Journal of Supramolecular Structure 9:363–371 (1978)
Transmembrane Signaling 347–355

# Resistance of the Intact and Reconstituted Adipocyte Hexose Transport System to Irreversible Inhibition by Sulfhydryl and Amino Reagents

**Michael P. Czech, Dennis J. Pillion, and Michael F. Shanahan**

*Section of Physiological Chemistry, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912*

Sensitivity of the adipocyte D-glucose transport system in intact plasma membranes or following solubilization and reconstitution into phospholipid vesicles to several protein-modifying reagents was investigated. When intact plasma membranes were incubated with N-ethylmaleimide (20 mM) or fluorodinitrobenzene (4 mM), D-glucose transport activity was virtually abolished. However, washing the membranes free of unreacted reagents restored transport activity, indicating that covalent interaction with the membranes did not mediate the transport inhibition. Reaction of [$^3$H]N-ethylmaleimide with plasma membranes under similar conditions resulted in extensive labeling of all protein fractions resolved on dodecyl sulfate gels. Similarly, addition of N-ethylmaleimide to cholate-solubilized membrane protein had no effect on transport activity in artifical phospholipid vesicles reconstituted under conditions where the membrane protein was free of unreacted N-ethylmaleimide. Transport activity in plasma membranes was also inhibited by both reduced and oxidized dithiothreitol or glutathione (15 mM) in a readily reversible manner, consistent with a noncovalent mode of inhibition. Thus, the insulin-responsive adipocyte D-glucose transport system differs from the red cell hexose transport system in its remarkable insensitivity to modulation by covalent blockade of sulfhydryal or amino groups by the reagents studied.

**Key words: cytochalasin B, insulin action, adipocytes, plasma membranes, D-glucose transport, protein reagents, membrane reconstitution**

Although the rapid activation of hexose transport in muscle and fat has long been known to be a major physiological effect of insulin, the molecular details of this effect remain unknown. The elusive nature of the mechanism involved is no doubt related to our complete ignorance of the biochemical structure of the hormone-sensitive D-glucose transport system itself. Although it seems generally agreed that binding of the hormone to specific cell surface receptor proteins initiates transport stimulation, it is not known whether the signal involves an intramembrane or cytoplasmic event or both. It is also not unequivocally established whether the increase in the apparent $V_{max}$ of hexose transport due to insulin [1–5] reflects an increase in the number of transport proteins available in the membrane [6, 7] or an increase in activity of the same number of carriers [8]. Furthermore, the possibility that certain cell membrane insulin receptors are also functional transport carriers cannot at present be ruled out.

Received April 11, 1978; accepted July 26, 1978.

0091-7419/78/0903-0363$02.00 

We have recently taken the direct approach to these general questions and have developed a highly reliable method to reconstitute a solubilized, partially purified membrane protein fraction into artifical phospholipid vesicles which then exhibit stereospecific hexose transport activity [9, 10]. The methodology developed involves selective elution of extrinsic membrane proteins from the adipocyte plasma membrane [9] followed by selective solubilization of the extracted membrane with sodium cholate [10]. The long-range strategy of such studies involves the complete purification and identification of membrane components that comprise the insulin-sensitive hexose transport system. Significantly, this feat may allow biochemical analysis of the transport system components derived from control versus insulin-activated cells in order to determine molecular alterations elicited by the hormone. In addition, detailed studies on the properties of the transport system under carefully defined conditions of membrane phospholipid composition should be feasible.

The present report documents several properties of the adipocyte D-glucose transport system that contrast sharply with those of the extensively studied red cell system, which is not hormone-sensitive. The data indicate that the adipocyte D-glucose transport system activity is remarkably resistant to large doses of sulfhydryl and amino blockers, either in the native plasma membrane or solubilized in sodium cholate prior to reconstitution. This property is also exhibited by the adipocyte hexose transport system after its solubilization and reconstitution into phospholipid vesicles.

