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SELECTIVE DIFFUSION OF NEUTRAL AMINO ACIDS ACROSS LIPID BILAYERS

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SUMMARY

1. Liposomes were formed from mixtures of cholesterol, phosphatidyl serine, and lecithins with differing degrees of acyl chain unsaturation.

2. The efflux of trapped amino acid from such liposomes was determined. The order of permeability was phenylalanine > methionine > leucine > isoleucine, norleucine > alanine, glycine > histidine.

3. The rate of amino acid efflux depended on the type of lecithin used. Amino acids diffused more quickly from liposomes formed from highly unsaturated lecithins. The increase was greatest for the smaller, hydrophilic amino acids.

4. There was a correlation between the water–*n*-octanol partition coefficients and the rates of efflux for all amino acids tested except histidine. The obvious features distinguishing histidine from the other amino acids are the positive charge carried on the imidazole ring, and the possibility of an extra hydrogen bond.

5. The lecithin-dependent change in the selectivity shown between glycine and phenylalanine is suggested as being brought about by entropic effects in the hydrocarbon core of the phospholipid bilayer.

INTRODUCTION

Membrane lipids may influence the passive permeability of cells to non-electrolytes. Experiments performed on red blood cells from different species have shown that a decreased haemolysis time, measured in isotonic solutions of non-electrolytes, may be correlated with an increased lecithin content[1,2]. The amount of lecithin, which contains a large proportion of unsaturated fatty acyl groups, is inversely correlated with that of sphingomyelin which contains only saturated acyl groups[3]; the permeability changes have been attributed to the changes in membrane unsaturated fatty acid content[4,5]. This correlation does not hold for all species, and some authors have questioned its significance[6]. Better evidence for a relation is provided by experiments which have changed the red cell fatty acid composition by altering the diet[7]. In an artificial membrane system a correlation has been firmly established[8]. More recently the permeability properties of the cell membrane, and of a model system derived from the total membrane lipids, have been compared directly[9]; the results suggest that changes in lipid composition produce comparable effects both in a biological and a model system.

Changes in membrane lipid unsaturation without noticeable change in the recovery of any of the phospholipid classes are observed when poikilotherms adapt to different temperatures. When such changes take place in the goldfish the intestinal transport of amino acids also changes, suggesting that the two events might be related[10].

A model membrane system designed to resemble the lipid component of cell membranes in both composition and charge[11] has been used in the present work to determine the permeabilities of different amino acids and to test whether these permeabilities are dependent on the type of lecithin used to form the bilayer. A preliminary account of some of this work has already been published[12].

METHODS

Large scale preparation of unsaturated lecithins

Highly unsaturated lecithins were separated from lipid extracts of the egg yolks of hens fed on a diet containing 10 % fish oil, by chromatography on a column of silicic acid. Details of this preparation have been described previously[13].

Preparation and use of liposomes

Liposomes were prepared from a mixture of lecithin, cholesterol and phosphatidyl serine. Chloroform solutions of lecithin (7.5 μmoles), cholesterol (10 μmoles) and the monopotassium salt of one of the two preparations of phosphatidyl serine shown in Table I (2.5 μmoles), made in this laboratory by N. MILLER[14], were mixed and dried *in vacuo* on a rotary evaporator. 1 ml of an aqueous solution containing 90 μmoles KCl, 5 μmoles potassium cacodylate and 5 μmoles of two different amino acids (pH 7.0) was added. One of these amino acids was labelled with 20 μC ^{3}H and the other with 2 μC of ^{14}C. The tube was flushed with N_2, stoppered and shaken mechanically until all the lipid adhering to the bottom had been dispersed. The preparation was left to equilibrate overnight at room temperature; under these conditions no change in the incorporation of amino acids occurs after 6 h. When determining the pH dependence of histidine permeability, [^{3}H]galactose was used as a measure of permeability to a non-electrolyte, and in these cases the pH was adjusted to the appropriate value with NaOH or HCl.

The liposomes were separated from free amino acids by gel filtration through 100 ml of G-50 coarse Sephadex equilibrated with a solution of 100 mM KCl and 5 mM potassium cacodylate, adjusted to the appropriate pH. Collection of effluent was

TABLE I

FATTY ACID COMPOSITION OF TWO SAMPLES OF PHOSPHATIDYL SERINE USED IN THE PREPARATION OF LIPOSOMES

Only fatty acids present in amounts greater than 1 % of the total fatty acids have been tabulated.

