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Membrane perturbation: Studies employing a calcium-sensitive dye, arsenazo III, in liposomes

(steroid hormones/lipid bilayers/metallochromes)

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ABSTRACT **A metallochromic dye, arsenazo III [2,7-bis-(2-arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid], has been incorporated into the aqueous interspaces of multilamellar liposomes. Addition of Ca produced no shift in the absorbance spectrum of dye captured by liposomes, whereas disruption of liposomes by Triton X-100, followed by Ca, produced the spectrum characteristic of the dye–Ca complex: evidence of latency. Addition of excess ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) reversed the spectal shift. Differences between spectra obtained in this sequence yielded *dye* efflux. To measure *Ca* efflux, difference spectra (±EGTA) were obtained from cationic liposomes containing Ca after detergent lysis (sensitivity <10 nmol/ml). Since liposomes were impermeable either to dye or Ca until perturbed, it was possible to test a variety of membrane-active steroids (diethylstilbesterol, deoxycorticosterone, etiocholanolone) for their capacity to provoke dye efflux from liposomes; preincorporation of cortisol stabilized liposomes against dye leak. Immunoglobulin-coated liposomes containing dye were taken up by phagocytes of *Mustelus canis*, and phagocytic vacuoles stained red-purple after ingestions. Liposomes containing the calcium-sensitive dye constitute a simple, accurate means for determining membrane perturbation and Ca fluxes; their uptake by cells or organelles remains to be exploited further.**

A variety of inorganic ions (1, 2), organic solutes (2, 3), drugs (4), and enzymes (4–6) can be captured in the aqueous interspaces of multilamellar liposomes (description: ref. 2). This has been done to determine these species' passive efflux as a measure of membrane perturbation (1–3), or to utilize liposomes as vectors for introducing trapped material into cells or organelles (4–6). Were it possible to achieve capture of a metallochromic dye by liposomes, both purposes might be fulfilled. The sizeable spectral shifts induced in such dyes by divalent cations (7) would permit spectrophotometric studies of dye or cation efflux from liposomes. Moreover, the introduction of metallochrome-laden liposomes into cells and/or organelles would offer a useful means of monitoring changes in activity of intracellular cations.

Abbreviations: As in previous communications (6, 14), a shorthand system of notation will be employed to describe liposomes. All externally adherent materials will be followed by a period, as in "aggIgM." to indicate heat-aggregated IgM. Next is written "L" for liposomes, followed by the molar ratios of membrane lipids as in L(PC 7:DCP 2:Chol 1) enclosed in parentheses, to indicate phosphatidylcholine, dicetylphosphate, and cholesterol in the molar ratios indicated above. All entrapped substances, such as proteins, ions, or organic solutes, are next written in square brackets, such as [AIII, glucose] to designate liposomes after capture of arsenazo III and glucose in aqueous compartments. SA, stearylamine; HC, hydrocortisone; DES, diethylstilbesterol; DOC, deoxycorticosterone; Etio, etiocholanolone; AIII, arsenazo III; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; TX-100, Triton X-100.

The dye arsenazo III (AIII), a bis(arylazo) derivative of chromotropic acid (Fig. 1) (7, 8), was chosen for capture by liposomes for the following reasons. (*1*) The dye undergoes a striking change of color from red to blue (absorption maximum at 560 nm → maxima at 605 and 660 nm) upon complexing the calcium (7, 9). (*2*) The molar extinction coefficient of its difference spectrum (with and without Ca) is on the order of 10^4 (7), and its selectivity for Ca over Mg is about 50-fold (10). (*3*) The dye forms stable, water-soluble complexes (1:1) with Ca at neutral pH (9). (*4*) AIII is nontoxic to cells at millimolar concentrations (10). (*5*) AIII has already been employed to monitor changes in the activity of intracellular Ca of tissues accessible to microinjection (10). Trivial reasons include that the dye is commercially available, cheap, and stable in the presence of tissue extracts.

