PHASE TRANSITIONS IN LIPID MEMBRANES INDUCED BY CARCINOGENIC AROMATIC HYDROCARBONS. A SPIN LABEL STUDY.

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SUMMARY: The interaction between a series of condensed aromatic hydrocarbons of different size and shape (with and without carcinogenic activity) with dipalmitoyl lecithin bilayers was investigated by the spin label method. The spectra of a cholestane spin probe in the bilayers were examined. The smaller non-carcinogenic hydrocarbons did not alter the degree of organization of the phospholipid molecules to a large extent. On the other hand, large, carcinogenic aromatic hydrocarbons interacted with the bilayers, promoting a gel to liquid crystal phase transition. These data indicate that the two types of molecules interact with the membrane in different ways.

There have been a number of discussions as to what are the molecular requirements for the carcinogenic activity of condensed aromatic hydrocarbons* (1). Explanations based on molecular size and shape, electron densities, molecular asymmetry, and combinations of these factors have been advanced. Numerous models assume that the mechanism of carcinogenesis involves intercalations or inclusion of these componds into hydrophobic regions of macromolecular cellular components such as nucleic acids and proteins. Evidently, one of the first steps in the mechanism of carcinogenesis is the penetration of the HC into the cell, which necessarily involves passage through the cellular membrane. It has been shown that permeation of the HC's into the cells obeys the law of passive diffusion (2). This strongly suggests that the medium traversed by these compounds could be

* Abbreviations used in this paper: HC-hydrocarbon, DPL-dipalmitoyl lecithin, esr - electron spin resonance, CSL-4',4'-dimethyl-N-oxyl oxazolidine derivative of 3-cholestanone.

the lipid bilayer of the membranes. In view of this possibility, we undertook the investigation of the interaction between aromatic condensed HC's and a phospholipid model membrane - dipalmitoyl lecithin. Previous studies report on the interaction of aromatic carcinogens with lipids in monomolecular films. For references concerning these studies, and also of these and other carcinogens with model and biological membranes, see (3).

The spin label method (4) has been extensively used in the study of the degree of motion and orientation of molecules in membranes (5). The esr spectra of a spin probe, CSL, in DPL bilayers to which varying amounts of aromatic HC's were added indicated a sharp difference between carcinogenic and non-carcinogenic compounds, the former being capable of inducing a gel to liquid crystal phase transition in DPL.

MATERIALS AND METHODS: DPL was purchased from Sigma Chemical Co., St. Louis, Missouri and used without further purification. 1,2-benzanthracene, 9-methylanthracene and 9,10-dimethyl-1,2-benzanthracene were obtained from Eastman Organic Chemicals, Rochester, N.Y. Naphtacene, 1,2,5,6-dibenzanthracene, 3,4-benzopyrene, and 20-methylcholanthrene were from L. Light & Co. Ltd., Colnbrook, England. Naphtalene, anthracene, pyrene, and phenanthrene were purified according to standard procedures (M.P.'s 79-80, 217-218, 156, and 99-100°C, respectively). CSL was from Syva, Palo Alto, Calif. DPL bilayers were prepared on the surfaces of flat aqueous esr cells (James Scanlon, Solvang, Calif.) as described by Smith (6). Chloroform solutions containing phospholipid, probe, and the amount of HC to give the desired DPL:HC ratio were evaporated under a stream of N_2, and dried under vacuum for 2 h. Samples were hydrated with 0.15 M NaCl. Spectra were run after 1 h, with the plane of the bilayers either parallel or perpendicular to the applied magnetic field. A Varian E-4 esr spectrometer was used, at room temperature (22 ± 2°C).

RESULTS: Fig. 1 shows the HC's employed in the present work in order of increasing size and approximate increasing carcinogenic potency. Thus, 1,2-benzanthracene is a very weak carcinogen, whereas the following compounds are much stronger ones.

