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ELECTROOPTICAL PHENOMENA IN BIMOLECULAR PHOSPHOLIPID MEMBRANES

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SUMMARY

1. Electrooptic phenomena were investigated both on unmodified bimolecular membranes and on bimolecular membranes with two different carriers.

2. On unmodified bimolecular membranes any optic effects under 100-mV voltage were not detected. In these experiments it was possible to detect the optic signal which was 30 times less than that revealed in axon membrane during action potential (under the same changes of electric field).

3. Under 200-mV voltage on the same membranes a decrease of the phase difference between ordinary and extraordinary rays by 0.1″ was detected which was 12 times less than the effect on the axon membrane in the peak of action potential (1.25″).

4. The electrooptic effect on the membrane with diphenylbarenylmercury (carrier of I^-) was the same as on unmodified bimolecular membrane; under 300-mV voltage it was 0.15″. In the presence of this carrier the conductivity of the membrane was 1500 $\Omega \cdot cm^2$ and the current–voltage characteristic of the membrane was practically linear.

5. In the presence of dibarenylmercury (carrier of I^-) providing the initial conductivity 1500 $\Omega \cdot cm^2$ and the current–voltage characteristic of N-type ("excitable" membrane) a decrease of phase difference up to 1″ under 300 mV voltage was revealed. The effect appeared only in the falling region of the current–voltage characteristic and increased approximately linearly with the field. Besides, a decrease of reflected light was detected.

Electrooptic effects on "excitable" bimolecular membranes had the same sign as similar effects on membranes of nerve fibres during the action potential and were close to the latter in value. It allowed us to suggest that optic effects during the action potential were due to the mechanism controlling the membrane conductivity.

7. The assumption was made that both in bimolecular membranes and in the axon membrane, optic effects resulted from the movement of like-charged carriers in membrane under the action of an electric field.

INTRODUCTION

Changes in birefringence of the nerve fibre were found during action potential[1–5]. This effect arose in the membranes of nerve fibres under the action of an electrical field[2].

It was not clear, however, in which of the membrane components the optical effect occurred and whether it was related to the mechanism being responsible for the permeability changes.

In most polymers a rather strong electrooptical effect is observed (Kerr effect). So it can be supposed that a similar effect arises in the lipid part of the membrane. In order to test this assumption bimolecular unmodified membranes of brain phospholipids were studied. These membranes are practically impermeable to ions[6]. On such membranes no changes were revealed in response to rectangular electrical impulses with an amplitude of 100 mV (refs. 3, 4). This means that the action of the electrical field directly on the lipid part of the membrane could not be responsible for the changes in the axon membrane.

In the presence of some substances, bimolecular membranes become permeable to ions[7]. Such substances are ion carriers, lipid-soluble molecules forming complexes with ions. In the presence of most ion carriers, the electric current through bimolecular membranes increases monotonically as the voltage applied rises. Some substances, however (for example dibarenylmercury), result in N-type voltage–current curves[8]. Similar voltage–current curves are observed on the nerve membrane for the initial sodium current[9] and in isotonic KCl solution[10].

It is hoped that the carrier's movement in a membrane induced by electrical field can result in some changes of membrane structure and optic parameters.

This paper is concerned with the electrooptical effects in bimolecular membranes, both impermeable to ions and in the presence of two types of carriers: one giving monotonous voltage–current characteristics and the other, falling ones. Some preliminary results have been published[11].

METHOD

The membranes were formed from phospholipids of bull brain[6] on the aperture diameter = 2 mm in a Teflon chamber (Fig. 1). The glass cell and the chamber were filled with aqueous solution of a suitable composition. The periodic sequence of rectangular or saw-tooth pulses was applied to the membrane through nonpolarizing electrodes (AgCl with an agar bridge). The resistance 11, of which the magnitude is two orders less than that of the membrane, enabled us to control the voltage–current characteristics during the experiment.

The membrane was placed at an angle of 45° to the incident beam of polarized light, the angle between incident plane and polarization plane of light being also 45°. The beam passed through the polarizer, the central region of the membrane, the phase plate, the analyzer crossed with the polarizer and the additional field diaphragm.

The optical part of the equipment involved a polarizing microscope (MIN-8), a photomultiplier (FEU-39) and a quartz–iodine lamp (KIM10-90) with a direct current source.

The direct current of the photomultiplier was measured by a microamperometer while the alternative component following amplification was fed to the input of a digital averager. This latter is made on the basis of the digital pulse analyzer (AI-256) (ref. 12). The signal store permits the increase of signal to noise ratio, which is tens of times less than unity at the average input in our experiments. The signal to noise ratio increases by the square root of sweeps averaged.

