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Permeability of Bimolecular Membranes Made from Lipid Extracts of Human Red Cell Ghosts to Sugars

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Summary. Spherical lipid bimolecular membranes of a large surface area separating two aqueous solutions were formed from the total lipid extracts of human red cell ghosts and from their individual lipid components. The isotopic permeabilities of these membranes to biologically important sugars and to a related polyol were measured. The observed D-glucose permeabilities of the bimolecular membranes of the total lipid, phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, and cholesterol were 2.35, 2.51, 2.23, 1.35, and 0.62×10^{-10} cm/sec, respectively. These permeabilities are about four to five orders of magnitude lower than that of the intact red cell membrane. The permeabilities of the bimolecular membrane made from an identical extract of the total lipid to different sugars varied: the values for D-glucose, D-mannose, D-ribose, D-fructose, 2-deoxy-D-glucose, 3-0-methyl-D-glucose, and D-mannitol were 2.3, 2.6, 8.9, 0.38, 16.1, 11.2, and 0.44×10^{-10} cm/sec, respectively. The pattern of the difference is neither parallel with nor as extensive as that observed with the intact red cell membrane. The observed permeabilities of the lipid membranes, however, agree qualitatively with what is predicted by an analysis of non-specific movements of nonelectrolytes across the cell membranes. The failure of the membrane lipids to reproduce the carrier function in a structure most closely approximating that of living membranes strongly suggests that some membrane components other than lipids are required for this function.

Movement of sugars across the human red cell membrane represents a typical example of the so-called "carrier"-mediated diffusion (Wilbrandt & Rosenberg, 1961; LeFevre, 1961). In understanding the molecular mechanisms of such a carrier mediation, it would be most desirable to isolate the specific membrane components which are responsible for the mediation, and from them to reconstitute the transport function *in vitro*. Because of its relative simplicity (non-electrogenic, non-metabolically coupled, and non-accumulating), the sugar transport system of human red cells appears to offer a particularly good opportunity for such an approach. Previously we have particularly examined lipid components of the cell

membrane for their possible reconstitution of the carrier function (Jung, Chaney & LeFevre, 1968; LeFevre, Jung & Chaney, 1968): the sugar movements across the lipid monolayers of a water-chloroform bilayer and of a water-chloroform-water trilayer were characterized. In the presence of the cell membrane phospholipids, but not the neutral lipids such as cholesterol, a marked enhancement of the sugar movements was observed. However, the systems failed to reproduce the main characteristics of the carrier mediation such as the high flux rate and the stereospecificity. It was not known, though, whether the observed failure was due to the fact that the hypothetical sugar carrier was not included in the lipid extracts examined, or that its characteristics were obscured by the model systems used. Seeking an answer to this question, we adopted in the present study a lipid bimolecular structure as a model system. This has been shown to approximate most closely biological membranes in many physical parameters, particularly those owing to the lipid structure (Tien & Diana, 1968). Spherical thin lipid membranes of a large surface area were formed from various lipid extracts of the red cell membrane, and permeabilities of these structures to various sugars were measured. It was shown that, when used alone, the lipid components in the structure failed to mimic the sugar-transport carrier function of living membranes. The results were further analyzed as molecular diffusion through a lipid lattice structure of the membranes. Preliminary reports of some of the sugar permeabilities of a bimolecular membrane made from an extract of the total crude lipids of human red cell membranes have been previously presented (Jung & Snell, 1968). In the present studies, the lipids components used were purified much more carefully in order to exclude any effect of protein contaminants.

Materials and Methods

Isolation and Purification of Lipids

Human blood bank blood, expired but not more than 25 days old, in the standard ACD units was used. The red cells were separated and washed by repeated centrifugation with isotonic saline. Ghosts were prepared from the washed packed cells by hemolysis at 4 °C with 20 volumes of acidified distilled water. Total lipids from the ghosts were extracted with a chloroform-methanol 2:1 mixture, and washed with water (20% v/v) in a bilayer system. They were further fractionated by passage through a silicic acid column (Ways & Hanahan, 1964). Chromatographically pure cholesterol, phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin were obtained by purifying the proper column fractions on a thin-layer chromatographic plate as a single streak. All of these procedures have been detailed elsewhere (Jung *et al.*, 1968). The lipids were analyzed for their total phosphorus by the method of Bartlett (1959), for total nitrogen according to Lang (1958), and for cholesterol by the ferric chloride method (Zlatkis,

Zak & Boyle, 1953). All of the silicic acid column fractions were recombined to get a mixture of the total protein-free lipids. The molar ratios of the mixture for cholesterol and total-N to lipid-P were 0.91 and 1.32, respectively. The total-N/lipid-P ratio before the column passage was 1.51.

