the most plausible explanation of the observed cellular reactivity of lymphocytes from glomerulonephritic patients is primary sensitization to the glomerular antigens. The observed reactivity to the streptococcal membranes would be merely a reflection of the known shared antigenicity between the two membranes. This type of cross-reactive sensitization might also explain the observed exacerbations in progressive glomerulonephritis. However, in view of the paucity of information regarding the nature of some of the immunizing antigens in progressive glomerulonephritis (*18*), it is conceivable that sensitization to the strepococcal membrane antigens might be the initiating factor in the glomerular damage. Continued release of . glomerular . membrane . antigens coupled with intercurrent streptococcal infections could be responsible for the progressive nature of the disease. A closer correlation between the nature and deposition of glomerular antibodies and the cellular reactivity to human glomerular membranes and streptococcal membranes in acute and progressive glomerulonephritis, as well as more precise characterization of these antibodies with respect to the antigens, will be needed to clearly separate the two hypotheses.

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Bimolecular (Black) Lipid Membranes: Study of Lipid-Protein Interactions

Abstract. *An experimental method has been devised for the study of the interaction of bimolecular (black) lipid membrane and protein in which 8-anilino-1-naphthalenesulfonic acid is used as a fluorescent probe. The presence of phospholipid in the membrane is necessary for the enhanced fluorescence.*

The properties and functions of cellular membranes undoubtedly are based upon the interaction of their lipids and proteins. Although lipid-protein interaction has been widely discussed, little information is available from direct examination of cellular membranes. Accordingly, we report here some qualitative observations of lipid-protein interactions in a bimolecular lipid membrane (BLM) system in which 8-anilino-1-naphthalenesulfonic acid (ANS) was used as a fluorescent probe.

This acid and its derivatives have been used in studies of changes in protein conformations and in studies of interactions (*1*), because the fluorescence properties of ANS depend upon the polarity of its environment (*2*). There is almost no emission when the acid is dissolved in water (quantum yield is 0.004) but it becomes fluorescent when stoichiometrically bound to specific sites in the nonpolar regions of the proteins. An enhancement of fluorescence accompanies the binding of ANS to albumin, an indication that in solution the nonpolar portions of this protein are available to ANS penetration. Recently ANS has been used in studies on biological membranes (*3*).

Black lipid membranes (*4*) are formed from solutions of (i) oxidized cholesterol prepared by bubbling oxygen through a 4 percent solution of cholesterol in *n*-octane at its refluxing temperature (*5*); or (ii) a mixture of oxidized cholesterol ($10^{-2}M$) and egg lecithin ($10^{-3}M$). All reagents (*6*) were C.P. grade and were used without further purification.

The experimental setup (Fig. 1) was modified from the one used for measurement of BLM thicknesses (*5*). A 100-watt low-pressure mercury lamp with a selected line filter provided excitation at 365 nm. The BLM was viewed with a low-power microscope provided with a side arm to accommodate a photomultiplier tube (EMI 9558Q). The output from the photomultiplier tube

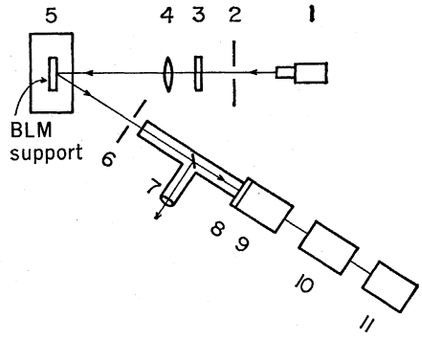

Fig. 1. Schematic diagram of the experimental setup used for studies of BLM-protein interaction; *1*, light source; *2*, slit; *3*, interference filter; *4*, condensing lens; *5*, cell assembly; *6*, slit; *7*, microscope and viewing tube; *8*, iris diaphragm; *9*, photomultiplier tube; *10*, microphotometer; *11*, recorder.

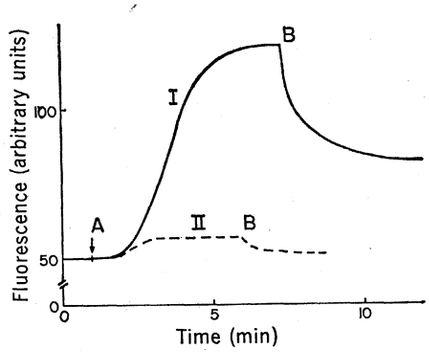

Fig. 2. Fluorescence of BLM treated with 8-anilino-1-naphthalenesulfonic acid and bovine serum albumin.

was measured on an Aminco microphotometer (American Instrument Company, Inc.), the signal of which was fed into a Health servo recorder (model EUW 20H).

