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Interaction of 3-Methylcholanthrene with Lecithin–Cholesterol Mixed Films

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Abstract □ The interaction of a carcinogenic hydrocarbon, 3-methylcholanthrene, with mixed films of various mole fractions of cholesterol and lecithin was investigated. The extent of interaction between 3-methylcholanthrene and cholesterol in the mixed films is greatly influenced by the competitive interaction between cholesterol and the phospholipid. At 50:50 cholesterol–lecithin molar ratios, where these lipids interact to the greatest extent, the interaction with 3-methylcholanthrene is weakest. These competitive interactions may account for the experimental observations that phospholipids retard while cholesterol accelerates the formation of tumors induced by polycyclic aromatic hydrocarbons.

Keyphrases □ 3-Methylcholanthrene interaction—lecithin–cholesterol mixed films □ Lecithin–cholesterol mixed films—competitive interaction □ Cholesterol–lecithin film competitive interaction effect—3-methylcholanthrene–film interaction □ Surface pressure—lecithin–cholesterol mixed film

Monomolecular films represent a relatively simple type of membrane model having a well-defined organized structure. They provide one of the most convenient and promising methods of studying molecules in a fixed orientation, as well as in a single layer where orientation can be changed by compression of the monomolecular film. As such, they constitute an important model system for the study of many natural phenomena that involve surfaces of an oriented array of molecules.

The production of cancer by pure hydrocarbons was discovered by researchers in England (1, 2). Since that time numerous substances have been demonstrated to have carcinogenic activity. These include polycyclic aromatic hydrocarbons, azo dyes, chemical agents of known and unknown composition, carcinogenic viruses, and physical agents (3).

Dickens and Weil-Malherbe (4, 5) observed that phospholipids retard, whereas cholesterol promotes, the formation and growth of tumors when injected simultaneously with the carcinogen, 3,4-benzpyrene.

Altman (6) also demonstrated that phospholipids retard the formation of tumors induced by a polycyclic hydrocarbon. He postulated that the protective activity of phospholipids must be due to their ability to form molecular associations with the polycyclic hydrocarbons, just as they do with cholesterol. Such associations promote the transportability of the carcinogen and thus prevent them from acting on the cell membrane. He felt that the question of why phospholipids only temporarily retard, and not permanently prevent, the formation of tumors must be seen in the scope of quantitative proportions in which phospholipids, on the one hand, and carcinogen plus cholesterol, on the other, occur in animal tissue.

Altman (7) suggested that carcinogenic substances induce cancer because they act as lipophilic sensitizers. They produce this effect either by occupying the available open spaces or by displacing the lipophilic sensitizers naturally occurring in the cell membrane. "Every change (even the slightest) in the structure of the membrane, imperatively leads to a change in its permeability and selectivity and consequently in the composition and properties of the whole cell." Therefore, a modification in the structure of the cell membrane can lead to the transformation of the original normal cell into another cell which accidently might be a cancer cell (6, 7).

Altman (6) felt that since phospholipids increase cell permeability while cholesterol decreases permeability, an excess of sterol (which condenses the cell membrane) promotes the fixation of carcinogenic molecules, while an excess of phospholipids (which expands the cell membrane) prevents such fixation. Absolute values of the ratio of phospholipids to cholesterol would then have a decisive influence on the incidence of cancer. Since values of phospholipids–cholesterol ratios are specific for each tissue, this situation offers an explanation of why one tissue is more accessible to tumor formation than another.

Finean (8) also explained the interactions of the polycyclic aromatic hydrocarbons on the basis of molecular association. Phospholipids and cholesterol both react with the carcinogenic compound; the association with the former promotes the motility of the carcinogen which is then prevented from sticking to the cell membrane.

