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Hormone Synthesis by Normal Human Thyroid Cells in Tissue Culture.* (29399)

WELLINGTON HUNG AND THEODORE WINSHIP (Introduced by John C. Houck)

Section of Endocrinology, Research Foundation of Children's Hospital of District of Columbia, Department of Surgical Pathology, Washington Hospital Center, and Departments of Pediatrics and Pathology, George Washington University School of Medicine, Washington, D.C.

The process of thyroid hormone synthesis is presumed to proceed via the concentration of iodide within the thyroid gland and its conversion into a form suitable for substitution onto either tyrosine or tyrosyl residues in peptide linkage to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Subsequently, these iodotyrosines couple to form the metabolically active thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). Which of these steps in the biosynthesis of thyroid hormone occur in the cell and which occur in the colloid is not known. We have previously shown that isolated normal human thyroid cells incubated in tissue culture are able to concentrate iodide and to incorporate the iodine into organically bound iodine(1). The location of iodination in the thyroid gland remains poorly defined. Results of studies utilizing I^{-131} and histologic radioautographs have been at variance. Several investigators have observed that the earliest localization of protein-bound radioactivity on radioautographs is in the apical portions of the epithelial cells adjacent to the follicular colloid in rats(2-4). Other studies have shown that some iodination of protein occurs within the cells, as evidenced by the appearance of cell particulate protein-bound iodine-^{131} (PBI^{-131}) in tissue slices(5-7). Wollman and Wodinsky(8), however, found by radioautography that PBI^{-131} occurred only in the colloid and not in the cells of mice. Meyer(9) observed by autoradiographic study of human thyroid tissue that the radioactivity was localized chiefly to the colloid. It is therefore not clear whether iodinated protein is normally produced in the cells and secreted rapidly as such into the colloid or whether it is produced in the colloid at the cell surface.

The present study was initiated to determine whether isolated normal human thyroid cells are capable of synthesizing iodotyrosines and iodothyronines.

Materials and methods. Normal thyroid tissue was obtained from radical neck dissections for cancer of the head and neck. Other normal thyroid tissue was obtained from lobectomies for solitary nodules of the thyroid gland. All patients (age 40-66 years) from whom thyroid tissue was obtained were females except for thyroid No. 20, which was obtained from a 40-year-old male. All tissue was examined histologically for verification of the presence of normal thyroid tissue. None of the tissues contained I^{-131} prior to these studies as determined by counting in a well-type scintillation counter.

The method for tissue culture of isolated thyroid cells was that of Irvine(10), with modifications as previously described(11). Further changes in our procedure will be described briefly. Thyroid tissue was obtained at surgery and immediately transported in cold sterile Delbeccos saline solution containing potassium penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml). Cells were obtained by trypsiniza-

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tion at pH 8.0 after trimming, mincing and washing of the thyroid tissue. The trypsinizing fluid consisted of 0.25% trypsin in isotonic saline. The freshly dispersed cells were collected by centrifugation at less than 1000 $\times$ *g*. The packed cells were washed twice with sterile Delbeccos saline to remove any serum or colloid which might have adhered to the dispersed cells during the trypsinizing process. The packed cells were then suspended in 100 volumes of tissue-culture medium 199 which contained 10% thermally inactivated human serum at pH 7.4. As a control, portions of the cell suspensions were incubated with Methimazole (0.02 M) to prevent the active incorporation of iodine into protein bound forms by the thyroid cells (12). The cells were incubated for 24 hours at 37°C in 160 ml "milk dilution" bottles and tissue culture tubes in a bacteriological incubator.

At the end of 24 and 48 hours of incubation the specimens were examined microscopically for viability. Intact follicles were never observed. Carrier-free I^{-131} was added to the culture medium to yield radioactivity levels of approximately 2 μc/ml together with 0.02 μg potassium iodide (KI) per ml of medium to insure constant iodide levels. These cell suspensions were then incubated for an additional 24 hours. In later experiments, I^{-131} and KI were added at the beginning of incubation and the cultures allowed to grow for 48 hours prior to protein hydrolysis. No apparent differences in viability were noted when this change was made.

