Interaction of Nitrogen Dioxide with Cholesterol Monomolecular Films: Effect of Initial Surface Pressure, Time of Exposure, and Concentration of Nitrogen Dioxide

AIDA M. KAMEL, ALVIN FELMEISTER, and NORMAN D. WEINER*

Abstract □ The interaction of NO_2 with cholesterol monomolecular films was studied as a function of the initial surface pressure, time of exposure, and concentration of NO_2. A condensation of the cholesterol films was observed that increased with the time of exposure and concentration of the NO_2. This effect apparently was due to the loss of cholesterol from the interface. A percent apparent loss of cholesterol was calculated. Films exposed at an initial surface pressure of 7 dynes/cm. exhibited a greater percent apparent loss of cholesterol than did those exposed at 0 dyne/cm. An explanation for these results based on the orientation of the cholesterol at the interface is offered, and the possible biological relevance of this work is considered.

Keyphrases □ Cholesterol monomolecular films—nitrogen dioxide interaction □ Films, cholesterol—initial pressure, exposure time effects □ Nitrogen dioxide, concentration effect—cholesterol film interaction □ Colorimetric analysis—spectrophotometer

Recently, the authors reported the interaction of a series of phospholipid films with the air pollutant nitrogen dioxide (1, 2). The data demonstrated that the unsaturated fatty acid moieties of phospholipids are sensitive to attack by NO_2. Similar results were reported *in vivo* by Thomas *et al.* (3), who postulated that some observed effects of air pollutants may be directly attributable to the interaction of NO_2 with unsaturated lipids.

Since cholesterol also is an important component of cell membranes, it was of interest to examine the interaction of NO_2 with cholesterol monomolecular films. In this study the effect of time of exposure, NO_2 concentration, and the physical state of the cholesterol film, as influenced by the initial surface pressure, were investigated.

EXPERIMENTAL

Materials and Equipment—Chromatographically pure cholesterol was obtained,[1] and spectroscopic grade hexane was used to prepare the cholesterol solutions. All other chemicals were of reagent grade. Previously deionized water was distilled from an all-glass still prior to use.

A mixture of 0.5% NO_2 (99.5% pure)[2] and 99.5% research grade nitrogen was used. The gas mixture was allowed to pass through a flow meter at a fixed rate and directed into a short length of perforated Teflon tubing which was affixed to the underside of an acrylic resin[3] trough cover as previously described (1). This served to maintain the desired gaseous atmosphere over the film.

The film balance used to study the surface pressure–surface area (π–A) characteristics of the film has been described previously (1). Surface pressures were measured by the Wilhelmy plate method (4).

Methods—Solutions of cholesterol in hexane were spread on a 0.065 M phosphate buffer at pH 7.0, and the gas was allowed to flow over the film for a fixed period of time. At the end of the time period, the flow of gas was discontinued; any NO_2 remaining over the surface was removed by use of the exhaust fan in the hood, in which the film-balance unit was set. Manual compression of the film was then initiated, and surface pressure readings were obtained at various film areas.

The concentration of the NO_2 over the subphase during exposure was determined as follows. At periodic intervals during each experiment, 10-ml. samples of the atmosphere under the acrylic resin cover were drawn into a gastight Hamilton syringe containing 10 ml. of NO_2-absorbing solution. The color of the absorbing solution was permitted to develop for 15 min., and the absorbance was measured at 550 mμ using a Spectronic-20 spectrophotometer. The concentration of NO_2 was determined from a standard nitrite curve (5).

RESULTS AND DISCUSSION

Effect of Concentration and Time of Exposure to NO_2—Figure 1 shows the π–A curves obtained after exposure of a cholesterol film (at an initial pressure of 0 dyne/cm.) to 175 ± 25 p.p.m. of NO_2 for various time periods. Under these conditions the condensation effects increased with the time of exposure up to 60 min. Exposure for periods longer than 60 min. had no further effect on the shape or position of the π–A curve. A similar limiting effect was observed also when the film was exposed at an initial surface pressure of 7 dynes/cm. Additional experiments were conducted at both

