Interaction of 3,4-Benzpyrene with Monomolecular Films

A. FELMEISTER*, D. TSAI†, and N. D. WEINER†▲

Abstract □ The interactions of the carcinogenic hydrocarbon, 3,4-benzpyrene [benz(*a*)pyrene], with cholesterol, phospholipid, protein, and lipid–protein films were investigated. Whereas the interaction between 3,4-benzpyrene and cholesterol was weak, the interaction between the carcinogen and dipalmitoyl lecithin was very strong, as determined from surface pressure–surface area data. The extent of the interaction between the phospholipid and 3,4-benzpyrene was dependent on the mole fraction of each component in the film. 3,4-Benzpyrene was shown also to interact strongly with protein films and mixed lipid–protein films. In the case of the latter films, the extent of interaction was dependent on whether or not the lipid was first allowed to interact with the protein before the carcinogen was added. The biological significance of these data is discussed.

Keyphrases □ 3,4-Benzpyrene—interaction with various monomolecular films, biological significance □ Monomolecular films—interaction of 3,4-benzpyrene with various cholesterol, phospholipid, protein, and lipid–protein films, biological significance □ Carcinogenic hydrocarbons—interaction of 3,4-benzpyrene with various monomolecular films, biological significance

The production of cancer by polycyclic aromatic hydrocarbons was reported by researchers more than 30 years ago (1, 2). Whereas the mechanism of tumor induction by these agents has not been elucidated, it has been suggested that they may interact with the cell membrane. These interactions may change the structure and permeability of the cell membrane, which can lead to the transformation of the original normal cell into another cell which accidently might be a cancer cell (3, 4).

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 involving surfaces of an oriented array of molecules.

Monolayer studies have shown that polycyclic aromatic hydrocarbons interact strongly with compounds normally associated with biological membranes, particularly phospholipids and cholesterol. These interactions may result in the formation of two-dimensional solutions or in the formation of association complexes (5, 6). The biological significance of these observations is supported by reports that phospholipids retard, whereas cholesterol promotes, the formation and growth of tumors when injected simultaneously with certain polycyclic aromatic hydrocarbons (3, 7, 8).

Weiner *et al.* (9) recently reported on the interactions of the carcinogenic hydrocarbon, 3-methylcholanthrene, with mixed films of various mole fractions of cholesterol and lecithin. Whereas 3-methylcholanthrene was found to interact strongly with cholesterol, its interaction with lecithin was minimal. Furthermore, 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.

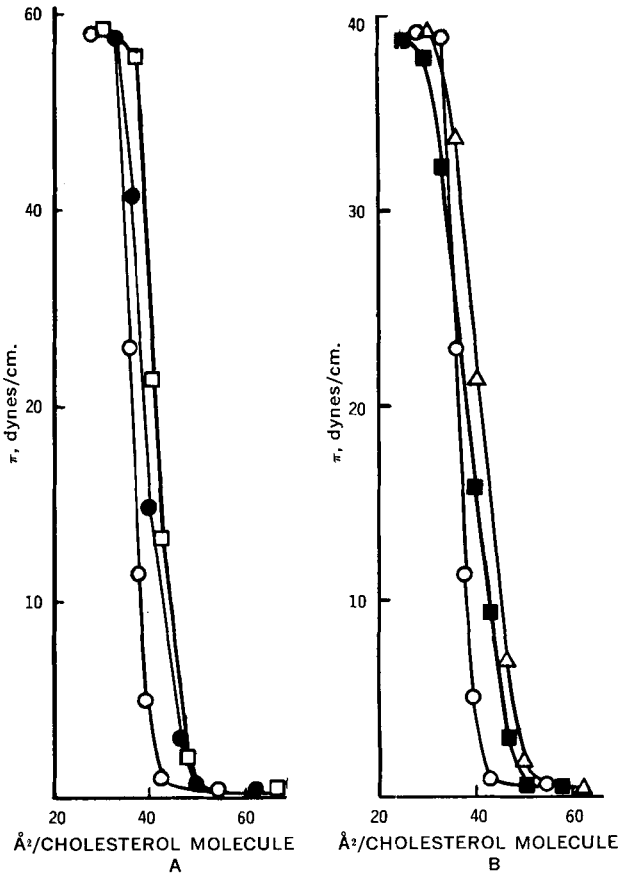

Figure 1—*Surface pressure* versus *surface area per cholesterol molecule* (π–A) *for various mole ratio mixtures of cholesterol and 3,4-benzpyrene. Key: cholesterol-3,4-benzpyrene mole ratios:* ○, *1:10;* □, *1:1;* △, *1:2;* ■, *1:3;* ■, *1:4; and* ●, *3:1.*