Phosphatidyl	*Fatty acid composition (mole %)*					
serine	$C_{18:0}$	$C_{18:1}$	$C_{18:4}$	$C_{22:4}$	$C_{22:5}$	$C_{22:6}$
Sample 1	39.5	31.1	3.6	2.5	5.0	13.6
Sample 2	41.6	37.1	4.3	2.1	3.5	7.0

started at the first signs of turbidity and finished when a further 12 ml had been eluted. The suspension was mixed and 2.9-ml aliquots transferred to dialysis bags of Visking 21/32 inch tubing. These were washed briefly, then transferred to flasks containing 10 ml of 100 mM KCl *plus* 5 mM potassium cacodylate at pH 7.0. The flasks were flushed with N_2, stoppered and shaken in a water bath at 30°. Timing began as the dialysis bag entered the incubation medium. In no case did more than 10 min elapse between the separation of liposomes from free amino acids and the beginning of incubation. Samples of the medium (0.5 ml) were taken at specified time intervals and put into vials containing 10 ml scintillation fluid[15]: 50-μl samples of the gel-filtered suspension and 10-μl samples of the radioactive suspending medium were treated similarly. ^{14}C and ^{3}H were assayed simultaneously in a Packard scintillation spectrometer.

Amino acid permeability of dialysis tubing

2.9-ml samples of an aqueous solution containing 95 μmoles/ml KCl, 5 μmoles/ml potassium cacodylate (pH 7.0) and 1 μmole/ml of each of the amino acids L-phenylalanine, L-methionine, L-alanine, glycine and L-histidine, were placed in dialysis bags of Visking 21/32 inch tubing. The bags were transferred to flasks containing 10 ml of a solution containing 100 μmoles/ml KCl, 5 μmoles/ml potassium cacodylate (pH 7.0) maintained at 30° in a shaking water bath. Samples of the incubation medium were taken over 20 min and assayed for amino acids in an automatic analyser (Locarte Co. Ltd., London).

Partition of amino acids between n-octanol and water

Equal volumes of *n*-octanol and a solution containing 90 μmoles/ml KCl, 5 μmoles/ml potassium cacodylate (pH 7.0) unless stated otherwise, and 10 μmoles/ml amino acid labelled with either 2 μC ^{14}C or 2 μC ^{3}H were shaken for 16 h at 30°, by which time equilibrium had been attained. The phases were allowed to separate for 30 min and samples of aqueous and organic phases taken for radioassay.

Analysis of fatty acyl chains

Lecithin β acyl chains were analysed by a method similar to that previously published[16]. Lecithin (10–20 mg) was dissolved in 5 ml diethyl ether and a solution of 2–5 mg of *Naja naja* phospholipase A in 5 ml ether added. 0.05 ml 5 mM $CaCl_2$ solution (pH 7.6) was added and the mixture left for 6 h at room temperature, then overnight at 4°.

The fatty acids free in ether solution were converted to their methyl esters by reaction with an ether solution of diazomethane freshly generated from Diazald. Methyl esters were separated in a stream of N_2 on a column of polyethylene glycol adipate at 197° in a Pye series 104 gas chromatograph fitted with a flame ionization detector. Components were identified by their retention times and by comparison with standards[17]. The amount of each was calculated from peak height and retention time using external standards[18]. All compositions are expressed as moles percent.

Reagents

Diazald was obtained from the Aldrich Chemical Co. Inc., Milwaukee, Wisc. 53233. *Naja naja* venom came from L. Light and Co., Colnbrook, Bucks. Cholesterol was the chromatographic standard grade supplied by the Sigma Chemical Co. St.

Louis, Miss. 63118. DL-[^{14}C]Norleucine was obtained from New England Nuclear, Boston, Mass. 02118 and DL-[^{14}C]isoleucine came from Schwartz BioResearch, Orangeburg, N.Y. 10962; both were a gift from Dr E. D. Korn. All other radioactive amino acids and galactose were purchased from The Radiochemical Centre, Amersham, Bucks. Non-radioactive amino acids and *n*-octanol, specially pure for chromatography, were obtained from British Drug Houses Ltd., Poole, Dorset. All reagents were of A.R. grade where available; water was twice distilled in glass apparatus the final distillation being from alkaline permanganate solution.

RESULTS

Separation of lecithins according to degree of unsaturation

The elution pattern of lecithins containing different proportions of unsaturated fatty acids from a column of silicic acid is shown in Fig. 1. The effluent was divided into four fractions A, B, C and D; the results of positional fatty acid analyses on these fractions are shown in Table II. The more unsaturated lecithins were eluted first. The percent of $C\beta_{22:6}$ fatty acyl chains varied from 80 % in Fraction A to 18 % in Fraction D. Before using these lecithins, all samples were checked for oxidation by ultraviolet spectroscopy; those giving a ratio $A_{233\,nm}/A_{217\,nm} > 0.07$, which corresponded to about 0.1 % oxidation[19], were rejected. Unoxidised fractions were taken either alone or mixed and used to make up solutions of lecithins of varying degree of unsaturation.

