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Metabolism of I-131 by Human Thyroid Tissue in Organ Culture.* (31069)

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Organ culture techniques have been used to study the metabolism of certain human endocrine glands(1-3). To our knowledge, only one study of human thyroid tissue in organ culture has been reported(2). Iodine metabolism by human thyroid tissue maintained in organ culture has not been studied extensively.

This report describes the metabolism of radioactive iodine by normal and pathological human thyroid explants maintained in organ culture for varying periods.

Material and methods. Normal thyroid tissue was obtained from radical neck dissection for cancer of the head and neck. Thyrotoxic tissue was obtained from a patient who had received an antithyroid drug and iodine solution prior to surgery. All of the thyroid tissues were obtained from adults. None of the tissues contained I-131 prior to these studies as determined by counting in a well-type scintillation counter.

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Thyroid tissue was collected in cold sterile Dulbecco's(4) phosphate buffered saline containing potassium penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml). The thyroid tissue was immediately dissected free of surrounding connective tissue and thinly cut, using micro dissecting scissors. Each slice measured approximately 10 $\times$ 5 $\times$ 1 mm. The watch-glass technique of Fell and Robison(5) and the stationary steel grid method of Trowell(6) were used for organ culture with some minor modifications. Stainless steel gauze grids, 4 mm in height with a platform measuring approximately 20 $\times$ 10 mm, were placed inside a 30 mm tissue culture dish. Two tissue culture dishes were placed in each 100 $\times$ 20 mm Petri dish which was lined on the bottom by absorbent paper moistened with sterile saline. One thyroid slice was placed on each stainless steel grid (Fig. 1). The Petri culture dishes were then placed in a jar and a cover was sealed in place. A mixture of 95% O_2 and 5% CO_2 was gassed into

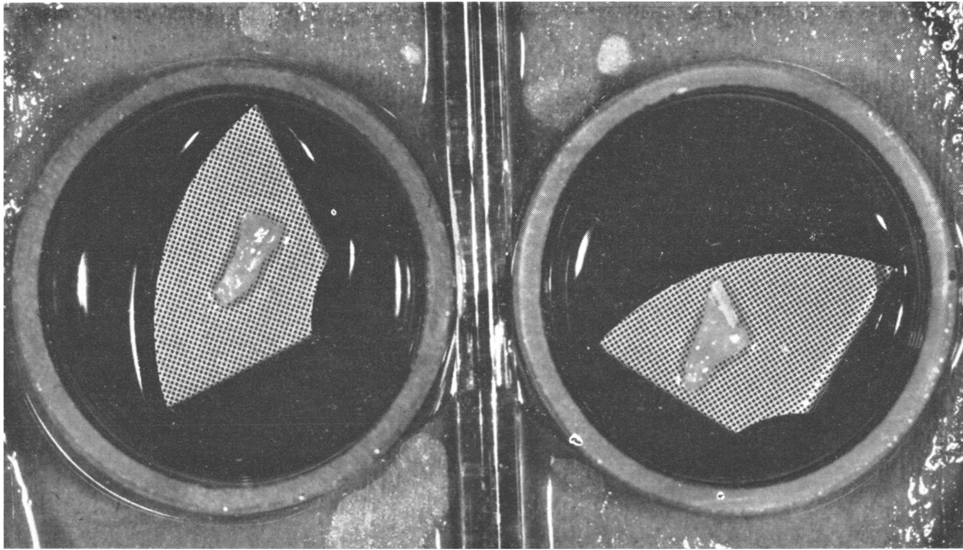

FIG. 1. Organ culture of human thyroid tissue in Petri culture dish.

the jar daily. The tissue was incubated at 37°C.

Tissue-culture medium 199(7) containing 10% of thermally inactivated human serum was employed at pH 7.4. Carrier-free I-131 was added to this culture medium to yield radioactivity levels of approximately 2 μc/ml. As a control for each tissue slice, methimazole (70 μg/ml) was added to the culture medium of one of the 2 tissue culture dishes in each Petri culture dish to prevent the active incorporation of iodine into protein-bound forms by the thyroid tissue(8). Between 3.5 and 4.0 ml of culture medium was used in each tissue culture dish which allowed the medium to come into contact with the lower surface of the thyroid tissue. Sterile 10% $NaHCO_3$ was added as necessary to maintain the pH at 7.4. The culture medium was removed at 24-hour intervals and replaced with fresh medium without I-131.

At the end of each 24-hour period, the tissue from one Petri culture dish was removed from the grids and washed with 10% Kl to remove any remaining culture medium and also to remove any adsorbed I-131 from the surface of the tissue. The tissue was weighed on a spiral spring balance and the total I-131 content in each tissue determined by counting in a well-type scintillation counter. Portions of each tissue were fixed in 10% neutral formalin, embedded in paraffin wax, sectioned and stained with H & E.