MATERIALS AND METHODS

Arsenazo III: Preparation and Spectroscopy. The metallochromic dye arsenazo III [2,7-bis(2-arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid] was obtained as a mixed, Ca-containing salt from Sigma Chemical Corp. Aqueous solutions of dye (30 mM) were brought to pH 7.5 by addition of KOH. AIII was converted to its K salt by passage through a cation exchange column of K-Chelex 100 (Bio-Rad, Richmond, Calif.). Dye was purified in the cold by addition of concentrated HCl to a final concentration of 6 M. After standing overnight in the cold the precipitated dye was collected by filtration (7). Beckman models 25 and DB twin-beam spectrophotometers were used for analysis.

Liposomes: Preparation and Capture of AIII. The preparation of liposomes and sources of lipids, etc. have been extensively described before (3, 5, 6, 11–13). After rotary evaporation from chloroform, liposomes (32 μmol of lipid per ml) were permitted to swell for 2 hr at room temperature in solutions (6 ml) containing AIII (3 mM), glucose (0.29 M), and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (5 mM) pH 7.4. Liposomes, phosphatidylcholine 7: dicetylphosphate 2: cholesterol 1 (PC 7:DCP 2:Chol 1), were applied (5 ml) to Sepharose 4B (Pharmacia, Stockholm) columns (2.5 × 28 cm); elution was in 0.145 M NaCl–KCl (equimolar) containing Hepes (5 mM) pH 7.4. To insure

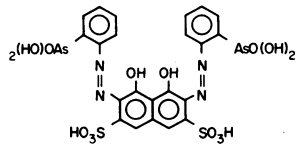

FIG. 1. Chemical structure of arsenazo III [2,7-bis(arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid] (8). Molecular weight = 776.

removal of trace Ca, each column was "pre-washed" with AIII (5 mM) until no trace of blue (AIII–Ca complex) was obtained. All reagents were prepared in Ca-free, deionized water, and disposable plastic tubes and pipettes (Falcon Plastics, Oxnard, Calif.) were utilized. Column fractions (0.5–1.0 ml) were assayed for AIII by its absorbance at 660 nm, after addition of Ca (1 mM), both in the presence and absence of Triton X-100 (TX-100) (0.5%, vol/vol). Liposomes were quantitated against appropriate standards by means of apparent absorbance at 750 nm (11–13), and glucose was determined by the glucose oxidase method (11–13). To correct for variations in apparent absorbance at 660 nm due to detergent-lysis of liposomes, liposome fractions rich in AIII were also treated with 10 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) and AIII was quantitated from the *decrements* in absorbance at 660 nm induced by EGTA. *Latency* is defined as those properties imparted to molecules by their physical sequestration within lipid barriers (5, 6). To test latency, aliquots (1.0 ml) of dye-containing liposomes, L[AIII, glucose], eluted from Sepharose 4B were exposed to Triton X-100 (0.5%, vol/vol) for 30 min at 23° and reapplied to smaller (1.0 × 18 cm), "pre-washed" columns of Sepharose 4B. After elution with NaCl–KCl (0.145 M) and Hepes (5 mM), eluates (0.5–1.0 ml) were again assayed for lipid, AIII and glucose.

Ca-Containing Liposomes. Since DCP formed precipitates in Ca salts, cationic liposomes (16 μmol of lipid/ml) were prepared with stearylamine (SA) as the charged component: L(PC 7:SA 2:Chol 1). They were permitted to swell in 0.29 M glucose containing $CaCl_2$ (3 mM) and Hepes (5 mM), pH 7.4. Subsequently, 0.75 ml were applied to small (1.0 × 18 cm) "pre-washed" Sepharose 4B columns and collected as 1.0 ml fractions, employing NaCl–KCl (0.145 M) and Hepes (5 mM), pH 7.4, as eluant. Fractions were assayed for liposomes and glucose as before, and for Ca by determining decrements in absorbance of AIII at 660 nm induced by 10 mM EGTA in fractions to which AIII (0.17 mM) and Triton X-100 (0.5%, vol/vol) had been added. Standard curves (absorbance decrements after EGTA) permitted detection of 2–10 nmol of Ca per ml, whether or not turbid liposomes (0–5 μmol of liposomal lipid per ml) were present.