In addition to the storage the second way of increasing signal to noise ratio was used–selecting the optimum frequency characteristic of amplifier. In the cases in which the fronts of electrooptical effect in response to rectangular voltage impulse were of interest, the upper limit frequency of the amplifier was taken to be high. If only the magnitude was studied then the upper limit frequency was selected as low as possible, in order that the electrooptical effect might reach the stationary value toward the end of the electrical impulse. As a result of such a reduction of frequency band, the noise level decreases and the storage time may be reduced.

The experiments indicate that the electrooptical effects on the membrane edge, the transitional zone between the bimolecular layer and the thick lipid film being illuminated, are 100 times more than the effects on the bimolecular membrane. The time-courses of these effects are different too[3]. Therefore in order to reduce to a minimum the edge effect contribution, only the central part of the bimolecular membrane (1/3 of the membrane diameter) was illuminated. For the contribution of light scattered by optics and passed through the membrane edge to be reduced, an additional field diaphragm 15 (in the plane of object image) was set (Fig. 1). This diaphragm selected only the light flux passed through the central part of the membrane.

The phase (birefringent) plate is an essential component of optics. Therefore we consider its functions in more detail.

The light is polarized in the plane, which divides in half the angle between the optical axes of the phase plate. The membrane is situated at about the polarization plane as indicated above. In this case the intensity of light W coming out from the analyzer and received by photomultiplier is expressed by[3]:

$$W = \frac{W_0}{2}(1-\alpha)\,[1-\cos(\varphi+\gamma)] \qquad (1)$$

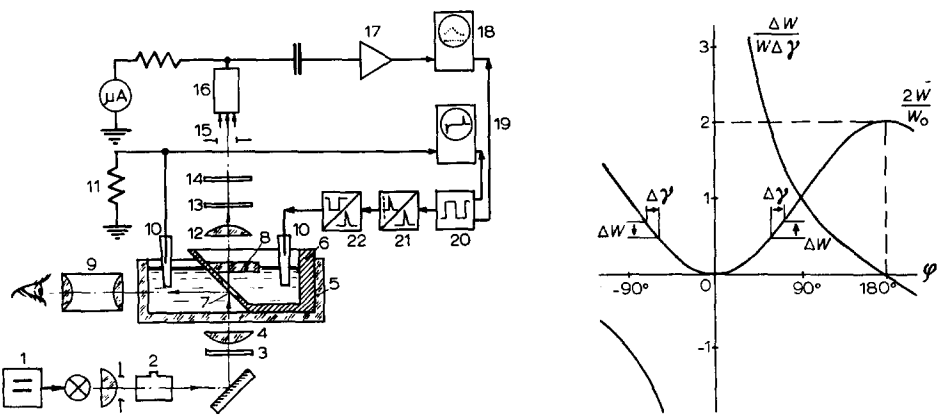

Fig. 1. Block diagram of the arrangement and the cross-section of the cell. 1, regulated power supply; 2, heat filter; 3, polarizer; 4, condenser; 5, glass cell; 6, Teflon chamber; 7, aperture, Φ = 2 mm, for the membrane; 8, cover glass; 9, arrangement for the observation of the membrane; 10, Ag–AgCl electrode; 11, resistance for measuring the current through the membrane; 12, objective; 13, phase plate; 14, analyzer; 15, additional field diaphragm; 16, photomultiplier; 17, amplifier with regulated gain factor and pass band; 18, signal averager; 19, oscillograph; 20, oscillator; 21, delayed pulse oscillator; 22, square pulse generator.

Fig. 2. The dependence of intensity of the light passed through the analyzer and of its relative change due to the increment of $\Delta\gamma$ on the phase difference φ in the phase plate.

where W_0 is the intensity of the incident polarized light, α, the effective membrane reflection coefficient equal to the sum of reflection coefficients for ordinary and extraordinary rays ($\alpha = \alpha_o + \alpha_e$), ($\alpha \ll 1$); γ and φ, the phase difference between ordinary and extraordinary rays arising when light beam passes through the membrane and the phase plate, successively. Hence for the relative increment of light flux $\Delta W/W$ due to the changing of optical parameters of the membrane we obtain:

$$\frac{\Delta W}{W} = \operatorname{ctg} \frac{\varphi + \gamma}{2} \cdot \Delta\gamma - \Delta\alpha \tag{2}$$

Turning a phase plate through an angle of 90° changes the sign of φ.

