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Alterations in the Organization of Phosphatidylcholine/Cholesterol Bilayers by Tetrahydrocannabinol*

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The interactions of Δ^9-tetrahydrocannabinol (THC) with various phosphatidylcholines (PCs) was studied in model membranes by differential scanning calorimetry. THC present in PC bilayers above a certain concentration complexed stoichiometrically with phospholipids containing both saturated and unsaturated fatty acids. When the bilayer PCs were sufficiently dissimilar for phase separation to occur, THC preferentially associated with the lower melting point lipid.

The presence of cholesterol below 20 mol % in dipalmitoylphosphatidylcholine bilayers enhanced THC·PC complex formation. Above 20 mol % cholesterol, there was no indication of THC·dipalmitoylphosphatidylcholine complex formation. This is in agreement with a phase rearrangement occurring in PC bilayers at concentrations of cholesterol of ~20 mol %.

These studies suggest several possible mechanisms for the modulation of membrane activities by hydrophobic drugs such as THC.

The cannabinoid THC[1] is a psychoactive drug (1) with a very high membrane/buffer partition coefficient (2, 3). Its mode of action is not known (1) but it is suggested to act in a similar fashion to the general anesthetics (2, 3). DSC of DPPC bilayers containing THC has been reported to give characteristic thermograms (4). These thermograms were different from those reported for PC bilayers containing the general anesthetics (5) and are suggestive of THC-induced phase separations or THC·PC complex formation or both (6).

This study was undertaken to explore the interaction of THC with membrane bilayers. In addition to serving as a model hydrophobic drug, it was hoped that THC might provide a DSC probe to aid in better understanding lipid-lipid interactions in membrane bilayers. It was of particular interest to use THC to investigate cholesterol/phospholipid interactions.

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[1] The abbreviations used are: THC, Δ^9-tetrahydrocannabinol; DSC, differential scanning calorimetry; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; PC, phosphatidylcholine; X_{THC}, mole fraction of THC against PC only; X_{chol}, mole fraction of cholesterol against PC only; ΔH, enthalphy of transition.

MATERIALS AND METHODS

DMPC, DPPC, DSPC, DOPC, egg yolk PC, and cholesterol were obtained from Sigma. All lipids were >99% pure as determined by thin layer chromatography and confirmed by an Iatroscan TH-10 TLC/FID (E. M. Becker Co., Bala Cynwyd, PA); they were used without further purification. The THC was a generous gift of Dr. Sumner Burstein (University of Massachusetts Medical School). THC, cholesterol, and all PCs were stored in solution in redistilled organic solvent under nitrogen. The precise concentration of each material was carefully determined before the preparation of each sample series by drying down known volumes of the solutions under vacuum (100 μM Hg) for 2 h at room temperature, and then weighing the residues. It was assumed that each mole of PC was hydrated with 1 mol of H_2O (7). To make up the PC, cholesterol, and THC mixtures, appropriate aliquots of solutions of each were mixed with extra chloroform added to ensure complete solubilization of all components. These solutions were then placed on clean, glass microscope slides and dried down overnight under vacuum (100 μM Hg) at room temperature. The dried lipids with cholesterol or THC or both were then scraped off the slide with a clean razor blade and placed in aluminum sample pans for DSC. Average sample mass was about 1 mg. Concentrations of THC and cholesterol in the PC bilayers, expressed as mole fractions against PC (X_{THC} and X_{chol}, respectively), are dependable to within ±5%. After weighing the sample, 5 μl of water were added to completely hydrate the lipids. When DOPC or egg yolk PC was one of the component lipids, 7 μl of a 1:1 (v/v) solution of ethylene glycol and water (50% ethylene glycol/H_2O) were added instead of pure water. The use of 50% ethylene glycol/H_2O instead of pure water eliminates the ice melt over the temperatures of interest and does not significantly affect the calorimetric scans of the PCs (8). The sample pans were hermetically sealed, and the encapsulated samples were scanned immediately or occasionally within 2-3 days, during which time the samples were kept at 4 °C. No difference in thermal behavior was observed for 1-month-old samples kept at −20 °C. Prior to scanning, samples were held above their transition temperature for 1-2 min to ensure complete hydration. All samples were scanned at least twice and the thermograms compared for any differences. In all cases, the rescans were identical with the original scan although the ΔH might vary slightly for samples having high X_{THC}. DSC was carried out on a DuPont 1090 Thermal Analysis System (DuPont, Wilmington, DE). For temperature calibration, benzene (m.p. 5.5 °C) and naphthalene (m.p. 80.3 °C) were used. Peak areas were determined using the DuPont Advanced DSC V1.0 program. Indium (ΔH = 28.4 J/g) was used to calibrate peak areas. Scans were usually performed at scan rates of 5 or 10 °C/min. Occasionally, scans were made as slowly at 1 °C/min.

