Insulin-induced Translocation of Glucose Transporters from Post-Golgi Compartments to the Plasma Membrane of 3T3-L1 Adipocytes

John Blok,* E. Michael Gibbs,‡ Gustav E. Lienhard,‡ J. W. Slot,* and Hans J. Geuze*

*Department of Cell Biology, State University of Utrecht, 3511 HG Utrecht, The Netherlands; and ‡Dartmouth Medical School, Department of Biochemistry, Hanover, New Hampshire, 03756

Abstract. A semiquantitative method using immunocytochemistry on ultrathin cryosections and the protein A-gold technique was performed to study the effect of insulin on the cellular distribution of the glucose transporters in cultured 3T3-L1 adipocytes. In basal cells a substantial portion of the label was present in a tubulovesicular structure at the *trans* side of the Golgi apparatus, likely to represent the *trans*-Golgi reticulum, and in small vesicles present in the cytoplasm. Treatment with insulin induced a rapid translocation of transporters from the tubulovesicular structure to the plasma membrane. The transporter labeling of the plasma membrane increased three-fold and that of the tubulovesicular structure decreased by half. There was no effect of insulin on the degree of label in the small cytoplasmic vesicles. Removal of insulin from stimulated cells rapidly reversed the distribution of transporters to that seen in basal cells. This study thus provides the first morphological evidence for the occurrence of transporter translocation in insulin action and identifies for the first time the intracellular location of the responsive transporters.

INSULIN rapidly stimulates glucose transport in fat and muscle cells. There is now considerable evidence that this stimulation is due, at least in part, to the translocation of glucose transporters from an intracellular storage site to the plasma membrane (2, 5, 6, 13, 16–18, 20, 22, 26, 29, 32, 34, 35). To date this evidence has been derived from subcellular fractionation methods in which the plasma membranes and a microsomal fraction have been isolated from basal and insulin-treated cells. Analysis of the glucose transporter content of these fractions by various methods has shown that insulin causes an increase in the amount of transporter in the plasma membranes and a corresponding decrease in the content of the microsomal fraction. For several reasons, it is important to investigate this translocation process in situ by an electron-microscopic method. First, the rigorous establishment of the phenomenon requires demonstration of its occurrence by a method entirely different from subcellular fractionation. Second, the intracellular membrane compartment(s) that store and translocate the transporters have not been identified. The microsomal fraction includes small vesicles derived from the Golgi apparatus and endoplasmic reticulum, as determined by the activities of marker enzymes (5, 26), and it probably also contains endosomal vesicles (6) and possibly membranes from other sources as well.

In this study, we have used the immunogold technique to localize the glucose transporters in basal and insulin-treated 3T3-L1 adipocytes. This cell line was chosen because it exhibits a rapid increase in glucose transport rate of ~15-fold in response to insulin (7). Using affinity-purified antibodies against the transporter (25), the distribution of glucose transporters was quantitated by counting immunogold particles on ultrathin cryosections (27). Our results indicate that insulin induced a threefold increase in the number of transporters in the plasma membrane and that these were recruited from an intracellular compartment located mainly at the *trans* side of the Golgi complex. This structure consists of a tubuloreticular system with small vesicles in its vicinity and in all likelihood represents the formerly described *trans*-Golgi reticulum (TGR)[1] (8, 10, 14). Further evidence for regulation by translocation is the observation that removal of insulin led to the rapid redistribution of the transporters to a state identical with the basal one.

Materials and Methods

Antibodies

A key reagent was the preparation of affinity-purified antibodies against the glucose transporter. Antiserum was obtained from a rabbit immunized with the purified glucose transporter from human erythrocytes. Antibodies against the transporter were isolated from the serum by adsorption onto the transporter in erythrocyte membranes and subsequent release with dilute acid. The details of this method and the characterization of the antibodies have been reported previously (25). On an immunoblot of total cellular protein from 3T3-L1 adipocytes these antibodies reacted to a significant extent only with a polypeptide of average M_r 55,000, which has previously been shown to be the glucose transporter of 3T3-L1 adipocytes (25) (Fig. 1). The immunoblot with these antibodies also shows a very weakly reactive band

1. *Abbreviations used in this paper*: CURL, compartment of uncoupling receptors and ligands; TGR, *trans*-Golgi reticulum.

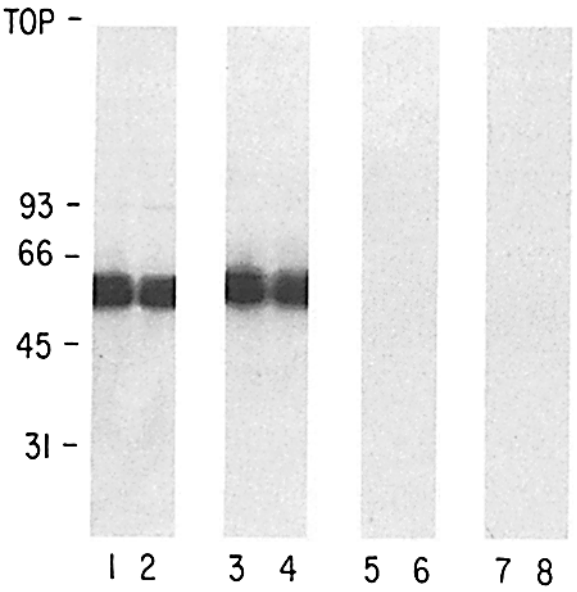


Figure 1. Immunoblotting of the glucose transporter. Single 35-mm plates of 3T3-L1 adipocytes were treated with or without 100 nm insulin for 10 min. The cells on each plate were solubilized in 600 μl of SDS sample buffer (1) containing protease inhibitors (1 mM diisopropylfluorophosphate, 1 μg/ml pepstatin A, and 10 μM L-*trans*-epoxysuccinyl-leucylamido-(3-methylbutane), and the samples were sheared by passage through 22- and 25-gauge needles. Samples (20 μl) were subjected to SDS-PAGE on a single slab gel and immunoblotting, as described in (20). Lanes *1*, *3*, *5*, and *7* received samples from basal cells; lanes *2*, *4*, *6* and *8* received ones from insulin-treated cells. Strips containing paired lanes were cut from the nitrocellulose and first treated with affinity-purified antibodies against the erythrocyte glucose transporter (lanes *1* and *2*), affinity-purified antibodies against the carboxy-terminal peptide of the transporter (lanes *3* and *4*), nonimmune rabbit IgG (lanes *5* and *6*), or no antibody (lanes *7* and *8*), and then treated with ^{125}I-labeled goat antibodies against rabbit IgG (all lanes). The first antibodies in each case were at 0.5 μg/ml. The M_r of standard proteins are given in thousands.

of M_r 93,000. We think that it is very unlikely that reaction with this polypeptide accounts for a significant portion of the labeling found in the immunocytochemistry (See Results), for two reasons. First, the amount of labeling in the immunoblot relative to that of the transporter is very low (Fig. 1). Second, a homogenate of basal 3T3-L1 adipocytes was separated into a fraction containing the plasma membranes and one containing the insulin-responsive intracellular transporters by centrifugation at 16,000 *g* (max) for 20 min, and then the two fractions were immunoblotted. Only the plasma membrane fraction showed the very weakly reactive band of M_r 93,000; none was present in the fraction containing the intracellular transporters (Brown, S. J., and G. E. Lienhard, unpublished results). Since intracellular membranes were the main region in which basal cells were labeled upon immunocytochemistry (see Results), the transporter must be the protein that was predominantly labeled by this method as well as by immunoblotting.

