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The Effect of Insulin on the Permeability of Phosphatidyl Choline Bimolecular Membranes to Glucose

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Summary. Insulin, on the outside of phosphatidyl choline bimolecular membranes, increased the permeability of the membranes to glucose. The magnitude of the increase in glucose permeability was dependent upon the insulin concentration and was independent of the glucose concentration over the range 2.7 to 14.4 mM. The increase in the permeability of the membranes to glucose was not accompanied by a change in the direct current electrical resistance of the membranes. Ovalbumin resulted in a smaller increase in the permeability of the membranes to glucose and no change in their electrical resistance, while phloretin changed neither the permeability to glucose nor the electrical resistance. Insulin, on both sides of the bimolecular membranes, did not change the permeability to glucose from that observed when insulin was present only on the outside, nor did it change the electrical resistance.

The earliest action of insulin on its target cell is probably an interaction between insulin and the plasma membrane. Rodbell (1966) proposed that the increase in glucose utilization and amino acid incorporation into protein observed when insulin acts on adipose cells is secondary to an interaction between insulin and the lipoproteins of the cell membrane. Cuatrecasas (1969) showed that insulin bound to sepharose beads and unable to enter adipose cells, increased glucose utilization and depressed lipolysis in these cells. Studying the binding of ^{125}I-insulin to the fat cell membrane, he suggested that as an initial step in insulin action, peptide insulin receptors in the membrane bind insulin (Cuatrecasas, 1972). Kono and Barham (1971*a*, 1971*b*) studying simultaneously the utilization of glucose by, and the binding of insulin to, fat cells, pointed out the correlation between the binding of insulin to a certain number of discrete sites and the increase in glucose oxidation by these cells.

The lipid bilayer membrane, the bimolecular lipid leaflet proposed by Davson and Danielli (1943) as the core of the biological membrane, shares many of the characteristics of cell membranes (Henn & Thompson, 1969;

Mueller & Rudin, 1969*a*), including relative impermeability to water-soluble substances, such as glucose and ions. A bilayer membrane of phosphatidyl choline, separating two aqueous compartments, was used as a model for the plasma membrane of the insulin target cell, one side of the bilayer to simulate the outer aspect of the plasma membrane in contact with the interstitial fluid ("outside") and the other to simulate the inner aspect of the plasma membrane in contact with the cell interior ("inside"). These studies were designed to examine whether insulin can increase directly the permeability of a phospholipid bilayer to glucose in the absence of protein, hence, of both the presumed "receptor" and "carrier" substances, and if so, whether the action of insulin changes directly the net flux of ions through the bilayer.

The results suggest that insulin on the outside of the phosphatidyl choline bimolecular membrane increases the permeability of the bilayer to glucose without a concommitant net flux of ions through the membrane. The magnitude of the permeability increase is dependent on the concentration of insulin in the outside aqueous compartment, but is independent of the glucose concentration in the system. Phloretin on the outside of the bimolecular membrane changes neither the permeability to glucose nor the electrical resistance, while ovalbumin, a protein without *in vivo* hormonal action, causes a smaller increase in the permeability of the bimolecular membrane to glucose, accompanied, as is the action of insulin, by no net flux of ions through the membrane. Insulin, on both sides of the bimolecular membrane, does not change the magnitude of the glucose permeability from that observed when insulin is present only on the outside, nor does it change the electrical resistance of the bimolecular membrane.

Materials

Egg yolk phosphatidyl choline, chromatographically pure, was obtained from Applied Science Laboratories (State College, Pa.) dissolved in chloroform or *n*-pentane and sealed under nitrogen in ampoules. The ampoules were kept at −23 °C until use, then brought to room temperature and the solvent evaporated to dryness in a stream of nitrogen. The phosphatidyl choline was redissolved in *n*-decane (2% w/v) and the solution maintained under nitrogen. Insulin (bovine, pancreatic, recrystallized, 26 U/mg) was purchased from Schwarz-Mann (Orangeburg, N.Y.), ovalbumin (3-times recrystallized) from Nutritional Biochemicals (Cleveland, Ohio), phloretin from K and K Laboratories (Plainview, N.Y.) and D-glucose-UL-^{14}C from ICN-Tracer Lab (Irvine, Calif.). The proteins were dissolved as a 0.5% solution in 5 mM HCl, pH 2.5, and stored at 3 °C. Immediately before use an aliquot of protein solution was adjusted to pH 7.0 with NaOH. Water was triple-distilled in a quartz still and stored in quartz flasks. D-glucose, $CaCl_2$, NaCl, Tris HCl, Tris Base, and disodium ethylendiamine tetraacetate (EDTA) were reagent grade.