It is seen from formulas given that both light flux magnitude W and its relative change $\Delta W/W$ (optical effect value to be measured) depend on phase difference φ, induced by the phase plate (Fig. 2). On the other hand, the value W is responsible for the fluctuation noise level of the photomultiplier. In order to take account of this factor when selecting the value φ, the relationship between signal to noise ratio and the value φ must be determined. Considering that the noise level is proportional to $\sqrt{W}$ and neglecting the value γ (10^{-4}) as compared to φ, we obtain the following:

$$\frac{\text{signal}}{\text{noise}} \sim \frac{\Delta W}{\sqrt{W}} \sim \cos \varphi/2 \cdot \Delta\gamma - \sin \varphi/2 \cdot \Delta\alpha \tag{3}$$

As seen from Eqn. 2, the contribution of $\Delta\gamma$ to the value $\Delta W/W$, as compared with $\Delta\alpha$, increases as φ decreases. The signal to noise ratio with small values of φ is practically unchanged and has the maximal value. The decrease of φ, however, is limited by "parasitic" light, always taking place because of inaccuracy of the crossed position of the analyzer and the polarizer, their not ideal polarization ability and other factors. At $\varphi \to 0$ the light flux $W \to W_{\text{parasitic}}$ and $\Delta W \to 0$ and hence, $\Delta W/W \to 0$.

For independent measuring $\Delta\alpha$, as it is clear from Eqns. 2 and 3, the optimal value $\varphi = \pi$. In this case $\Delta W/W = -\Delta\alpha$. It is, however, not the maximal effect value. For an ordinary ray the reflection coefficient of the membrane α_o is much more than α_e for the extraordinary ray. Therefore it is more profitable to illuminate the membrane only with an ordinary ray, then $\Delta W/W = -2\Delta\alpha_o$. The analyzer and the phase plate are then not necessary.

The given consideration shows that the phase plate allows the changing to be revealed and the absolute value $\Delta\gamma$ of the membrane to be estimated, the value γ of the membrane being unknown. In studies of the axon, its axoplasm plays the role of a phase plate.

In summary, the use of the phase plate with small φ considerably reduces requirements for the noise level of the light source.

To correlate data obtained on bimolecular membranes with the electrooptical effect in nerve, parameters must be chosen which are independent of geometrical forms of the model and the object and of the optical scheme of the experiment. Those parameters are refraction coefficients of the membrane for ordinary and extraordinary rays (n_o and n_e) as well as membrane thickness d. The calculations show, however, that in the axon membrane the birefringence ($n_e - n_o$) changes while d is practically unchanged[3]. The birefringence change is related to the change of γ: $\Delta\gamma = A \cdot \Delta n$, where A is the coefficient depending on optic-geometrical conditions of the experiment and $\Delta n = \Delta n_e - \Delta n_o$. In our experiment on bimolecular membranes $A = 4 \cdot 10^{-2}$

(ref. 3), the maximal increase of birefringence Δn during the action potential calculated for axon is equal to $1.5 \cdot 10^{-4}$. So the birefringence change of the model membrane will be the same as that of the axon, when $\Delta\gamma = 6 \cdot 10^{-6}$, or in angle seconds, $\Delta\gamma = 1.25''$. Therefore, $\Delta\gamma$ can serve as an intermediate parameter when comparing the model and the object.

In our experiments $\Delta\alpha$ was measured while the membrane was illuminated only by the ordinary ray, and $\Delta\gamma$ was estimated by means of the phase plate with $\varphi = 10°$. In earlier experiments the measurements were carried out with a quarterwave plate ($\varphi = 90°$). Then $\Delta\gamma$ and $\Delta\alpha$ were calculated from two measurements (the plate in one fixed position and turned through a 90° angle).

When investigating the unmodified bimolecular membranes (without carrier) a Teflon chamber and an external cell were filled with 0.1 M NaCl solution in one set of experiments and 10 mM KI solution with 5 mM $Na_2S_2O_3$ in the other one (pH 7). These membranes are practically impermeable to ions.

Membranes from the same phospholipids with the addition of two types of carriers, diphenylbarenylmercury and dibarenylmercury served as models of membranes permeable to ions. The chamber and the cell were filled with 10 mM KI solution with 5 mM $Na_2S_2O_3$. The carriers were added into lipid solution in heptane. Both these substances carried I^- through the membrane. The voltage–current characteristic of the membrane with diphenylbarenylmercury was monotonic. The voltage–current characteristic of the membrane with dibarenylmercury had a region with a negative resistance where the voltage increase caused the current to decrease. Examples of such characteristics are given in Fig. 3.

RESULTS

The unmodified bimolecular membrane

As it was reported earlier, the effects on unmodified bimolecular membranes are absent at the level of $5 \cdot 10^{-7}$. However, the subsequent development of the method: the application of the plate $\lambda/36$ ($\varphi = 10°$) instead of $\lambda/4$ and the use of a brighter light source allowed the changes in unmodified bimolecular membranes to be revealed. More than 100 experiments were carried out on such membranes.