Some experiments were also performed with affinity-purified rabbit antibodies against the carboxy-terminal peptide (residues 477-492) of the human erythrocyte and rat brain glucose transporter. The preparation and characterization of these has been reported (3). On an immunoblot of total cellular protein from 3T3-L1 adipocytes the anti-carboxy-terminal antibodies also reacted to a significant extent only with the 55,000-M_r polypeptide (Fig. 1).

Cell Culture

3T3-L1 fibroblasts were cultured on 10-cm plates and differentiated into adipocytes by treatment with dexamethasone, 3-isobutyl-1-methylxanthine, and insulin as described previously (7). Adipocytes were used on day nine after the initiation of differentiation, at which time they are fully differentiated. The fibroblasts examined by EM were ones at 2 d post confluency (7 d after plating) that had not been treated with any of the differentiating agents. Just before use, cells were placed in 10 ml serum-free DME for 2 h. Insulin-treated cells were exposed for 10 min to 100 nM insulin, which was added from a stock 160 μM solution. For reversal of the insulin effect, insulin-treated adipocytes were washed twice at 37°C with 10 ml Krebs-Ringer salts (136 mM NaCl, 4.7 mM KCl, 1.25 mM $MgCl_2$, and 1.25 mM $CaCl_2$) containing 5 mM sodium phosphate, pH 7.4, and incubated for 1 h at 37° in 10 ml Krebs-Ringer salts containing 1 mg/ml BSA and 10 mM 2-(N-morpholino)ethane sulfonic acid (MES), pH 6.0.

Cell Processing for Electron Microscopy

After the treatments described above, the cells were rapidly washed twice with 10 ml Krebs-Ringer phosphate buffer at 37°C and then fixed for 1 h at 4°C in 10 ml 1% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate, pH 7.4. The fixed cells were washed with 10 ml 0.1 M sodium phosphate, scraped off the plate in 2 ml 1% paraformaldehyde in 0.1 M sodium phosphate, and shipped from Hanover to Utrecht.

For routine EM, the cells were postfixed in 1% OsO_4 in 0.1 M sodium phosphate, pH 7.4, for 1 h at 4°C, pelleted in agar, dehydrated, and embedded in Epon. Ultrathin sections were stained briefly with uranyl acetate and lead hydroxide and examined with a Philips EM 301. For EM-immunocytochemistry, the cells were embedded in 10% gelatin and stored at 4°C in 1% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4.

Immunocytochemistry

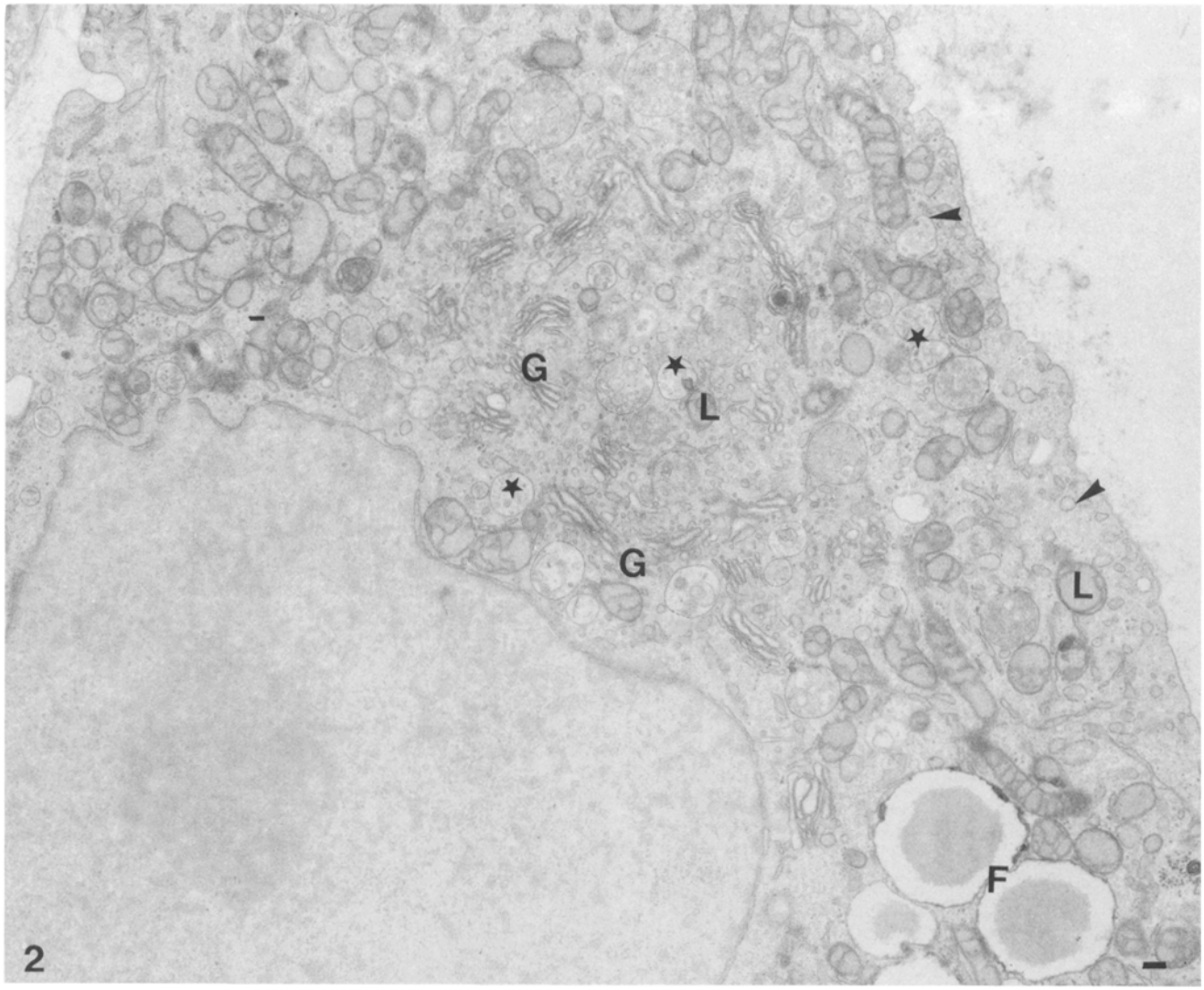
Details of the procedure have been described previously (9, 27). Ultrathin (~100 nm) cryosections were prepared according to Tokuyasu (31). The sections were incubated for 30 min with the primary antibody at 25 μg/ml. The sections were then treated with swine anti-rabbit IgG to enhance the signal (28). The immunoglobulins were visualized by the protein A-gold technique, using 8-nm gold particles (28). In control sections, incubation with the primary antibody was omitted. The sections were stained with uranyl acetate and embedded in methyl cellulose (31).

