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INTERACTION OF BUTYLATED HYDROXYTOLUENE (BHT) WITH PHOSPHOLIPID BILAYER MEMBRANES: EFFECT ON ^{22}Na PERMEABILITY AND MEMBRANE FLUIDITY

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Abstract—Butylated hydroxytoluene (BHT) is a small lipophilic molecule that is widely used as a food preservative. The interaction of this chemical with phospholipid bilayer membranes was monitored by measuring changes in ^{22}Na transport and hydrocarbon chain motion. Vesicles composed of saturated phospholipids display a marked increase in ^{22}Na permeability in the temperature region of the phase transition temperature of the component phospholipid. BHT greatly reduces this permeability increase. When a series of structural analogues was examined, there was found to be a poor correlation between lipid solubility and the capacity to decrease ^{22}Na transport. However, the presence of a hydroxyl group appeared to be an important structural requirement for the permeability change. In a parallel set of experiments, a spin labelled fatty acid ester was incorporated into similar vesicles and the mobility of the label used as a measure of lipid hydrocarbon chain motion. The phase transition temperature of a phospholipid is associated with a marked increase in fatty acyl chain motion. BHT lowers the temperature at which the lipid chains display significant motional freedom (i.e. melt). Since this change in membrane fluidity cannot account for the capacity of BHT to reduce ^{22}Na permeability some additional perturbation must be occurring. It is proposed that this additional perturbation involves an alteration at the interface.

The chemical butylated hydroxytoluene (BHT) enjoys widespread use as a food preservative presumably because of its antioxidant properties. The average daily intake of BHT per person in U.S.A. has been estimated at 2 mg and since this molecule is not readily excreted it tends to accumulate in the body [1]. Despite its presumed non-toxicity, BHT has significant 'biological effects'. Snipes *et al.* [1] examined the effect of this agent on the infectivity of various viruses. BHT readily inactivated viruses containing lipids whereas those containing no lipids were comparatively insensitive. Metcalfe [2] noted that BHT reversibly inhibited the growth rate of cultured monkey kidney cells. A comparison of the effects of analogues of BHT, indicated that the degree of inhibition was related to the lipid solubility of the test molecule. Metcalfe concluded that BHT and its analogues act via a non specific mechanism which depends primarily on the concentration of the perturbing agent within a hydrophobic environment. She further proposed that the most likely site of action is the cell membrane and that the perturbation induced by these molecules involves a decrease in permeability of the membrane to essential metabolites. In addition, Metcalfe drew certain comparisons between the 'membrane stabilizing' effects of anesthetics and the action of BHT. Further evidence that BHT can significantly perturb a 'hydrophobic environment' is the observation of Eletr *et al.* [3] that this molecule lowers the transition temperature of asolectin vesicles by about 10° as measured by the spin label technique.

The above observations suggest that BHT can significantly alter membrane function probably as a result of a perturbation of the hydrocarbon core. In this paper, the interaction between BHT and a model phospholipid membrane is examined in detail and the properties of BHT in this system are compared to those of a local anesthetic in view of Metcalfe's suggestion of a similar mode of action.

MATERIALS AND METHODS

Materials. Dimyristoyl and dipalmitoyl phosphatidylcholine (PC) were purchased from Calbiochem, California. Each of these phospholipids showed a single spot on thin-layer chromatography and was used without further purification. Both of these lipids were stored as stock chloroform solutions under N_2 at −20°. Dicetylphosphate (DCP) was obtained from Sigma Chemical Co., St. Louis, MS and ^{22}Na as the chloride salt from Amersham/Searle. Dibucaine was purchased from K & K laboratories, while the following chemicals were obtained from Aldrich Chemical Co.; 2,6,-di-*t*-butyl-4-methylphenol (butylated hydroxytoluene, BHT), 2-*t*-butyl-4-methylphenol, 2,6-di-*t*-butylphenol, 2,4,6,-tri-*t*-butylphenol, 2-*t*-butylphenol, *t*-butylbenzene. All other chemicals were of reagent grade wherever possible. Twice distilled water was used for all experiments.

Preparation of lipid vesicles (liposomes). Lipid vesicles were prepared by previously described methods [4]. Briefly, appropriate aliquots of stock chloroform solutions of dimyristoyl or dipalmitoyl PC and DCP to give a mole ratio of 95% PC, 5% DCP were dried under vacuum in a glass tube.

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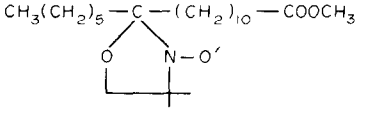
The required salt solution in a volume of 1 ml/15 μmoles lipid and containing tracer amounts of ^{22}NaCl if efflux measurements were to be made, was then pipetted into the tube and the mixture mechanically shaken on a rotamixer. Since these saturated phospholipids form characteristic liposomes only when the temperature exceeds their phase transition temperature (23° for dimyristoyl PC, 41° for dipalmitoyl PC), the salt solution, in which the lipid was dispersed, was preheated to 50° and the tube, when not applied to the rotamixer, was immersed in a water bath also at 50°. The final suspensions were left overnight under N_2 at either room temperature for dipalmitoyl PC–DCP vesicles or at 5° for dimyristoyl PC–DCP vesicles.