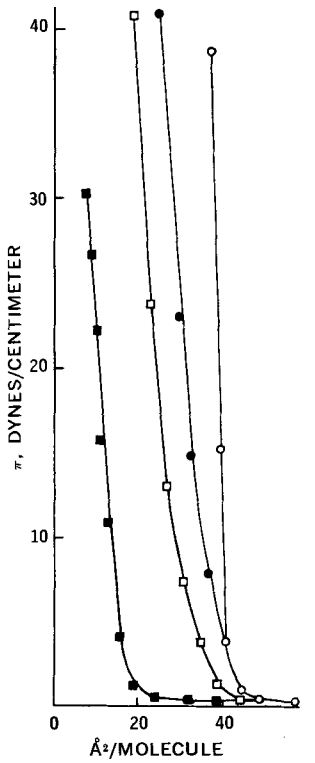

Figure 1—π–A *curves of cholesterol films exposed to 175 ± 25 p.p.m. of NO_2 at an initial surface pressure of 0 dyne/cm. for various time periods. Key:* ○, *control;* ●, *20 min.;* □, *35 min.; and* ■, *60 and 90 min.*

[1] Mann Research Laboratories, N. Y.
[2] Matheson Co., East Rutherford, N. J.
[3] Lucite, E. I. du Pont de Nemours, Wilmington, Del.

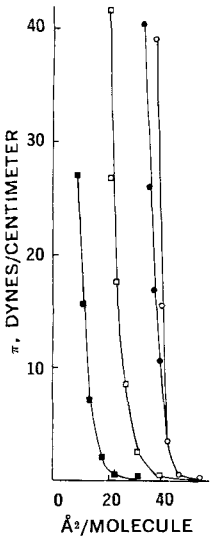

Figure 2—π–A *curves of cholesterol films at an initial surface pressure of 0 dyne/cm. exposed to various concentrations of* NO_2 *for 60 min. Key:* ○, *control;* ●, *25 ± 10 p.p.m.;* □, *95 ± 20 p.p.m.; and* ■, *175 ± 25 p.p.m.*

initial pressures using a concentration of 95 ± 20 p.p.m. of NO_2. In these cases, maximum condensation was observed after a 90-min. exposure.

The reason for this limiting effect could not be explained on the basis of the data obtained in this study. However, Bergstrom and Wintersteiner (6) previously reported such a limiting effect during the autoxidation of aqueous dispersions of cholesterol, which they attributed to an inhibitory effect exerted by the oxidation products. A maximum loss of 70% of the cholesterol was reported by these workers, which corresponds to the maximum percentage losses observed under varying conditions in the present study.

The effect on the π–A curves of films exposed at zero initial pressure for 60 min. to different concentrations of NO_2 is shown in Fig. 2. The condensation effect clearly increases with increasing concentration of NO_2. When films were exposed at an initial pressure of 7 dynes/cm. for the same period of time, the results were essentially the same.

The apparent condensation effect observed in these experiments appears to be due, at least in part, to an NO_2–cholesterol interaction. This reaction, in turn, results in the formation of relatively polar products and in their subsequent desorption or dissolution. This effect can be represented by the percent apparent loss (PAL) of cholesterol calculated from the following equation:

$$PAL = (1 - A'/A)\,100 \qquad \text{(Eq. 1)}$$

where A and A' are the area/molecule of cholesterol at 30 dynes/cm. before and after exposure to NO_2, respectively. PAL is not the actual percent loss of cholesterol from the surface, but rather a complex function which takes into account such factors as desorption, dissolution, changes in molecular orientation, and chemical modification of the cholesterol molecules. However, PAL is a useful means of comparing the effects of NO_2 concentration and initial surface pressure on the stability of NO_2-exposed cholesterol monomolecular films.

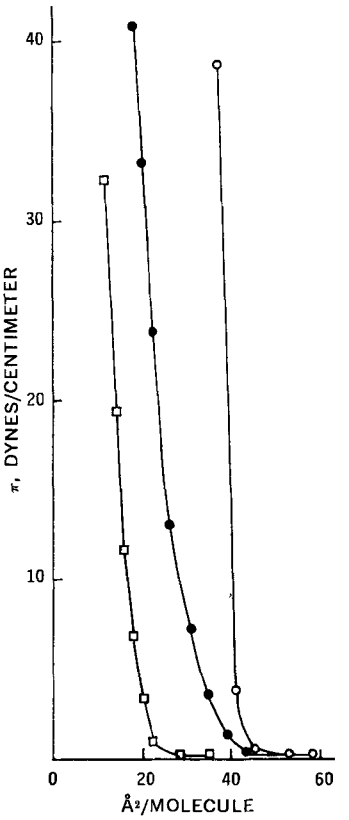

Figure 4—π–A *curves of cholesterol films at various initial surface pressures exposed to 175 ± 25 p.p.m.* NO_2 *for 35 min. Key:* ○, *control;* ●, *0 dyne/cm.; and* □, *7 dynes/cm.*

The curves shown in Fig. 3 were constructed from the calculated values of PAL of cholesterol from films exposed at an initial pressure of 0 dyne/cm. to 25 ± 10, 95 ± 20, and 175 ± 25 p.p.m. of NO_2 for periods of time ranging from 20 to 90 min.

