STRUCTURE-FUNCTION RELATIONSHIPS IN THE ADIPOSE CELL

II. Pinocytosis and Factors Influencing Its Activity in the Isolated Adipose Cell

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ABSTRACT

Pinocytic activity in the adipose cell has been examined by measuring the uptake of colloidal gold. Pinocytic activity occurs in the isolated adipose cell under all experimental conditions; a portion of the vesicular elements of the cell can be identified by electron microscopy as pinocytic in origin. The isolated adipose cell appears to take up serum albumin by pinocytosis. Pinocytic activity in the isolated adipose cell is enhanced by epinephrine, but not by insulin. The relationship between pinocytosis and the metabolic activity of the adipose cell has been studied by measuring simultaneously the uptake of radioactive colloidal gold, the incorporation of ^{14}C-counts from U-glucose-^{14}C into CO_2, total lipid, triglyceride glycerol and triglyceride fatty acids, and the release of nonesterified fatty acids in the absence of hormones and in the presence of insulin or epinephrine. Correlations between hormone-produced alterations in lipid metabolism and in pinocytic activity suggest that intracellular nonesterified fatty acid levels are a factor in the regulation of both the cell's pinocytic activity and its metabolism and that pinocytosis in the adipose cell functions in the extracellular-intracellular transport of nonesterified fatty acids.

INTRODUCTION

The first paper in this series of studies described a technique for high resolution ultrastructural characterization of the isolated adipose cell (1). The results from this study suggested that any hormonally produced alterations in the adipose cell's structure, if such occur, must be dynamic and/or quantitative rather than qualitative in nature (1). In addition, they suggested that the extensive system of vesicles and smooth-surfaced membranous elements, so characteristic of the adipose cell's cytoplasm, resembles the membrane-bound transport and metabolic systems so well characterized in other cell types and that such a vesicular transport system might well respond to hormones in just this fashion (1).

Vesicles of pinocytic nature are a typical feature of the adipose cell and of the endothelial cells of adipose tissue capillaries (1–5). A number of investigators have noted these invaginations of the cell membrane and the vesicles which occupy the region of the cytoplasm adjacent to the cell membrane. One study purports to describe an increase in the number of invaginations and vesicles in adipose tissue incubated in vitro in the presence of insulin (6). Several reports describe increases in the number of these structures in

adipose tissue removed from experimental animals which have been starved for long periods (2, 3, 7, 8). A recent report suggests that such invaginations and vesicles represent a bidirectional lipid transport system in the adipose cell (8). All such reports, however, have been based on nonquantitatively analyzed morphological observations; no direct evidence is available to support the claim that the observed invaginations and vesicles represent pinocytic activity or that their number is increased or decreased under any experimental conditions (1).

This paper describes the uptake of nonradioactive colloidal gold by isolated adipose cells as observed by electron microscopy. Pinocytic activity in the adipose cell is thereby documented, and at least some of those structural elements of pinocytic origin are identified. In addition, the uptake of radioactive colloidal gold and radioactive serum albumin by the cells is described; the pinocytic process is thereby quantitated, enabling an examination of some of the metabolic and hormonal factors which influence it.

METHODS

Isolated adipose cell suspensions were prepared from rat epididymal fat pads and incubated experimentally as described in the preceding and following papers (1, 9). Glucose and defatted serum albumin were present throughout each experiment unless alterations in their concentrations were to be examined as experimental variables. When cells were to be incubated in the presence of fatty acid, palmitic acid was added back to stock defatted serum albumin (1) to the desired fatty acid to albumin molar ratio, and the albumin so treated was added to the incubation vessels. U-glucose-^{14}C, insulin, and epinephrine were added to the incubation vessels as previously described (1).

A stock solution of nonradioactive colloidal gold was prepared fresh weekly. Approximately 3 ml of commercial colloidal gold (Abbott Laboratories, North Chicago, Ill.) containing 2.5 mg gold, 10 mg sodium acetate, 5 mg ascorbic acid, 3 mg gelatin, and 9 μl benzyl alcohol per ml, were dialyzed in the cold for 5–48 hr against 2 1-liter volumes of Krebs-Ringer-bicarbonate (KRB) buffer. The dialyzed colloidal gold was diluted to 200 μg per ml with 0.9% NaCl in water yielding the stock solution. Daily the stock solution was diluted with KRB-albumin buffer. To the diluted solution of nonradioactive colloidal gold was added radioactive colloidal gold (Aurcoloid-198, Abbott Laboratories), of a composition identical to that of the nonradioactive material, to yield 0.05 μCi per μg colloidal gold. The diluted solution was then added to the incubation vessels to yield 1–20 μg gold per ml. Where higher colloidal gold concentrations were desired, portions of the dialyzed commercial preparation were used directly. Only nonradioactive colloidal gold was added to incubation vessels containing cells to be analyzed by electron microscopy. A commercial solution of radio-iodinated (^{131}I) human serum albumin (RISA-121-H, Abbott Laboratories), containing 10 mg albumin, 4.5 mg sodium chloride, and 9 μl benzyl alcohol per ml and sodium bicarbonate as buffer, was diluted with KRB-albumin buffer, containing bovine serum albumin, and the diluted solution was added to the incubation vessels to yield 0.05 μCi per mg albumin in the final incubation medium.