## METHODS

### Isolation of Fat Cells

White fat cells were obtained by enzymatic digestion of the parametrial adipose tissue of 200–700 gm female rats (Charles River CD strain) fed laboratory chow ad libitum [11]. For each experiment involving isolated cells and plasma membranes, the parametrial adipose tissue (40–80 gm) from 10 or more rats was pooled and cut into small pieces with scissors, blotted, and added to small plastic bottles. Each bottle normally contained from 5 to 7 gm of tissue and 8 ml of Krebs-Ringer phosphate buffer containing 3% bovine serum albumin (Armour Lot N10101) and 1 mg/ml of crude collagenase (Clostridium hisolyticum, Worthington), and was incubated for 60 min at 37°C. The Krebs-Ringer phosphate buffer (pH 7.4) contained 128 mM NaCl, 1.4 mM $MgSO_4$, 1.4 mM $CaCl_2$, 5.2 mM KCl, and 10 mM $Na_2HPO_4$. At the end of the digestion period, cells were filtered through one layer of nylon chiffon, washed twice with albumin buffer, and once with warm 0.25 M sucrose, 1 mM EDTA, 5mM Tris at pH 7.5 (buffer A).

### Preparation of Plasma Membrane Fraction

The experiments described herein were performed with a crude fat cell plasma membrane preparation as well as a purified plasma membrane preparation [12]. The crude preparations of plasma membranes were prepared by homogenization of cells in ice-cold buffer A in a loose-fitting glass homogenizing tube with seven up and down strokes of a Teflon pestle. The homogenate was centrifuged at 8500g for 10 min, and the sueprnatant as well as a small amount of fluffy white material, collected from the surface of the brown (mitochondrial) pellet were then centrifuged at 40,000g for 30 min; the resulting pellet was resuspended in ice-cold 1 mM EDTA, 5 mM Tris (pH 7.5).

### Membrane Extractions

Dimethylmaleic anhydride-extracted membranes were prepared [9] by adding 1 volume of plasma membranes (1 to 2 mg/ml) to 15 volumes of water, and 2 mg/ml of solid 2,3-dimethylmaleic anhydride was added with constant stirring while maintaining the pH at 8.0 with 2 N NaOH. After acid ceased to evolve, the suspension was centrifuged at 40,000g for 45 min, and the pellet was resuspended and frozen overnight in 5 mM Tris buffer, 1 mM EDTA, pH 6.8.

For sodium cholate extractions, frozen dimethylmaleic anhydride-extracted membranes were thawed and sodium cholate was added to a final concentration of 2% or 4% (w/v) in a total volume of 1 ml containing 1 to 2 mg of protein and incubated 60 min on ice. The material was then centrifuged as above, and the clear supernatant was carefully removed and stored on ice for reconstitution with exogenous phospholipids.

### Reconstitution of D-Glucose Transport Activity

Solubilization and reconstitution of adipocyte hexose transport activity was achieved as described in detail previously [10]. Briefly, phosphatidylcholine (10 mg) in benzene and phosphatidylethanolamine (10 mg) in chloroform were mixed in a test tube and dried under $N_2$. One milliliter of $Mg^{2+}$- free buffer (buffer A) containing 100 mM NaCl, 10 mM Tris/HCl (pH 7.5) was added to the dried phospholipids and mixed vigorously under $N_2$. Sodium cholate was added as a solid to a final concentration of 2% (w/v), and the mixture was sonicated under $N_2$ in a Bransonic 220 ultrasonic bath at 25° for 5 min to disperse the phospholipids.

One milliliter of cholate-solubilized membrane protein (0.2 to 1.0 mg/ml) was added to 1 ml of cholate-dispersed phospholipids (20 mg/ml). Liposomes were prepared from this mixture using the method of Brunner et al [13] by applying the sample to a column (20 × 1.5 cm) containing Sephadex G-50, equilibrated, and eluted with a buffer containing 100 mM NaCl, 10 mM Tris/HCl (pH 7.5), 2 mM $MgSO_4$ (buffer B) at 4° (flow rate 12 ml/h). These conditions were such that a milky dispersion of phospholipid vesicles appeared at the void volume. After collecting 3–4 ml, this suspension was rapidly frozen in dry ice/acetone, thawed under cold tap water, and sonicated in the ultrasonic bath for 20 sec.