The rate of loss of cholesterol appears to be dependent on the concentration of NO_2 and independent of the amount of cholesterol remaining in the films up to the time at which the limiting effect is observed.

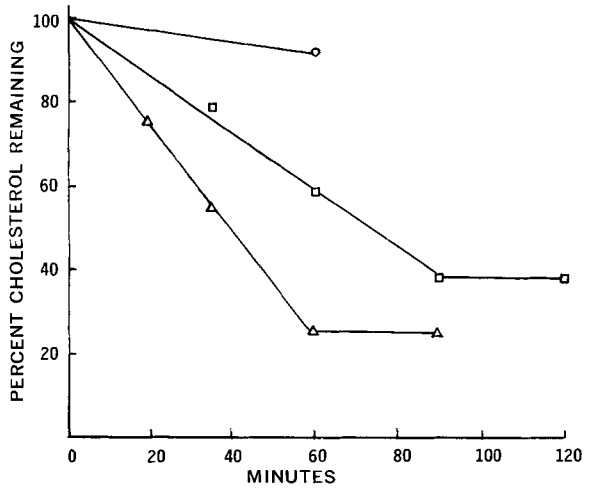

Figure 3—*Rate of loss of cholesterol from films at an initial surface pressure of 0 dyne/cm. exposed to various concentrations of* NO_2. *Key:* ○, *25 ± 10 p.p.m.;* □, *95 ± 20 p.p.m.; and* △, *175 ± 25 p.p.m.*

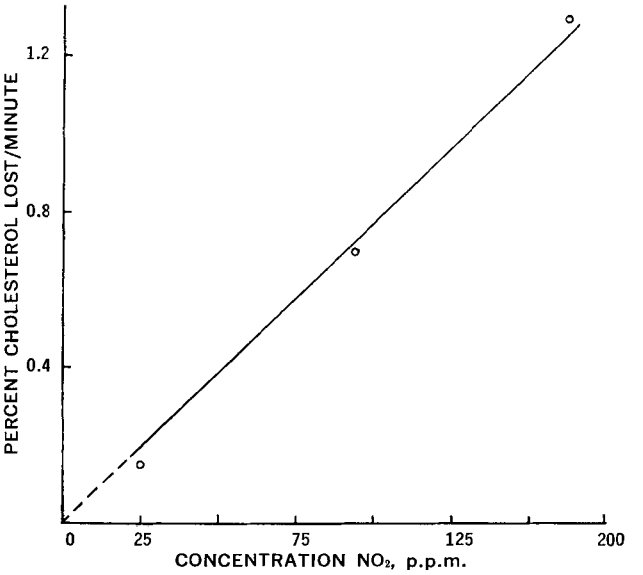

Figure 5—*Rate of loss of cholesterol as a function of* NO_2 *concentration for films at an initial surface pressure of 0 dyne/cm.*

Effect of Initial Surface Pressure—Figure 4 shows the π–A curves of cholesterol films at initial surface pressures of 0 and 7 dynes/cm. prior to and following a 35-min. exposure to 175 ± 25 p.p.m. nitrogen dioxide.

Exposure to NO_2, in the case of the film adjusted to an initial surface pressure of 7 dynes/cm., resulted in a steady decrease in pressure, reaching a value of zero in about 20 min. Furthermore, following a 35-min. exposure to NO_2, the film exposed at this higher surface pressure exhibited a greater PAL of cholesterol.

The orientation of cholesterol molecules in a monomolecular film is markedly influenced by the surface pressure of the film. At zero pressure the molecules would be expected to be essentially flat on the surface, occupying relatively large areas. At 7 dynes/cm., the cholesterol molecules are closely packed and occupy an area that indicates they are in a vertical position with the 3-hydroxy groups anchored in the subphase. Thus, at zero pressure, both polar sites of the cholesterol molecule (*i.e.*, the 3-hydroxy group and the 5–6 double bond) would likely be associated with the aqueous subphase. At 7 dynes/cm., only the 3-hydroxy group would be associated with the subphase, while the double bond would be in the gas phase.