^{14}C-counts incorporated by the isolated adipose cells from U-glucose-^{14}C into CO_2 were monitored, and isolated cells were prepared for and analyzed by electron microscopy, as described in the preceding paper (1). Fixed cells were examined microscopically at all levels of the embedded pellets, with no apparent inconsistencies in the experimental results; contamination of the isolated adipose cells with other cell types was negligible. ^{14}C-counts incorporated from U-glucose-^{14}C into total lipid, triglyceride fatty acids, and triglyceride glycerol, and nonesterified fatty acids released into the incubation medium by the isolated adipose cells were monitored as described in the following paper (9). The uptake of radioactive colloidal gold and radioactive serum albumin by the isolated adipose cells was measured as follows: A portion of incubated cell suspension was transferred to a plastic vessel containing the same volume of cold, 2% OsO_4 in KRB buffer. Cells were fixed in the cold for 1 hr, the fixation medium was aspirated, and the cells were washed with 10-ml portions of cold KRB buffer. Seven washes reduced cell-associated gold radioactivity to a constant level; nine washes were required for the stabilization of albumin radioactivity. Fixed cells were transferred to a tared, nylon Millipore filter (Millipore Filter Corp., Bedford, Mass., NCWP02500) with a final portion of cold KRB buffer, added in two successive 5-ml volumes. The funnel of the glass Millipore filtration apparatus (Millipore Filter Corp., XX1002500) was lined with a cut polyethylene tube. The filter with its fixed cells was dried and reweighed, filter and cells were transferred to a γ scintillation tube, and colloidal gold or albumin counts were obtained in a Nuclear Chicago automatic γ scintillation spectrometer (Nuclear Chicago Corp., Des Plaines, Ill.). Results were expressed as colloidal gold ^{198}Au-counts or as serum albumin ^{131}I-counts per minute per mg fixed cell weight. Specific activity data permitted the calculation of mμg colloidal gold or mμg albumin per mg fixed cell weight.

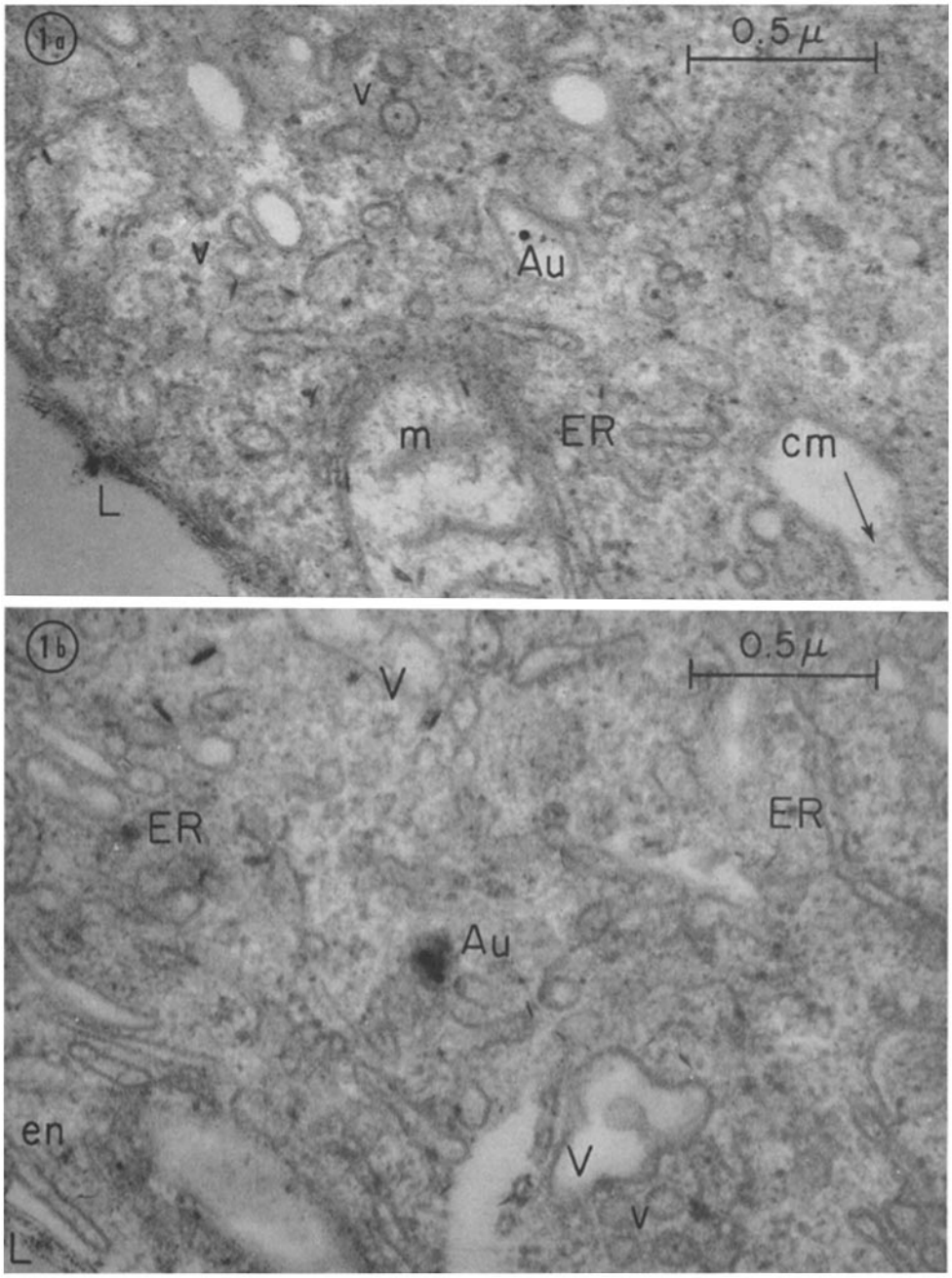

FIGURE 1 Isolated adipose cells incubated 240 min in KRB-albumin buffer in the presence of 1000 μg colloidal gold per ml and in the absence of hormones. *L*, large, central lipid droplet; *ER*, endoplasmic reticulum; *v*, vesicle. Fig. 1 *a*. *Au*, single gold particle in small vacuole; *cm*, cell membrane; *m*, mitochondrion; ↓, electron dense debris. × 51,000. Fig. 1*b*. *Au*, four gold particles in vesicle; *V*, vacuole; *en*, fenestrated envelope. × 51,000.