When operating with the plate $\lambda/4$ it was noted that sometimes oscillations of the light flux at the level $5 \cdot 10^{-7}$–$7 \cdot 10^{-7}$ were observed which occurred rather slowly and existed not only at the time of the action of the electric field on the membrane but also when the field was removed. The examples of this effect are shown in Figs. 4a and 4b. These oscillations were revealed to take place particularly distinctly in cases when the membrane was a little sagged. On the edges of the membrane where the bimolecular layer turned into a thick one the electric field produced a shifting of the intermediate zone towards the edges. That is why it is naturally to also expect small displacements in the central area of the membrane due to the movement of the intermediate zone, especially if the membrane is not plane. It is also natural that both the amplitude and the character of the oscillations depend upon the properties of the edge zone (its uniformity, its own resonance frequencies), the sagging degree of the whole membrane and also the frequency of electric impulses. This is illustrated in the Figs. 4c and 4d where records of the light flux oscillations are presented for cases when the light flux illuminated whole membrane together with the edges, the repetition rate of electric impulses being different.

In order to eliminate the influence of these oscillations, the phase plate $\lambda/36$ was used which increased the contribution of phase changes about 10 times as large without changing that of the amplitude changes (see Eqn. 2) to which the oscillations mentioned above were basically related. As a consequence, it was possible to detect the changes of light fluxes. These changes altered their sign with the turning of the phase plate. The typical record is presented in Fig. 5. When the phase plate and the membrane were placed so that they gave the initial phase difference between ordinary and extraordinary rays in one direction (φ and γ are of the same sign), during the action of the electric field the decrease of the light flux was observed (Fig. 5b); when turning the phase plate through the 90° angle, the same increasing of the light flux occurred (Fig. 5a). These experiments show unambiguously that the phase difference in the membrane decreased under the action of the electric field. The average value of the reduction under 200-mV voltage was about 0.1″ (the result from the data of 40 experiments carried out after development of the technique). As the membrane was illuminated by one ordinary ray the change of the reflection coefficient ($\Delta\alpha_0$) at the level $1.5 \cdot 10^{-7}$ was absent.

In cases where the membrane did not break for a long time (for some hours), gradual reduction of the effect up to its full disappearance at the level 0.04″ (Fig. 6a) was observed. The effect was not revealed on the membranes which were formed from the lipids, heptane solutions of which had been stored for 17 days. In this case the effect was absent at the level 0.05″ (Fig. 6b). Both the pH change of the solution over the range 6.3–7.5 and the substitution of the NaCl solution by the KI with $Na_2S_2O_3$ did not influence the optic effect.

In contrast to the membranes with carriers where measurements were taken at

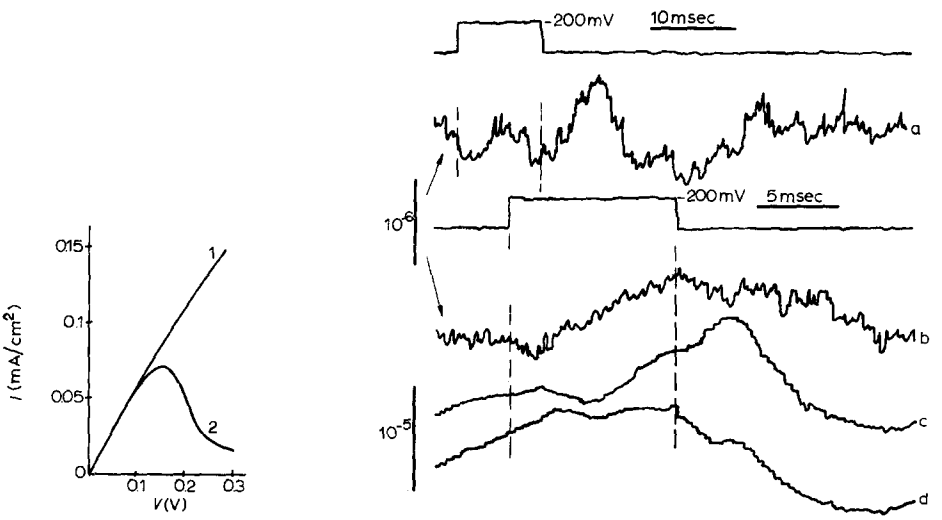

Fig. 3. Current–voltage characteristics of bimolecular membranes with carriers. 1, diphenylbarenylmercury; 2, dibarenylmercury. Solution: 10 mM KI, 5 mM $Na_2S_2O_3$.