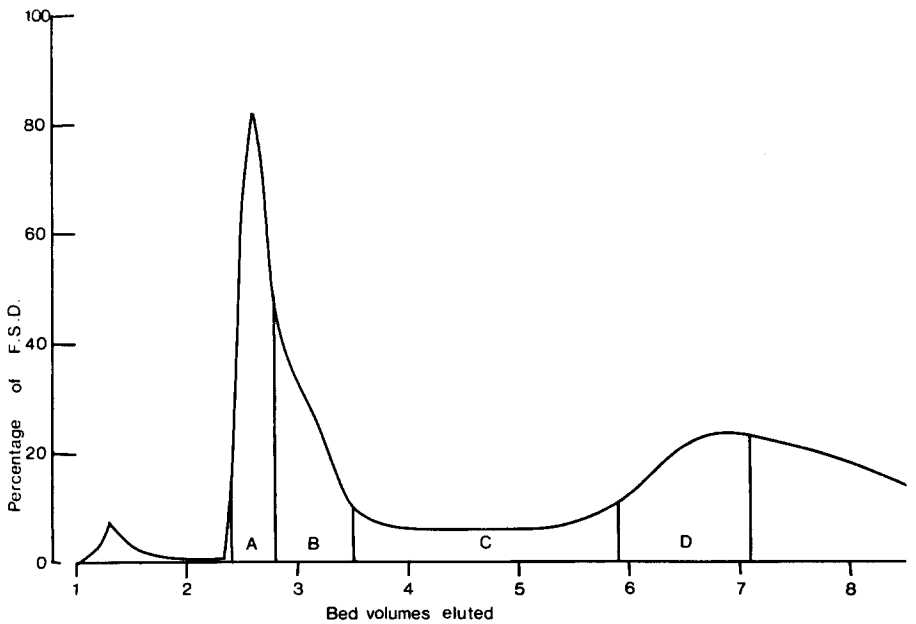


Fig. 1. Silicic acid chromatography of unsaturation-enriched lecithins[13]. Crude egg lecithin, prepared from egg yolks collected from chickens fed a fish oil-containing diet, was chromatographed on a silicic acid column using chloroform–methanol (2:1, v/v). The effluent was monitored on a Pye Liquid Chromatograph with Argon Ionization Detector. The relative amount of lecithin in each fraction, based on lipid phosphorus determinations, was: A, 350; B, 475; C, 333 and D, 745. F.S.D. = full scale deflection.

TABLE II

FATTY ACYL CHAIN ANALYSIS OF LECITHIN FRACTIONS OBTAINED BY SILICIC ACID CHROMATOGRAPHY OF EGG YOLK LECITHINS FROM CHICKENS FED A DIET CONTAINING FISH OILS

The fatty acid compositions were determined by gas–liquid chromatography of the methyl esters as described in the text. A, B, C and D are different fractions of lecithins numbered as eluted from silicic acid (see Fig. 1). Only the major fatty acyl chains are shown.

Fatty acid	*Fatty acid composition (mole %) (β-acyl chains)*			
	A	*B*	*C*	*D*
$C_{18:1}$	0.9	15.1	38.2	59.4
$C_{18:2}$	0.8	11.6	17.9	9.1
$C_{20:5}$	10.6	15.7	8.5	5.0
$C_{22:6}$	78.8	46.7	25.7	18.1

Amino acid efflux from liposomes

Five amino acids were studied. One tritiated and one ^{14}C-labelled amino acid were incorporated in an experiment; a series of ten experiments allowed each amino acid to be compared in one experiment with each of the others. This design was adopted to allow for the determination of relative permeabilities as the surface area of these preparations was unknown and probably varied between experiments. The term "efflux rate constant" used throughout this paper is, in each case, the true permeability coefficient multiplied by the unknown surface area and is expressed in units of μl/min. From the amount of radioactivity in samples of the liposome suspending medium and of the gel-filtered liposome suspension, both the total amount of trapped radioactivity and the volume of trapped solution that this represented could be calculated for each experiment. From these quantities, and the amount of radioactivity that had escaped at each sampling time, the fraction of the original radioactivity

