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Utilization of Model Membranes in a Test for the Mechanism of Ethylene Action

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Summary. Reversible alteration of the surface tension of thin films of lipids, proteins and mixtures of both resulted when the thin films were treated with ethylene and other aliphatic gases. This effect appeared to be a nonspecific surface effect related to the molecular size of the gases. Ethylene produced no change which would ascribe to it any specific properties in this test system. The conductivity of an egg lecithin-cholesterol bilayer membrane separating two electrolytes was unaffected by all the test gases (including ethylene), but chloroform vapors markedly altered the conductivity in a reversible manner. In each of the test systems employed, there was no specificity exhibited by ethylene, either qualitatively or quantitatively, indicating the mechanism of ethylene action cannot be explained as a simple physical effect on membranes.

The physiology of ethylene as a plant growth regulator is well established (*cf.* [13]); however, elucidation of the mechanism by which ethylene effects its hormonal control is still obscure. Support for the concept that ethylene induces some change in membrane permeability or in the enzmye system associated with the membranes has been derived from studies which have shown that ethylene can induce an accelerated rate of swelling in isolated mitochondria [6, 10, 12]. However, recent experiments [11] have shown that other aliphatic gases (both saturated and unsaturated) are as effective as ethylene in causing a more rapid mitochondrial swelling. Thus, because the same specificity shown by ethylene in causing changes in intact plant tissues has not been demonstrated in systems using mitochondrial swelling, no direct evidence for an effect of ethylene on membranes exists.

Evidence correlating the effects of physiologically active agents and membrane phenomenon has been derived from experiments with model

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membrane systems. For example, a decrease in surface tension of inter facial films caused by narcotic agents was determined to occur at the sam partial pressure needed to produce anesthesia in mice, suggesting that thi membrane effect was the basis for their narcotic action [3]. Psychoactiv compounds were shown to decrease the surface tension of monomolecula lipid films, and it was suggested that these psychoactive compounds ma be bound to lipids of natural membranes [4]. Additional support for th proposal of narcotic agents acting upon the lipid moiety of membranes wa demonstrated for a series of n-alkyl alcohols (C_4 to C_8) which altered ar egg phosphatidylcholine-dicetylphosphoric acid mixed monolayer [1]. Whe treated with the alcohol series (and other narcotic agents), labeled potas sium (^{42}K) was shown to leak from swollen phospholipid liquid crystals. A measurable response of the model membranes to all the compounds at o about their effective *in vivo* concentrations was reported. Narcosis whic is induced by these types of compounds was suggested to be related to th change in permeability to cations which results from the alteration in th lipid component of biological membranes.

Another model membrane system which has been employed in the stud of membrane physiology is the "black lipid membrane" (BLM), a lipi bilayer membrane separating two aqueous phases. When applied to a smal orifice separating two aqueous media, amphiphilic lipids spontaneousl form thin membranes which have electrical properties similar to those o biological membranes. Reviews of the bimolecular lipid membrane techniqu have been published [7, 8], and Tien and Diana [14] detail the usefulness o the formation of *in vitro* bimolecular lipid membranes which have mad possible the systematic study of biological-like membrane properties.

In the study reported here, monolayer and bilayer model systems wer used to evaluate the influence of ethylene on membrane components. Othe aliphatic gases and organic compounds were also tested for comparativ purposes.

Materials and Methods

The apparatus used consisted of a 1-liter trough of Teflon, 23 cm long and 15 cn wide, with a movable bar across the top for compressing the film. Surface tension an relative tension measurements were made, using a rectangular platinum blade, 0.7 cn high, 4 cm long and 0.001 cm thick, supported so that the long edge penetrated th surface of the water. The platinum blade was drawn up until the meniscus clung t either side, and the base of the blade was positioned parallel to the surface of the water The maximum resultant tension was used to calculate the surface tension (dynes/cm) The platinum blade was connected to a Statham Model G10B Transducer, and th signal was amplified by a UR4 Statham precision readout amplifier and recorded on 1-mV recorder. The trough was filled with unbuffered water, doubly distilled fron

alkaline permanganate in a glass still. The apparatus was housed in a plastic container to protect the film from dust and atmospheric contaminants and to restrict the gas within the system.

Stearic, oleic and linolenic acids (Applied Science Laboratories – grade A) and cholesterol (CalBiochem Co.) were dissolved to a concentration of 0.05% in isooctane (Phillips Petroleum Co. – spectral grade). Twenty-μliter samples of these solutions were dropped onto the water and allowed to spread over the surface. The isooctane was then allowed to evaporate for 5 min before readings were taken on the resultant film. One ml of methanol containing 1.0% chromatographically pure egg lecithin (obtained from Dr. R. C. MacDonald, University of California, Berkeley) and 0.17% cholesterol was diluted with 99 ml of n-decane and 300 ml of isooctane and spread in the manner described above.

