

Observations on the Growth and Metabolic Functions of Cultured Cells Derived from Human Adipose Tissue¹ (38846)

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(Introduced by Jules Hirsch)

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Studies have indicated that the development of human obesity may be due to an increase in the number of adipose cells and to a lesser degree, to their enlargement (1, 2). This is especially true in childhood-onset obesity, where it has been shown that greater increases in cell number occur, than in adults whose obesity begins in adult life (1, 3). It has been postulated that this adipose tissue hypercellularity may be due to a greater proliferation of adipose cells in obese children early in life. Thus, in order to understand the problem of obesity, it is necessary to learn more about the factors controlling the multiplication and enlargement of adipose cells and how they may be modified during development. The considerable variability in the metabolic activity of adipose cells due to such external influences as the donor's nutritional status has hampered studies of adipose tissue slices and isolated cells. Furthermore, the osmic acid method normally used for adipose cell sizing and counting can not detect small adipose cells (containing < 0.01 μg lipid/cell) or pre-adipocytes (4). For these reasons, it is desirable to be able to grow adipose cells in tissue culture where they would be exposed to a specific, controllable milieu in which the factors controlling their growth and differentiation could be investigated. The present study reports the establishment of cultures of cells derived from human adipose tissue (CAT cells) and observations on their growth and metabolic activity which appear to differentiate them from skin fibroblasts.

Materials and Methods. Cell cultures. Adipose tissue samples were obtained from in-

dividuals undergoing abdominal surgery. Cultures of isolated fat cells were prepared by collagenase treatment using the method of Rodbell (5). After filtration through a 250- μm nylon sieve, the cell suspension was centrifuged at 100g for 1 min. The infranatant was discarded, and the isolated fat cells floating on top were washed three times with culture medium. The fat cell suspension was transferred to 30-ml plastic culture flasks containing a total of 5 ml RPMI-1640 medium (Grand Island Biological Co.), 20% fetal calf serum, 0.292 mg/ml l-glutamine, 100 U/ml penicillin, and 100 μg /ml streptomycin. The flasks were gassed with 10% CO_2 to pH 7.4 and incubated at 37°. The culture medium was changed biweekly, and subculturing was done with trypsin (2.5% in 0.2% EDTA). Abdominal skin specimens were obtained at surgery, and skin fibroblast cultures were established in a standard manner. All culture conditions for the fibroblast cultures were identical to those used for the CAT cells.