Liposomal Perturbation and Stabilization. Liposomes, L(PC 7:DCP 2:Chol 1) [AIII], prepared with 32 μmol of lipid per ml were swollen in NaCl–KCl (0.145 M) and Hepes (5 mM) containing 3 mM AIII. Samples (5.0 ml) were applied to Sepharose 4B columns as above and peaks of dye-containing liposomes (4 μmol of lipid per ml) were pooled. Two spectrophotometric methods were employed to detect membrane perturbation. In the "twin-beam" method, two cuvettes were prepared to contain L[AIII] (0.5 ml) in 0.145 M KCl and 10 mM Hepes (2.0 ml). Cuvette 1 (L[AIII] + TX-100) was placed in the "reference" chamber after addition of Triton X-100 (0.2 ml, 0.2%, vol/vol) or steroids; cuvette 2 (L[AIII]) was placed in the "sample" chamber with added buffer (0.2 ml). Absorbance differences (cuvette 2 − 1) represented turbidity, and were proportional either to the concentration of liposomes or of perturbant (1–6). After Ca (0.1 ml) was added to both cuvettes at a final concentration of 10 mM, cuvette 2 (L[AIII] + Ca) was transferred to the "reference" chamber, becoming cuvette 3. Cuvette 1 (L[AIII] + TX-100 + Ca) was now transferred to the "sample" chamber, becoming cuvette 4. The absorbance difference (4 − 3) represents dye–Ca complex *minus* the apparent absorbance after Ca addition. Addition of the two readings, (4 − 3) + (2 − 1) yielded true values for dye rendered accessible to Ca, by detergent or steroid, independent of changes in apparent absorbance.

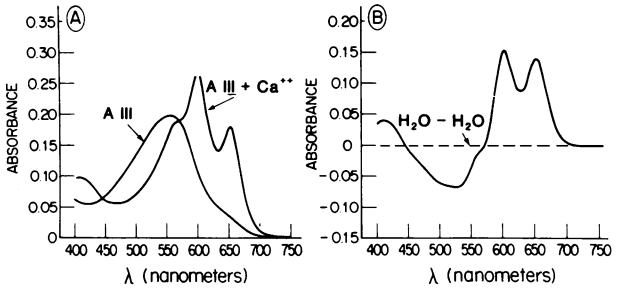

FIG. 2. Absorbance spectra of arsenazo III (8 μM), dissolved in a solution containing KCl (0.145 M) and Hepes (5 mM), pH 7.4. (A) AIII = arsenazo III without added divalent cation; AIII + Ca = arsenazo III with $CaCl_2$ (3 mM); distilled water as reference. (B) Difference spectrum of AIII ± Ca. $H_2O - H_2O$ = distilled water in both cuvettes.

In the "single-beam" method, steroids, dissolved in absolute ethanol, were added as 0.1–0.2 ml aliquots to Ca-free tubes containing 0.5 ml of L[AIII] in 2.0 ml of NaCl–KCl (0.145 M) and Hepes (5 mM), pH 7.4. To controls, ethanol alone was added. After 15 min of incubation in a shaking water bath at 37°, 0.3 ml of Ca (final concentration 1 mM) were added, and the absorbances at 750 nm (reading 1) and 660 nm (reading 2) were determined. Next, 0.2 ml of EGTA (final concentration 10 mM) were added and the absorbancy at 660 nm (reading 3) was again determined. The dye–Ca complex (= dye efflux from liposomes) was quantitated by subtracting reading 3 from reading 2 and by comparison with standard curves prepared with AIII in its Ca-form ± EGTA; these permitted detection of less than 2 nmol/ml of dye–Ca complex. Since addition of EGTA did not affect turbidity (apparent absorbance at 750 nm, or reading 1), the final absorbance decrement (2 − 3) measures dye leak independent of steroid-induced changes in turbidity. Comparison between the "single-beam" and the "twin-beam" methods (using various concentrations of liposomes and of detergent, >200 samples), showed the two to differ by less than 5% with respect to dye leak.

