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The Curative Action of Iodine on Soybean Goiter and the Changes in the Distribution of Iodoamino Acids in the Serum and in Thyroid Gland Digests

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Rats were fed iodine-deficient rations (0.7–2.3 μg. iodine/100 g. diet) based upon raw soybeans, solvent-extracted soy flour, isolated soy proteins, or soybean infant formulas with or without added iodide.

Significant enlargement of the thyroid gland occurred on the iodine-deficient rations in 1 or 2 weeks. The addition of 160 μg. iodine as KI/100 g. diet caused the hypertrophied gland to return to normal size in 2 or 3 weeks.

Although the lack of iodine is the principal cause of soybean goiter, raw soybeans, which contain more iodine than either solvent-extracted soy flour or glycinin, produce greater thyroid hypertrophy. This observation suggests that raw soybeans have a goitrogenic activity (goitrogen?) which is removed or destroyed during processing.

The methodology used to estimate the iodoamino acids in serum and in thyroid digests is discussed. Evidence is presented that neither thiouracil nor propylthiouracil, added as a preservative to serum, is responsible for the reported presence of appreciable quantities of iodotyrosines in rat and human sera.

The quantities and distribution of the iodoamino acids in the sera and in digests of the thyroid glands were used as additional criteria to study the changes produced by soybean goiter and its prevention by the inclusion of KI in the iodine-deficient regimens.

Besides the very marked decrease in organic iodine in the serum and in the thyroid glands of the animals on the iodine-deficient rations, the ratios of iodotyrosines to iodothyronines differed from those found in rats receiving soybean diets with added KI or from those on the usual laboratory foods. Thus the ratio of iodotyrosines to iodothyronines in the sera of animals on iodine-deficient diets is approximately 1:1, while it is in the range of 1:2 or 1:4 in the sera of animals containing an adequate quantity of KI. The situation with respect to the distribution of iodoamino acids in digests of the glands is reversed from that of the serum. In this case, the ratio of iodotyrosines to iodothyronines is much higher in thyroid glands of euthyroid rats than in the glands from iodine-deficient animals. Tentative explanations of these changes in the distribution of iodoamino acids are offered.

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INTRODUCTION

The recognition that certain foods interfere with the normal metabolism of iodine began with the finding of Chesney *et al.* in 1928 (1) that rabbits fed exclusively on cabbage developed large goiters. These results were soon confirmed in other laboratories, and a number of foodstuffs which were found to be goitrogenic included other members of the *Brassicae* such as cauliflower, brussels sprouts, turnips, and their seeds [cf. (2, 3)].

Sharpless (4) in 1938 reported that soybeans produce a goiter in rats which could be completely reversed by the administration of iodide. Sharpless' results have been confirmed by a number of investigators [cf. (3)]. However, if soybean goiter is treated with excessive quantities of iodide, iodine thyrotoxicosis may develop [cf. (3)]. In contrast to the studies on soybeans, McCarrison (5) and Kennedy and Purves (6) were unable by feeding iodide to reverse completely the goiter produced in rats by feeding the seeds of various members of the *Brassicae* family. The differences in the results of the administration of iodide on the goiters produced by the feeding of *Brassicae* seeds and soybeans appear to have thrown some doubt on the efficacy of iodide as a preventative and curative agent for goiter produced by feeding soybeans.

The objects of this investigation were to ascertain the goitrogenicity of several varieties of raw soybeans, of isolated soy proteins, and of several soybean infant foods as well as the effects of the addition of iodide to soybean diets. The rate at which the thyroid gland of the rat responded to iodine lack or to its administration was also studied. Besides the effect of the diet on the weight of the thyroid glands, the protein-bound iodine (PBI) and the distribution of the iodoamino acids in the serum and the thyroid glands of the rats were used as additional criteria.

TABLE I

COMPOSITION OF DIETS

All animals were also given three drops weekly of an oil containing 4000 units of vitamin A, 800 units of vitamin D, and 4.0 mg. of *d*-α-tocopherol acetate/ml. Deionized water, presumably devoid of iodide, was given *ad libitum*.