Bovine serum albumin (BSA; Fraction V, CalBiochem Co.) and cytochrome c (C. F. Bockringer and Sochne) were dissolved in water at a concentration of 45 mg/100 ml. These films were spread by a method similar to that described by Trurnit [15], employing a frosted glass rod placed vertically in the trough so that it extended above the water surface by approximately 1 inch. The protein solutions were dripped onto the glass rod from a syringe at a rate of approximately two drops per second, so that the protein solution spread out over the surface of the rod and very slowly moved down the sides until, upon contact with the surface of the water in the through, it spread out in all directions from the rod.

Mixtures of BSA with egg lecithin and cholesterol, and of cytochrome c with egg lecithin and cholesterol were used. These films were constructed by first spreading the protein in the usual fashion and then forming the lipid film over the protein film in the manner previously described for lipids. Packing of the films was accomplished by the standard procedure described by Gaines [5].

To test the effects of gases on the films, the chamber was purged with the following undiluted gases at atmospheric pressure after bubbling them through water: ethylene, ethane, propylene, propane, 1-butene and butane (CP grade, Matheson Chemical Co., 99.0 to 99.5% purity). The purity of the gases was checked by flame-ionization gas chromatography (Beckman GC-4), and contamination by physiologically active gases was insignificant. Chloroform, hexane and methanol vapors were carried into the chamber by bubbling air saturated with water vapor through the solvents.

After the films were spread and time allowed for the isooctane to evaporate as described, the films were packed and the surface tension vs. area curves were determined at 21 °C. The air inside the chamber was purged to equilibrium by the test gas or vapor (causing a decrease in surface tension), the gas was turned off, and the air saturated with water vapor was passed into the chamber, displacing the test gas (Fig. 1). When the surface tension of the system returned to its original value, the next gas was introduced. This procedure was repeated until all the aliphatic gases had been passed over the surface. Hexane, chloroform and methanol vapors followed the aliphatic gases because of the slow recovery time. These gases dissolve in the water and film to a considerable concentration, and thus a longer period was required for the film to return to its original tension.

To study the conductance of a lipid bilayer, a lucite apparatus was constructed, similar to that described by Huang, Wheeldon and Thompson [9]. This consisted of two cells containing an aqueous media separated by a 1/32-inch lucite plate with a 1.5-mm pore in which the lipid bilayer was spread. Approximately 5 ml of 0.1 M NaCl was added to each cell, and the membrane formed by brushing over the pore a solution containing 1% egg lecithin – 0.17% cholesterol in 100 ml of n-decane. The membrane constructed in this manner permitted a current of 0.5×10^{-12} to 3×10^{-12} amp as measured by a

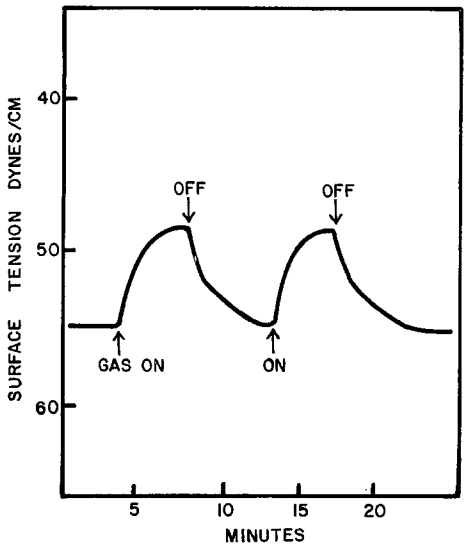

Fig. 1. Reversible decrease in surface tension of a thin film of oleic acid when treate with butane. Notations on the figure indicate time at which gas treatment began and ende

Hewlett Packard 425 AR DC-micro volt ammeter at an applied voltage of 150 mV Various test gases were passed over the surface of the cell, and changes in conductivity wer measured.

Results and Discussion

Comparison of the values in the Table showing the decrease in surfac tension of thin films treated with aliphatic gases and organic vapors indi cates that each of the test compounds could alter the model membrane

Table. *The decrease in surface tension of various thin films induced by aliphatic gases and organic vapors*

Gas or vapor	Decrease in surface tension (dynes/cm ±0.7)				
	Oleic acid	Linolenic acid	BSA	Cyto-chrome c	BSA- egg lecithin-cholesterol
Ethylene	0.5	0.6	1.3	0.5	2.7
Propylene	2.0	1.7	3.0	2.0	–
Propane	2.1	1.7	2.9	2.6	7.5
l-Butene	6.1	5.3	9.5	6.5	21.3
Butane	6.5	5.1	9.6	5.9	26.0
Hexane	10.5	11.2	13.9	10.0	30.7
Chloroform	8.0	10.1	11.3	6.8	28.0
Methanol	7.0	7.0	5.5	5.9	5.5

