Interaction of Cholesterol with Dibenzanthracenes at Air–Water Interface

ALBERT A. BELMONTE and JAMES SWARBRICK▲

Abstract □ A film balance approach using continuous compression was used to study the interaction of the carcinogen 1,2:5,6-dibenzanthracene and the noncarcinogen 1,2:3,4-dibenzanthracene with insoluble monolayers of cholesterol. Surface pressure and surface potential of mixed dibenzanthracene–cholesterol films spread on water and saline were studied. Results showed an association of 1,2:5,6-dibenzanthracene and no association of 1,2:3,4-dibenzanthracene with cholesterol. Surface potential data support the concept of carcinogen–cholesterol association. The data show a useful difference between a carcinogenic and a noncarcinogenic isomer at the air–water interface.

Keyphrases □ Cholesterol–dibenzanthracene—interaction at air–water interface, film balance approach, comparison of carcinogenic and noncarcinogenic dibenzanthracene isomers □ Dibenzanthracene–cholesterol—interaction at air–water interface, film balance approach, comparison of carcinogenic and noncarcinogenic dibenzanthracene isomers □ Carcinogens, 1,2:5,6-dibenzanthracene—interaction with cholesterol at air–water interface, compared to noncarcinogenic isomer, 1,2:3,4-dibenzanthracene □ Films—mixed dibenzanthracene–cholesterol, surface pressure and surface potential, air–water interface

Carcinogens, materials known to cause cancer, pose an interesting area of cancer research. While many investigations have been conducted on carcinogen interactions with nucleic acids, only a few studies have been directed toward carcinogen–cell membrane interactions. Wallach (1) proposed that oncogenic agents may act to introduce an inappropriate protein into cell membranes. Numerous membrane functions may be affected as a result of such an alteration. Weissmann *et al.* (2) reported the effects of tumor-promoting agents on biological and artificial membrane systems. Their results suggested membrane disruption as a prime tumor-initiating step. Selkirk *et al.* (3) compared the phospholipid profiles from plasma membranes of solid hepatoma cells and liver cells in an attempt to understand cancer–membrane phenomena; differences in composition and degree of fatty acid saturation were found. All of these studies support the premise that cell membranes are affected by neoplasia. Since cell membranes can control passage of materials into the cell, membrane interactions, particularly penetration of cell membrane by carcinogens, may be extremely important in understanding the cancer-initiating process.

Many materials have been shown to be carcinogenic *in vivo*, yet the ability to correlate carcinogenicity to *in vitro* behavior is often difficult. In an attempt to differentiate between closely related polycyclic aromatic hydrocarbons, it was decided to utilize a film balance technique. This approach is based on the well-recognized fact that insoluble monolayers can be employed to simulate biological interfaces (4). Snart (5), in 1967, made a preliminary study of some aromatic hydrocarbons in monolayers, showing some association of hydrocarbons with cholesterol and lecithin. Weiner *et al.* (6) showed an interaction occurring with 3-methylcholanthrene and lecithin–cholesterol mixed films. The present investigation was conducted to study some surface interactions of dibenzanthracene with cholesterol.

The polycyclic aromatic hydrocarbon dibenzanthracene was chosen for study for several reasons. These include the facts that 1,2:5,6-dibenzanthracene was the first synthetic chemical compound to induce cancer and that considerable information has been compiled on its properties (7). In addition, 1,2:5,6-dibenzanthracene has a structural isomer that is noncarcinogenic or weakly carcinogenic, 1,2:3,4-dibenzanthracene (8, 9). Cholesterol was chosen because of its function in cell membrane structure.

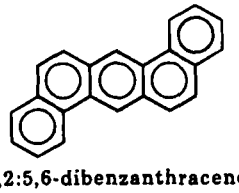
The intent of this report is to relate the monolayer properties of mixed films of cholesterol with dibenzanthracene. The properties will be characterized by: (*a*) the effect of concentration of dibenzanthracene on surface pressure and surface potential parameters, and (*b*) the effect of each dibenzanthracene isomer with respect to their different behavior *in vivo*.

EXPERIMENTAL

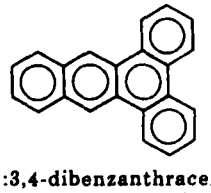
Materials—The mixed films were prepared by dissolving the cholesterol (S.C.W.)[1] in chloroform (nanograde)[2] and the dibenzanthracene[1] in benzene (nanograde)[2]. The appropriate molar ratios of dibenzanthracene–cholesterol were then prepared with the aid of a calibrated syringe[3]. This premixed solution was used as the film-forming material. Double-distilled water from an all-glass still was used as the subphase. The specific conductance of the water was not greater than 1.5 μmhos/cm. When appropriate, normal saline was prepared by adding analytical reagent grade sodium chloride[2].

