Biochemical Pharmacology, Vol. 37, No. 20, pp. 3823–3828, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

ALCOHOL ABUSE INCREASES THE LIPID STRUCTURAL ORDER IN HUMAN ERYTHROCYTE MEMBRANES

A STEADY-STATE AND TIME-RESOLVED ANISOTROPY STUDY

FRANÇOISE BEAUGÉ,*† JACQUES GALLAY,‡ HELENA STIBLER§ and STEFAN BORG‖

*INSERM U.26, Hôpital Fernand Widal, Paris, France, ‡Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Université Paris-Sud, Orsay, France, § Department of Neurology, Karolinska Hospital, Stockholm, and ‖ Department of Psychiatry, St. Göran Hospital, Stockholm, Sweden

(*Received* 23 *December* 1987; *accepted* 14 *April* 1988)

Abstract—The effect of ethanol abuse on the lipid ordering of the human erythrocyte membranes was studied by steady-state and time-resolved fluorescence anisotropy measurements of DPH and its polar analogue TMA-DPH, which probe different membrane regions. Steady-state anisotropy values with DPH as a probe were slightly but significantly increased (+3%) in erythrocyte membranes from alcoholic patients. A resistance to the ethanol fluidizing effect was evidenced in these membranes with DPH and TMA-DPH. No difference in the probe lifetimes was detected between the control and the alcoholic subjects. In the alcoholic patients as compared to the healthy controls, the residual anisotropy for DPH was significantly increased (+7%) corresponding to an increase in the orientational order parameter of 4%; a decrease of the apparent correlation time value was also observed. Nevertheless, no differences between the two erythrocyte populations were observed with TMA-DPH.

Experimental evidence has shown that ethanol exerts its pharmacological effects mainly by altering the physiochemical properties of biological membranes by changing the "fluidity" [1]. Although most of the results have been obtained in animal models, recent studies [2–5] have documented similar perturbations in human alcoholic patients. A previous work [2] indicated a slight but significant increase in steady-state fluorescence anisotropy of DPH together with a reduced sensitivity to ethanol addition *in vitro* in erythrocyte membranes from alcoholic patients as compared to normal subjects. However, differences in fluorescence excited state lifetime, apparent correlation time and residual anisotropy affect the steady-state anisotropy values. Therefore, in the present study, lifetime and time-resolved fluorescence anisotropy measurements were carried out in order to delineate the respective effect of alcohol on each parameter, especially on the residual anisotropy [6, 7]. In order to get better insight of the ethanol effect, two fluorophores, i.e. DPH¶ and its cationic derivative TMA-DPH labelling different transverse regions of the erythrocyte membrane, were used [8, 9].

MATERIALS AND METHODS

Clinical subject group. Venous blood samples were collected from 10 thoroughly selected male alcoholic patients aged 32–60 years who had been admitted to hospital for detoxication treatment and from 10 healthy control persons matched for age and sex and with an alcohol consumption of 0–23 g of ethanol per day. The daily alcohol consumption in the patients was estimated at 125–500 g of ethanol per day during the preceding 8–21 days. The duration of the alcohol dependence was at least 10 years and all the patients fulfilled the DSM III criteria for alcohol dependence [10]. All the patients had stopped drinking within 24 hr before sampling and no one had positive blood or breath tests for ethanol. No patient showed any clinical signs of complicating organic disease, and no one had received any drugs during the preceding week. Blood samples were collected in the morning with the patients fasting.

Apart from analyses of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma glutamyl transferase (GT), alkaline phosphatase (ALP) and bilirubin, the samples were examined for routine peripheral haematological values. The concentration of CDT in serum was also determined according to Stibler *et al.* [11] in order to monitor alcohol abuse in alcoholic patients. Samples from the controls were analysed as well. All reagents for CDT analysis were provided by Pharmacia Research and Development (Uppsala, Sweden).

