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In vitro Formation of Free Iodotyrosines and Iodothyronines by Rat Thyroid Lobes or Isolated Follicular Cells.* (30267)

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Roche *et al*(1) have reported that free thyroid hormones can be detected in the medium 18 to 24 hours after addition of radioactive iodine to tissue cultures of young rat thyroid tissue. Kerkof *et al*(2) have also noted that isolated sheep thyroid follicle cells in monolayer tissue culture can release free amino acids, including thyroxine, into the culture medium after prolonged incubation with radioiodine. However, no studies have been reported on the formation and liberation of free iodinated amino acids during short-term *in vitro* incubations of thyroid tissue.

In studies of iodine metabolism of rat thyroid lobes and isolated beef thyroid cells during short-term *in vitro* incubations we have found that free iodothyronines can be detected both in the medium and in the thyroid tissue itself.

Materials and methods. 1. Rat thyroids. Male Sprague-Dawley rats weighing 150-300 g were fed a low iodine diet (LID) containing 30 μg ^{127}I/kg for up to 60 days before autopsy. Two groups were additionally fed 0.15% propylthiouracil (PTU) in the low iodine diet for 10 days. One PTU group was killed at the end of this period; the other was fed LID without PTU for 6 days after autopsy (PTU-withdrawal). At autopsy the thyroid lobes were carefully removed and individually incubated in 50 ml Erlenmeyer flasks containing 4 ml of Earle's balanced salt solution containing 25 μc carrier-free $^{131}I^-$ at 37°C in a shaking water bath. Room air was used as the gas phase. One lobe from each rat was incubated 3 hours and the opposite lobe was incubated 6 hours. At least 2 lobes from different animals were used for the analysis at each time interval. The lobes from animals fed propylthiouracil until autopsy were pre-incubated in 2 fresh changes of Earle's solution for 15 minutes each before being placed in the radioiodine solution.

2. Isolated beef thyroid follicle cells. The isolated thyroid cells were prepared by the method of Tong(3). Approximately 20 μl of cells were suspended in 3 ml of Earle's solution containing 100 μc $^{131}I^-$. Incubations were carried out as with the rat thyroid lobes for a period of 3 hours. At the end of incubation the cells were separated from the medium by centrifugation at 50 *g* for 10 minutes.

3. Analysis of thyroid tissue and media. After separation from the media, the thyroid tissue was homogenized in 1 ml of pH 8.5 phosphate buffer containing 100 μg thiouracil. Chromatography of the homogenates and of the medium before and after digestion with 1% pancreatin for 20 hours was performed in both n-butanol-absolute alcohol-0.5% ammonia (5:1:2) and in butanol-acetic acid-water (4:1:5). The identification of radioactive components was determined by comparing their location with suitable standards added to the chromatographed material at time of application to the paper. The amount of radioactivity corresponding to each iodinated material was determined with a windowless gas-flow strip scanner and quantified by planimetry.

Results. As documented in Table I, no organic radioiodine-labeled materials were found in the medium at the end of 3 hours incubation in any of the groups fed a low

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TABLE I. Chromatographic Analysis of Medium in Which Rat Thyroid Lobes Were Incubated for 3 to 6 Hours.

Group	No. of exp	Pancreatin digestion	% of total medium radioactivity					
			Origin	DIT	MIT	I^-	T_4	T_3
			3 Hours incubation					
LID 10 days	5	(—)	0	0	0	100	0	0
		(+)	0	0	0	100	0	0
LID 16 "	4	(—)	0	0	0	100	0	0
		(+)	0	0	0	100	0	0
LID 60 "	3	(—)	2.9 ± .9	0	0	97.2 ± 2.2	0	0
		(+)	.2 ± .1	.4 ±.1	1.9 ± .6	97.4 ± .7	0	0
PTU	2	(—)	1.8	0	0	98.2	0	0
		(+)	.1	.1	.9	88.8	.1	0
PTU-withdrawal	2	(—)	2.9	0	.4	96.7	0	0
		(+)	.2	.4	1.8	97.6	0	0
			6 Hours incubation					
LID 10 days	5	(—)	4.9 ±1.0	0	0	95.1 ± 1.0	0	0
		(+)	.7 ± .2	.9 ±.3	1.3 ± .3	97.1 ± 1.2	0	0
LID 16 "	4	(—)	1.1 ± .2	0	0	98.9 ± .2	0	0
		(+)	0	.1 ±.01	.5 ± .2	99.4 ± .1	0	0
LID 60 "	3	(—)	14.1 ±2.8	.7 ±.2	1.0 ± .2	76.9 ± 3.2	4.4 ± .7	1.5 ±.7
		(+)	.7 ± .2	2.2 ±.3	8.8 ±2.3	84.2 ± 3.5	2.6 ±1.0	1.6 ±.5
PTU	2	(—)	9.0	.7	.8	88.1	1.3	.2
		(+)	1.0	3.3	7.0	87.2	1.0	.5
PTU-withdrawal	2	(—)	7.5	.2	.4	91.1	.6	.2
		(+)	.3	1.4	3.7	92.3	.7	.5