Formation of Spherical Lipid Membranes

An experimental assembly used for formation of the lipid membranes and measurements of flux is illustrated in Fig. 1. A micrometer syringe with a fine glass tubing attached was filled with an aqueous solution (inside bathing solution). About 5 μliters of the lipid extract in an appropriate solvent (membrane-forming solution) was applied at the tip of the tubing, and immersed into an aqueous solution (outside bathing solution) in a glass container. The aqueous solution inside the syringe was gently advanced by using a thumb-screw-operated shaft which advances the plunger of the syringe via the stem of a direct-reading dial-micrometer gauge. A spherical lipid membrane was thus formed surrounding the inside bathing solution and suspended in the outside bathing solution, which was supported by the glass tubing. The solvents for the membrane-forming solutions used were a chloroform-methanol 2:1 mixture containing 10 to 20% (w/v) of -tocopherol for the total lipid and each phospholipid tested, and *n*-dodecane (Eastman Organic Chemicals, Rochester, N.Y.) containing 20% of di-n-octadecyl phosphite (Aldrich Chemical Co. Inc., Milwaukee, Wisc.) for cholesterol. The membrane-forming solutions contained 1 to 4% of the lipids. The bathing solutions were a balanced salt solution (Na^+, 125 mM; K^+, 5 mM; Ca^{++}, 3.75 mM; Mg^{++}, 2.5 mM, all as chloride salts) of pH 7.4 buffered with Tris-HCl (1 mM), containing the permeant tested at a fixed concentration.

Flux Measurements

The unidirectional flux of sugars across the lipid membrane preparation was measured by a tracer technique using ^{14}C-tagged permeants. The tracer at a specific activity of 25 to 50 μc/ml was added only to the inside bathing medium before membrane formation.

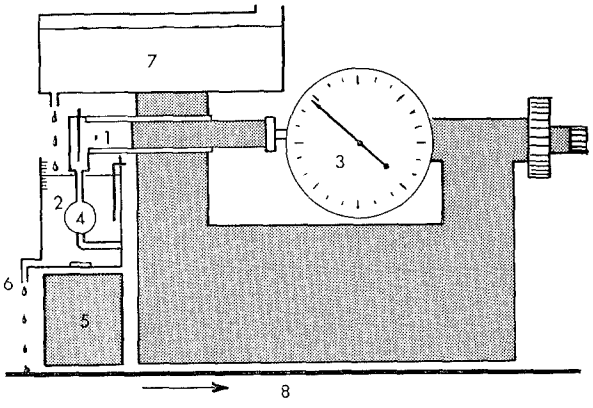

Fig. 1. Experimental assembly for the spherical membrane-formation and flux measurements. *1* A micrometer syringe with a fine glass tip; *2* outside bathing solution in a glass container; *3* micrometer gauge; *4* spherical membrane and inside bathing solution; *5* magnetic stirrer; *6* an outlet; *7* reservoir for outside bathing solution; *8* continuous sample-collector

The chemical concentration of the permeants, however, was identical (5 mM) in the inside and outside bathing solutions. The outside bathing solution was kept well stirred by a magnetic stirrer at a constant temperature of 25 °C (±0.5 °C). The outside bathing solution was continuously sampled through an outlet at a prefixed rate, while the radioactivity-free bathing solution was continuously infused from a reservoir to maintain the bathing volume constant. Usually 18 to 24 samples of 5-min aliquots were collected, and the radioactivities were counted in a Nuclear-Chicago automatic liquid scintillation counter using 10 ml of Bray's solution (Bray, 1960). The data were converted into cumulative quantities of fractional loss from inside to outside at the end of each sampling (N_i) according to the equation:

$$N_i = \left(b\, n_i + c \sum^{i-1} n_i\right)/a\, m_0 \tag{1}$$

where m and n are the activities (counts/min/ml) of the inside and the outside bathing solution, respectively; subscripts 0 and i denote the initial and ith samplings, respectively; and a, b and c are the volumes (ml) of the inside bathing solution, the outside bathing solution, and each sample, respectively (a was varied in a range of 0.03 to 0.12 ml in order to produce membranes of different surface areas). All of these values, however, were fixed constant within an experiment, and so adjusted that the value of N_i at the end of the experiment and the ratio of a/b were less than 10^{-4} and 3×10^{-2} respectively.