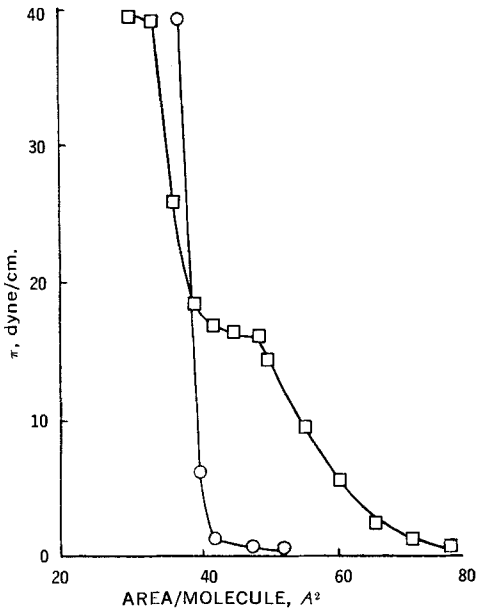

Figure 1—*Surface pressure–surface area (π–A) curves of cholesterol alone (○) and of cholesterol–3-methylcholanthrene (50:50 molar ratio) mixture (□).*

Clowes *et al.* (9, 10) studied the interactions of a variety of polycyclic hydrocarbons with cholesterol, β-dihydrocholesterol, and ergosterol spread as monomolecular films. Their work showed that the hydrocarbons fall into two classes with respect to the families of pressure–area curves obtained with the sterol films. Analysis of their data indicated that most of the hydrocarbons, in spite of their inability to spread on water, displayed sufficient reactivity toward sterol molecules to be held between the sterol ring system at an air–water interface.

The first class of interactions results in the formation of two-dimensional solutions from which the hydrocarbon can enter or leave at some particular surface pressure. The second class of interactions can be explained in terms of association complexes formed with the sterol molecules. It was suggested that this type of interaction may have significance in the transport of the hydrocarbon in the animal organism and in modifying biological structures.

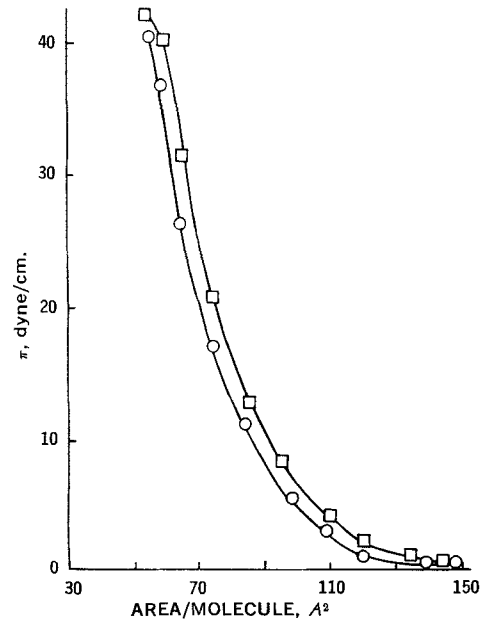

Figure 2—*Surface pressure–surface area (π–A) curves of egg lecithin alone (○) and of egg lecithin–3-methylcholanthrene (50:50 molar ratio) mixture (□).*

Snart (11) studied the monolayer behavior of various aromatic hydrocarbons with lecithin and cholesterol. He reported that hydrocarbons associate with cholesterol and lecithin in these films to a limited extent, after which additional hydrocarbon does not contribute to the film area but instead forms an excess solid phase. The association was treated in terms of solubility of the hydrocarbon in a two-dimensional solution and compared with data obtained from phase equilibria studies.

Phospholipids and cholesterol are major components of biological membranes composed of bimolecular leaflets or lipoprotein subunits (12). Mixed films of lecithins and cholesterol were first studied by de Bernard (13), who noted that cholesterol apparently was able to condense lecithin to a smaller cross-sectional area than it can exhibit alone. This condensation effect may be due to either a molecular interaction or a cavity effect (14).

The extent of the interactions of the carcinogenic hydrocarbons with cholesterol and lecithin would be modified by preexisting intermolecular interactions at the cell membrane between cholesterol and lecithin. The purpose of this investigation was to study the interaction of a carcinogenic hydrocarbon, 3-methylcholanthrene, with mixed films of various mole fractions of cholesterol and lecithin in order to gain some insight into the effect of membrane composition on the carcinogen–membrane interaction.

EXPERIMENTAL

The egg lecithin (mol. wt. 790)[1] and cholesterol (mol. wt. 386.7)[2] used were specified as chromatographically pure. Methylcholanthrene (mol. wt. 268.4)[3] and *n*-hexane, spectrograde[4], were also used.