Erythrocyte membrane preparation. Erythrocyte membranes were prepared from blood samples anticoagulated with EDTA as described previously [2]. The membranes obtained were suspended in 10 mM phosphate buffer (pH 7.4) and stored at −20° until fluorescence measurements were done. The protein concentration was determined according to Lowry *et al.* [12] with human serum albumin as a standard (Behringwerke, Marburg, F.R.G). Membrane prep-

† Author to whom correspondence should be addressed at: INSERM U.26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France.

¶ Abbreviations: DPH, all*trans*,1,6-diphenyl-1,3,5-hexatriene; THF, tetrahydrofuran; TMA-DPH, 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene; CDT, carbohydrate deficient transferrin.

Table 1. Peripheral haematological values and concentration of carbohydrate-deficient transferrin (CDT) in serum in 10 alcoholic patients and 10 healthy controls

Test	Alcoholics	Controls
Haemoglobin conc. (g/l) (140–170)	150 ± 6	143 ± 7
Mean corpuscular haemoglobin conc. (g/l) (320–360)	328 ± 1*	349 ± 3
Mean corpuscular volume (fl) (76–96)	99 ± 2*	85 ± 6
Sedimentation rate (mm) (1–15)	11 ± 3	8 ± 2
CDT (mg/l) [<74, (11)]	121 ± 12*	49 ± 2

The values are mean values ±SEM. The normal range of each test is given in brackets.
* $P < 0.001$.

arations and all membrane analyses were made paired on the same day.

Membrane labelling. Diluted samples from thawed aliquots of the native membranes were used. To 1 ml of membrane sample, 0.5 μl of DPH dissolved in THF or TMA-DPH in THF/water 1:1 was added to give a final probe/lipid molar ratio lower than 1/200. DPH and TMA-DPH were from Molecular Probes (Eugene, Or, U.S.A.). After thorough mixing, the membranes were incubated for 1 hr at 37°.

Fluorescence measurements. The steady-state anisotropy (r_s) was recorded at 25.0 ± 0.2° using a SLM 8000 spectrofluorimeter (Urbana, IL, U.S.A.) as described previously [2]. r_s was measured before and after addition of ethanol from 0.35 to 1.05 M and the slope (Δr_s) of the regression line, r_s–ethanol concentration, was calculated as a measure of the fluidizing effect of ethanol [2]. For each concentration, 5–10 determinations were carried out.

Excited state lifetime and nanosecond anisotropy measurements were performed at the same temperature on a time-correlated single photon counting instrument [13] at an excitation wavelength of 357 nm, fluorescence emission being collected through a Schott KV 418 cut-off filter. The apparatus response function was obtained with a short lifetime standard (tetraphenylbutadiene in cyclohexane, $\tau = 1.76$ nsec) [14] according to Wahl *et al.* [15]. Analysis of the data was performed as previously described [11, 13]. Wobbling angle (θ_{max}) of probe rotation was calculated according to Kinosita *et al.* [16]. Intrinsic anisotropy values (r_0) were 0.384 and 0.390 for DPH [17] and TMA-DPH [8], respectively.

Statistical analyses. Comparisons between patients and controls were evaluated by one-tailed Student's *t*-test (membrane parameters) or Mann–Whitney *U*-test (peripheral and serum blood parameters) to determine significant differences ($P < 0.05$) between means.

RESULTS

Table 1 summarizes the peripheral haematological values in the patient group compared to healthy controls. Mean corpuscular volume is increased in 6 of the 10 patients. In no instance are there any signs of infection as judged by sedimentation rate and white cell count. Slightly or moderately elevated values of GT are present in 5 of the 10 patients, of ALAT and/or ASAT in three cases and ALP and bilirubin are somewhat elevated in one patient. In three alcoholic patients all of the liver test values are normal. Nevertheless, CDT levels in serum are clearly increased in the alcoholic patients compared to the controls ($P < 0.001$) and to the previously obtained normal values [11], confirming the alcohol abuse and that the abuser group was homogeneous and representative [11].