24% (w/w) protein diet	
	g.
Soybeans, raw	60.0
Soy oil	17.0
Dextrose	5.0
Corn sirup solids	5.0
Sucrose	11.0
$Ca_3(PO_4)_2$	1.5
NaCl	0.5
Ferric citrate	0.12
Vitamin mixture	0.44
Vitamin mixture	
	mg.
2-Methyl-1,4-naphthoquinone	1.0
Thiamine.HCl	0.8
Riboflavine	1.6
Pyridoxine.HCl	0.8
Niacin	4.0
D-Calcium pantothenate	4.4
p-Aminobenzoic acid	4.0
Inositol	21.6
Folic acid	0.1
Vitamin B_{12}	0.0033
Biotin	0.03
Choline bitartrate	400.0
18% (w/w) protein diet	
	g.
Glycinin (Promine)	22.0
Soy oil	30.0
Corn sirup solids	5.3
Dextrose	5.3
Sucrose	33.4
Minerals, U.S.P. XIII No. 2	4.0
Ferric citrate	0.12
Vitamin mixture	0.44

EXPERIMENTAL

DIETS

Because of the nature of the problem, the diets employed were divided into two major categories. Whole raw-milled beans or commercial soybean infant foods were fed at a level of 24% (w/w) protein. Those diets based upon isolated proteins (glycinin, casein) or defatted soy flour contained sufficient protein to contribute 15% of the total calories, corresponding to 18% of the ration by weight. A typical diet in each series is given in Table I.

FEEDING EXPERIMENTS

Five or more male rats weighing approximately 50 g. each and an equal number of female rats of

the same initial weight were used for each test unless otherwise indicated. The animals were weighed individually, exsanguinated, and thyroidectomized at the end of 5 or 6 weeks. The serum was removed from the pooled blood of each group as soon as possible after bleeding, and a small quantity (15 mg.%) of thiouracil was added in order to decrease the opportunity for deiodination of the iodoamino acids (7). The serum was kept frozen until processed. The glands from each animal were weighed individually, and those in the separate groups were pooled and stored at −20°C.

In another study designed to investigate the rate of thyroid enlargement and the rate of recovery when KI was added, 84 male weanling rats (average weight 65.4 g.) were started on the soybean diet (24% w/w protein) and 48 rats on a control stock diet (Purina Chow). Six of the experimental animals and four of the control rats were sacrificed each week.

Estimation of Iodine and Iodoamino Acids in Serum and in Thyroid Digests

A. Serum

The serum was thawed, adjusted to pH 3–4 with dilute H_2SO_4, and immediately poured into 10 vol. of boiling 1-butanol during rapid stirring (7). The butanol solution was boiled under reflux for 3 min., cooled rapidly in an ice bath, and filtered to remove the proteins. The precipitate was thoroughly washed with 1-butanol. The combined filtrate and washings were immediately made alkaline with concentrated NH_4OH, and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml. of 0.2 *M* sodium acetate buffer, pH 4.6, containing 0.5% (v/v) Tween 20 (Atlas Powder Co.) and clarified by filtration (8). The insoluble residue was washed with two 5-ml. portions of buffer, and the combined filtrate and washings were passed through a 1 × 5 cm. column of Dowex 50 W-X8, 200–400 mesh, in the NH_4^+ cycle (pH 4.6 with 0.2 *M* ammonium acetate) under slight pressure. The resin was washed twice with 5-ml. portions of sodium acetate buffer and three times with water. In order to remove any particulate matter (Tween, fat globules, etc.), the resin was thoroughly slurried with each addition of the buffer before pressure was applied. Inorganic iodide, chloride, thiouracil, and many other impurities were separated from the iodoamino acids by this procedure.

The iodoamino acids were then eluted from the Dowex 50 W-X8 with 50 ml. of 3.5 *N* NH_4OH. Five drops of a mixture of 0.1% (w/v) phenol red and 0.1% (w/v) thymol blue in ethanol were added to the elutriate, and the solution was concentrated to dryness *in vacuo*.