Uptake of aggIgM.L[AIII] by Phagocytes of *Mustelus canis*. Phagocytes and purified IgM of the smooth dogfish were obtained by procedures previously described (6). L[AIII] were collected after chromatography on Sepharose 4B in a total volume of 10 ml (4 μmol of lipid per ml) and centrifuged for 30 min at 100,000 × *g* in a Spinco model L ultracentrifuge. The pellet, now containing 40 μmol of lipid per ml, was incubated with either native or heat-aggregated (62°, 10 min) dogfish IgM for 30 min at 37°. Phagocytes (10^7) which had been permitted to adhere to glass slides for 30 min at 20° were exposed to 0.05 ml of IgM.L[AIII], aggIgM.L[AIII], or L[AIII] for various times. Both the number of cells which had formed phagocytic vacuoles and the number of vacuoles per cell were determined.

RESULTS

Spectroscopic Studies of Dye and Its Ca Complex. Absorption spectra of arsenazo III in the presence and absence of Ca are shown in Fig. 2A. Upon addition of Ca the peak absorbance shifted from 560 nm to two peak at longer wavelengths: 605 and 660 nm, with minimum absorbance by either form of the dye at 750 nm. The difference spectrum,

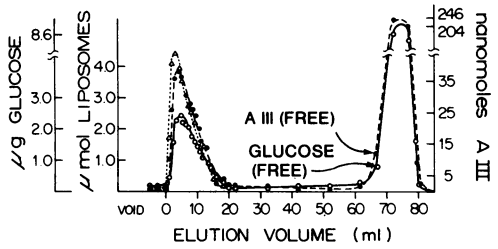

FIG. 3. Sepharose 4B chromatography of liposomes, L(PC 7:DCP 2:Chol 1)[AIII, glucose]. Suspensions (5 ml) were applied to the column (2.5 × 28 cm) and eluted with 0.145 M NaCl–KCl (equimolar) containing Hepes (5 mM) pH 7.4. Fractions from the column (0.5–1.0 ml) were collected and assayed for glucose (○——○), AIII (●– – ●), and lipid (△- - -△). AIII and glucose captured in liposomes were distinguished from the *free* solutes as described in the *text*.

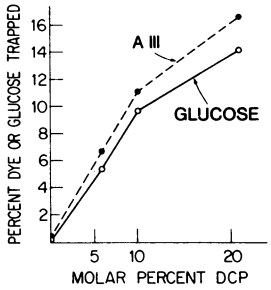

FIG. 5. Trapping of arsenazo III and glucose by liposomes as a function of molar percentage of charged components (dicetylphosphate, DCP). Liposomes were prepared with DCP in amounts varying from 0 to 20 molar percent. Liposomes were swollen in a solution containing glucose (0.29 M) and AIII (3 mM). Results are presented as percent of dye or glucose in the swelling solution which was trapped.

(AIII + Ca) − AIII, is shown in Fig. 2B. Although the absorbance difference was slightly greater at 605 nm, the absorbance of the uncomplexed dye was considerably less at 660 nm than at 605 nm.

Trapping of Dye in Liposomes: Chromatography. Exclusion chromatography by means of Sepharose 4B (Fig. 3) clearly resolved free dye from AIII associated with liposomes. Dye eluted together with glucose (dissolved in the aqueous compartments of multilamellar liposomes) both associated with liposomes and as free dye. Addition of Ca to fractions 1–20 did not produce the red → blue color shift until Triton X-100 was added; in contrast, Ca produced an immediate color change in fractions containing free dye. Percentage of AIII trapping (versus free dye) was 16.5 ± 3.2 (n = 4); glucose was 15.2 ± 4.1 (n = 4), data comparable to results with $CrO_4^=$, glycine, and glucose (1, 3, 5).

Latency of [AIII, Glucose]. Untreated and Triton-X-100-treated fractions (from exclusion chromatography) were reapplied to smaller columns of Sepharose 4B. Dye and glucose in untreated liposomes emerged in the void volume (Fig. 4). However, detergent-treated liposomes released AIII and glucose, which now emerged with elution patterns of free dye or glucose (Fig. 4). Therefore, Ca-induced changes in absorbance (i.e., red → blue color change) in detergent-treated samples of L[AIII, glucose] represent leakage of free dye across disrupted bilayers.