Film Balance—The film balance was a modified Wilhelmy type which was described previously (10). Surface potential was measured using the ionizing air-gap technique. The apparatus also was described previously (11).

Procedure—Surface pressure was measured continuously using a thin mica plate, of known perimeter, attached to a recording electrobalance. Upon compression, the change in mass of the mica



1,2:5,6-dibenzanthracene (carcinogen)



1,2:3,4-dibenzanthracene (non- or weak carcinogen)

[1] Nutritional Biochemical Corp., Cleveland, Ohio. Purity was checked by TLC using silica gel G and H plates and petroleum ether–ether–acetic acid (90:30:1) and chloroform–methanol–water (65:25:4) as solvents. In all cases, only one spot was found when the plates were developed with bromthymol blue and ammonia gas.

[2] Mallinckrodt, St. Louis, Mo.

[3] Digi-Pet, Manostat Corp., New York, N. Y.

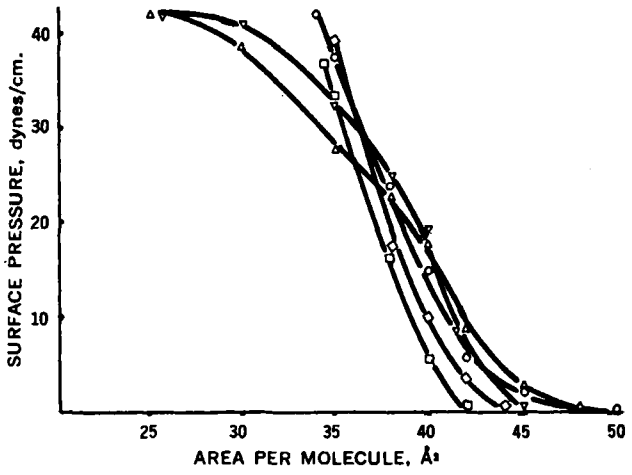


Figure 1—*Surface pressure–area relationship for 1,2:3,4-dibenzanthracene on water. Key: ○, cholesterol; ◇, 1:10 1,2:3,4-dibenzanthracene–cholesterol; □, 1:1 1,2:3,4-dibenzanthracene–cholesterol; ▽, 3:1 1,2:3,4-dibenzanthracene–cholesterol; and △, 5:1 1,2:3,4-dibenzanthracene–cholesterol.*

plate yields a direct measurement of surface pressure. Simultaneous recording of surface potential afforded constant monitoring of monolayers at all stages of compression.

Since preliminary results showed that dibenzanthracene itself does not form surface films, the effect when allowed to react with cholesterol may be evaluated by surface pressure–area per molecule relationships (π–A) directly. Surface potential (ΔV) was also monitored since this can be helpful in interpreting subphase film interactions (4).

Dibenzanthracene–cholesterol films were prepared from solutions containing varying mole fractions. The mixed film solution was pipeted onto the aqueous surface using a micropipet[4] and allowed to spread undisturbed for 10 min. After this equilibration, compression was initiated at a rate of 2.54 cm./min., and data taken from simultaneous recordings of surface pressure and surface potential were used to produce the characteristic π–A isotherms. All runs were performed in triplicate at 25°.

RESULTS

Surface Pressure–Area per Molecule—Figure 1 represents the relationship between surface pressure and area per molecule, expressed in terms of cholesterol, for mixed films of 1,2:3,4-dibenzanthracene–cholesterol. Also shown is the isotherm for pure cholesterol. Similar curves were obtained when saline was substituted as the subphase. The extrapolated area per molecule for cholesterol, which depends on the method of compression, is comparable to that found by Kwong *et al.* (12), who also used continuous compression to generate the surface pressure–area isotherm.

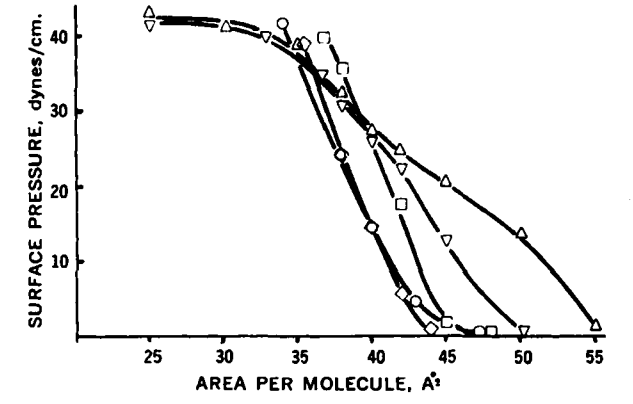


Figure 2—*Surface pressure–area relationship for 1,2:5,6-dibenzanthracene on water. Key: ○, cholesterol; ◇, 1:10 1,2:5,6-dibenzanthracene–cholesterol; □, 1:1 1,2:5,6-dibenzanthracene–cholesterol; ▽, 3:1 1,2:5,6-dibenzanthracene–cholesterol; and △, 5:1 1,2:5,6-dibenzanthracene–cholesterol.*

[4] Bio-Rad Laboratories, New York, N. Y.