Other Characterization of the Membrane Preparation

The thinning process and eventual blackening of the membrane were followed by observing the interference color generated by illumination with a white light using a wide-field stereoscope at 40× magnification. The DC conductance of the membrane was continuously recorded throughout the flux measurement with a DC Amplifier and Electrometer (General Radio, Type 1230-A). A precisely matched pair of Ag–AgCl electrodes was used, and usually 100 mV of potential was applied across the electrodes. Resistance was calculated from the potential drop across the membrane. The membrane capacitance was measured using a Universal Impedance Bridge (Model 710B, John Fluke Mfg. Co., Inc., Seattle, Wash.). The continuous conductance recording also provided a sensitive means of detecting any damage in membrane integrity. The membrane preparations assumed in all cases virtually a perfect sphere with a small upper spherical segment missing because of the supporting tip, when the proper amounts of the membrane-former solution were used. Accordingly, the volume (V) of the free segment of the sphere (whose radius was r) under the supporting tip (whose radius was q), and the corresponding free surface area (S) of the free spherical segment were calculated according to the equation:

$$V = \tfrac{1}{3}\pi\left[(r^2 - q^2)^{\frac{1}{2}}(q^2 + 2r^2) + 2r^3\right], \qquad S = 2\pi r\left[r + (r^2 - q^2)^{\frac{1}{2}}\right].$$

They were then correlated graphically. From the volume of the inside bathing solution of the membrane which was read on the dial micrometer gauge with a proper calibration, the free surface area of the membrane was read from the graph.

Paper Chromatography and its Radioactive Scanning of Samples

A portion of the last sample of the flux measurements was mixed with a relatively large amount of the non-radioactive permeant being tested, then chromatographed on a Whatman No. 4 paper strip using an isopropanol-water 4:1 solvent system (Smith, 1960). The sugar spot was localized by aniline reagent. The radioactivities were counted in a Packard Paper Chromatographic Radioactive Strip Scanner (Model 6200).

Chemicals

D-glucose-^{14}C(UL), D-fructose-^{14}C(UL), D-mannitol-1-^{14}C, D-ribose-1-^{14}C, and 2-deoxy-D-glucose-1-^{14}C were obtained from Tracerlab Technical Products (Waltham, Mass.). Purity of these radiochemicals was routinely checked on appropriate paper chromatographies, and verified to be better than 99%.

Calculation of Permeability Constants from the Flux Data

The conditions employed in the flux measurements as detailed above justify analyzing the flux data simply as a unidirectional efflux (also *see* the Results section). Accordingly,

$$\frac{dm}{dt} = -P\frac{A}{a}m \tag{2}$$

where t, P, and A are time, permeability constant and free surface area of the membrane, respectively, with other denotations being those of Eq. (1). Upon integration, Eq. (2) gives:

$$\log_e \frac{m}{m_0} = -P\frac{A}{a}t. \tag{3}$$

The value of m/m_0 at the ith sampling is $(1-N_i)$. Substituting this equality and noting the fact that $N_i < 10^{-4}$, Eq. (3) is approximated, without introducing any significant (less than 0.01%) error, to:

$$N_i = P\frac{A}{a}t. \tag{4}$$

The value of the permeability constant P was calculated from the slope of the curve relating N_i to time (Fig. 2) according to Eq. (4).