The purpose of this investigation was to study the interaction of the carcinogenic hydrocarbon, 3,4-benzpyrene [benz(*a*)pyrene], with cholesterol and phospholipid films. Furthermore, since proteins are an integral part of membrane structures, the interactions of 3,4-benzpyrene with protein and lipid–protein films were investigated.

EXPERIMENTAL

The lipids used in this study, egg lecithin, dipalmitoyl lecithin[1], and cholesterol[2], were chromatographically pure. The protein used

[1] Mann Research Laboratories, New York, N. Y.
[2] Eastman Organic Chemicals, Rochester, N. Y.

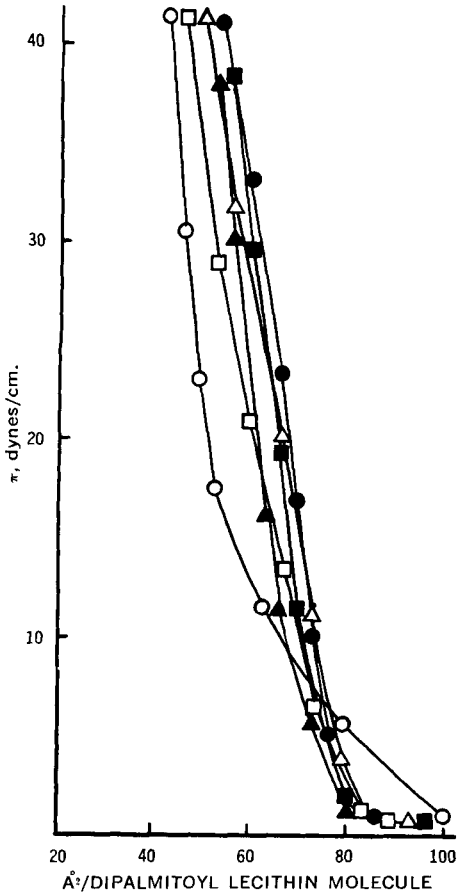

Figure 2—*Surface pressure* versus *surface area* (π–A) *plots of spread films of dipalmitoyl lecithin alone and in the presence of various amounts of 3,4-benzpyrene. Key: dipalmitoyl lecithin–3,4-benzpyrene mole ratio:* ○, *1:0;* □, *1:1, 1:2, and 1:3;* △, *2:1;* ●, *3:1;* ■, *4:1; and* ▲, *5:1.*

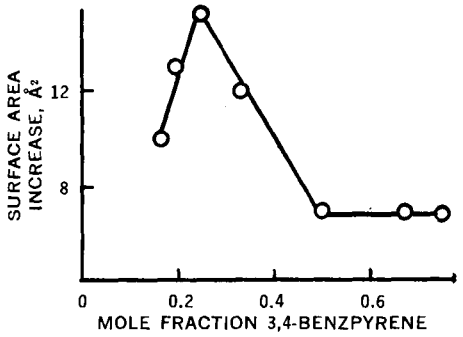

Figure 3—*Increase in area per molecule of dipalmitoyl lecithin in the presence of various amounts of 3,4-benzpyrene* (ΔA) versus *mole fraction of 3,4-benzpyrene for mixed dipalmitoyl lecithin–3,4-benzpyrene films.*

was bovine serum albumin[3], and the polycyclic aromatic hydrocarbon was 3,4-benzpyene[4]. Organic solvents used in the experiments were all spectrograde, and all inorganic chemicals used were of reagent grade. The water used in this study was deionized by being passed through a Bantam demineralizer and was then distilled in an all-glass still. Glassware was cleaned in chromic acid solution and rinsed in hot distilled water prior to use.

