A class of inositol phosphate-containing oligosaccharides (IPG) derived from a membrane glycan-phosphatidylinositol precursor (GPI) has been identified as a possible mediator of insulin action. Saltiel’s laboratory has recently communicated an in vitro assay for the synthesis of GPI in rat liver microsomes. Herein we have established this method in rat and human liver microsomes, it being our end point to evaluate if the pool of GPI was normal in diabetes and if failure of insulin to generate IPG from GPI could be involved in the mechanism of insulin resistance in Type II diabetes. However, subsequent to the detailed study of $[^3H]$myoinositol incorporation into phospholipids in liver microsomes from our study subjects, we demonstrated by gas chromatography/mass spectrometry analysis that the material reported to be GPI is a mixture of lysophospholipids that does not contain hexosamine, ethanalamine, or amino acids.

The concept of chemical mediators in the mechanism of insulin action was first proposed by Larner’s laboratory in 1974 (1). Jarett’s laboratory (2), working independently and following different experimental approaches, also concluded that insulin elicits at least some of its biological effects by promoting the generation and/or release of unique intracellular mediators. The glycophosphate nature of the mediator(s) was suggested by Larner et al. (3); however, little progress was made in the elucidation of its structure until the discovery in 1986 by Saltiel and Cuatrecasas (4) that a class of inositol phosphate-containing oligosaccharides (IPG) mimicked in insulin action in the regulation of several enzymes. These data have been confirmed independently by several laboratories (5-9). These results suggest that insulin stimulates a plasma membrane phospholipase C with substrate specificity for a phospholipid that does not contain hexosamine, ethanolamine, or amino acids.

The LACK OF SYNTHESIS OF GLYCOSYLPHOSPHATIDYLINOSITOL, PRECURSOR OF THE INSULIN MEDIATOR INOSITOL PHOSPHATE GLYCAN*

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‡ The abbreviations used are: GPI, glycosyl-phosphatidylinositol; EGTA, [ethylenebis(oxyethylenenitrilo)tetraacetic acid; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PIP$_1$, phosphatidylinositol phosphate; PIP$_2$, phosphatidylinositol diphosphate; VSG, variant surface glycoprotein.

Phospholipase C has been recently described by Fox et al. (10) in rat liver. Saltiel’s laboratory (11-14) has recently developed an in vitro assay for the synthesis of the GPI precursor from which IPG is released in response to insulin. Furthermore, using this method, the authors studied the structure of IPG and suggested the presence of 7 5 sugar residues (12, 13). Herein, we have characterized this method in human liver because of our objective to explore if a defect(s) in this insulin messenger system is involved in the mechanism of insulin resistance in liver from patients with Type II diabetes mellitus. This method offers several advantages over previously published methods: (a) using a very small amount of liver, it is possible to test if the pool of GPI is normal in Type II diabetes; and (b) partially purified GPI from human liver can be used as a substrate of GPI-specific phospholipase C. Therefore, it would be possible to examine the effect of insulin on this specific phospholipase C in the membranes prepared from normal human liver and to determine if this enzyme is resistant to insulin in Type II diabetes.

EXPERIMENTAL PROCEDURES

Materials—Phosphatidylinositol (PI)-specific phospholipase C from culture supernatants of Bacillus thuringiensis was generously provided by Dr. Martin Low of Columbia University. GPI-specific phospholipase C from Trypanosoma brucei (VSG-lipase, $[^3H]$myristate-labeled VSG substrate, and $[^3H]$mannoselabeled glycolipid A were generously supplied by Dr. Paul T. Englund of Johns Hopkins University. $[^3H]$Myoinositol (specific activity, 20 Ci/mmol) and $[^3H]$glucosamine hydrochloride (specific activity, 27 Ci/mmol) were purchased from Du Pont-New England Nuclear. Reverse phase thin layer chromatography (RP-8 F254S) and Silica Gel 60 thin layer chromatography plates were purchased from VWR Scientific. Dipalmitoyl CDP-diglyceride, phospholipid standards, and other reagents were obtained from Sigma.