RESULTS

Effect of THC on Saturated Single Species PC Bilayers—All bilayers formed from single disaturated PC species containing THC showed similar phase properties by DSC. The behavior of DPPC was studied most intensively, and its calorimetric scans, as well as the phase behavior deduced from these scans, are representative of the other disaturated PC species. Thermograms of DPPC as X_{THC} increases from 0 to 1.00 are shown in Fig. 1. For pure DPPC (X_{THC} = 0), the pretransition is centered at 35 °C and the onset of the main transition occurs at 41 °C. The only effect of low concentrations of THC in DPPC bilayers is to eliminate the pretran-

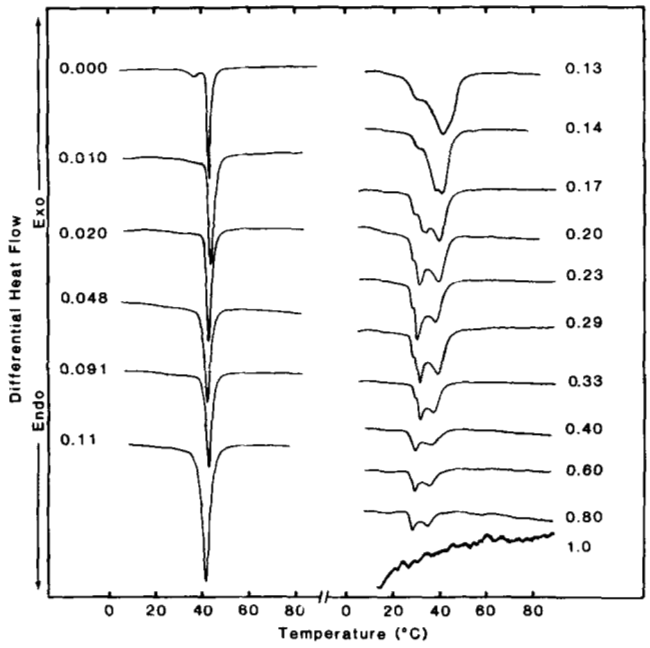

FIG. 1. **Increasing THC in DPPC bilayers.** Thermograms are of DPPC bilayers with increasing concentrations of THC. The scaling of the thermograms is not precise, but represents approximately equal amounts of DPPC. The mole fraction of THC is indicated *beside* each thermogram. The sensitivity of the thermogram of THC (X_{THC} = 1.00) is about 20 times greater than the other thermograms. All scans were performed at 10 °C/min, except for the thermogram of THC, which was performed at 5 °C/min.

sition. At $X_{THC} = 0.048$, the main transition begins to broaden while the onset temperature is essentially unchanged. At $X_{THC} = 0.11$, the onset of the transition moves down in temperature and the appearance of a new peak around 30 °C is just visible. This new peak appears as a shoulder on the main transition at $X_{THC} = 0.13$, while the area of the main transition broadens considerably. As X_{THC} increases to 0.14, the main peak of the transition begins to split into two peaks, both slightly lowered in temperature compared to the previous single peak. As X_{THC} increases to 0.23, the lower temperature peak (or shoulder), centered at 30 °C, does not change significantly. It appears less distinct, however, because both higher temperature peaks are lowered in temperature with the middle peak becoming the largest and most clearly defined. Above $X_{THC} = 0.23$, there is essentially no change in the thermograms until $X_{THC} = 1.00$. THC itself goes through no transition in the temperature range studied, so the scan of $X_{THC} = 1.00$ is flat, with only some noise apparently contributed by the presence of THC.

The only other significant trend in this series is ΔH. Although the ratios of ΔH for the different peaks within a given thermogram are always highly reproducible, values for total ΔH at high X_{THC} could vary by as much as ±5 J/g. A trend in total ΔH is nonetheless clearly apparent as THC concentration in the bilayer is increased. Up to $X_{THC} = 0.23$, ΔH does not change significantly but remains at 49.5 J/g of DPPC. Above $X_{THC} = 0.23$, ΔH falls slowly until at $X_{THC} = 0.90$, ΔH = 25 J/g of DPPC.

