Proc. Natl. Acad. Sci. USA
Vol. 77, No. 2, pp. 915–918, February 1980
Biochemistry

Coordinate modulation of D-glucose transport activity and bilayer fluidity in plasma membranes derived from control and insulin-treated adipocytes

(insulin action/membrane structure/fatty acids/transport regulation)

PAUL F. PILCH, PETER A. THOMPSON, AND MICHAEL P. CZECH

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

Communicated by Donald F. Steiner, November 26, 1979

ABSTRACT **The *cis*-monoenoic fatty acids vaccenate and oleate stimulate D-glucose transport when partitioned into isolated plasma membranes from rat adipocytes. The magnitude of hexose transport stimulation due to these agents is equal to that observed in plasma membranes derived from insulin-treated adipocytes. Addition of *cis*-unsaturated fatty acids to plasma membranes derived from insulin-treated cells results in no further stimulation of glucose transport over that due to the hormone alone. In contrast, treatment of membranes exhibiting insulin-activated D-glucose transport activity with saturated fatty acids reduces transport activity to control levels. No effect of the saturated fatty acids was observed on D-glucose transport in control membranes. Because *cis*-unsaturated fatty acids fluidize plasma membranes under the conditions used in these experiments, these data demonstrate a positive correlation between membrane fluidity and adipocyte D-glucose transport system activity. In addition, the results suggest that enhanced bilayer fluidity or increased affinity of the glucose transporter for fluid microenvironments of the membrane may play a key role in transport regulation by insulin.**

A major obstacle in determining the mechanism by which insulin stimulates glucose transport in fat and muscle tissue has been the absence of a cell-free system for studying this phenomenon. Glucose transport in isolated adipocyte plasma membranes, which possess both high-affinity insulin receptors and a stereospecific D-glucose transport system, is unresponsive to the direct addition of insulin. Moreover, agents that mimic the ability of insulin to stimulate glucose transport in intact adipocytes, such as lectins (1, 2), oxidants (3), and antibodies against membrane (4), are also without effect on glucose transport when added directly to membrane vesicles. However, Avruch *et al.* (5) reported that plasma membrane vesicles obtained from insulin-treated adipocytes exhibit elevated levels of glucose transport compared to membrane vesicles derived from untreated adipocytes. These results suggest that insulin treatment in the intact cell confers a stable change in the transport system or its environment that is retained in the isolated membrane.

Recently, we reported that the activity of the adipocyte glucose transport system, when reconstituted into artificial phospholipid vesicles, is markedly temperature sensitive and correlates with the fluidity of the phospholipid bilayer (6, 7). These data led us to suggest the working hypothesis that insulin stimulates glucose transport in fat cells by increasing membrane fluidity (6, 7). Accordingly, in the present studies we have attempted to alter membrane fluidity in native adipocyte plasma membranes and have measured the effect of this alteration on glucose transport. We found that fatty acids such as oleic and vaccenic acids, which fluidize membranes (8–10), are able to stimulate glucose transport in isolated membrane preparations to the same degree as insulin treatment of the intact adipocytes. Saturated fatty acids such as stearic and palmitic acids have no such effect on glucose transport when added to membranes from untreated adipocytes but, rather, inhibit the elevated glucose transport rates in membranes isolated from insulin-treated fat cells. These findings further support the notion that the adipocyte glucose transporter is sensitive to bilayer fluidity changes in its native membrane.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Preparation of Fat Cells and Membranes. Fat cells were isolated from the epididymal fat pads of 125- to 175-g, male Sprague–Dawley rats by collagenase (Worthington) digestion according to Rodbell (11). Prior to membrane preparation, isolated adipocytes were incubated for 15 min at 37°C in the presence or absence of 24 milliunits of porcine insulin per ml (gift from R. Chance, Eli Lilly). Purified plasma membranes were then prepared from adipocytes by the protocol of McKeel and Jarett (12) with modifications as described (13, 14).

