fashion to the intracutaneous injection of old tuberculin. This reaction is similar to that which can be transferred passively by the viable cells from which the cell-free supernatant fluid is derived.

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Iodide Concentrating Ability of Normal Human Thyroid Cells in Tissue Culture.* (28918)

WELLINGTON HUNG AND THEODORE WINSHIP (Introduced by John C. Houck)
Section of Endocrinology, Research Foundation, 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.

Studies on the iodide-concentrating ability of isolated thyroid cells have recently been published. Pulvertaft *et al*(1) reported that cells obtained from pathologic human thyroid cells were able to concentrate iodide, although no values for cell-to-medium iodide concentration ratios were given. Pastan(2) found that isolated calf thyroid cells were able to incorporate I-131 into iodinated proteins, although no data concerning iodide-concentration ability were reported. Tong and coworkers(3) reported cell-to-medium iodide concentration ratios of 6-9, using isolated sheep thyroid cells. This study was initiated to investigate the iodide-concentrating ability of normal human thyroid cells in tissue culture.

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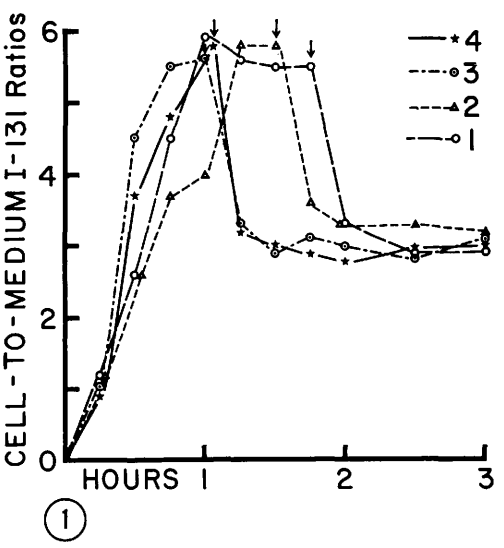
Materials and methods. Normal thyroid tissue was obtained from radical neck dissections for cancer of the head and neck. Other normal thyroid tissue was obtained from lobectomies performed because of solitary nodules of the thyroid gland. All tissue was examined histologically for verification of the presence of normal thyroid tissue.

The method for tissue culture of isolated human thyroid cells was that of Irvine(4), with modification as previously published(5). 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 trypsinization at pH 8.0 after trimming, mincing and washing of the thyroid tissue. The trypsinizing fluid consisting of 0.25%

trypsin in isotonic saline. The freshly dispersed cells were collected by centrifugation at less than 1000 × *g*. The packed cells were suspended in 50 volumes of tissue-culture medium 199 which contained 10% thermally inactivated human serum at pH 7.4 and incubated at 37° in a bacteriological incubator. In each experiment the cell suspension was divided into 2 portions. Two microcuries of I-131 were added to each milliliter of cell suspension. In addition, 0.002 M methimazole was added to one of the portions of cell suspension to prevent the incorporation of iodine into protein-bound forms by the thyroid cells (6). Therefore, the cell-to-medium I-131 concentration ratios in the cell suspensions incubated with methimazole are a direct determination of the iodide-concentrating ability of these cells. Each cell suspension was incubated at 37°, with gentle shaking. Two milliliter aliquots of the cell suspension were removed every 15 minutes for 2 hours, and then every 30 minutes for an additional hour. The thyroid cells were sedimented by centrifugation and the cells and supernatant media were counted separately in a well-type scintillation counter to yield a cell-to-medium I-131 ratio (I-131/ml cells)/(I-131/ml medium). Sodium perchlorate (0.002 M) was added to the cell suspensions when the cell-to-medium I-131 ratio reached a plateau. This drug inhibits the iodide-concentrating ability of thyroid cells and permits discharge of any iodide trapped by the thyroid but not yet organically bound(7,8).

In 3 experiments, the percent of organically-bound I-131 of "unblocked" cells at the end of 24 hours 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). The cells were 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 as previously described(9). Following chromatography, the strips were scanned in a strip-scanner and the areas under the radioactive peaks were identified and measured(9).