The HC's were intercalated in DPL bilayers at compositions of

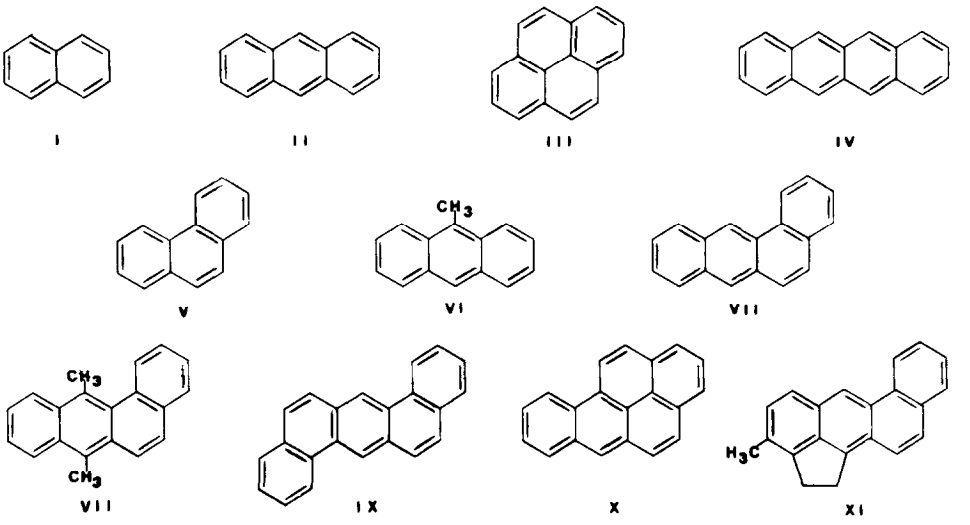

Fig. 1 - Aromatic hydrocarbons used in the present work: I- naphtalene, II- anthracene, III- pyrene, IV- naphtacene, V- phenanthrene, VI- 9-methylanthracene, VII- 1,2-benzanthracene, VIII- 9,10-dimethyl-1,2-benzanthracene, IX- 1,2,5,6-dibenzanthracene, X- 3,4-benzopyrene, XI- 20-methylcholanthrene.

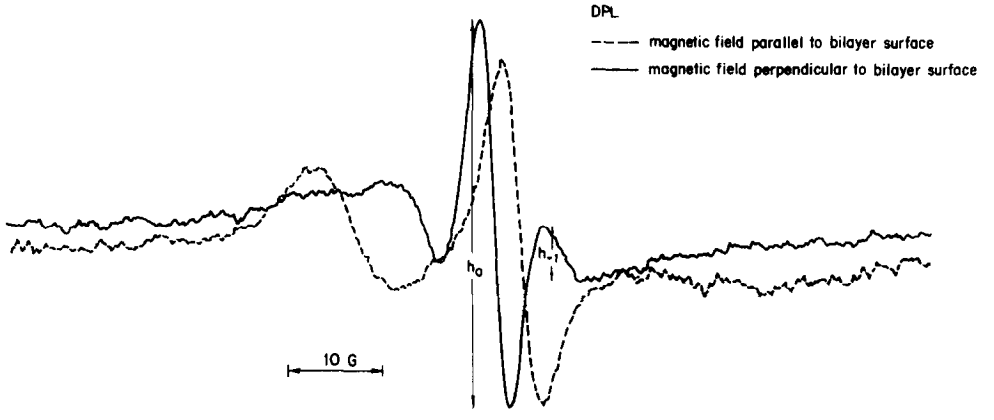

Fig. 2 - Spectra of CSL in DPL bilayers. Magnetic field parallel (---) and perpendicular (——) to bilayer surface.

5, 10, 15, 20 and 25 mole %. The spectra of CSL in DPL alone present a very low degree of anisotropy (Fig. 2), that is, very little difference is observed between the spectra taken with the magnetic field parallel and perpendicular to the surface of the