Quantitation of Immunocytochemistry

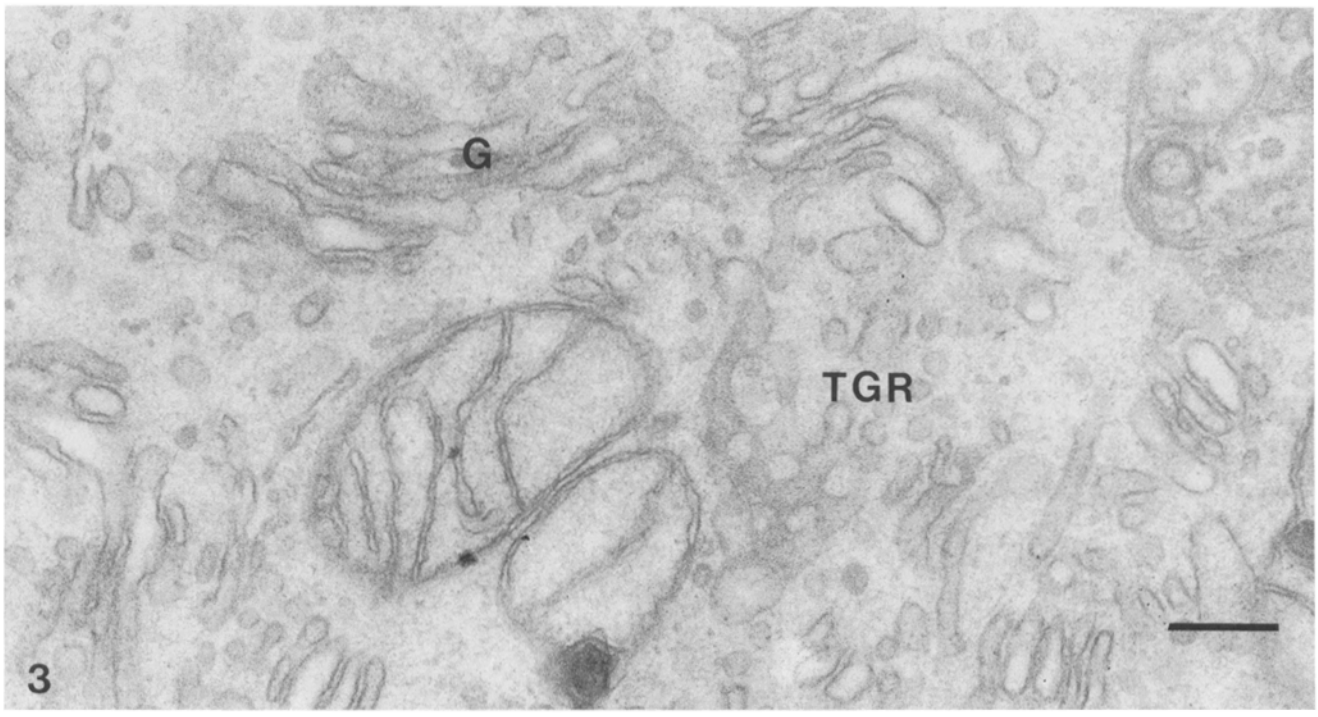
The distribution of the gold particles was assessed over the seven organelles listed in Table II, which contained more than 95% of the cellular labeling. Since the distinction between the *trans*-Golgi reticulum (TGR) and the compartment in which ligands uncouple from receptors (CURL) on morphological grounds is not always sharp (8), and since no markers of these organelles compatible with the labeling method have been established for 3T3-L1 adipocytes, the quantitative data have been presented as a joint item (TGR/CURL). As discussed in (8), TGR is located in close proximity to the *trans* side of the Golgi stacks and often exhibits fenestrated lamella, whereas CURL is located in a 1-μm wide zone of peripheral cytoplasm and invariably contains short, branched tubules and associated vacuoles, most of which are multivesicular vacuoles. On this basis, an attempt has been made to separate TGR and CURL labeling as accurately as possible.

The experiments that were quantitated in this study are listed in Table I. For each experiment 20 blocks were cut from the pellet of cells from a single 10-cm plate. Three blocks were randomly selected and cut to sections. Three grids among the many from each block were selected on the basis of good morphology at 850×; cells in these that showed a nuclear profile

Figures 2 and 3. (Fig. 2) Routine EM of paranuclear cytoplasm of 3T3-L1 adipocyte. An extensive Golgi complex consisting of several curved stacks of Golgi cisternae (*G*) is present close to the nucleus. In the central area of the complex, at the concave (*trans*) side of the stacks occur numerous vesicles and tubules of TGR. Multivesicular bodies (*asterisks*) and lysosomes (*L*) are also present in this area. Just beneath the plasma membrane occur smooth vacuoles and tubular profiles which presumably belong to CURL (*arrowheads*). *F*, fat droplets. (Fig. 3) Higher magnification of a central Golgi area as in Fig. 2, showing the fenestrated appearance of TGR. *G*, Golgi cisternae. Bars, 0.25 μm.



2



3

Table I. Experiments Performed and Gold Particles per Cell Profile

Cell type	Number of experiments*	Number of cells quantified per experiment	Average number of gold particles per cell profile‡ ±SEM
Adipocytes			
basal	2	24	59.1 ± 3.7
insulin-treated	2	24	62.9 ± 2.8
insulin-treated, reversed	1	24	52.4 ± 5.5
Fibroblasts			
basal	1	20	23.3 ± 1.0
insulin-treated	1	20	24.9 ± 1.0

* Each experiment was performed with cells from a single 10-cm plate.
‡ In each case, control experiments in which the anti-transporter antibodies were omitted showed 5% or less gold particles per cell profile.

were randomly chosen and used for counting at 15,000×. For each experiment 20–24 profiles were scored.

Hexose Transport

The method for measuring hexose transport has been described in detail previously (7). Cells that had been treated in the various ways described above were washed quickly with Krebs–Ringer phosphate at 37°C, and the uptake of [^{3}H] 2-deoxyglucose (0.1 μCi/ml, 50 μM) in Krebs–Ringer phosphate at 37°C with or without 100 nM insulin over a 10-min period was measured.