B. Thyroid Glands

The frozen glands were dropped into 10–20 vol. of hot 1% (v/v) acetic acid and placed in boiling water for 5 min. to inactivate the deiodinases and to facilitate the subsequent digestion of the thyroid proteins. The tissue was ground in a Ten Broeck homogenizer. The proteins were digested with pancreatin (Viokase) at 39°C. in 0.05 *M* Na borate buffer, pH 8.6, prepared from 12.00 ml. of 0.1 *N* NaOH and 50 ml. of 0.1 *M* H_3BO_3 in 0.1 *N* KCl. A trace of Mn^{++} (0.0002 *M*) was added to improve the digestion (9). At the end of the hydrolysis, the digest was adjusted to pH 4.6 and the iodoamino acids were separated from inorganic iodine, peptides, and other amino acids by adsorption on Dowex 50 W-X8 at pH 4.6 as described for serum.

C. Separation of Iodothyrosines from Iodothyronines

The dry elutriate from either sec. *A* (serum) or sec. *B* (thyroid digest) was dissolved in a minimum volume of 1-butanol–1.5 *N* NH_4OH, 315:65 (v/v), and this solution was chromatographed on a 1 × 20 cm. column of cellulose powder (Whatman) which had been previously saturated with the 1-butanol–1.5 *N* NH_4OH solution (10). After the amino acid solution had entered the paper, the column was developed with 20 ml. of the 1-butanol–1.5 *N* NH_4OH mixture and then with 30 ml. of ethanol–1.5 *N* NH_4OH, 3:1 (v/v). The iodothyronines were collected in the fraction beginning with the appearance of the thymol blue and stopping just *before* the phenol red band leaves the column. The iodotyrosines were in the remaining elutriate. The separate fractions were concentrated *in vacuo* to dryness, and the residues were dissolved in 100–200 μl. of ammoniacal methanol (10).

D. Separation of the Iodoamino Acids by Paper Chromatography

Diiodotyrosine (DIT), monoiodotyrosine (MIT), thyroxine (T_4), and triiodothyronine (T_3) were separated from each other in distinct tight spots on one-dimensional chromatograms by developing with 2-butanol–3% (w/v) aqueous NH_3, 3:1 (10).

E. Estimation of Iodoamino Acids

Although the iodoamino acids in the thymol blue and the phenol red fractions (sec. *C*) or after elution of the paper chromatograms (sec. *D*) can be estimated directly by their catalytic action on

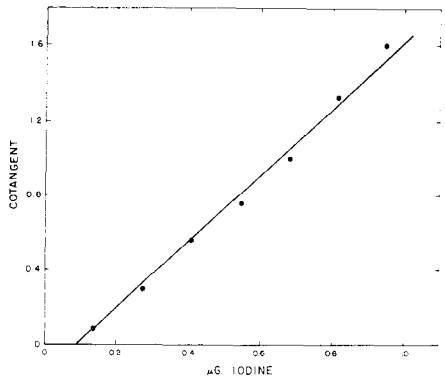

FIG. 1. The rate of fading of the ceric sulfate color is proportional to the quantity of iodine.

the reduction of ceric sulfate by arsenious acid (8, 10), it is preferable to oxidize the organic iodine to elemental iodine and then to reduce the latter to iodide. The usual method for carrying out this conversion of organic iodine to inorganic iodide is tedious and requires rather elaborate equipment. Therefore, the combustion of the sample in an atmosphere of oxygen as described by Schöniger (11) has been adapted to the ultramicro quantities of iodine encountered in this investigation.