Assay of Glucose Transport and Fatty Acid Incorporation. Purified suspensions of adipocyte plasma membrane [0.5 ml of membrane protein (3 mg/ml) in 10 mM Tris·HCl/1 mM EDTA, pH 7.4] were incubated with 12 μl of an ethanolic solution of 100 mM fatty acid (all from Sigma) for 30 min at 0°C (unsaturated fatty acids) or 23°C (saturated fatty acids). Ethanol alone was used for the controls. The final concentrations of ethanol and fatty acid were 2.4% and 2.4 mM, respectively. The membranes were then collected by centrifugation and were resuspended in Krebs–Ringer phosphate buffer for measurement of glucose transport or of labeled fatty acid incorporation. Both processes were measured by a rapid filtration technique previously described (15). For fatty acid incorporation, [^{3}H]oleic acid or [^{14}C]stearic acid (New England Nuclear) was used in the incubation with membranes. Stereospecific D-[^{3}H]glucose (New England Nuclear) uptake was measured in the presence or absence of 20 μM cytochalasin B (Aldrich). Hexose uptake in the presence of cytochalasin B was attributed to simple diffusion and was subtracted from hexose uptake in the absence of cytochalasin B to give a net transport value. The data presented are the means of triplicate experimental points, and all experiments were performed two to six times. Protein was determined by the method of Lowry *et al.* (16).

RESULTS

cis-Vaccenic acid is a most effective compound in increasing isoproterenol stimulation of adenylate cyclase concomitant with increasing membrane fluidity, as measured by fluorescence polarization techniques (8, 9). Fig. 1 shows the effect of incorporating 2.4 mM *cis*-vaccenic acid into adipocyte plasma membranes on D-[^{3}H]glucose uptake. In Fig. 1*A*, D-[^{3}H]glucose

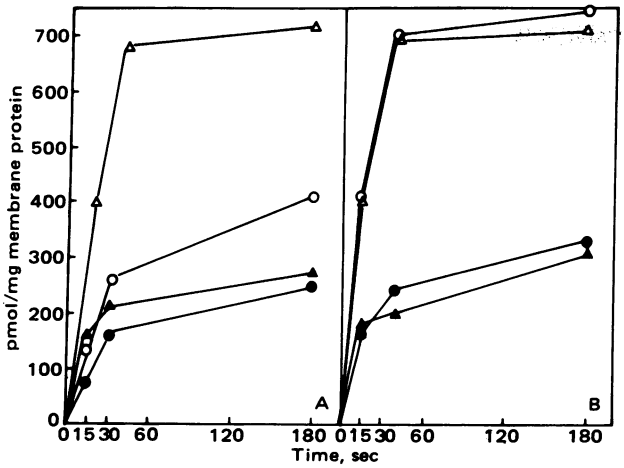

FIG. 1. Effect of *cis*-vaccenic acid incorporation on D-glucose uptake by purified plasma membranes from rat adipocytes. Purified membranes from insulin-treated (*B*) or untreated (*A*) adipocytes were each divided into two parts (1–2 mg of protein per condition) and incubated in the presence or absence of 2.4 mM *cis*-vaccenic acid. The membranes were pelleted and resuspended in Krebs–Ringer phosphate buffer from which 90-μl aliquots (50–120 μg of protein) were removed and assayed for D-[^{3}H]glucose uptake in the presence and absence of 20 μM cytochalasin B by a rapid filtration technique. ○, No addition; △, plus *cis*-vaccenic acid; ●, plus cytochalasin B; ▲, plus *cis*-vaccenic acid and cytochalasin B.

uptake was measured as a function of incubation time with membrane vesicles obtained from untreated fat cells. Vesicles that had been incubated with *cis*-vaccenic acid exhibited a marked stimulation of glucose uptake at the earliest time points measured. Diffusion of D-[^{3}H]glucose, measured as uptake in the presence of the glucose transport inhibitor cytochalasin B, was also slightly elevated by *cis*-vaccenic acid, but to a much smaller degree. The cytochalasin-B-sensitive transport system is therefore markedly stimulated by *cis*-vaccenic acid (see Table 1). This stimulation is not due to a change in vesicle size because uptake at 60 min is the same for both untreated and *cis*-vaccenic-treated membranes (data not shown).

In Fig. 1*B*, D-[^{3}H]glucose uptake was measured in plasma membrane vesicles obtained from insulin-treated adipocytes, which exhibit elevated hexose uptake compared to control membranes. This insulin-stimulated hexose transport rate is similar to that induced by *cis*-vaccenic acid in untreated membrane vesicles (Fig. 1*A*). Moreover, the addition of *cis*-vaccenic acid to the insulin-stimulated membranes resulted in no further stimulation of hexose uptake rates, as seen at 15 sec of uptake (Fig. 1*B*). Again, intravesicular space was determined to be the same in all membrane preparations illustrated in Fig. 1*B* (data not shown). In other experiments, fluorescence polarization measurements using 1,6-diphenyl-1,3,5-hexatriene confirmed a decreased viscosity of *cis*-vaccenic-treated adipocyte plasma membranes compared to control membranes (L. Cantley and M. Czech, unpublished data).