Methods

A slight modification of the apparatus described by Mueller and Rudin (1969*b*) used in these experiments was housed in a Faraday cage resting on a rubber pad. A Teflon cup with an aperture 1 mm in diameter was fitted tightly into a plexiglass boat, and both boat and cup filled with a buffer solution similar to that of Okhi (1970) at pH 7.0, and containing NaCl (150 mM), EDTA (0.05 mM), Tris (0.2 mM), and $CaCl_2$ (1 mM). A phosphatidyl choline bimolecular membrane was formed across the cup aperture. A matched pair of Ag – AgCl electrodes placed in the solution on each side of the bimolecular membrane was used both to apply a potential difference across the membrane and to conduct the current resulting from the potential difference. Fig. 1 is a diagram of the electrical circuit used in these studies.

Protein solutions at pH 7.0, consisting of ^{14}C-glucose, buffer, and insulin or ovalbumin, contained in a volume of 50 μliters, were added to the cup (the outside aqueous compartment) with 30 stirs, sufficient to achieve complete mixing, as previously measured by the completeness of dispersal of a dye within the cup. The final concentration of protein in the outside compartment was 16 μg/ml, and that of ^{14}C-glucose, 25 μC/ml

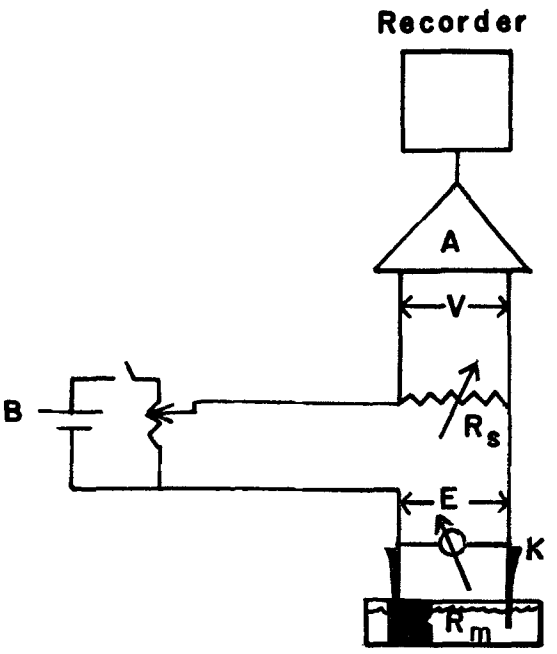

Fig. 1. The circuit used for measuring electrical resistance. A d–c potential difference (E), whose magnitude within the ohmic range, 0 to 50 mV, is displayed on a Keithley 610C Electrometer (K), is generated by a battery (B) and imposed across a known resistance (R_s) and across the resistance of the bimolecular membrane (R_m). The resulting potential difference (V) across R_s is amplified 20-fold with a high impedance differential amplifier (A) and the amplifier output fed into the input module (Hewlett-Packard 17504A, range 100 mV full scale) of a one-channel recorder (Hewlett-Packard 7127A). As E and V are measured, and R_s is known, the resistance of the bimolecular membrane R_m can be calculated from the following relation: $\frac{V}{E} = \frac{R_s}{R_m}$

(S.A. 1.7 to 9.3 mC/mM). In experiments in which protein was absent, an equal volume of protein-free solution was added to the outside compartment. A Monel stirrer in the inside compartment was rotated continuously with a magnetic stirrer at a rate sufficient to insure complete mixing, as previously measured by the completeness of dispersal of dye. The bimolecular membrane was examined repeatedly during the experiment and buffer added when needed to prevent bulging. All experiments were carried out at 25 ± 1 °C. The possibility of the presence of a shunt was examined and eliminated by finding that no ^{14}C-glucose penetrated a cup without an aperture. Samples from the aqueous compartments were dissolved in 15 ml of Aquasol (New England Nuclear, Boston, Mass.) at the end of an experiment and counted in a Packard Tri-Carb Scintillation Counter (Downers Grove, Ill.). The count-rate error was less than $\pm 17\%$, $p < 0.05$. The area of the bimolecular membrane was calculated from measurements made by viewing the membrane through a grid placed between the lenses of the magnifying system.

The flux of glucose across the phosphatidyl choline bimolecular membrane, and the glucose permeability coefficient of diffusion, P_d, for this membrane were calculated as follows:

$$\text{Flux (moles cm}^{-2}\text{ sec}^{-1}) = \frac{(^{14}C_i)(V_i)}{\frac{(^{14}C_o)(T)(A)}{(C_o)}} \tag{1}$$

where $^{14}C_i$ and $^{14}C_o$ are the average concentrations of ^{14}C-glucose in the inside and outside compartments, respectively, during the interval of time T; C_o is the concentration of nonradioactive glucose in the outside compartment; V_i, the volume of the inside compartment; and A, the area of the membrane. In these experiments, the initial value of $^{14}C_i = 0$ and $^{14}C_i \ll {}^{14}C_o$; therefore, $^{14}C_o - {}^{14}C_i \approx {}^{14}C_o$, and the final value of $^{14}C_o \approx$ the initial value of $^{14}C_o$.

$$\text{Permeability Coefficient, } P_d(\text{cm sec}^{-1}) = \frac{\text{Flux}}{C_o}. \tag{2}$$