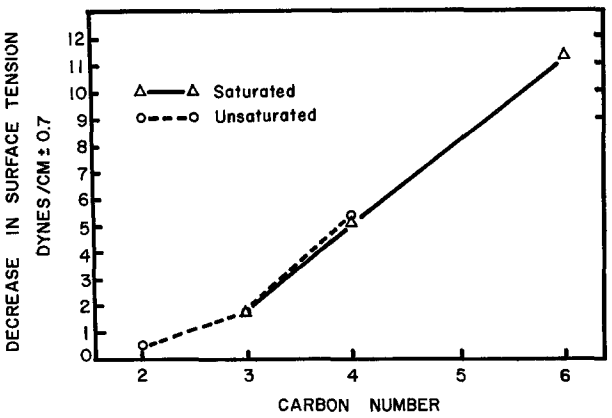

Fig. 2. The effect of molecular size (carbon number) of aliphatic gases on the decrease in surface tension of a thin film of linolenic acid

tested. Across all of the thin films tested, there appears to be general correlation between the molecular size within the homologous series of hydrocarbon gas and the resultant decrease in surface tension observed. As the molecular size increased, the decrease effected in surface tension similarly increased. Small differences in these effects on decreasing surface tension between the unsaturated and saturated gases were observed; however, these differences were not significant within the error of the test system. Fig. 2 shows the relationship between molecular size of the aliphatic gas and its resultant effect on reducing surface tension of a thin film of linolenic acid. A similar relationship between molecular size and decrease in surface tension was observed for the other thin films although the magnitude of the change differed between the films.

The effects for any single test gas on thin films of oleic acid, linolenic acid, BSA and cytochrome c were very similar (Table). It is readily apparent that the treatment of a lipoprotein complex (BSA-egg lecithin-cholesterol) induced a greater decrease in surface tension than did treatment of the fatty acids or the proteins alone. These results are consistent with those of Clements and Wilson [3] who showed that the effect of narcotic agents was greater on a lipoprotein membrane isolated from beef lungs than on a lipid thin film alone.

To extend these findings with another test system, the change in current of an egg lecithin-cholesterol bilayer over an orifice separating chambers of electrolyte was measured while exposing the electrolyte surfaces to the undiluted test gases.

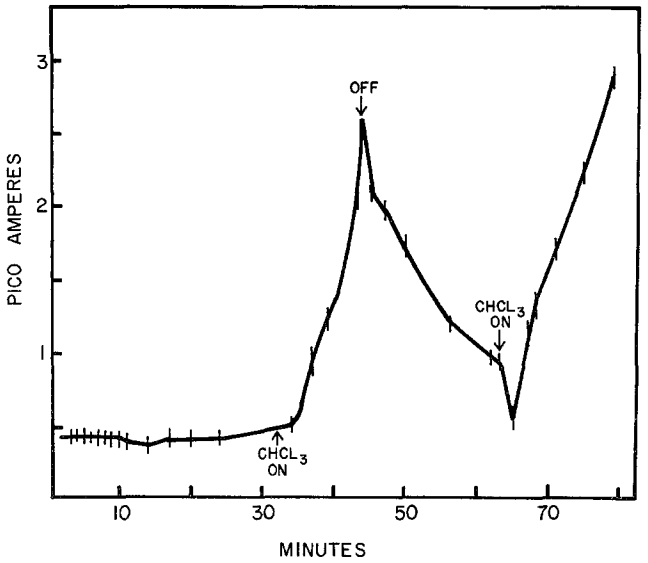

Fig. 3. The reversible effect of chloroform vapors on increasing conductance of egg lecithin-cholesterol bilayer. Notations on the figure indicate time which vapor treatment began and ended. The vertical lines indicate the error of the measurements

The vapors from chloroform showed that the system was functioning, and the results are shown in Fig. 3. The stability of this bilayer system is demonstrated by the ability of the conductance to return to nearly the initial value after the chloroform vapors are allowed to dissipate. Because of the high affinity of lipids for chloroform, it is apparent that significant changes in a lipoprotein membrane can occur from such a treatment. On the other hand, none of the aliphatic hydrocarbon gases tested had such an effect even after 60 min of gassing.

From these results with the model membrane systems described, it is apparent that, although the aliphatic hydrocarbon gases can induce a reversible decrease in surface tension of the films tested, there is no evidence that ethylene has any unique properties which might allow one to explain its physiological effects in terms of a physical action on subcellular membranes. It is still possible that low concentrations of ethylene could influence membrane permeability *in vivo*, but it would have to be the result of some indirect and secondary effect, perhaps by binding to an active metal group in a key enzyme [2]. These studies further indicate that, when investigating the potential influence of ethylene on isolated systems [6, 10], test gases other than ethylene must also be compared in order to demonstrate a specificity for ethylene.

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