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PREPARATION OF STABLE AND SOLVENT-FREE MODEL MEMBRANES

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SUMMARY. A method for the preparation of solvent-free , phospholipid-impregnated filters is described.Polycarbonate filters of 13 mm diameter,5 µm thickness and 0.1 µm pore size were employed, and 20 to 48 nmol phospholipid per filter was incorporated.Passive permeation of polar substances across the filter was determined by a flow-dialysis procedure.The presence of phospholipid led to a decrease in passive permeability by 96 - 99%.Due to their size and stability, and a partially preferential lipid orientation, these phospholipid-impregnated filters may serve as an alternative type of model membrane.

INTRODUCTION. Single- or multilamellar liposomes (1) and black lipid films (2) are currently widely used as bilayer model membranes.These systems are rather small and fragile, and alkane or other organic solvents are usually present in the formation of black lipid films (2).Much more stable model membranes have been obtained by impregnating Millipore-type filters with the solutions used for black lipid films (3-7).Light-induced photopotentials could be obtained by use of such filters which also contained bacteriorhodopsin (4-6) or certain pigments (7).The potential use of such filters in energy conversion devices has been stressed (7).A method has now been developed to prepare stable phospholipid-impregnated filters which were free of organic solvent and largely impermeable to polar substances.

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MATERIALS AND METHODS.

Materials. Polycarbonate filters (13 mm diameter,5 μm thickness,0.1 μm pore size) were purchased from Nuclepore Corporation,Pleasanton,California.Soybean phospholipid (Asolectin) was purchased from Associated Concentrates,Woodside,NY, and used after acetone extraction (8).Crude egg lecithin and hexadecane (99% pure) were purchased from Sigma,St.Louis.The following pure lipid species were obtained commercially and gave single spots on thin-layer chromatography with appropriate solvents (9,10), egg lecithin,bovine cardiolipin,dioleoyl phosphatidylethanolamine,cholesterol,dicetyl phosphate, and dimyristoyl lecithin.

Impregnation of filters.The dry polycarbonate filter was placed into organic solvent containing the desired phospholipid (for example,35 mM egg lecithin in propanol-2).After soaking for 10 - 30 min at 25°C the filter was removed by means of teflon-coated tweezers,excess lipid solution was removed with the aid of tissue paper and the filter was completely dried in vacuo.It was then transferred into 20-100 ml 0.1 M potassium phosphate,pH 6.6 (conditioning step).After 1-20 h at 25°C the filter was washed once in 20 ml of the same buffer and placed into a flow-dialysis cell.

Assay for passive permeability. Flow-dialysis cells were built according to a published workshop drawing (Fig.3 in ref.11). The volume of the lower chamber was 0.16 ml,and both the lower and the upper chamber were equipped with magnetic stirring bars (8 x 1 mm).The filter was positioned onto the buffer-filled lower chamber,between two O-rings of 125 μm thickness which were made from polyvinylchloride sheets.The buffer routinely used throughout the procedure was 0.1 M potassium phosphate,pH 6.6.The upper part of the flow-dialysis apparatus (11)was then tightly clamped onto the lower chamber containing the filter.Buffer (500 μl)was pipetted into the upper chamber. A constant flow of buffer (160 μl/min)was pumped through the lower chamber,with magnetic stirring in both chambers.One of two types of permeants was then added to the upper chamber. This was either (1-^{14}C)-lactose (0.68 x 10^6 cpm in 20 μl of a 12 mM solution) or $^{86}Rb^+$ chloride (1.1 x 10^6cpm in 10 μl of a 40 mM solution). Effluent fractions of 800 μl were collected every 5 min,and their radioactivity was determined by liquid scintillation counting.The rate of leakage of radioactivity was constant for 30 to 90 min,depending on the degree of leakiness of the filter.

RESULTS AND DISCUSSION.

Filters impregnated with alkane solutions.Impregnation with soybean phospholipid dissolved in hexadecane led to a marked reduction in the passive permeability of polycarbonate filters (Table 1).This observation was consistent with the reported high electrical resistance of such filters (4-6).However,a

TABLE 1. Rates of passive permeation across polycarbonate filters impregnated with alkane solutions. Filters were soaked in the indicated alkane solvent for 10 min (25°C), followed by conditioning in 1 litre 0.1 M potassium phosphate, pH 6.6 (3-6 h, 25°C). The filters were then placed into the flow-dialysis cell as described under Materials and Methods.