Results

Morphology of 3T3–L1 Adipocytes

Novikoff et al. (21) have previously described the morphology of 3T3–L1 fibroblasts and adipocytes in detail, and our observations generally agree with theirs. Upon differentiation, the cells rounded up, and numerous fat droplets appeared in the cytoplasm. The adipocytes contained prominent Golgi stacks. At the concave (*trans*) side of the stacks occurred a system of tubules, vesicles, and fenestrated lamellae designated TGR (Figs. 2 and 3), which was morphologically identical to GERL (21). The TGR often appeared as a fenestrated lamella with smooth or coated buds (Fig. 3). Adjacent to the plasma membrane occurred numerous tiny vesicles and attached tubules (Fig. 2) which were very reminiscent of CURL (9). In the following we have tentatively used the term CURL for all the tubulovesicle profiles present in a 1-μm wide zone of peripheral cytoplasm. No obvious effect of insulin on the general morphology of the adipocytes was detected. The cells contained abundant multivesicular bodies, mostly located in the Golgi areas (Fig. 2).

Distribution of Glucose Transporters in 3T3–L1 Adipocytes

The distribution of glucose transporters was determined by counting the number of gold particles in the various cell organelles. The data have been expressed as percent of the total number of particles per cell profile to normalize for differences in labeling efficiency from plate to plate of cells, although in fact the average number of gold particles per cell profile for adipocytes in the various states did not vary much (Table I). In basal adipocytes, most of the transporters were located intracellularly, with 55% of the total in the TGR/CURL (Fig. 4, Table II). Intracellular small vesicles of unknown nature also contained a substantial portion (26%) of the transporter. Only 14% was in the plasma membrane.

Previously, others as well as ourselves (7, 11, 12) have reported that treatment of 3T3–L1 adipocytes with 100 nM insulin for 10 min stimulates hexose transport, as measured by 2-deoxyglucose uptake, by 10–20-fold. Responses in this range were observed with plates assayed for transport over the course of this study. Treatment of the adipocytes with insulin under these conditions caused a marked redistribution of the glucose transporter (Table II, Figs. 4–7). The percentage of the transporters in the TGR/CURL decreased by half, whereas that in the plasma membranes increased three-fold. The decrease in the percentage of transporters in the TGR/CURL in response to insulin (55 − 28 = 27%) was almost the same as the increase in the percentage of transporters in the plasma membranes (43 − 14 = 29%). Therefore TGR/CURL most likely provided for the pool of the transporters that appeared in the plasma membrane. As noted in the Material and Methods, the TGR and CURL has been presented

Table II. Distribution of Glucose Transporters in 3T3-L1 Adipocytes

Cell type	Total gold particles	Percentage in					
		PM	TGR/CURL	G	MVB	VES	LYS
Adipocytes							
basal	2,838	14.5 ± 2.3	54.9 ± 3.7	2.2 ± 0.5	1.5 ± 0.3	25.5 ± 2.9	1.5 ± 0.5
insulin-treated	3,019	43.1 ± 8.7	28.6 ± 5.1	1.0 ± 0.3	1.7 ± 0.1	23.6 ± 3.5	2.0 ± 0.6
insulin-treated, reversed	1,048	17.9 ± 2.2	50.9 ± 3.5	1.2 ± 0.4	0.6 ± 0.2	20.8 ± 2.8	0.6 ± 0.3
Fibroblasts							
basal	463	30.4 ± 1.4	22.8 ± 2.8	4.0 ± 2.1	1.8 ± 0.5	38.1 ± 3.5	2.9 ± 1.3
insulin-treated	497	29.6 ± 3.2	27.0 ± 3.1	3.0 ± 0.9	3.0 ± 0.6	34.8 ± 2.8	2.5 ± 0.8

Values are expressed as percentages of the total number of gold particles per cell profile ± SEM. The abbreviated headings are: PM, plasma membranes; G, Golgi; MVB, multivesicular bodies; VES, vesicle of undefined nature; LYS, lysosomes.

as a single category because it was not always possible to distinguish with certainty between the two. Nevertheless, tentative assignment of gold particles to one or the other organelle was made. On this basis in basal cells most of the transporters in this category were located in the TGR (~45% out of the 55%); moreover, the 27% decrease in the transporter content in response to insulin occurred entirely in the TGR. The percentages of the transporters in the other cell organelles were not significantly affected by insulin, with the exception of a decrease in the small percentage of transporter in the Golgi apparatus (Table II).

Because the glucose transporters are almost certainly inserted into the plasma membrane by an exocytotic process, it was of interest to examine whether they were clustered or randomly located in the plasma membrane. Although this question has not been evaluated in a quantitative way, our impression from the many cell profiles examined is that the distribution of gold particles along the plasma membrane was random. The small clusters of particles that are predominant in Figs. 3–7 are due to the binding of several swine antibodies against rabbit IgG to a single anti-transporter antibody, since these clusters were not observed when the intermediate antibodies were omitted.

The results shown in Table II were obtained with affinity-purified antibodies raised against the human erythrocyte glucose transporter. Labeling of the transporter in basal and insulin-treated adipocytes was also performed with affinity-purified antibodies against the carboxy-terminal peptide of the glucose transporter. Since the amount of labeling was less than 10% of that obtained with the antibodies against the entire transporter, the distribution of label was not quantitated. However, our impression was that the distributions in the two states were roughly similar to those in Table II. The much lower reactivity of the antibodies against the carboxy terminus in the immunocytochemistry is likely to be the result of the modification of lysine 477 and histidine 484 in this exposed region of the protein by the formaldehyde/glutaraldehyde used for fixation (3, 15).

We have previously shown that incubation of insulin-stimulated 3T3–L1 adipocytes in the absence of insulin at pH 6, where the affinity of the receptor for insulin is substantially reduced, results in a return of hexose transport to the basal rate in 30 min (11). Insulin-stimulated adipocytes that had been returned to the basal state by 60-min incubation in this way were examined for the distribution of transporters. The distribution in these deactivated adipocytes was the same as that in the basal adipocytes, which had been cultured for 5 d in the absence of insulin (Table II).