Steady-state anisotropy values, r_s, with DPH as probe increases in nine patients and is at the upper limit in one patient with a statistically significant difference compared to the healthy control (+3%, $P < 0.001$) (Table 2). r_s Values are less sensitive to ethanol addition *in vitro* (−30%) in alcoholic patients. With TMA-DPH, r_s values are much higher than for DPH and do not change between patients and controls, but his parameter is also less sensitive

Table 2. Steady-state fluorescence anisotropy of DPH and TMA-DPH and effect of ethanol *in vitro* in human erythrocyte membranes from healthy controls and alcoholic patients

Origin of the membranes	Probe	r_s	Δr_s
Controls	DPH	0.244 ± 0.005 (0.234 − 0.254)	0.0149 ± 0.0024 (0.0125 − 0.0200)
Alcoholics	DPH	0.251 ± 0.002* (0.249 − 0.254)	0.0103 ± 0.0017* (0.0076 − 0.0125)
Controls	TMA-DPH	0.280 ± 0.006 (0.277 − 0.289)	0.0095 ± 0.0010 (0.0076 − 0.0113)
Alcoholics	TMA-DPH	0.284 ± 0.004 (0.266 − 0.287)	0.0071 ± 0.0016* (0.0044 − 0.0096)

Results are expressed as means ± standard deviation (range is given in parentheses).
* $P < 0.001$ (compared to healthy controls).

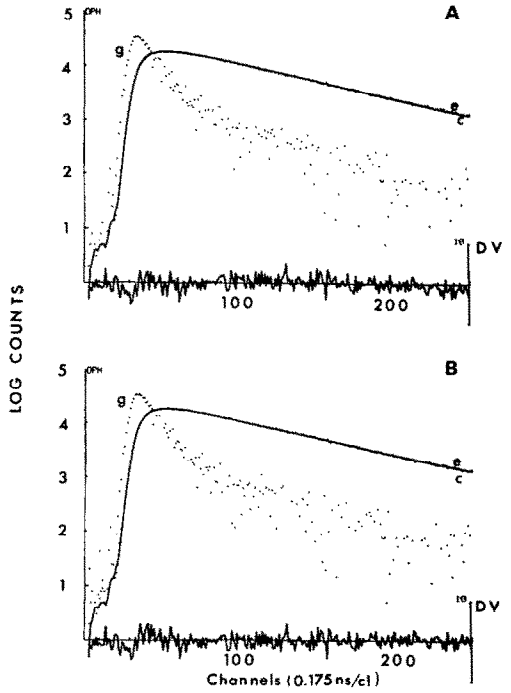

Fig. 1. Total fluorescence decay of DPH in erythrocyte membranes from healthy subjects. (A) Monoexponential model: $\tau = 10.90$ nsec; $\chi^2 = 2.66$; (B) biexponential model; $\tau_1 = 13.43$ nsec; $a_1 = 0.35$; $\tau_2 = 9.37$; $a_2 = 0.65$; $\chi^2 = 2.41$; $<\tau> = 11.16$ nsec. Symbols: (g) apparatus response function; (e) experimental data; (c) reconvoluted curve.