Measurement of ^{22}Na efflux. After overnight equilibration, a given lipid dispersion was passed down a column of Sephadex G-50 coarse (Pharmacia, Montreal) to remove excess tracer not trapped within the liposomes. Dimyristoyl PC–DCP vesicles are very leaky in the temperature region of their phase transition, which is close to room temperature. Therefore, these liposomes were eluted from 'chilled' Sephadex using a salt solution previously cooled to 5°. This was done to reduce the loss of trapped isotope while this particular lipid was still on the column. One ml portions (about 1 μmole) of eluted lipid were pipetted into dialysis bags, which were then sealed and dropped into stoppered glass tubes containing 10 ml of aqueous solution. All tubes were placed in a shaking water bath maintained at the desired temperature. The efflux rate was measured over a three hour period. At the termination of each experiment, the ^{22}Na content of the various tubes and bags was counted on a Nuclear-Chicago gamma scintillation counter. Effluxes are expressed as the percentage of initial trapped radioactivity lost over the 180 min. This three hour flux period was chosen primarily to allow sufficient counts to collect in the bulk aqueous phase in those experiments performed at low temperatures and hence involving small leakage rates.

Electrophoretic measurements. The electrophoretic mobility of different liposome populations was measured at various temperatures in a cylindrical microelectrophoresis chamber [5]. Under the influence of an electric field, the vesicles will migrate toward the electrode of opposite polarity. For a given lipid particle, the mobility is expressed as the velocity ($\mu m\ sec^{-1}$)/unit potential gradient ($V\ cm^{-1}$). For each sample, the mobility of at least ten to fifteen liposomes was measured and the results were averaged. The range of values was never greater than ±10 per cent of the mean.

Electron spin resonance (esr) spectroscopy. The spin label probe 12-doxyl stearate methyl ester hereafter referred to as I (5,10) was obtained from Syva Corporation, Palo Alto, CA.



The label in a stock chloroform solution was added to the lipid prior to drying at a concentration of 1 mole per 100 moles total lipid. Hence, the composition of the liposomes by mole ratio was PC 94%, DCP 5%, probe 1%. Electron spin resonance spectra were recorded on a Varian E 3 X-band spectrometer with a 100 kHZ modulation frequency using a special quartz flat cell placed in a custom-designed quartz temperature control probe [4]. The temperature of the sample was maintained at better than ±1° during the measurement.

Stock solutions of BHT and its analogues in methanol were prepared freshly each week. In the permeability and electrophoretic experiments an appropriate aliquot of these stock solutions was added to the aqueous phase bathing the liposomes to give the required concentration. Equal volumes of methanol alone, added to the aqueous phase, had no effect on ^{22}Na efflux or electrophoretic mobility. Since these agents have a high oil solubility, they will partition strongly into the lipid phase. In order to allow comparison between experiments, the test molecule was always added to the same amount of lipid. In the spin label experiments, BHT or one of its analogues in methanol was added to the lipid prior to drying. The amount of test molecule added in relation to the quantity of lipid was kept as close as possible to the conditions used in the permeability and electrophoretic experiments. Since the methanol is subsequently removed when the mixture is dried, any changes in the esr spectra can be attributed to the test molecule alone.

RESULTS

Permeability experiments. Figures 1 and 2 (upper half) illustrate the temperature dependence of ^{22}Na permeability in liposomes composed of dimyristoyl PC–DCP, dipalmitoyl PC–DCP, or an equimolar mixture of these two lipids. In all cases ^{22}Na efflux displays a maximum in the region of the phase transition temperature of the constituent phospholipid or phospholipid mixture.* As previously described [4], the local anesthetic dibucaine lowers the temperature at which the permeability maximum occurs consistent with its ability to decrease the phospholipid transition temperature. In contrast, BHT reduces ^{22}Na efflux in these same liposomes and in the case of dimyristoyl PC–DCP and dimyristoyl-dipalmitoyl PC–DCP vesicles completely eliminates the permeability maximum.

Figure 2 (lower half) summarizes the effect of different concentrations of BHT on ^{22}Na efflux from dimyristoyl PC–DCP liposomes. The maximum reduction in ^{22}Na permeability occurs at an aqueous BHT concentration of approximately 0.22 mM. Higher concentrations do not depress ^{22}Na transport any further. By comparison, Metcalfe [2] found that BHT at a concentration of 0.136 mM caused an 80 per cent inhibition of cell growth and a 58 per cent inhibition of RNA synthesis. However, these aqueous phase concentrations must be viewed as nominal only. Due to its high oil solubility† most of the BHT will partition into the lipid phase resulting in true

* An equimolar mixture of dimyristoyl and dipalmitoyl PC possesses a single transition temperature intermediate between that of the two individual phospholipids [6].

† The hexane to water partition coefficient of BHT measured in this laboratory exceeded 2,000 to one.