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 cm.^{2}. The barrier could be disengaged from the lead screw for rapid sweeping of the surface. Surface pressure values were measured by the Wilhelmy plate method (10). The platinum plate, roughened to ensure wetting, was suspended from a torsion balance[6]. The balance can measure surface tension changes of 0.1 dyne/cm.

The trough was filled with distilled water for experiments utilizing lipid films or with phosphate buffer (pH 7.2) for experiments utilizing protein or lipid–protein films. The surface was then swept several times with the barrier to clean it, and suction was used to adjust the level of the subphase and to remove any remaining traces of dust or other insoluble contaminants.

The platinum plate was then lowered into position and allowed to remain beneath the surface while the films were applied from their appropriate spreading solvents 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. *n*-Hexane was used as the spreading solvent for cholesterol films, egg lecithin films, 3,4-benzpyrene films, and the various mixed films of these components. An ethanol–*n*-hexane mixture (5:95 v/v) was used as a spreading solvent for dipalmitoyl lecithin films and various mixed films containing dipalmitoyl lecithin.

Solutions of bovine serum albumin were spread by the method of Trurnit (11, 12). The method consists of allowing the drops to fall at a slow, steady rate onto the hemispherical top of a clean glass rod placed in the trough. The syringe was always held directly above the

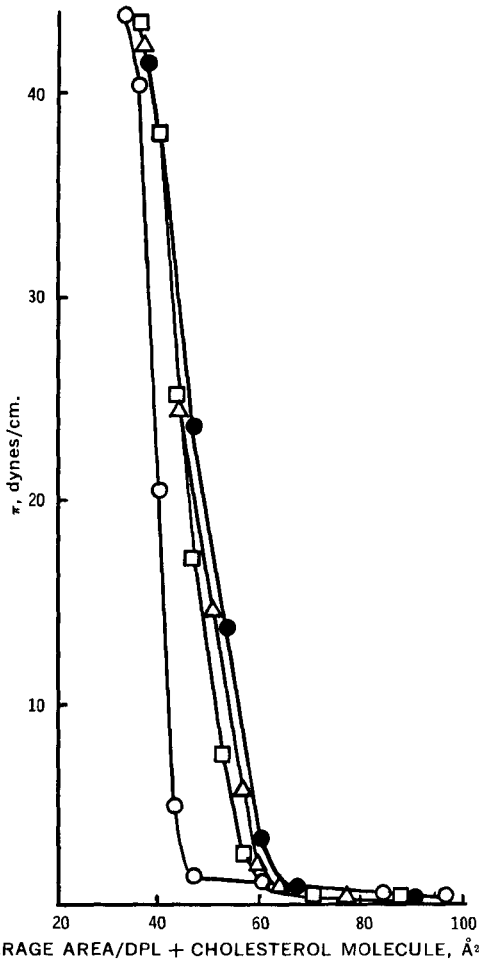

Figure 4—*Surface pressure* versus *surface area per lipid molecule* (π–A) *for 1:1 dipalmitoyl lecithin (DPL)–cholesterol films alone and in the presence of various amounts of 3,4-benzpyrene. Key: total lipid–3,4-benzpyrene mole ratio:* ○, *1:0;* □, *1:1;* △, *2:1; and* ●, *4:1.*

[3] Pierce Chemical Co., Rockford, Ill.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Frater Instrument Co., Corona, N. Y.
[6] Bethlehem Instrument Co., Bethlehem, Pa.