Human Subjects—Liver tissue were obtained from three brain-dead organ donors. After procurement, the liver was immediately cut into small pieces, frozen in liquid nitrogen, and stored at -70 °C until ready to use. We also studied five morbidly obese patients with Type II diabetes and five morbidly obese patients without diabetes who were admitted to the hospital to undergo gastric bypass for the treatment of morbid obesity and four nonobese nondiabetic patients admitted for electric cholecystectomy. With the exception of obesity and Type II diabetes, none of the subjects had any diseases or had taken any medications known to alter carbohydrate or lipid metabolism. Detailed clinical information on this patient population has been published elsewhere (15, 16). Written consent was obtained from all patients after they were informed about the nature and potential risk of the study. The patients underwent surgery after an overnight fast. Only saline was given intravenously before the liver biopsy. After opening the abdomen a 1-2-g liver biopsy specimen was obtained from the left lobe of the liver, frozen immediately, and stored at -70 °C until ready to be used.

Preparation of Human Liver Microsomes—Human and rat liver microsomes were prepared by modification of the method of Taraschi
et al. (17). Briefly, liver tissue was minced and homogenized (25% suspension, w/v) at 4 °C in potassium phosphate buffer, pH 7.4 (glass/ Teflon). The homogenate was filtered through cheesecloth and centrifuged, 8,000 rpm for 15 min in an SS34 rotor. The supernatant was collected and centrifuged at 100,000 x g for 1 h. The resulting pellet was washed with 100 mM Tris-Cl, pH 7.5, and resuspended in the same buffer at a protein concentration of 7–10 mg/ml. The samples were further purified from EDTA and 80% ethanol, and 80% ethanol was used as substrates rather than peak II phospholipid. Termination and extraction of phospholipids were performed as described in GPI-specific phospholipase C assays.

**VSG Lipase Assays**—These assays were performed as described by Herold et al. (21). The assay was performed in a 25-μl volume containing 2 μg (about 6000 cpm) of [3H]myristate-labeled membrane binding form VSG, 0.04% sodium dodecyl sulfate, 1% Nonidet P-40, 5 mM EDTA, and 50 mM Tris-Cl, pH 8.0. The enzyme (VSG lipase) was diluted in 1% Nonidet P-40, 5 mM EDTA, and 50 mM Tris-Cl, pH 8.0. After addition of the enzyme and incubation at 37 °C for 30 min, the mixture was thoroughly mixed with 0.5 ml of H2O-saturated n-butyl alcohol. The phases were separated by brief centrifugation, and 0.4 ml of the upper phase was counted for radioactivity using Aquasol.

**Nitrous Acid Deamination of Peak II Phospholipid**—This was performed as described by Ferguson et al. (23). Briefly, a sample of partially purified peak II phospholipid was dried under a stream of nitrogen and resuspended in 0.1 ml of 50 mM sodium acetate, pH 3.5. After the addition of 0.1 ml of 0.33 M NaNO2, the sample was incubated for 1 h at room temperature. At the end of this period, lipids were extracted with chloroform/methanol/6 N HCl and compared with the authentic sample of peak II phospholipid on reverse phase TLC plates using the ethanol/H2O (60:40) solvent system. The bands corresponding to peak II phospholipid were cut from the plate and suspended in 5 ml of chloroform/methanol (2:1). The suspension was vigorously vortexed and left on ice for 15 min. Following brief centrifugation at 2000 rpm for 5 min, the supernatant was transferred to another tube and organic solvent evaporated under N2. The lipids were reextracted from the remaining silica gel as described in the isolation section. From scintillation counting data, >99% of the radioactivity was extracted during the first extraction.

**Gas Chromatography/Mass Spectrometry Analysis**—All samples were hydrolyzed with 2 N trifluoroacetic acid at 100 °C for 6 h to hydrolyze all possible anomeric linkages and subsequently silylated. Each sample was pertrimethylsilylated with a mixture of pyridine/trimethylchlorosilane/triethylsilylimidazole (10:1:2) ( Pierce Chemical Co.) and then analyzed on a Varian 3400 gas chromatograph with a Finnigan Incas 50B mass spectrometer. An injection of 1 μl was made into a split/splitless injector with a 0.8-min split time. The samples were analyzed on a 30 meter DB 5 column (J and W Chromatography) with a 0.25-mm inner diameter and a flow rate of 11 ml/min. The detector was operated at a temperature of 300 °C, the injection port was held, followed by a 10 °C/min ramp to 250 °C. The final temperature was held for 10 min. The mass spectrometer was operated in the electron impact mode with a 70-eV input.