RESULTS

Electron microscopic examination of isolated adipose cells incubated in the presence of nonradioactive colloidal gold particles demonstrates an uptake of such particles by the cell. All colloidal gold particles taken up are localized to membrane-bound structures (Fig. 1): vesicles attached and adjacent to the cell membrane (Fig. 1 *a*); vesicles localized to the interior of the cytoplasm (Fig. 1 *b*); and small vacuoles. A large proportion of such structures remain, however, unlabeled. Colloidal gold particles are most frequently observed singly within vesicles and only rarely as aggregates; gold particles also appear, but do not accumulate, within lysosomes. Few gold particles are localized to the cell membrane or to the debris adhering to it.

The uptake of radioactive colloidal gold by the isolated adipose cells has permitted a quantitation of the nonradioactive gold uptake described by electron microscopy. Colloidal gold has no detectable effect on the metabolism of the cells as determined by the examination of several metabolic parameters (data not shown). At least one metabolic parameter, most often the production of total lipid-^{14}C, has been monitored during each experiment.

The uptake of colloidal gold particles by isolated adipose cells under basal metabolic conditions increases with increasing gold concentration in the extracellular medium until a maximum level of uptake is reached (Fig. 2). When the quantity of colloidal gold taken up during 60 min of incubation is used as an approximation of the velocity of uptake and the weight concentration of gold in the medium as the concentration of substrate, a

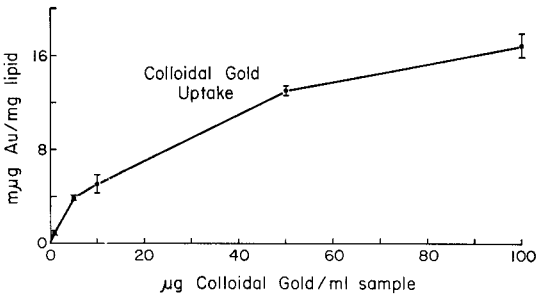

FIGURE 2 Colloidal gold uptake *versus* colloidal gold concentration. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the absence of hormones. Values ± standard deviation.

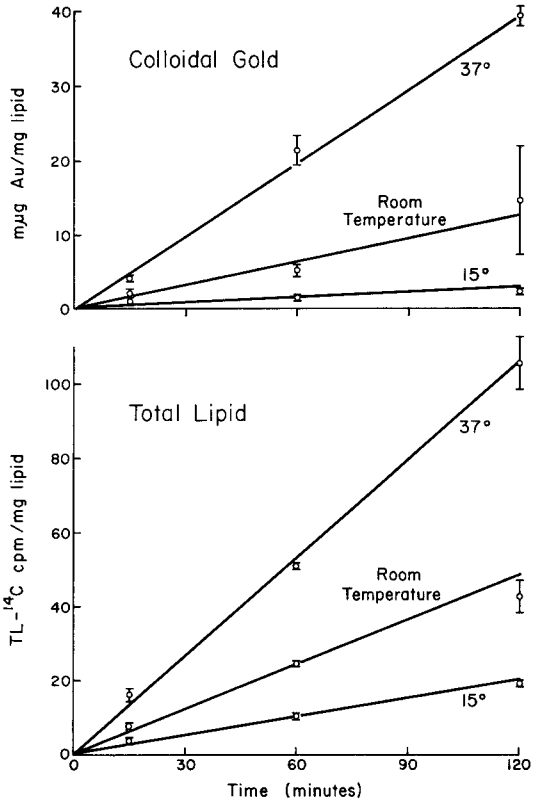

FIGURE 3 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* time at 15°C, room temperature, and 37°C. Isolated adipose cells incubated in KRB-albumin buffer in the presence of 100 μg colloidal gold and 1.6 mg glucose per ml and in the absence of hormones.

Lineweaver-Burk plot can be constructed (Fig. 9), as has been done in great detail for gold uptake in another cell type (10). The similarity between this plot for the isolated adipose cell and that for the rabbit peritoneal macrophage (10) supports the suggestion that colloidal gold uptake is a biphasic process consisting of a rapid, reversible binding of gold particles to sites at or on the cell membrane followed by a slower, irreversible internalization (10).