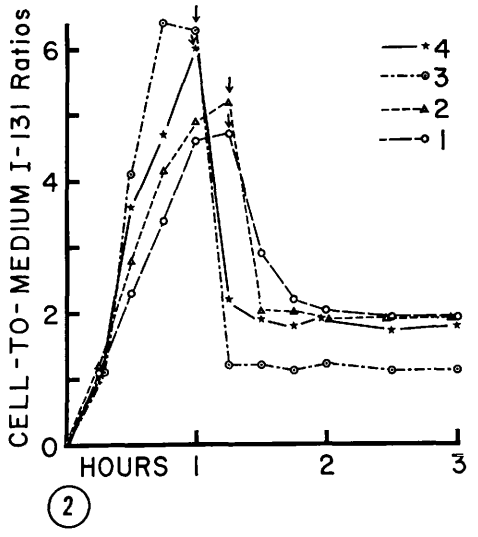


FIG. 1. Cell-to-medium I-131 ratios for thyroid cells incubated without methimazole. Sodium perchlorate was added to media at times indicated by arrows.

FIG. 2. Cell-to-medium I-131 ratios for thyroid cells incubated with methimazole. Sodium perchlorate was added to media at times indicated by arrows.

Results and discussion. The data of Fig. 1 and 2 show that the thyroid cells concentrated I-131 rapidly. The maximum iodide-concentration occurred between 60 and 90 minutes after incubation of the thyroid cells with I-131. The cell-to-medium I-131 concentration ratios ranged between 4.7 and 6.4. No significant differences in iodide-concen-

TABLE I. Thyroidal Organic Iodine at the End of 24 Hr (% of I-131 Present in Cells).

Thyroid No.	Precipitation with TCA	Radio-chromatography
6	22.6	24.5
8	25.1	28.7
11	21.7	26.4

trating ability were noted between those cells incubated with or without methimazole. The cell suspensions containing methimazole reflect the maximal iodide-concentrating ability of these cells. Addition of sodium perchlorate to the cell suspensions was followed by marked decreases in the cell-to-medium I-131 concentration ratios, thus further substantiating the fact that these cells had concentrated I-131.

Radiochromatograms of the "unblocked" cells revealed radioactivity only at the origin and in the iodide area. Isolated normal human thyroid cells can incorporate I-131 into iodinated proteins. The amount of organically-bound iodine after 24 hours' incubation with I-131 is given in Table I. The slight differences between the results obtained by the two methods is probably due to small amounts of adsorbed inorganic iodine.

It is apparent from these data that normal human thyroid cells are able to concentrate iodide and to incorporate the iodine into organically bound iodine in the absence of intact thyroid follicles and colloid. Microscopic examination of the cell suspensions obtained by the technique used in these studies have revealed the presence mainly of isolated thyroid follicular cells and absence of intact follicles(4,5). This work confirms previously published reports on the ability of isolated pathologic human thyroid cells and sheep thyroid cells to concentrate iodide and to incorporate it into organic form.

Summary. Normal human thyroid cells were dispersed by trypsinization and incubated in tissue-culture medium 199. When I-131 was added to the culture medium, the isolated cells were shown to retain their ability to concentrate iodide. This indicates that intact thyroid follicles and colloid are not essential for iodide-concentration and organic binding of the trapped iodine by normal human thyroid cells.

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Role of Cell Proliferation in Hepatic Carcinogenesis.* (28919)

EMANUEL RUBIN, KAZUO MASUKO, STANLEY GOLDFARB, AND FREDERICK G. ZAK
(Introduced by Hans Popper)
Department of Pathology, Mount Sinai Hospital, New York City

Hepatocellular carcinoma develops through stages of nodular regeneration as described in rats following the feeding of Aramite(1). By contrast it was demonstrated that during the induction of cirrhosis and hepatic carcinoma by an azo-dye, cell proliferation in the non-carcinomatous portions of the liver is not increased, while the tumor takes up more tritiated thymidine(2) and has a high mitotic index(3). This was taken to indicate that hepatic carcinoma does not develop over in-

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