The transition of a THC/DPPC mixture is not isothermal but takes place over a range of temperature. Scans at 1 °C/min differ from scans at 5 or 10 °C/min only in endotherms with slightly higher end temperatures. Even at slow scan rates, scans never return to the base-line during the transition. Downscans performed on samples at the same rate as upscans are virtually identical with the upscans.

A temperature-composition phase diagram for the THC/DPPC system is presented in Fig. 2. The data are from scans of THC/DPPC mixtures taken at 10, 5, or 2 °C/min. While the starting temperatures of the transitions are independent of scan rate, the end temperatures differ slightly. A correction has been made for these end temperatures by subtracting from the end of the transition of a THC/DPPC mixture obtained at a given scan rate the temperature range of the main temperature of pure DPPC bilayers obtained at the same scan rate (9).

The outstanding feature of all the THC/PC systems studied is the appearance of a new, sharp peak with increasing THC. A scan of a THC/DMPC mixture is shown in Fig. 3. The low temperature peak usually present as a shoulder in THC/DPPC mixtures is completely obscured in THC/DMPC mixtures. All other features of the transition are identical with those of THC/DPPC mixtures. A temperature-composition phase diagram for the THC/DMPC system (not presented) was constructed from the calorimetric data and is similar to the phase diagram for the THC/DPPC system. The behavior of THC/DSPC mixtures does not differ from THC/DMPC mixtures or THC/DPPC mixtures (data not presented).

Effect of THC on DOPC Bilayers—THC added to bilayers of DOPC gives thermograms differing from those obtained with saturated chain PCs. Fig. 4 presents scans of THC/DOPC mixtures. When THC is added to DOPC bilayers, the original transition is broadened and its onset shifted down in temperature from an initial onset of −22 °C. At $X_{THC} = 0.091$, the broadening of the endotherm and downshift in onset is seen to be the result of a new peak which at $X_{THC} = 0.17$ is clearly visible as a shoulder on the original peak. Up to $X_{THC} = 0.091$, the original peak gradually moves down in temperature. The new peak is centered at −19.5 °C and with increasing X_{THC} continues to grow at the expense of the original peak. At $X_{THC} = 0.33$, only one peak remains, that centered at −19.5 °C. Above $X_{THC} = 0.33$, the appearance of the transition does not change. During this series, ΔH decreases slowly from 67 J/g of DOPC for pure DOPC to 51 J/g of DOPC when $X_{THC} = 0.33$.

Effect of THC on Mixed PC Bilayers—The effect of THC on bilayers composed of mixed PCs agrees with our findings on single species PC bilayers. Thermograms for THC added to bilayers composed of a 1:1 (mole:mol) mixture of DPPC and DSPC are shown in Fig. 5. With no THC added, the mixed saturated chain PC bilayers act very much like bilayers made of a single saturated chain PC species. A pretransition is present, centered about 6 °C below the main transition. The main transition has an onset at 45.5 °C and is somewhat broader than that obtained from bilayers composed of a single PC species. When X_{THC} is increased to 0.091, the pretransition disappears and the main transition onset is lowered. This broadening is the result of a new peak. When $X_{THC} = 0.17$, the new, sharp peak is obvious and, as in the THC/DPPC system, a low temperature shoulder is present. Except for temperature, the thermogram of $X_{THC} = 0.23$ DPPC/DSPC bilayers is indistinguishable from that of the same concentration of THC in DPPC bilayers (Fig. 1). Above $X_{THC} = 0.23$, the transition does not change. The ΔH remains constant through the series (at least until $X_{THC} = 0.33$): about 50 J/g of PC, the same as the ΔH of the pure DPPC/DSPC bilayer.

Bilayers of a 1:1 (mole:mol) mixture of DOPC and DSPC yield different results (Fig. 6). Pure DOPC/DSPC bilayers show a single transition extending from −22 °C to 51 °C. The peak at −13 °C results from the melting of a molecular population highly enriched in DOPC, while the peak at 46 °C is that of a population enriched in DSPC. The addition of THC to these bilayers strongly affects the low temperature peak. When $X_{THC} = 0.17$, the low temperature peak is split into two