Both the basal uptake of colloidal gold and the basal production of total lipid-^{14}C by isolated adipose cells increase linearly with time for at least 120 min (Fig. 3) but remain detectable for up to 8 hr. The Q_{10}'s, 27°–37°C, for the two activities (Fig. 3) are virtually identical at values of 1.8 for gold uptake and 1.6 for total lipid-^{14}C production. After 60 min of incubation at 37°C

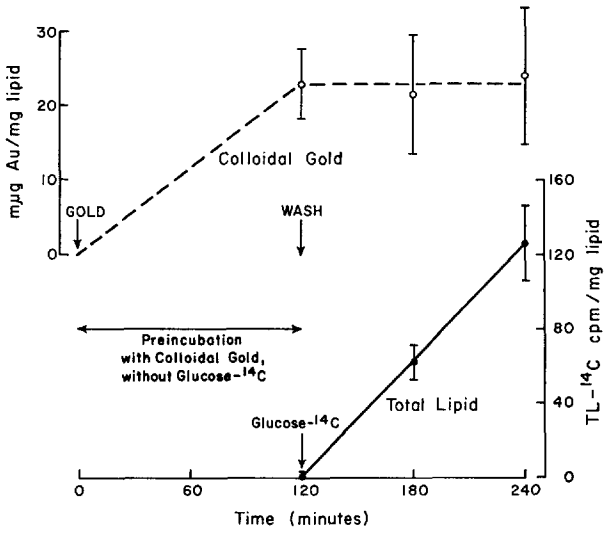

FIGURE 4 Release of colloidal gold taken up and total lipid (*TL*)-^{14}C production *versus* time. Isolated adipose cells incubated in KRB-albumin buffer in the presence of 10 μg colloidal gold and 1.0 mg glucose per ml and in the absence of hormones; reincubated in KRB-albumin buffer in the presence of 1.0 mg glucose per ml and in the absence of colloidal gold and hormones.

in the presence of 100 μg of colloidal gold, 50 mg of cells take up roughly 1% of the gold initially present. The quantity of gold taken up is, however, variable among experiments. Background levels of colloidal gold adsorbed nonspecifically to the cell surface are constant at approximately 10% of the quantity of colloidal gold taken up in 60 min of incubation.

The basal uptake of colloidal gold by isolated adipose cells is irreversible within the limits of the analytical procedure (Fig. 4). Cells have been incubated for 2 hr in the presence of radioactive colloidal gold and nonradioactive glucose, washed, and reincubated for an additional 2 hr in fresh medium lacking colloidal gold but containing U-glucose-^{14}C. During the reincubation, the cells produce total lipid-^{14}C in a characteristic fashion; the colloidal gold content of the cells remains unchanged.

Several substances known to influence the metabolic activity of the adipose cell have been tested for their effects on the uptake of colloidal gold by the isolated cells. Over a physiological range, increasing glucose concentrations have little effect on colloidal gold uptake (Fig. 11). Increasing serum albumin concentrations in the incubation medium have no effect on or slightly inhibit gold uptake but enhance the production of total lipid-^{14}C (Fig. 5).

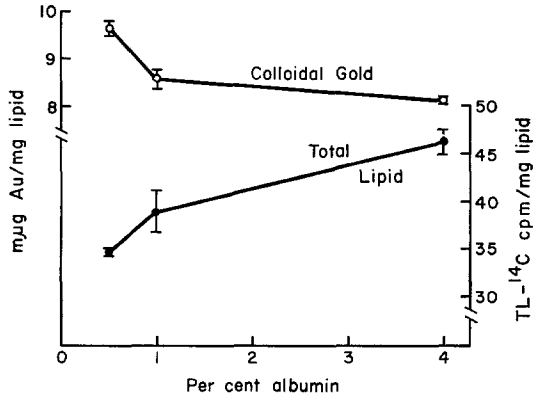

FIGURE 5 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* serum albumin concentration. Isolated adipose cells incubated 60 min in KRB buffer in the presence of 10 μg colloidal gold and 1.28 mg glucose per ml and in the absence of hormones.

Palmitic acid added to the incubation medium has a dual effect on colloidal gold uptake and total lipid-^{14}C production by the isolated adipose cells (Fig. 6). Over the lower range of fatty acid to albumin molar ratios, increasing ratios have no effect on gold uptake but considerably enhance the production of total lipid^{14}-C. Over the higher range of ratios, increasing ratios enhance the production of total lipid-^{14}C no further, but stim-

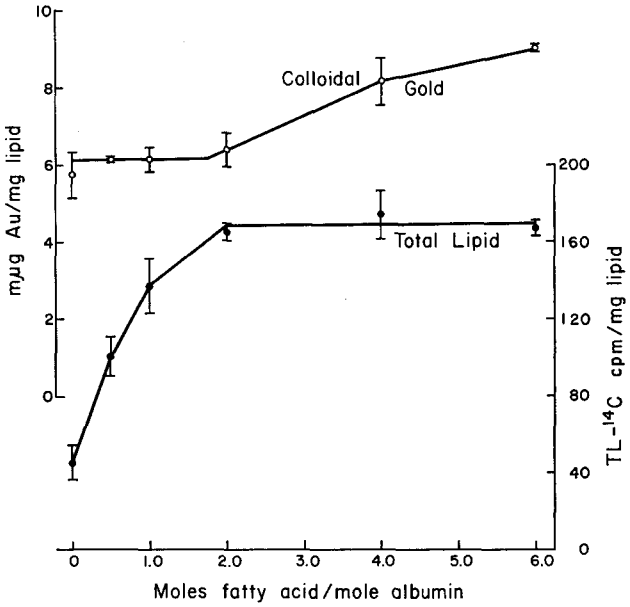

FIGURE 6 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* fatty acid to albumin molar ratio. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 10 μg colloidal gold and 0.98 mg glucose per ml and in the absence of hormones.

ulate the uptake of gold particles. A ratio of 6 moles fatty acid to 1 mole albumin stimulates gold uptake roughly 50%.