Results

General Characteristics of the Lipid Membranes

By the procedure detailed in the previous section, *in vitro* spherical thin lipid membranes were made from the total lipid extracts, as well as from each of the major lipid constituents – phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, and cholesterol – of human red cell membranes. All of these membrane preparations underwent a thinning process lasting about 10 to 20 min as revealed by interference colors typical of white light reflected from a thin film, followed by a rapid blackening in spots at first and then ultimately of the entire free surface. The preparations withstood gentle stirring and were stable for 1 to 2 hr. The DC resistance of these preparations was initially relatively low, then increased gradually with time reaching a stable value of 0.1 to 1.5×10^8 $\Omega - \text{cm}^2$ at about 20 min. The capacitance of the membranes at the completion of the blackening

was measured to be 0.3 to 0.9 $\mu F/cm^2$. Both the conductance and the capacitance were increased linearly as the membrane area was increased. All of these findings agree closely with those reported by others for other bimolecular lipid membranes (Tien & Diana, 1968).

The Time Course of the Isotopic Sugar Flux

Fig. 2 represents a typical result of the flux measurements, where a fractional loss (N_i) of ^{14}C-D-glucose from inside to outside of a lipid membrane was plotted against time [*see* Eq. (4)]. As evident in this figure, the rate of efflux (that is, the slope of the curve) was in general initially somewhat greater, then gradually slowed down to the final steady rate at about 20 min. Since the total depletion of the ^{14}C-sugar in the inside bathing solution owing to the translocation at the end of the 90 min experiment did not exceed 10^{-4} of that originally added, and since the volume ratio of outside to inside bathing solutions was around 10^2, the tracer concentration difference across the membrane was practically constant and the size of influx of the tracer was in no case greater than 10^{-6} of that of efflux throughout the experiment. Consequently, the steady-state rate obtained from the linear portion of the curve would represent a unidirectional efflux. From the slope and the volume and surface area of the membrane,

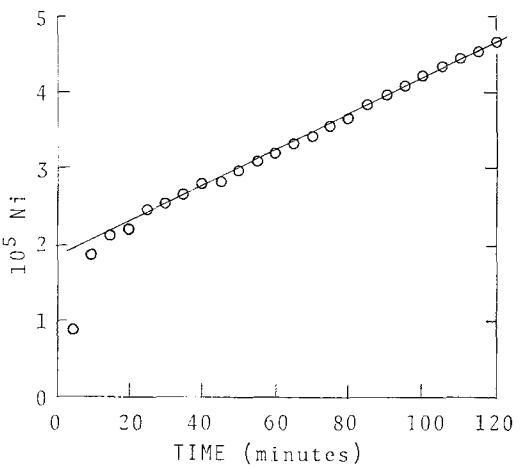

Fig. 2. Fraction (N_i) of ^{14}C-D-glucose translocated across a lipid bimolecular membrane as a function of time. The membrane was made from a protein-free total lipid mixture of human red cell ghosts. The volume and calculated free surface area of the membrane were 0.040 ml and 0.56 cm^2, respectively. The volume of the outside bathing solution was 4.0 ml. The solid line represents the final steady slope of 3.85×10^{-9} sec^{-1}. Time zero indicates the time of completion of the sphere formation. Computation of N_i is shown in Eq. (1)

a permeability constant of 2.75×10^{-10} cm/sec was obtained by Eq. (4). A membrane conductance recording was also included in these flux experiments; the electrical conductance decreased in parallel with the decrease in the sugar permeability. The time courses of these decreases corresponded roughly with the thinning process of the membrane. The significance of these correlations is not clear at this time.

Validity of the Membrane Permeability Measurement

Fig. 3 provides experimental evidence that the observed translocation of the radioactivity in the flux experiment is totally accounted for by that of unaltered D-glucose, the permeant studied. Sample of the flux sample was chromatographed after mixing with proper amounts of non-radioactive glucose. It is evident that a single radioactive peak precisely superimposes on the chemical spot of the sugar. Wood, Worth and Morgan (1968), in studying the permeability of lipid bimolecular membranes to glucose using a planar membrane preparation and tracer methods, noted that nearly all ($>98\%$) of the radioactivity which penetrated the lipid membrane was found to be not glucose but some unknown chemical entity. The present study did not suffer from such a problem of radioisotopic impurity.