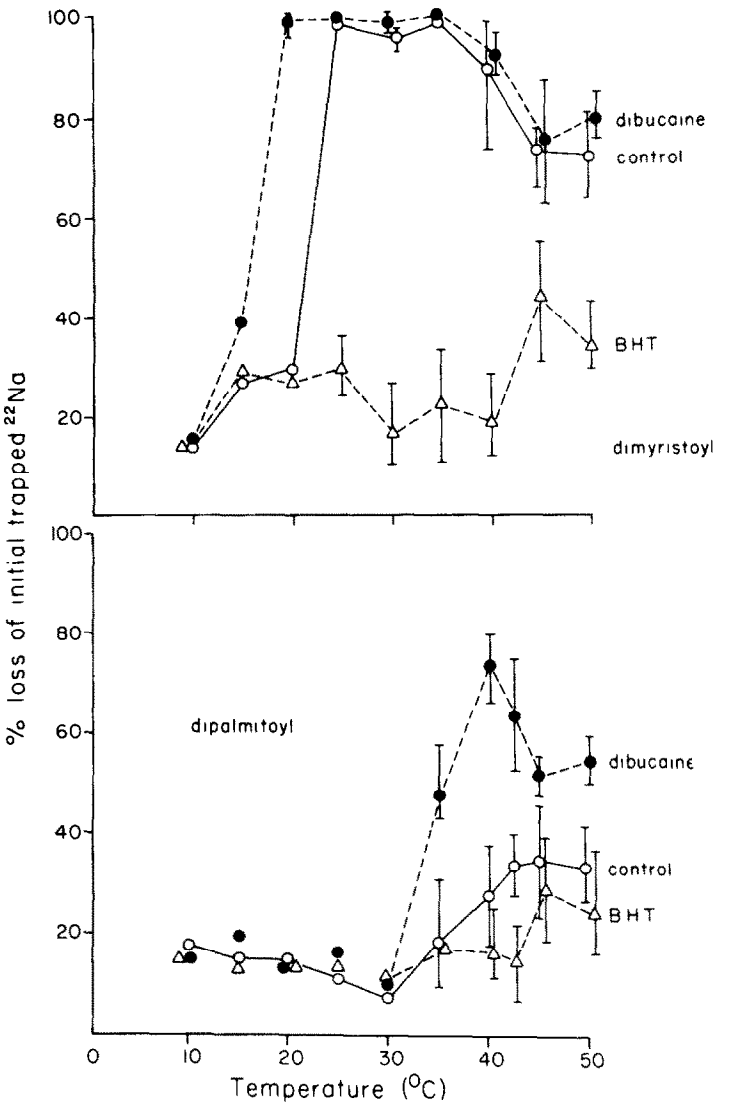
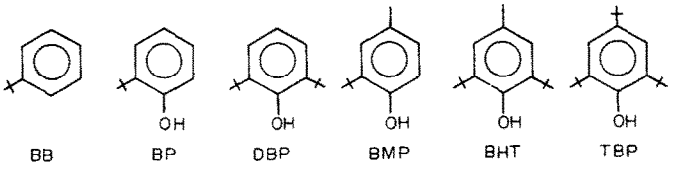


Fig. 1. Effect of BHT and dibucaine on ^{22}Na efflux. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5% (upper half) or dipalmitoyl PC 95%, DCP 5% (lower half) were dispersed in 50 mM NaCl, $^{22}NaCl$, and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into three portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control), and in the presence of dibucaine (1 mM) and BHT (0.22 mM). The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points, for each temperature, represent two separate experiments each performed in quadruplicate. The results illustrated are the means with error bars indicating the range of values obtained. To simplify the figure error bars have been given for only some of the points.

bulk aqueous concentrations less than that noted in Fig. 2.

A series of experiments were performed comparing the capacity of structural analogues of BHT to decrease the ^{22}Na permeability of dimyristoyl PC–DCP liposomes (Fig. 3). The analogues used in these experiments are illustrated below. A comparison of the properties of the two compounds *t*-butylbenzene (BB) and 2-*t*-butylphenol (BP) establishes the role of the hydroxyl group while a comparison between BP and the other analogues defines the effects of adding extra butyl and methyl groups onto the BP molecule.