Distribution of Glucose Transporters in 3T3–L1 Fibroblasts

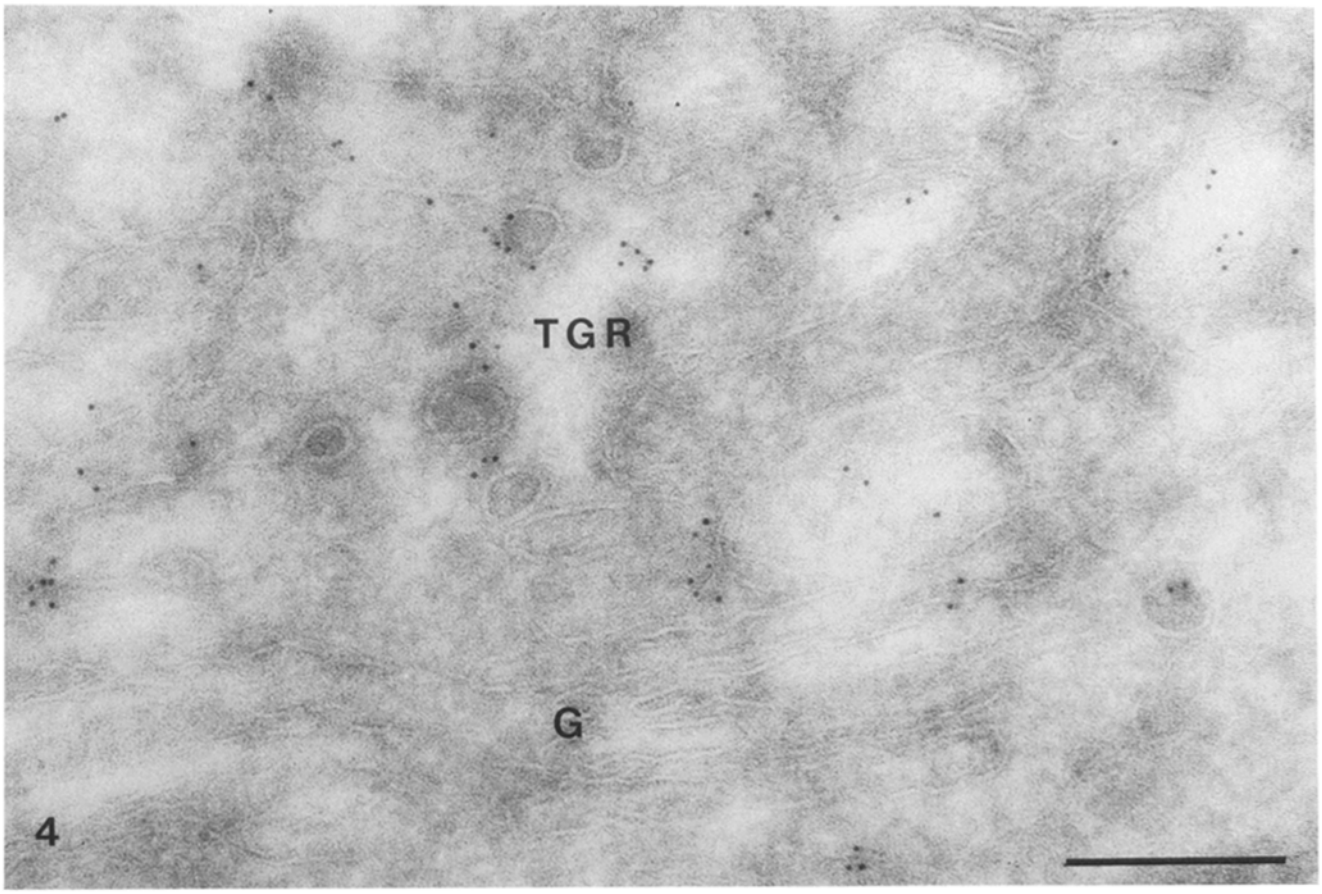
It has been shown previously that insulin stimulates hexose transport in the 3T3–L1 fibroblasts much less markedly than in the adipocytes (24). In this study we found that the rate of 2-deoxyglucose uptake by fibroblasts was increased only 1.8-fold by treatment with 100 nM insulin for 15 min. In agreement with the absence of a large effect on transport, insulin did not alter the distribution of the glucose transporters in fibroblasts (Table II). Both basal and insulin-treated fibroblasts contained ~30% of the transporters in the plasma membrane, which is twice as much as in basal adipocytes. The fraction of transporters in the TGR/CURL was about half that in basal adipocytes. The absence of a large pool in this location may be one of the reasons for the small effect of insulin on hexose transport in fibroblasts.