Alkane solution	Leakage rate (cpm in effluent/5 min) $1\text{-}^{14}C$-lactose	$^{86}Rb^{+}$
None (control filter)	80,000	130,000
Hexadecane	1,000	3,500
Hexadecane plus soybean phospholipid (150 mg/ml, about 200 mM, see ref. 6).	100	400

drastic reduction of passive permeability was also brought about by the hexadecane solvent itself (Table 1). Furthermore, upon transfer of filters soaked with soybean phospholipid in hexadecane into aqueous buffer, most (about 90%) of the phospholipid initially bound was released as lipid droplets. These data are not shown here in detail, and were obtained by inclusion of di-(1-^{14}C)- palmitoyl lecithin as a radioactive marker for phospholipid. These tracer experiments also indicated that about 52 nmol phospholipid remained associated with the soybean phospholipid/hexadecane filter after ten consecutive conditioning steps in aqueous buffer. It was concluded that the hexadecane-containing lipid filters were structurally unstable and that the alkane solvent by itself gave rise to a permeability barrier.

Solvent-free filters.

Phospholipids were then employed as solutions in methanol or propanol-2, and these solvents were removed *in vacuo* prior to the conditioning of the lipid-soaked filters in aqueous buffer. There was a marked reduction of the leakage of lactose

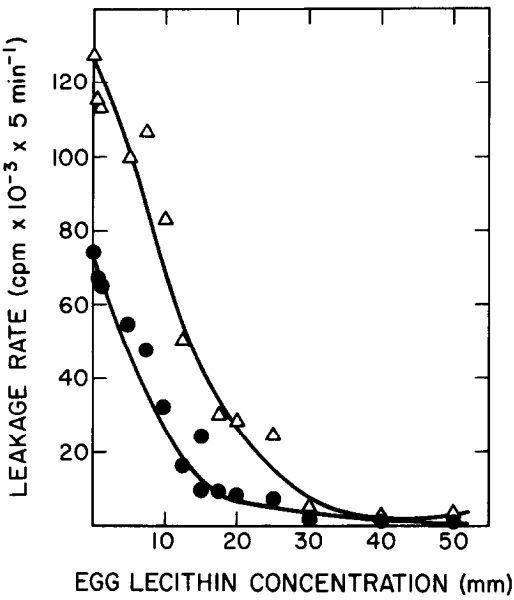

Fig.1. The dependence of passive permeation rates of lactose (●) and Rb^+ (Δ) on the concentration of egg lecithin used for the impregnation of polycarbonate filters. The indicated concentrations of egg lecithin in propanol-2 were employed in the soaking step (45 min,25°C), followed by removal of solvent in vacuo.Each filter was conditioned in 20 ml 0.1 M potassium phosphate,pH 6.6 (1-6 h,25°C, followed by the flow-dialysis procedure described under Materials and Methods.The amount of radioactivity (cpm)per 5 min fraction of effluent is plotted against the initial concentration of egg lecithin (mM).

or Rb^+ ,as shown in Fig. 1 for egg lecithin.To achieve low leakage rates of 1 to 4% of the control filters,initial lipid concentrations of 28-35 mM were required.The following phospholipid preparations were similarly effective, crude egg phospholipid (30 mM), egg lecithin (33 mM)/cholesterol/dicetyl-phosphate (molar ratio, 4/3/0.4,see ref.1), egg lecithin (20 mM)/ E.coli phosphatidylethanolamine (5 mM)/bovine cardiolipin (1 mM), dioleoyl phosphatidylethanolamine (28 mM) or dimyristoyl lecithin (35 mM).These various solvent-free phospholipid filters retained their low leakage rates after storage in the conditioning buffer for at least 80 h at 25°C.The amounts of

phospholipid were between 20 and 48 nmol per filter , depending on the particular phospholipid preparation used.Very little phospholipid was released after the initial conditioning step (use of a di-(1-^{14}C)- palmitoyl lecithin marker,data not shown).

An electron spin resonance label,2-(2-carboxyethyl)-2-tetradecyl-4,4-dimethyl-N-oxazolidinyloxyl,has been added at about 3 mol % to an egg lecithin solution (30 mM)in propanol-2,and filters were impregnated with this solution (see Materials and Methods).The spectra obtained with the magnetic field either parallel or perpendicular to the plane of the filter suggested that the filter contained both non-oriented bilayer domains and a significant fraction of lipids oriented with their fatty acid chains perpendicular to the plane of the filter.No such preferential orientation was observed with alkane-containing filters (R.Mehlhorn, University of California at Berkeley,Dept. of Physiology, personal communication).

In conclusion,the solvent-free phospholipid filters described above may serve as alternative model membranes with the advantages of a convenient size,of stability over several days and of a preferential lipid orientation.It remains to be investigated whether the above method can be exploited for a functional reconstitution of biological transport systems with membrane proteins dissolved in the organic lipid solution used for the impregnation of the filters.A proline transport system of E.coli has been solubilized in butanol-1 (12),and the lactose permease system (13)and the phage lambda receptor protein (14)of E.coli have been solubilized in another organic solvent, hexamethylphosphoric triamide.The latter solvent also

dissolved bacteriorhodopsin in high yield (H.Sandermann, unpublished results).

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