Insulin and epinephrine both stimulate the production of total lipid-^{14}C by isolated adipose cells in a characteristic fashion; only epinephrine, however, enhances the uptake of colloidal gold (Fig. 7). Insulin does not affect gold uptake. The epinephrine dose response curve for total lipid-^{14}C production slightly precedes that for gold uptake but the shapes of the curves are similar.

The uptake of colloidal gold by isolated adipose cells in the presence of epinephrine parallels that in the absence of hormones with increasing colloidal gold concentration in the incubation medium (Fig. 8). The straight line obtained in a Lineweaver-Burk plot for colloidal gold uptake in the presence of epinephrine suggests that similar first order mechanisms can explain gold uptake both in the presence of epinephrine and in the absence of hormones (Fig. 9). Epinephrine appears both to elevate the maximum velocity and to lower the apparent binding constant.

The enhancement of colloidal gold uptake and of total lipid-^{14}C production by epinephrine depends differentially upon incubation time (Fig. 10). At 15 min, epinephrine stimulates total lipid-^{14}C production but not gold uptake. At 60 min, epinephrine continues to stimulate total lipid-^{14}C production as at 15 min; however, at the same time, it not only stimulates gold uptake, but stimulates it maximally. At 120 min, epinephrine stimulates total lipid-^{14}C production by an additional 20%; its stimulation of gold uptake, however, has practically disappeared.

Colloidal gold uptake in the absence of hormones is not affected by glucose concentration, while total lipid-^{14}C production is. The degree of enhancement of colloidal gold uptake by epinephrine, however, varies with glucose concentration at the same time that the degree of stimulation of total lipid-^{14}C production by epinephrine remains fairly constant (Fig. 11). Over the lower range, increasing glucose concentrations reduce the stimulation of colloidal gold uptake by epinephrine. Over the higher range, increasing glucose concentrations have little, if any, additional effect. The glucose-dependent reduction in epinephrine-stimulated gold uptake does, however, correspond to the phase of rapidly increasing basal and epinephrine-stimulated total lipid-^{14}C production observed over the lower range of glucose concentrations.

The enhancement of nonesterified fatty acid release, colloidal gold uptake, and total lipid-^{14}C

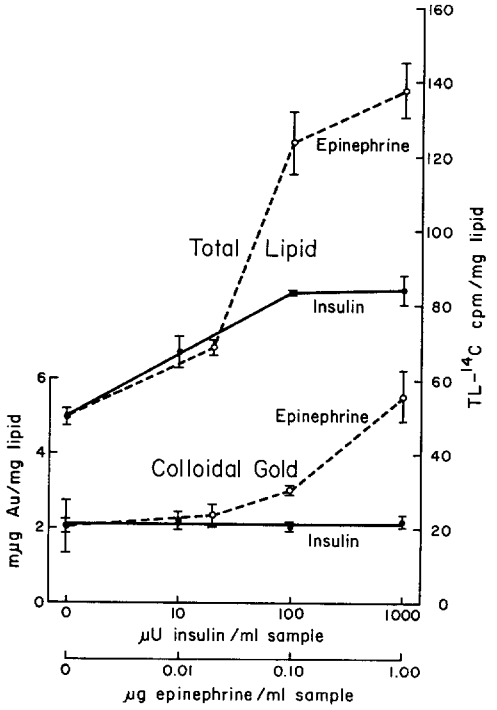

FIGURE 7 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* insulin and epinephrine concentration. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 10 μg colloidal gold and 0.75 mg glucose per ml.

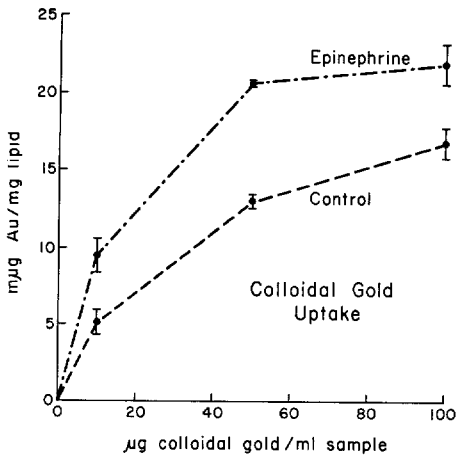

FIGURE 8 Colloidal gold uptake *versus* colloidal gold concentration in the presence of 10 μg epinehprine per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB-albumin buffer.

production by epinephrine depends upon the serum albumin concentration in the incubation medium (Table I). The molar ratio of fatty acids released to albumin present in the medium is similarly dependent. As the quantity of fatty acids released in the presence of epinephrine increases with increasing albumin concentrations, the fatty acid to albumin ratio decreases. Decreases in the stimulation of gold uptake and total lipid-^{14}C production by epinephrine parallel this decrease in fatty acid to albumin ratio.

The enhancement of triglyceride glycerol-^{14}C production, of nonesterified fatty acid release, and of colloidal gold uptake, and the inhibition of triglyceride fatty acid-^{14}C production by epinephrine depend upon the epinephrine dose (Fig. 12). With increasing epinephrine concentrations in the incubation medium, the appearance of an epinephrine-produced stimulation of triglyceride glycerol-^{14}C production slightly precedes the simultaneous appearance of epinephrine-produced alterations in the other parameters. With further increases in epinephrine concentration, the largest change in effect between consecutive hormone doses occurs first in triglyceride glycerol-^{14}C and triglyceride fatty acid-^{14}C production simultaneously, then in fatty acid release, and finally, in colloidal gold uptake. The maximum epinephrine-produced alteration in each parameter occurs first in triglyceride glycerol-^{14}C production, then in triglyceride fatty acid-^{14}C production, and finally, in fatty acid release and colloidal gold uptake simultaneously. The highest dose of epinephrine tested restimulates triglyceride glycerol-^{14}C production; the other parameters remain at their plateau levels.