At the beginning of an experiment, phosphatidyl choline in *n*-decane was applied to the aperture of the Teflon cup to create a hydrophobic surface. The cup, with aperture facing forward, was fitted into the boat resting on a stand in the Faraday cage. Both aqueous compartments were filled with buffer, the magnetic stirrer started in the front of the boat, the Ag – AgCl electrodes clamped in place, and phosphatidyl choline brushed across the surface of the aperture. Formation of the bimolecular membrane was monitored visually and electrically. Fifteen minutes after the membrane had formed, the electrical resistance was measured and recorded, and a sample of solution was withdrawn from the inside compartment for the measurement of radioactivity at time zero. An aliquot of unlabeled glucose was added to the 4-ml inside compartment and an aliquot of labeled glucose to the 3-ml outside compartment, so that the final concentration of unlabeled glucose was the same on both sides of the membrane. Protein solution, or buffer solution without protein, was added to the outside compartment and the compartment covered with a lid. After 60 min, the electrical resistance was again measured and a 200-μliter sample for counting was drawn from the inside compartment with buffer replacement to maintain the hydrostatic pressure across the bimolecular membrane. Additional 200-μliter samples were taken from the inside compartment as long as the bimolecular membrane remained intact. At the end of the experiments, 10-μliter aliquots for counting were taken from the outside compartment.

Results

In a typical experiment, the radioactivity from the flux of D-glucose-UL-^{14}C across the phosphatidyl choline bimolecular membrane into the inside compartment increased linearly with time (Fig. 2). In the presence of insulin (16 μg ml^{-1} outside compartment) the increase, still linear, was greater (Fig. 2).

The P_d for glucose across the phosphatidyl choline bimolecular membrane was independent of the concentration of glucose in the aqueous compartments over the range of 2.7 to 14.4 mM, in the presence or in the absence of insulin (Fig. 3). The P_d for glucose in the absence of insulin was 2.8 ± 0.44 (SEM) $\times 10^{-7}$ cm sec^{-1}, and in the presence of insulin, 7.0 ± 0.44 (SEM) $\times 10^{-7}$ cm sec^{-1}, an increase of 150% (Table 1). The P_d for glucose across the phosphatidyl choline bimolecular membrane in the presence of insulin increased from 2.8×10^{-7} cm sec^{-1} to 7.0×10^{-7} cm sec^{-1} as the concentration of insulin in the outside compartment increased from 0 to 16 μg ml^{-1} (Fig. 4). When insulin was present in equal concentration (16 μg ml^{-1}) on both sides of the bimolecular membrane, the P_d for glucose was 6.8 ± 0.88 (SEM) $\times 10^{-7}$ cm sec^{-1}, a value not significantly different from the P_d observed when insulin was present only on the outside (Table 1). When phloretin (0.1 mM final concentration) was present in the outside compart-

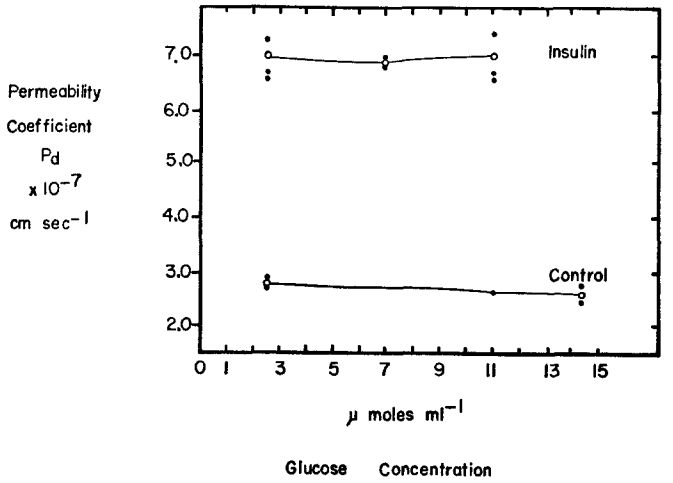

Fig. 2. The ^{14}C-glucose radioactivity in the inside aqueous compartment as a function of time. Each curve represents a single bimolecular membrane experiment typical of 6. The area of the membranes is 0.3 mm^2; the concentration of nonlabeled glucose, the same in both compartments, is 2.4 mM. In the insulin experiment, ^{14}C-glucose, buffer, and insulin (16 μg ml^{-1} final concentration) were added to the outside aqueous compartment. In the control experiment, an equal volume of the same solution, without insulin, was added