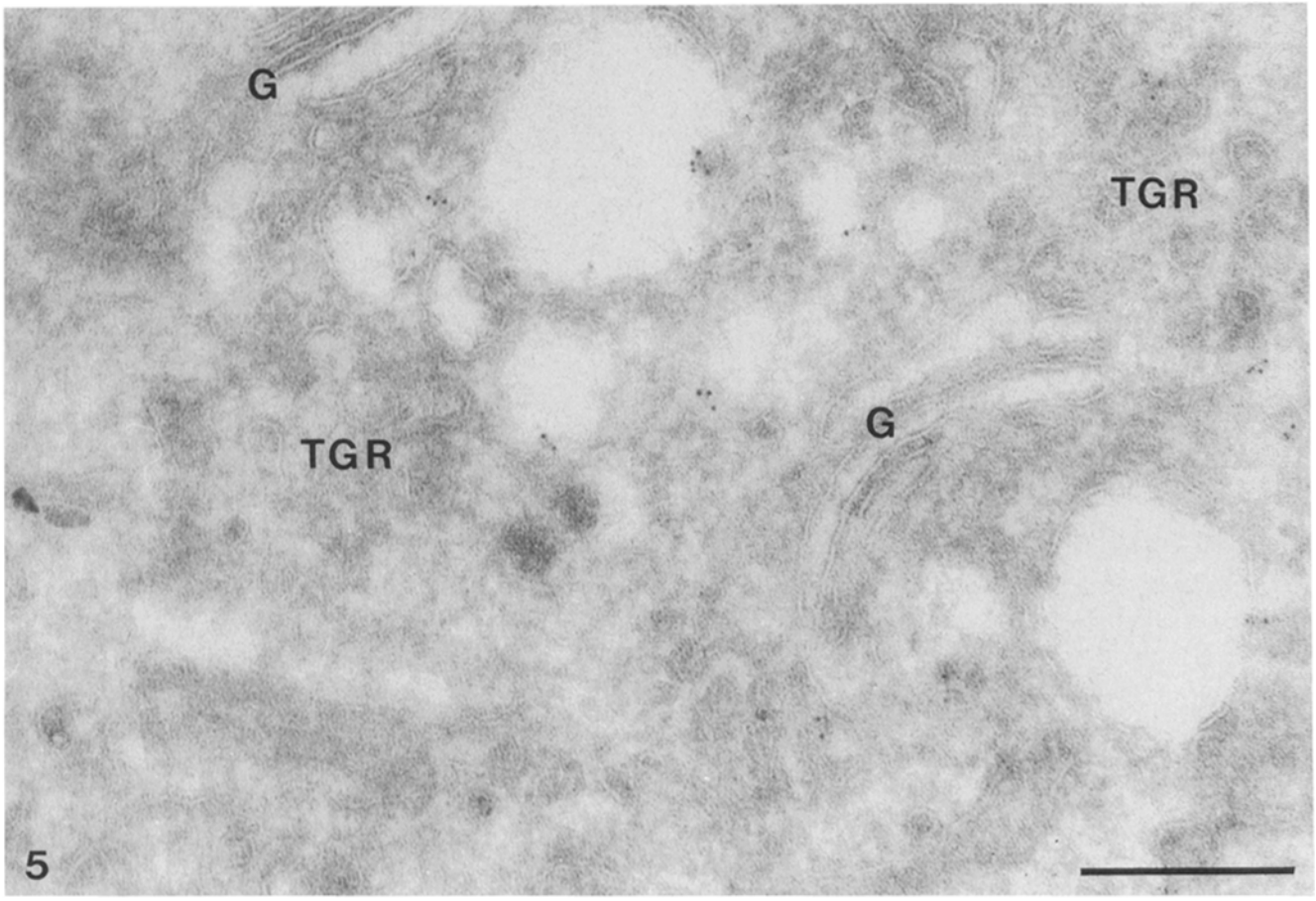
Discussion

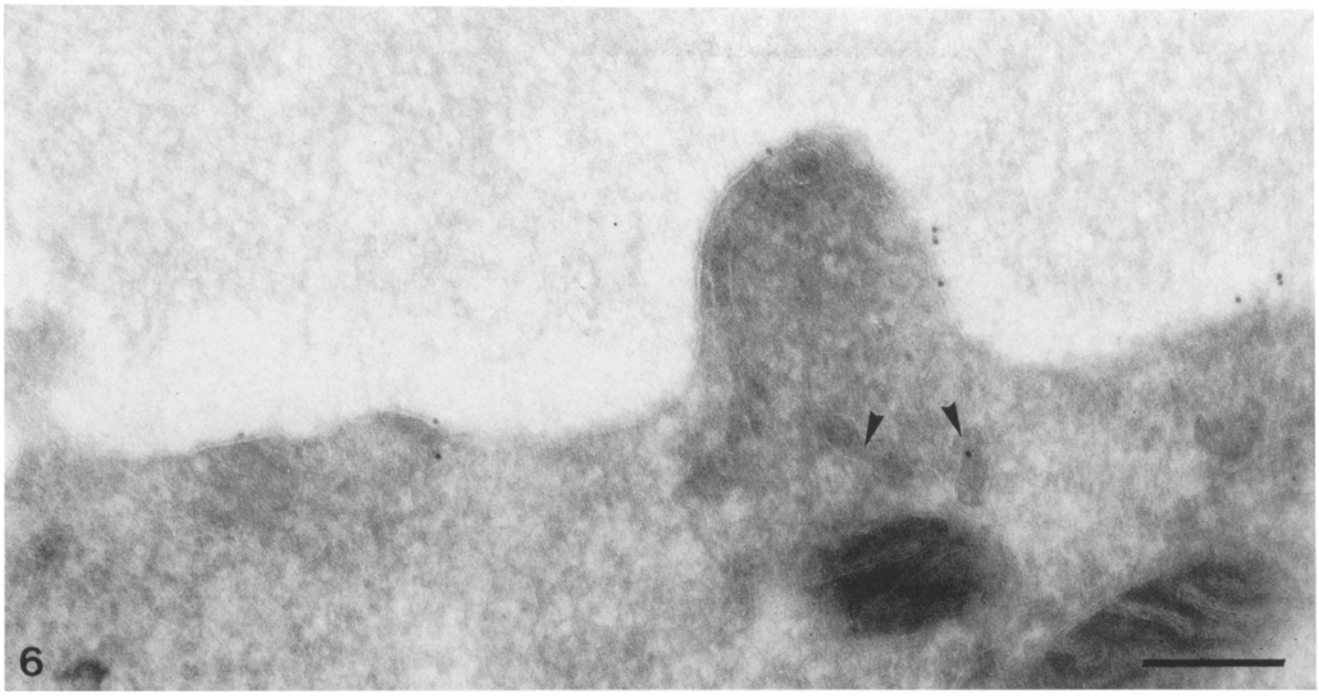
The results of the present immunocytochemical study show that in cultured adipocytes, the distribution of glucose transporters over cellular profiles is strictly dependent on the presence of insulin. The hormone induced a rapid translocation of transporters from the TGR/CURL to the plasma membrane, and removal of the hormone caused a rapid translocation of the transporters in the reverse direction. These findings thus establish by immunocytochemistry the occurrence of the translocation processes that had previously been deduced by subcellular fractionation (see Introduction). In addition, they show for the first time that the insulin-responsive intracellular transporters are located in the TGR/CURL. Most probably the site of these transporters is the TGR, a compartment in which membrane proteins are sorted and through which plasma membrane components cycle (14). Recently, we have developed a method for the isolation of vesicles containing the insulin-responsive intracellular transporters from 3T3–L1 adipocytes by immunoadsorption (1), and it will be of interest to determine whether these vesicles are derived from the TGR.

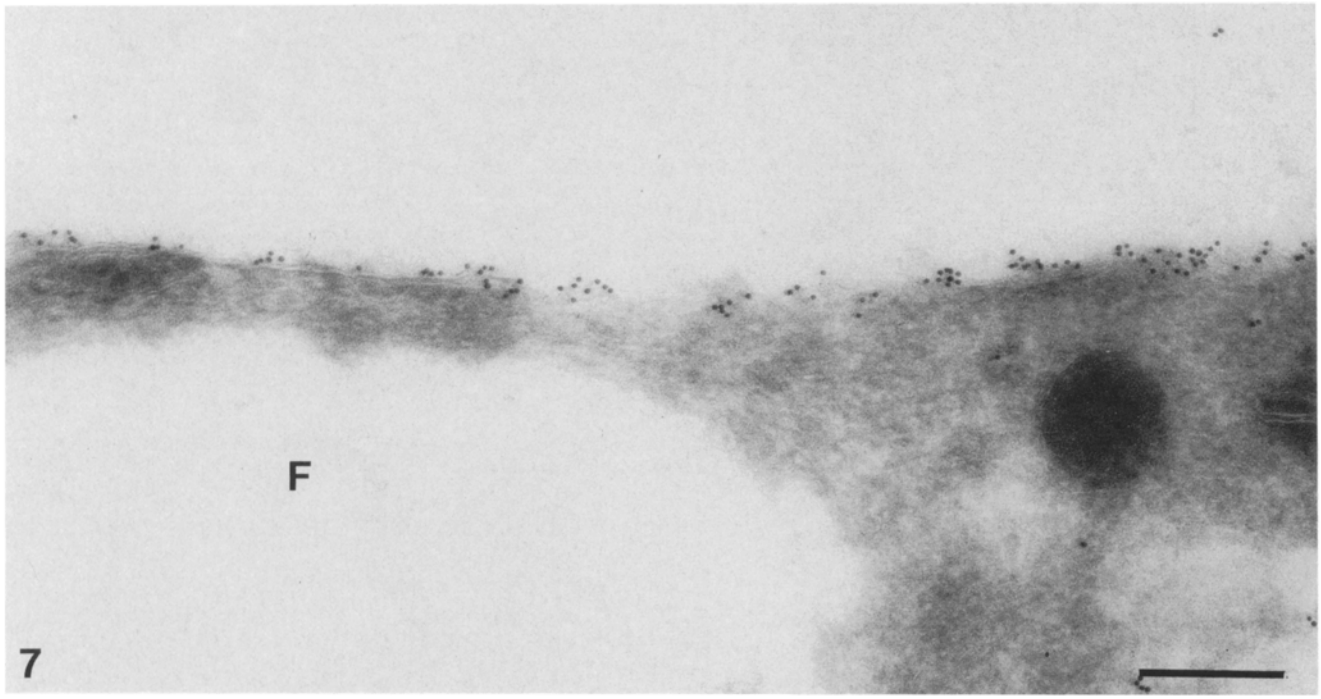
The magnitudes of the increase in transporter content of the plasma membrane (threefold) and the decrease in the content of the TGR/CURL (50%) in response to insulin measured by quantitation of the gold particles agree well with the values that we have previously obtained by subcellular fractionation (1). In this earlier study, subcellular fractions that possessed characteristics expected for enriched plasma membranes and intracellular membranes were isolated from basal and insulin-treated 3T3–L1 adipocytes and assayed for transporter content by quantitative immunoblotting. Insulin caused a 2.5-fold increase in the transporter content of the plasma membrane fraction and a 48% decrease in the content of the intracellular membrane fraction.