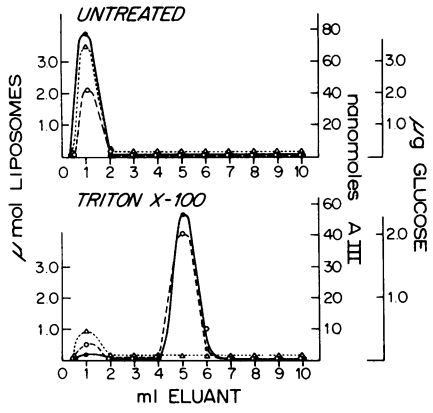

FIG. 4. Rechromatography of purified liposomes untreated and treated with detergent. Liposomes, L(PC 7:DCP 2:Chol 1)[AIII, glucose] were eluted from a Sepharose 4B column (first peak, Fig. 3). One portion was treated for 30 min with Triton X-100 (0.5%, vol/vol) at 23°. The *Triton X-100* treated sample and an *untreated* sample (1.0 ml each) were rechromatographed on Sepharose 4B columns (1.0 × 18 cm) and eluted with NaCl–KCl (pH 7.4 in Hepes) as before. Fractions (0.5–1.0 ml) were assayed for lipid (△- - -△), AIII (●——●), and glucose (○ – – ○).

Increments in AIII Trapping with Increments in Surface Charge. As the net surface charge on liposomes increases, so does the interlamellar volume (V_{H_2O}) available for trapping (2, 5, 6). When the molar percentage of dicetylphosphate (Fig. 5) was increased from 0 to 20 molar percent, increments in trapping of both AIII and glucose varied with the net anionic charge.

Spectroscopic Studies of Arsenazo III Entrapment by Liposomes. Absorption spectra were determined for suspensions of liposomes untreated and after sequential addition of Ca, Triton X-100, and EGTA (Fig. 6). Fig. 6A shows that addition of Ca to AIII-containing liposomes (L[AIII] + Ca) did not change the absorption spectrum for L[AIII]. At shorter wavelengths, Ca produced slight reductions in absorbance, presumably reflecting interactions of anionic liposomes with Ca (15). Fig. 6B shows the absorption spectrum for L[AIII] + Ca, and that of the same suspension after rupture of liposomes by Triton X-100. To demonstrate that the AIII–Ca complex in L[AIII] + Ca + TX-100 is free in solution, the effect was determined of an excess of EGTA. Fig. 6C shows that addition of EGTA (7.5 mM) reversed the characteristic absorption peaks of the AIII–Ca complex to that of the Ca-free dye.

Perturbation and Stabilization of Liposomes as Detected by Means of Arsenazo III. The spectrophotometric studies suggested that agents which perturb membranes less drastically than Triton X-100 might also provoke dye efflux from L[AIII]. Consequently, L[AIII] were treated with diethylstilbesterol (DES), a non-steroid hormone surrogate which possesses membrane activity (1, 3, 11), deoxycorticosterone (DOC), and etiocholanolone (Etio). Since addition of steroids in ethanol produced changes in apparent absorbance at 750 nm, dye efflux in these experiments was determined by the "single-beam" method, employing EGTA reversal (*Materials and Methods*). Each of the steroids produced dose-dependent (0.5–1.0 mM) increments in AIII efflux (Table 1). Kinetic studies with DOC and Etio indicated that leak of AIII was linear with respect to time for the first 15 min of incubation.

Since the liposome model can also be used to demonstrate *stabilization* of lipid bilayers (1, 3), we preincorporated 1 molar percent of hydrocortisone (HC) into liposomes. The spectrophotometric method employing AIII was as useful in demonstrating stabilization of liposomes (Table 1) by hydrocortisone as it was for demonstrating perturbation. Lipo-