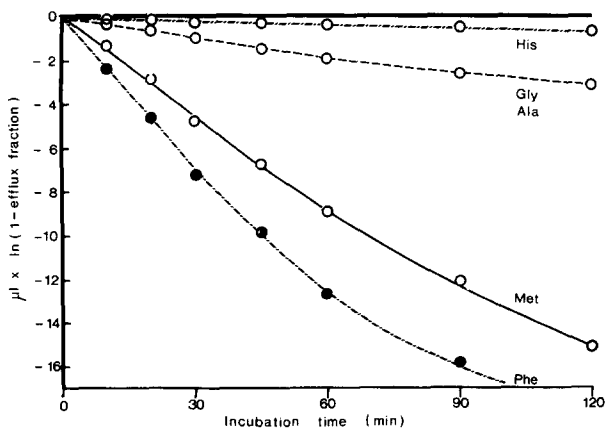


Fig. 2. Amino acid efflux from liposomes. The conditions for formation and incubation of liposomes were as described in the text. Liposomes were formed from an unsaturated lecithin (80% $C_{22:6}$ fatty acid in the β-position) and incubated at 30° for 150 min in oxygen-free buffer. Samples were taken for assay at known times during incubation. Each point was derived from 3 determinations; the initial slope of the lines gives the rate constants for efflux in μl/min.

remaining in the liposomes was calculated, multiplied by the volume of trapped solution, and plotted against time.

This mode of presenting the results would, if the efflux were purely first order, yield a straight line with a negative slope equal to the efflux rate constant. Typical efflux plots for the five amino acids L-histidine, glycine, L-alanine, L-methionine and L-phenylalanine, from liposomes rich in unsaturated lecithins, are shown in Fig. 2. These are linear for the first 50 min of the experiment; the slope of this portion was taken as the efflux rate constant.

TABLE III

AMINO ACID EFFLUX FROM TWO PREPARATIONS OF LIPOSOMES

Liposomes were prepared from cholesterol and phosphatidyl serine as described in the text using lecithins containing different proportions of $C_{22:6}$ fatty acid in the β-position. The efflux of each amino acid was compared with each of the others in turn, one being labelled with ^{3}H and the other with ^{14}C. L-Amino acids were used throughout. Each series of experiments was treated as a balanced incomplete block and mean efflux rates calculated.

Amino acid	*Efflux rate constant (μl/min)*		*Ratio b/a*
	(*a*) 5% $C_{22:6}$	(*b*) 80% $C_{22:6}$	
Phenylalanine	0.13	0.23	1.8
Methionine	0.052	0.16	3.0
Alanine	0.011	0.032	3.0
Glycine	0.011	0.033	3.0
Histidine	0.0036	0.0047	1.3

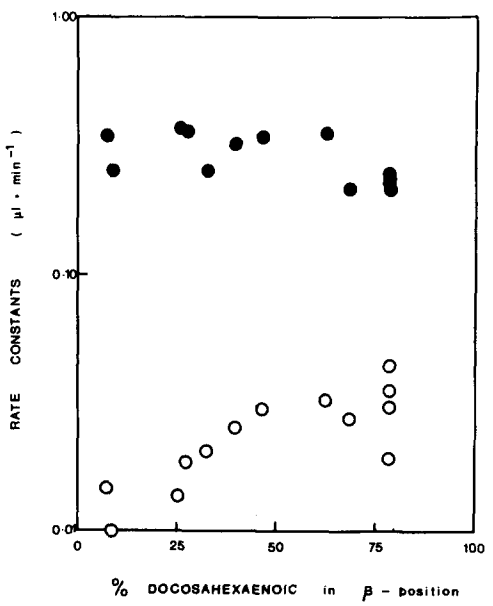


Fig. 3. Glycine and phenylalanine efflux from liposomes prepared from lecithins of different compositions. The conditions of incubation were as in Fig. 2. The lecithin used contained from 7 to 80% $C_{22:6}$ fatty acid in the β-position. Radioactively labelled phenylalanine and glycine were present in each preparation of liposomes. Efflux rate constants are given for phenylalanine (●) and for glycine (○).

Effect of varying lecithin composition on amino acid efflux

The results of two series of experiments, similar except for the degree of unsaturation of the lecithin used and using all five amino acids, are presented in Table III. The efflux rate constants may only be directly compared within each block. Unsaturation increased the efflux rate constants of all five amino acids but by differing amounts: 3 times for alanine, glycine and methionine, 1.8 times for phenylalanine and 1.3 times for histidine. The ranking order remained unchanged.

The effect of changing lecithins on the efflux of phenylalanine and glycine is shown in greater detail in Fig. 3. The efflux rate constant for glycine increased steadily with unsaturation, while that for phenylalanine remained constant within the limits 7–80 $\%C_{22:6}$ acyl groups in the β-position of the lecithin. Reduction of the $C_{22:6}$ content to 5 % or less resulted in a considerable drop in phenylalanine efflux which could be a consequence of the highly saturated membrane solidifying.