### Glucose Transport Assay

Assay of glucose uptake was routinely performed by addition of 45 μl of liposomes or 100 μl plasma membranes to glass test tubes (12 × 100 mm) as described in detail previously [9, 10]. All inhibitors were added prior to glucose to the appropriate concentrations. Transport was initiated by the addition of 5 μl of a solution containing 2.5 mM D[$^3$H] glucose (1 to 2 μCi/ tube). The tubes were vigorously shaken and incubated at room temperature (22°) for the appropriate time. Transport was stopped by the addition of 1 ml of ice-cold buffer B, and the suspension was then decanted onto Millipore (0.22 μm pore size, 25 mm diameter) filters attached to a rotary Doerr pump and quickly washed with 1 ml of ice-cold buffer. Filters were air-dried and immersed in 4 ml of ACS (Amersham/Searle) liquid scintillation fluid for counting.

### Analytical Methods

Membrane protein was estimated by the method of Lowry et al [14] using bovine serum albumin as a standard. Sodium dodecyl sulfate-gel electrophoresis (5% acrylamide) was performed on membrane proteins as previously described [15].

### Reagents

Cytochalasin B was obtained from Aldrich Chemical Co. and N-ethylmaleimide, fluorodinitrobenzene, reduced and oxidized dithiothreitol, and glutathione were obtained from Sigma. D[$^3$H]glucose was obtained from New England Nuclear. Dimethylmaleic anhydride, L-alpha-phosphatidylcholine (Type III-E), L-alpha-phosphatidylethanolamine (Type III), sodium cholate, and Sephadex G-50-150 were obtained from Sigma.

## RESULTS

Table I illustrates the ability of 20 mM dithiothreitol, 20 mM N-ethylmaleimide, and 4 mM fluorodinitrobenzene to modulate D-glucose transport activity in a plasma membrane preparation from isolated adipocytes. Uptake of label was measured after incubation of membranes with D[$^3$H]glucose for 20 sec, which gives an estimate of the transport system activity [16, 17]. The former two protein modifiers when incubated with the membrane vesicles markedly inhibited D[$^3$H]glucose uptake, whereas the latter inhibited uptake to the same extent as did 50 $\mu$M cytochalasin B. Since previous results showed that L[$^3$H]glucose uptake was similar to that of cytochalasin B-inhibited D[$^3$H]-glucose uptake, simple diffusion is assumed to be the mode of uptake under the latter condition. In the present experiments, little or no difference was observed in the uptake of D[$^3$H]glucose in the presence of cytochalsin B, due to the three protein reagents employed, indicating that simple diffusion of glucose was essentially constant under all these conditions. Net stereospecific D-glucose transport activity, however, was markedly inhibited by dithiothreitol and N-ethylmaleimide and abolished by fluorodinitrobenzene, consistent with a previous report that adipocyte plasma membrane hexose transport was markedly inhibited by N-ethylmaleimide [16]. However, upon washing the membranes free of unreacted reagents, D-glucose transport activity approached control levels in all three cases. However, in the membranes treated with fluorodinitrobenzene, a very small degree of residual inhibition was observed. Untreated membranes exhibited similar transport rates before and after the washing procedure (not shown), although the data presented are normalized in the various experiments by use of percents.