**RESULTS AND DISCUSSION**

The right panel of Fig. 1 shows that when rat liver microsomes were incubated with [3H]myoinositol and CDG-diglyceride under the identical conditions described by Saltiel's laboratories, the major [3H]inositol incorporation occurred into a phospholipid that stayed at the origin (peak I) and in a phospholipid with an Rf value of 0.4 (peak II). Approximately 90% of the total radioactivity was incorporated in peak I, which consists of a mixture of phosphoinositides, e.g. P1, PIP, and PIP2. The second peak (peak II) has been identified by Saltiel's laboratory (11–14) as GPI, the precursor of IPG. Peak II in Fig. 1 has the same Rf value as that reported by Saltiel's laboratory (11–14) and also shown in the left panel of the same figure. Peak II is readily hydrolyzed by PI-specific phospholipase C, one of the criteria used (11–14) to identify peak II as GPI. Fig. 2 demonstrates that human liver microsomes incubated under identical conditions as rat liver microsomes result in the biosynthesis of both peaks. Because of our interest in further study of GPI in humans with and without type II diabetes, we carried out a
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**Fig. 1.** Incorporation of \[^{3}H\]myoinositol into phosphoinositides by rat liver microsomes. *Left panel,* rat liver microsomes were incubated in the presence of CDP-diglyceride and \[^{3}H\]myoinositol as described under "Experimental Procedures." The reaction was terminated by the addition of chloroform/methanol/6 N HCl (100:50:1, v/v/v). The organic phase (OR) was collected and evaporated under N\(_2\). The residue was resuspended in 50 \(\mu\)l of chloroform/methanol (2:1) and spotted on a reverse phase thin layer chromatography plate which was preactivated by heating at 100 °C for 30 min. The chromatogram was developed in 60:40 ethanol/H\(_2\)O. One lane was cut into 1-cm pieces and counted for radioactivity following the addition of 0.5 ml of ethanol.

**Right panel,** partially purified peak II phospholipid was incubated with the indicated dilutions of PI-phospholipase C (PLC) from *B. thuringiensis* at 37 °C for 30 min. The reaction was stopped by adding chloroform/methanol/1 N HCl, and the material was processed and quantitated as described above.

**Fig. 2.** Incorporation of \[^{3}H\]myoinositol into phosphoinositides by human liver microsomes. Human liver microsomes were incubated in the presence of CDP-diglyceride and \[^{3}H\]myoinositol as described under "Experimental Procedures." The reaction was terminated by the addition of chloroform/methanol/6 N HCl (100:50:1, v/v/v). The organic phase (OR) was collected and evaporated under N\(_2\). The residue was resuspended in 50 \(\mu\)l of chloroform/methanol (2:1) and spotted on a reverse phase thin layer chromatography plate which was preactivated by heating at 100 °C for 30 min. The chromatogram was developed in 60:40 ethanol/H\(_2\)O. One lane was cut into 1-cm pieces and counted for radioactivity following the addition of 0.5 ml of ethanol. Right panel, partially purified peak II phospholipid was incubated with the indicated dilutions of PI-phospholipase C (PLC) from *B. thuringiensis* at 37 °C for 30 min. The reaction was stopped by adding chloroform/methanol/1 N HCl, and the material was processed and quantitated as described above.

Detailed characterization of the biosynthesis of peak II phospholipid in normal human liver microsomes.

The *middle* and *upper panels* of Fig. 3 show that the incorporation of \[^{3}H\]myoinositol into peaks I and II was linear with respect to time and protein concentration. The *lower panel* of Fig. 3 demonstrates that the incorporation of \[^{3}H\]myoinositol into peaks I and II showed a broad pH range with optimal incorporation at pH 7.5. At low pH, there was no incorporation of \[^{3}H\]inositol into phospholipids.

**Fig. 3.** Incorporation of \[^{3}H\]myoinositol into peak I and II phosphoinositides as a function of protein concentration (upper panel), time (middle panel), and pH (lower panel) by human liver microsomes. The *in vitro* biosynthesis assays were performed as described in the legend to Fig. 2. Each point is an average of triplicate determinations from three different experiments.

**Fig. 4.** Effect of CDP-diglyceride (DG) on synthesis of peak I and II phosphoinositides in human liver microsomes. The microsomes were incubated in the presence or absence of CDP-diglyceride, and the assays were performed as described in the legend to Fig. 2. The results are from duplicate determinations from three different experiments.