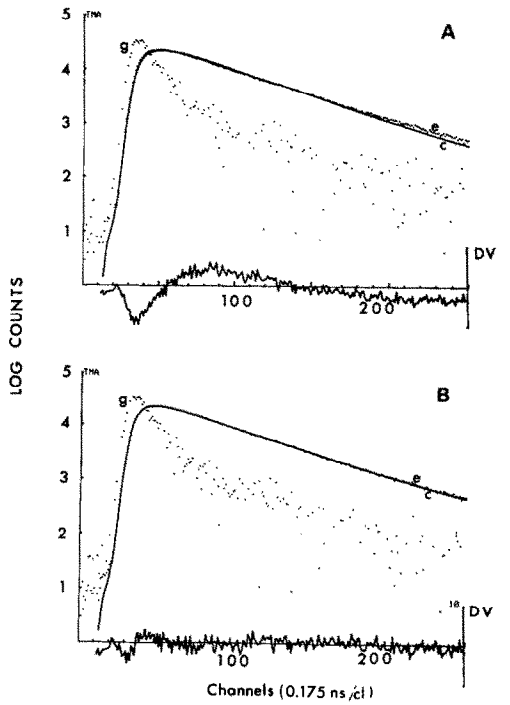

Fig. 2. Total fluorescence intensity decay of TMA-DPH in erythrocyte membranes from healthy subjects. (A) Monoexponential model: $\tau = 7.03$ nsec; $\chi^2 = 20.19$; (B) biexponential model: $\tau_1 = 7.81$ nsec; $a_1 = 0.69$; $\tau_2 = 2.34$ nsec; $a_2 = 0.31$; $\chi^2 = 2.23$, $<\tau> = 7.16$ nsec. Symbols as in Fig. 1.

to *in vitro* ethanol addition in membrane from alcoholic subjects (−25%) (Table 2).

The total fluorescence decay of both probes was measured in the two groups of erythrocyte ghost membranes to delineate the phenomenon. For DPH, which labels the hydrophobic region of the lipid bilayer, the total intensity decay can be adequately fitted by a monoexponential function (Fig. 1A). However, when a biexponential model is tried, the values of the two lifetime components are close to each other and the chi-square (χ^2) value is only slightly decreased (Fig. 1B). The time-resolved fluorescence emission of DPH in these membranes therefore suggests that their hydrophobic interior as probed by this dye is more homogeneous in terms of polarity and presence of quenching groups, than in other biological membranes studied so far [9, 17, 21]. The high degree of hydrophobicity of the lipid core in these membranes is emphasized by the high value of the probe excited state lifetime which can be compared to the values measured in hydrocarbon solvent [18]. Such high values were also found to be related to a high ordering of the probe environment [19, 20]. By contrast, the total fluorescence intensity decay for TMA–DPH is definitely best fitted by a biexponential (Fig. 2A,B). The environment of this probe, which is most likely the membrane region of intermediate polarity near the lipid polar head groups, is more heterogeneous than the hydrophobic

Table 3. Total fluorescence intensity decay parameters of DPH and TMA-DPH in human erythrocyte membranes from alcoholic patients and normal subjects.

	DPH	TMA-DPH			
Samples	$<\tau>$ (nsec)*	τ_1(nsec)	τ_2(nsec)	a_1	$<\tau>$ (nsec)*
Controls	10.98 ± 0.08	7.82 ± 0.14	2.27 ± 0.46	0.70 ± 0.01	7.16 ± 0.11
Alcoholics	10.80 ± 0.10	7.80 ± 0.08	2.94 ± 0.21	0.69 ± 0.06	7.07 ± 0.23

Biexponential model: $i_{(t)} = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$.
Mean (±SD) over five determinations.
* Mean lifetime calculated from $<\tau> = \Sigma_i a_i \tau_i^2 / \Sigma_i a_i \tau_i$.

