show that retinol dissolved in Tween 40 or Tween 80 causes little isotope release from adult parasites although retinol is taken up into the surface and other membranes. If, however, the retinol is presented to the adult dissolved in Tween 20, considerable damage occurs to the membrane. This damage represents a synergism between Tween 20 and retinol (Table III). The Tweens are polyoxyethylene sorbitol esters of fatty acids. The fatty acid found in Tween 20 is lauric acid; that in Tween 80 is oleic acid (Schick, 1967). The synergism between Tween 20 and retinol is evident only when the two compounds are presented simultaneously. We therefore suggest that the mixed micelle (Tween 20/retinol) has the ability to penetrate areas of membrane unavailable to mixed micelles of other composition.

It may be that the inner bilayer of the complex pentalaminate membrane is perturbed by the Tween 20/retinol micelle. Certain properties (hydrophobicity, size) of this micelle may determine its effect, but the causes of the synergism are unknown. We suggest that the presentation of membrane-active drugs to this complex membrane may be facilitated by understanding why certain mixed micelles are particularly damaging and others are not so.

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^{31}P NMR INVESTIGATION OF RHODOPSIN-PHOSPHOLIPID INTERACTIONS IN BOVINE ROD OUTER SEGMENT DISK MEMBRANES

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The interaction between the integral membrane protein, rhodopsin, and the surrounding phospholipids in bovine rod outer segment disk membranes was investigated using ^{31}P NMR. Disks were prepared as described in (1). ^{31}P NMR intensity measurements were obtained as described in reference 2 using spectra as shown in Fig. 1 and a standard curve as shown in Fig. 2. Intensity measurements indicated that ~ 20% of the phospholipid molecules in the disk membrane were immobilized. This is not to imply they are absolutely immobile or frozen, but it does imply that the immobilized phospholipids as reflected by the phosphate headgroup are in slow exchange ($\tau < 10^{-4}$ s) with the bulk phospholipids. These measurements are to be distinguished from those which detect motional properties of the hydrocarbon chain. It is reasonable to hypothesize a certain amount of independence with respect to the motional freedom experienced by the headgroup and by the hydrocarbon chain.

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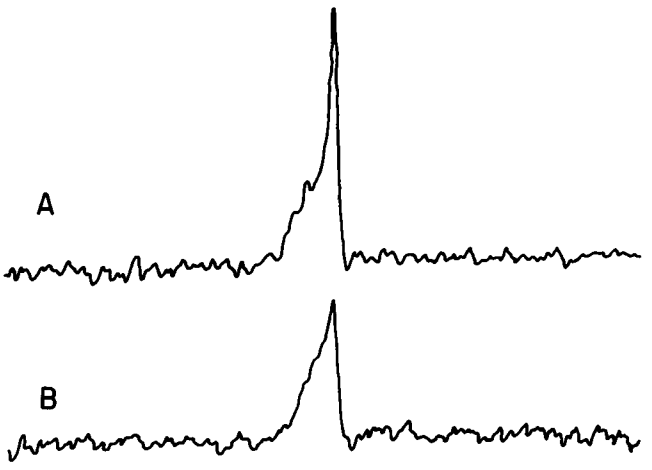

FIGURE 1 81 MHz ^{31}P NMR spectra of membranes obtained in 10 mm tubes at 30°C. The ^{1}H decoupler was gated to remove the ^{31}P (^{1}H) nuclear Overhauser effect. 90° pulses (11 μ s) separated by 4–5 T_1, were used and 500 scans collected with 2048 data points over a 50 KHz spectral width. *A*, Unsonicated egg phosphatidylcholine in 100 mM NaCl, 10 mM TRIS, pH 7.5; *B*, bovine rod outer segment disks in 100 mM NaCl, 10 mM TRIS, pH 7.5.

RESULTS AND DISCUSSION

The disk membrane contains ~ 75–100 phospholipid mol/rhodopsin molecule. 19% of these phospholipids experience restricted motion presumably by interaction with a protein, the most likely protein (Table I) being rhodopsin, as it comprises at least 80% of the disk membrane. This would suggest that each rhodopsin molecule can interact

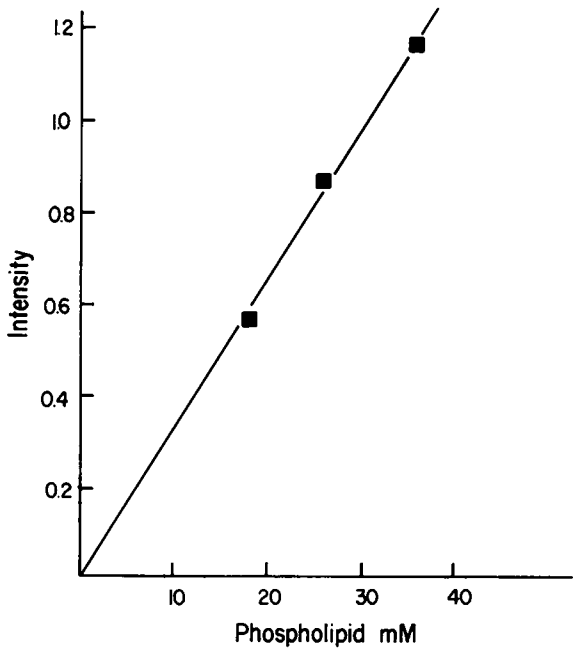

FIGURE 2 Intensity of the ^{31}P NMR resonance of unsonicated egg phosphatidylcholine dispersions in 100 mM NaCl, 10 mM TRIS, pH 7.5, as a function of phospholipid concentration. Intensities were evaluated by cutting and weighing photocopies of the spectra.