Since the PAL of cholesterol upon exposure to NO_2 is greater when the molecules are in the vertical position, it appears that association of both polar sites with the subphase inhibits the oxidation of cholesterol by NO_2. Such a postulation is supported by Altshuller and Cohen (7), who reported that the oxidation of olefins by NO_2 occurred in the gas phase along with some nitration, while little or no oxidation was observed (though nitration did occur) in the aqueous phase reaction.

CONCLUSIONS

While it appears that the rate of loss of cholesterol from a monomolecular film is dependent on the concentration of NO_2, the rate of loss at levels of NO_2 normally found in polluted air ($\approx$0.5 p.p.m.) would be very slow. Thus, only at relatively high levels of NO_2 would the loss of cholesterol be significant, even over several hours. This can be seen from the extrapolated portion of the plot in Fig. 5 of the rate of loss of cholesterol *versus* the concentration of NO_2.

Whether these results can be related to the effect of NO_2 on cell membranes or to the *in vivo* exposure of humans or animals to NO_2 has not been established. However, the work of Steadman *et al.* (8), in which animals were exposed to wide ranges of concentrations of NO_2 for varying periods of time, does show some correlation. These workers noted that animals exposed to NO_2 concentrations of 70 p.p.m. for 8 hr. suffered pulmonary edema and vascular congestion with high mortality rates. On the other hand, exposure to a concentration of 0.5 p.p.m. even for as long as 90 consecutive days produced no apparent untoward effects. Thus, it may be that the loss of cholesterol from surface films, which would be very slow at a NO_2 concentration of 0.5 p.p.m. and relatively fast at a concentration of 70 p.p.m., gives some indication of the loss of this essential lipid from cell membranes and of the observed clinical effects of NO_2.

REFERENCES

(1) A. Felmeister, M. Amanat, and N. D. Weiner, *Environ. Sci. Technol.*, **2**, 40(1968).

(2) N. D. Weiner, M. Amanat, and A. Felmeister, *Arch. Environ. Health*, **18**, 636(1969).

(3) H. V. Thomas, P. K. Mueller, and R. L. Lyman, *Science*, **159**, 532(1968).

(4) H. W. Adamson, "Physical Chemistry of Surfaces," 2nd ed., Interscience, New York, N. Y., 1967, p. 26.

(5) B. E. Saltzman, *Anal. Chem.*, **26**, 1949(1954).

(6) S. Bergstrom and O. J. Wintersteiner, *J. Biol. Chem.*, **141**, 597(1941).

(7) A. P. Altshuller and I. Cohen, *Int. J. Air Water Pollut.*, **4**, 55(1961).

(8) B. L. Steadman, R. A. Jones, D. E. Rector, and J. Siegel, *J. Tox. Appl. Pharmacol.*, **9**, 160(1966).

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Crystal Pseudopolymorphism of Cephaloglycin and Cephalexin

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Abstract □ The systematic investigation of the crystallization of cephaloglycin and cephalexin leads to a better understanding of the part that pseudopolymorphic crystal transitions play in the analysis, processing, and formulation of these and many other pharmaceutical compounds. The employment of solubility *versus* solvent composition diagrams to detect various crystal forms of compounds is discussed. This appears to be a convenient and sensitive method for detecting new crystalline phases. It should find application whenever crystallizations are performed with more than one solvent, and particularly when instability of the compound at elevated temperatures prevents the use of conventional thermal methods or when poor crystal development limits the use of microscopic methods. Interpretation of vapor pressure–composition relationships for various crystal forms of these compounds points to the advisability of obtaining such data for all pharmaceutical solids.

Keyphrases □ Crystal pseudopolymorphism—cephaloglycin, cephalexin □ Cephaloglycin, cephalexin—pseudomorphic crystallization □ UV spectrophotometry—analysis □ X-ray powder diffraction—analysis □ NMR spectroscopy—analysis

It is the responsibility of the pharmaceutical chemist to become familiar with the crystallizing properties of drugs in order to control the crystal form, habit, size, size distribution, degree of crystallinity, and state of aggregation of the drug particles. These parameters often determine the acceptability of bulk properties (mixing, tableting, filling, dusting, *etc.*) and pharmaceutical performance (dissolution, biological availability, chemical and physical stability, suspendibility, rheology, *etc.*). Haleblian and McCrone (1) adequately documented the broad aspects of this argument in a recent review article on polymorphism, wherein they