The solution containing the iodoamino acids (100–200 μl.) is applied to a 10.5 sq. cm. piece of Whatman No. 50 filter paper in 25-μl. aliquots by means of a micropipet, or an area of the paper chromatogram approximately of this size containing an individual iodoamino acid is burnt in the Schöniger apparatus (11, 12). The flask contains 1.5 ml. of 1% (w/v) aqueous Na_2AsO_3 (8) and 27.0 ml. water to trap and to reduce the resulting iodine to iodide. At the completion of the combustion, the flask is shaken vigorously to scrub the gases, the stopper is loosened and 1.5 ml. of 2% (w/v) ceric ammonium sulfate in N H_2SO_4 is added. The contents are mixed and decanted into a colorimeter tube. The rate of fading of the ceric sulfate color as measured on a recording galvanometer is proportional to the quantity of iodine present (8, 10, 13). The values shown in Fig. 1 are the average points of three replicate determinations.

RESULTS

Table II summarizes the results of the first group of experiments. The ingredients, except for the ferric citrate and vitamins, of Diets 2, 4, 5, 6, 7, and 12 were mixed with an equal volume of water and autoclaved for approximately 15 min. at 126.7°C. The autoclaved diets were then spray dried and the vitamins and iron added. Diets 1 and 10 were dry blended using c.p. grade rather than commercial sources of carbohydrates and fats. The commercial materials apparently contained more iodine than the c.p. reagents.

Numerous attempts were made to determine the iodine contents of the final

TABLE II

EFFECT OF THE ADDITION OF POTASSIUM IODIDE TO A SOYBEAN INFANT FOOD ON THE SIZE AND ON THE DISTRIBUTION OF IODOAMINO ACIDS IN PANCREATIC DIGESTS OF THE THYROID GLANDS OF RATS[a]

Diet No.	Protein source	Iodine content of diet/100 g.	Thyroid weight per animal	Thyroid weight/100 g. of body weight	Thyroidal iodine/kg. of body weight	Ratio of thyroidal iodoamino acids calculated as iodine $\frac{MIT + DIT}{T_4 + T_3}$
		μg.	mg.	mg.	μg.	
10	Soy flour, raw	0.8	110	50	0.7	3.5
1	Soy flour, toasted	0.7	135	63	2.1	6.2
2	Soy flour, toasted, diet suspended in water and autoclaved	2.1	103	54	0.6	3.6
12	Diet 2 + 0.1% w/v thiouracil	2.1	86	78	1.5	3.6
4	Diet 2 + 40 μg.% I as KI	36.2	16	9	19.5	19.0
5	Diet 2 + 80 μg.% I as KI		15	8	20.4	10.0
6	Diet 2 + 160 μg.% I as KI		14	7	20.7	9.0
7	Diet 2 + 320 μg.% I as KI	279	13	6	18.0	7.3
9	Purina Dog Chow	78.6	15	7	15.9	5.6

[a] The iodine determinations (standard deviation ±7% relative) in the diets were carried out in the Endocrinology Laboratories, Slovak Academy of Science, through the kindness of Dr. V. Štole.

TABLE III

EFFECT OF SOYBEANS AND VARIOUS COMMERCIAL SOYBEAN INFANT FOODS ON THE THYROID GLAND AND ON THE DISTRIBUTION OF THE IODOAMINO ACIDS IN THE SERUM AND IN DIGESTS OF THE THYROID GLAND[a]