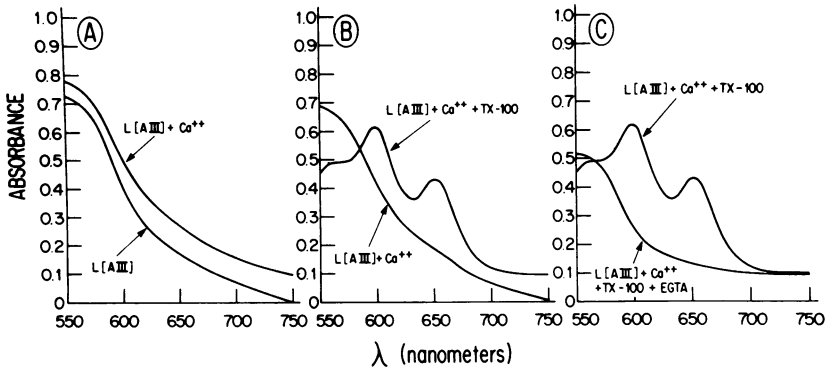

FIG. 6. Absorbance spectra of liposomes containing arsenazo III. Spectra were determined for untreated liposomes and for liposomes after sequential addition of Ca, Triton X-100, and EGTA. Liposomes containing dye were prepared and collected from a Sepharose 4B column (Fig. 3), and suspended in a solution containing KCl (0.145 M) and Hepes (5 mM) at pH 7.4 (0.2 ml of liposomes from the column + 2.0 ml of salt solution). (A) L[AIII] = untreated liposomes; L[AIII] + Ca = the same suspension of liposomes + $CaCl_2$ (0.5 mM); for clarity the plot of absorbance at 750 nm after addition of Ca was raised by 0.1 absorbance units. (B) L[AIII] + Ca = spectrum of liposomes + Ca redetermined; L[AIII] + Ca + TX-100 = the same suspension with Triton X-100 (0.3%, vol/vol). For the latter spectrum the absorbance at 750 nm was arbitrarily reset to 0.1. (C) L[AIII] + Ca + TX-100 = spectrum of liposomes + Ca + TX-100 redetermined; L[AIII] + Ca + TX-100 + EGTA = the same suspension with EGTA (7.5 mM). The absorbance at 750 nm was not reset for the second spectrum.

somes containing HC released on the order of 2-fold less dye in 15 min than did control liposomes exposed to perturbing steroids.

Detection of Ca Efflux from Liposomes. It was of equal interest to determine whether AIII could be used to measure Ca efflux from liposomes. Cationic liposomes: L(PC 7:SA 2:Chol 1)[Ca, glucose] were eluted from Sepharose 4B; the liposome-associated peak was found to have captured 12% of added Ca and 13% of glucose (Table 2). The bulk of Ca and glucose emerged as free solutes unassociated with liposomes. Peak fractions of liposomes were pooled, and Ca was determined by addition, sequentially, of AIII, Triton X-100, and EGTA. Addition of detergent to Ca-containing liposomes caused the expected red → blue shift of the indicator AIII, the decrement in reading 1 − 2 representing detergent-induced loss of turbidity *plus* increments in true absorbance of the dye–Ca complex. Addition of EGTA resulted in further absorbance decrements due only to reversal of the dye–Ca complex. In contrast, when detergent was added to Ca-containing fractions eluting after liposomes, no shift of the spectrum of AIII was observed. Again, as expected, EGTA caused a blue → red shift; residual absorbance of dye at 660 nm after EGTA represents only of free dye.

Uptake of aggIgM.L[AIII] by Phagocytes of *Mustelus canis*. Phagocytes (10^7) were incubated with 0.05 ml of L[AIII], IgM.L[AIII], and aggIgM.L[AIII] for periods up to 2 hr. By 30 min, over 70% of cells exposed to aggIgM.L[AIII] had developed three to four vacuoles approximately 10–14 μm in diameter, colored a pale red-purple. By 1 hr, close to 90% of cells had developed these inclusions; some cells (approximately 10–15%) had as many as five or six such vacuoles, and some of the vacuoles appeared distinctly bluish, suggesting access to dye of intracellular Ca. Degranulation of lysosomes into the vacuoles was regularly observed. Cells exposed to L[AIII] did *not* form vacuoles, and no more than 5–8% of cells exposed to IgM.L[AIII] formed red-purple vacuoles.