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The human liver microsomes were incubated in the absence or presence of indicated concentrations of specific metal ions, and [3H]inositol incorporation assays were performed as described in the legend to Fig. 2. The data represent the average of duplicate determinations from three different experiments.

The next group of experiments was performed to attempt to answer our first question. Is the biosynthesis of peak II altered in Type II diabetes? For these experiments, we used previously established optimum conditions, e.g. 100 μg of liver microsome protein, 15-min incubation in the presence of CDP-diglyceride, and appropriate metal ions at pH 7.6. As shown in Fig. 6, we found no statistical differences among lean controls, obese controls, and obese noninsulin-dependent diabetes mellitus in the incorporation of [3H]myoinositol into peak II. However, [3H]myoinositol incorporation was higher in peak I phospholipids in lean controls compared with obese controls or obese noninsulin-dependent diabetes mellitus (p < 0.01). It is possible that endogenous levels of inositol in the microsome preparation from different patients may have a profound effect on the incorporation of [3H]myoinositol in the phospholipids. However, in our experiments we have used 10 mM final concentration of inositol, and the endogenous level of free inositol may not change the inositol concentration substantially to affect [3H]myoinositol incorporation. Although these data cannot be extrapolated to in vivo conditions, under the optimal in vitro experimental conditions there are no statistically significant differences in the biosynthesis of peak II phospholipid in human liver microsome preparations from normal patients and obese patients with type II diabetes.

The next group of experiments was directed to attempt to answer our second question. Is GPI-phospholipase C stimulated by insulin in human liver plasma membranes? If so, is insulin stimulation of GPI-phospholipase C altered in Type II diabetes? For this purpose, we needed to partially purify large quantities of peak II phospholipid from human liver to be used as a substrate and liver plasma membranes as a source of GPI-phospholipase C; in an analogous way we (19) and others (24) have evaluated the effect(s) of hormones on phosphatidylinositol 4,5-bisphosphate (PIP2) specific phospholipase C using exogenous [3H]PIP2. To this end, stability of the phospholipids during the purification and extraction procedure needs to be demonstrated. Fig. 7 shows that when peak I and II phospholipids were extracted from TLC plates and rechromatographed in an identical way, the purified lipids show identical migration properties. These data indicate the stability of peak I and II phospholipids during the extraction process.
Central to this study are the data shown in Table I which demonstrate, under several experimental conditions, the failure of insulin and liver plasma membranes to stimulate hydrolysis of peak II phospholipid. The membranes used in this study contain a catalytically competent insulin receptor kinase capable of phosphorylating the β-subunit of insulin receptor and three membrane-associated endogenous substrates (25). When peak II phospholipid was used as a substrate and liver plasma membranes as a source of GPI-phospholipase C, no breakdown of peak II phospholipid was observed under the assay conditions, i.e. up to 200 μg of membrane protein and 1 h of incubation. These experiments were performed by suspending the peak II phospholipid in an appropriate buffer followed by short sonication. Saltiel et al. (20) reported that substrate works optimally if incorporated into micelles prepared from phosphatidylethanolamine/phosphatidylcholine (PE/PC) mixture. Therefore, we repeated the above set of experiments by incorporating the purified peak II phospholipid in PE/PC micelles. Under these conditions, hydrolysis of peak II phospholipid was not observed either. Also, the presence of Ca2+ may have a significant effect on the enzymatic activity. When GPI-phospholipase C assays were performed in the presence of 0.1–5.0 mM Ca2+, no hydrolysis of the peak II phospholipid was observed. Furthermore, when insulin was added in the above assays, no effect of insulin was observed.

If peak II phospholipid is a true substrate of GPI-phospholipase C, insulin might cause the breakdown of newly synthesized GPI, i.e. in the presence of insulin, the level of GPI will be decreased in biosynthesis assays. As shown in Table II, when the biosynthesis of peak II phospholipid was examined in the presence and absence of insulin (10−7 M), there was no difference in the level of peak II phospholipid, indicating the failure of insulin to hydrolyze peak II phospholipid. There are at least three possible explanations for these negative results: (a) in spite of our several protocols, we might have failed to physically couple the exogenous substrate with the plasma membrane enzyme; (b) an intermediary between the activation of the insulin receptor and the activation of GPI-phospholipase C might be missing in the plasma membrane preparation; (c) peak II is not GPI. Clearly, of these three possibilities, the most feasible to test is the last one.

