available dietary iodine. Mice were studied during the ingestion of diets containing 5.0 to 3000 ng I/g diet.

Only 34.2% of the iodine of dietary T_4 was available to equilibrate with the thyroid iodine pool of iodine-deprived mice. This result can be compared with the observation of Hall *et al.* (6) who showed that only 60 to 64% of dietary T_4 was absorbed from the intestine of normal rats. Therefore, if animal products should be incorporated into experimental diets, there would probably be traces of T_4 in the diet, and the T_4 iodine would probably appear as part of a total elemental analysis of the diet; but only 34.2% of the T_4 iodine would be available to the thyroid iodine pools. It is reasonable to suggest that there may be natural forms of dietary iodine, other than T_4, which are not readily available as iodide.

It is concluded that dietary materials undergo significant contamination or changes in their iodine content, and, to study iodine deficiency, the total available iodine in the environment of experimental animals should be studied. Even so, other nutritional deficiencies or contamination in natural products regulate the degree of goitrogenesis resulting from limited iodine intake. These factors in cereal diets are unidentified.

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