Results are expressed as ± standard error where results of 3 or more experiments are combined.

iodine diet for 16 days or less. However, by the end of 6 hours, organic radioiodine was found in the medium in all groups fed LID for 10 days or longer. These iodinated components remained at the origin of the chromatograms of the undigested materials of the 10- and 16-day LID groups but separated into diiodotyrosine (DIT) and monoiodotyrosine (MIT) after digestion.

Lobes from rats fed LID for 60 days did not release significant amounts of free thyroxine into the medium during a 3-hour incubation. However, by the end of 6 hours, free thyroxine (T_4), triiodothyronine (T_3), MIT, and DIT were found in the medium. Hydrolysis of the peptide-bound, iodinated material at the origin of all chromatograms apparently liberated only labeled MIT and DIT since the relative proportion of T_4 and T_3 in the medium did not significantly change or decreased slightly after pancreatin hydrolysis whereas the quantity of MIT and DIT markedly increased, in proportion to the decrease of material at the origin. The lobes from animals fed PTU also liberated free iodinated amino acids but to a less marked degree than those from animals fed a low iodine diet for 60 days.

Free radioiodinated amino acids were not found in any thyroid lobes except those from animals fed the iodine-deficient diet for 60 days. In these lobes free MIT, DIT, T_3 and T_4 were detectable (Table II). In the lobes of this group incubated for 6 hours it was of considerable interest that there was more free T_3 than T_4 within the thyroid lobes whereas there was a much greater quantity of free T_4 than free T_3 in the medium. However, more T_4 than T_3 was found in the digests of the thyroid lobes.

Free radioiodinated amino acids were also consistently found in both the medium and cells of the isolated beef thyroid cells at the end of 3 hours incubation (Table III). In only one experiment out of 5 was free T_4 present; in the others the free organic material was primarily MIT. Only rarely could free DIT be found although it was always clearly detectable in both cells and medium after digestion. Triiodothyronine, either pep-

TABLE II. Distribution of Radioactive Iodinated Substances Found in Thyroid Lobes from 60-Day LID Rats Incubated with ^{131}I.

No. of exp	Incubation time (hr)	Pancreatin digestion	% of total medium radioactivity					
			Origin	DIT	MIT	I^-	T_4	T_3
3	3	(—)	90.5 ± 1.8	.3 ± .1	.5 ± .1	8.7 ± 1.7	0	0
		(+)	4.4 ± 1.2	23.1 ± 3.0	52.1 ± 7.9	14.1 ± 3.9	4.2 ± .2	2.0 ± .1
	6	(—)	94.2 ± .7	.5 ± .1	.9 ± .1	2.4 ± .1	.8 ± .1	1.2 ± .2
		(+)	4.9 ± 1.1	23.7 ± 1.8	48.5 ± 5.5	5.3 ± 1.3	10.8 ±1.3	6.8 ± .1

Results are expressed as ± standard error where results of 3 or more experiments are combined.

tide-bound or free, was never detected in either cells or medium during a 3-hour incubation. Free MIT was always found in greater concentration in the medium than within the cells, as was free T_4 in the one experiment in which it was detectable. No free iodinated amino acids were found in the medium or in the tissue of slices from the same thyroid glands from which the isolated cells were prepared and incubated under identical conditions.

Discussion. These studies indicate that thyroid lobes from rats chronically fed an iodine-deficient diet or PTU are capable of liberating free iodinated amino acids into the incubation medium. Similar results can be obtained with isolated thyroid follicular cells. All 3 preparations have in common a marked diminution in the amount of follicular colloid present compared to that of intact thyroid lobes or slices from animals fed an adequate iodine intake.

Free T_4 apparently can reach the medium from the rat thyroid lobes more easily than can free T_3 since the free T_4/T_3 ratio was much higher in the medium than in the thyroid tissue itself. Apparently iodothyronines are released into the medium from the rat lobes only in the free form since the relative proportion of these substances did not appreciably change after digestion of the peptide-bound material contained in the medium. In contrast, MIT and DIT reached the medium primarily in peptide-bound form and the relative proportion of these materials increased significantly after hydrolysis of the peptide material in the medium.