It has often been questioned if the permeability measured for such a system indeed represents that of the membrane itself (Cass & Finkelstein, 1967). The problem of a so-called unstirred water layer would be readily dismissed in the present study since a complete omission of stirring from the outside bathing solution did not significantly reduce the flux. The diffusion constant of D-glucose in the balanced salt solution used in the flux

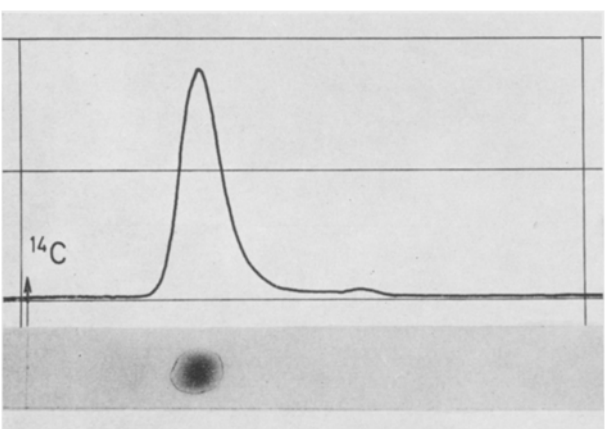

Fig. 3. Comparison of paper chromatography and its radioactive scanning of translocated ^{14}C-tagged D-glucose during the flux measurement

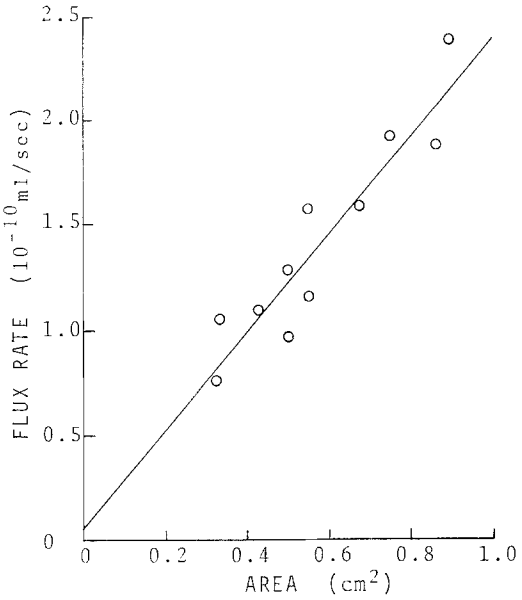

Fig. 4. Flux rate of ^{14}C-D-glucose across a lipid bimolecular membrane as a function of membrane area. Eleven membranes of various sizes were made from three different batches of protein-free total lipid mixture of human red cell ghosts. The flux rate, as ml cleared per sec, was obtained by multiplying the final slope of N_i vs. time such as shown in Fig. 2, by the volume (a) of the spherical membrane, for each measurement. Calculation of free area of the spherical membranes is shown in Materials & Methods. The solid line represents the least-squares solution; $y = 0.041 + 2.336\,x$

experiments was found to be 2.3×10^{-6} cm^2/sec at 24 °C. Using this value and an expression of diffusional resistances in series (Cass & Finkelstein, 1967), it can be easily shown that, to suffer an error of 1% in flux rate of this rate, more than 10 cm of an unstirred water layer should be assumed. Since the spherical membranes used in this study were only a few tenths of 1 cm in radius, the contribution of the unstirred water layer to the overall resistance would not be more than 1%, and probably much smaller.

The contribution of the portion of the flux through the torus around the supporting tip to the overall flux was shown to be insignificant in a series of experiments in which different sizes of spherical membranes were used. As evident in Fig. 4, the flux rate is a linear function of the free surface area of the membrane, and extrapolation to the vertical axis indicates a relatively small flux, (0.04 ml/sec was obtained by the least-squares solution) at zero surface area. It should be mentioned at this point that the torus of a bulk lipid layer could be progressively minimized to a negligible amount without apparently affecting the membrane stability.

The Permeability of Different Lipid Membranes to D-*Glucose*

The permeability constants to D-glucose of various lipid membranes are summarized in the Table. It is interesting to note that the observed permeability to D-glucose of the membrane made from the protein-free total lipids of the ghosts in the present study, 2.3×10^{-10} cm/sec, is some 100-fold lower than that of the membrane made from the crude total lipids of the ghosts (Jung & Snell, 1968). Although this difference may be due to the protein contaminant, the exact nature is yet to be determined. On the other hand, the glucose permeability in the present study is slightly higher than the maximum value, 10^{-10} cm/sec of Wood *et al.* (1968). The differences between the glucose permeabilities of the membranes of phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin and that of the total lipid are insignificant (P values were less than 0.8, 0.9, and 0.1, respectively). The permeability of the membranes made from cholesterol, however, is significantly lower ($P<0.01$).