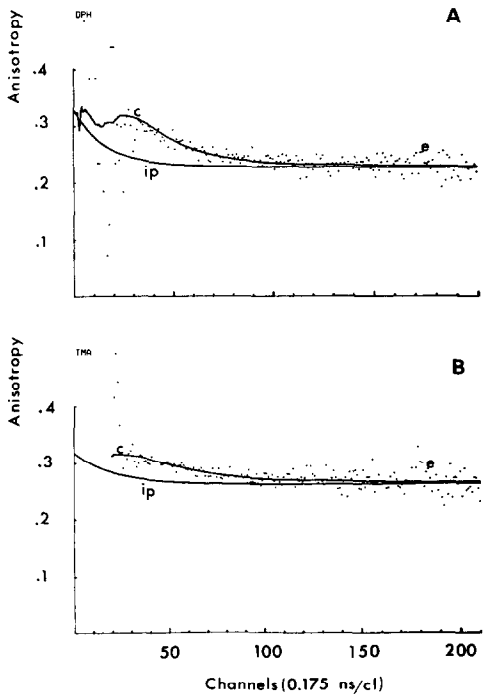

Fig. 3. Time-resolved fluorescence anisotropy decays of DPH (A) and TMA-DPH (B) in erythrocyte membranes. (A) DPH: $\Phi = 2.77$ nsec; $r_\infty = 0.230$; $r_{t=0} = 0.326$. (B) TMA-DPH: $\Phi = 4.33$ nsec; $r_\infty = 0.254$; $r_{t=0} = 0.318$. Symbols as in Fig. 1; (ip) impulse response function.

core. As for DPH, the lifetime values for TMA–DPH are higher than in other membrane systems which can be related to the high degree of rotational constraints imposed by the environment [8, 9, 18, 21]. The decay parameters for both probes do not exhibit any significant change between the two erythrocyte membrane populations (Table 3).

For both probes, the anisotropy decay curves were fitted by a biexponential function of the form:

$$r_{(t)} = \beta_1 \exp(-t/\Phi_1) + \beta_2 \exp(-t/\Phi_2).$$

The second correlation time values were found to be > 100 ns and therefore a function of the form:

$$r_{(t)} = (r_{t=0} - r_\infty) \exp(-t/\Phi) + r_\infty$$

was used in all analyses [16], where $r_{t=0}$ is the zero time value of the anisotropy, r_∞ is the residual anisotropy and Φ the apparent correlation time. An example of the experimental data is given in Fig. 3A, B. The r_∞ parameter was used to calculate the average orientational order parameter (S) and the wobbling angle (θ_{max}) of the probe [6, 16, 22]. The results are presented in Table 4.

For both probes, the r_∞ value is high and reflects the high value of the order parameter. As in model membrane systems [8, 18] and in biological membranes [9, 23], the motion of TMA-DPH is more constrained than that of DPH. This charge-bearing probe is tightly anchored in the membrane–water interface in a manner similar to fatty acids. Recent experiments (F. Beaugé, unpublished results) with propionyl-DPH showed similar effects. Therefore, the rigid-body order parameter [24] as sensed by these rod-shaped probes is much higher in the polar half of the erythrocyte membrane than in the hydrophobic interior.

Comparison of the data obtained in the two erythrocyte ghost populations shows that membranes from chronic alcoholic patients display a significant ($P < 0.001$) higher ordering in the hydrophobic region of the bilayer (Table 4). This is accompanied by a shorter apparent correlation time parameter although the data are more scattered. There is absolutely no effect within the experimental errors either on the order parameter or on the apparent correlation time for TMA-DPH.

DISCUSSION

The steady-state anisotropy results from DPH in erythrocyte membranes appear to be extremely reproducible as observed in this and other laboratories [2, 5, 25, 26]. Although less studied, this is also the case for TMA-DPH [5]. From these steady-state data and time-resolved anisotropy results, the erythrocyte ghost membranes appear to be highly ordered in the two membrane regions probed by both fluorophores. However, in the case of TMA-DPH, due to the additivity rule of the anisotropies [27], the measured order parameter is a weighted average over different populations since the total intensity decay is multiexponential. We can speculate that these populations may correspond (i) for the

Table 4. Anisotropy decay parameters of DPH and TMA-DPH in human erythrocyte membranes. A function of the form: $r_{(t)} = (r_{(t=0)} - r_\infty) \exp(-t/\Phi) + r_\infty$ was used to fit the anisotropy decay data*

Samples		Φ(nsec)	$r_{(t=0)}$	r_∞	S‡	θ_{max} (°)§
DPH	Controls	5.2 ± 1.0	0.305 ± 0.009	0.215 ± 0.003	0.746 ± 0.005	34.8 ± 0.4
	Alcoholics	3.0 ± 0.6†	0.332 ± 0.013†	0.231 ± 0.009†	0.773 ± 0.015†	32.8 ± 1.2†
TMA-DPH	Controls	5.2 ± 1.1	0.323 ± 0.016	0.256 ± 0.01	0.810 ± 0.002	29.8 ± 0.1
	Alcoholics	4.8 ± 1.9	0.324 ± 0.006	0.257 ± 0.002	0.812 ± 0.004	29.7 ± 0.3