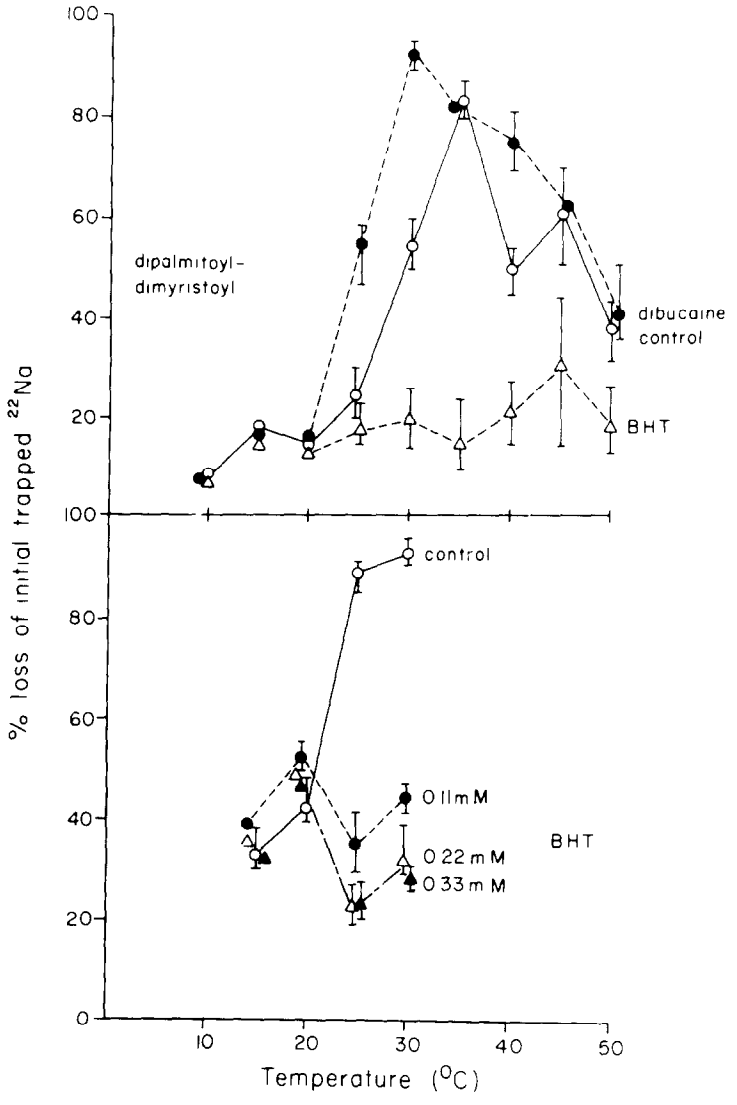


Fig. 2. (upper half). Effect of BHT and dibucaine on ^{22}Na efflux. Liposomes composed (by mole ratio) of dimyristoyl PC 47.5%, dipalmitoyl PC 47.5% and DCP 5% were dispersed in 50 mM NaCl, $^{22}NaCl$, and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into three portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and in the presence of dibucaine (1 mM) and BHT (0.22 mM). The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points, for each temperature, represent two separate experiments each performed in quadruplicate. The results illustrated are means with the range of values indicated by error bars. (lower half). Effect of different concentrations of BHT. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5% were dispersed in 50 mM NaCl, $^{22}NaCl$, and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into four portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and presence of BHT (0.11 mM, 0.22 mM and 0.33 mM). Experimental points for each temperature represent a separate experiment performed in quadruplicate. Results are expressed as means plus range of values obtained.

Both BP and BB are less effective than BHT at a nominal test concentration of 0.22 mM. However, BP is more effective in decreasing ^{22}Na movement than BB, especially at 25°, at which temperature the latter compound is without effect. Figure 3 also illustrates the comparative effects of BHT, 2,6-di-*t*-butylphenol (DBP), 2-*t*-butyl-4-methylphenol (BMP), and 2,4,6-tri-*t*-butylphenol (TBP) at this same test concentration. Among these compounds BHT appears the most potent and BMP the least, with DBP and TBP falling somewhere in between depending upon the temperature.

It is of interest to compare these permeability effects with the relative lipid solubilities of these molecules. The substituted phenols display the sequence TBP > BHT > DBP > BMP > BP in terms of their partition coefficients between liquid paraffin and 0.005 M Tris–HCl buffer [7]. At 25°, for example, these same compounds rank in the order BHT = DBP > TBP = BMP > BP in terms of their capacity

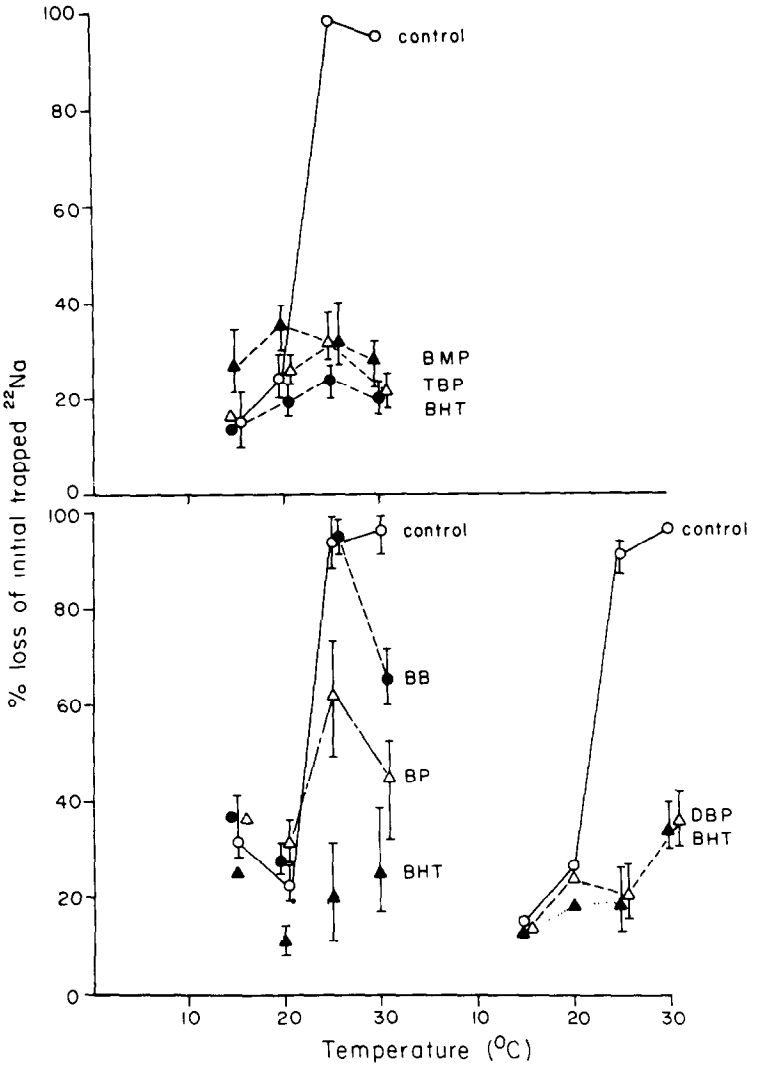


Fig. 3. Effect of different analogues of BHT. Liposomes composed (by mole ratio) of dimyristoyl PC 95%, DCP 5% were dispersed in 50 mM NaCl, ^{22}NaCl, and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into three or four portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and presence of BHT and one or two of its analogues. The abbreviations BMP, TBP, BB, BP, DBP are defined in the text. Test molecules were present at a concentration of 0.22 mM. The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points for each temperature represent a separate experiment performed in quadruplicate. The results illustrated are the means plus range of values obtained.

to decrease ^{22}Na efflux. BB has a higher octanol to water partition coefficient than BP* yet is much less effective in reducing ^{22}Na movement. These observations suggest that there is not a strict correlation between the permeability effects of these compounds and their lipid solubility. This statement requires qualification however, in that a certain degree of lipid solubility (eg. BMP or DBP vs BP) appears necessary for maximum reduction of ^{22}Na efflux. Furthermore, the fact that BP has a greater 'permeability effect' than BB indicates that the hydroxyl group is an important structural requirement for the reduction in ^{22}Na efflux.