**TABLE I. Reversible Effects of Sulfhydryl and Amino Reagents on Adipocyte Plasma Membrane D-Glucose Transport Activity**

| | % Control D-glucose uptake | | | |
|---|---|---|---|---|
| | First incubation Plus cytochalasin B | | Second incubation (washed) Plus cytochalasin B | |
| Treatment | Control | (50 $\mu$M) | Control | (50 $\mu$M) |
| None | 100 | 42 ± 3 | 100 | 55 ± 5 |
| Dithiothreitol (20 mM) | 52 ± 5 | 33 ± 4 | 101 ± 7 | 50 ± 8 |
| N-ethylmaleimide (20 mM) | 52 ± 2 | 35 ± 2 | 99 ± 9 | 56 ± 11 |
| Fluorodinitrobenzene (4 mM) | 40 ± 4 | 38 ± 3 | 87 ± 8 | 57 ± 8 |

Plasma membrane vesicles (~ 1 mg protein/ml) were prepared from adipocytes and incubated with various reagents for 15 min at 22°C; one aliquot of each sample was immediately used for transport studies while the remainder was diluted with 20 volumes of cold Krebs-Ringer phosphoate buffer, pH 7.4. These samples were then centrifuged for 40 min at 40,000g; the pellet was again resuspended with Krebs-Ringer phosphate buffer, sedimented, and resuspended in Krebs-Ringer phosphate buffer (~ 1 mg protein/ml). D-glucose uptake was then monitored over a 20-sec period in the presence or absence of 50 $\mu$M cytochalasin B. The values are normalized as percents of the values obtained from untreated membranes and represent the means ± standard error for six observations.

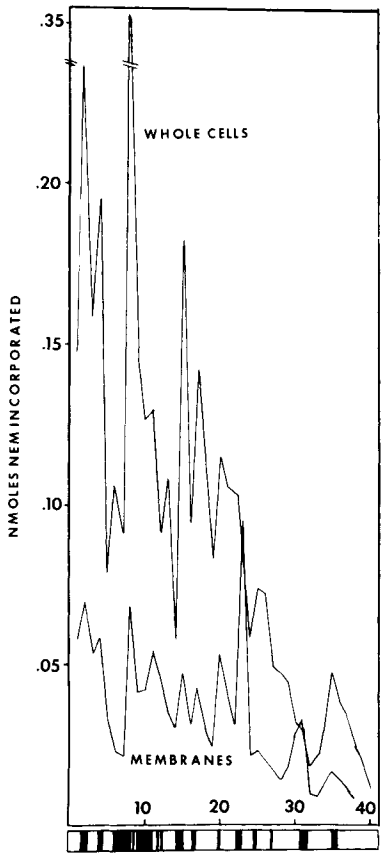


Fig 1. Reaction of [$^3$H] N-ethylmaleimide with plasma membrane proteins following incubation with intact cells and isolated membranes. Fat cells were isolated from 30 gm of white fat, washed twice with Krebs-Ringer phosphate buffer containing 0.5% albumin, and resuspended in a total volume of 40 ml 0.5% albumin buffer. The cells were divided into two groups of 20 ml each and incubated with or without 0.42 mM [$^3$H] ethylmaleimide (specific activity 15.4 $\mu$Ci/$\mu$mole) for 15 min at 37°C. Incubation was terminated by the addition of 0.42 mM mercaptoethanol; the cells were washed twice and homogenized, and plasma membranes were prepared. The membranes from the maleimide-treated cells were resuspended in 1 mM EDTA (pH 7.5) and stored in ice. The membranes from the untreated cells were resuspended in 0.5 ml Krebs-Ringer phosphate buffer plus 0.42 mM [$^3$H] N-ethylmaleimide (specific activity 15.4 $\mu$Ci/$\mu$mole) and incubated at 37°C for 15 min. Incubation was terminated by the addition of 0.42 mM mercaptoethanol, the membranes sedimented, and the pellet resuspended in 1 mM EDTA (pH 7.5). Membranes (100 $\mu$g protein/gel) were solubilized in 2% SDS-8M urea and electrophoresed on 10% acrylamide-0.2% SDS gels.

In order to document whether covalent reaction of N-ethylmaleimide and dinitrofluorobenzene with membrane proteins actually takes place under conditions similar to those in these experiments, dodecyl sulfate gels were used to resolve membrane proteins following reaction with labeled reagents. Figure 1 presents the data from a representative experiment performed with [$^3$H] N-ethylmaleimide. Extensive labeling of all membrane protein bands following electrophoresis was observed whether intact fat cells or isolated plasma membranes were incubated with the labeled N-ethylmaleimide. Similar uniform

labeling patterns were obtained when [$^3$H] fluorodinitrobenzene was incubated with adipocyte plasma membranes (not shown). Since it is likely that all or most of the membrane protein bands on such dodecyl sulfate gels contain several protein species, it is of course not possible to conclude that hexose transport system components are necessarily labeled in these experiments.