These results are presented again in Figs. 4 and 5, this time as the phenylalanine to glycine rate-constant ratio, since this is independent of surface area. Changes in β-acyl composition resulted from replacement of $C_{22:6}$ by $C_{18:1}$ and $C_{18:2}$, and *vice versa*; these were the only fatty acids showing major changes. There are high correlations

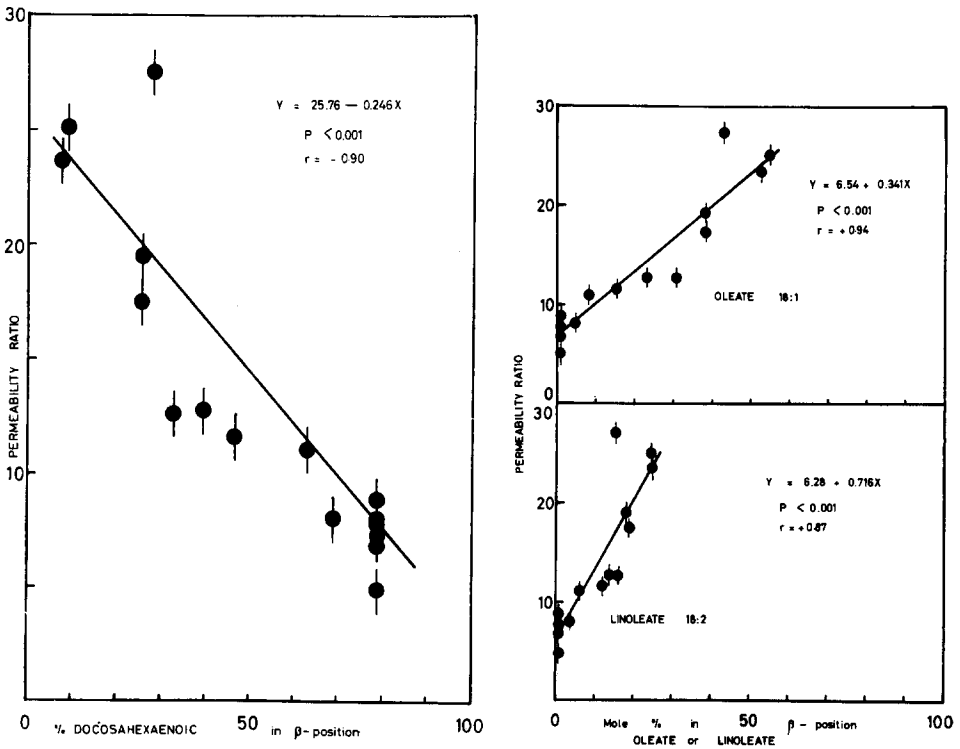


Fig. 4. The relative permeability of liposomes of varying lecithin composition to phenylalanine and glycine. The relative permeability is plotted as the ratio of the efflux rate constants phenylalanine to glycine. The lines across each point give the range of results expected assuming a 5 % error in the calculation of individual efflux rate constants.

Fig. 5. The relative permeability of liposomes to phenylalanine and glycine compared with the percentage of oleate and linoleate present in the β-position of the lecithin molecule. The assumed error is the same as that shown in Fig. 4.

between the permeability ratio and the proportions of the three fatty acyl groups $C_{22:6}$, $C_{18:1}$ and $C_{18:2}$.

The temperature dependence of glycine and phenylalanine efflux

The efflux rate constants for glycine and phenylalanine were determined over the temperature range 25–40°. Experiments were performed on liposomes containing either the highly unsaturated or the less unsaturated lecithins, the whole temperature range being covered for each preparation of liposomes. The Arrhenius plots were linear for both amino acids within this temperature range, the slopes were independent of the type of lecithin used. The heats of activation for glycine and phenylalanine were 17.6 ± 2.5 (3) and 12.7 ± 2.0 (4), respectively, means ± S.E. (No. of determinations). These values were significantly different ($P < 0.05$).

Amino acid partition in a water–n-octanol system

It was found that amino acids were not measurably soluble (partition coefficients greater than 10^4–10^5) in the organic solvents benzene, pentane, decane, or carbon tetrachloride. *n*-Octanol was chosen because this solvent gave measurable partition coefficients, and because it possessed both a hydrophilic and hydrophobic part to the molecule. The means of four determinations of the water–*n*-octanol partition coefficients, determined with an accuracy of ± 2 % were: phenylalanine, 22.5; leucine, 33.0; isoleucine, 48.5; methionine, 74.5; alanine, 520; glycine, 991; and histidine, 330. These partition coefficients were all determined at pH 7.0 in cacodylate buffer containing 100 mM KCl. There was no significant temperature dependence of the partition coefficients ($\Delta H \ll 1$ kcal).