Water used in this study was deionized by passing through a Bantam demineralizer and then distilled in an all-glass still. Glassware was cleaned in chromic acid solution and rinsed in hot-distilled water prior to use. All inorganic chemicals used were of reagent grade.

In the surface balance[5] used in these experiments, the Teflon-coated trough was removable to facilitate cleaning. The precision lead screw, which drives the reinforced Teflon barrier, allows for changes in surface area of the trough as small as 0.0125 cm². The barrier could be disengaged from the lead screw for rapid sweeping of the surface.

Surface pressure values were measured by the Wilhemy plate method (15). The platinum plate, roughened to ensure wetting, was suspended from a Torsion balance[6]. The balance can measure surface pressure changes of 0.1 dyne/cm.

The trough was filled with 0.9% sodium chloride solution, and the surface was swept several times with the barrier to clean it. Suction was then used to adjust the level of the subphase and to remove any remaining traces of dust or other insoluble contaminents.

The platinum plate was then lowered into position and allowed to remain beneath the surface while a known volume of mixtures of phospholipid–cholesterol–hydrocarbons dissolved in *n*-hexane

[1] Obtained from the Sylvania Chemical Co., Milburn, N. J.
[2] Obtained from Applied Science Laboratories, Inc., State College of Pennsylvania.
[3] Eastman Organic Chemicals, Rochester, N. Y.
[4] Obtained from Fisher Scientific Co., Fairlawn, N. J.
[5] Frater Instrument Co., Corona, N. Y.
[6] Supplied by Federal Pacific Electric Co.

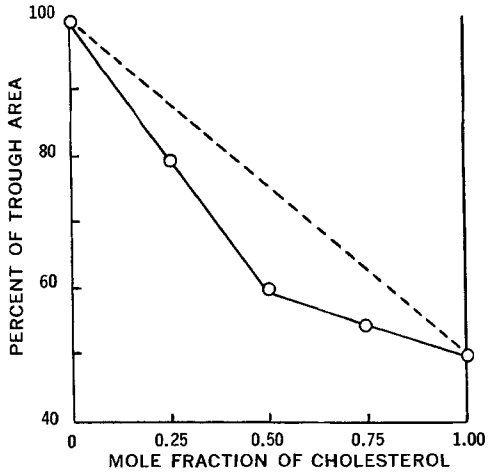

Figure 3—*Percent trough area of egg lecithin–cholesterol monolayers at a surface pressure of 10 dynes/cm. The broken line represents the additivity rule.*

was spread with the aid of an Agla micrometer syringe. This syringe can deliver accurately volumes as small as 0.001 ml. Generally, 0.07 ml. of the spreading solution was applied onto the subphase by allowing small drops to fall from the syringe held a few millimeters away from the subphase surface. At least 5 min. was allowed for the system to reach equilibrium before compression was initiated. The area available to the film molecules was then reduced in small increments, and surface pressure readings were taken 1 min. after each area change. Compression was continued until surface pressure no longer changed with area, indicating collapse of the film.

Each surface pressure–surface area (π–A) curve is the result of three separate experiments. The curves were always superimposable, with deviations of less than 0.2 dyne/cm. at all areas tested.

RESULTS AND DISCUSSION

Two-Component Systems—Figure 1 shows the surface pressure–surface area (π–A) plots of spread films of cholesterol alone and in combination with 3-methylcholanthrene. Alone, cholesterol does not exhibit any resistance to the barrier until each molecule occupies an area of about 40A^2. A further slight reduction in area then causes a sharp increase in pressure until the film collapses. These results are in agreement with those reported earlier by Clowes (16) and Demel and Joos (17).

Since 3-methylcholanthrene contains no hydrophilic groups, it has no tendency, when alone, to spread at the air–water interface. When 3-methylcholanthrene was spread alone, no surface pressure was observed upon compression at all areas tested.