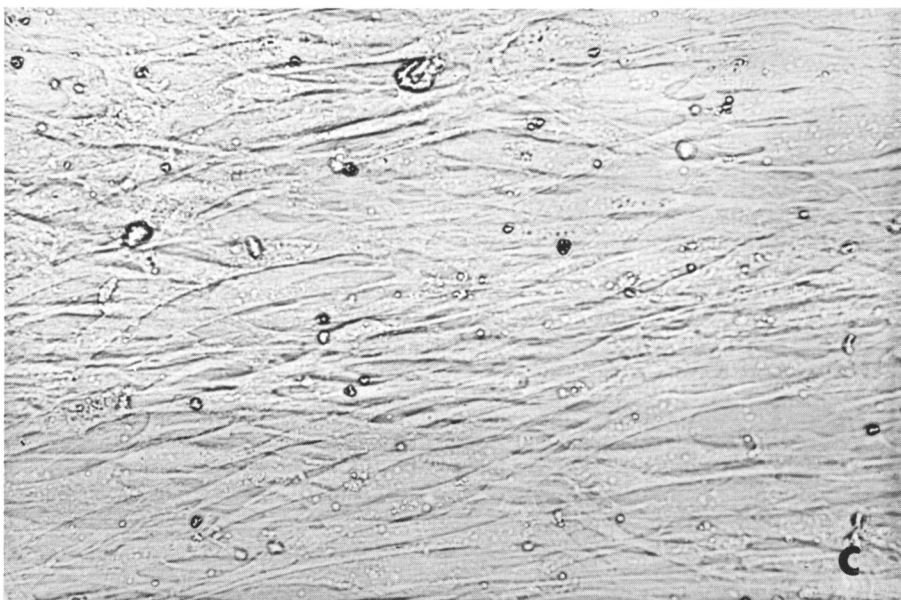
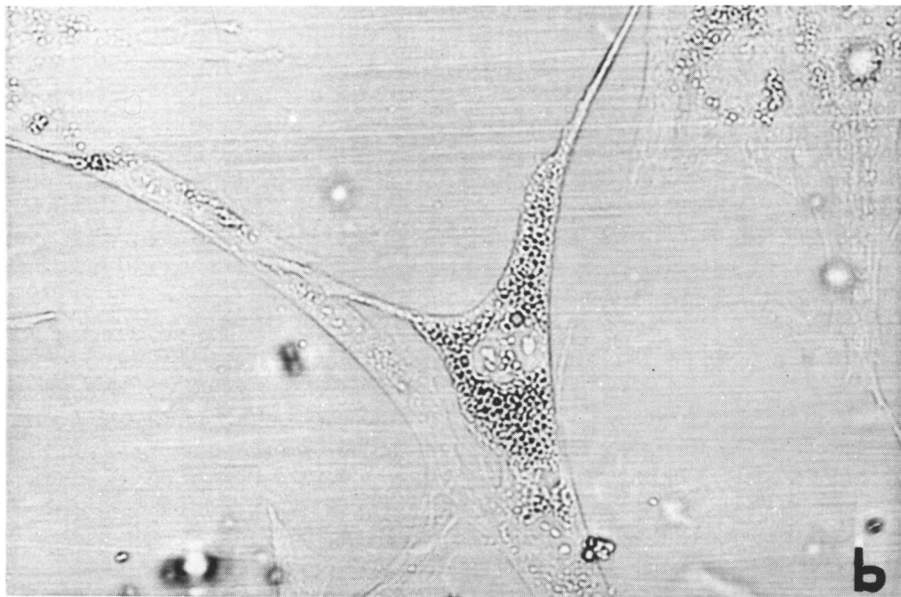
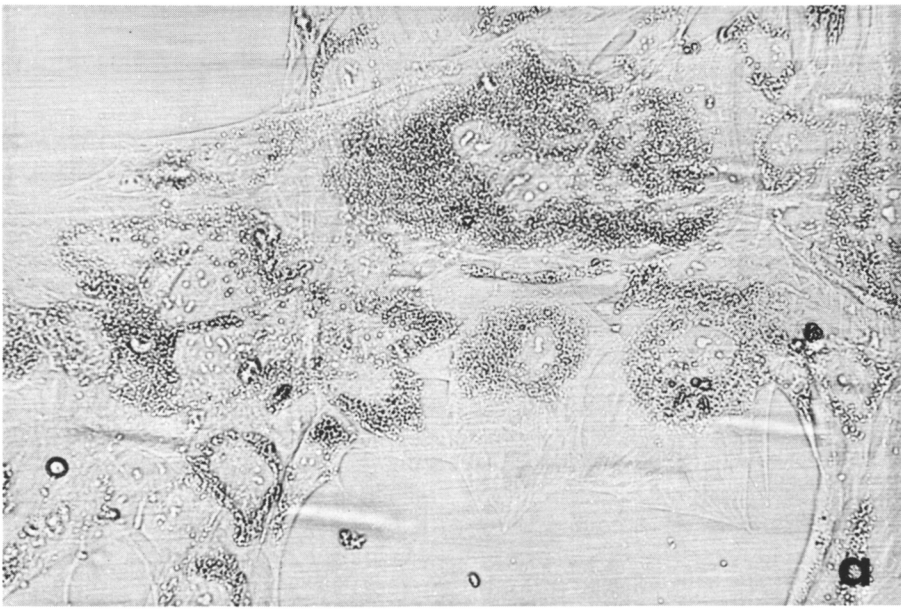
Metabolic studies. ³H-Thymidine incorporation into DNA was measured by incubating the CAT and fibroblast cultures for 24 hr with 0.1 Ci/ml of methyl-³H-thymidine (6 Ci/mole) in complete medium. For experiments with insulin, medium containing 5% fetal calf serum was used. The medium was removed, and the cells were washed and harvested by trypsinization. An aliquot of cells was removed for DNA determination, and the remaining cells were treated with cold 10% TCA, and the acid-insoluble material was collected by filtration on Millipore filters (0.45 μm). The filters were washed with cold 5% TCA, dried, and counted in Liquifluor (Packard Co.) in a liquid scintillation spectrometer. Cellular DNA was determined by the fluorometric method of Kissane and Robbins (6).

To measure lipogenesis, cell cultures were

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incubated with 0.04 $\mu\text{Ci/ml}$ $1\text{-}^{14}\text{C}$ -glucose (48.6 mCi/mole) in complete medium for 10–12 days. The incubation medium was removed, and the cells were washed with isotonic saline. The cells were scraped off, pelleted, and lipids were extracted with Dole's M solution (isopropanol: *n*-heptane: IN H_2SO_4 , 78:20:2). The cell residue was treated with cold 20% TCA and analyzed for DNA. The M solution was rectified with heptane and water, and a suitable aliquot of the heptane layer was dried and counted in a liquid scintillation counter.