Fig. 4. Examples of artifacts due to mechanical action of the edge zone of a membrane. Unmodified bimolecular membranes, curves of voltages and electrooptic effects. a, b. Responses to rare and frequent voltage impulses, respectively. The middle part of the bimolecular membrane is illuminated, the phase plate, $\lambda/4$. c, d. Responses for two frequencies of impulses, 31 and 28 impulses/sec. The whole membrane together with edges is illuminated.

voltages of 300–350 mV, the unmodified bimolecular membrane had broken up already at 200 mV as a rule.

Bimolecular membrane with diphenylbarenylmercury

10 experiments were performed on these membranes. The same concentration of carrier was preset as in the experiments with dibarenylmercury described below. The concentration equivalence in the set of experiments with dibarenylmercury and diphenylbarenylmercury was controlled over the initial specific resistance of the membrane. For this purpose in all the experiments its current–voltage characteristic was recorded. Electrooptic effects revealed on the membranes with diphenylbarenylmercury did not differ from those on unmodified bimolecular membranes both in sign and in value. The typical record is presented in Fig. 7. Under 300-mV voltage the average value of the decrease of phase difference in the membrane was 0.14″. The specific resistance of the membrane was 1500 $\Omega \cdot cm^2$.

As in the experiments on unmodified bimolecular membrane the effect under 300-mV voltage sometimes was not observed at the level 0.07″.

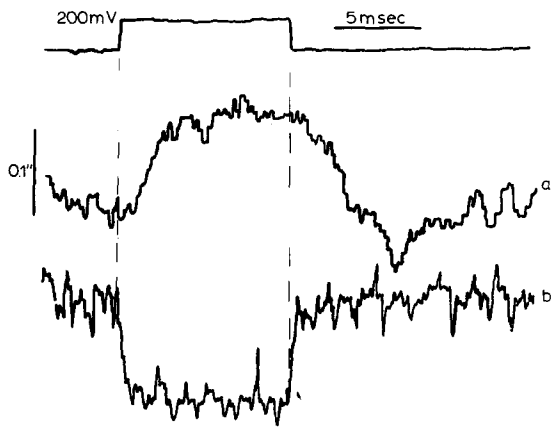

Fig. 5. Electrooptic effect on unmodified bimolecular membrane. The phase plate, $\lambda/36$. a, γ and φ are of opposite signs, the smoothing time $T_f = 2.2$ msec; b, γ and φ are of the same sign, $T_f = 0.1$ msec.

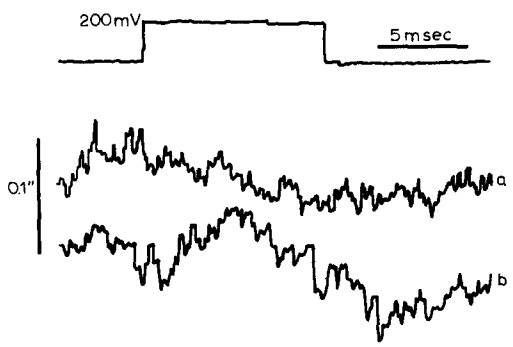

Fig. 6. Records of light fluxes when a voltage impulse is applied to unmodified bimolecular membrane (examples of absence of electrooptic effect). a, 2,5 h after the forming of a membrane; b, the membrane is formed from lipids the solution of which in heptane was stored for 17 days.

Experiments were carried out on membrane with the voltage changing linearly from 0 to 300 mV. As a result, the volt–optic characteristic (the dependence of the value of the optic effect upon the voltage) was obtained. Typical records for two reciprocally perpendicular positions of the phase plate are presented in Fig. 8. The results show that the optic effect increased monotonically with the voltage. It began with rather small voltages on the membrane which did not exceed 50 mV (the noise hinders the more exact determination of the low limit).

Bimolecular membrane with dibarenylmercury

After the improvement of the method, 23 experiments were carried out on such bimolecular membranes. During all the experiments the current–voltage characteristic of the membrane was recorded.

Always changes of the light flux were observed. The optic effect varied sign

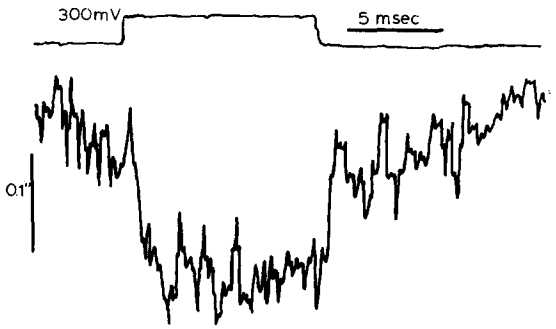

Fig. 7. The electrooptic effect on a membrane with diphenylbarenylmercury. γ and φ are of the same sign. T_f = 0.1 msec.