When a 50:50 molar ratio mixed film of cholesterol and 3-methylcholanthrene was spread, the film area exceeded that of pure cholesterol at low surface pressures (Fig. 1), indicating that the hydrocarbon interacts strongly with cholesterol. At a surface pressure of about 17 dynes/cm., a further increase in pressure produced a gradual decrease in film area. This indicates removal of hydrocarbon molecules from an area-determining position to a non-area-determining position, *i.e.*, to an excess phase outside of, but in very close contact with, the film (16).

The fact that the area/molecule of the mixed film in the high pressure region is less than that of the pure cholesterol film at equal surface pressures is characteristic of mixed films in which the excess component, 3-methylcholanthrene, solubilizes the other (18).

Figure 2 shows the π–A curve of a spread film of egg lecithin. In the presence of 3-methylcholanthrene, the curves obtained for the mixed film were displaced only slightly. Similar results were reported by Clowes (16), who concluded that no discernible interaction between polycyclic hydrocarbons and lecithin could be found.

Three-Component Systems—Figure 3 shows the average area/molecule (percent trough area) *versus* the mole fraction of cholesterol for mixed films of cholesterol and egg lecithin at a surface pressure of 10 dynes/cm. While a condensation effect is observed at all mole fractions (the average area/molecule is always less than that predicted by the additivity rule), this effect is most striking at a

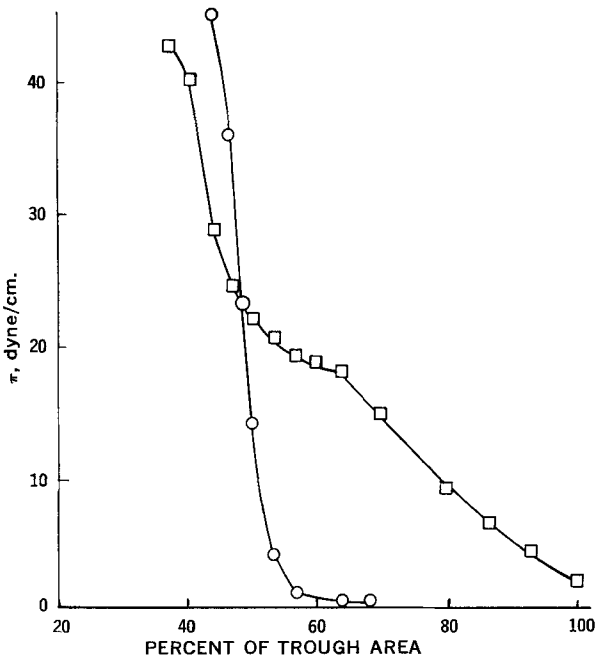

Figure 4—*Surface pressure–surface area* (π–A) *curves of cholesterol–egg lecithin (75:25 molar ratio) mixture alone* (○) *and in the presence of 3-methylcholanthrene* (□).

50:50 mole ratio. The results obtained are in agreement with those reported by other investigators (19, 20).

Figures 4–6 show the effect of 3-methylcholanthrene on the π–A curves of lecithin–cholesterol mixed films at various mole fractions. In each case the number of molecules of 3-methylcholanthrene added was equal to the total number of lipid molecules in the film. Since 3-methylcholanthrene interacts to a much greater extent with cholesterol as compared to egg lecithin, one would expect the degree of interaction of the carcinogen with the mixed films to be proportional to the mole fraction of cholesterol in the film. However, whereas the degree of interaction (as measured by comparing the increase on surface pressure at a given surface area) is greatest for the 75:25 cholesterol–lecithin mixed film, there is no discernible difference between the effect of 3-methylcholanthrene on the 50:50