In order to better assess whether the stimulatory effect of *cis*-vaccenic acid might be related to a membrane-fluidizing effect, we determined the effects of the saturated fatty acid, stearic acid, on glucose uptake in plasma membrane vesicles from untreated and insulin-treated adipocytes (Fig. 2). Neither basal D-[^{3}H]glucose uptake nor diffusion of glucose in the presence of cytochalasin B was affected by stearic acid in untreated membranes (Fig. 2*A*). However, the stimulated glucose transport observed in membranes from insulin-treated fat cells was abolished by stearic acid incorporation into membrane vesicles (Fig. 2*B*). Thus, D-[^{3}H]glucose uptake in stearic-acid-treated membranes derived from insulin-activated cells is equal

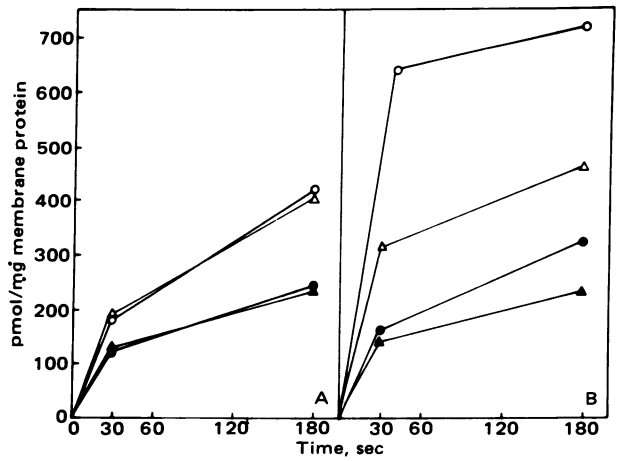

FIG. 2. D-Glucose uptake in purified plasma membranes containing stearic acid. Plasma membranes were prepared from untreated (*A*) and insulin-treated (*B*) cells. Stearic acid was incorporated into these membranes, and hexose uptake was measured as described in the legend to Fig. 1 and in *Materials and Methods*. ○, No addition; △, plus stearic acid; ●, plus cytochalasin B; ▲, plus stearic acid and cytochalasin B.

to that of control membranes. Similarly, the slight but significant increase in cytochalasin-B-insensitive glucose uptake into membrane vesicles seen as a result of insulin treatment (Figs. 1–3, $P < 0.01$) is abolished by stearic acid (Fig. 2). This apparent slight stimulation of diffusion by insulin is also observed when L-[^{3}H]glucose uptake is monitored (data not shown).

We have also tested the effects of *trans*-vaccenic acid on glucose uptake in adipocyte plasma membranes (Fig. 3). This fatty acid possesses a *trans*-double bond which, when incorporated into membrane vesicles, should introduce only a minor perturbation to membrane structure compared to *cis*-vaccenic acid. Hexose uptake in membranes derived from both control and insulin-treated cells was unchanged by treatment with *trans*-vaccenic acid (Fig. 3).

Two additional fatty acids, oleic and palmitic acids, were tested for their effects on glucose transport in adipocyte plasma membranes. The results are summarized in Table 1 together

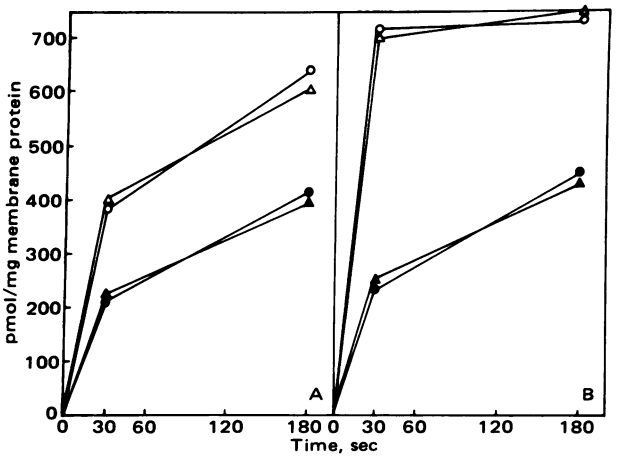

FIG. 3. Effect of *trans*-vaccenic acid incorporation on D-glucose uptake by purified plasma membranes from rat fat cells. Plasma membranes were prepared from insulin-treated (*B*) or untreated (*A*) fat cells. Fatty acid was incorporated into the membrane and D-[^{3}H]glucose uptake was assayed as described in the legend of Fig. 1 and *Materials and Methods*. ○, No addition; △, plus *trans*-vaccenic acid; ●, plus cytochalasin B; ▲, plus *trans*-vaccenic acid and cytochalasin B.