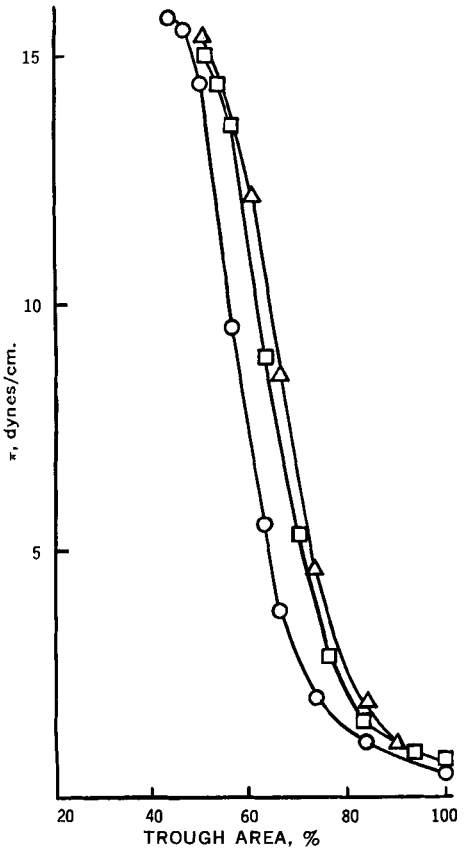

Figure 5—*Surface pressure* versus *percent trough area (100% = 1 m.²/mg. of bovine serum albumin) for bovine serum albumin films alone and in the presence of various amounts of 3,4-benzpyrene. Key: ○, no 3,4-benzypyrene; □, 0.0132 mg. 3,4-benzpyrene; and △, 0.0264 mg. 3,4-benzpyrene.*

top of the glass rod, which was positioned so that it could remain in place during the compression cycle. The spreading solution consisted of 45 mg. protein/100 ml. phosphate buffer (pH 7.2). The amount spread corresponded to a surface area of 1 m.²/mg. protein at the full trough area. In experiments using protein–carcinogen mixed films, 5 min. was allowed to elapse before the 3,4-benzpyrene was spread over the bovine serum albumin film.

Mixed films of lecithin–bovine serum albumin were prepared by using the Trurnit method to spread the protein onto the previously spread phospholipid. Experiments utilizing three-component films (phospholipid–protein–carcinogen) were performed by two different methods. The first involved spreading the lipid–protein film as already described, allowing 5 min. for equilibration, and then spreading the carcinogen. The second method involved spreading the carcinogen together with the phospholipid (in the same spreading solvent) and then spreading the protein as previously described.

In each case, at least 5 min. was allowed for the system to reach "equilibrium" before compression was initiated. It is apparent that true equilibrium in these systems is not achieved during the course of the experiments since the π–A curves are dependent on the order of addition of components to the film. However, for each system studied, 5-, 15-, and 30-min. equilibration times produced identical π–A curves. 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.

RESULTS AND DISCUSSION

When the hexane solution of 3,4-benzpyrene was spread alone, no surface pressure was observed upon compression at all areas tested. Since polycyclic aromatic hydrocarbons contain no hydrophilic groups, they have no tendency, when alone, to spread at the air–water surface (9).

Figures 1*a* and 1*b* show the surface pressure–surface area (π–A) plots of spread films of cholesterol alone and in combination with various amounts of 3,4-benzpyrene. When the various molar ratios of the cholesterol–3,4-benzpyrene mixed films were spread, the film area per cholesterol molecule slightly exceeded that of pure cholesterol at low surface pressures, indicating that the hydrocarbon interacts with cholesterol. The areas per molecule of the mixed films containing 1:3, 1:1, and 2:1 mole ratios (3,4-benzpyrene–cholesterol) at pressures near the collapse pressure of the film approached that of pure cholesterol itself. This indicates removal of hydrocarbon molecules at high pressures from an area-determining position to a nonarea-determining position, *i.e.*, to an excess phase outside of, but in very close contact with, the film (13). The fact that the areas per molecule of the mixed films containing higher mole ratios of 3,4-benzpyrene–cholesterol (3:1 and 4:1) are less than that of the pure cholesterol film in the higher pressure region is characteristic of mixed films in which the excess component, 3,4-benzpyrene, solubilizes the other (14). However, the degree of interaction between cholesterol and 3,4-benzpyrene is much weaker than that of cholesterol and 3-methylcholanthrene (9).