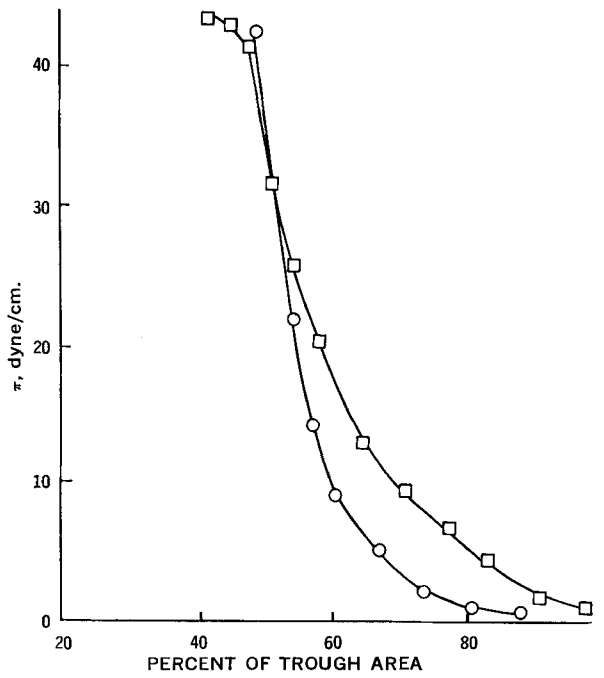

Figure 5—*Surface pressure–surface area* (π–A) *curves of cholesterol–egg lecithin (50:50 molar ratio) mixture alone* (○) *and in the presence of 3-methylcholanthrene* (□).

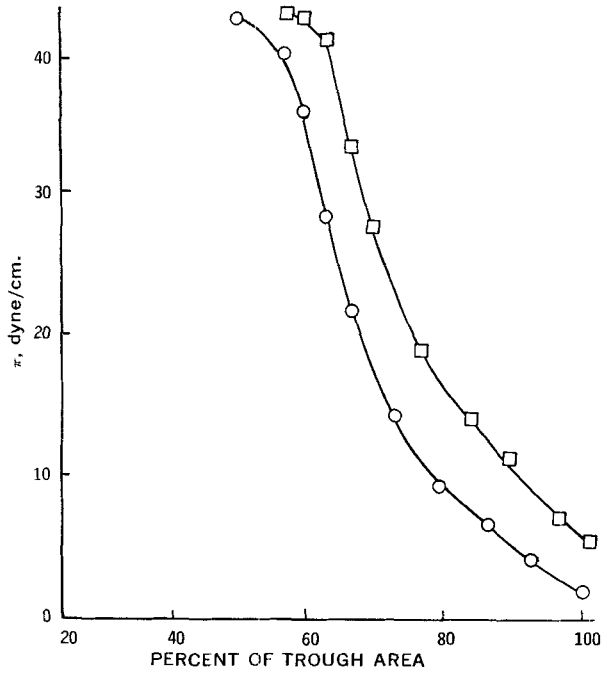

Figure 6—*Surface pressure–surface area (π–A) curves of cholesterol–egg lecithin (25:75 molar ratio) mixture alone (○) and in the presence of 3-methylcholanthrene (□).*

and 25:75 cholesterol–lecithin mixed films at low surface pressures, while the degree of interaction appears to be greater in the case of the 25:75 cholesterol–lecithin film at higher pressures.

It appears that the extent of interaction between 3-methylcholanthrene and cholesterol in the three-component mixed films is greatly influenced by the competitive interaction between cholesterol and the phospholipid. Therefore, at 50:50 cholesterol–lecithin molar ratios, where these lipids interact to the greatest extent, the interaction with 3-methylcholanthrene is weakest.

Altman (6, 7) demonstrated that phospholipids actually do retard the formation of tumors induced by a polycyclic hydrocarbon, when administered subcutaneously. He postulated that this effect could be explained on the basis of greater affinity of cholesterol to phospholipid than to 3-methylcholanthrene due to the presence of the OH group in the cholesterol molecule. This enables cholesterol to release 3-methylcholanthrene from its association with phospholipid, a process he expressed by the following equation:

phospholipid – 3-methylcholanthrene + cholesterol →
phospholipid – cholesterol + 3-methylcholanthrene (Eq. 1)

Based on this reaction, he postulated that the value of the ratio of phospholipid–cholesterol may have a decisive influence on the incidence of cancer.

Whereas the data support the postulation that the absolute value of the ratio of phospholipid to cholesterol influences the interaction with carcinogen, it appears that cholesterol, rather than the phospholipid, is the major interactor with 3-methylcholanthrene. This postulation is more direct than the one suggested by Altman (6, 7) to account for the experimental observation that phospholipids retard, while cholesterol accelerates, the formation of tumors induced by polycyclic aromatic hydrocarbons (4, 5).

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