In view of the contrasting effects of BHT and dibucaine on ^{22}Na efflux the interaction between these two molecules was examined. When both agents are added to the aqueous phase, dimyristoyl PC–DCP and dipalmitoyl PC–DCP liposomes fail to display a temperature dependent increase in ^{22}Na efflux (Fig. 4, lower half). In fact both types of liposomes behave as if BHT alone was present. The capacity of BHT to block the effect of dibucaine could be due to failure of the local anesthetic to adsorb onto BHT treated liposomes. This possibility was examined by means of the following type of experiment. Liposomes of identical composition to those used in the permeability experiments were electrophoresed at different

* The octanol–water partition coefficients for butylbenzene and butylphenol were calculated from the data given in Ref. 8.

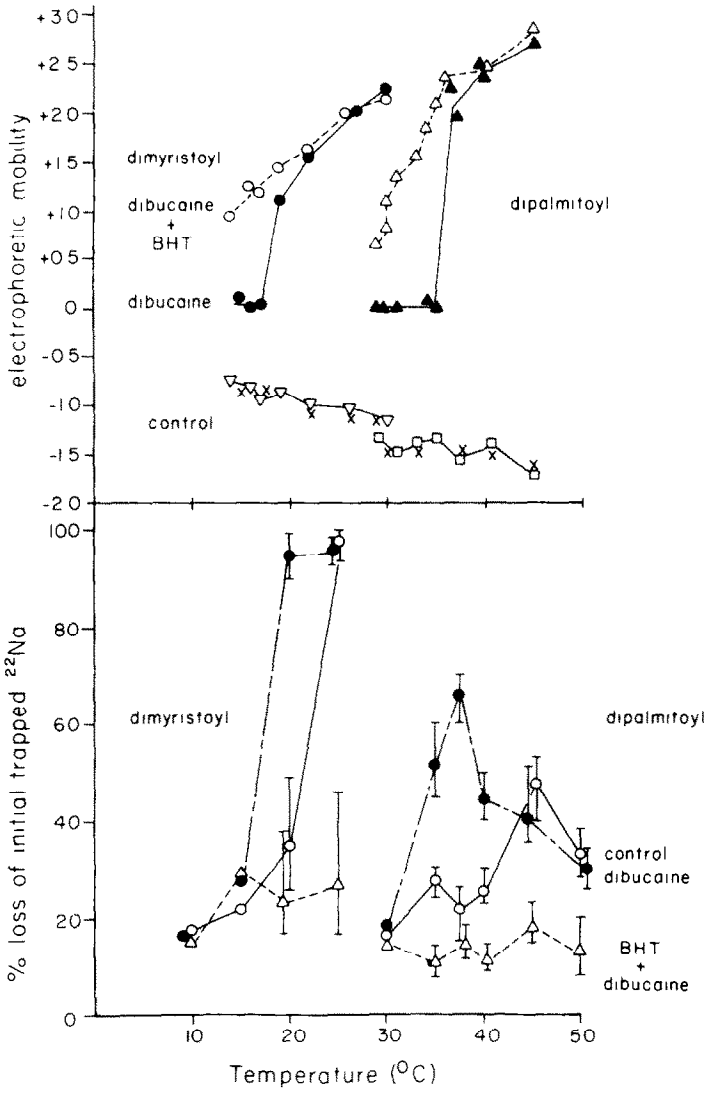


Fig. 4. (lower half). Interaction of dibucaine and BHT. Liposomes composed (by mole ratio) of either dipalmitoyl PC 95%, DCP 5% or dimyristoyl PC 95%, DCP 5% were dispersed in 50 mM NaCl, ^{22}NaCl, and 6 mM Tris–HCl, pH 7.5. Each liposome preparation was divided into three portions to measure ^{22}Na efflux, at a specific temperature, in the absence (control) and presence of dibucaine (1 mM) and dibucaine (1 mM) plus BHT (0.22 mM). The ordinate refers to the percentage of initial trapped isotope lost over 3 hr. Experimental points for each temperature represent a separate experiment performed in quadruplicate with results expressed as the means and range of values. (upper half). Liposomes of identical composition to those used in the permeability experiments were dispersed in 50 mM NaCl, 6 mM Tris–HCl, pH 7.5. The electrophoretic mobility of the lipid vesicles was measured, at different temperatures, both in the absence (control) and presence of dibucaine (1 mM) and dibucaine (1 mM) plus BHT (0.22 mM). The electrophoretic mobility of liposomes exposed to BHT alone is indicated by the crosses and was the same as that of control vesicles. Each point represents the mean of at least ten to fifteen measurements with the range of values being within ± 10 per cent of the mean. The ordinate has the units $\mu m\ sec^{-1}\ V^{-1}\ cm$.

temperatures both in the presence and absence of dibucaine and BHT (Fig. 4, upper half). Both dimyristoyl and dipalmitoyl PC–DCP 'control' liposomes become slightly more negatively charged as the temperature is increased. However, no significant discontinuities occur over the temperature ranges examined. In the presence of dibucaine alone, dimyristoyl PC–DCP and dipalmitoyl PC–DCP vesicles develop a significant positive surface charge at 18° and 36° respectively due to an abrupt increase in surface concentration of dibucaine molecules. In the presence of both BHT and dibucaine, no such abrupt change in electrophoretic mobility occurs, but rather dimyristoyl and dipalmitoyl PC–DCP vesicles display a significant and increasing positive surface charge over the whole temperature range studied. Since BHT itself has no effect on liposome electrophoretic mobility this positive charge must reflect surface adsorption of anesthetic molecules. In other words, although BHT blocks anesthetic induced permeability changes