On the other hand, there is a considerable discrepancy between the effect of insulin on the transporter content of the plasma membrane, as assayed by these two methods, and its effect on the rate of transport. It has been reported that insulin increases the V_{max} for 2-deoxyglucose uptake in 3T3–L1 adipocytes by a factor of 15, without any effect on K_m; the rate of transport of the nonphosphorylated hexose 3-0-methylglucose is also stimulated 15-fold (17). Under our conditions we typically find that 2-deoxyglucose uptake is stimulated 10–15-fold by insulin. Thus, it may be that in addition to causing an increase in the transporter content of the plasma membrane insulin also acts to enhance the intrinsic activity of each transporter. Alternatively, we cannot exclude the possibility that the electron microscopic and subcellular fractionation methods underestimate the increase in plasma membrane transporter content. With regard to fractionation, since there is no established marker for transporter-containing intracellular membranes, these may contaminate the plasma membrane fraction. Concerning the immunocytochemistry, implicit in our discussion of the results has been the assumption that the efficiencies of labeling of the transporter in the several organelles under the various conditions are the same. This assumption is supported by the finding that even though the transporter undergoes a dramatic redistribution in response to insulin, the total number of gold par-


TGR
G
4


G
TGR
TGR
G
5





Figures 6 and 7. (Fig. 6) Plasma membrane of basal adipocyte showing glucose transporter labeling. The arrowheads indicate CURL profiles. (Fig. 7) Plasma membrane of insulin-stimulated cell showing increased density of glucose transporter labeling. This section is more heavily labeled than a typical one, and the increase in labeling seen here, relative to Fig. 6, is thus even larger than the threefold increase found from examining many sections (see Table II). *F*, fat droplet. Bars, 0.25 μm.

Figures 4 and 5. (Fig. 4) Ultrathin cryosection of basal 3T3-L1 adipocyte immunolabeled with 8-nm protein A-gold particles for the demonstration of glucose transporters. The micrograph shows a stack of Golgi cisternae (*G*) with TGR. Most of the label is associated with TGR elements, some of which bear coats at their cytoplasmic surface. This field was selected to demonstrate the label in the TGR elements and is more heavily labeled than a typical one (an average complete cell profile contained ~60 particles [Table I]). (Fig. 5) Cryosection of insulin-stimulated adipocyte showing reduction of glucose transporter labeling in TGR (cf. Fig. 4). *G*, Golgi stacks. Bars, 0.25 μm.

ticles per cell profile is the same for basal and insulin-treated adipocytes (Table I). However, since the plasma membrane of basal cells contains only 15% of the transporters (Table II), the possibility that the transporters in this organelle are labeled at somewhat higher efficiency in the basal state than in the insulin-treated state cannot be excluded. Studies on labeling efficiencies in immunoelectron microscopy have in fact just begun (Passthuma, G., J. W. Stot, and H. G. Geuze, manuscript submitted for publication), and we do not have an estimate of the efficiency with which the transporter is labeled.

There is evidence that insulin also causes the translocation of intracellular receptors for transferrin and insulin-like growth factor II to the plasma membrane in adipocytes (4, 23, 30, 33). Immunocytochemical double labeling with gold particles of different size may provide insight into whether these receptors are stored and translocated together with the glucose transporters.

The pathway(s) by which the redistribution of the glucose transporter between the plasma membranes and the TGR/CURL in response to insulin addition and withdrawal occurs is not known. A reasonable hypothesis is that the glucose transporter, similar to the transferrin receptor (30, and references therein), is a membrane protein that continuously recycles between the plasma membrane and the TGR/CURL. Insulin could then increase the proportion of the total cellular transporter in the plasma membrane by either increasing the rate constant for its externalization and/or decreasing the rate constant for its internalization (19). It has recently been shown that in the case of the transferrin receptor the basis of the insulin-induced increase in plasma membrane receptors is an increase in the rate constant for externalization of intracellular receptors (30).

We are deeply indebted to Antony Davies and Stephen A. Baldwin of the Royal Free Hospital School of Medicine for providing the antiserum against the carboxy-terminal peptide of the glucose transporter. We thank Susan J. Brown for excellent technical assistance.

This research was supported by a grant from the National Institutes of Health (AM 25336) to G. E. Lienhard and a Career Development Award from the Juvenile Diabetes Foundation International to E. M. Gibbs.

Received for publication 17 July 1987, and in revised form 5 October 1987.