The effects of insulin on the enhancement of total lipid-^{14}C and triglyceride glycerol-^{14}C production, of nonesterified fatty acid release, and of colloidal gold uptake, and on the inhibition of triglyceride fatty acid-^{14}C production by epinephrine, vary (Fig. 13). Insulin completely inhibits the stimulation of fatty acid release and colloidal gold uptake by epinephrine. Insulin without epinephrine has no effect on either parameter. Insulin completely reverses the inhibition of triglyceride fatty acid-^{14}C production by epinephrine; in fact, triglyceride fatty acid-^{14}C production in the presence of epinephrine plus insulin is roughly identical to that in the presence of insulin alone. Total lipid-^{14}C and triglyceride glycerol-^{14}C production in the presence of epinephrine plus insulin are considerably greater than that in the

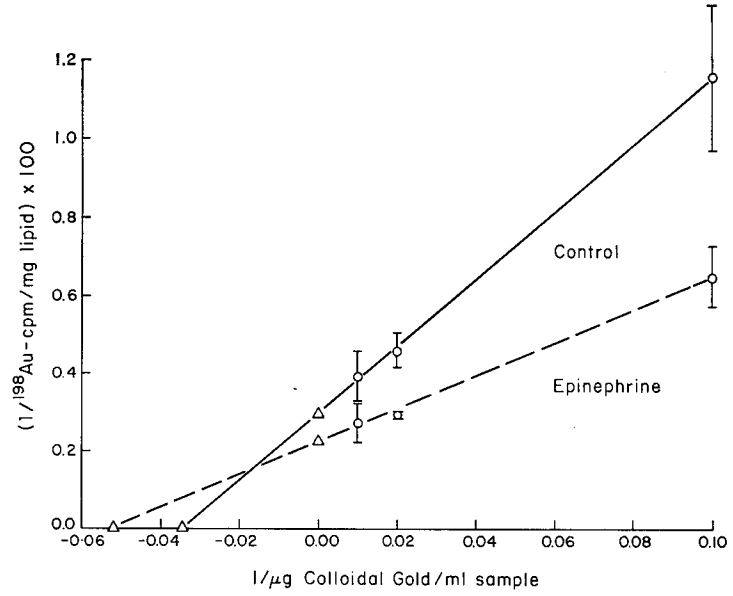

FIGURE 9 Reciprocal of colloidal gold uptake *versus* reciprocal of colloidal gold concentration in the incubation medium in the presence of 10 μg epinephrine per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB-albumin buffer. ○, experimental point; △, calculated point.

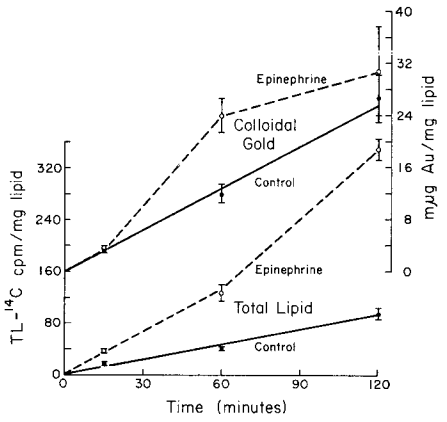

FIGURE 10 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* time in the presence of 10 μg epinephrine per ml or in the absence of hormones. Isolated adipose cells incubated in KRB-albumin buffer in the presence of 35 μg colloidal gold per ml.

presence of either hormone alone or in the absence of hormones.

When isolated adipose cells are incubated under basal conditions in the presence of human serum albumin-^{131}I, a roughly linear uptake of ^{131}I-counts with time occurs (Table II, part A). Epinephrine enhances this albumin-^{131}I uptake by the cells with a dose dependence virtually identical to that for the uptake of colloidal gold (Table II, part B). However, in contrast to the reliability with which background counts can be reduced to constant and negligible levels when colloidal gold has been used, background counts remain high and somewhat variable when the cells have been incubated with albumin-^{131}I. Presumably these reflect a strong, nonspecific adsorption of albumin to the cells. In the representative experiments reported here, this high background (325 and 252 mμg albumin/mg cells, respectively) has been subtracted. Such background levels have prevented the use of albumin-^{131}I uptake as a routine monitor of pinocytic activity.