Table 1. Effect of fatty acid incorporation on net uptake of D-glucose by purified plasma membrane vesicles from rat adipocytes

Condition	Net D-glucose uptake*	
	Untreated	Insulin-treated
Control	81	400
cis-Vaccenic acid (Δ11-12, 18:1*c*)	403	417
Control	67	445
trans-Vaccenic acid (Δ11-12, 18:1*t*)	75	432
Control	100	552
Oleic acid (Δ9-10, 18:1)	429	519
Control	54	485
Stearic acid (18:0)	48	135
Control	89	246
Palmitic acid (16:0)	102	99

Membrane vesicles were prepared from insulin-treated or untreated fat cells. The membrane preparations were suspended in 10 mM Tris·HCl/1 mM EDTA and incubated with fatty acids (2.4 mM) at 0°C (unsaturated) or 23°C (saturated) for 30 min. The membranes were then pelleted, resuspended in Krebs–Ringer phosphate buffer, and assayed for D-[^{3}H]glucose uptake. Values given for net uptake were obtained by subtracting uptake values in the presence of 20 μM cytochalasin B from total uptake in the absence of cytochalasin B.
* pmol D-glucose per mg of protein per 30 sec.

with the combined data obtained from several experiments using *cis*-vaccenic, stearic, and *trans*-vaccenic acids. The *cis*-unsaturated fatty acid, oleic acid, mimicked the action of *cis*-vaccenic acid to stimulate hexose uptake in adipocyte plasma membrane vesicles. This stimulation was equal, but not additive, to the stimulation of glucose uptake observed in membranes derived from insulin-treated cells. Furthermore, the saturated fatty acid, palmitic acid, did not affect glucose uptake in vesicles isolated from untreated fat cells, but abolished the elevated rate of hexose transport activity in membrane vesicles from insulin-treated adipocytes, as did stearic acid (Fig. 2 and Table 1).

In order to demonstrate that the effects of the various fatty acids described above were not artifactual, we measured incorporation of labeled fatty acid into membranes. The results of these experiments are shown in Table 2. Most of the added label was incorporated into the adipocyte membrane for both oleic (70%) and stearic (80%) acids. When the data were expressed as μmol of fatty acid incorporated per mg of membrane protein, there was little if any difference in incorporation between the saturated and the unsaturated fatty acid. Also, treatment of fat cells with insulin did not alter subsequent partitioning of fatty acid into the plasma membranes. These data are similar to those previously reported for incorporation of oleic acid into turkey erythrocyte plasma membranes (8).

Table 2. Incorporation of labeled fatty acids into plasma membrane vesicles

Fatty acid	Untreated		Insulin-treated	
	μmol fatty acid/mg protein	%	μmol fatty acid/mg protein	%
[^{3}H]Oleic acid	0.19	69	0.18	68
[^{14}C]Stearic acid	0.19	85	0.16	79

Membranes prepared from insulin-treated or untreated adipocytes were incubated for 30 min with either [^{3}H]oleic acid at 0°C or [^{14}C]-stearate at 23°C. The membrane pellet was then separated from free fatty acid by centrifugation (30°C, 30,000 × *g*) prior to assessing incorporation of label by rapid filtration. The amount of label absorbed on the filter in the absence of membranes was subtracted from the total label incorporated as a blank.

DISCUSSION

The present finding, that unsaturated fatty acids stimulate glucose transport when added directly to adipocyte plasma membranes, represents a demonstration of an insulin-like effect on hexose transport in a cell-free system. All other agents that activate the D-glucose transport system in intact adipocytes, including insulin itself, were ineffective when added directly to isolated plasma membranes (unpublished data). Previous studies on the hormonal activation of adenylate cyclase have shown that *cis*-vaccenic and oleic acids increase bilayer fluidity in cholesterol-containing native plasma membranes as measured by fluorescence polarization (8, 9). We have recently demonstrated that the activity of the hexose transport system from adipocytes can be modulated by the physical state of the phospholipid bilayer (6, 7). Also, a temperature-dependent change in the fluidity of adipocyte ghost membranes, as measured by electron spin resonance techniques, has been correlated with a change in adipocyte hexose transport (17). Stereospecific D-glucose transport activity in reconstituted vesicles depends on a fluid phospholipid matrix, which can be monitored by differential scanning calorimetry (6). As the temperature rises, the activity of the hexose transporter rises in parallel with bilayer melting until the fully melted state is reached. At this point, a further increase in temperature causes little further increase in transport activity (6, 7).