* Mean (±SD) over 5–10 determinations.
† $P < 0.05$ (alcoholics compared to controls).
‡ Calculated from $S = (r_\infty/r_0)^{1/2}$.
§ Calculated from $\theta_{max} = \text{arc cos} \dfrac{[1 + 8(r_\infty/r_0)^{1/2}]^{1/2} - 1}{2}$.

long lifetime emitting species to the probe dissolved in the lipidic part of the membrane and (ii) for the short lifetime one to the probe close to membrane proteins. However, the contribution of the probe population emitting with the shortest lifetime to the residual anisotropy must be extremely weak [17] and, therefore, the residual anisotropy must mainly result from the long lifetime emitting probe.

The order parameter of DPH computed from the residual anisotropy value is among the highest as compared to other biological membranes [9, 17, 28, 29]. The ghost lipid ordering is significantly increased in alcoholic subjects in the hydrophobic region probed by this dye. The r_∞ variation is even greater than the r_s one due to a conversed changed in the apparent correlation time (Φ). Contrary to other investigations which have shown insensitivity of this parameter to a number of changes that dramatically altered the residual anisotropy [30], chronic alcohol intoxication impairs both parameters. Therefore, if the use of steady-state anisotropy measurements is feasible to assess ethanol-induced membrane perturbations, it must be noted that this technique provides underestimated results. The increase in lipid ordering in alcoholic patients compared to healthy controls is equivalent to a drop in temperature of 6 ± 1° (data not shown) and is pharmacologically relevant since the normal body temperature in humans cannot vary by more than 2° over a period of a day or during exercise [31]. A similar change in temperature would induce large effects on membrane-associated enzyme activities.

Comparison of the results obtained with DPH and TMA-DPH indicates that the ethanol-induced increase in the lipid order parameter is specifically localized in the hydrophobic part of the phospholipid acyl chains.

The observed effect with DPH must be based on chemical modifications of the lipids, probably more subtle than an overall decrease of the global degree of unsaturation of the fatty acid chains and/or an increase in the cholesterol content of the membrane [1, 32]. It has been observed that the compositional lipid changes complement one another, buffering against external stresses [33]. Therefore, more specific changes in the acyl chains are probably occurring, such as migration of the double bonds either towards the methyl end or the polar head [34]. With respect to the cholesterol content, the absence of modifications at the TMA-DPH level argues against any changes. Beside the pure adaptive phenomenon against the fluidizing effect of acute ethanol, consequences of ethanol oxidative metabolism may also be involved. We have previously suggested that alterations of surface-bound glycans might participate in the maintenance of the membrane structure in alcoholic subjects [2, 5].

Beside the higher acyl chain ordering observed in erythrocyte membranes from alcoholic patients, chronic alcohol consumption induces a membrane resistance or tolerance to the *in vitro* fluidizing effect of ethanol addition. A high lipid order would indicate strong acyl chain interactions, less prone to disruption by ethanol molecules partitioned within the membrane. However, the resistance to the fluidizing effect of ethanol is also evidenced in the polar membrane region where no change in lipid ordering was observed. As ethanol molecules are known to be preferentially partitioned in this polar region [1], an alternative explanation for the membrane resistance to the *in vitro* effect can be a decrease in its local concentration. We have actually shown that the partition coefficient of ethanol into the membranes from alcoholic patients was decreased [5] and correlated with the fluidizing efficacy of ethanol [35, 36].

In conclusion, chronic ethanol consumption causes differential disturbances in lipid organization at different levels of the human erythrocyte membrane. The exact molecular basis of the alteration in intrinsic lipid ordering as well as in the sensitivity to ethanol *in vitro* remains to be clarified. However, it can be concluded that the resulting changes in membrane lipid ordering are sensitive correlates and indicators of alcohol abuse.