The ability of bacterial phospholipase C to release cell surface protein with a GPI membrane anchor has been widely appreciated, and some of these proteins, of which over 30 have been identified (26). As previously stated, this enzyme physically couples the exogenous substrate with the plasma membrane enzyme and 1 h of incubation. These experiments were performed by suspending the peak II phospholipid in an appropriate buffer followed by brief sonication. Saltiel et al. (20) reported that substrate works optimally if incorporated into micelles prepared from phosphatidylethanolamine/phosphatidylcholine (PE/PC) mixture. Therefore, we repeated the above set of experiments by incorporating the purified peak II phospholipid in PE/PC micelles. Under these conditions, hydrolysis of peak II phospholipid was not observed either. Also, the presence of Ca2+ may have a significant effect on the enzymatic activity. When GPI-phospholipase C assays were performed in the presence of 0.1–5.0 mM Ca2+, no hydrolysis of the peak II phospholipid was observed. Furthermore, when insulin was added in the above assays, no effect of insulin was observed.

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The ability of bacterial phospholipase C to release cell surface protein with a GPI membrane anchor has been widely used to identify these unique proteins, of which over 30 have already been identified (26). As previously stated, this enzyme has been frequently used to identify GPI, the precursor of insulin mediator IPG (11–14). The lower panel of Fig. 8 demonstrates that peak II from human liver microsomes like that from rat liver microsomes (Fig. 1) is readily hydrolyzed by bacterial phospholipase C. However, the upper panel of the same figure also demonstrates the complete hydrolysis of peak I. Although hydrolysis of peak II phospholipid occurred by PI-phospholipase C, the fact is that the phospholipase C characterized by Low's laboratory is specific for PI rather than GPI (27). Thus, this enzyme is not specific to elucidate the structure of GPI or to study the functions of GPI in insulin action. In contrast, a GPI-specific phospholipase C from T. brucei (21) and a phospholipase D with a specificity for GPI from human and bovine plasma (22) have been recently identified. As shown in Table III, neither of these enzymes hydrolyzed to any extent peak II phospholipid under conditions that were able to hydrolyze [3H]mannose-labeled GPI that have been purified from T. brucei (21). The presence of these enzymes during biosynthesis of peak II had no effect on [3H]myoinositol incorporation into peak II phospholipid (Table III). Also, nitrous acid deamination has been used to demonstrate the presence of glucosamine residues in this glycolipid (28). When the purified phospholipid from peak II was subjected to nitrous acid deamination and the resulting product was isolated, it co-migrated with the original peak II phospholipid indicating the absence of glucosamine residue (Table III). Finally, when liver microsomes were incubated as before but with UDP glucosamine, we were unable to demonstrate any incorporation of labeled glucosamine into the peak II (not shown). Gaulton et al. (29) showed a distinct migration of glucosamine-labeled glycosyl-PI from lyso-PI on silica gel TLC plates. Using a similar method, we compared the relative mobility of peak II phospholipid with GPI and lyso-PI. As can be seen in Fig. 9, by two different chromatography methods, peak II co-migrated with lyso-PI and peak I with PI. Thus, the data strongly suggest that peak II phospholipid is not GPI but an inositol-containing phospholipid that co-migrates with lyso-PI.

To further demonstrate that peak II phospholipid is not GPI and exclude the possibility that our negative results in a human liver do not represent species-dependent differences between man and rat, we studied peak II phospholipid from human and rat liver by gas chromatography/mass spectrometry analysis.

The samples were analyzed with the goal of determining if a GPI anchor was present or not. Traditional carbohydrate analysis techniques were used for this purpose. Pertrimethylsilyl ethers were formed on all available hydroxyl groups to afford volatile materials. Rat and human biopsy samples were analyzed. The results were the same for both species. Each of the samples was analyzed for any trace amounts of GPI constituents such as amino acids, ethanolamine, hexosamine, neutral carbohydrates, and inositol. Amino acid analysis failed to identify amino acids. None of the nitrogen-containing species of the GPI (i.e. amino acids, ethanolamine, or hexosamine) were identified in the samples by the gas chromo-