A strong correlation existed between partition coefficient and permeability for all the amino acids except histidine; the pH dependence of histidine's partition coefficient and permeability was therefore investigated. The results are presented in Fig. 6. The partition coefficient was measured as before but with the aqueous phase adjusted to a pH of 5, 6, 7 or 8. A straight line relationship was obtained between $\log_{10}$ (partition coefficient) *versus* "fractional" positive charge on the imidazole ring, calculated from the p*K* for this group.

Histidine efflux was measured at pH 6, 7 and 8 and compared with that for

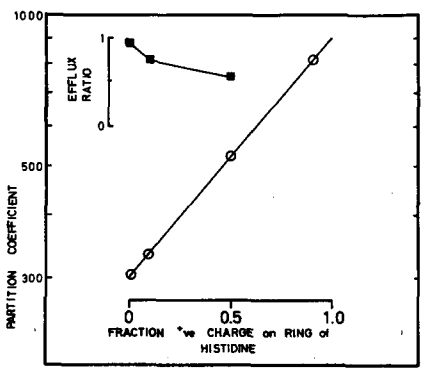


Fig. 6. Fractional positive charge on the histidine imidazole ring against water–*n*-octanol partition coefficient (○) for histidine at pH 5,6,7,8, and against efflux ratio (■) (histidine efflux/galactose efflux) from liposomes for pH 6,7,8.

galactose, which was included as an internal permeability standard. It was assumed that galactose, being a non-electrolyte, could be used as a reference over this small pH range. The permeability to histidine fell as the amount of charge carried by the imidazole group increased (as the pH fell). This change in permeability could be accounted for by the pH dependence of the partition coefficient for histidine. However, this still did not explain why histidine should have such a low permeability at pH 7.0, relative to the other amino acids.

Limits of discrimination of the liposome to amino acid diffusion

Stereospecific diffusion was investigated by examining the efflux of L-[^{3}H]alanine and D-[^{14}C]alanine from liposomes prepared from a moderately unsaturated lecithin. No difference between the efflux rate constants was found.

The effect of chain branching was tested by investigating the homologous series DL-leucine, DL-norleucine and DL-isoleucine in two blocks of three experiments, one with a highly unsaturated lecithin, one with a less unsaturated lecithin. The efflux rate constants for the less unsaturated preparation were DL-leucine 0.094; DL-norleucine 0.086 and DL-isoleucine 0.084 μl/min. The rates in the highly unsaturated preparation were 0.11, 0.12 and 0.12 μl/min, respectively.

DISCUSSION

Kinetic behaviour and justification of the model system

The kinetic analysis of the liposome system is complicated both by the presence of the dialysis bags and by the multiple and unknown number of compartments within the liposome. Since an exact mathematical analysis is impracticable, the properties of a mathematical model were studied to determine the order of error involved in assuming that the dialysis bags did not affect the measured efflux kinetics significantly, and that the initial part of the efflux was a first order process.

The model chosen is illustrated in Fig. 7. The system has five compartments: the external medium, the medium inside the dialysis bag, and the liposomes which are treated for simplicity as three compartment bodies. The flow of permeant molecules across an interface between two compartments A and B is described by the equation

$$\frac{dN_{AB}}{dt} = k_{AB}(c_A - c_B)$$

where N_{AB} is the number of molecules, k_{AB} the "efflux rate constant" incorporating the interfacial area and the absolute permeability, and c_A and c_B are the concentrations in compartments A and B.

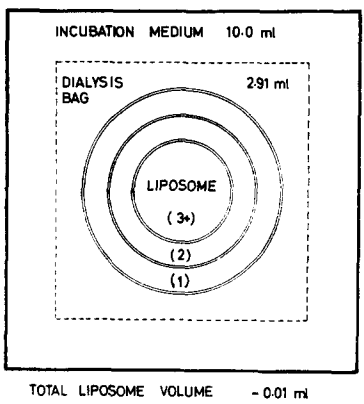
The change in concentration in compartment A is given by

$$V_A \cdot \frac{dc_A}{dt} = \frac{dN_{AB}}{dt}$$

as the contribution for permeant flow across the interface AB. The total change in concentration for a compartment with a number of interfaces is given by

$$V_1 \cdot \frac{dc_1}{dt} = \sum \frac{dN_{12}}{dt} + \frac{dN_{13}}{dt} + \frac{dN_{14}}{dt} \cdots$$

A model was constructed on an IBM 1130 computer using the IBM continuous system modelling programme set up to solve the appropriate simultaneous differential equations for the five compartments. It was assumed for simplicity that the liposome bilayers could be represented as spherical shells of equal thickness, and of comparable absolute permeability; also that the size distribution could be represented as a number of "average" sized particles. Various combinations of liposome compartment size were investigated: as stated previously, the "efflux rate constant" k_{AB} involves the interfacial area term and the absolute permeability. Variation of these parameters, using reasonable values of the same order as those found experimentally, had little qualitative effect on the shape of the efflux curve.