Diet No.	Protein source	Iodine content of diet/100 g.	Thyroid wt. M	Thyroid wt. F	Thyroid wt./100 g. body weight	PBI in 100 ml. serum[b]	Iodoamino acids in 100 ml. of serum[b] MIT DIT	Iodoamino acids in 100 ml. of serum[b] T_4 T_3	Iodotyrosine iodine/g. thyroid	Iodothyronine iodine/g. thyroid	Ratio of thyroidal iodoamino acids calculated as iodine $\frac{MIT + DIT}{T_4 + T_3}$
		μg.	mg.	mg.	mg.	μg.	μg.	μg.	μg.	μg.	
1A	Raw soy, Lincoln	1.04	64	54	39	0.9	0.68	0.22	2.5 ± .27	1.7 ± .01	1.5
2A	Raw soy, Harsoy	1.45	65	56	37	1.1	0.42	0.68	4.6 ± .25	<1	...
3A	Raw soy, Hawkeye	2.28	72	65	39	0.8	0.10	0.70	1.2 ± .19	<1	...
4A	Raw soy, Clark	1.03	61	55	36	0.8	0.42	0.38	2.5 ± .28	1.1 ± .08	2.3
6A	Soy flour, toasted	0.71	39	31	19	0.7	0.54	0.16	8.1 ± 1.29	1.6 ± .15	5.1
8A	Glycinin (Promine)	0.86	31	24	16	3.2	1.76	1.44	16.1 ± 1.85	3.9 ± .44	4.1
13A	Soy infant foods[c]	2.36	15	16	15	0.7	0.44	0.26	36.8 ± 5.00	9.7 ± .49	3.8
11A	Soy infant food[d] iodized	40.0	15	12	7	4.6	3.04	1.56	106.0 ± 11.4	31.9 ± 4.1	3.3
14A	Soy infant food[e] iodized	42.6	19	13	8	1.5	0.63	0.87	143.0 ± 20.6	19.3 ± 1.9	7.4
12A	11A + 10 μg. I/g. protein	186	14	12	7	2.8	0.84	1.96	196.0 ± 11.2	82.0 ± 6.3	2.4
5A	1A + 10 μg. I/g. protein	196	12	13	8	3.1	0.56	2.54	174.0 ± 8.4	16.4 ± 1.5	10.6
7A	6A + 10 μg. I/g. protein	199	15	14	8	8.3	0.42	7.88	186.0 ± 19.8	34.7 ± 3.6	5.4
10A	Soy infant food[f] iodized	169	13	12	8	4.6	0.28	4.32	133.0 ± 13.6	34.1 ± 3.2	3.9
15A	Dog Chow (Purina)	78.6	15	13	6	1.8	0.61	1.19	191.0 ± 21.0	32.7 ± 2.2	5.4
9A	Casein (Labco vitamin-free)	30	15	13	7	Lost	Lost	Lost	166.0 ± 18.1	34.0 ± 2.8	4.9

[a] The iodine determinations (standard deviation ±7% relative) in the diets were carried out in the Endocrinology Laboratories, Slovak Academy of Sciences, through the kindness of Dr. V. Štole.

[b] Single determinations.

[c] Soyalac, liquid.

[d] Sobee, liquid.

[e] Sobee, powder.

[f] Mullsoy, liquid.

TABLE IV

EFFECT OF THE ADDITION OF KI TO A DIET BASED UPON RAW SOYBEANS (LINCOLN) ON THE DISTRIBUTION OF IODOAMINO ACIDS IN THE SERUM AND IN DIGESTS OF THE THYROID GLAND[a]

Diet No.	1B	5B
Number of animals	55	18
Protein source	Lincoln soybeans	1B + 10 μg. I/g. protein
Iodine content/100 g. diet	1.09 μg.	251 μg.
Average gain in weight per rat in 7 weeks	98.2 g.	95.7 g.
Thyroid weight per animal	63.5 mg.	12.5 mg.
Thyroid weight/100 g. of body weight	42.8 mg.	8.6 mg.
PBI in 100 ml. serum	0.9 μg.	5.5 μg.
Iodotyrosine iodine/100 ml. serum	0.46 μg.[b]	1.7 μg.[b]
Iodothyronine iodine/100 ml. serum	0.46 μg.[b]	3.8 μg.[b]
Iodotyrosine iodine/g. of thyroid tissue	0.35 ± .04 μg.	110 ± 17 μg.
Iodothyronine iodine/g. of thyroid tissue	0.19 ± .01 μg.	9.2 ± 1.8 μg.
Ratio of iodine in iodotyrosines:iodothyronines in the thyroid	1.8	11.9

[a] The iodine determinations (standard deviation ±7% relative) in the diets were carried out in the Endocrinology Laboratories, Slovak Academy of Sciences, through the kindness of Dr. V. Štolc.