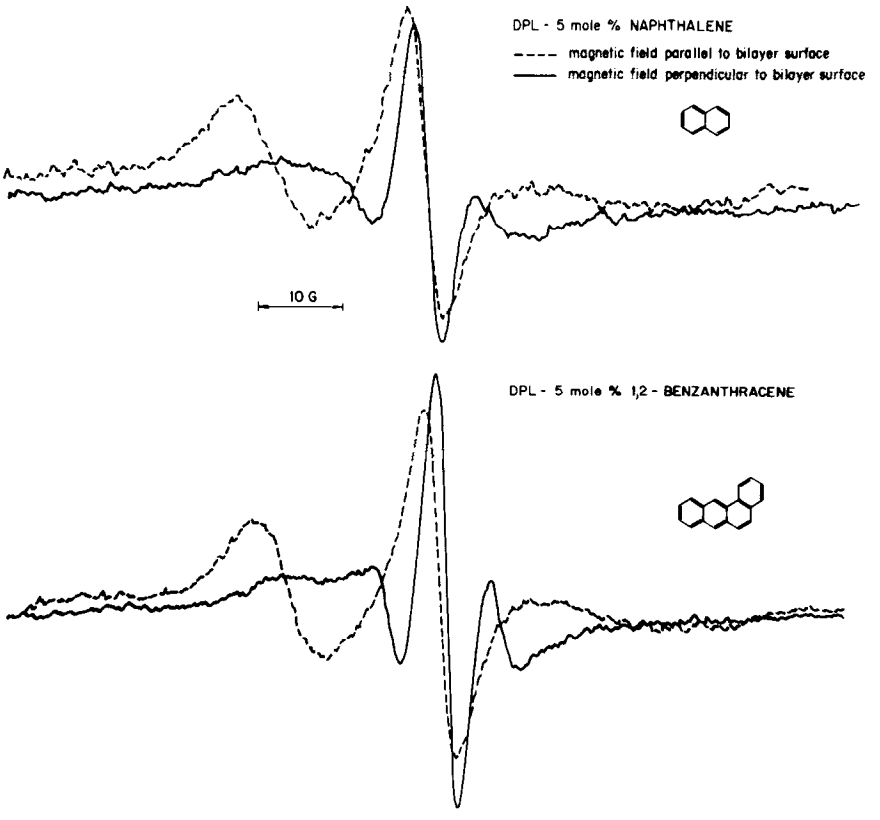

Fig. 3 - Spectra of CSL in DPL bilayers containing 5 mole % of naphtalene (top) and 1,2-benzanthracene (bottom). Magnetic field parallel (---) and perpendicular (——) to bilayer surface.

bilayers. The degree of anisotropy in the spectra was evaluated by the ratios h_{-1}/h_o (Fig. 2). The larger h_{-1}/h_o, the higher the degree of anisotropy in the spectra. The interpretation of spectra similar to the present ones is fully discussed in (7).

The small HC's caused virtually no change in the spectra of CSL when compared to DPL alone. Fig. 3 illustrates the results for 5 mole % of naphtalene and 1,2-benzanthracene. As the size of the HC increased, a larger degree of anisotropy was obtained (large h_{-1}/h_o). This can be clearly seen in Fig. 4 for 5 mole % of 9,10-dimethyl-1,2-benzanthracene and 20-methyl cholanthrene. The order of increasing h_{-1}/h_o ratios was: naphtalene $\tilde{=}$ pyrene < anthracene

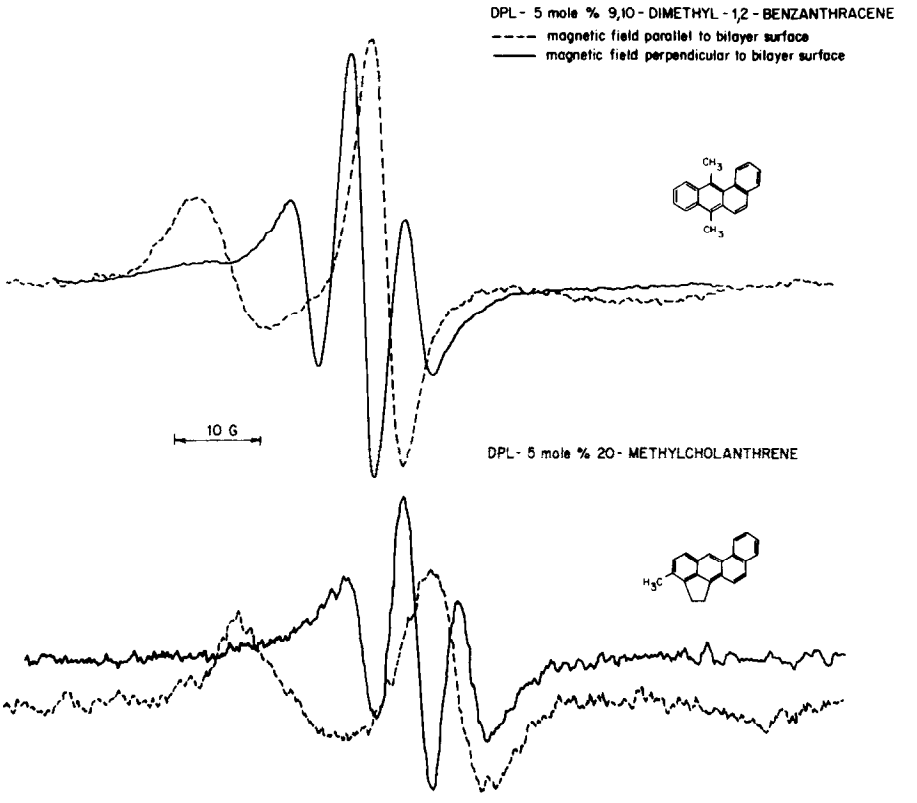

Fig. 4 - Spectra of CSL in DPL bilayers containing 5 mole % of 9,10-dimethyl-1,2-benzanthracene (top) and 20-methylcholanthrene (bottom). Magnetic field parallel (---) and perpendicular (——) to bilayer surface.