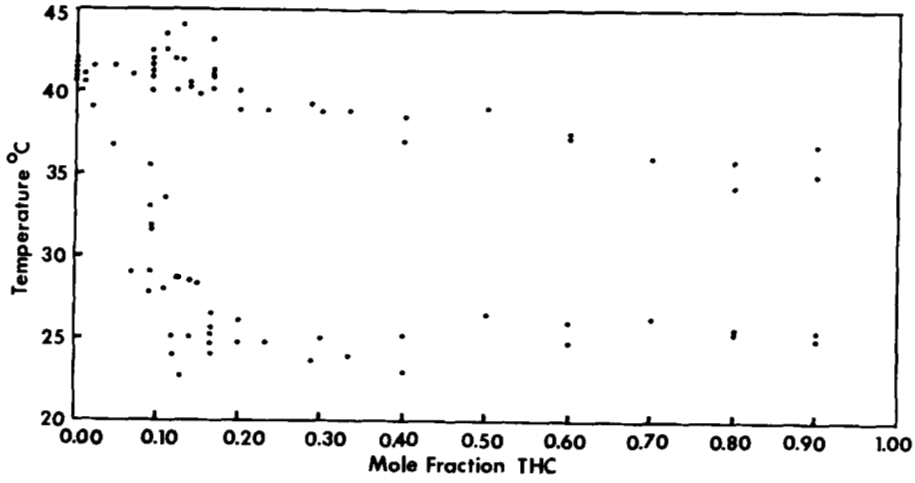

FIG. 2. **Temperature-composition phase diagram of DPPC and THC.**

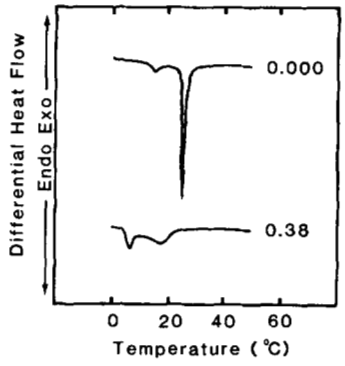

FIG. 3. **THC in DMPC bilayers.** Thermograms are of DMPC bilayers containing or not containing THC. See Fig. 1 for details. Scans were performed at 5 °C/min.

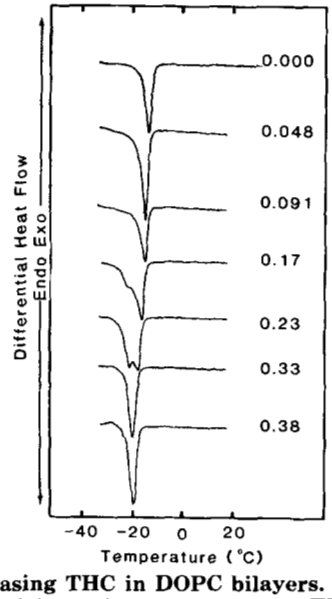

FIG. 4. **Increasing THC in DOPC bilayers.** Thermograms of DOPC bilayers with increasing concentrations of THC. See Fig. 1 for details. Scans were performed at 5 °C/min.

peaks, one centered at the temperature of the original peak, the other centered at −20 °C. The effect is equivalent to THC added to pure DOPC at $X_{THC} = 0.23$. It appears that most of the THC associates with the DOPC (about 75% of the THC) rather than partitioning equally between the DOPC and the DSPC. The effect of the THC not associated with the DOPC-rich phase can be seen in the approximate 3 °C depression of the upper peak. When $X_{THC} = 0.29$, there is only one low

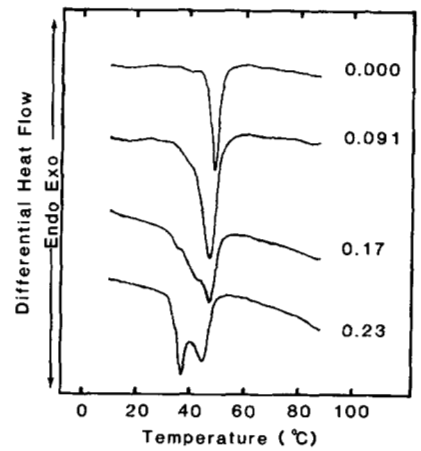

FIG. 5. **Increasing THC in DPPC/DSPC bilayers.** Thermograms are of 1:1 (mole/mol) DPPC/DSPC bilayers with increasing concentrations of THC. Other details are the same as in Fig. 1. Scans were performed at 10 °C/min.

temperature peak centered at −19 °C. The high temperature peak remains as one broad peak, but the temperature depression is large. Although THC above $X_{THC} = 0.29$ in DOPC bilayers produces no change in the transition, when THC is added to DOPC/DSPC mixture above $X_{THC} = 0.29$, the low temperature peak decreases in size. This is obvious when $X_{THC} = 0.44$. Still, the only effect on the upper peak is the temperature depression, which at this point is about 15 °C lower than is the DOPC/DSPC mixture with no added THC.