Acknowledgements—This work was supported by grants from INSERM and CNRS (France) and MFR (Sweden).

REFERENCES

1. Goldstein DB, The effects of drugs on membrane fluidity. *Ann Rev Pharmacol Toxicol* **24**: 43–64, 1984.
2. Beaugé F, Stibler H and Borg S, Abnormal fluidity and surface carbohydrate content of the erythrocyte membranes in alcoholic patients. *Alcohol Clin Exp Res* **9**: 322–326, 1985.
3. Benedetti A, Birabelli AM, Brunelli E, Curatola G, Ferreti G, Jezequel AM and Orlandi F, Effect of chronic ethanol abuse on the physico-chemical properties of erythrocyte membranes in man. *Pharmacol Res Comm* **48**: 1003–1014, 1986.
4. Wood WG, Lahiri S, Gorka C, Ambrecht HJ and Strong R, *In vitro* effects of ethanol on erythrocyte membrane fluidity of alcoholic patients: an electron spin resonance. *Alcohol Clin Exp Res* **11**: 332–335, 1987.
5. Beaugé F, Stibler H and Borg S, Alterations of erythrocyte membrane organization in alcoholics. *Alcohol Alcoholism* Suppl **1**: 561–564, 1987.
6. Jähnig F, Structural order of lipids and proteins in membranes. Evaluation of fluorescence anisotropy data. *Proc Natl Acad Sci USA* **76**: 6361–6365, 1979.
7. Araiso T, Shindo Y, Arai T, Nitta J, Kikuchi Y, Kakinchi Y and Koyama T, Viscosity and order in erythrocyte membranes studied with nanosecond fluorometry. *Biorheology* **23**: 467–483, 1986.
8. Prendergast FG, Haughland RP and Callahan PJ, 1-(4-(Trimethylamino)phenyl)-6-phenylhexa-1,3,5-triene: synthesis, fluorescence properties and use as a fluorescent probe of lipid bilayer. *Biochemistry* **20**: 7333–7338, 1981.
9. Stubbs CD, Kinosita K Jr, Munkonge F, Quinn P and Ikegami A, The dynamics of lipid motion in sarcoplasmic reticulum membranes determined by steady-state and time-resolved fluorescence measurements on 1,6 diphenyl 1,3,5 hexatriene and related molecules. *Biochim Biophys Acta* **775**: 374–380, 1984.
10. *Diagnostic and Statistical Manual of Mental Disorders.* American Psychiatric Association, 3rd ed. APA, Washington, DC, 1980.
11. Stibler H, Borg S and Joustra M, Microanion exchange chromatography of carbohydrate-deficient transferrin in serum in relation to alcohol consumption. *Alcohol Clin Exp Res* **10**: 535–544, 1986.