Table I—Limiting Surface Potential on Water and Saline[a]

System	Ratio	ΔV, mv. Water	ΔV, mv. Saline
Cholesterol[b]		401 (2.6)[c]	406 (6.1)[c]
1,2:3,4-DBA–chol	1:10	399 (3.1)	411 (7.2)
1,2:3,4-DBA–chol	1:1	403 (8.0)	424 (9.5)
1,2:3,4-DBA–chol	3:1	396 (3.6)	410 (6.6)
1,2:3,4-DBA–chol	5:1	397 (4.7)	427 (4.0)
1,2:5,6-DBA–chol	1:10	378 (13.7)	433 (20.1)
1,2:5,6-DBA–chol	1:1	383 (2.6)	412 (5.5)
1,2:5,6-DBA–chol	3:1	439 (13.6)[d]	464 (1.5)[d]
1,2:5,6-DBA–chol	5:1	436 (18.6)[d]	482 (5.0)[d]

[a] At collapse. [b] DBA = dibenzanthracene; chol = cholesterol. [c] Standard deviation. [d] Significantly different at $p < 0.01$.

Figure 2 illustrates the isotherm resulting from the interaction of the potent carcinogen 1,2:5,6-dibenzanthracene and cholesterol on water. Identical isotherms were observed when experiments were conducted on saline. This figure may be contrasted to Fig. 1, or the noncarcinogenic isomer with cholesterol. The expansion of the carcinogen film at low surface pressures is significant in view of the noncarcinogen film results.

Surface Potential—Surface potential data are shown in Table I. Surface potential was taken at collapse or when the potential failed to change and reached a limiting value. In either case, this generally corresponded to a surface potential of the film at cross-sectional areas per molecule of 35–40 Å². A one-way analysis of variance performed on the data for each subphase showed statistical significance at $p < 0.01$. The individual means within each subphase were then tested using the sequential Q test (13). The 3:1 and 5:1 carcinogenic 1,2:5,6-dibenzanthracene–cholesterol films showed significance with $p < 0.01$ and regardless of subphase.

DISCUSSION

The steric rearrangement of a benzene ring in dibenzanthracene changes this compound from a potent carcinogen to a very weak carcinogen or a noncarcinogen. Although Snart (5) reported some surface interactions of 1,2:5,6-dibenzanthracene, the study did not include 1,2:3,4-dibenzanthracene. The ability to contrast these two compounds *in vitro* may have added importance, since Heidelberger and Moldenhauer (14) showed the noncarcinogen to bind more strongly to protein than the carcinogenic isomer. This binding behavior of dibenzanthracene is generally recognized to be contrary to the usual behavior wherein carcinogens bind more strongly than noncarcinogens.

In the surface interactions with cholesterol, no correlations could be drawn between concentration of noncarcinogenic dibenzanthracene and the isotherms produced. No trend was evident, and the slight differences noted are relatively insignificant when compared to changes evident in the carcinogen films. Mixed films of the strong carcinogenic isomer showed a very different effect with cholesterol. No difference was observed between the cholesterol isotherm and the 1:10 carcinogen–cholesterol film. However, with the films containing higher carcinogen–cholesterol ratios (1:1, 3:1, and 5:1), a significant difference in behavior was noted. There was a dramatic expansion of the film at low surface pressures, attributable to the presence of carcinogen at the interface. There was also a rank correlation between the concentration of 1,2:5,6-dibenzanthracene and the expansion of the film, and this was related to the extrapolated area per molecule.

The extrapolated area per molecule, by tradition, is obtained by continuing a portion of the π–A isotherm to zero film pressure. The assumption was made that, from steric factors and for maximum interaction to occur, the cross-sectional area per molecule of dibenzanthracene was 40–50 Å². By evaluating the expansion effect of the carcinogen–cholesterol films from extrapolated areas per molecule, it was possible to estimate the final molecular association