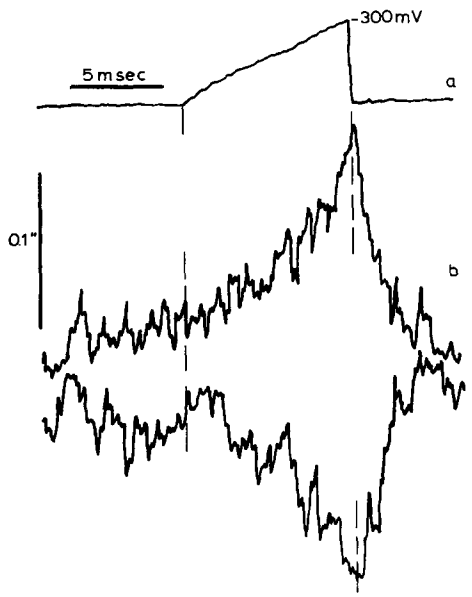

Fig. 8. Volt–optic characteristics of a membrane with diphenylbarenylmercury. T_f = 2.2 msec. a, voltage on a membrane; b, optic effects when γ and φ are of the opposite sign and of the same sign, respectively.

when the phase plate turned through a 90° angle. Typical records are presented in Fig. 9.

The effect was of the same sign as in experiments on other membranes. The aver-

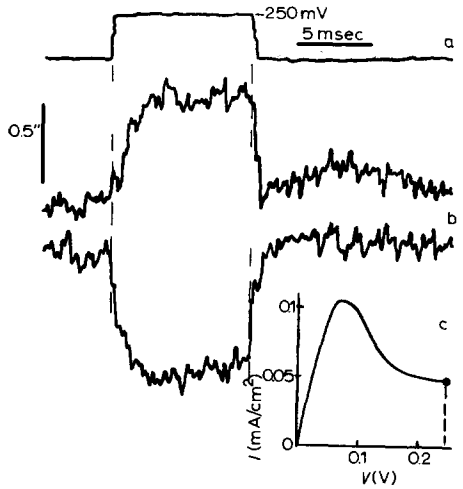

Fig. 9. Electrooptic effect on a membrane with dibarenylmercury. T_f = 0.1 msec. a, voltage on a membrane; b, optic effects when φ and γ are of the opposite sign, and of the same sign, respectively; c, current–voltage characteristic of a membrane.

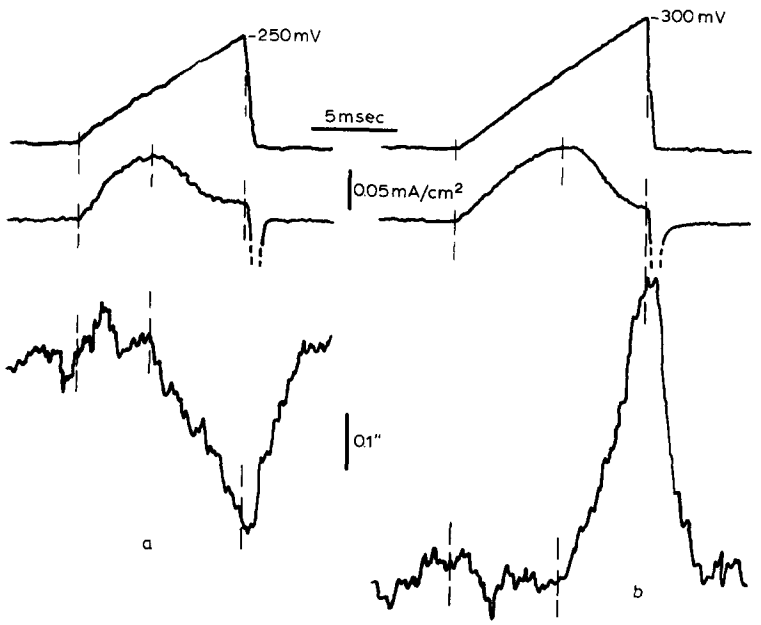

Fig. 10. Volt–optic characteristics of membranes with dibarenylmercury. T_f = 2.2 msec. Above, voltage on the membrane; in the middle, current through the membrane; below, electrooptic effect. a, φ and γ are of the same sign; b, φ and γ are of the opposite sign.

age value of the decrease of phase difference under 300-mV voltage was 0.56″. In contrast with experiments on unmodified bimolecular membranes and on bimolecular membranes with diphenylbarenylmercury where all the results were near to the average value, significant deviations from the average value were observed: $\Delta\gamma$ varied from 0.22″ to 1.0″. It is necessary to note that the current–voltage characteristics of such membranes also varied very much, *i.e.* the falling region appeared under different voltages, the values of initial and final conductivity changed (from one experiment to the other).