[b] Duplicate determinations.

diets, but without success either in our laboratories or in any of the best-known commercial laboratories in the United States. The iodine values shown in Table II were carried out in the laboratory of Dr. V. Štolc, Institute of Endocrinology, the Slovak Academy of Sciences, Bratislava, Czechoslovakia. Although Dr. Štolc did not have any idea of the iodine content of the diets, it is apparent that his results are in excellent agreement with the thyroid weights.

Although the absolute weight of the thyroid glands in the animals on the iodine-deficient regimens (Diets 1, 10, 2, and 12) varied considerably, when the thyroid weights were calculated per 100 g. of final body weight, this variation was reduced. The largest glands were produced by the rats fed soy proteins plus thiouracil.

The quantity of iodine in the thyroid glands/kg. of body weight and the distribution of the iodoamino acids in digests of the glands are also presented in Table II. The goitrous glands contain less than one-tenth the amount of total iodine/kg. of body weight of the normal glands.

Table III summarizes the results obtained on feeding four varieties of unprocessed, raw soybeans, soy flour, soy protein (glycinin), four commercial soybean infant formulas, casein, and dog chow. The effect of the different diets on the thyroid size and the distribution of the iodoamino acids in the sera and the thyroid gland digests are presented. The thyroid weights were obtained from the average weight of six male and six female rats. The PBI and thyroidal iodine values were calculated from the quantity of iodotyrosine and iodothyronine iodine in the elutriates from the cellulose columns and consequently are subject to the errors of the methods used. In those cases where the standard deviation is given, the values are based upon three replicate oxidations. It is seen that the standard deviation of the Schöniger method (11, 12) as adapted to the small quantities of iodine involved in this study is approximately 10% of the absolute value.

Table IV shows that although the addition of iodide to a diet based upon raw, unprocessed soybeans did not improve the rate of growth, there were marked changes in the size of the thyroid gland as well as in the distribution of the iodoamino acids.

Table V summarizes the results obtained when the rats were placed on an iodine-deficient soybean ration (Diet 2) and when this regimen was replaced by the same diet with added KI (Diet 6). The rapid response

of thyroid size to the presence or absence of sufficient iodine is apparent. Although Diet 2 in this experiment did not result in as large goiters as in the earlier experiment (Table II), it was sufficiently goitrogenic to show that the glands reached their maximum size, per 100 g. of body weight, in about 2 weeks and that the thyroid gland regressed to "normal" in 3–4 weeks when the ration was supplemented with iodide.

TABLE V

PRODUCTION OF GOITER ON A SOYBEAN DIET AND ITS ALLEVIATION BY THE ADDITION OF KI

Weeks on diet	Thyroid weight/100 g. of body weight			
	Diet 2	Diet 6[a]	Diet 2	Diet 9[a]
	mg.	*mg.*	*mg.*	*mg.*
0	9.2	—	—	
1	11.6	—	—	7.2
2	16.5	—	—	7.0
3	13.8	13.8[b]	—	6.7
4	15.5	9.1	—	5.6
5	13.8	8.8	—	6.1
6	14.7	7.3	—	5.2
7	14.8	6.3	—	6.0
8	—	6.1	6.1[c]	4.9
9	—	6.7	8.3	4.9
10	—	6.7	10.2	5.7
11	—	5.5	10.7	5.4

[a] Diet 6 is Diet 2 plus 160 μg.% iodide as KI. Diet 9 is the control ration.

[b] KI introduced.

[c] KI removed.

DISCUSSION

GOITROGENICITY OF SOYBEANS

In agreement with the findings of Sharpless (4) and others (14–16), the feeding of soybeans to rats in the absence of added iodide produces goiter. Goiter is also produced when raw or processed soybean meal or isolated soy proteins are fed as the sole source of protein in an iodine-deficient diet. In fact, the wet weight of the thyroid glands doubles or triples in size when the animals are fed an autoclaved diet based upon toasted, solvent-extracted soybean meal. The addition of approximately 0.2 mg. iodine as KI/100 g. diet quickly restores the gland to its normal size (Table V). These findings are in agreement with the majority of papers in the literature. The only exception which came to our attention is the report by McCarrison (17) that the ingestion of 1 g. soybeans daily would produce thyroids three times the normal size even though the growing rats ingested approximately 1.5 mg. iodine/day. The failure of McCarrison to cure thyroid enlargement after feeding KI may be ascribed to a dietary inadequacy other than iodine, to excessive dosage of iodine, or to both. It should be noted that McCarrison fed approximately 100 times the quantity of iodine per day found adequate in these experiments (Table V).

