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The Effect of Electric Fields on Brain Lipid-Cholesterol Films

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Summary. The effect of a direct-current electric field on films of brain cerebroside and cholesterol and mixtures with brain phosphatidylethanolamine (PE) using infrared spectral techniques was studied. The intensities of the spectral bands assigned to the vibration of the phosphate and the -$(CH_2)_n$-group of the fatty acid chain of the phospholipid increased to a maximum as the applied voltage increased. The changes were similar to those previously observed with films of the phospholipid, but higher voltages were required to reach the maximum than with the phospholipid films. The spectral changes are related to conformational changes occurring in the phospholipid component of the films containing cerebroside and cholesterol. The infrared spectra of the mixtures indicate that there is hydrogen bonding between the phosphatidylethanolamine and cholesterol in the films and that the phospholipid exists in the nonpolar form in the films. As found previously with films of brain cephalin and lecithin, the electric field strength at which the maximum intensities of spectral bands are observed varies inversely with the thickness.

During the excitation of the neuron, changes occur in the permeability of the membrane to ions. These changes may be associated with alterations in the molecular configuration of the membranal constituents some of which are lipids. The behavior of films of lipids may provide information concerning the changes that occur in these substances in the neuronal membrane during excitation. Films are used because they can be considered as models for the lipid structures of the membrane.

Previous observations on the effect of direct-current electric fields on films of the brain phospholipids, cephalin and lecithin, have shown that the intensities of certain infrared absorption bands were altered [7]. Since the phospholipids exist in the membrane in combination with cerebroside (CEB) and cholesterol (CHOL), the effect of the interaction between these lipids could be determined by performing similar spectral measurements with mixtures of the lipids and CHOL. A mixture of PE–CEB–CHOL with the molar ratio of 1:0.5:1 was selected since this represented the composition that might be considered representative of the lipid constituents in the neuronal membrane. The results of the effect of electric fields on films of CHOL, brain CEB, and mixtures with brain PE are reported in this paper.

Materials and Methods

All lipids used in these studies were tested for purity using thin-layer chromatography (TLC) and purified by standard procedures when necessary. Brain PE (Pierce Chemical Co.) was purified on silicic acid columns [9]. The purified fraction gave one spot on TLC (Silica gel G) using three different solvent systems: $R_F = 0.30$ in chloroform-methanol-water (65:25:4, v/v/v); $R_F = 0.23$ in chloroform-methanol-35% ammonia (14:6:1, v/v/v) [6]; and $R_F = 0.44$ in *n*-butanol-acetic acid-water (3:1:1, v/v/v) [8]. Bovine brain CEB (Pierce Chemical Co.) showed two spots on TLC, which is typical of all CEB reported by other investigators [6]. CHOL (Baker Chemical Co.) showed one spot after TLC.

The films were prepared by dropping concentrated chloroform-methanol (1:1, v/v) solutions onto silver chloride plates. The average thickness of a film was evaluated from the weight of the lipid or mixture on the plate and the measured area of the film [7]. A density of 1.07 g/ml was used. The areas of the film were reproducible to 0.5%.

The arrangement and procedure for measuring the effect of the field was identical to that used previously [7]. The direct-current voltage was applied across a cell consisting of two silver chloride plates with the film between the plates. The infrared spectra were recorded on Perkin-Elmer Models 13 and 21 infrared spectrophotometers using a screen in the reference beam. The Model 13 was used in the voltage studies. The intensities of the spectral band were evaluated by measuring the areas under the band with a planimeter. The areas of a band were reproducible to 2%.