Figure 2 shows the π–A plots of spread films of dipalmitoyl lecithin alone and in combination with various amounts of 3,4-benzpyrene. At all mole ratios tested, the areas per molecule of dipalmitoyl lecithin in the mixed films greatly exceeded those of dipalmitoyl lecithin alone at all values of π above 7 dynes/cm., indicating a strong interaction between dipalmitoyl lecithin and 3,4-benzpyrene. Furthermore, the areas per molecule at the collapse pressure for all of the mixed films were greater than that of dipalmitoyl lecithin alone, indicating that 3,4-benzpyrene was not completely squeezed out of the film even at high pressures. The

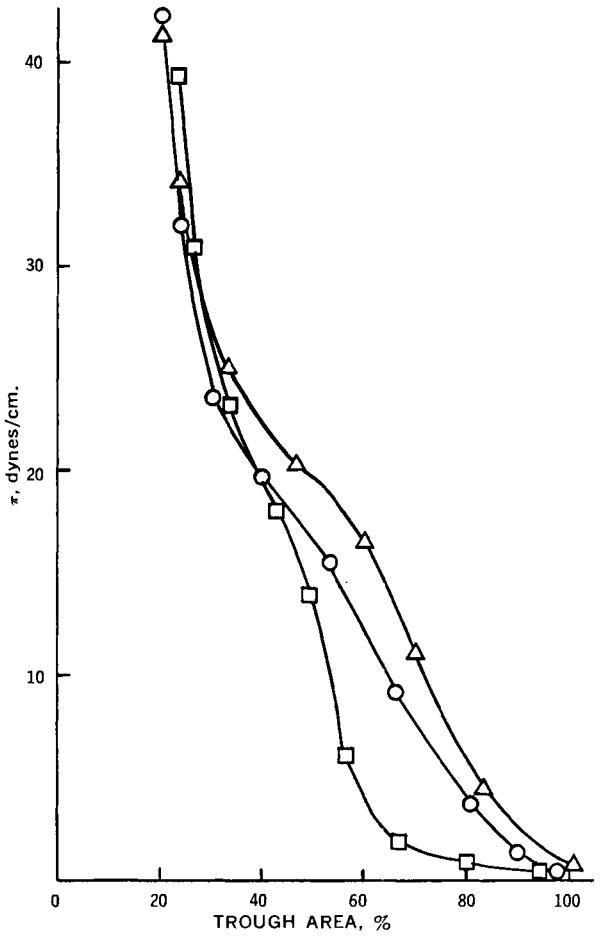

Figure 6—*Surface pressure* versus *percent trough area for mixed dipalmitoyl lecithin–bovine serum albumin films alone and in the presence of 0.0066 mg. 3,4-benzpyrene. Key: ○, no 3,4-benzpyrene; □, bovine serum albumin added to dipalmitoyl lecithin–3,4-benzpyrene film; and △, 3,4-benzpyrene added to dipalmitoyl–bovine serum albumin film.*

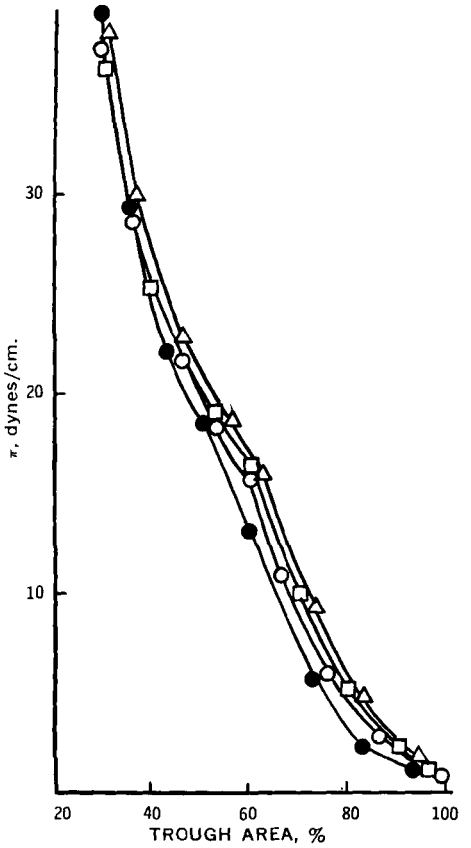

Figure 7—*Surface pressure* versus *percent trough area for mixed egg lecithin–bovine serum albumin films alone and in the presence of various amounts of 3,4-benzpyrene. Key:* ○, *no 3,4-benzpyrene;* □, *0.0066 mg. 3,4-benzpyrene added to egg lecithin–bovine serum albumin film;* △, *0.0132 mg. 3,4-benzpyrene added to egg lecithin–bovine serum albumin film; and* ●, *bovine serum albumin added to egg lecithin–3,4-benzpyrene film.*

presence of 3,4-benzpyrene appears to have a condensing effect on the dipalmitoyl lecithin monolayers at very low pressures. The reason for this effect is uncertain, and a two-dimensional solubilization mechanism is not likely.