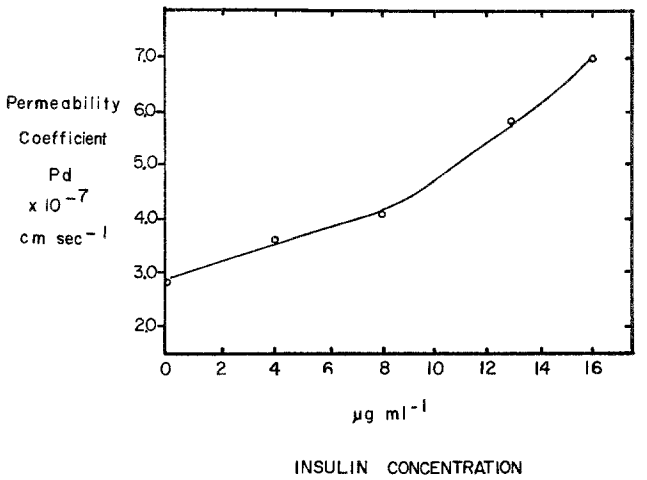

Fig. 3. The glucose permeability coefficient P_d for the phosphatidyl choline bimolecular membrane as a function of the glucose concentration in the aqueous compartments. The nonlabeled glucose concentration is the same in both compartments. In the insulin experiments, ^{14}C-glucose, buffer, and insulin (16 μg ml^{-1} final concentration) were added to the outside compartment. In the control experiments, an equal volume of solution, without insulin, was added

Table 1. The glucose permeability coefficient of phosphatidyl choline bimolecular membranes

	Reactants		Permeability coefficient of diffusion, P_d ($\times 10^{-7}$ cm sec^{-1})
	Concentration	Compartment	
Control	0		2.8 ± 0.17
Insulin	16 μg ml^{-1}	outside	7.0 ± 0.44, $p < 0.01$
Ovalbumin	16 μg ml^{-1}	outside	4.9 ± 0.01, $p < 0.01$
Insulin	16 μg ml^{-1}	outside and inside	6.8 ± 0.88
Phloretin	0.1 μM ml^{-1}	outside	3.7 ± 0.35, $p > 0.2$

Each P_d represents the mean ± SEM of 6 experiments, except for the P_d for phloretin (3 experiments). The significance of the differences between the P_d in control experiments and the P_d in the presence of protein or phloretin is shown. In addition, the p value for the difference in P_d between insulin and ovalbumin, <0.05, and that for the differences between insulin and the outside compartment and insulin in both compartments, >0.8, is shown.

ment, the P_d for glucose across the bimolecular membrane was 3.7 ± 0.35 (SEM) $\times 10^{-7}$ cm sec^{-1}, a value not significantly different from the P_d when no phloretin was present (Table 1). Ovalbumin in the same concentration

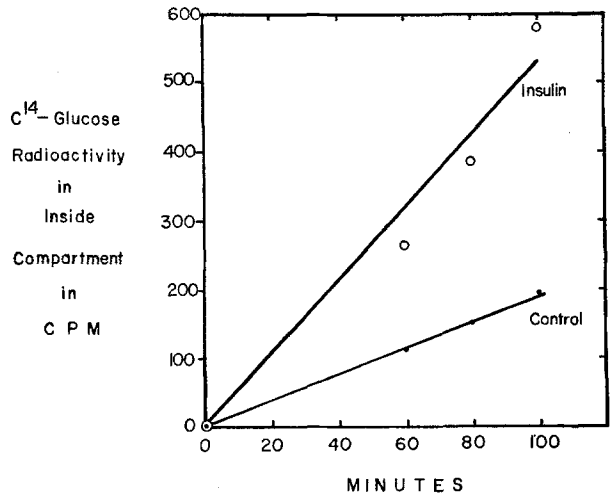

Fig. 4. The glucose permeability coefficient P_d for the phosphatidyl choline bimolecular membrane as a function of the concentration of insulin in the outside aqueous compartment

Table 2. The direct current electrical resistance of phosphatidyl choline bimolecular membranes

	Reactants		Electrical resistance ($\times 10^9\ \Omega\ cm^2$)	
	Concentration ($\mu g\ ml^{-1}$)	Compartment	Initial time	60 min
Control	0	outside	5.0	2.0 ± 36
Insulin	16	outside	5.0	0.9 ± 3.0
Ovalbumin	16	outside	5.0	5.0 ± 39
Insulin	16	outside and inside	5.0	1.0 ± 1.3

All values represent the mean $\pm$ SEM. Differences between the means are not significant, $p > 0.5$.

(w/v) as insulin (16 $\mu g\ ml^{-1}$ outside compartment) increased the P_d for glucose across the phosphatidyl choline bimolecular membrane to 4.9 ± 0.01 (SEM) $\times 10^{-7}$ cm sec^{-1}, an increase of 75% (Table 1).

The change in the direct current electrical resistance of the phosphatidyl choline bimolecular membrane was small, and not significantly different in the presence or absence of insulin or ovalbumin in the outside compartment, or of insulin in both compartments (Table 2). There was no change in electrical resistance when phloretin was present in the outside compartment.