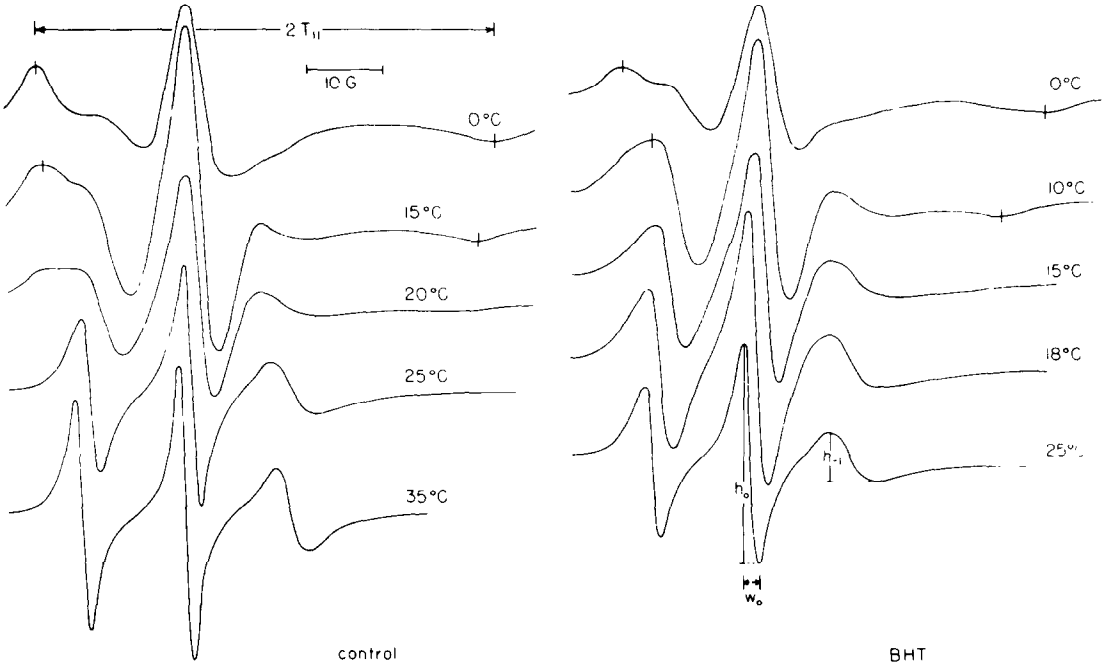


Fig. 5. Effect of BHT on spin labelled vesicles. Control liposomes were composed (by mole ratio) of dimyristoyl PC 94%, DCP 5%, spin label I (5,10) 1% and dispersed in 50 mM NaCl, 6 mM Tris–HCl, pH 7.5. As described in the text, BHT treated liposomes were prepared by adding the BHT (in methanol) to the lipid mixture prior to drying. Electron spin resonance spectra were recorded at the indicated temperatures.