DISCUSSION

Electron microscopic analysis of nonradioactive colloidal gold particle uptake demonstrates a pinocytic activity in the adipose cell and identifies directly as pinocytic in nature a portion of the invaginations of the cell membrane and of the vesicular structures in the cytoplasm. Contrary to expectation, however, in the isolated adipose cell gold particles label only a small proportion of each of those subcellular elements which in other cell types have been characterized in detail as of

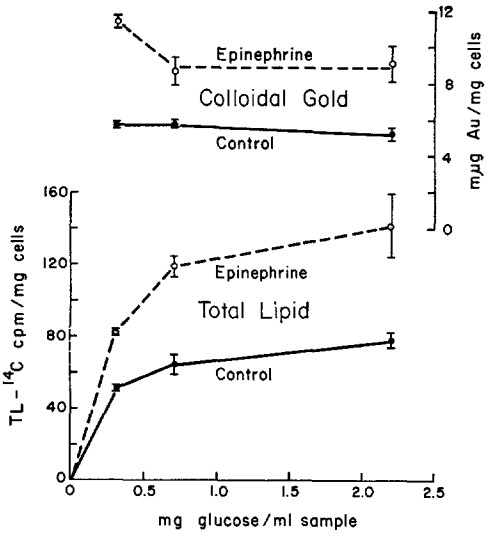

FIGURE 11 Colloidal gold uptake and total lipid (*TL*)-^{14}C production *versus* glucose concentration in the presence of 1.0 μg epinephrine per ml and in the absence of hormones. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 10 μg colloidal gold per ml.

TABLE I

Response to Epinephrine versus Serum Albumin Concentration

Responses to epinephrine of nonesterified fatty acid release, molar ratio of fatty acids released to albumin in the medium, colloidal gold uptake, and total lipid-^{14}C production *versus* serum albumin concentration. Isolated adipose cells incubated 60 min in KRB buffer in the presence of 1.23 mg glucose and 10 μg colloidal gold per ml and in the presence of 1.0 μg epinephrine per ml or the absence of hormones.

	Albumin, %		
	0.50	1.00	4.00
μ equiv.* FA released/ml (epi.-con.)	0.35	0.55	1.15
Moles FA released/mole albumin	4.73	3.74	1.96
mμg Au, epi./mμg Au, con.	1.61	1.44	1.25
TL-^{14}C-cpm, epi./TL-^{14}C-cpm, con.	3.24	3.05	2.46

* equiv., equivalents; FA, fatty acid; epi., epinephrine; con., control; TL, total lipid.

pinocytic origin (11–14). This low level of labeling may reflect a limitation in the capacity of the adipose cell's pinocytic system specifically for gold uptake (15). Alternatively, such a labeling pattern may indicate the existence in the adipose cell of an exocytic, as well as pinocytic, system, the two containing morphologically similar components (16, 17). If vesicular transport systems are functioning simultaneously in opposite directions, however, a direct pathway connecting them appears unlikely since colloidal gold uptake is irreversible, at least over short incubation times (11, 14).

Pinocytic activity in the adipose cell, as reflected in the uptake of radioactive colloidal gold, is temperature-dependent and, therefore, probably energy-dependent (18). Alterations in the rate of glucose uptake and metabolism, as effected by alterations in the external glucose concentration, have, however, little, or no, effect on the basal pinocytic rate over the time intervals observed. Increasing serum albumin concentrations partially inhibit basal pinocytic activity, but, at the same time, stimulate the rate of glucose uptake and metabolism, and of fatty acid esterification, as reflected in the incorporation of ^{14}C-counts from U-glucose-^{14}C into total lipid (9). Increasing palmitic acid to albumin molar ratios at a constant albumin concentration in the incubation medium stimulate pinocytic activity (19) but only after that ratio, which stimulates maximally the cell's capacity to esterify those fatty acids taken up, has been surpassed.

Insulin has no effect on pinocytic activity in the isolated adipose cell, as quantitated in radioactive colloidal gold uptake. This lack of effect contrasts with the reported stimulatory effect of insulin on pinocytosis in the adipose cell of the intact fat pad, as reflected in a qualitative evaluation of the number of invaginations of the cell membrane (6).

Epinephrine, on the other hand, stimulates pinocytic activity in the adipose cell, again as quantitated in gold uptake. In spite of the lack of morphological evidence for increased pinocytosis in isolated cells treated with epinephrine under relatively physiological conditions (1), this stimulation by epinephrine is clearly compatible with the reported increase in the number of invaginations and vesicles in the adipose cell of the fat pad of experimental animals which have undergone prolonged starvation (2, 3, 7, 8).

The only metabolic parameter with which the

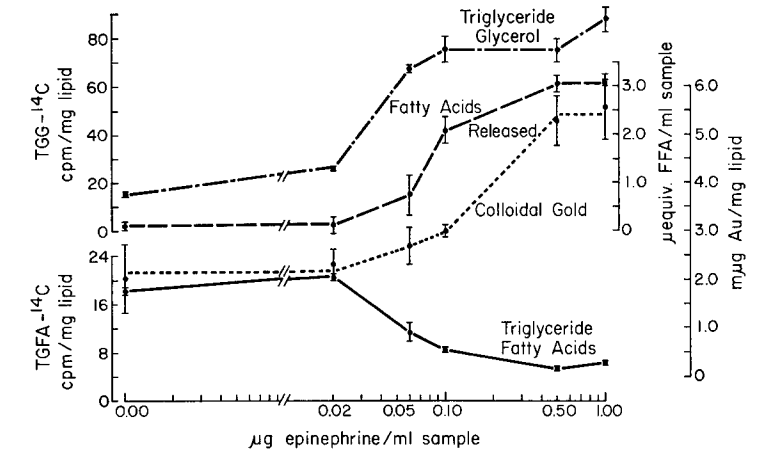

FIGURE 12 Triglyceride glycerol (*TGG*)-^{14}C and triglyceride fatty acid (*FFA*) (*TGFA*)-^{14}C production, colloidal gold uptake, and nonesterified fatty acid release *versus* epinephrine concentration. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 1.28 mg glucose and 10 μg colloidal gold per ml.