The above-average value $\Delta\gamma$ was related primarily to the experiments where the falling region on the current–voltage characteristic of the membrane began from about 150 mV, the current density being equal to 0.1 mA/cm^2 (specific resistance is 1500 $\Omega\cdot$cm^2). When the falling region began from 100 mV and 200-mV voltage was applied, $\Delta\gamma = 0.26''$ and $\Delta\alpha_0 = -3\cdot10^{-7}$.

From volt–optic characteristics it is seen (Fig. 10) that the optic effect began only from the moment when the falling region arose on the current–voltage characteristic of the membrane; after the falling region this effect increased linearly with the voltage in the first approximation (at least valid up to 300 mV).

It was necessary to test whether in the experiments on bimolecular membranes with dibarenylmercury the optic effect related to the availability of the falling region on the current–voltage characteristic or to some critical voltage (about 120–150 mV). The experiment was carried out on the membrane where the beginning of the falling region was displaced up to the 350-mV voltage with the increasing of dibarenylmercury concentration (Fig. 11). It is evident that at the 250-mV voltage the effect was absent at the level 0.07″. This points to the close relationship between the optic effect and availability of the falling region on the current–voltage characteristic.

DISCUSSION

It has been mentioned above that the same structural changes in the model membrane as in the living one must result in the decreasing of the phase difference by 1.25″ on the model membrane. On unmodified bimolecular membranes formed from fresh prepared lipids, the decreasing of γ under the 200-mV voltage was 12 times as small as the value mentioned above. This supports the former conclusion[3,4] that the phenomena under observation can not explain changes in the living membrane. Furthermore, the gradual disappearance of the effect after long-term storing of the membrane and its absence on the membranes, which have been formed from the 17-day-long solution of lipids in heptane, suggest the effect on unmodified bimolecular membranes to have been induced by some admixtures. The latter may go out of long-stored membrane or fall out in the solution.

The experiments on the bimolecular membranes with diphenylbarenylmercury have given results which agree with those on unmodified bimolecular membranes. Under the 200-mV voltage the effect on these membranes does not differ from the changes on unmodified membranes; as there, the effect is not always observed. But as distinct from unmodified bimolecular membranes, there is a rather high conductivity here. Thus, there is no direct relationship between the optic effect and the conductivity.

The average value of the effect on membranes with dibarenylmercury is 4 times as much as on the membranes with diphenylbarenylmercury, after applying equal

voltages and with equal initial specific resistances. Under 300-mV voltage it is half as great as on the cell membrane; in some experiments it is practically equal to the latter. The sharp increase of the effect after the introduction of the carrier, which results in the appearance of the falling region on the current–voltage characteristic, points to the close relation of the optic effect to the processes which control the membrane conductivity on the falling region of the characteristic. Good evidence of this are volt–optic characteristics (Fig. 10).

Two carrier forms, bound and unbound with a transported ion are distinguished. In the case of diphenylbarenylmercury the unbounded form seems to be electrically neutral, while the bounded one is negatively charged. In the presence of dibarenylmercury, forms having one and two negative charges predominate in the above mentioned solution. The process of transport of an ion through the membrane is assumed to consist of three successive stages[8,13]. On one membrane surface the complex of the ion with the carrier is formed, then under the action of electrical field and of concentration gradient the complex formed moves to the other surface where the ion passes into the solution. Now the carrier returns to the first surface and so the process is repeated.

The falling region is assumed to be connected with the fact that with an increasing of voltage the two charged forms of a carrier are pressed by electrical field against one surface of a membrane. This results in a decreasing of current through the membrane. It is not inconceivable that this process may be responsible for optic changes under observation. It is expected that the number of carriers and the degree of their pressing may have an influence on the value of the effect. All the above information gives evidence that the effects in the cell membrane may be explained by the movement of the similar carrier. In the cell membrane the sodium current density during the action potential is one order higher than in our model system. The falling region on the current–voltage characteristic of the cell membranes begins earlier than in the model membrane. That is why under smaller voltages the optic effect on the cell membrane may be greater than on the model.

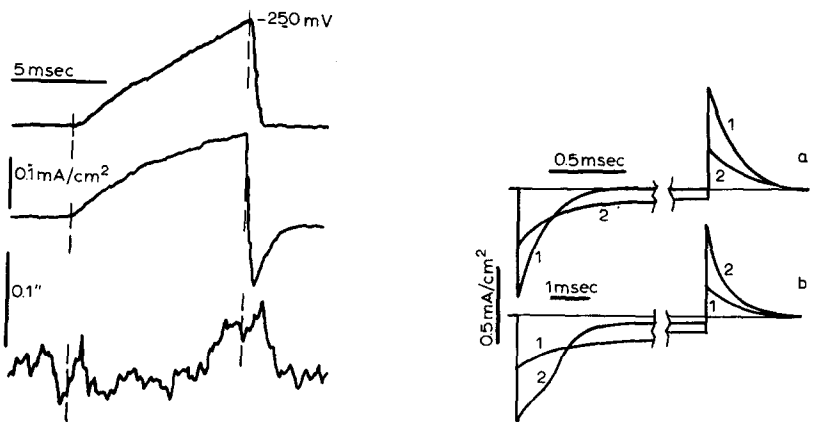

Fig. 11. Records of light fluxes (below) when the electric field (above)is applied to bimolecular membrane with dibarenylmercury. In the middle, current through the membrane.