Results

No changes in the intensities of any bands were observed when voltage was applied to films of CHOL, CEB, or CEB–CHOL mixture. The bands whose intensity changes in the spectra of the binary mixtures (PE–CEB, PE–CHOL) and the ternary mixture (PE–CEB–CHOL) are shown in Fig. 1 and Table 1. With some mixtures the measurements of the areas included several bands. In these cases, the bands were too close together to permit differentiation between the bands. Of the four bands that change with applied voltage in the spectra of PE and the mixtures, three bands were located near the same frequencies in the spectra of all films examined. The band at 760 cm^{-1} in the spectrum of the PE film does not appear to change with the voltage in the spectra of any of the films of the mixtures. This may be due to the lack of sensitivity in the measurements because the band is very weak in the spectra of the mixtures. The bands near 880 cm^{-1} in the spectra of the mixtures change intensity with applied voltages, but

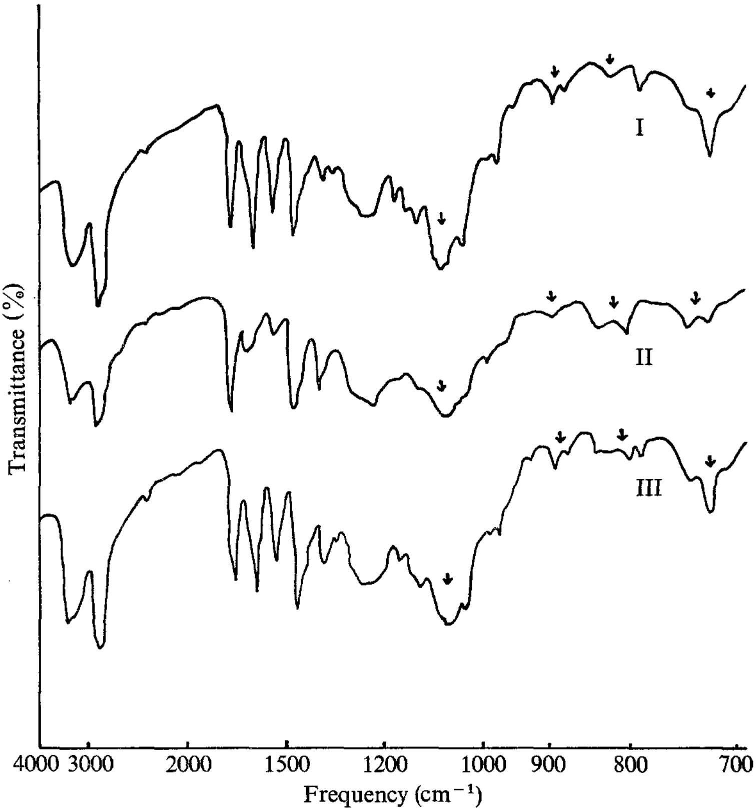

Fig. 1. Infrared spectra of films of mixtures. Arrows indicate the band or regions whose intensities change in an electric field. I: PE–CEB (1:0.5); II: PE–CHOL (1:1); and III: PE–CEB–CHOL (1:0.5:1)

Table 1. Infrared absorption bands of films of PE, CEB and CHOL affected by an electric field

No.	Bands (cm^{-1})	Vibration	Films
1	1070	P–O–C stretch	PE[a]
	1068		PE–CHOL
	1050		PE–CEB, PE–CEB–CHOL
2	885, 870	P–O–C stretch	PE–CEB
	880		PE–CHOL
	880, 865		PE–CEB–CHOL
3	830, 810	P–O–C stretch	PE–CHOL, PE–CEB–CHOL
	825		PE–CEB
	820		PE[a]
3	760	P–O–C stretch	PE[a]
4	740, 720	–$(CH_2)_n$–rock	PE–CHOL
	725		PE[a], PE–CEB, PE–CEB–CHOL

[a] From Ref. [7].