The data presented here provide further support for the hypothesis that insulin may stimulate hexose transport in adipocytes by enhancing membrane fluidity (6, 7). According to this hypothesis, the glucose transport system in the basal state may be regarded as existing in a relatively immobilized phospholipid environment. Thus, insertion of *cis*-monoenoic fatty acids increases overall membrane fluidity and D-glucose transport activity, whereas saturated fatty acids do not have these capabilities. Conversely, the fully fluid membrane state induced by insulin treatment results in maximal glucose transport which cannot be further increased by addition of *cis*-unsaturated fatty acids (Fig. 1). Addition of saturated fatty acids to stimulated membranes would be expected to decrease membrane fluidity and consequently reduce glucose transport (Fig. 2). The temperature sensitivity of hexose transport in intact adipocytes agrees with this proposal. Workers in several laboratories have noted that basal glucose transport in fat cells is essentially constant when the temperature is varied whereas the insulin-stimulated hexose transport increases as the temperature rises (17–20). This observation would be expected if insulin treatment of adipocytes altered the phospholipid environment of the glucose transport protein from a relatively immobile basal state to a more fluid stimulated state.

The major metabolic action of insulin in the fat cell is to inhibit lipolysis and to promote the conversion of free fatty acid into triglycerides. It is therefore highly unlikely that insulin enhances membrane fluidity in intact adipocytes by causing insertion of *cis*-unsaturated fatty acid into the plasma membrane. However, many possible mechanisms are known by which membrane fluidity might be enhanced as a result of insulin action. For example, insulin increases the degree of unsaturation in phospholipid fatty acyl groups,* which could result from stimulation of an insulin-responsive fatty acid desaturase (21). Additional mechanisms by which the fluidity of biological membranes may be modulated include phospholipid methylation (22), cholesterol depletion or enrichment (23), and calcium binding (24). These fluidity changes in turn affect various enzymatic or transport activities.

* Murray, D. K. & Nelson, D. H. (1979) *61st Annual Meeting of the Endocrine Society*, June 13–15, Anaheim, CA, abstr. 6, p. 74.

An alternative hypothesis consistent with the present findings is the possibility that insulin increases the affinity of the transporter for regions of the membrane characterized by maximum fluidity. According to this concept, the adipocyte plasma membrane is visualized as a heterogeneous entity, with widely different viscosities residing in various microenvironments. This hypothesis predicts that the basal glucose transport system partitions into the more ordered regions of the membrane, a prediction that is consistent with our previous results (6). These results showed the reconstituted basal glucose transport system is completely inhibited under conditions where only 50% of the bilayer is in the ordered state, indicating its residence in this phase of the membrane (6). However, further experiments must be performed to determine whether insulin alters membrane microenvironment or glucose transporter affinity for fluidized membrane regions, or both.

Direct addition of insulin to isolated liver plasma membranes has been reported to result in decreased membrane fluidity, as measured by fluorescence polarization (25). These data are seemingly at variance with our present findings, but may be rationalized by several possibilities. For example, the fluorescent probe used by Luly and Shinitzky (25) may selectively partition into an area of the liver membrane that is unrepresentative of the larger structure. The present data (Figs. 1–3) and previous observations (26) showing that insulin treatment of adipocytes results in enhanced D-glucose or L-glucose diffusion support the concept that insulin exerts a general fluidizing effect on the adipocyte membrane. It is also difficult to compare our results with those of Luly and Shinitzky because of the different methodology used. These workers added insulin directly to isolated liver membranes, which do not respond physiologically to the hormone. Glucose transport in intact hepatocytes is also unaffected by insulin. It is therefore noteworthy that we observe no effect on glucose transport when *cis*-vaccenic acid is added to isolated liver membranes (data not shown).