In one experiment adenomatous tissue was cultured for 4 days as outlined above except that carrier-free I-131 was again added to the culture medium on the third day. In this experiment duplicate cultures were incubated. In addition, thyroid stimulating hormone (Bovine NIH-TSH-B2)† was added to the culture medium in one of the duplicate sets to provide a concentration of 0.2 U per ml. The solution of TSH was sterilized by passage through a millipore filter with an average porosity of 0.45 μ. At the end of the fourth day of incubation the tissue and media were studied as outlined below.

The bulk of the cultured thyroid tissue was then minced and incubated with a 2.5 mg% solution of papain for 16 hours at 37°C. In this fashion the tissue was completely solubilized. The culture medium was extracted 3 times with butanol and chromatographed. Papain-solubilized samples of the tissue were also chromatographed in parallel in n-butyl alcohol:2 N acetic acid (1:1, v/v) and n-butyl alcohol:2 N ammonium hydroxide (4:5, v/v) systems using the descending technique. Following chromatography, the strips

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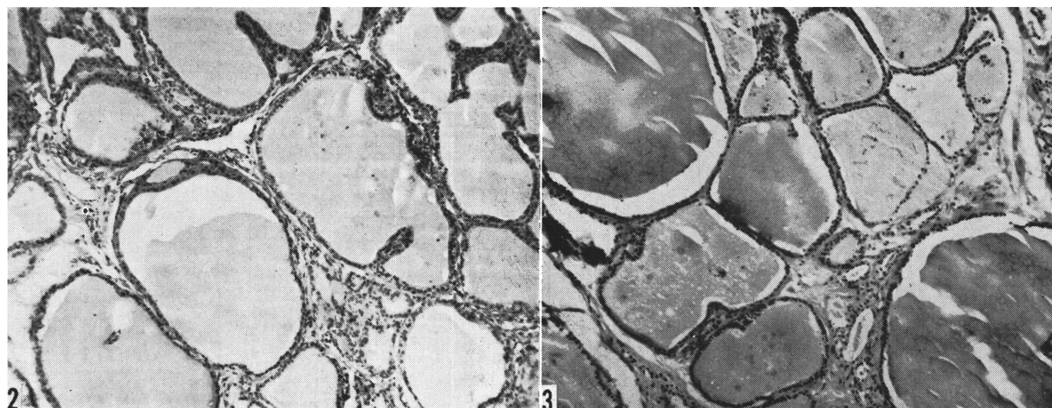

FIG. 2. Photomicrograph of surgical biopsy of toxic thyroid. (× 60).
FIG. 3. Photomicrograph of same tissue as in Fig. 2, but on 7th day of organ culture. (× 60.)

were scanned in a strip-scanner (Nuclear-Chicago Actigraph 11) and the areas under the radioactive peaks were identified and quantified by planimetry(9). Location of labeled compounds was achieved by addition of carrier iodoamino acids and stable KI.

Results. Histological evidence of viability was observed in all of the thyroid explants up to 6 and 7 days of incubation. No attempt was made to maintain these cultures beyond 7 days. These tissues were considered to be viable because of their almost identical histological appearance to the same tissue obtained for surgical pathologic diagnosis (Fig. 2 and 3). Interestingly enough, when sterile normal saline was substituted for human serum in the culture medium in several experiments, almost complete necrosis of the tissues appeared within 24-48 hours.

The I-131 content of the tissues observed in our experiments is listed in Table I. Values reported include those obtained with methimazole used as a control. In Table I the effect of methimazole and TSH upon I-131 concentration in various pieces of normal, adenomatous and toxic thyroid is demonstrated. These results indicate first, that unusually large amounts of methimazole employed in these studies inhibit markedly the accumulation of I-131 in both normal and pathologic human thyroid tissue, and secondly, addition of TSH to the media increased profoundly the accumulation of I-131 by adenomatous tissue. Finally, the considerable variation in cpm/g of particularly the adenomatous thyroid tissue may well be an expression of heterogeneity of the tissues selected into the samples during dissection of the abnormal thyroid.

The results of the analysis of the papain

TABLE I. Radio-Iodide Content of Human Thyroid Expressed as cpm per g of Tissue.