The upper peak of the DOPC/DSPC system is broad, representing a continuously changing ratio of unmelted DOPC and DSPC. Another heterogeneous PC system containing both saturated and unsaturated fatty acids is egg yolk PC. This natural mixture has a single, broad transition centered at −5 °C. When THC is added to this bilayer system, the only effect is to lower the temperature of the transition by about 8 °C (see Fig. 7).

Effect of Cholesterol on PC Bilayers Containing THC—Adding increasing amounts of cholesterol to THC-containing DPPC bilayers significantly affects the calorimetric behavior of the bilayers (Fig. 8). There are two aspects to this, $X_{chol} < 0.20$ and $X_{chol} > 0.20$. Low concentrations of bilayer cholesterol, $X_{chol} < 0.20$, enhance the usual effects of THC. Fig. 8 presents scans of DPPC bilayers containing THC to which increasing amounts of cholesterol were added. In this series, $X_{THC} = 0.20$; the behavior of THC and DPPC with no added cholesterol was previously described. Addition of a small amount of cholesterol greatly enhances the THC effect (com-

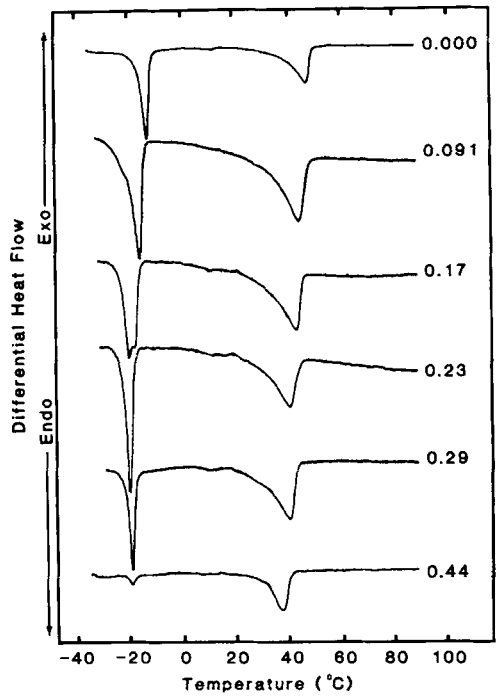

FIG. 6. **Increasing THC in DOPC/DSPC bilayers.** Thermograms are of 1:1 (mole/mol) DOPC/DSPC bilayers with increasing concentrations of THC. Other details are the same as in Fig. 1. Scans were performed at 5 °C/min.

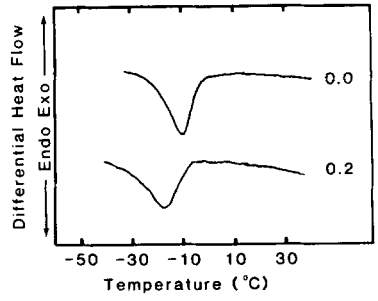

FIG. 7. **THC in egg yolk PC bilayers.** Thermograms are of egg yolk PC bilayers that do or do not contain THC. The indicated mole fraction of THC is approximate. A molecular weight of about 750 was assumed for the egg yolk PC. See Fig. 1 for other details. Scans were performed at 5 °C/min.

pare Fig. 1). At $X_{chol} = 0.048$, a large, sharp peak centered at 29 °C is superimposed on a broad peak centered at 38 °C. The broad peak apparently derives from the original bilayer transition, and the sharp peak is attributed to an association between THC and DPPC. The presence of cholesterol depresses the temperature of the sharp peak while only moving the center of the broad peak slightly down in temperature. No further significant changes take place until $X_{chol} = 0.23$ where the broad peak begins to increase in temperature. At $X_{chol} = 0.20$, the broad peak is centered at 38 °C. As X_{chol} increases to 0.23, it increases in temperature until it is centered at 43 °C. Above $X_{chol} = 0.23$, the remnants of the sharp peak disappear. At $X_{chol} = 0.33$, only a broad peak centered at 43 °C is present. This is true even in the presence of $X_{THC} = 0.23$.