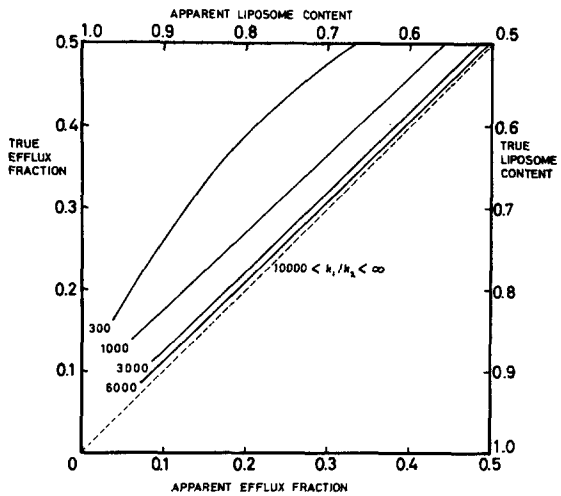


Fig. 7. Diagrammatic representation of the liposome system used in the kinetic analysis of amino acid efflux experiments. The liposome suspension is represented as a system containing three compartments (see text) and the total liposome volume is the calculated amino acid capture volume.

Fig. 8. Curves obtained from the computer simulated model, showing the relation between true and apparent efflux fraction and liposome content, for values of dialysis bag efflux constant (k_1) to liposome efflux constant (k_2) of 300–6000. The dotted line represents the ideal situation of equality and is obtained for $10000 < k_1/k_2 < \infty$. Estimated efflux fraction and liposome content are obtained using the approximation described in the text.

BANGHAM *et al.*[11] have likened the kinetic behaviour of the liposome suspension to that of heat flow from a solid sphere in a well-stirred medium (CARSLAW AND JAEGER[20]). The differences between this model and the one presented in this paper, are that in the solid sphere the medium through which heat (*i.e.* the permeant) diffuses, is homogeneous both in composition and capacity to retard diffusion, whereas our model is based on several concentric spherical shells which limit diffusion, separated by layers of fluid through which, it is assumed, the permeant diffuses at a far greater rate than through the spherical shells. The true situation is likely to be something of a compromise between the solid sphere and the concentric spherical shells, since the fluid-filled regions between the phospholipid bilayers are likely to contain unstirred layers.

The model predicted that, after an initial period of non-linearity, the rate of appearance of permeant in the external phase would follow apparent first-order kine-

tics; the data for Na^+ efflux (when plotted semilogarithmically) from liposomes presented by MOORE *et al.*[21] demonstrate this point.

The error caused by ignoring the presence of the dialysis bag was determined for various ratios of liposome to dialysis bag permeability; these results are presented in Fig. 8 for the case in which the first (outermost) liposome compartment was taken as 15 % of the total volume, the second 10 % and the third and subsequent compartments 75 % of the total liposome compartment volume. These theoretical results can be compared with experimentally determined ratios. The worst case, phenylalanine, gives a ratio of about 1 : 1000, resulting in an error in the estimation of the slope of the efflux plot of less than 5 %. Since this is of the same order as that due to the rest of the experiment, it was concluded that the dialysis bag could be ignored as a barrier to amino acid diffusion.

The gradient of the logarithmic efflux curve is not a simple function of the absolute permeability as it depends also on the relative volumes and interfacial areas of all contributing compartments: it became clear from the model that the apparent permeability constant underestimated the true permeability constant by about 50 %. However, changes in relative compartment sizes and permeabilities had a small effect on the magnitude of this underestimate. Experiments were designed to give comparisons in all cases.

Sampling the external phase sufficiently quickly to detect the initial non-linearity in efflux was not practicable: all the efflux curves appeared linear until about half of the trapped amino acid had escaped. The flattening out of the curves after this point may be attributed to the size heterogeneity of the liposomes, although it is not possible to distinguish mathematically between this and the effects of low-permeability compartments.

Mechanism of selectivity

Selective permeability has been analyzed in terms of size, steric configuration, membrane-water partition coefficients, hydrogen-bonding capacity and membrane charge[22–24]. The hydrogen-bonding capacity of all the amino acids is, with the exception of histidine, similar. No correlation existed between the experimentally determined diffusion rates of the amino acids and size, whether this was represented as a molecular spheroid[25] or as the radius of a minimum enclosing cylinder[26]. Steric configuration and chain branching were found not to influence diffusion. Correlations did exist between efflux rate constants and the water–*n*-octanol partition coefficients. The relation between partition coefficients, molecular weights and permeability has been analyzed for the case of polymer membranes by LIEB AND STEIN[23]. They derive the equation