	Normal		Adenomas				Toxic	
	Experimental	Methimazole	Experimental	Methimazole	Experimental	Methimazole	Experimental	Methimazole
Day 1	—	—	452,000	209,000	492,000	106,000	440,000	476,000
2	1,360,000	17,300	63,900	52,000	900,000	5,070	102,000	59,400
3	1,760,000	12,600	126,000	6,600	135,000	8,090	141,000	37,100
4	1,270,000	8,250	124,000	4,700	400,000	4,150	196,000	27,300
5	570,000	6,430	110,000	6,100	112,000	3,310	183,000	36,600
6	753,000	6,440	65,700	5,160	243,000	3,590	103,000	12,600
7	687,000	1,020					125,000	5,390

	Adenoma			
Day 4	I-131	Methimazole + I-131	TSH + I-131	TSH + methimazole + I-131
	199,000	18,000	2,490,000	118,000

TABLE II. Chromatographic Analysis of Human Thyroid Tissue in Organ Culture (% of Total Radioactivity).

		Origin	I-131	MIT	DIT	T_4	MIT/DIT
				Normal			
Day	1	10.5	73.8	12.5	3.2	0	3.8
	2	10.0	32.3	32.5	25.2	0	1.3
	3	11.5	17.7	36.2	34.6	0	1.1
	4	13.9	13.4	40.5	33.2	0	1.3
	5	10.1	24.7	32.8	26.7	5.8	1.2
	6	10.4	19.5	41.5	22.7	5.9	1.8
	7	4.9	22.7	31.3	35.1	6.0	0.9
				Adenoma 1			
	1	10.2	77.7	7.1	1.4	3.6	5.0
	2	19.6	68.5	8.9	3.0	0	3.0
	3	14.6	35.8	33.2	16.4	0	2.0
	4	12.0	17.8	49.7	16.0	4.5	3.0
	5	8.3	12.0	51.9	26.3	1.5	2.0
				Adenoma 2			
	1	10	58	9.5	20.8	1.7	0.5
	2	11.5	46.1	24.3	18.1	0	1.3
	3	9.2	32.8	35.9	22.1	0	1.6
	4	11.0	17.0	47.6	22.6	1.8	2.1
	5	10.5	10.4	39.9	37.3	1.9	1.1
	6	17.6	6.8	46.5	29.1		1.6
				Toxic			
	1	17.2	69.8	8.9	1.8	2.3	4.9
	2	18.8	38.2	28.6	8.8	5.6	3.2
	3	16	29.5	32.1	8.9	13.5	3.6
	4	13.5	21.4	35.1	20.0	10	1.8
	5	9.6	21.6	45.9	16.9	6	2.7
	6	6.3	18.9	42.9	20.7	10.2	2.0
	7	9.9	13.2	44.7	24.2	8.0	1.8
				Adenoma receiving TSH			
Day 4	Control	6.6	60.6	18.7	11.2	2.9	1.6
	TSH	4.6	52.1	7.3	17.1	19.4	0.4

produced hydrolysis of the thyroid tissues are presented in Table II. Monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T_4) were detected in the tissues. Triiodothyronine (T_3) was not detected in any of the tissues. Table II shows that with time both normal and pathologic tissue contained increasing percentages of MIT and decreasing percentages of I-131. The normal tissue also demonstrates a decreasing MIT/DIT ratio and an increasing amount of T_4 with time. The pathologic tissues contained significant amounts of T_4 but the MIT/DIT ratios did not change consistently with time. TSH appears to increase the amount of T_4 by a factor of 5 and also decrease the MIT/DIT ratio by a factor of 4. In the methimazole controlled studies, at least 85% of the total radioactivity in the tissues was represented by inorganic iodide. The remaining radioactivity was represented by origin material (*ca.* 10%) or I-131 labeled iodotyrosines (less than 3%).

In the butanol extracts of the culture media which contained TSH, 6.7% of the radioactivity was represented by labeled T_4. The remaining radioactivity was iodide. Only radioactive iodide was detected in the butanol extracts of the medium containing TSH and methimazole. No radioiodinated amino acids were detected in the butanol extracts of the culture media which did not contain TSH.

In the thyroid tissue incubated with methimazole and methimazole plus TSH at least 90% of the radioactivity was present as iodide and the remaining radioactivity was present at the origin (*ca.* 5%) or as iodotyrosines (less than 5%).

Discussion. Our studies show that human thyroid tissue in organ culture can concentrate iodide. These findings are consistent with published reports of iodide concentration by thyroid tissue of embryonic rat(10),

embryonic chick(11), postnatal rats(12), mice(13) and pathologic human(2) thyroid tissue in organ culture. The percentage of iodide in the tissues is high during the first 48 hours of culture but gradually decreases as the iodide is utilized for protein bound iodine formation.