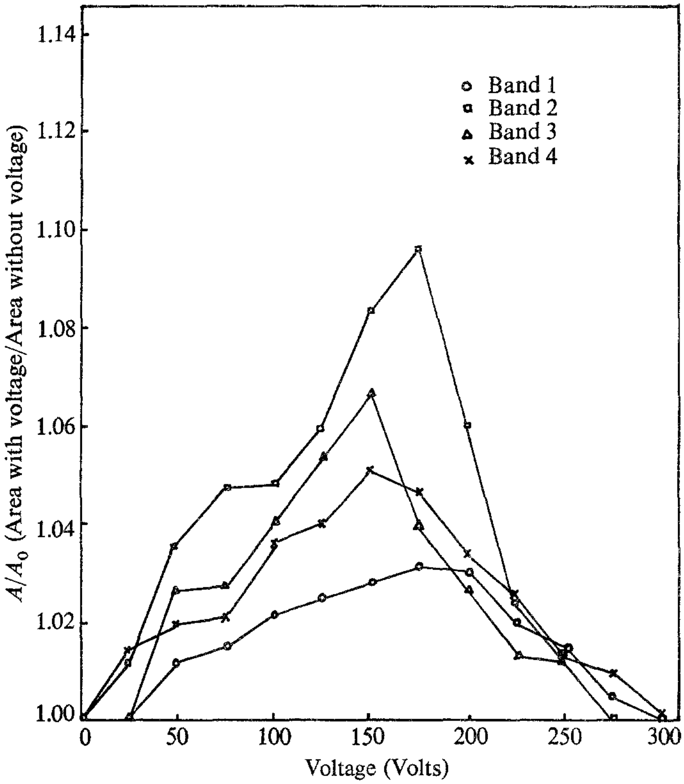

Fig. 2. Variation of the intensities of the bands in the spectrum of a film of PE–CEB (1:0.5) with the applied electric field. Bands are listed in Table 1. A/A_0 is the ratio of area of band to the area with no field

this band does not change its intensity in the spectra of the phospholipids where it appears as a very weak band [7].

The areas of the spectral bands increased with the applied field in the same manner as found with films of the pure PE [7]. Typical results are shown for films of the various mixtures in Figs. 2 through 4. The ordinate (A/A_0) is the ratio of the area of the band at an applied voltage to the area of the band when no voltage is applied. As the voltage increased above the voltage where the maximum change was observed, the ratio of the areas decreased to one. The rate of return to the initial area varied with the film and the band being examined. The maximum voltage $F_{\max}$ (electric field strength, V/mm at which maximum change in area of the spectral band occurs) was not always the same for all bands nor for all mixtures. The values of the area ratios were not the same. For example, the area ratio with peak

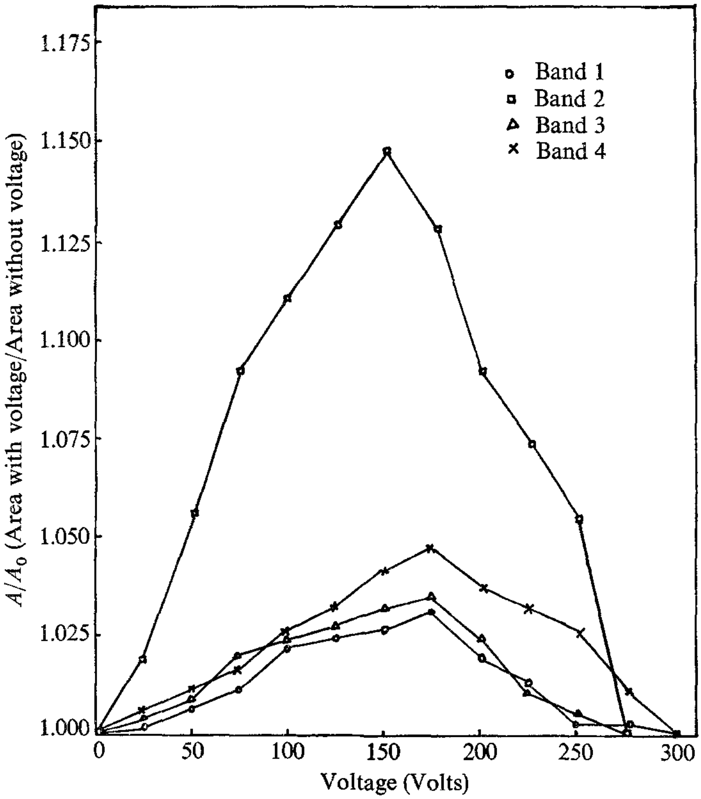

Fig. 3. Variation of the intensities of the bands in the spectrum of a film of PE-CHOL (1:1) with the applied electric field. Bands are listed in Table 1. A/A_0 is the ratio of area of band to the area with no field

No. 2 reached the maximum at the lowest applied voltage of the four peaks, but the change in the area ratio was the largest for the four peaks (Fig. 3).