Since the results in Table I demonstrated reversible inhibition of transport activity by dithiothreitol, it seemed possible that this agent was acting via reduction of protein disulfides. Reversibility might therefore simply reflect spontaneous oxidation of the resulting protein sulfhydryls back to the native disulfide form. We therefore tested the effects of the oxidized forms of both dithiothreitol and glutathione on hexose transport activity in these membranes. As shown in Table II both reduced and oxidized forms of these reagents were equally effective in inhibiting D[$^3$H] glucose uptake in the absence of cytochalasin B.. In addition, both reduced and oxidized forms of glutathione inhibited D[$^3$H] glucose uptake in the presence of cytochalasin B, indicating that a decrease in simple diffusion also occurred. These effects were maximal at the concentrations presented in Table II, but smaller inhibitory effects were observed with concentrations of these reagents as low as 1 mM (not shown). Furthermore, the effects of the oxidized forms of these agents were readily reversible upon washing membranes with buffer prior to assaying transport (not shown).

**TABLE II. Inhibition of D-Glucose Transport in Plasma Membrane Vesicles by Reduced or Oxidized Thiol Reagents**

| | Percent of control [$^3$H] D-glucose uptake | |
|---|---|---|
| Additions | Control | Plus cytochalasin B |
| None | 100 | 48 ± 11 |
| Dithiothreitol (15 mM) | 53 ± 2 | 40 ± 4 |
| Oxidized dithiothreitol (15 mM) | 66 ± 11 | 49 ± 6 |
| Glutathione (30 mM) | 57 ± 7 | 21 ± 5 |
| Oxidized gluthathione (30 mM) | 42 ± 16 | 10 ± 3 |

Plasma membrane vesicles were suspended in Krebs-Ringer phosphate buffer, and 100 μl aliquots were incubated with or without the various reagents shown for 15 min at 22°C. Cytochalasin B (100 μM) was added to one-half the samples 5 min before transport was assayed. Transport was initiated with 5 μl of [$^3$H] D-glucose, and terminated 20 sec later by rapid filtrations. The values are the means ± standard error for 4 experiments, with triplicate determinations in each experiment. The data are normalized as the percent of values obtained with untreated membranes.

It appeared possible that the adipocyte hexose transport system is sensitive to covalent modification by these protein modifiers, but that the reactive groups are protected from reaction due to a hydrophobic environment or steric hindrance by neighboring groups. We therefore incubated N-ethylmaleimide (15 mM) with sodium cholate-solubilized intrinsic adipocyte plasma membrane proteins before addition of cholate-dispersed phospholipids and preparation of reconstituted vesicles. Since formation of reconstituted vesicles from cholate-solubilized material is achieved by passage through a Sephadex G-50 column, the vesicles eluted in the void volume are freed of unreacted N-ethylmaleimide which is retarded on the column due to its low molecular weight. The data in Figure 2 show that reconstituted vesicles exhibit cytochalasin B-sensitive D-glucose-transport