$^{14}\text{CO}_2$ production was measured by incubating cell cultures with 0.2 $\mu\text{Ci/ml}$ of $1\text{-}^{14}\text{C}$ -glucose (48.6 mCi/mole) in 2 ml Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 mg/ml glucose and 2 mg/ml gelatin. Incubation time was 3 hr at 37° in a gas phase of 95% O_2 –5% CO_2 . Control flasks contained no cells. The incubation was stopped by addition of 1 ml of 6 N H_2SO_4 , and the flasks were shaken at 37° for 1 hr. $^{14}\text{CO}_2$ was trapped in 0.2 ml of hyamine hydroxide contained in a plastic cup suspended inside the flask and counted in a scintillation counter. The cells were scraped from the flasks after removal of the medium, and the total flask DNA was determined.

Results. Cultures of CAT cells were established from both omental and subcutaneous adipose tissue obtained from five adult male subjects (aged 22–68) and six adult female subjects (aged 28–72).

After approximately 5–7 days in culture numerous cells were found to be growing on the flask bottoms. The CAT cells were polymorphic in form. Some cells had a polyhedral shape while others assumed a more fibroblast-like appearance. The presence of a large number of small fat droplets which stained with oil red O could be observed in these cells. There was considerable variation in cell size, but the average cell had a mean diameter of about 25 μm . The morphological appearance of the CAT cells (Fig. 1a, b) clearly distinguishes them from the fibroblast cultures (Fig. 1c). The CAT cells divided and grew, but at a much slower rate than

did the corresponding fibroblast cultures. To date we have maintained viable cultures for up to 6 mo.

The basal rates of ^3H -thymidine incorporation into DNA shown by nonconfluent cultures of CAT cells and fibroblasts are shown in Table I. The average growth rate of the CAT cultures was approximately 20 times less than that of fibroblasts under the same culture conditions. The addition of insulin generally stimulated the amount of ^3H -thymidine incorporation in both CAT cells and fibroblasts (Table I). ^3H -Thymidine incorporation in both CAT cells and fibroblasts was significantly increased with the lower insulin concentration (5 mU/ml). Insulin at 100 mU/ml stimulated ^3H -thymidine incorporation in CAT cells but was inhibitory in the fibroblast cultures.

The incorporation of $1\text{-}^{14}\text{C}$ -glucose into total cell lipid was measured in a number of different CAT and skin fibroblast cell lines. Under the conditions used, long incubation periods (10–12 days) were necessary to accurately measure the formation of ^{14}C -labeled lipid. Replicate cultures were treated with insulin (1000 $\mu\text{U/ml}$) every third day. The total amount of ^{14}C -label incorporation into lipid was very small (usually < 1%). CAT cells in comparison with fibroblasts, showed an approximately 16-fold increase in the ability to incorporate glucose- ^{14}C into cell lipid (Table II). We were unable to demonstrate any insulin stimulation of triglyceride formation by either CAT or fibroblast cultures.

$1\text{-}^{14}\text{C}$ -Glucose was added to CAT and fibroblast cultures, and the formation of $^{14}\text{CO}_2$ was measured with and without insulin addition. Fibroblast cultures had a higher metabolic rate (3-fold greater) than CAT cells as measured by CO_2 production (Table III). There was no consistent stimulatory effect of insulin on CO_2 production in either CAT or fibroblast cultures.

Discussion. One of the most important considerations in the study of adipose cells in tissue culture is the ability to demonstrate the retention of some of their characteristic

FIG. 1. a. Typical appearance of unstained CAT cells in culture. Note the presence of intracellular lipid. $\times 200$. 1b. Single isolated CAT cell in culture. $\times 200$. 1c. Confluent culture of human fibroblasts (unstained) $\times 200$.

TABLE I. ³H-THYMIDINE INCORPORATION IN CULTURED CELLS AND ITS STIMULATION BY INSULIN.

Incubation conditions	CAT cells	Fibroblasts
No insulin	3.11 ± 1.41 ^a (7) ^b	61.8 ± 27.0 (6)
+ Insulin (5 mU/ml)	8.60 ± 0.70 ^c (2)	111 ± 4.00 (2)
+ Insulin (100 mU/ml)	36.8 ± 6.35 (2)	-23.4 ± 3.75 (2)

^a Results are expressed as mean percentage conversion of ³H-thymidine/μg DNA × 10⁻² ± SEM.