The effect of the electric field with varying thicknesses of the films was also investigated. The relationship between $F_{\max}$ and the inverse of the thickness was linear for all the mixtures as was found previously with PE and lecithin [7]. Fig. 5 presents the data for the films of PE–CEB–CHOL. The equations of the lines for the mixtures were fitted by the method of least squares and were found not to be different from each other nor from the equation for the results published previously for the phospholipid films [7]. An F test confirmed this at the 1% probability level. There were between five and nine different thicknesses for each mixture. The equation of the line fitting all the data for the mixtures was $F_{\max} = 144\,(1/t)$ with a SD of 78 as compared to the equation for PE of $F_{\max} = 122\,(1/t)$ with a SD of 22.

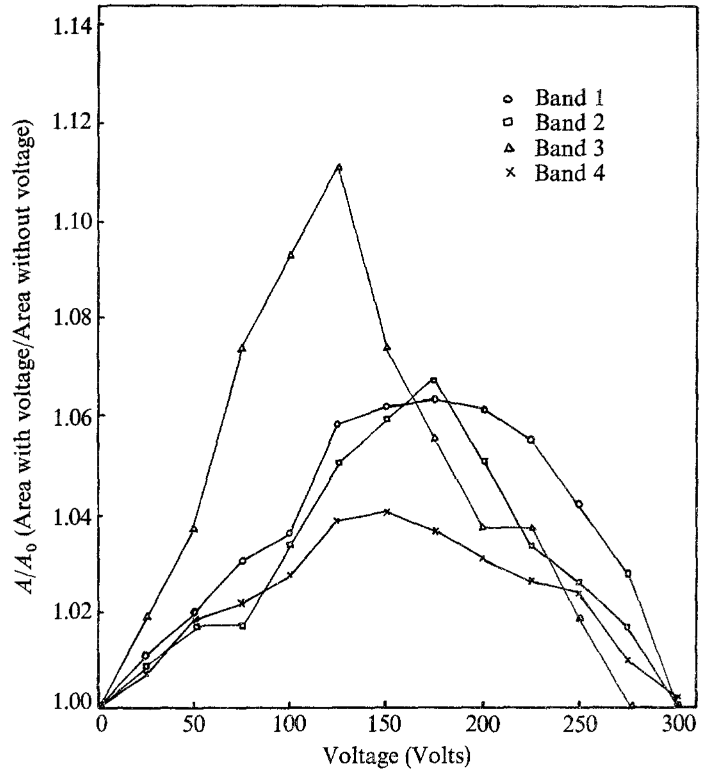

Fig. 4. Variation of the intensities of the bands in the spectrum of a film of PE–CEB–CHOL (1:0.5:1) with applied electric field. Bands are listed in Table 1. A/A_0 is the ratio of area of band to the area with no field

Discussion

The bands that change intensity in the spectra of the mixtures with the application of an electric field can be attributed to the phosphate and $-(CH_2)_n$-groups in the PE (*see* Table 1). This is confirmed by the observations that the spectral bands of films of CEB, CHOL, and CEB–CHOL are not affected by the applied voltage. The assignment of the bands have been given previously [7]. The different voltages required to reach the maximum spectral intensity change in the various mixtures reflects the difference in the bonding between PE molecules in the PE film and the bonding between PE and its neighbors in the binary and ternary mixtures.