The effect of increasing cholesterol concentration in THC/DPPC bilayers is not restricted to DPPC bilayers at $X_{THC} = 0.20$. As an example, Fig. 9 presents a similar experiment with DPPC bilayers at $X_{THC} = 0.091$. For comparison, a scan of a DPPC bilayer mixture at $X_{THC} = 0.091$ and no cholesterol may be found in Fig. 1. Again, both cholesterol effects are

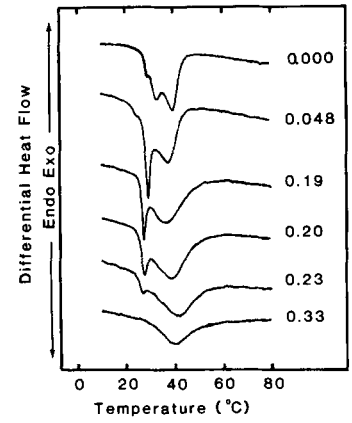

FIG. 8. **Increasing cholesterol in THC/DPPC bilayers.** Thermograms are of THC/DPPC bilayers ($X_{THC} = 0.20$) with increasing concentrations of cholesterol. The mole fraction of cholesterol is expressed *beside* each thermogram. In the thermogram of $X_{chol} = 0.33$, the molar fraction of THC is $X_{THC} = 0.23$. The mole fractions of THC and cholesterol are expressed against PC only. Other details are the same as in Fig. 1. Scans were performed at 5 °C/min.

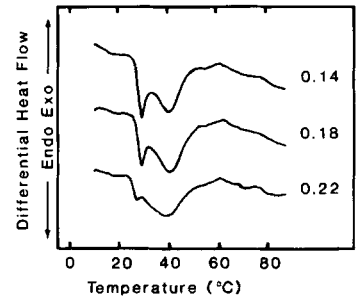

FIG. 9. **Increasing cholesterol in THC/DPPC bilayers.** Thermograms are of THC/DPPC bilayers ($X_{THC} = 0.091$) with increasing concentrations of cholesterol. See Figs. 1 and 8 for other details. Scans were performed at 5 °C/min.

present: enhancement of the THC effect at low cholesterol concentrations and elimination of the THC effect when $X_{chol} = 0.20$. Differences between the series of mixtures containing $X_{THC} = 0.091$ compared to the series containing $X_{THC} = 0.20$ can be attributed to the lower concentration of THC. For instance, a cholesterol concentration of $X_{chol} = 0.14$ is necessary in the $X_{THC} = 0.091$ series before complete enhancement of the THC effect occurs. In addition, the sharp, low temperature peak resulting from the presence of THC is not as large as that in the series containing $X_{THC} = 0.20$. However, all other calorimetric aspects of this series containing $X_{THC} = 0.091$ are identical with the series containing $X_{THC} = 0.20$ upon addition of cholesterol.

DISCUSSION

Our thermograms of THC/DPPC bilayers agree well with an earlier published work (4). A few points stand out for bilayer systems containing various individual PC species. 1) The addition of THC broadens the main transition and gradually lowers its temperature. 2) The addition of THC creates a sharp, distinct peak several degrees below the original main transition. 3) The ΔH of the entire transition is equal to or less than that of the main transition of pure PC. 4) Above a certain characteristic mole fraction of THC, the appearance of the transition does not change significantly.

When THC is present in PC bilayers at low concentration (*i.e.* $X_{THC} < 0.10$), it acts much as an impurity; eliminating the pre-transition, broadening the main transition, and lowering its onset temperature. At higher concentrations, how-

ever, adding THC to PC bilayers is similar to adding a lower melting lipid, resulting in bilayers with some degree of solid-solid immiscibility (9). This behavior is best described by a temperature-composition phase diagram as shown in Fig. 2 (for reviews, see Refs. 10 and 11) for the THC/DPPC system. Here the added component is THC, and the transition temperature of the lower melting species is centered 8 °C below the main transition peak. The shape of the phase diagram indicates some degree of solid-solid immiscibility. If the first component is DPPC, the second may be some association or complex of THC and DPPC in the bilayer, and this contributes the sharp middle peak in the calorimetric scans. This second component cannot be pure THC, since THC itself has no transition at that temperature (Fig. 1). Furthermore, in all cases, the second peak resulting from an addition of THC to any PC species is always centered 3–10 °C below the PC main transition.

If there is a simple association or complex between THC and PC as X_{THC} increases, one might expect the higher temperature peak remaining from the transition of pure PC bilayers to disappear at the expense of the low, sharp peak. This does not happen with the saturated PCs studied up to $X_{THC} = 0.90$. Above $X_{THC} = 0.23$, thermograms of THC/DPPC do not change shape, although the heat of the transition decreases. A possible explanation for this is that above $X_{THC} = 0.23$ the bilayers are saturated and more THC does not enter the bilayers (15). It is possible that the excess of THC removes some of the DPPC from a bilayer conformation, thus explaining the decrease in ΔH.