TABLE I
PHOSPHOLIPIDS IMMOBILIZED IN ROD OUTER SEGMENT DISKS AT 30°C.

Systems	No. prep-arations	% Phospholipids immobilized	Phospholipid/ rhodospin immobilized
Normal disks	8	19 ± 2	14 ± 2
Papain-treated disks	2	20 ± 4	15 ± 3
O G solubilized disks	2	0 ± 2	0 ± 1

*Based on 75 phospholipids per rhodopsin.

with 14–19 phospholipid molecules in such a manner as to restrict the phosphate motional freedom or rate of motion. Proteolytic cleavage by papain of rhodopsin in the disk membrane has been shown to produce little or no change in the spectral properties of the protein (3, 4). Papain proteolysis was carried out as described in (4). The effect of papain proteolysis on the phospholipid immobilization was examined. In contrast to findings in the papain proteolyzed sarcoplasmic reticulum membrane (Albert and Yeagle, manuscript in preparation), no decrease in immobilized component was observed after proteolysis. This is consistent with previous conclusions that the rhodopsin conformation and position in the membrane are unaltered by proteolytic cleavage. To show that all the phospholipid can be observed by ^{31}P NMR, the disks were solubilized in 250 mM octyl glycoside to disrupt the protein-phospholipid interactions. Intensity measurements using solubilized phospholipids as standards indicate all the phospholipid in the solubilized disk sample could be observed.

It was of interest to determine whether proteolytic cleavage would disrupt phospholipid immobilization in the

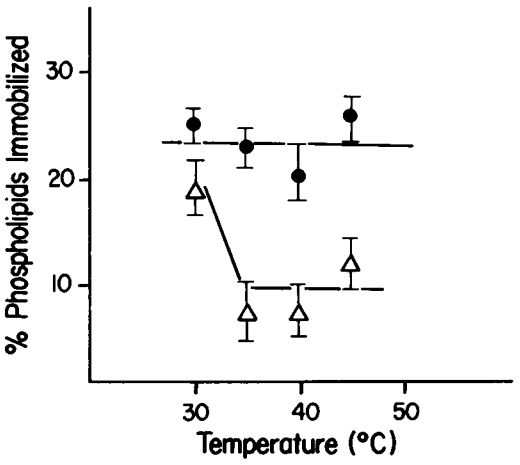

FIGURE 3 Temperature dependence of the immobilized component of bovine rod outer segment disk membranes. Samples and standards were allowed to equilibrate at each new temperature for 10 min before accumulating data. (●) normal disks; (△) papain-treated disks.

TABLE II
^{31}P SPIN LATTICE RELAXATION TIMES AT 81 MHz.

System	T_1 (±10%)	No. of preparations
Unsonicated egg phosphatidylcholine	1.3s	2
rod outer segment disks	1.4 s	2

disk membrane. As shown in Fig. 3, just above 30°C the immobilization of phospholipids by proteolyzed rhodopsin undergoes a dramatic reduction, while no temperature effect is observed for native, intact disks in the temperature range studied. This result indicates that the protein-phospholipid complex of the proteolyzed rhodopsin is not as thermally stable as the original complex.

Both ^{31}P NMR spectral width and T_1 data indicate that the phospholipids which were observed exhibited behavior similar to phospholipids in a pure phospholipid bilayer, shown in Fig. 1 and Table II. This is significant in that it suggests the loss of spectral intensity is not due to an average property of the membrane bilayer, but rather that a component has been removed from an otherwise typical phospholipid bilayer.

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MEMBRANE LIPID FLUIDITY AND PHYSICAL STATE AND THE ACTIVITY OF THE Na^+,Mg^{2+}-ATPASE OF *ACHOLEPLASMA LAIDLAWII* B

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Nonlinear Arrhenius plots are often obtained when the temperature dependence of membrane enzymes and transport systems is investigated. Such plots are conventionally analyzed as sets of two (or sometimes more) straight-line segments, the slopes of which are assumed to represent reaction activation enthalpies of different states of the enzyme or transport system. Apparently sharp changes in the slopes of these Arrhenius plots are usually ascribed to thermotropic membrane structural alterations, especially lipid phase transitions. We have recently studied in a quantitative and systematic way the temperature dependence of the membrane Na^+,Mg^{2+}-ATPase of the simple, cell wall-less prokaryote *Acholeplasma laidlawii* B (1). By utilizing *A. laidlawii* B membranes enriched or made homogeneous in a variety of exogenous fatty acids, both the temperature and the cooperativity of the gel to liquid-crystalline lipid phase transition can be markedly altered (2, 3), permitting a rigorous investigation of the influence of lipid fluidity and physical state on the temperature dependence of the activity of this enzyme in intact membranes. The results thus obtained were analyzed using the theoretical rate-temperature relationships derived for a variety of physically plausible membrane rate processes described in detail elsewhere (4).

METHODS

The organism used in this study, the growth medium and conditions employed, the determination of ATPase activity, and the preparation and differential thermal analysis of isolated membranes, have all been previously described (1–3, 5).

RESULTS

The temperature dependence of the Na^+,Mg^{2+}-ATPase in two *A. laidlawii* B membranes whose lipids exist exclusively in the liquid-crystalline state at physiological temperatures are shown in Fig. 1. These Arrhenius plots are clearly smooth curves and their slopes decrease as a nearly linear function of temperature. Similar behavior is observed for membranes enriched in other fatty acids exhibiting lipid phase transitions below 0°C, or at temperatures above the phase transition midpoint temperature in membranes exhibiting phase changes above 0°C. Thus the temperature dependence of this ATPase activity is not

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