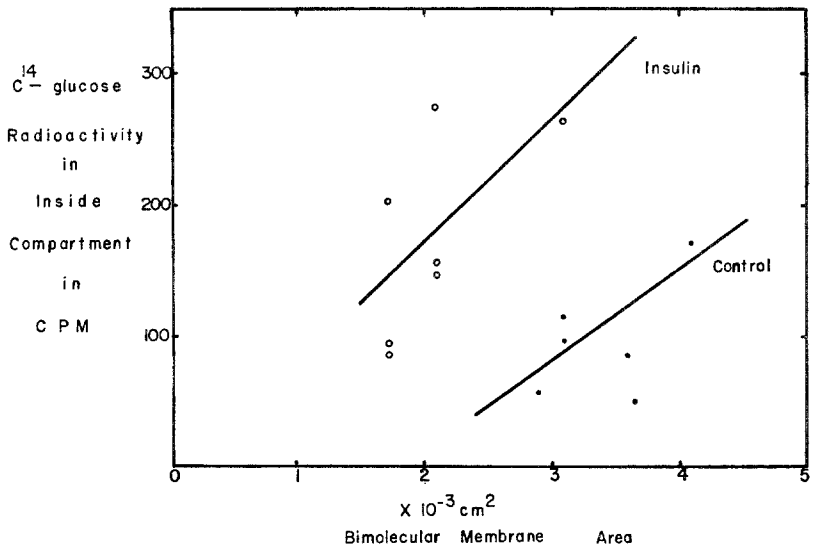

Fig. 5. The ^{14}C-glucose radioactivity in the inside aqueous compartment as a function of the bimolecular membrane area. In the insulin experiments ^{14}C-glucose, buffer, and insulin (16 μg ml^{-1} final concentration) were added to the outside aqueous compartment. In the control experiments an equal volume of solution, without insulin, was added. The regression lines are fitted by the method of least squares

The radioactivity from D-glucose-UL-^{14}C passing the bimolecular membrane increased linearly with the area of the membrane in the presence or absence of insulin (Fig. 5).

As aliquots of inside compartments, outside compartments, and D-glucose-UL-^{14}C (as purchased) gave single spots, identifiable as D-glucose (Trevelyan, Procter & Harrison, 1950) and coinciding with single peaks of radioactivity on chromatography with Whatman #1 paper in *n*-butanol/ethanol/water, 52:18:30 (v/v/v), radioactivity measured in these experiments originated from D-glucose.

Discussion

The glucose permeability coefficient at the phosphatidyl choline bimolecular membranes measured in these studies, 2.8×10^{-7} cm sec^{-1}, is larger than the glucose permeability coefficient of 10^{-10} cm sec^{-1} estimated by Wood, Wirth and Morgan (1968) in planar bimolecular membranes made from a total phospholipid extract of human red blood cells in a solution of chloroform, methanol, α-tocopherol, and cholesterol. Jung (1971) making spherical bimolecular membranes from lipids dissolved in a similar solution,

measured glucose permeability coefficients of 2.35×10^{-10} cm sec^{-1} in membranes made from total lipid extracts of human red blood cells, 2.23×10^{-10} cm sec^{-1} in membranes formed from phosphatidyl ethanolamine, and 2.51×10^{-10} cm sec^{-1} in membranes formed from phosphatidyl choline, the last two in the absence of cholesterol. He estimated that although the glucose P_d's measured in the lipid bimolecular membranes were lower by a factor of about 10^{-4} than the glucose P_d's measured for red blood cells or cell membranes with functional carriers, the P_d's measured in bimolecular membranes were similar to those observed in red blood cell preparations in which the carrier was inactivated and to that which characterizes nonspecific glucose permeation of living cell membranes. Papahadjopolous, Nir and Okhi (1972) found that the glucose permeability coefficient in phosphatidyl serine liposomes was 4×10^{-11} cm sec^{-1}, and Lossen (1972) studying soybean phosphatidyl choline liposomes found a glucose permeability coefficient 6.8×10^{-12} cm sec^{-1}.

The most probable explanation for the differences in permeability coefficients measured lies in the differences in composition of the several bimolecular membranes and liposomes used. The solutions from which the membranes were formed differed not only in lipid composition but also in the composition of their other organic constituents, which were present to some extent in the membranes. Wood *et al.* (1968) and Jung (1971) found similar permeability coefficients using bimolecular membranes formed from solutions similar in lipid and other organic constituents. The permeability coefficients measured in liposomes were lower, and those found in the current studies, employing a solution consisting only of egg-yolk phosphatidyl choline and *n*-decane, were consistently higher.