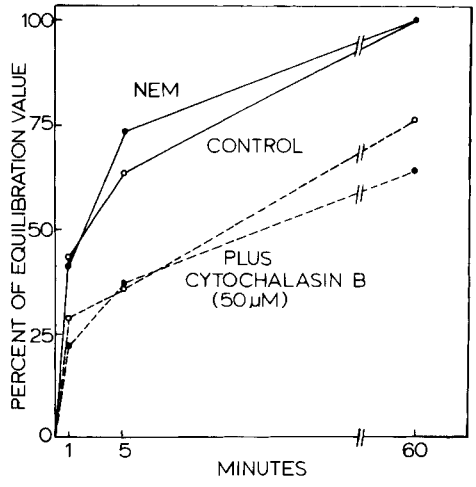


Fig 2. Normal hexose transport activity in reconstituted vesicles prepared with N-ethylmaleimide-treated membrane proteins. Reconstituted vesicles were prepared in duplicate with solubilized dimethylmaleic anhydride-extracted membranes by the standard procedure. In one group, 15 mM N-ethylmaleimide was incubated with the solubilized membrane protein for 10 min at 22° prior to mixing with the cholate-dispersed phospholipids, while the other group was incubated for 10 min at 22°without addition. Following the reconstitution procedure D-glucose uptake was monitored in quadruplicate at the times indicated, and the values presented are those of a representative experiment. The data are normalized as percent of the equilibration values for each group of vesicles which are obtained after a 60 min incubation with D [$^3$H] glucose.

activity which is similar whether or not the protein was first reacted with N-ethylmaleimide before reconstitution. In other experiments not illustrated, assay of hexose transport in reconstituted vesicles in the continued presence of N-ethylmaleimide at 15 mM showed marked inhibition, similar to that observed in Table I with intact plasma membranes.

## DISCUSSION

Several reports have appeared which demonstrate potent irreversible inhibitory effects of N-ethylmaleimide [18–20] and fluorodinitrobenzene [21–23] on D-glucose transport in red blood cell membranes. In addition, the presence of D-glucose consistently modulates the extent or rate of inhibition of transport activity by these agents. This specific effect of substrate for the transport system has been employed in attempts to label differentially membrane protein components of the transport system using $^{14}$C- and $^3$H-labeled inhibitory reagents [20, 23]. We initiated the present studies in an attempt to document the feasibility of performing similar experiments in adipocytes. The present data clearly illustrate the surprising reversibility of D-glucose transport inhibition by N-ethylmaleimide and fluorodinitrobenzene in adipocyte plasma membranes. This lack of covalent modulation of transport activity in intact adipocyte plasma membranes or solubilized, reconstituted membrane protein is consistent with our previous observations that low concentrations of N-ethylmaleimide had no effect on hexose transport in intact adipocytes

[24]. It should be noted that the data presented here do not allow us to distinguish whether the transport system is indeed reacting with the agents used and that its activity is unaffected by such reaction, or whether no such reaction takes place under the conditions employed.

In light of the absence of marked irreversible effects on transport of the sulfhydryl and amino agents used in these studies, it is striking that such severe reversible inhibitory effects are found with these covalent protein modifiers. It seems possible that the agents may act as weak anesthetics and are solubilized to some degree in the membrane matrix. This may lead to a substantial degree of membrane expansion with potential restraint of molecular mobility within the membrane. Since this same reversible inhibitory property is observed with agents as diverse as oxidized glutathione and fluorodinitrobenzene, it would seem that simple binding to a common, specific site on the transport system is unlikely. It should also be noted that, although it could be argued that the dithiothreitol-mediated inhibition of transport indeed does involve reduction of sulfhydryls, we have found that addition of this agent followed by high doses of N-ethylmaleimide does not render the inhibitory effect irreversible.

The data presented here contrast sharply with studies by many laboratories on erythrocyte D-glucose transport and suggest a dramatic difference in the molecular nature of the adipocyte versus erythrocyte hexose transport systems. Table III illustrates these and other reported characteristics of erythrocyte hexose transport which appear to differ from those observed with adipocyte plasma membrane D-glucose transport activity. Kasabara and Hinkle [25, 26] developed a procedure to reconstitute D-glucose transport activity from erythrocyte membranes solubilized with Triton X-100. In contrast, we have been unable to use this methodology successfully to reconstitute the adipocyte transport system and have found that exposure of cholate-solubilized adipocyte membrane proteins to 0.5% Triton X-100 blocks transport activity in subsequently reconstituted vesicles (unpublished data, 2/78). Similarly, D-glucose transport activity appears to respond very dif-