$$\log P = \log P_0 + s_k \log k_{\text{est}} - s_m \log M$$

where P/P_0 is the relative permeability, k_{est} is the partition coefficient for a system modelling the membrane-aqueous system, M is the molecular weight of the permeant and s_k and s_m are constants. If the water–*n*-octanol system is taken as a reasonable model of the water-lipid bilayer, the permeabilities of the amino acids, excluding histidine, may be correlated with the *n*-octanol–water partition coefficients, giving values of s_k of 0.49 ($r = 0.99$) for highly unsaturated lecithins and 0.70 ($r = 0.94$) for less unsaturated lecithins, r being the correlation coefficient. These high positive values

suggest that the phospholipid bilayers act as hydrophobic diffusion barriers. Histidine diffuses more slowly than this relation would suggest.

In contrast, the value of s_k for dialysis tubing was —0.15 ($r = 0.85$); but the value for s_m was also significant + 0.78 ($r = 0.99$). This behaviour is typical of that of a hydrophilic polymer membrane where smaller molecules diffuse more quickly than larger molecules.

Since the bilayers carry net negative charge by virtue of the phosphatidyl serine content, they would be expected to show increased permeability to the more positively charged molecules[11]. The opposite was found to be the case for histidine. Furthermore histidine shows a permeability between five and eight times less than would be expected from the water–*n*-octanol partition coefficient. The importance of the hydrophobic region of the membrane in determining amino acid permeability has already been stressed and one possible explanation for the anomalous behaviour of histidine would be that it has to pass through the hydrophobic region in the non-polar rather than the highly polar zwitterionic form. Similar mechanisms are thought to occur for NH_4^+ in both mitochondria[27], and liposomes[28]. Although histidine did show a pH dependent efflux this was insufficient to explain the exceptionally low permeability. Similarly the explanation that the low permeability is due to the positively charged ring becoming immobilized at the negatively charged interface, as has been reported for red blood cells with polyelectrolytes[29], becomes untenable in view of the small effect pH had on the efflux rate.

The presence of a single extra hydrogen bond with the secondary amine of the imidazole ring (not present in the other amino acids), with a ΔF^* of approx. 1 kcal (ref. 30), might account for the difference.

Mechanism of selectivity between phenylalanine and glycine

Increasing the unsaturation of the lecithin component of the liposomes increased the efflux rate constants for all amino acids. Similar increases in non-electrolyte permeability through liposomes have been observed and attributed to an increase in membrane fluidity[8]. But the increases found in the present work were not all of the same magnitude and operation of the theory of absolute rate processes[31–33] gives some idea as to how this might occur. The relevant expression for the permeability coefficient P is given by the equation

$$P \cdot f(x) = \lambda^2 \cdot \frac{kT}{h} \cdot e^{-\Delta H^*/RT} \cdot e^{\Delta S^*/R}$$

where f(x) is a function of membrane thickness and ΔH^* and ΔS^* are the enthalpy and entropy changes for the activated state. The permeability ratio between two species a and b is therefore

$$\frac{P_a}{P_b} = e^{(\Delta H_b^* - \Delta H_a^*)/RT} \cdot e^{(\Delta S_a^* - \Delta S_b^*)/R}$$

ΔH^* was found to be 17.6 kcal for glycine and 12.7 kcal for phenylalanine. The difference is too large to account for the observed permeability ratios; it therefore appears that the entropy of activation for glycine is from 9.7 to 13.1 cal·mol^{-1}·degree^{-1} greater than that for phenylalanine, using experimentally determined permeability ratios, and the above equation.

Efflux rate constants for glycine were found to be more susceptible to membrane compositional change than those for phenylalanine. It therefore seems probable that changes in hydrocarbon core composition change the value of the entropy of activation, although small changes in enthalpy could account for the observed changes in permeability ratio; the required change, $\delta(\Delta H^*) = RT \ln 4.17 = 840$ cal, lies within the error of the estimation. The water–*n*-octanol partition coefficients for amino acids were found to be independent of temperature ($\Delta H \ll 1$ kcal). The observed differences in partition coefficients must therefore be entropic in origin[34]. The partition coefficient for phenylalanine is 45 times that for glycine; the entropy difference $\Delta S_{Phe} - \Delta S_{Gly}$ is therefore $+7.6$ cal·mol^{-1}·degree^{-1}. The comparison with values of $\delta\Delta S^*$ from -9.7 to -13.1 cal·mol^{-1}·degree^{-1} found for the permeation process is tempting but not strictly justifiable; ΔS^* is an entropy change of activation and ΔS an equilibrium entropy change.

It is not possible to identify with certainty all the factors controlling the relative permeability changes of phospholipid bilayers, but the results discussed above suggest that entropic effects in the hydrocarbon core are probably the most significant.

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