Fig. 12. Oscillograms of currents through a membrane in response to a rectangular voltage impulse. a, voltage 200 mV; 1, unmodified bimolecular membrane; 2, bimolecular membrane with diphenylbarenylmercury. b, bimolecular membrane with dibarenylmercury; 1, voltage 200 mV; 2, 400 mV.

The possibility that the dynamics of the optic changes in the cell membrane copy that of the electric field on the membrane[2,3] has not been investigated on bimolecular membranes. The electric scheme for feeding the field on the membrane has not been designed for this purpose. For a first approximation, however, our data also confirm this possibility with respect to bimolecular membranes. The time it takes for the optic effect to reach its maximum after the voltage jump is rather long: approx. 0.5 msec on unmodified bimolecular membranes (Fig. 5b), approx. 0.6 msec with diphenylbarenylmercury (Fig. 7a) and approx. 2.5 msec with dibarenylmercury (Fig. 9). These times are determined by the setting time of the electric field itself. In order to illustrate this, the oscillograms of currents for all kinds of bimolecular membranes are presented in Fig. 12. The setting process of the current and hence that of the electric field in the membrane lasts around as long as the light flux setting process.

The changes of γ on the membranes with dibarenylmercury may be caused by varying the birefringence or the thickness of the membrane. The calculations[14] show that as it is in the cell membrane, the change of γ must be connected with sufficient authenticity to the changes of birefringence of the membrane.

COHEN *et al.*[2] and COHEN AND KEYNES[15] have revealed the change in light scattering in the giant axon of a squid during excitation. The voltage clamp experiments have shown that one of the components of the effect under observation is closely connected with the action of the electric field. The authors suppose that it is induced by structural changes in the axon membrane. At the same time it has been demonstrated that these changes differ from the structural rearrangements inducing birefringence changes of the membrane. Namely, the treating with tetrodotoxin did not influence the effect of light scattering changes in the axon, changes of light-scattering rate being one-third that of birefringence after the voltage jump on the membrane. During the action potential the light flux which has passed through the axon decreases, while the flux scattered at 90° increases.

Our calculations[14] have shown that these light-scattering changes can be connected to the increase of the membrane thickness by $2 \cdot 10^{-3}$–$3 \cdot 10^{-2}$ Å during the action potential or with the increase of both n_e and n_o by $1 \cdot 10^{-5}$–$1.5 \cdot 10^{-4}$. The increasing of reflection coefficients can be induced for example by some carriers entering the membrane when the electric field is removed. It is necessary to note that the value of the light scattering component associated with the electric field must not be dependent upon the axon dimensions. The variety of effects obtained by COHEN *et al.*[2] and COHEN AND KEYNES[15] can be associated with the aperture changes of illumination and light gathering in various experiments (the increasing of the aperture must result in the decreasing of the optic effect).

The changes of the light reflected from the bimolecular membrane with dibarenylmercury may be connected with the decreasing of the membrane thickness by $1 \cdot 10^{-2}$ Å or with the decreasing of n_o by $4 \cdot 10^{-5}$ under the 200-mV voltage[14]. It is interesting that on unmodified bimolecular membranes the changes of reflected light were not detected under the same conditions.

Thus, the changes of light flux reflected from the axon membrane and bimolecular membrane are induced by similar changes of the parameters of both membranes.

The electrooptic effects in the falling region of the current–voltage characteristic on bimolecular membrane with dibarenylmercury are observed to be very close to those in the axon membrane during the action potential: (1) In both cases an increase

of the electric field results in the decrease of phase difference between the ordinary and extraordinary rays in the membrane ($\Delta\gamma$). (2) The analysis shows that in both cases the decreasing of γ is associated with the decreasing of membrane birefringence during the action of the electric field. (3) The values of birefringence changes of bimolecular membrane with dibarenylmercury and those of an axon membrane are rather close. (4) In both cases increasing the electric field induces a decreasing of the light flux reflected from the membrane. (5) The result obtained is in favour of one of the possible mechanisms of ion permeability of the membrane: the mechanism of the carrier which was suggested in 1949 by HODGKIN *et al.*[16].

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