**TABLE III. Contrasting Properties of the Erythrocyte vs Adipocyte Hexose Transport System**

| Property | Erythrocyte transport system | Adipocyte transport system |
|---|---|---|
| Insulin responsive | No | Yes |
| Irreversible inhibition by N-ethylmaleimide | Yes[a] | No[b] |
| Irreversible inhibition by fluorodinitrobenzene | Yes[c] | No[b] |
| Stable in Triton X-100 | Yes[d] | No[e] |
| Inhibited by trypsinization of membranes | No[f] | Yes[g] |

[a] References 18–20.
[b] The present study.
[c] References 21–23.
[d] References 25, 26.
[e] Shanahan, M.F., and Czech, M.P., unpublished data.
[f] Reference 27.
[g] Czech, M.P., unpublished experiments.

ferently to trypsinization of adipocyte membranes versus red cell membranes. Carter et al [27] demonstrated the complete resistance of hexose transport activity in red cell ghosts to extensive proteolytic attack by 100 μg/ml trypsin, whereas we have observed marked inhibition of adipocyte plasma membrane hexose transport activity by trypsin under similar conditions (unpublished data, 1/78). It will therefore be of great interest to compare the molecular weights and other molecular properties of the two transport systems once further characterization of the adipocyte system has been performed. It is tempting to speculate that the different properties of the two systems may be related in an important way to the fact that the adipocyte transport system but not the red cell system is rapidly activated by low doses of insulin.

## ACKNOWLEDGMENTS

We wish to thank Lynne Rozek and Diane Goldman for expert technical assistance. This work was supported by grants from the United States Public Health Service (AM-17893) and the Juvenile Diabetes Association. D. P. and M. S. are Postdoctoral Fellows (AM-05563 and AM-05430, respectively) of the United States Public Health Service, and M.C. is a Research Career Development Awardee (AM-00249) of the United States Public Health Service.

## REFERENCES

1. Vinten J, Gliemann J, Osterlind K: J Biol Chem 251:794, 1976.
2. Czech MP, Lawrence JC Sr, Lynn WS: J Biol Chem 249:5421, 1974.
3. Czech MP: Mol Cell Biochem 11:51, 1976.
4. Vineten J: Biochim Biophys Acta 511:259–273, 1978.
5. Foley JE, Cusman SW, Salans LB: Am J Physiol 3:E112–E119, 1978.
6. Narahara HT, Ozand P: J Biol Chem 238:40–49, 1963.
7. Olefsky JM: Biochem J (in press).
8. Czech MP: J Cell Physiol 89:661, 1976.
9. Shanahan MF, Czech MP: J Biol Chem 252:6554, 1977.
10. Shanahan MF, Czech MP: J Biol Chem 252:8341, 1977.
11. Rodbell M: J Biol Chem 239:375, 1964.
12. McKeel DW, Jarrett L: J Cell Biol 44:417, 1970.
13. Brunner J, Skrabal P, Hauser H: Biochim Biophys Acta 445:322, 1976.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
15. Weber K, Osborn M: J Biol Chem 244:4406, 1969.
16. Carter JR Jr, Avruch J, Martin DB: J Biol Chem 247:2682, 1972.
17. Pillion DJ, Shanahan MF, Czech MP: J SUpramol Struct (in press).
18. Dawson AC, Widdas WF: J Physiol 168:644, 1963.
19. LeFevre PG: Pharmacol Rev 13:39, 1961.
20. Batt EP, Abbott RE, Schater D: J Biol Chem 251:7184, 1976.
21. Krupka RM: Biochemistry 10:1148, 1971.
22. Jung CY: J Biol Chem 249:3568, 1974.
23. Shanahan MF, Jacquez JA: Membrane Biochem 1:239, 1978.
24. Czech MP: J Biol Chem 251:1164, 1976.
25. Kasahara M, Hinkle PC: Proc Natl Acad Sci USA 73:396, 1976.
26. Kasahara M, Hinkle PC: J Biol Chem 252:7384, 1977.
27. Carter JR Jr, Avruch J, Martin DB: Biochim Biophys Acta 291:506, 1973.