^b () = n

^c Duplicate flasks were incubated with and without insulin, and the results are expressed as the mean percentage change with insulin over basal values ± SEM.

TABLE II. THE FORMATION OF ¹⁴C-LABELED LIPID IN CULTURED CELLS FROM 1-¹⁴C-GLUCOSE.

Incubation conditions	CAT cells	Fibroblasts
No insulin	5.55 ± 0.86 ^a (12) ^b	0.35 ± 0.35 (2)
+ Insulin (1000 μU/ml)	6.12 ± 1.78 (12)	0.20 ± 0.20 (2)

^a All results are expressed as mean percentage conversion to ¹⁴C-labeled lipid/μg DNA × 10⁻³ ± SEM.

^b () = n.

TABLE III. ¹⁴CO₂ PRODUCTION IN CULTURED CELLS FROM 1-¹⁴C-GLUCOSE.

Incubation conditions	CAT cells	Fibroblasts
No insulin	3.27 ± 1.13 ^a (7) ^b	9.42 ± 0.81 (7)
+ Insulin (1000 μU/ml)	3.76 ± 1.89 (7)	9.91 ± 1.79 (4)

^a Results are expressed as mean percentage conversion of 1-¹⁴C-glucose to ¹⁴CO₂/μg DNA × 10⁻² ± SEM.

^b () = n.

functions under culture conditions. Our results have been only partially successful in this regard. The cultured cells from adipose tissue appear to be morphologically different from skin fibroblasts, the most likely contaminant of adipose cell cultures. The

CAT cells grow and divide, but at a significantly lower rate than fibroblasts. The amount of ³H-thymidine incorporation by CAT cells was increased by insulin at all concentrations tested. The production of lipid from added glucose is much greater in the CAT cell than in fibroblasts, but the characteristic rise in CO₂ production with insulin shown by adipose tissue (7) was not consistently demonstrated in the CAT cell. It has been reported that the level of insulin stimulation of these metabolic pathways is inversely correlated with age (8); therefore, our failure to demonstrate any consistent insulin stimulation of either lipogenesis or CO₂ production may be due to the relatively older age of our subjects or the lack of the necessary incubation conditions.

The culture of adipocytes from a human neonate (9) and of human adipose stromal cells (10) have been reported. The preadipocytes (stromal cells) synthesized cell lipid at a greater rate than fibroblasts, but the adipocytes from the child did not accumulate intracellular lipid. Recently Green and Meuth (11) reported the establishment of a preadipose murine cell line which is capable of accumulating intracellular fat under certain conditions.

Insulin has been reported to increase the growth and cell division rate of mouse mammary-gland tissue *in vitro* (12), but other investigators have found no effect of insulin on growth of cultured mammalian cells (13, 14). With regard to adipose tissue, it has been reported that insulin increased DNA synthesis and adipose tissue cellularity in man and the rat (15, 16), although the necessary separation of adipose from stromal cells was not always done. The results of our investigations have shown that insulin stimulates DNA synthesis in both CAT cells and skin fibroblasts. The only exception was the inhibition of DNA synthesis exhibited by fibroblast cultures treated with high insulin concentrations.

The proper culture conditions necessary for the full expression of characteristic adipose cell metabolism are currently under investigation in our laboratory. Although we have not proved that the CAT cells are indeed true adipocytes, we have demonstrated marked differences between these

cells and skin fibroblasts derived from the same individual. Cultures of CAT cells derived from obese and nonobese subjects can be established, and their study may reveal differences pertaining to a basic defect in obesity.

Summary. The growth and metabolic activity of cultured cells derived from human adipose tissue (CAT cells) were studied and compared to cultured skin fibroblasts. The morphological appearance of the CAT cells was distinctly different from that of fibroblasts. The growth rate of CAT cells as measured by ^3H -thymidine incorporation was much slower than the fibroblast growth rate. Cultured CAT cells synthesized significantly more ^{14}C -lipid from ^{14}C -glucose, while fibroblast cultures had a higher metabolic rate as measured by CO_2 production. Insulin stimulated ^3H -thymidine incorporation in both CAT and fibroblast cultures. The CAT cells did not show a consistent insulin response of lipid or CO_2 production, but this may be a reflection of donor age or nutritional status. Even though the CAT cell may be a type of stromal cell peculiar to adipose tissue rather than a preadipocyte or adipocyte, it may prove useful in studies of human obesity.

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