An examination of the bands in the spectra of the mixtures with CHOL confirms that there has been an interaction between the components. For example, the bands in the 3,100 to 3,300 cm^{-1} region are shifted in the spectra

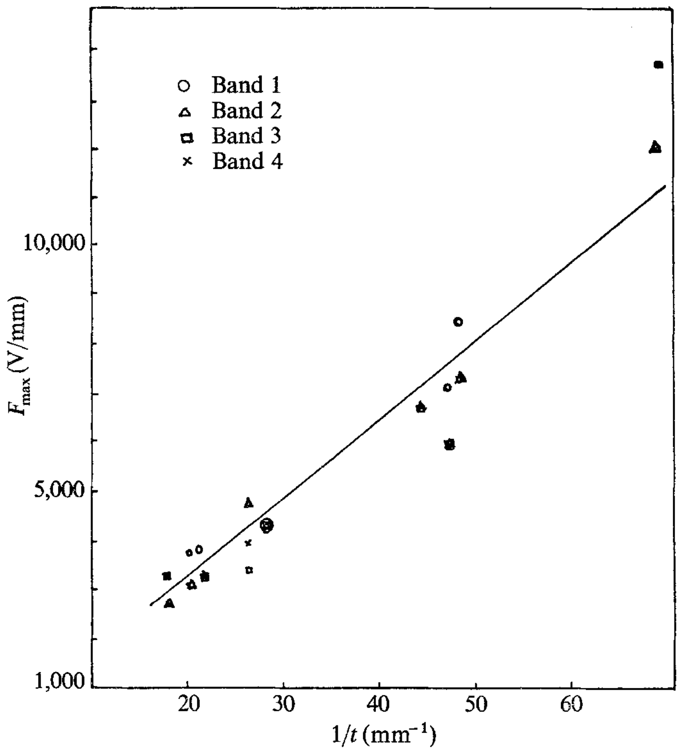

Fig. 5. Variation of the electric field strength at the maxima of change of intensities of spectral bands F_{max} with thickness (t) of films of PE–CEB–CHOL (1:0.5:1). Bands are listed in Table 1

of the different mixtures. The 3,310 cm^{-1} band of CHOL does not appear in the spectra of the mixtures containing PE. However, the band at this frequency in the spectrum of CEB does not shift when this lipid is mixed with PE. In the mixtures of CHOL and the lipids, a band appears near 3,260 cm^{-1} indicating there is hydrogen bonding that causes the band to shift to lower frequency, which occurs when the OH group becomes bonded. A band in the spectrum of the PE film appears near this frequency indicating that the amine group in PE is also hydrogen bonded in these films. The PE molecule is in the neutral form since bands appear in the spectrum that are characteristic of the P–OH group; a weak band near 2,670 cm^{-1}, which frequency is assigned by Bellamy and Beecher [3] to bonded P–OH, and a band at 1,020 cm^{-1}, which Abramson, Norton and Katzman [1] have assigned to the vibration of P–OH. In addition, the bands normally assigned to $-NH_3^+$ are missing in the spectrum: for example, a series of bands between

2,100 and 2,720 cm^{-1} and the bands at 1,560 and 1,645 cm^{-1} [4]. The amine group of PE is in the form $-NH_2$ and is bonded to the P–OH in the phosphate through hydrogen bonding in PE films. In films containing CHOL and PE, CHOL is bonded through its OH group to the phospholipid since the spectra of the mixtures with PE and CHOL have no absorption in the 3,300 cm^{-1} region, which frequency is characteristic of unbonded OH groups, but there is an absorption band near 3,260 cm^{-1}, which is assigned to the absorption of hydrogen-bonded OH and NH groups. In the spectrum of both CEB and PE–CEB, a band appears near 3,370 cm^{-1}. Hence, there is no bonding between the OH of CEB and PE, which would be reflected in the shifting of this band in the spectrum of the mixture. In the spectrum of the ternary mixture, the band in this region appears at a position assigned to hydrogen-bonded –OH or –NH (3,260 cm^{-1}). However, the CEB is present at a concentration of one-half that of the other two components, so that the absorption of CEB at 3,370 cm^{-1} may be obscured. Thus, the CHOL forms a hydrogen bond to the phospholipid through its –OH group possibly to the amine. There appears to be little change in the positions of the 2,665 cm^{-1} band assigned to the P–OH vibration in the spectra of the phospholipid alone and the mixtures with CHOL and CEB. This suggests that there is no difference in the bonding to the P–OH in all the mixtures, and the phospholipid is nonionic in all the films. The –NH deformation is shifted to higher frequencies when the spectra of the mixtures are compared: PE 1,640, PE–CEB 1,650, PE–CHOL 1,700, and PE–CEB–CHOL 1,690 cm^{-1}. These changes indicate that there is a change in the bonding of the $-NH_2$ in the various mixtures. The deformation frequency for this group generally appears between 1,590 and 1,650 cm^{-1}, and the increase of deformation frequency is generally considered indicative of the bonding of the groups [2].