< naphtacene ≃ 1,2-benzanthracene ≃ phenanthrene < 1,2,5,6-dibenzanthracene ≃ 9,10-dimethyl-1,2-benzanthracene < 9-methyl-anthracene ≃ 3,4-benzopyrene < 20-methyl cholanthrene. It parallels the increasing size of the HC's, the angular ones being more efficient than the linear ones. The order also parallels closely the carcinogenic potency of the compounds, with the exception of 9-methylanthracene. We do not know exactly the reason for the behaviour of this HC, although it could be speculated that the methyl group is important in promoting the observed effect. Approximately similar results were obtained for all concentration of the HC's. It is possible that some or all

of the hydrocarbons are not totally soluble in the lipid phase(3). However, samples with the spin probe and the hydrocarbon alone did not yield spectra ressembling those of an oriented probe, as obtained with the carcinogens and DPL.

DISCUSSION: Spin probes intercalated in planar lipid bilayers can exhibit esr spectra which depend on the angle between the bilayer surface and the applied magnetic field (8, 9). Schreier-Muccillo et al. (10) have shown that, whereas CSL displays angular-dependent spectra in egg lecithin, very little angular dependence is obtained for DPL. This was ascribed to the fact that DPL is in the gel state at room temperature (11), whereas egg lecithin is in the liquid crystal state. Additional evidence came from studying the effect of cholesterol. Cholesterol has been proposed to promote the gel to liquid crystal phase transition in DPL at temperatures at which the phospholipid alone is in the gel phase (11). The sterol caused a large increase in the anisotropy of the spectra of CSL in DPL (10). Amounts as low as 10 mole % cholesterol were able to produce similar spectra to those corresponding to 30 mole % in Fig. 8, ref. 10. These spectral alterations were related to the phase transition induced by cholesterol.

Applying a similar criterion to the present results, it is seen that the efficiency of the aromatic HC's in inducing a phase transition in DPL follows approximately the order: naphtalene $\simeq$ $\simeq$ pyrene < anthracene < naphtacene $\simeq$ 1,2-benzanthracene $\simeq$ $\simeq$ phenanthrene < 1,2,5,6-dibenzanthracene $\simeq$ 9,10-dimethyl-1,2-benzanthracene < 9-methylanthracene $\simeq$ 3,4-benzopyrene < 20-methylcholanthrene. Except for 9-methylanthracene, which is not a carcinogen this sequence is very close to that of increasing degree of carcinogenic power.

The ability of the larger, carcinogenic HC's to cause spectral changes that resemble those of a phase transition strongly suggests that these compounds intercalate between the phospholipid acyl chains, thereby decreasing phospholipid-phospholipid interactions. The smaller, non-carcinogenic HC's are much less capable of promoting such an effect. It is possible that these molecules are dissolved in the central region of the bilayer, close to the acyl terminal methyl groups, where the fluidity of the environment is probably maximal. This difference between the two groups of compounds may be of considerable importance in two ways: first, it indicates that the smaller HC's are solubilized by the membrane in a different way from the carcinogens, the latter aligning themselves parallel to the acyl chains of the phospholipid. As a consequence, the permeation of these compounds through the membrane could also differ, and this may constitute one of the characteristics that confer on them the carcinogenic power. Second, whereas the small HC's do not seem to alter the packing properties of the membrane, the carcinogens induce a transition to a state which is characterized by a different degree of packing. In this manner, the larger HC's may be able to alter the permeability properties of the membrane. It has been shown that permeability increases in going from the gel to the liquid crystal state (12). This aspect could also be related to the mechanism of carcinogenesis (13).

Finally, it should be noted that the results we have obtained are a consequence of hydrophobic and possibly other non-covalent interactions by which the large aromatic HC's intercalate between the phospholipid acyl chains. It is likely that this intercalation resembles those that take place in nucleic acids and in hydrophobic regions of proteins once the HC is inside the cell.

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