To account for the high rate at which glucose penetrates cell membranes and for the degree of selectivity among sugars exhibited by natural membranes, it has been postulated that glucose permeates cell membranes through a process of facilitated diffusion with stereospecific protein carriers (Hoos, Tarpley & Regen, 1971; Leib & Stein, 1971). Several workers have presented evidence that glucose enters adipose cells via a carrier (Hernandez & Sols, 1963; Morgan, Regen & Park, 1964; Rodbell, 1966; Crofford, 1967); others, that glucose entry into muscle (Regen & Morgan, 1964) and red blood cells (Regen & Morgan, 1964; Jung, 1971) is carrier-mediated. Further, Jung (1971) has estimated the P_d for glucose across the human red blood cell membrane and the lipid bimolecular membrane, and concluded that the latter is smaller by several orders of magnitude than the former, which presumably depends in part on the presence of a protein carrier. Adipose cells (Rodbell, 1966), muscle cells (Levine, 1965), and perhaps red

blood cells (Gavin, Roth, Jen & Freychet, 1972; Zipper & Mawe, 1972), are *in vivo* target cells of insulin. Calculations from the data of Rodbell (1964), who measured the removal of glucose from the medium by adipose cells in suspension, indicate that the rate of uptake of glucose by the cells increased 68% in the presence of insulin. Zipper and Mawe (1972) found that insulin increased by 47% the maximal net flux of glucose in human red blood cells. The current studies have shown that insulin in the outside aqueous compartment of the phosphatidyl choline bimolecular membrane increases the permeation of glucose from the outside into the inside compartment by 150%. The actual flux of glucose across adipose and red blood cell membranes was, respectively, 1 and 2 orders of magnitude greater than that across the bimolecular membrane, but the ratios of the rates of glucose permeation in the presence and absence of insulin were similar. As the entry of glucose into cells is considered the rate-limiting step in the utilization of glucose by cells (Rodbell, 1966; Hoos *et al.*, 1971), and as insulin increases about twofold the glucose permeation rate across the phosphatidyl choline bimolecular membrane and the cell membrane, the action of insulin at the bimolecular membrane may resemble the action of insulin at the plasma membrane of the target cell. The smaller glucose fluxes through the bimolecular membrane compared with the cell membrane might be explained if the mechanism of glucose transport in bimolecular membrane and cell membrane is the same, but fewer units of transport are present in the bimolecular membrane. It is also possible that insulin may interact with the phospholipids of cell membranes, doubling the rate of glucose permeation but that the rate-limiting interaction is between insulin and the carrier.

The magnitude of the insulin acceleration of the passage of glucose through the bimolecular membrane is related to the concentration of insulin in the outside aqueous compartment, the glucose P_d increasing with increasing concentrations of insulin. The concentration of insulin necessary to produce an increase of 68% in the rate of glucose permeation through the bimolecular membrane is greater than that used in the adipose cell suspension, whereas the concentration of insulin required to increase the bimolecular membrane P_d by 47% is about the same as that utilized in the red blood cell suspension. As adipose cells show a greater "specific binding" of insulin than red blood cells (Cuatrecasas, 1972; Gavin *et al.*, 1972), reflecting, perhaps, a greater affinity of adipose cell receptor sites for insulin, a lower concentration of insulin with adipose cells may lead to the same increase in the rate of glucose permeation as a higher concentration with red blood cells.

The electrical resistance of the bimolecular membranes in these studies was similar to that measured for many kinds of lipid bimolecular membranes in the literature (Mueller & Rudin, 1969*a*). The mean electrical resistance of the phosphatidyl choline bimolecular membranes used in these studies was 5×10^9 Ω cm^2; the range 1×10^9 to 1×10^{10} Ω cm^2. Membranes whose initial resistance was less than 10^9 Ω cm^2 were eliminated from the studies. The electrical resistance did not change significantly in these studies while glucose was permeating the bimolecular membrane, nor while insulin was interacting with the bimolecular membrane to increase the permeability of the membrane to glucose. This suggests that the process of glucose permeation of the phosphatidyl choline bimolecular membrane, including that accelerated by the presence of insulin, occurs without a net flux of ions through the bilayer. These findings are compatible with those in the literature (Wood *et al.*, 1968; Jung, 1971) in which no change in the electrical resistance of lipid bimolecular membranes occurred during glucose permeation, and none (Hanai, Haydon & Taylor, 1965; Barfort, Arquilla & Vogelhut, 1968) when insulin was added.

Ovalbumin, a protein without hormone action, increases the permeability of the phosphatidyl choline bimolecular membrane to glucose by an amount less than does insulin, even though the concentration of protein in solution is the same. The increase in glucose P_d when insulin is present does not, then, depend solely on the presence of protein but is related to the structure of the insulin molecule. This finding extends previous observations (Kafka & Pak, 1969*a*, *b*, 1972*a*, *b*) that interactions between peptide and protein hormones and their analogues and lipid monolayers depend on the specific structures of the hormones. As with insulin, the presence of ovalbumin resulted in no significant change in the electrical resistance of the bimolecular membrane. These data are compatible with those of Hanai *et al.* (1965), who found no change in the electrical resistance of lipid bimolecular membranes in the presence of ovalbumin, but differ from those of Tsofina, Liberman and Babakov (1966) who observed a decrease in the electrical resistance in the presence of ovalbumin in lipid bimolecular membranes formed by the apposition of two monolayers.