References

1. Biber, J. W., and G. E. Lienhard. 1986. Isolation of vesicles containing insulin-responsive, intracellular glucose transporters from 3T3-L1 adipocytes. *J. Biol. Chem.* 261:16180–16184.
2. Cushman, S. W., and L. J. Wardzala. 1980. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. *J. Biol. Chem.* 255:4758–4762.
3. Davies, A., K. Meeran, M. T. Cairns, and S. A. Baldwin. 1987. Peptide-specific antibodies as probes of the orientation of the glucose transporter in the human erythrocyte membrane. *J. Biol. Chem.* 262:9347–9352.
4. Davis, R. J., S. Corvera, and M. P. Czech. 1986. Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. *J. Biol. Chem.* 261:8708–8711.
5. Ezaki, O., and T. Kono. 1983. Sedimentation characteristics of subcellular vesicles associated with internalized insulin and those bound with intracellular glucose transport activity. *Arch. Biochem. Biophys.* 231:280–286.
6. Ezaki, O., M. Kasuga, Y. Akanuma, K. Takata, H. Hirano, Y. F. Yamaguchi, and M. Kasahara. 1986. Recycling of the glucose transporter, the insulin receptor, and insulin in rat adipocytes. *J. Biol. Chem.* 261: 3295–3305.
7. Frost, S. C., and M. D. Lane. 1985. Evidence for the involvement of vicinal sulfhydryl groups in insulin-inactivated hexose transport by 3T3-L1 adipocytes. *J. Biol. Chem.* 260:2646–2652.
8. Geuze, H. J., J. W. Slot, G. J. Strous, A. Hasilik, and K. von Figura. 1985. Possible pathways for lysosomal enzyme delivery. *J. Cell Biol.* 101:2253–2262.
9. Geuze, H. J., J. W. Slot, G. J. Strous, H. F. Lodish, and A. L. Schwartz. 1983. Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. *Cell.* 32:277–287.
10. Geuze, H. J., J. W. Slot, K. von Figura, A. Hasilik, A. Helenius, S. Green, and I. Mellman. 1986. Simultaneous localization of the 215 kD mannose 6-phosphate receptor and a lysosomal membrane protein in hepatoma cells. *J. Cell Biol.* 103:354*a* (Abstr.).
11. Gibbs, E. M., W. J. Allard, and G. E. Lienhard. 1986. The glucose transporter in 3T3-L1 adipocytes is phosphorylated in response to phorbol ester but not in response to insulin. *J. Biol. Chem.* 261:16597–16604.
12. Gibbs, E. M., G. E. Lienhard, J. R. Appleman, M. D. Lane, and S. C. Frost. 1986. Insulin stimulates fluid-phase endocytosis and exocytosis in 3T3-L1 adipocytes. *J. Biol. Chem.* 261:3944–3951.
13. Gorga, J. C., and G. E. Lienhard. 1984. One transporter per vesicle: determination of the basis of the insulin effect on glucose transport. *Fed. Proc.* 43:2237–2241.
14. Griffiths, G., and K. Simons. 1986. The trans Golgi network: sorting at the exit site of the Golgi complex. *Science (Wash. DC).* 234:438–442.
15. Habeeb, A. F. S. A., and R. Hiramoto. 1968. Reactions of proteins with glutaraldehyde. *Arch. Biochem. Biophys.* 126:16–26.
16. Karnieli, E., M. J. Zarnowski, P. J. Hissin, I. A. Simpson, L. B. Salans, and S. W. Cushman. 1981. Insulin-stimulated translocation of glucose transport systems in the isolated rat adipose cell. *J. Biol. Chem.* 256: 4772–4777.
17. Kono, T., F. W. Robinson, T. L. Blevins, and O. Ezaki. 1982. Evidence that translocation of the glucose transport activity is the major mechanism of insulin action on glucose transport in fat cells. *J. Biol. Chem.* 257: 10942–10947.
18. Kono, T., K. Suzuki, L. E. Dansey, F. W. Robinson, and T. L. Blevins. 1981. Energy-dependent and protein synthesis-independent recycling of the insulin-sensitive glucose transport mechanism in fat cells. *J. Biol. Chem.* 256:6400–6407.
19. Lienhard, G. E. 1983. Regulation of cellular membrane transport by the exocytotic insertion and endocytic retrieval of transporters. *Trends Biochem. Sci.* 8:125–127.
20. Lienhard, G. E., H. H. Kim, K. J. Ransome, and J. C. Gorga. 1982. Immunological identification of an insulin-responsive glucose transporter. *Biochem. Biophys. Res. Comm.* 105:1150–1156.
21. Novikoff, A. B., P. M. Novikoff, O. M. Rosen, and C. S. Rubin. 1980. Organelle relationships in cultured 3T3-L1 preadipocytes. *J. Cell Biol.* 87:180–196.
22. Oka, Y., and M. P. Czech. 1984. Photoaffinity labeling of insulin-sensitive hexose transporters in intact rat adipocytes. *J. Biol. Chem.* 259:8125–8133.
23. Oka, Y., C. Mottola, C. L. Openheimer, and M. P. Czech. 1984. Insulin activates the appearance of insulin-like growth factor II receptors on the adipocyte cell surface. *Proc. Natl. Acad. Sci. USA.* 81:4028–4032.
24. Resh, M. D. 1982. Development of insulin responsiveness of the glucose transporter and the (Na+, K+)-adenosine triphosphatase during in vitro adipocyte differentiation. *J. Biol. Chem.* 257:6978–6986.
25. Schroer, D. W., S. C. Frost, R. A. Kohanski, M. D. Lane, and G. E. Lienhard. 1986. Identification and partial purification of the insulin-responsive glucose transporter from 3T3-L1 adipocytes. *Biochim. Biophys. Acta.* 885:317–326.
26. Simpson, I. A., D. R. Yver, P. J. Hissin, L. J. Wardzala, E. Karnieli, L. B. Salans, and S. W. Cushman. 1983. Insulin-stimulated translocation of glucose transporters in the isolated rat adipose cells: characterization of subcellular fractions. *Biochim. Biophys. Acta.* 763:393–407.
27. Slot, J. W., and H. J. Geuze. 1984. Gold markers for single label and double immunolabeling of ultrathin cryosections. *In* Immunolabeling for Electron Microscopy. J. M. Polak and I. M. Varndell, editors. Elsevier Science Publishers/North Holland, Amsterdam. 129–142.
28. Slot, J. W., and H. J. Geuze. 1985. A new method of preparing gold probes for multiple-labeling cytochemistry. *Eur. J. Cell Biol.* 38:87–93.
29. Suzuki, K., and T. Kono. 1980. Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc. Natl. Acad. Sci. USA.* 77:2542–2545.
30. Tanner, L. I., and G. E. Lienhard. 1987. Insulin elicits a redistribution of transferrin receptors in 3T3-L1 adipocytes through an increase in the rate constant for receptor externalization. *J. Biol. Chem.* 262:8975–8980.
31. Tokuyasu, K. T. 1978. A study of positive staining on ultrahin frozen sections. *J. Ultrastruct.* 63:287–307.
32. Wardzala, L. J., and B. Jeanrenaud. 1981. Potential mechanism of insulin action on glucose transport in the isolated rat diaphragm. *J. Biol. Chem.* 256:7090–7093.
33. Wardzala, L. J., I. A. Simpson, M. M. Rechler, and S. W. Cushman. 1984. Potential mechanism of the stimulatory action of insulin on insulin-like growth factor II binding to the rat adipose cell. *J. Biol. Chem.* 259: 8378–8383.
34. Watanabe, T., M. M. Smith, F. W. Robinson, and T. Kono. 1984. Insulin action on glucose transport in cardiac muscle. *J. Biol. Chem.* 259: 13117–13122.
35. Wheeler, T. J., I. A. Simpson, D. C. Sogin, P. C. Hinkle, and S. W. Cushman. 1982. Detection of the rat adipose cell glucose transporter with antibody against the human red cell glucose transporter. *Biochem. Biophys. Res. Comm.* 105:89–95.