Following incubation of the cells with I^{-131} for 24 or 48 hours the culture medium was decanted and the cells gently washed with 10% KI to remove any remaining culture medium and also to remove any adsorbed I^{-131} from the surface of the cells prior to protein hydrolysis. Microscopic examination at this time showed that most of the cells were in a monolayer. Pangestin^{R} (Pancreatin enzymes) was then added to the cells at pH 8.0 to accomplish protein hydrolysis overnight. Samples were then chromatographed in parallel in n-butyl alcohol:2N acetic acid (1:1, v/v) and n-butyl alcohol:2N ammonium hydroxide (4:5, v/v) systems using the descending technique as previously described (13). Following chromatography, the strips were scanned in a strip-scanner (Nuclear-Chicago Actigraph II) and the areas under the radioactive peaks were identified(13). Location of labeled compounds was achieved by addition of carrier amino acids and stable KI. It is assumed that all of the iodoproteins detected were from within the cells since care was taken to wash the cells free of the tissue culture medium prior to protein hydrolysis.

In some experiments, the percent of organically-bound I^{-131} of undigested cells at the end of 24 hours growth was determined by radiochromatography (protein-bound iodine remaining at the origin of the chromatogram) and compared with that estimated by precipitation of the cells with trichloroacetic acid (TCA).

Results. Histologic evidence of viability was observed after 24 and 48 hours of incubation. The cells are considered to be viable because of their similarity to those seen in three micron sections of normal thyroid tissue. They have a delicate cell membrane and finely granular cytoplasm. The nuclei approximate the size of those in the normal thyroid, have a distinct capsule, and the nucleoplasm is coarsely granular. Most of them have a prominent nucleus. To obtain further evidence that these cells were viable at the end of 48 hours incubation, most of the cells were recultured for 6 days in tissue culture tubes. An example of one such monolayer of cells at the end of 6 days is shown in Fig. 1.

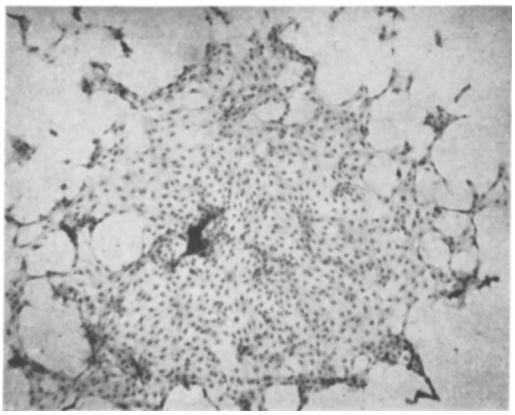

FIG. 1. Monolayer of human thyroid cells grown in tissue culture for 6 days. ($\times$ 60)

TABLE I. Distribution of Radioiodinated Amino Acids After Pancreatic Hydrolysis of Normal Human Thyroid Cells.*

24 hr growth with I^{131}	Butyl alcohol: 2N acetic acid						Butyl alcohol: 2N ammonium hydroxide			
	Origin	I	MIT	DIT	T_3T_4	MIT/DIT	Origin, MIT, DIT	I	T_3	T_4
9	4.9	54.4	18.9	11.8	10.0	1.4	33.3	54.1	7.7	4.9
10	3.9	61.5	15.8	5.8	13.0	2.7	21.7	62.2	6.8	9.3
11	0	76.7	8.8	6.8	7.7	1.3	14.9	77.4	4.0	3.7
15	8.2	57.3	28.7	4.3	1.5	6.7	32.1	63.1	0	4.8
16	7.0	61.4	15.8	5.3	10.5	3.0	29.9	64.5	0	5.6
Mean	4.8	62.3	17.6	6.8	8.5	3.0	26.4	64.3	3.7	5.6
48 hr growth with I^{131}										
18	16.0	52.3	12.8	14.1	4.8	.9	40.6	47.4	3.4	8.6
20	4.7	61.9	5.9	19.4	8.1	.3	31.5	56.7	7.1	4.7
21	3.4	68.1	10.7	11.1	6.7	.9	20.5	72.2	2.6	4.7
24	7.5	67.9	8.6	8.8	7.2	.9	30.7	62.5	2.4	4.4
43	10.2	59.4	10.4	14.1	5.9	.7	28.7	58.4	4.5	8.4
Mean	8.3	61.9	9.7	13.5	6.5	.7	30.4	59.4	4.0	6.1

* Percentage of total radioactivity of chromatogram.

Isolated normal human thyroid cells can incorporate I^{131} into iodinated proteins. The per cent of iodine as PBI^{131} found in these cells after 24 hours incubation with I^{131} as determined by precipitation with TCA ranged between 22.6 and 24.8%. The per cent of iodine as PBI^{131} determined by radiochromatography was between 24.5 and 28.9%.