Within the range of glucose concentrations employed in these studies, 2.7 to 14.4 mM, a range roughly commensurate with mammalian circulating blood levels (48 to 259 mg per 100 ml), the P_d for glucose is independent of the glucose concentration in the aqueous compartments. The increase in glucose P_d observed when insulin is present is similarly independent of the glucose concentration over the range 2.7 to 11 mM. Rodbell (1964) observed that glucose utilization in adipose cells was independent of glucose con-

centration over a similar range, and further, that the increased glucose utilization in the presence of insulin was independent of glucose concentration in the medium. In red blood cells, on the other hand, the kinetics of glucose transport does not show first-order behavior (Stein, 1967). Because phloretin does not decrease the P_d for glucose through phosphatidyl choline bimolecular membranes, it is unlikely that the bimolecular membranes contain a saturable carrier mechanism.

In the current studies, the circumference of the bimolecular membrane plus the torus surrounding it is constant. The area of the torus, then, decreases as the area of the bimolecular membrane is increased. As the radioactivity from glucose entering the inside compartment increases linearly with the area of the bimolecular membrane, the data suggest that glucose permeates through the membrane and not through the torus.

The addition of insulin to the inside compartment of the bimolecular membranes does not alter the increased P_d for glucose that occurs when insulin is present only in the outside compartment. These data would be compatible with, although they do not prove, an action of insulin which, occurring on the outside of the membrane where glucose enters, results in an increased P_d for glucose through the membrane, but when occurring on the inside, neither increases the rate at which glucose leaves the bimolecular membrane and enters the inside compartment nor decreases that rate.

Previous studies (Kafka & Pak, 1969*a*, *b*, 1972) have shown that insulin without penetrating, acts at lipid monolayers to decrease the specific resistance of the monolayers to the evaporation of water. The decrease in the specific resistance, in turn, was shown by La Mer, Healy and Aylmore (1964) to depend on a decrease in the free energy of activation of the lipid molecules. Insulin, then, in condensed lipid monolayers, decreases the free energy of activation of the lipid molecules, with the result that water permeates through the monolayers at an increased rate. As the free energy of activation in such monolayers depends largely on attractive forces between the long chains of the lipid (La Mer *et al.*, 1964), insulin may act by decreasing the attractive forces between the long chains of the lipid molecules.

Although studies of lipid monomolecular films cannot be compared directly with those of lipid bimolecular membranes, the action of insulin in increasing the glucose permeation of the phosphatidyl choline bimolecular membrane also may result from an action of insulin which lowers the energy of activation of the lipid molecules of the bilayer. Sweet and Zull (1969) presented evidence that a protein decreased the energy of activation of glucose diffusion from liposomes. Insulin, in a system in which phospholipids accelerated glucose transfer from water into a less polar phase (LeFevre,

Jung & Chaney, 1968), formed a complex with the phospholipid, resulting in the release of glucose into the water (Perry, Tampion & Lucy, 1970).

The phosphatidyl choline bimolecular leaflet may serve as a simplified model for the insulin target cell plasma membrane. An action of insulin at the membrane may be to interact with the phospholipid molecules of the core to increase the passage of glucose (and perhaps water and other water-soluble metabolites) through the membrane.

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References

Barfort, P., Arquilla, E. R., Vogelhut, P. O. 1968. Resistance changes in lipid bilayers: Immunological applications. *Science* **1960**:1119

Crofford, O. B. 1967. Countertransport of 3-O-methyl glucose in incubated rat epididymal adipose tissue. *Amer. J. Physiol.* **212**:217

Cuatrecasas, P. 1969. Interaction of insulin with the cell membrane: The primary action of insulin. *Proc. Nat. Acad. Sci.* **63**:450

Cuatrecasas, P. 1972. Isolation of the insulin receptor of liver and fat-cell membranes. *Proc. Nat. Acad. Sci.* **69**:318

Davson, H., Danielli, J. F. 1943. The Permeability of Natural Membranes. Cambridge University Press, London

Gavin, J. R., III, Roth, J., Jen, P., Freychet, P. 1972. Insulin receptors in human circulating cells and fibroblasts. *Proc. Nat. Acad. Sci.* **69**:747

Hanai, T., Haydon, D. A., Taylor, J. 1965. Polar group orientation and the electrical properties of lecithin bimolecular leaflets. *J. Theoret. Biol.* **9**:278

Henn, F. A., Thompson, T. E. 1969. Synthetic lipid bilayer membranes. *Annu. Rev. Biochem.* **38**:241

Hernandez, A., Sols, A. 1963. Transport and phosphorylation of sugars in adipose tissue. *Biochem. J.* **86**:166

Hoos, R. T., Tarpley, H. L., Regen, D. M. 1971. Sugar transport in beef erythrocytes. *Biochim. Biophys. Acta* **266**:174

Jung, C. Y. 1971. Permeability of bimolecular membranes made from lipid extracts of human red cell ghosts to sugars. *J. Membrane Biol.* **5**:200

Kafka, M. S., Pak, C. Y. C. 1969*a*. Effects of polypeptide and protein hormones on lipid monolayers. I. Effect of insulin and parathyroid hormone on monomolecular films of monooctadecyl phosphate and stearic acid. *J. Gen. Physiol.* **54**:134