Zull, Greanoff and Adam [12] found that CHOL interacts with lecithin using an infrared total reflectance method. There was a shift of the –OH stretching frequency at 3,400 cm^{-1} in the spectrum of CHOL to the region of 3,250 cm^{-1} in the spectrum of the mixture of the CHOL and lecithin. In the spectra of the mixtures with CHOL and lipids (Fig. 1) the band appears near 3,260 cm^{-1}. The results from both studies indicate that the –OH group of CHOL is involved in interacting with the phospholipid.

This bonding between the lipids would lead to a tighter structure than is found in the films in which PE is the only molecule. It would require more energy in the films of the mixtures with PE than in the films of only PE for the changes to occur in the PE molecules when the voltage is applied. This could explain why higher voltages are found for the maxima in the intensity-voltage curves of the mixtures (Figs. 2–4) than are found with PE films (100 V) [7].

Since the shapes of the intensity-voltage curves are the same with all films including PE films and the PE molecules only are being affected by the electric field, the same mechanism proposed for the effect of the electric field on PE films would be operating in the films of the mixtures. The stronger dipole, the phosphate group, tends to align itself with the field at a lower applied voltage than the weaker dipole, the base, ethanolamine. As the applied potential increases, this second dipole begins to align itself with the field. This causes the P–O–C group to return to its original conformation. The conformational changes in the $-(CH_2)_n$-groups follow the changes in the phosphate group.

The variation of $F_{\max}$ with the films of the mixtures with the inverse of the thickness of the film is identical to that found with PE films [7]. Extrapolation to the thickness that is postulated to be that of the neuronal membrane (100 Å) yields a value of about 10^6 V/mm for $F_{\max}$. Assuming that the nerve impulse is about 100 mV, the electric field strength in the membrane is 10^4 V/mm. This value is less than the extrapolated value of $F_{\max}$ indicating the effect of the field on the PE in the membrane is much less than observed in these *in vitro* studies. Hence, only the phosphate and $-(CH_2)_n$-groups would undergo conformational changes. Since extrapolations from measurements on thick films to those on thin films are not always valid, this should be considered only speculation. However, these results suggest that although the PE is strongly bound in the complex with CEB and CHOL, there would be similar conformational changes in the membrane where it is bound to other cellular constituents.

Sherebrin and coworkers [10] observed changes in the infrared spectrum of frog sciatic nerve during stimulation with 50 mV/mm. The procedure involved signal averaging of a number of spectra. The largest signals with the least number of spectra averaged were found with the bands in the region of the spectrum (980 to 710 cm^{-1}) where the changes in the bands were observed with the ternary films (PE–CEB–CHOL). The band near 1,050 to 1,070 cm^{-1} (P–O–C stretch) in the nerve spectrum required more spectra averaging and was less intense than the other bands below 980 cm^{-1}. This band also includes contributions from nucleic acids [5], which are probably not affected by the electric field, so that it is more difficult to detect changes in this band than the others that arise mainly from phospholipid components.

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