Table. *Permeability constants of different lipid bimolecular membranes to different sugars and a related polyol*

Membrane lipids	Permeants	Permeability constants[a] ($10^{-10} \times$ cm/sec)
Phosphatidyl choline	D-glucose	2.51 ± 1.22 (8)
Phosphatidyl ethanolamine	D-glucose	2.23 ± 0.32 (6)
Sphingomyelin	D-glucose	1.35 ± 0.34 (6)
Cholesterol	D-glucose	0.62 ± 0.21 (6)
Total lipids	D-glucose	2.35 ± 1.31 (24)
Total lipids	2-deoxy-D-glucose	16.1 ± 5.23 (6)
Total lipids	3-0-methyl-D-glucose	11.2 ± 3.46 (7)
Total lipids	D-mannose	2.63 ± 1.56 (6)
Total lipids	D-ribose	8.94 ± 3.95 (4)
Total lipids	D-fructose	0.38 ± 0.15 (5)
Total lipids	D-mannitol	0.44 ± 0.13 (5)

[a] Mean ± SEM (no. of exp.).

The Permeabilities of the Total Lipid Membrane to Different Sugars

The bimolecular membrane made from an identical extract of the total lipids of the ghosts had a different permeability to different sugars and a related polyol, as shown in the lower part of the Table. D-ribose, an aldopentose, was transferred four times as fast as D-glucose, an aldohexose, whereas D-fructose, a ketohexose, was transferred six times more slowly

than D-glucose. D-mannose, an aldohexose and an epimer of D-glucose, was transferred as fast as D-glucose, whereas D-mannitol, a polyol closely related to the sugars, was transferred five times more slowly than glucose. Another interesting comparison is for D-glucose and its derivatives: the 2-deoxy and 3-0-methyl sugars were transferred 13 and 8 times, respectively, as fast as D-glucose.

Discussion

A serious effort has been directed in a number of laboratories toward isolation and identification of the membrane components responsible for the carrier-mediated transport of various cell membranes (Fox & Kennedy, 1965; Pardee, 1966; Kaback, 1968). Such an effort inevitably involves in principle at least two major difficult experimental steps: an isolation of the components intact, and a proper identification of the materials as the transport carrier. It appears that the more serious problem is the identification of the carrier. In principle, this would be done most positively by studying a possible reconstitution of the carrier function in a well-defined *in vitro* membrane structure relevant to living membranes. The recent availability of bimolecular lipid membranes *in vitro* (Mueller, Rudin, Tien & Wescott, 1962) has provided a definite advance in such a reconstitution. Results of extensive studies have shown that the lipid bimolecular membrane preparation closely approximates living membranes in many physical properties such as thickness, electrical capacitance, refractive index and interfacial tension (Tien & Diana, 1968). On addition of some macromolecules, these membranes also mimic some aspects of the more complicated functions of living membranes such as excitability (Mueller & Rudin, 1963), selective ion transport (Andreoli, 1967), and active transport of sodium (Jain, Strickholm & Cordes, 1969). All of these observations support the notion (Davson & Danielli, 1952) that the lipid bimolecular structure indeed represents at least some portion of the molecular structure of the lipid components of living membranes. It seems clear then that this structure provides the best available system for the *in vitro* identification of the carrier molecules, in particular when the lipid components are to be examined.

The planar bimolecular membrane originally described by Mueller *et al.* (1962) has been extensively used for the study of ionic permeability. The use of such a preparation for studies on nonelectrolyte permeability, particularly those of biologically important molecules such as sugars and amino acids, has been limited by technical difficulties. The major problem is its very small membrane area which allows passage of insufficient amounts of these slowly moving solutes to be accurately measured. Even with the

sensitive fluorescence technique used by Bean, Shepherd, and Chen (1968), the preparation permitted a flux analysis only for those molecules with permeabilities in the range of 10^{-6} cm/sec or higher. The spherical bimolecular membrane used in the present study with free membrane area 30 times as large as that of the planar membrane, using a tracer technique, provides a means for a precise and direct analysis of permeability to many biologically important neutral molecules having permeabilities of 10^{-7} to 10^{-11} cm/sec. Although measurement of the thickness of the lipid membrane was not attempted in this study, its observed optical properties (thinning with ultimate blackening) and electrical properties (resistance and capacitance) leave little doubt that it is a true bimolecular structure (Tien & Diana, 1968). In this respect the preparation was very similar to the spherical lipid bilayer membrane reported by Pagano and Thompson (1967), and is essentially identical to the one reported by Tien (1967).