12. Lowry OH, Rosebrough MJ, Farr AC and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
13. Gallay J and Vincent M, Cardiolipin–cholesterol interactions in the lipid crystalline phase: a steady-state and time-resolved fluorescence anisotropy study with *cis* and *trans*parinaric acids as probes. *Biochemistry* **25**: 2650–2656, 1986.
14. Berlman IB, *Handbook of Fluorescence Spectra of Aromatic Molecules*, 2nd ed. Academic Press, New York, 1971.
15. Wahl P, Auchet JV and Donzel B, The wavelength dependence of the response of a pulse fluorimeter using the single photon electron counting method. *Rev Sci Instrum* **45**: 28–32, 1974.
16. Kinosita K Jr, Kawato S and Ikegami A, A theory of fluorescence polarization decay in membranes. *Biophys J* **20**: 289–305, 1977.
17. Gallay J, Vincent M and Alfsen A, Dynamic structure of bovine adrenal cortex microsomal membranes studied by time-resolved fluorescence anisotropy of all-*trans*-1,6-diphenyl-1,3,5-hexatriene. *J Biol Chem* **257**: 4038–4041, 1982.
18. Cranney M, Cundall RB, Jones GR, Richards JT and Thomas EW, Fluorescence lifetime and quenching studies on some interesting diphenylhexatriene membrane probes. *Biochim Biophys Acta* **735**: 418–425, 1983.
19. Hare F, Simplified derivation of angular order and dynamics of rodlike fluorophores in models and membranes. *Biophys J* **46**: 205–218, 1983.
20. Ameloot M, Hendrieux H, Herreman W, Pottel H and Van Cauwelaert F, Effect of orientational order on the decay of the fluorescence anisotropy in membrane suspensions. *Biochem J* **46**: 525–539, 1984.
21. Engel LW and Prendergast FG, Values for and significance of order parameters and "cone angles" of fluorophore rotation in lipids bilayers. *Biochemistry* **20**: 7338–7345, 1981.
22. Heyn MP, Determination of lipid order parameters and rotational correlation times from fluorescence depolarization experiments. *FEBS Lett* **108**: 359–364, 1979.
23. Illsley NP, Lin H and Verkan AS, Lipid domaine structure correlated with membrane protein function in placental microvillus vesicules. *Biochemistry* **28**: 446–454, 1987.
24. Jähnig F, Vogel H and Best L, Unifying description of the effect of membrane proteins on lipid order. Verification for the melittin dimyristoyl phosphatidylcholine system. *Biochemistry* **21**: 6790–6798, 1982.
25. Owen JS, Bruckdorfer KR, Day RC and McIntyre N, Decreased erythrocyte membrane fluidity and altered lipid composition in human liver disease. *J Lipid Res* **23**: 124–132, 1982.
26. Henry O, Maire C, Donner M and Stoltz JF, Comparative study of fluorescent molecules probing human erythrocytes and platelets. *Innov Tech Biol Med* **6**: 420–433, 1985.
27. Weber G, Polarization of the fluorescence of macromolecules. *Biochem J* **51**: 145–155, 1952.
28. Hildenbrand K and Nicolau C, Nanosecond fluorescence anisotropy decays of 1,6 diphenyl-1,3,5 hexatriene in membranes. *Biochim Biophys Acta* **553**: 365–377, 1979.
29. Kinosita K, Kataoka R, Kimura Y, Gotoh O and Ikegami A, Dynamic structure of biological membrane as probed by 1,6-diphenyl-1,3,5 hexatriene. A nanosecond fluorescence depolarization study. *Biochemistry* **20**: 4270–4277, 1981.
30. Amler E, Teisinger J, Svobodová J and Vyskočil F, Vanadyl ions increase the order parameter of plasma membranes without changing the rotational relaxation time. *Biochim Biophys Acta* **863**: 18–22, 1986.
31. Wright S, Notes on experiments illustrating normal temperature regulation in young men. *Br Med J* **1**: 610–613, 1949.
32. Hitzemann RJ, Schueler HE, Graham-Brittain C and Kreishmann GP, Ethanol-induced changes in neuronal membrane order. A NMR study. *Biochim Biophys Acta* **859**: 189–197, 1986.
33. Thompson GA, Jr, Metabolism and control of lipid structure modification. *Biochem Cell Biol* **64**: 66–69, 1986.
34. Barton PG and Gunstone FD, Hydrocarbon chain packing and molecular motion in phospholipid bilayers formed from unsaturated lecithins. *J Biol Chem* **250**: 4470–4476, 1975.
35. Kelly-Murphy S, Waring AJ, Rottenberg H and Rubin E, Effects of chronic ethanol consumption on the partition of lipophilic compounds in erythrocyte membranes. *Lab Invest* **50**: 174–183, 1984.
36. Leguicher A, Beaugé F and Nordmann R, Concomitant changes of ethanol partitioning and disordering capacities in rat synaptic membranes. *Biochem Pharmacol* **36**: 2045–2048, 1987.