### Table I

**Effect of Ca2+ and insulin on the hydrolysis of peak II phospholipid by human liver plasma membranes**

| Plasma membranes (protein) | 50 μg | 100 μg | 200 μg |
|---------------------------|------|-------|-------|
|                           | 0    | 0.1 mM Ca2+ | 5.0 mM Ca2+ | 0 | 1 × 10−7 M insulin | 0 | 1 × 10−7 M insulin |
| Peak II phospholipid (dpm) | 19,362 ± 288 | 18,887 ± 497 | 18,843 ± 427 | 19,125 ± 715 | 18,850 ± 626 | 19,850 ± 656 | 19,450 ± 691 |
| Peak II phospholipid in PE/PC micelle (dpm) | 19,217 ± 325 | 18,975 ± 500 | 19,050 ± 250 | 19,000 ± 700 | 18,975 ± 425 | 19,750 ± 550 | 19,500 ± 475 |
Effect of insulin on synthesis and breakdown of peak II phospholipid

Human liver microsomes (100 µg of protein) were incubated with [3H]mioinositol as described under "Experimental Procedures" in the presence or absence of insulin (10^{-7} M) for 30 min at 37 °C. The radioactivity in the peak II phospholipid was analyzed as described in the legend to Fig. 1. The results are expressed as mean ± S.E. from four different experiments performed in duplicate.

| Treatment | Peak II phospholipid biosynthesis (dpm) |
|-----------|----------------------------------------|
| Control   | 9,775 ± 96                            |
| PI-PLC    | 406 ± 37                               |
| PLD       | 9,725 ± 371                            |
| GPI-PLC   | 9,737 ± 20                             |
| HNO3      | 19,500 ± 468                           |
| 1 x 10^{-7} M insulin | 19,500 ± 468  |

Peak II phospholipid biosynthesis (dpm) was significantly higher in the presence of insulin compared to the control. The table data are mean ± S.E. from three different experiments performed in duplicate.

Effect of PI-phospholipase C (PLC) from B. thurigensis on the hydrolysis of peak I (upper panel) and peak II (lower panel) phosphoinositide. The partially purified peak I and II phospholipids were incubated with the indicated concentrations of PI-phospholipase C. The reactions were stopped by adding chloroform/methanol/6 N HCl and processed as described in the legend to Fig. 1. The results are average from three different experiments performed in duplicate.

FIG. 9. Analysis of peak I and II phospholipids by thin layer chromatography. Synthesis of peak I and II phospholipids from human liver microsomes was performed as shown in the legend to Fig. 1. Relative mobilities of these lipids were compared with the authentic samples of several phospholipids run in parallel on the same TLC plates. The spots of authentic samples were visualized by exposure to iodine and radioactive bands by counting the radioactivity as described under "Experimental Procedures." A, comparison using reverse phase TLC used in this study. B, comparison using silica gel plates and chloroform/methanol/NH4OH/H2O (45:45:3.5:10, v/v/v/v) solvent system as described by Mato et al. (5). The open bars represent the chromatography of peak I and the closed bars, peak II.

Matography/mass spectrometry technique. Inositol and neutral carbohydrates were identified. Glucose was a component in all samples and trace concentrations of mannose in one sample; both were most likely derived by the purification procedure since thin layer chromatography plate use a binder for the silica which contains cellulose, and upon hydrolysis, glucose. The materials isolated were a mixture of lysophospholipids. Of four samples analyzed, all contained lysophosphatidylserine, lyso-PC, and lyso-PI. The mioinositol was identified from both its retention time in comparison with standards and from the characteristic mass fragments of the pertrimethylsilylated compound, m/e 305, 318, 507. With the characteristic fragments of the inositols, chiroinositol was also identified in the samples from both rat and human biopsies.

In summary, our data does not exclude the presence of GPI as the precursor of the insulin mediator IGP nor question the novel hypothesis of insulin action put forward by Saltiel and Cuatrecasas (4) and supported by experimental data from several laboratories (7-8,30). Our data only demonstrate that under the experimental in vitro conditions described by Saltiel's laboratories, GPI is not synthesized. However, it is important to recognize that Saltiel's laboratory originally reported this method in abstract form (11) and although it has been enthusiastically communicated in several scientific meetings and review articles (12-14) it has not been published yet in a peer review journal. However, the general acceptance that a phosphatidylinositol glycan anchor of membrane protein could be the precursor of an insulin mediator has made us and probably other laboratories recognize the several potential advantages of an in vitro method to study the metabolism and structure of this precursor in normal and pathological states. However, we demonstrate here that GPI is not synthesized in vitro.

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