It is possible that free iodothyronines are normally formed within or at the apical border of the thyroid follicular cell and are then bound to follicular colloid in peptide form. In the absence of adequate colloid to insure such peptide binding, the materials can escape into the medium. These studies suggest that basic differences in the mechanism of formation of iodotyrosines and iodothyronines may exist and that these mechanisms may be different in intact thyroid tissue than in isolated thyroid follicular cells.

Summary. Free radioiodinated amino acids

TABLE III. Chromatographic Analysis of Homogenized Cells and Medium After 3 Hr Incubation of Isolated Beef Thyroid Follicle Cells with ^{131}I.

	Pancreatin digestion	% of total radioactivity in cells or medium						
		Origin	DIT	MIT	I^-	T_4	T_3	Front
A) Cells	(—)	36.1	1.1	5.3	28.2	0	0	30.0
	(+)	4.0	1.7	25.2	28.1	.8	0	40.4
Medium	(—)	16.1	.1	13.2	52.4	1.9	0	15.6
	(+)	1.8	.3	19.3	63.4	.7	0	14.9
B) Cells	(—)	22.5 ±6.1	0	2.2 ± 1.2	53.2 ± 14.6	0	0	22.0 ± 7.3
	(+)	2.5 ± .5	2.5 ± .6	16.6 ± 4.8	57.1 ± 13.8	.6 ±.2	0	20.7 ± 8.8
Medium	(—)	5.6 ± .3	0	3.1 ± 1.8	85.1 ± 5.8	0	0	6.3 ± 2.6
	(+)	1.1 ± .5	.5 ± .1	8.9 ± 4.0	82.4 ± 9.3	0	0	7.1 ± 2.8

A—The one experiment in which free T_4 was found. B—Mean ± S.E. of 4 experiments in which no free T_4 was found.

Results are expressed as ± standard error where results of 3 or more experiments are combined.

are released into the medium during a 3- to 6-hour period of incubation with ^{131}I of intact thyroid lobes from severely iodine-deficient rats or of isolated beef thyroid follicular cells. MIT and DIT are found in the medium primarily in peptide-bound form, but T_4 and T_3 are apparently found only as free amino acids. T_4 enters the medium more readily than T_3.

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Effect of an Amyloid Inducing Regimen on Phagocytosis of Carbon Particles.* (30268)

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While the pathogenesis of amyloidosis is uncertain, much speculation has centered on its relationship to the producton of humoral antibody, and several workers in the past have suggested that in fact amyloid deposits are the result of the interaction of antigen and antibody. Light microscopic immunofluorescent studies seemed to offer indirect support to this hypothesis(1,2). Other studies, however, including those utilizing the electron microscopically distinct component of amyloid, the fibril, suggest that this unique element at least is not undenatured gamma globulin(3-7).

The relationship between amyloidosis and phagocytosis, the other major host defense mechanism has until now been little studied, and is the subject of the present report.

Materials and methods. 1) A modified version of the reticuloendothelial system (R.E.S.) evaluation technique described by Benacerraf, McCluskey, and Patras(8) was used. A commercial preparation of carbon ('Pelikan,' Gunther, Wagner, Hanover) was suspended in a 6% gelatin solution such that each milliliter of solution contained 120 mg of carbon. White female New Zealand rabbits were given injections of this carbon gelatin solution in the dose of 4 mg of carbon/100 g of body weight by ear vein. The rate of carbon clearance was established by taking measured samples of arterial blood (0.2 ml) from the opposite ear every 2 minutes after injection for 10-12 minutes (*i.e.*, 5-6 points). Each sample was dissolved in 2 ml of 0.1% sodium carbonate. The carbon concentration of each sample was determined spectrophotometrically on a Coleman Junior Spectrophotometer at wavelength 675.

2) Phagocytic indices were determined according to the formulae described by Biozzi, Benacerraf, and Halpern(9). It has been shown that the clearance from the blood of intravenously injected colloidal solutions, such as the carbon gelatin solution described above, follows an exponential equation:

$$\frac{\log C_1 - \log C_2}{T_2 - T_1} = K$$

where C is the serum concentration of the colloid at time T. K therefore measures the activity of the reticuloendothelial system (R.E.S.) for a given dose of colloid and serves as the index of phagocytosis.

3) The phagocytic indices (K values) of 3 separate groups of 7-8 animals were determined at 3-4-week intervals over the course of several months. The *first group* consisted of young rabbits which were simply allowed to mature and gain weight. These served as

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