Kafka, M. S., Pak, C. Y. C. 1969*b*. The effect of polypeptide hormones on lipid monolayers. II. The effect of insulin analogues, vasopressin, oxytocin, thyrocalcitonin, adrenocorticotropin, and 3′,5′-cyclic AMP on the uptake of Ca^{2+} by monomolecular films of monooctadecyl phosphate. *Biochim. Biophys. Acta* **193**:117

Kafka, M. S., Pak, C. Y. C. 1972*a*. The effect of polypeptide hormones on lipid monolayers. III. The effect of insulin, vasopressin, oxytocin, albumin, and prostaglandin E_1 on the specific resistance to the evaporation of water through monomolecular films of monooctadecyl phosphate, stearic acid, and stearyl alcohol. *J. Colloid Interface Sci.* **41**:148

Kafka, M. S., Pak, C. Y. C. 1972*b*. Peptide hormones at cell membranes: Studies in a model system. *J. Colloid Interface Sci.* **41**:388

Kono, T., Barham, F. W. 1971*a*. Insulin-like effects of trypsin on fat cells. *J. Biol. Chem.* **246**:6204

Kono, T., Barham, F. W. 1971*b*. The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin. *J. Biol. Chem.* **246**:6210

La Mer, V. K., Healy, T. W., Aylmore, L. A. G. 1964. The transport of water through monolayers of long-chain *n*-paraffinic alchols. *J. Colloid Sci.* **19**:643

LeFevre, P. G., Jung, C. Y., Chaney, J. E. 1968. Glucose transfer by red cell membrane phospholipids in $H_2O/CHCl_3/H_2O$ three-layer systems. *Arch. Biochem. Biophys.* **126**:677

Levine, R. 1965. Cell membrane as a primary site of insulin actions. *Fed. Proc.* **24**:1071

Lieb, W. R., Stein, W. D. 1971. New theory for glucose transport across membranes. *Nature, New Biol.* **230**:108

Lossen, O. 1972. A sequential dialysis method for measuring permeability coefficients of phospholipid vesicles. *Biochim. Biophys. Acta* **282**:31

Morgan, H. E., Regen, D. M., Park, C. R. 1964. Identification of a mobile carrier-mediated sugar transport system in muscle. *J. Biol. Chem.* **239**:368

Mueller, P., Rudin, D. O. 1969*a*. Translocators in bimolecular lipid membranes: Their role in dissipative and conservative bioenergy transductions. *In:* Current Topics in Bioenergetics. D. R. Sanadi, editor. Vol. 3, p. 157. Academic Press, New York

Mueller, P., Rudin, D. O. 1969*b*. Bimolecular lipid membranes: Techniques of formation, study of electrical properties, and induction of ionic gating phenomena. *In:* Laboratory Techniques in Membrane Biophysics. H. Passow and R. Stampfli, editors. pp. 141–143. Springer-Verlag, Berlin

Okhi, S. 1970. Effect of local anesthetics on phospholipid bilayers. *Biochim. Biophys. Acta* **219**:18

Papahadjopoulos, D., Nir, S., Okhi, S. 1972. Permeability properties of phospholipid membranes: Effect of cholesterol and temperature. *Biochim. Biophys. Acta* **266**:561

Perry, M. C., Tampion, W., Lucy, J. A. 1970. Interaction of insulin with phospholipid in a membrane model. *Biochem. J.* **119**:50P

Regen, D. M., Morgan, H. E. 1964. Studies of the glucose-transport system in the rabbit erythrocyte. *Biochim. Biophys. Acta* **79**:151

Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**:375

Rodbell, M. 1966. Metabolism of isolated fat cells. II. The similar effects of phospholipase C and of insulin on glucose and amino acid metabolism. *J. Biol. Chem.* **241**:130

Stein, W. D. 1967. Facilitated diffusion—The kinetic analysis. *In:* The Movement of Molecules Across Cell Membranes. p. 134. Academic Press, New York

Sweet, C., Zull, J. E. 1969. Activation of glucose diffusion from egg lecithin liquid crystals by serum albumin. *Biochim. Biophys. Acta* **1973**:94

Trevelyan, W. E., Procter, D. P., Harrison, J. S. 1950. Detection of sugars on paper chromatograms. *Nature* **166**:444

Tsofina, L. M., Liberman, E. A., Babakov, A. V. 1966. Production of bimolecular protein-lipid membranes in aqueous solution. *Nature* **212**:681

Wood, R. E., Wirth, E. P., Jr., Morgan, H. E. 1968. Glucose permeability of lipid bilayer membranes. *Biochim. Biophys. Acta* **1963**:141

Zipper, H., Mawe, R. C. 1972. The exchange and maximal net flux of glucose across the human erythrocyte. I. The effect of insulin, insulin derivatives and small proteins. *Biochim. Biophys. Acta* **282**:311