Bilayers formed from pure DOPC to which THC has been added behave somewhat differently from THC containing bilayers composed of pure saturated PCs. When the bilayers are formed from DOPC at $X_{THC} = 0.29$, the only peak remaining is the lower one, presumably a THC·DOPC complex. At $X_{THC} = 0.29$, the molar fraction of the complex to DOPC is $X_{complex} = 1.00$. The addition of THC above this concentration produces no visible effect on the calorimetric scans.

THC has a high membrane/water partition coefficient (2, 3). Our studies suggest that THC locates at or near the membrane interface since the behavior of THC/PC depends little on the nature of the PC's acyl chains (also see Ref. 5). This is obvious for single PC species' bilayers and is consistent with the behavior of the mixed PC bilayers. The localization of THC at the membrane interface is most likely due to its phenol group. Differences in lateral packing may be enough to explain the difference between DOPC bilayers and saturated PC bilayers when X_{THC} is high; otherwise, their behavior is quite similar.

DPPC and DSPC mixed in a 1:1 molar ratio form a bilayer system that behaves like a bilayer formed from a single PC species. This is no doubt due to the similarity of the two species of molecules. When THC is added, the usual effect is seen. THC thus does not associate preferentially with one lipid species over another. If THC preferentially associated with one of the two component lipids, thermograms would show the emergence of an endotherm at the transition temperatures of the noncomplexed lipids. In contrast, Fig. 6 shows the thermogram of a 1:1 (mole/mol) mixture of DOPC and DSPC. The transition temperatures of pure DOPC and pure DSPC are widely separated (onset temperature of DOPC = −22 °C; onset temperature of DSPC = 54 °C), and in a 1:1 mixture there is partial solid-solid immiscibility. In this mixture, the lower peak is greatly enriched in DOPC and hence, is sharp and centered near the transition temperature of pure DOPC. A temperature-composition phase diagram constructed for DOPC/DSPC would be similar to the one in Fig. 2; the solidus would be a nearly horizontal line extending over a wide range of DOPC/DSPC composition (see Refs. 9 and 12–14 for similar examples; also Ref. 11). In DOPC/DSPC bilayers, it appears that THC preferentially associates with DOPC. It cannot be stated whether this is an actual specific preference of the THC molecule for DOPC in a fully fluid bilayer or a consequence of the bilayer transition. That is, as DSPC-enriched membrane domains solidify, the process excludes THC from the gel phase into fluid DOPC-enriched areas.

Although the interaction of THC with PC bilayers is interesting in itself, THC may be used as a probe to elucidate the behavior of cholesterol in bilayers. Recent evidence supports the occurrence of an alteration of the physical properties of PC/cholesterol bilayers at $X_{chol} = 0.20$ (16–19). Below and above $X_{chol} = 0.20$, different types of cholesterol/PC associations appear to exist. It is suggested (16, 18, 19, 20) that below $X_{chol} = 0.20$ a two-phase system exists, one phase being pure or nearly pure DPPC and the other phase being a 4:1 association of DPPC/cholesterol. Above $X_{chol} = 0.20$, some sort of organizational change or phase change occurs within the bilayer and cholesterol is incorporated into the bilayer nonstoichiometrically (21). Our studies with THC in DPPC bilayers containing different X_{chol} support these ideas. For example, low concentrations of cholesterol enhance the THC effects. Cholesterol, by associating with DPPC, removes that DPPC from possible interaction with THC, thereby increasing the effective concentration of THC with respect to unassociated DPPC. In our study of THC/DPPC bilayers, it was found that the THC effect is complete and constant above $X_{THC} = 0.23$ (Fig. 1). When $X_{THC} = 0.20$ in DPPC bilayers, the concentration of cholesterol required to fully bring about the THC effect was $X_{chol} = 0.048$. If, when $X_{chol} < 0.20$, cholesterol associates with four DPPC molecules, then the concentration of THC in DPPC not associated with cholesterol is $X_{THC} = 0.24$. When $X_{THC} = 0.091$ in DPPC bilayers, the X_{chol} needed for full enhancement was 0.14. This gives an effective concentration of THC in free DPPC of $X_{THC} = 0.22$.

When $X_{chol} > 0.20$, THC no longer produces its usual effects; not only does the sharp, low temperature peak disappear, but the broad peak moves up in temperature to 43 °C. The resulting transition is characteristic of DPPC bilayers that contain $X_{chol} > 0.20$ with no THC present (16, 18, 19). This may be a consequence of a new phase, composed of associated cholesterol and DPPC. In any case, it is clear that the bilayer organization changes abruptly when the concentration of cholesterol in the bilayer reaches $X_{chol} = 0.20$.