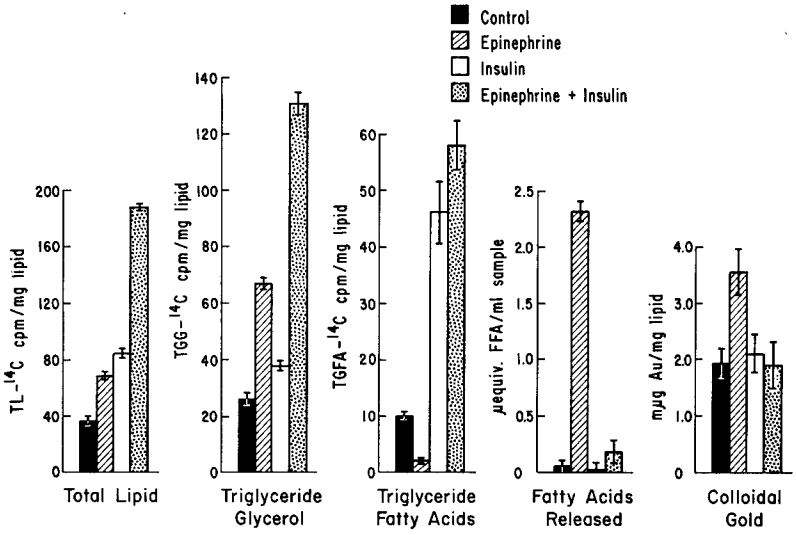

FIGURE 13 Total lipid (*TL*)-^{14}C, triglyceride glycerol (*TGG*)-^{14}C, and triglyceride fatty acid (*TGFA*)-^{14}C production, nonesterified fatty acid (*FFA*) release, and colloidal gold uptake in the presence of 1.0 μg epinephrine, 100 μU insulin, or 1.0 μg epinephrine plus 100 μU insulin per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB-albumin buffer in the presence of 0.35 mg glucose and 10 μg colloidal gold per ml.

adipose cell's pinocytic response to epinephrine correlates in time and with hormone dose is that phase of epinephrine-stimulated nonesterified fatty acid release, during which released fatty acids nearly saturate the binding capacity of the extracellular albumin present (20–22). Insulin inhibits completely both epinephrine-stimulated fatty acid release (23, 24) and pinocytic activity; at the same time, insulin and epinephrine together raise the rate of esterification to a level well above that observed in the presence of either hormone alone. Insulin also completely reverses epinephrine-inhibited fatty acid synthesis (23, 24).

In contrast to the basal rate of pinocytosis, the

TABLE II

Serum Albumin Uptake versus Time and Epinephrine Concentration

A. Serum albumin uptake *versus* time. Isolated adipose cells incubated in KRB buffer in the presence of 1.0 mg glucose and 50 mg serum albumin per ml and in the absence of hormones. B. Serum albumin uptake and nonesterified fatty acid release *versus* epinephrine concentration. Isolated adipose cells incubated 60 min in KRB buffer in the presence of 1.0 mg glucose and 50 mg serum albumin per ml.

	A Time, min			
	0	30	60	120
mμg albumin/mg cells	00.0	26.5	34.5	101.3

	B Epinephrine, μg/ml		
	0.0	0.1	1.0
μ equiv.* FA released/ml	0.00	1.50	2.70
mμg albumin/mg cells	30.7	33.6	67.9

* equiv., equivalents; FA, fatty acid.

pinocytic response of the adipose cell to epinephrine depends considerably upon the glucose and serum albumin concentration in the incubation medium. A decrease in the pinocytic response to epinephrine with increasing glucose concentrations correlates with a reported decrease in the efficiency of fatty acid release, that is, a decrease in the ratio of fatty acids released to glycerol released (25). These decreases occur in spite of the increases in the overall rates of lipolysis and fatty acid release (25). A decrease in the pinocytic response to epinephrine with increasing albumin concentrations correlates with decreases in the epinephrine-stimulated rate of esterification and in the proportion of albumin binding sites occupied by released fatty acids (9). These decreases occur in spite of the increase in the rate of fatty acid release (9).

These data extend to pinocytic activity the concept that intracellular nonesterified fatty acid levels play an important role in the coordination of adipose cell metabolism (20, 25–28). The experimental conditions which give rise to an increase or decrease in pinocytic activity correspond to those which are thought to give rise to an increase or decrease in the intracellular level of fatty acids (20, 25–27). The alterations in glucose metabolism, observed under these same conditions and reported here, are likewise compatible with those alterations in fatty acid and triglyceride metabolism observed by others and explained as reflections of variable intracellular fatty acid levels (20, 25–27). These correlative relationships suggest a role for pinocytic activity in the transport of fatty acids by the adipose cell (8, 19, 29).

The similarities between the time courses and between the epinephrine dose dependencies for the uptake of serum albumin and of colloidal gold indicate that the adipose cell can and does take up albumin from the extracellular space by pinocytosis. Such uptake has been reported in detail for other cell types (30, 31). The possible role of pinocytosis in fatty acid transport, the potential capacity of pinocytosis for serum albumin uptake, and the well established function of albumin as a fatty acid carrier in serum suggest that fatty acid metabolism in the adipose cell is mediated by a vesicular fatty acid transport system within which nonesterified fatty acids are bound to albumin of extracellular origin. The effects of serum albumin on the metabolism of glucose and on the release of nonesterified fatty acids and glycerol by the isolated adipose cell, as reported in the following paper (9), support this suggestion.

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