The MIT/DIT ratios of greater than 1.0 found in our normal thyroid tissue differ from most of the previously reported values of less than 1.0(14-17). In these latter studies, the iodoamino acids in the thyroid tissue were labeled *in vivo* pre-operatively. Arosenius(18), however, has reported MIT/DIT ratios ranging from 0.49 to 1.12 which were determined without the use of radioactive iodine. His values were obtained by using an anion exchange resin and high voltage paper electrophoresis. Published studies on MIT/DIT ratios in human thyrotoxic tissue are rare. Braasch *et al*(15) reported a mean MIT/DIT ratio of 0.63 in the thyroid tissue of patients who had been treated medically prior to surgery. Yamazaki *et al*(17) found a mean MIT/DIT ratio of 2.2 in patients who had received methylthiouracil followed by Lugol's solution prior to *in vivo* labeling with I-131 and surgery. Arosenius(18) found a mean MIT/DIT ratio of 2.8 in the thyroid tissue of patients who had received anti-thyroid drugs before surgery. The relative increase of MIT, as compared to that of DIT, might partly be due to an iodine deficiency in the gland. The degree of iodine deficiency cannot be stated, as the amount of inorganic iodine in the tissue in our studies was not determined. Braasch *et al*(15), however, could not demonstrate any differences between normal, untreated thyrotoxic and treated thyrotoxic glands. It seems unlikely that iodine deficiency explains the differences in the MIT/DIT ratios obtained by Yamazaki *et al*(17), Arosenius(18) and ourselves compared to those obtained by Braasch *et al* (15). Another possibility is that the conversion of MIT to DIT is more sensitive to antithyroid agents than is the formation of MIT (19).

The MIT/DIT ratios of greater than 1.0 and the percentage of T_4 found in the adenomas in our studies are similar to those values reported by Pitt-Rivers *et al*(20). They also showed that the content of T_4 is much less in adenomas than in normal thyroid tissue. Other investigators have also demonstrated that the T_4 content of adenomas of the thyroid is 10 to 20% that of normal thyroid tissue(21-22).

The percentage of T_4 present in our normal thyroid tissue is similar to some previous reports(14,17) but is lower than other reports (15,16). The percentage of T_4 recovered in the thyrotoxic tissue is similar to that demonstrated by other investigators(17-18).

The addition of TSH to the culture medium greatly improved the uptake of I-131 and apparently accelerated the conversion of MIT to DIT as well as coupling of DIT to form T_4. TSH also apparently caused release of T_4 from the thyroid into the medium since we were unable to detect any labeled amino acids in the culture media which did not contain TSH. These findings are in accord with previous observations of the action of TSH on embryonic rat thyroid glands in organ culture(10).

Finally, the data of Table I indicate that methimazole decreased *markedly* the I-131 content of both normal and pathologic human thyroid tissue. This finding is consistent with the known actions of the thiocarbamide drugs on iodine metabolism in the thyroid gland. As larger amounts of thiocarbamides are given, iodination of DIT, and then of MIT, is prevented(17,23). In addition, the release of iodide is increased from a gland blocked by thiocarbamides(24,25).

Summary. The incubation of human thyroid tissue in tissue culture medium containing I-131 by an organ culture technique is described. The histology of the gland was preserved up to 7 days. Labeled MIT, DIT, and T_4 were present following papain hydrolysis of the tissue. Addition of TSH to the culture medium apparently accelerated the conversion of MIT to DIT as well as the rate of coupling of DIT to form T_4 and released T_4 from the thyroid tissue into the medium. No labeled amino acids were detected in the culture medium when TSH was not added.

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The Feed-Back Mechanism in Immunoglobulin Synthesis.* (31070)

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The amount of antibody formed in response to an antigen does not exceed a critical upper limit. It is therefore clear that some mechanisms must exist which do not allow the concentration of antibody to increase indefinitely. Two such mechanisms may operate independently of each other, having two different targets: the antigen and the antibody forming apparatus. The *first* of these mechanisms may operate by eliminating antigen molecules as a consequence of combination with antibody, thus depriving them of their immunogenicity. It has been known since 1901 that antigens lose their immunogenicity when injected as antigen-antibody complexes(1). Recently Uhr and Baumann(2) have described and analyzed this phenomenon. Their results may be interpreted as indicating that the loss of the immunogenicity is a consequence of the shielding of antigenic determinants by antibody molecules. Circulating antibody can, therefore, be regarded as a barrier, protecting the antibody forming cells from overexposure to antigens. If this barrier is broken by larger quantities of antigen, the antigen reaches the antibody synthesizing apparatus, which, as a response to this stimulation, increases its antibody output. With a rising concentration of antibody a *second* regulatory mechanism my become noticeable. This mechanism, in which the concentration of the product of the reaction (in our case

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