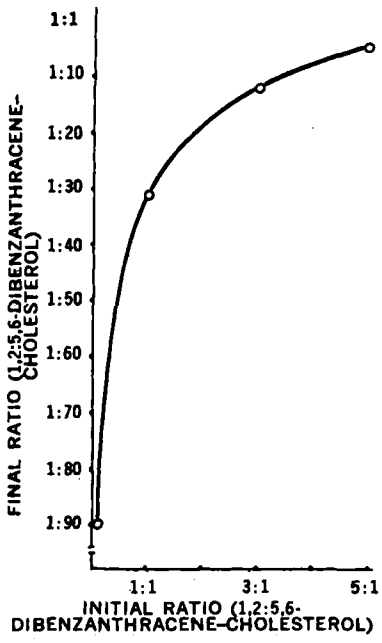


Figure 3—*Degree of association of 1,2:5,6-dibenzanthracene to cholesterol estimated from extrapolated area per molecule.*

present in the film. The molar ratio of carcinogen to cholesterol initially prepared *versus* the association estimated from expansion of the resulting films is plotted in Fig. 3. The data show that after spreading a solution containing a 5:1 carcinogen–cholesterol initial ratio, a 1:4 molecular association results at the interface. Although this association appears to be self-limiting as carcinogen concentration is increased in the film, the limiting value and its significance are still unknown.

Surface potential data reinforced the concept of carcinogen association at the interface. The 3:1 and 5:1 carcinogenic films were statistically different from the noncarcinogenic films regardless of subphase. These results were not unexpected in view of their π–A isotherms, since the 3:1 and 5:1 carcinogen–cholesterol films showed the greatest expanding effect.

These results demonstrate a very useful difference existing between a carcinogenic and a noncarcinogenic or weakly carcinogenic isomer at the air–water interface. This method conceivably may be useful in discriminating between other carcinogenic and noncarcinogenic isomers. Furthermore, cell membrane integrity or disruption at the molecular level may be a cancer-initiating step prior to interaction with other critical targets within the cell.

REFERENCES

(1) D. F. H. Wallach, *Proc. Nat. Acad. Sci. USA*, **61**, 868(1968).

(2) G. Weissmann, W. Troll, B. L. van Duuren, and G. Sessa, *Biochem. Pharmacol.*, **17**, 2421(1968).

(3) J. K. Selkirk, J. C. Elwood, and H. P. Morris, *Cancer Res.*, **31**, 27(1971).

(4) G. L. Gaines, Jr., "Insoluble Monolayers at Liquid–Gas Interfaces," Interscience, New York, N. Y., 1966, pp. 188, 348.

(5) R. S. Snart, *Biochim. Biophys. Acta*, **144**, 10(1967).

(6) N. D. Weiner, I. Chawdry, and A. Felmeister, *J. Pharm. Sci.*, **60**, 425(1971).

(7) J. W. Cook, I. Hieger, E. L. Kennaway, and W. V. Mayneord, *Proc. Roy. Soc.*, **B111**, 455(1932).

(8) C. Heidelberger, *Eur. J. Cancer*, **6**, 161(1970).

(9) E. Huberman, J. K. Selkirk, and C. Heidelberger, *Cancer Res.*, **31**, 216(1971).

(10) J. W. Munden, D. W. Blois, and J. Swarbrick, *J. Pharm. Sci.*, **58**, 1308(1969).

(11) D. W. Blois and J. Swarbrick, *ibid.*, **61**, 393(1972).

(12) C. N. Kwong, R. E. Heikkila, and D. G. Cornwell, *J. Lipid Res.*, **12**, 31(1971).

(13) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 6th ed., Iowa State University Press, Ames, Iowa, 1967, p. 271.

(14) C. Heidelberger and M. G. Moldenhauer, *Cancer Res.*, **16**, 442(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 3, 1972, from the *Pharmaceutics Section, School of Pharmacy, University of Connecticut, Storrs, CT 06268*

Accepted for publication October 5, 1972.

Presented in part to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

Supported by Grant 117 from the University of Connecticut Research Foundation, Storrs, CT 06268

▲To whom inquiries should be directed. Present address: Sterling-Winthrop Research Institute, Rensselaer, NY 12144

Constituents of *Mammea americana* L. XII: Biological Data for Xanthones and Benzophenones

R. A. FINNEGAN▲, K. E. MERKEL, and J. K. PATEL

Abstract □ Sarcoma 180 inhibition data are presented for a number of related xanthones and benzophenones; a few showed significant activity. One of these was also tested *in vivo* against Ehrlich ascites tumor. Four hydroxyxanthones isolated from a polar extract of *Mammea americana* L. (Guttiferae) seeds did not account for the activity of the extract. The results of two antibacterial assays for a few of these compounds are also presented.

Keyphrases □ *Mammea americana* L.—xanthone constituents and related benzophenones, antitumor and antibacterial activities □ Xanthones—from *Mammea americana* L., antitumor and antibacterial activities □ Benzophenones—antitumor and antibacterial activities

A previous paper (1) described the isolation and structure determination of a variety of coumarin derivatives from mamey oil (the dewaxed petroleum ether extract of *Mammea americana* L. seeds) and reported the significant antitumor activity (against Sarcoma 180) of the extract as well as that of a number of the constituent coumarins. During these studies the more polar extract, obtained by extraction of the seed residue with