The presence of a true goitrogen in soybeans has not, as yet, been demonstrated. Greer (3) and Shepard *et al.* (18) suggest that the goitrogenicity of soybean diets may be simply the result of iodine deficiency. This may be the case and is, undoubtedly, a major cause of soybean goiter when processed soy products are fed. However, in agreement with Sharpless *et al.* (14), the evidence presented in Table III indicates that raw soybeans produce larger glands than either toasted soy flour (Diet 6A) or isolated soy proteins (Diet 8A) even though there appears to be more total iodine in the raw soy diets.[2] A factor which may contribute to goitrogenicity of raw and processed soybeans is found in the reports of Van Middlesworth (19) and of Beck (20) who observed an increased fecal loss of thyroxine when rats were fed soy.

The absence of thyroid enlargement, when purified casein (Labco vitamin-free) was fed, is in accord with the findings in the literature [cf. (2)] that MIT and traces of DIT are present in milk proteins. It is interesting to note that although the purified casein (Diet 9A) contributed only 30 μg. iodine/100 g. diet, no evidence of goiter is apparent.

IODOTYROSINES IN RAT SERUM

Tables III and IV show the quantities of iodotyrosines and iodothyronines found in

[2] This conclusion is apparently not justified on the basis of the thyroid weights given in Table II but is confirmed by the quantity of thyroidal iodine and the ratio of thyroidal iodoamino acids.

the pooled sera from the rats on each diet. The apparent presence of appreciable quantities of iodotyrosines in serum is in agreement with previous studies on human and rat blood (8, 21) which have been confirmed on human sera by Beale and Whitehead (22) using double isotope dilution chromatography. The ratio of iodothyronines to iodotyrosines in the serum of the rats on Diet 15A, which is the only one that may be considered a usual stock diet, is the same as that which we reported previously (8) for rats reared at the College of Physicians and Surgeons. Kono *et al.* (23) and Dimitriadou *et al.* (24) were unable to find iodotyrosines in human serum. Their failure to do so may be due to differences in the methods of separation of the iodoamino acids from the other constituents in serum employed by these investigators and by ourselves (7, 8, 10).

Kono *et al.* (23) and Dimitriadou *et al.* (24) found that thiourea, thiouracils, and chloride ions reduce the ceric sulfate–arsenious acid reagent on paper chromatograms and that the sulfhydryl compounds reduce this reagent *in vitro* at a rate similar to that of the iodoamino acids (24). Both groups believe that the presence of a thiouracil in our preparations led us to an erroneous conclusion. However, thiouracil, chloride, and iodide are separated from the iodoamino acids by absorption of the latter on Dowex 50W at pH 4.6 (10). The separation of thiouracil from the iodoamino acids was confirmed in the following manner: A large excess (15 mg.) of thiouracil containing approximately 70,000 counts/min. thiouracil-S^{35} was added to 20 ml. serum, and the mixture was processed as described above; over 99% of the thiouracil was removed as indicated by the distribution of S^{35} in the effluent and in the eluate from the ion-exchange column. Paper chromatography of the iodoamino acids in the eluate with 2-butanol–3% (w/v) aqueous NH_3, 3:1 (v/v), on Whatman No. 3 paper (for chromatography) showed the presence of $MI^{127}T$ and $DI^{127}T$ (10) and the complete absence of thiouracil-S^{35}. Although thiouracil (R_{BCP} 0.71–0.76), if present, might be confused on paper chromatograms developed with 2-butanol–3% (w/v) NH_3, with MIT (R_{BCP} 0.64–0.71), its location on the paper is far removed from DIT (R_{BCP} 0.31–0.38). Furthermore, thiouracil is only one-fortieth as effective as sodium iodide in the catalyses of the reduction of ceric sulfate by sodium arsenite under the conditions employed in this study. Kono *et al.* (23) and Dimitriadou *et al.* (24) also report that although thiouracil may move in the iodotyrosine area on paper chromatograms, propylthiouracil (PTU) moves in the iodothyronine area. We have reported the apparent presence of MIT and DIT in human sera either after the addition of thiouracil, PTU, or without the inclusion of any reducing agent (8, 21). Although it was not made clear in the previous publications (8, 21), the great majority of the human sera studied were stabilized with PTU, the rat sera with thiouracil. Furthermore, sera from ten apparently normal human beings as well as sera from the many rats used in this experiment were found to yield a substance, presumably iodine, which catalyzes the ceric sulfate–arsenious acid reagent after combustion of the iodotyrosine fraction. Thiouracil in amounts which ranged from 10 to 50 times that of the iodotyrosines did not reduce the ceric sulfate after combustion.