DISCUSSION

The data indicate that arsenazo III, which is potentially useful for the detection of changes in intracellular calcium activity, can be captured in aqueous interspaces of multilamellar liposomes. The change in absorption spectrum of AIII in the presence of Ca permitted use of L[AIII] in a sensitive method for measuring perturbation or stabilization of lipid bilayers. Liposomes were impermeable to external Ca until the integrity of bilayers was impaired. Dye efflux could, therefore, be measured either by determining *increases* in absorbance at 660 nm caused by formation of the dye–Ca

Table 1. Perturbation by steroid hormones of liposomes, and their stabilization upon preincorporation of hydrocortisone, as detected by means of arsenazo III

Additions to liposomes	Concentration (mM)	nmol AIII released at 15 min/μmol of lipid	
		Control liposomes*	Liposomes with 1% hydrocortisone†
None		10.4	8.7
Ethanol (solvent control)	(0.6%, vol/vol)	11.3	9.5
Diethylstilbesterol	0.5	35.6	27.8
	1.0	40.9	28.6
Deoxycorticosterone	0.5	15.8	10.3
	1.0	23.9	19.0
Etiocholanolone	0.5	13.3	10.3
	1.0	21.6	12.7

* L(PC 70:DCP 20:Chol 10) [AIII] containing 40.8 nmol of AIII per μmol of lipid. Mean of three experiments.
† L(PC 70:DCP 20:Chol 9:HC 1) [AIII] containing 40.4 nmol of AIII per μmol of lipid.

Table 2. Detection of Ca efflux from positively charged* liposomes by means of arsenazo III after Sepharose 4B chromatography

Reading no.	Additions† to L[Ca, glucose]	Δ Absorbance (660 nm)	nmol of Ca
	Liposome-associated peak (percent lipid: 100, glucose: 12, Ca: 13)		
1	AIII	2.67	—
2	AIII, TX-100	2.02	—
3	AIII, TX-100, EGTA	1.67	350
	"Free" peak (percent lipid: 0, glucose: 88, Ca: 87)		
1	AIII	2.90	—
2	AIII, TX-100	2.90	—
3	AIII, TX-100, EGTA	0.64	2260

* Liposomes were prepared as L(PC 7:SA 2:Chol 1) [Ca, glucose].
† AIII added at 1.7×10^{-4} M, Triton X-100 as 0.5% (vol/vol), EGTA at 10 mM.

complex, or by determining *decreases* in Ca-induced absorbance at 660 nm when excess EGTA dissociated the complex. The latter maneuver permitted rapid determinations of liposomal integrity in the face of changes in apparent absorbance (turbidity) induced by various perturbants. The dye method, using L[AIII], yielded results entirely comparable to less sensitive and more cumbersome procedures previously described (1, 3, 11). However, each of these earlier experiments required dialysis of liposomes, analyses both of dialysate and liposomes, and steroid concentrations of >5 mM (1, 3, 11), and did not permit direct spectrophotometric analysis of liposomes.

Two general methods have been previously employed to determine the perturbation of lipid bilayers by steroids, drugs, proteins, etc. In the *first*, leak or passive efflux of previously captured ions (1, 2, 11, 12), neutral molecules (2, 11, 12, 16), fluorochromes (17), or enzymes (5, 16) has been determined. Such studies yield information as to the functional "lesions" induced by various perturbants. The *second* method employs fluorescent (18) or spin-labelled (19) reporter molecules incorporated into liposomal bilayers. Spectral signals generated by the reporter molecules indicate changes in fluidity or motion in the immediate environment of the molecule (20), but have not generally (exception, ref. 21) determined the functional consequences to the vesicles of treatment with perturbants.

Both of the above general methods for the analysis of bilayer integrity have validated our earlier suggestion (1, 3, 11) that some steroid hormones or surrogates (e.g., DES) can enter into and perturb lipid bilayers (22). In contrast, hydrocortisone and its analogues stabilize liposomes as they do natural membranes (1, 3, 11), and we have suggested that some pharmacologic effects of these steroids may be mediated by this property (23); further such studies will be facilitated by the L[AIII] method.

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