No fluorescence was detected when either the albumin alone or the ANS alone was adsorbed to either type of BLM. However, after flushing the membrane with 0.1*M* KCl or 0.1*M* acetate buffer solution and subsequently adding the other one of the pair of compounds (Fig. 2, point A), we observed enhanced fluorescence. The intensity of the fluorescence response (Fig. 2) depends on which of the two compounds is adsorbed onto the phospholipid membrane first. For example, when ANS is adsorbed onto the membrane first, and then bovine serum albumin (BSA), is added (curve I), the fluorescence response is three times larger than in the case (curve II) where BSA is adsorbed onto the BLM before ANS is added. If the phospholipid BLM is again flushed with 0.1*M* KCl solution (point B), the fluorescence decreases to half its original intensity in the first case, whereas, after a second flushing with KCl solution in the second case (curve II), the fluorescence returns almost to the base line. The maximum increase in fluorescence for the situation depicted in curve I occurs when the *p*H of the surrounding solution is between 5.6 and 7.

When the BLM is prepared from either of the solutions of oxidized cholesterol saturated with ANS, subsequent addition of BSA does not cause a significant change in the fluorescence. Moreover, BLM formed from oxidized cholesterol alone shows no increased fluorescence, regardless of the order of addition of ANS and BSA.

Three important conclusions can be drawn from these results. First, ANS must adsorb to the phospholipid portion of the BLM, but not to the cholesterol or oxidized cholesterol portion, or both. However, the orientation of the ANS bound to the lecithin must be such that the emitting moiety projects into the aqueous phase, since there is no enhancement of fluorescence over that observed in water alone.

Second, either the enhanced fluorescence observed upon the addition of H_2O has been excluded from the ANS or the protein surrounds the emitting moiety with hydrophobic groups upon adsorption. The latter explanation seems most likely since the enhancement of fluorescence is greatest in the *p*H range 5.6 to 7 (if the *p*H at the interface is about 2 *p*H units lower than that of the solution, then this corresponds to the maximum *p*H range for BSA-ANS interaction in solution). Finally, the lack of enhanced fluorescence when ANS is added to the BLM after the addition of BSA indicates that the protein adsorbs to the BLM in such a way that the hydrophobic regions are not available for ANS penetration.

Changes in fluorescence dependent on *p*H can be related to the conformational changes in serum albumin itself. Enhancement of fluorescence was observed around *p*H 3.35 (*7*) because of an increase in the number of apolar sites in albumin. However, thus far we have not been able to perform experiments on the enhancement of fluorescence at low *p*H because of the instability of BLM at low *p*H.

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Chlorinated Hydrocarbon Insecticides: Root Uptake versus Vapor Contamination of Soybean Foliage

Abstract. *The major environmental source of DDT (dichlorodiphenyltrichloroethane) residues in soybean plants arises from vapor movement from contaminated soil surfaces. In contrast, the presence of dieldrin, endrin, and heptachlor results primarily from root uptake and translocation through stems to leaves and seeds.*

The chlorinated hydrocarbon insecticides have been among the most useful pesticides for the protection of agricultural crops during the last two decades. Nevertheless, advances in instrumentation during the last 6 to 8 years have demonstrated that minute quantities of these insecticides may contaminate our foods and feeds.

Crops may become contaminated

Table 1. Soybean sorption of [^{14}C]DDT, [^{14}C]dieldrin, [^{14}C]endrin, and [^{14}C]heptachlor residues from soils and percentage of residue as insecticide. Values for the insecticides have been adjusted so that direct comparison among insecticide residues is possible. All values are the mean of four replications, except those for dieldrin and heptachlor sorbed through roots which are based on three replications. Values in parentheses represent the percentage of total residues as parent insecticide.

Plant part	Insecticide [counts per minute (in thousands) per gram of dry weight]			
	DDT	Dieldrin	Endrin	Heptachlor
		Through roots		
Upper leaves	0.8	1.1	34.1	26.6
Lower leaves	1.4 (0.3)	6.2 (64.6)	62.4 (19.0)	36.6 (0.1)
Upper stem	0.6	8.2	84.5	14.7
Lower stem	0.8 (Trace)	61.7 (91.1)	139.8 (57.3)	33.9 (4.5)
Pods	0.1	0.3	4.1	3.9
Seeds	0.1 (None)	0.2 (117.2)	1.3 (2.7)	1.8 (0.7)
		Through vaporization		
Upper leaves	4.5	10.2	6.2	4.7
Lower leaves	9.2 (77.9)	16.6 (51.9)	13.1 (51.6)	9.8 (2.3)
Upper stem	0.8	0.9	0.9	1.0
Lower stem	0.7 (46.2)	0.6 (107.2)	0.6 (41.3)	1.1 (21.3)
Pods	1.1	1.2	1.1	1.2
Seeds	0.1 (None)	0.3 (91.4)	0.4 (31.0)	0.6 (5.1)