On intact human red cell membranes, the permeability[1], at the infinitesimal concentration, to D-glucose of 10^{-5} to 4×10^{-4} cm/sec has been estimated on the basis of kinetic analysis of the transport affinity (K_m) and the maximum rate (V_{max}) (LeFevre & McGinniss, 1960; Miller, 1968; Jung, 1971). This high permeability was also shown to be drastically lowered to a level of practical impermeability in the presence of $HgCl_2$ (Britton, 1964). In our recent studies (Jung, 1971), we showed that a purified human red cell membrane, prepared under carefully controlled conditions, retains the glucose carrier function intact, with a high permeability to D-glucose of 2×10^{-5} cm/sec (at 24 °C), which decreased to about 10^{-8} cm/sec in the presence of $HgCl_2$. Moreover, under a certain condition of purification, a carrier-inactive membrane was obtained whose permeability to glucose was as low as 10^{-8} cm/sec. Comparing these results with the glucose permeability of the lipid bimolecular membrane of the present study, we see that the glucose permeability of the lipid membrane is some 10^4-fold lower than that of the carrier-active intact cell or purified membrane, but roughly comparable to that of the carrier-inhibited or carrier-inactive preparations.

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1 For sugars which are transported by a saturable path, a true permeability P cannot be calculated. Permeability calculated at an infinitesimal concentration of the sugars, where no saturation is effective, however, would provide a unique measure of relative permeation by different permeants. This permeability at an infinitesimal concentration of the permeant is obtainable by taking the first derivative of the classical Michaelis-Menten expression of the carrier-mediated flux (Wilbrandt & Rosenberg, 1961) with respect to the permeant concentration, at the condition of zero permeant concentration, (V_{max}/K_m), then dividing by the total surface area of the cells. The values of 1.55×10^{-6} cm^2 and 8.7×10^{-11} cm^3 were adopted for the surface area and the volume, respectively, of a single human red cell (Whittam, 1964).

It is well known that living human red cell membranes transfer various sugars with a distinct stereospecificity (LeFevre & Marshall, 1958) which parallels relative stability of the Cl conformation of Reeves (1950). The relative affinity to the carrier-mediation ($1/K_m$) for 2-deoxy-D-glucose: D-glucose: D-mannose: D-ribose: D-fructose: D-mannitol are roughly 10,000:5,000:1,500:100:10:1 (LeFevre & Marshall, 1958). Since the maximal flux rate (V_{max}) for each of these sugars is known to be practically identical (LeFevre, 1962), the relative rate, in terms of permeability at the infinitesimal concentration (*see* footnote 1), would be identical to the relative affinity quoted above. Practically identical relative rate sequences were observed with the carrier-active preparation of purified human red cell membrane (Jung, 1971). The sequence of relative permeabilities of the lipid membrane studied here as calculated from the Table, to the same group of sugars – 2-deoxy-D-glucose: D-ribose: D-mannose: D-glucose: D-mannitol: D-fructose – is roughly 40:22:7:6:1:1. Comparing these two sequences, the following two points can be made. (1) The specificity sequence of the lipid membrane is not identical to that of the living membrane, and is not in any way correlated with the conformation of sugars. (2) The living membrane can discriminate these sugars by up to 10,000-fold, whereas the lipid membrane can do so only up to 40-fold. It should be noted at this point that the great discrimination seen with the living membrane results entirely from selective enhancement of the transfer of specific sugars, rather than any retardation of the others.