Because of the small quantity of iodine present in the sera of the goitrous rats, the values given in Table III are based upon single oxidations. The error of an individual determination is approximately 25%. In spite of this error, it is apparent and expected that the PBI of the normal rats is considerably higher than that of the animals with goiter. Differences in the ratios of iodotryosines:iodothyronines in the serum of normal and goitrous animals are less clear cut. However, the average ratio of iodotyrosines:iodothyronines is approximately 1:4 for the normal animals and approximately 1:1 for the goitrous rats. Differences in the distribution of iodoamino acids in the sera of normal and of goitrous rats are confirmed by the data in Table IV. The decrease, both absolute and relative, in the circulating iodothyronines in the goitrous animal may be explained by the assump-

tion that the tissues of the iodine-deficient animal are avid for whatever thyroid hormone is available and consequently remove as much T_4 and T_3 as possible from the circulation. The absolute quantities of circulating thyroid hormone in the goitrous and the euthyroid animals are in the range reported by Whitehead and Beale (25) for hypothyroid and euthyroid individuals.

Iodoamino Acids in Digests of Thyroid Glands

The distribution of iodotyrosines and iodothyronines in pancreatic digests of the rat thyroid glands is given in Tables II, III, and IV. The results are in agreement with the literature (26–28), that thyroid digests contain more iodotyrosines than iodothyronines. The data demonstrate that the absolute quantity of thyroidal iodine per gram of tissue in the goitrous animals is but a small fraction of that in euthyroid glands (Tables III and IV). The ratios of iodotyrosines to iodothyronines vary considerably among the different groups presumably due in large part to the experimental error; however, the results suggest that the iodothyronines may account for a greater proportion of the thyroidal iodine in the goitrous animals than in the iodine-fed group. The shift in this ratio on addition of KI to the raw soybean diets is striking; thus digests prepared from the glands of the animals on Lincoln soybeans (Diets 1A and 1B) show ratios of iodotyrosines:iodothyronines of 15:10 and 18:10 respectively; when KI is included in the diets (5A and 5B), the ratios are 106:10 and 119:10. The results on the chronic iodine-deficient rat differ from the effect of acute stimulation of the thyroid of animals on a diet adequate in iodine with thyroid-stimulating hormone (10 units T.S.H./day/guinea pig). Costa *et al.* (29) found that the iodotyrosine:iodothyronine ratio rose from 3.8 to 9.8 after 10 days' treatment. These changes in the iodotyrosine:iodothyronine ratios may be explained as follows: T.S.H. causes the liberation of T_4 and T_3 at a faster rate than they can be synthesized from MIT and DIT in the gland. The shift in the opposite direction of the iodotyrosine:iodothyronine ratio on chronic iodine deficiency may, likewise, be explained on the assumption that the production of MIT and DIT is limiting due to the lack of iodine in the diet.

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