The behavior of cholesterol in PC bilayers has been studied intensively but is still not understood. This study is in agreement with a 4:1 DPPC·cholesterol complex when $X_{chol} < 0.20$ (16, 18, 19). At $X_{chol} = 0.20$, our results indicate that a reorganization of membrane lipids, or even a phase change takes place. What sort of change this is cannot be said, but there can be little doubt of its occurrence.

The mode of action of THC is unknown. Since THC has a high membrane/water partition coefficient, an association between THC and membrane lipids may be biologically significant. A complex between THC and a specific membrane lipid could serve as a substrate for enzyme action, initiating a cascade of events. Alternatively, the association of THC with bilayer lipids may somehow alter membrane organization, for instance alter membrane domains (22, 23). While model bilayer studies are not necessarily germane to native membrane systems, our results suggest specific possibilities for the *in vivo* mode of action of THC. The effect of cholesterol is particularly interesting. If THC·phospholipid complex formation is important for the action of THC, then high membrane cholesterol concentrations could block or modulate

THC activity, while low cholesterol concentrations would enhance THC activity. Alternatively, if THC·phospholipid complex formation inhibits THC action, high concentrations of cholesterol by preventing THC·phospholipid complex formation could enhance its activity by allowing it to act as a free molecule. The concentrations of THC used in this study may or may not be relevant to *in vivo* action since physiological concentrations within specific cells are unknown. Further, THC could preferentially segregate into specific membrane domains to produce high local concentrations of THC.

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REFERENCES

1. Burstein, S., and Hunter, S. (1981) *Rev. Pure Appl. Pharmacol. Sci.* **2,** 155–226
2. Roth, S. H., and Williams, P. J. (1979) *J. Pharm. Pharmacol.* **31,** 224–230
3. Seeman, P., Chau-Wong, M., and Moyyen, S. (1972) *Can. J. Physiol. Pharmacol.* **50,** 1193–1200
4. Bach, D., Raz, A., and Goldman, R. (1976) *Biochim. Biophys. Acta* **436,** 889–894
5. Jain, M. K., and Wu, N. M. (1978) *J. Membr. Biol.* **34,** 157–201
6. McElhaney, R. N. (1982) *Chem. Phys. Lipids* **30,** 229–259
7. Hauser, H., and Phillips, M. C. (1979) *Prog. Surf. Membr. Sci.* **13,** 297–413
8. Ashe, G. B., and Steim, J. M. (1971) *Biochim. Biophys. Acta* **233,** 810–819
9. Maybrey, S., and Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 3862–3866
10. Lee, A. G. (1977) *Biochim. Biophys. Acta* **472,** 285–344
11. Melchior, D. L., and Steim, J. M. (1979) *Prog. Surf. Membr. Sci.* **13,** 211–296
12. Phillips, M. C., Ladbrooke, B. D., and Chapman, D. (1970) *Biochim. Biophys. Acta* **196,** 35–44
13. Shimshick, E. J., and McConnell, H. M. (1973) *Biochemistry* **12,** 2351–2360
14. Wu, S. H., and McConnell, H. M. (1975) *Biochemistry* **14,** 847–854
15. Lawrence, D. K., and Gill, E. W. (1975) *Mol. Pharmacol.* **11,** 595–602
16. Mabrey, S., Mateo, P. L., and Sturtevant, J. M. (1978) *Biochemistry* **17,** 2464–2468
17. Rubenstein, J. L. R., Owicki, J. C., and McConnell, H. M. (1980) *Biochemistry* **19,** 569–573
18. Estep, T. N., Mountcastle, D. B., Biltonen, R. L., and Thompson, T. E. (1978) *Biochemistry* **17,** 1984–1989
19. Melchior, D. L., Scavitto, F. J., and Steim, J. M. (1980) *Biochemistry* **19,** 4828–4834
20. Rechtenwald, D. J., and McConnell, H. M. (1981) *Biochemistry* **20,** 4505–4510
21. Presti, F. T., Pace, R. J., and Chan, S. I. (1982) *Biochemistry* **21,** 3831–3835
22. Klausner, R. D., and Kleinfeld, A. H. (1982) in *Cell Surface Phenomena* (Delise, K., Wiegel, F., and Perelson, A. S., eds) Marcel Dekker, Inc., New York
23. Wolf, D. E. (1983) in *Development in Mammals*, (Johnson, M. H., ed) Vol. 5, Elsevier/North-Holland, New York