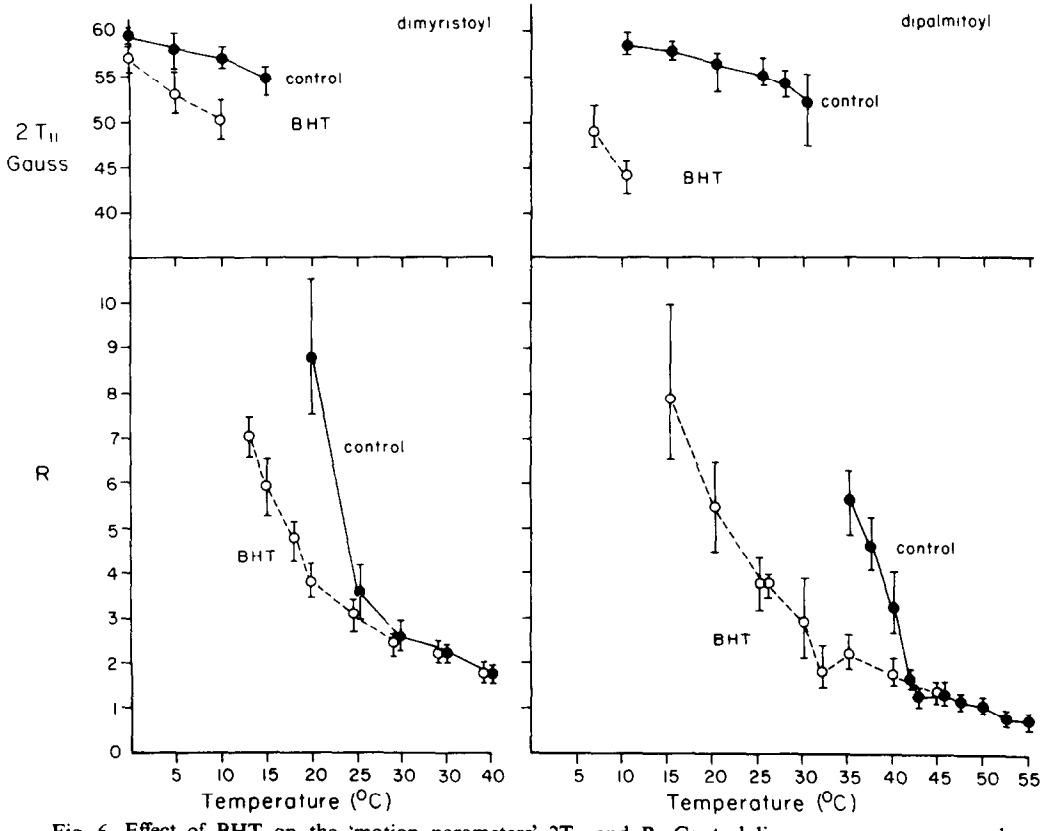


Fig. 6. Effect of BHT on the 'motion parameters' $2T_{\parallel}$ and R. Control liposomes were composed (by mole ratio) of either dimyristoyl 94%, DCP 5%, spin label I (5,10) 1% or dipalmitoyl PC 94%, DCP 5%, spin label I (5,10) 1% and dispersed in 50 mM NaCl, 6 mM Tris–HCl, pH 7.5. BHT treated liposomes were prepared by adding the BHT (in methanol) to the lipid mixture prior to drying. The parameters $2T_{\parallel}$ and R are described in the text. Each point is the mean obtained from ten to fifteen separate spectra. Error bars denote the range of values obtained.

it actually facilitates the surface adsorption of this molecule.

Spin probe experiments. The main information sought from the esr spectra was the effect of BHT on probe, and hence fatty acyl chain motion. Since only relative changes in mobility were examined, two empirical parameters were used to semi-quantitate spin label motion. For esr spectra performed at low temperatures the separation between the low and high field extrema ($2T_{\parallel}$) was measured. Increasing molecular motion is characterized by a narrowing of this separation [9]. With sufficient probe motion, however, the high field deflection disappears making it impossible to measure $2T_{\parallel}$. For these spectra an empirical motion parameter R was calculated according to the following formula [3].

$$R = Wo\{(h_0/h_{-1})^{\frac{1}{2}} - 1\}$$

Where Wo, ho, h − 1 are all defined in Fig. 5. Increasing probe motion is associated with a smaller value for R.

Figure 5 illustrates some representative spectra obtained with spin labelled dimyristoyl PC–DCP vesicles and measured in the presence and absence of BHT. The 'motion parameters' $2T_{\parallel}$ and R for all the spectra are summarized in Fig. 6.

In the case of dimyristoyl PC–DCP liposomes, probe motion increases modestly between 0° and 15°. Above this temperature the upper field deflection disappears and R displays a significant decrease between 20° and 25°. This large increase in probe motion between 20° and 25° corresponds closely to the phase transition temperature of this phospholipid (23°). In the presence of BHT, spin label mobility, as measured by the separation $2T_{\parallel}$, is enhanced compared to 'control' liposomes, over the temperature range 0°–10°. Above 10°, $2T_{\parallel}$ is no longer measurable, but the motion parameter R shows a large decrease between 13° and 20°. By 25°, control and BHT treated liposomes display no difference in the motional freedom of the incorporated spin probe.

Dipalmitoyl PC–DCP vesicles display qualitatively similar results. The motion of the spin label in control liposomes increases moderately over the temperature span 10°–30°. Above this temperature, the upper field deflection disappears and R demonstrates a significant decrease between 35° and 42°. This marked change in spin probe mobility between 35° and 42° corresponds reasonably well with the phospholipid transition temperature (41°). Dipalmitoyl PC–DCP spin labelled vesicles, treated with BHT, display a large reduction in $2T_{\parallel}$ between 6° and 10°. Above 10°, $2T_{\parallel}$ can no longer be measured. The parameter R however demonstrates a significant decrease between 15° and approximately 32°. By 45° spin probe motion appears equivalent in both untreated and BHT treated liposomes.

Finally, as illustrated in Fig. 7, BHT and BP have comparable effects on spin probe motion in labelled dimyristoyl PC–DCP liposomes whereas BB is less effective than these two substituted phenols.

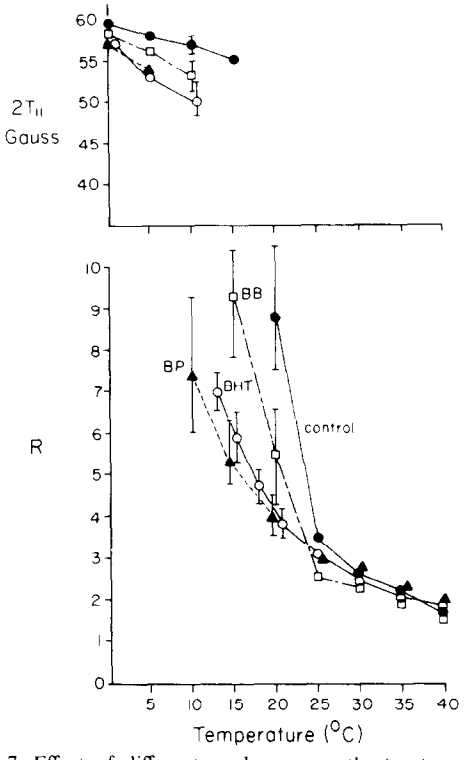


Fig. 7. Effect of different analogues on the 'motion parameters' $2T_{\parallel}$ and R. Control liposomes were composed (by mole ratio) of dimyristoyl PC 94%, DCP 5%, spin label I (5,10) 1% and dispersed in 50 mM NaCl, 6 mM Tris–HCl, pH 7.5. BHT, BP, and BB, in methanol, were added to the lipid prior to drying. Each point is the mean obtained from ten to fifteen separate spectra. Error bars denote the range of values obtained.

DISCUSSION

The model system used in these experiments consists of an aqueous suspension of lipid vesicles or liposomes. The physical characteristics and properties of this system have recently been reviewed by Bangham *et al.* [10]. Each of these vesicles consists of a series of concentric unbroken bimolecular phospholipid membranes separated by discrete and isolated internal aqueous compartments.