In a systematic analysis of the observed permeability data of biological membranes to various water-soluble organic molecules, Stein (1967) has advanced an empirical theory which correlates the non-specific permeability of living membrane with the molecular parameters of the penetrants. This theory predicts that the rate of non-specific diffusion of a neutral molecule across biological membranes would: (1) decrease linearly with an increase in the 1/2 power of its molecular weight (for those with a molecular weight less than 1000); (2) increase some two-fold for each bare – CH_2 – residue of the molecule; and (3) decrease 6- to 10-fold for each hydrogen-bond-forming group in the molecule. The data of the Table were tested against this theoretical prediction in Fig. 5. It is apparent in this comparison that the present data of the lipid membrane are in fair agreement with the theoretical correlation. This agreement would indicate that the molecular mechanism of the sugar permeation of the lipid membrane may represent that of the non-specific sugar permeation of the living cell membranes. Some deviations of the present data from the prediction are also apparent in the figure. The permeabilities are somehow higher than what

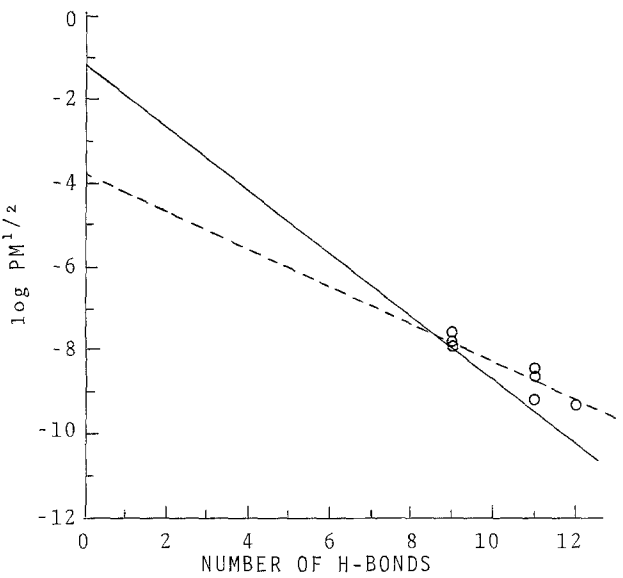

Fig. 5. Permeability of a bimolecular membrane to different sugars, as a function of the number of hydrogen-bond formation. The membrane was made from a protein-free total lipid mixture of human red cell ghosts. The number of hydrogen-bond formations of each permeant was calculated according to the Table 3.2 of Stein (1967). The ordinate is the common log of a value which is the permeability constant multiplied by the 1/2 power of the molecular weight. The effect of apolar chain length was not corrected in this plot. The solid line, log $PM^{1/2} = -1.15 - 0.75\,N$, is the correlation based on the permeability data of the human red cell membrane to 28 different nonelectrolytes as appeared in Fig. 3.14 of Stein (1967). The dotted line was drawn to fit best to the present data by least-squares method, and represents log $PM^{1/2} = -3.75 - 0.45\,N$

the theory predicts. Furthermore, the dependency on the number of hydrogen-bond formations is significantly less than the prediction. The significance of these deviations is not clear at this time.

Lipid components of biological membranes have often been examined for their possible key role in biological transport carrier functions (Jung *et al.*, 1968). The present study has shown that the membrane lipids of human red cells in a reconstituted artificial membrane, which closely approximates living membranes in many aspects, failed to mimic the two main features of the sugar transport carrier function, i.e., the rapidity and the stereo-specificity. This failure strongly supports the notion that some membrane proteins in addition to membrane lipids are required for function, a tentative conclusion drawn from our previous studies with water-chloroform and water-chloroform-water systems (Jung *et al.*, 1968; LeFevre *et al.*, 1968). This conclusion is also in accord with that of Wood *et al.* (1968) in their lipid bilayer study. Indeed, a number of membrane proteins have been

isolated with a claim that they are responsible for certain membrane transport systems (Fox & Kennedy, 1965; Pardee, 1966; Kaback, 1968). In a preliminary study (Jung & Snell, 1968), we have seen that the spherical lipid membranes made from crude total lipid extracts of human red cell membranes are about 100 times more permeable to glucose than are the lipid membranes made from more purified lipids studied here. This enhanced permeability could be due to the protein contents of the crude lipid preparation. In the same study we have also seen that the spherical lipid membranes are capable of incorporating some protein molecules with reasonable stability. Thus, it appears that the preparation would provide a valuable experimental system in which various protein components of living membranes can be studied for their effect on solute movements across lipid barriers.

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