Insulin has been shown to cause an increase in the apparent V_{max} of adipocyte hexose transport without affecting the Michaelis constant (18, 20, 27, 28). This observation has been generally interpreted to support two possible mechanisms of insulin action. Insulin may stimulate hexose transport either by increasing the number of glucose carriers in the plasma membranes (27, 28) or by increasing the activity of a constant number of transporters (20). The former hypothesis is compatible with data showing no apparent difference in the temperature sensitivity between basal and insulin-stimulated hexose transport rates (27, 28). An insulin-induced increase in carrier protein number has also been inferred from the observation that D-glucose-sensitive [^{3}H]cytochalasin B binding sites are increased in plasma membranes derived from insulin-treated cells (29). However, several studies have shown a markedly greater temperature sensitivity of the insulin-stimulated glucose transport system (17–20) compared to control transport activity. These data indicate that different properties characterize basal in contrast to insulin-activated hexose transport. This notion is strengthened by the present findings that an increase in membrane fluidity stimulates basal glucose transport activity whereas the insulin-activated transporter is unaffected. Furthermore, in reconstitution studies with constant glucose carrier numbers, transport activity correlated directly with bilayer fluidity (6, 7). Our data therefore indicate that hexose carrier activity rather than carrier number is increased by insulin. However, we cannot rule out the possibility that insulin increases the number of transporters, but these differ from the basal transport system in responsiveness to bilayer fluidity.

This work was supported by Grants AM-17893 and HD-11343 from the National Institutes of Health. M.P.C. is a Research Career Development Awardee (AM-00249) and P.F.P. is a Postdoctoral Fellow of the U.S. Public Health Service.

1. Czech, M. P. & Lynn, W. S. (1973) *Biochim. Biophys. Acta* **297,** 368–377.
2. Cuatrecasas, P. & Tell, G. P. E. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 485–489.
3. Czech, M. P., Laurence, J. C. & Lynn, W. S. (1974) *J. Biol. Chem.* **249,** S1001–S1006.
4. Pillion, D. J. & Czech, M. P. (1978) *J. Biol. Chem.* **253,** 3761–3764.
5. Avruch, J., Carter, J. R. & Martin, D. B. (1972) *Biochim. Biophys. Acta* **288,** 27–42.
6. Melchior, D. L. & Czech, M. P. (1979) *J. Biol. Chem.* **254,** 8744–8747.
7. Czech, M. P., Melchior, D. L., Thomson, P. A., Pilch, P. F. & Carter-Su, C. (1979) in *Proceedings of the 10th Congress of the International Diabetes Federation* (Excerpta Medica, Amsterdam, Holland), in press.
8. Orly, J. & Chramm, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3433–3437.
9. Hanski, E., Riman, G. & Levitzki, A. (1979) *Biochemistry* **18,** 846–852.
10. Schaeffer, B. E. & Zadunaisky, J. A. (1979) *Biochim. Biophys. Acta* **556,** 131–143.
11. Rodbell, M. (1964) *J. Biol. Chem.* **239,** 375–380.
12. McKeel, D. W. & Jarett, L. (1970) *J. Cell Biol.* **44,** 417–432.
13. Avruch, J., Leone, G. R. & Martin, D. B. (1976) *J. Biol. Chem.* **215,** 1505–1510.
14. Pilch, P. F. & Czech, M. P. (1979) *J. Biol. Chem.* **254,** 3375–3381.
15. Shanahan, M. F. & Czech, M. P. (1977) *J. Biol. Chem.* **252,** 6554–6561.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
17. Amatruda, J. M. & Finch, E. D. (1979) *J. Biol. Chem.* **254,** 2619–2625.
18. Czech, M. P. (1976) *Mol. Cell Biochem.* **11,** 51–63.
19. Kono, T., Rosinson, F. W., Sarver, J. A., Vega, F. V. & Pointer, R. H. (1977) *J. Biol. Chem.* **252,** 2226–2233.
20. Whitesell, R. R. & Gliemann, J. (1979) *J. Biol. Chem.* **254,** 5276–5283.
21. Prasad, M. R. & Joshi, V. C. (1979) *J. Biol. Chem.* **254,** 997–999.
22. Hirata, F. & Axelrod, J. (1978) *Nature (London)* **275,** 219–220.
23. Madden, T. D., Chapman, D. & Quinn, P. J. (1979) *Nature (London)* **279,** 538–554.
24. Puskin, J. S. & Martin, T. (1979) *Biochim. Biophys. Acta* **552,** 53–65.
25. Luly, P. & Shinitzky, M. (1978) *Biochemistry* **18,** 445–450.
26. Pillion, D. J., Shanahan, M. F. & Czech, M. P. (1978) *J. Supramol. Struct.* **8,** 269–277.
27. Vinten, J. (1978) *Biochim. Biophys. Acta* **511,** 259–273.
28. Olefsky, J. M. (1978) *Biochem. J.* **172,** 137–145.
29. Wardzala, L. J., Cushman, S. W. & Salans, L. B. (1978) *J. Biol. Chem.* **253,** 8002–8005.