Liposomes composed of the saturated phospholipids dimyristoyl PC, dipalmitoyl PC, or an equimolar mixture of these two, display a large increase in ^{22}Na efflux in the vicinity of the phase transition temperature (Tc) of the component lipids. Although the molecular mechanism of this permeability increase is not completely clear, it has been postulated that enhanced diffusion occurs across discontinuities between co-existing liquid crystal and solid lipid domains [11]. The local anesthetic dibucaine lowers the temperature of onset of the permeability increase. As previously described [4], the permeability effects of dibucaine are in good agreement with the effects of this agent on the physical state of the phospholipids, since dibucaine also lowers the temperature at which the lipid chains melt. In contrast, BHT causes a reduction in ^{22}Na efflux and in dimyristoyl PC–DCP and dimyristoyl—dipalmitoyl PC–DCP vesicles actually eliminates the permeability maximum.

The effect of BHT on the molecular motion of lipid hydrocarbon chains was measured by the technique

of electron spin resonance spectroscopy. The underlying assumptions of this technique are that the label is distributed randomly throughout the membrane and that the mobility of the spin label is a valid measure of the molecular motion of the surrounding phospholipid fatty acyl chains. In the case of dimyristoyl PC–DCP liposomes, hydrocarbon chain motion increases significantly between 20° and 25°, in good agreement with the Tc of this phospholipid of 23°. In these vesicles BHT causes the lipid fatty acyl chains to 'melt' at a temperature significantly below the Tc. In addition the melting process appears to occur over a broader temperature range than that observed in control vesicles. By 25° however hydrocarbon chain motion becomes identical in both treated and untreated liposomes. Dipalmitoyl PC–DCP vesicles display qualitatively similar results. In control liposomes the lipid chains show a large increase in motion between 35° and 42°, the Tc of this lipid being 41°. In the presence of BHT, fatty acyl chain mobility begins to increase significantly above 15° and again the melting process appears to extend over a wider temperature span. By 45°, however, both control and treated vesicles possess the same degree of hydrocarbon chain motion. Thus BHT induces qualitatively similar changes in lipid chain mobility to dibucaine, despite their marked difference in terms of effects on ^{22}Na permeability. It is unlikely, then, that the reduction in ^{22}Na efflux caused by BHT is due to its effect on fatty acyl chain mobility.

The experiments involving the interaction of liposomes with both BHT and dibucaine support the observation that BHT increases hydrocarbon chain motion. Since adsorption of anesthetic molecules leads to the development of a positive membrane surface charge, this adsorption process can be monitored by measuring changes in liposome electrophorectic mobility. In essence, the charged end of the dibucaine molecule is used in these experiments as a 'marker' for the whole molecule. As previously described, the surface concentration of dibucaine is a function of the physical state of the phospholipid [4]. Liposomes exposed to this anesthetic, develop an abrupt and large positive charge at a characteristic temperature, as noted in Fig. 4. This enhancement in the adsorption process is due to a melting of the lipid bilayer which would permit more hydrophobic interactions between the non-polar part of the anesthetic molecule and the membrane interior. In the presence of BHT *and* dibucaine, both dimyristoyl PC–DCP and dipalmitoyl PC–DCP vesicles develop a significant positive surface charge at temperatures below that which occurs in the absence of BHT. In other words, BHT increases the surface concentration of dibucaine molecules most probably as a result of its capacity to fluidize the bilayer interior at these lower temperatures. Despite the fact that BHT facilitates the adsorption of dibucaine, itself a 'fluidizing' agent, the ^{22}Na permeability of these liposomes remains low. This observation supports the contention that the permeability properties and the fluidity of the BHT treated membrane display a poor correlation.

It is obvious that the interaction of these substituted phenols with phospholipid bilayer membranes is quite complex and the following model is proposed as a working hypothesis only. As a result of its high oil solubility, BHT will partition strongly into the lipid phase. However, rather than being 'buried' deep within the bilayer BHT, by virtue of its hydroxyl group, would probably be localized close to the interface.

Presumably, this molecule would be intercalated between phospholipid molecules with its hydroxyl group anchored to the membrane–water interface by polar interactions. In this position, BHT could easily disrupt phospholipid packing resulting in the alterations in fatty acyl chain motion observed in the spin label experiments. The other substituted phenols such as BP would have a similar orientation within the bilayer. On the other hand, BB does not possess a hydrophilic group and would probably be found deeper within the membrane interior. If this model is correct, then the observation that BP causes a greater fluidizing effect than BB suggests that a molecule localized close to the interface will cause a larger disruption in lipid packing than a similar sized molecule 'floating freely' within the membrane interior. Since the permeability effects of BHT correlate poorly with its capacity to increase lipid chain motion, some additional perturbation must be invoked to account for the reduction in ^{22}Na efflux. As noted above, the hydroxyl group appears to play a pivotal role in bringing about this reduction. Most likely this additional perturbation involves some change at the interface. The presence of a number of BHT derived hydroxyl groups could alter the surface dipole potential or possibly the organization of interfacial water itself. These two possibilities are not necessarily mutually exclusive nor are they conceivably the only ones. It is of interest that one of the mechanisms by which cholesterol reduces cation permeability is by making the membrane interior more electropositive with respect to the bulk aqueous phase most likely through an alteration in the surface dipole potential by the sterol hydroxyl group [12, 13]. On the basis of this model, BP should be less potent than BHT since it is less lipid soluble and therefore would have a smaller membrane concentration and consequently present a smaller number of hydroxyl groups at the interface.

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