The degree of interaction of 3,4-benzpyrene with dipalmitoyl lecithin seems to be quite dependent on the mole fraction of hydrocarbon spread at the surface. Figure 3 shows a plot of ΔA *versus* mole fraction 3,4-benzpyrene for the various mixed films at a surface pressure of 25 dynes/cm. ΔA is the area per molecule of the mixed film minus the area per molecule of the pure dipalmitoyl lecithin film at 25 dynes/cm. It can be seen that the area per molecule increases with the addition of 3,4-benzpyrene up to a mole fraction of 0.25, after which there is a decrease in area until a mole fraction of 0.5 is reached. Further addition of hydrocarbon beyond a mole fraction of 0.5 yields superimposable curves. These results indicate that the mechanism of interaction is quite complex. One possible interpretation of the data might be that at low mole fractions of hydrocarbon, penetration of the carcinogen into the film is observed, yielding increased areas per molecule. At higher mole fractions of carcinogen, a solubilization effect is observed in addition to the penetration effect, causing some of the dipalmitoyl lecithin to leave the film. Finally, at a mole fraction of 0.5, equilibrium is achieved, and further addition of carcinogen will no longer affect the remaining dipalmitoyl lecithin in the film. In any event, the interaction between 3,4-benzpyrene and dipalmitoyl lecithin is far greater than that of the carcinogen with cholesterol.

Mixed films of cholesterol–dipalmitoyl lecithin at different mole ratios with various amounts of 3,4-benzpyrene also showed an expansion effect (larger areas per molecule in the presence of 3,4-benzpyrene). The degree of interaction, as determined by changes in the π–A curves, was approximately intermediate to those between 3,4-benzpyrene–cholesterol and 3,4-benzpyrene–dipalmitoyl lecithin. A typical curve is shown in Fig. 4.

The π–A curves of mixed films of egg lecithin–3,4-benzpyrene were indistinguishable from those of egg lecithin alone, indicating a lack of discernible interaction. The reason why dipalmitoyl lecithin should interact so strongly with 3,4-benzpyrene while egg lecithin shows no interaction cannot be determined from these data. However, it was reported (15) that dipalmitoyl lecithin has a greater tendency than egg lecithin to engage in intermolecular interactions at the air–water surface.

Figure 5 shows the π–A plots of spread films of bovine serum albumin alone and in combination with various amounts of 3,4-benzpyrene. In all cases, the mixed films yielded more expanded curves than that of bovine serum albumin alone, indicating a significant interaction between bovine serum albumin and the carcinogen.

Figures 6 and 7 show the π–A curves for the three-component systems (lecithin–bovine serum albumin–carcinogen). While the addition of 3,4-benzpyrene significantly affects the lipid–protein π–A curves, the method of addition of carcinogen to the film is extremely important. In the first case the carcinogen is added to the already formed lipid–protein film. This results in expanded curves, indicating that the carcinogen is penetrating into the lipid–protein mixed films. In the second case, the carcinogen–lipid curves are more condensed than those of the pure lipid–protein mixed film curves, indicating that the carcinogen is interfering with the formation of the lipid–protein mixed films. This may be due to either a competitive interaction, solubilization of the lipid, or a combination of these.

It was reported (16) that 3,4-benzpyrene can interact with proteins (hydroxylating enzymes). Since many enzymes are strongly associated with lipids (particularly at the membrane surface), the mechanism of action of 3,4-benzpyrene could strongly depend on the sequence of events at the biomembrane, *i.e.*, whether or not the enzyme was already associated with the lipid prior to exposure to the carcinogen.

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