After incubation with I^{131} for 24 or 48 hours, protein hydrolysis revealed the formation of MIT, DIT, T_3 and T_4 (Table I). The mean MIT/DIT ratio of the tissue cultures incubated with I^{131} for 24 hours was 3.0, while that of tissue cultures incubated for 48 hours was 0.7. No iodotyrosines or iodothyronines were found in the tissue cultures incubated with I^{131} and methimazole.

Discussion. Which steps in the biosynthesis of thyroid hormones occur within the thyroid cells and which occur in the colloid is not known. We have previously shown that normal human thyroid cells incubated in tissue culture medium were able to concentrate iodide(1). This finding was consistent with the previously published data concerning the ability of isolated pathologic human thyroid cells(5) and sheep thyroid cells(6) to concentrate iodide.

The present data indicate that isolated normal human thyroid cells incubated in tissue culture medium are also able organically to bind iodine. The possibility that a nonspecific exchange of I^{131} with the iodoproteins in the thyroid cells occurred is rendered unlikely by the failure to find any iodotyrosines or iodothyronines in the thyroid cells incubated with methimazole, while nonspecific adsorption was excluded by the failure to remove the I^{131} label with large amounts of added stable iodide. Recently published studies on the functional ability of isolated thyroid cells also appear to suggest that nonspecific exchange of I^{131} is unlikely to play a significant role in our experiments(14-16).

Although Pulvertaft *et al*(14) have reported that isolated pathologic human thyroid cells grown in tissue culture are able to form iodoamino acids, this is the first report concerning the ability of normal human thyroid cells to accomplish this. There are several reports concerning the ability of isolated animal thyroid cells to form iodoamino acids(15,16).

The ratio of DIT to MIT tended to increase with time. These results suggest two possibilities: 1. The activity of the enzyme controlling the formation of MIT may decrease during the second 24 hour period of incubation while during the same period the activity of the enzyme controlling the further iodination of MIT to form DIT may increase or remain the same. It has not been definitely shown whether one or more enzymes control the formation of both MIT and DIT.

2. Since increases in T_3 and T_4 were not found with time, DIT might be an end product of the iodination reaction. Therefore, the increase in DIT/MIT with time could be an expression of the accumulation of the end product rather than of a decrease in iodinating enzyme activity.

The failure to demonstrate larger amounts of T_3 and T_4 in our studies suggests that most of the enzymes necessary for formation of these compounds lies within the colloid. Another reason for this failure might be that trypsinization of the thyroid cells may have damaged the necessary enzymes of the coupling process.

Summary. Normal isolated human thyroid cells were incubated in tissue culture medium containing I^{-131} for 24 to 48 hours. The nature and distribution of labeled compounds were determined. Labeled MIT, DIT, T_3 and T_4 were present following protein hydrolysis of the incubated cells. The MIT/DIT ratios ranged from 1.3 to 6.7 in the tissue cultures incubated with I^{-131} for 24 hours, while that for tissue cultures incubated for 48 hours ranged from 0.3 to 0.9. These results indicate that isolated normal human thyroid cells are able to synthesize iodotyrosines and iodothyronines in the absence of intact follicles and colloid.

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Triglyceride Accumulation and Release in the Rare-Earth Fatty Liver.* (29400)

FRED SNYDER AND G. C. KYKER
(With the technical assistance of Nelson Stephens and E. A. Cress)
Oak Ridge Institute of Nuclear Studies, Medical Division, Oak Ridge, Tenn.

Our previous work(1,2) has demonstrated that the acute infiltration of hepatic cells by triglyceride after intravenous rare earths is influenced by hormones, a fact which led us to investigate lipid mobilization in animals exposed to cerium. The elevation of free fatty acids in plasma from cerium-treated rats strengthened the idea that increased mobilization of lipid from extrahepatic sites(3) and a depressed oxidation of fatty acids in the liver(4) were the likely explanations for their massive accumulation in liver. Recknagel *et al*(5) recently determined the release of triglycerides in the CCl_4-fatty liver by using Triton (p-iso-octyl polyoxyethylene phenol polymer). This detergent blocks the exit of triglyceride from plasma, and Byers and Friedman(6) estimated that after Triton is given the bulk of the triglycerides found

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