Biochimica et Biophysica Acta, 597 (1980) 263—273

BBA 78690

PLASMA MEMBRANE CHANGES ASSOCIATED WITH RAT LIVER REGENERATION

GIOVANNELLA BRUSCALUPI [a], GIOVANNA CURATOLA [b], GIORGIO LENAZ [b], SILVIA LEONI [a], M. TERESA MANGIANTINI [a], LAURA MAZZANTI [b], SILVANA SPAGNUOLO [a] and ANNA TRENTALANCE [a]

[a] *Institute of General Physiology, University of Rome, 00185 Rome, and* [b] *Institute of Biochemistry, University of Ancona, Ancona (Italy)*

(Received July 19th, 1979)

Key words: Regeneration; Fluidity; Temperature dependence; Membrane-bound enzyme; Spin-label mobility; (Rat liver)

Summary

The lipid composition and fluidity of plasma membranes have been studied at different stages of liver regeneration (4, 15 and 24 h after surgery).

The phospholipid and fatty acid composition is not modified, whereas the cholesterol/phospholipid ratio is lower with respect to control membranes.

The modification of the physical properties of the membranes has been studied directly by EPR analysis and indirectly by temperature dependence and cooperativity of some membrane-bound enzymes (Mg^{2+}-ATPase, ($Na^+ + K^+$)-ATPase and 5′-nucleotidase). Surgical operation or anaesthesia alone causes an early increase in fluidity; such an effect appears to be markedly reduced at a later stage. There seems to be a marked effect of regeneration on plasma membrane fluidity 15 h after partial hepatectomy when several parameters — surface fluidity, cholesterol/phospholipid ratio, and 5′-nucleotidase activity in the presence of concanavalin A — are modified and indicate an increase in membrane fluidity.

It is suggested that this modification of membrane properties could be related to the proliferative process.

Introduction

The plasma membrane undergoes several alterations during cell proliferation and the modification of lipid fluidity appears to be of major interest [1]. The

Abbreviations: 5NS, 16NS, 5-, 16-*N*-oxyl-4′,4′-dimethyloxazolidine derivatives of stearic acid, respectively.

presence of particular phospholipids [2], the fatty acid composition [3], and the cholesterol/phospholipid ratio play a crucial role in membrane fluidity regulation. Changes either in the cholesterol/phospholipid ratio or in fatty acid composition of plasma membranes have been observed under different physiological and pathological conditions [4—6]. Plasma membrane fluidity appears to be involved in the control of those membrane functions linked to the proliferative process, such as permeability [7], receptor and antigen mobility [8], enzyme activities [9] and lectin agglutination [10].

In the case of regenerating liver, the plasma membrane is known to undergo biochemical and enzymatic modifications [11—15], some of which have been correlated with the cell cycle [16]. The aim of the present study was to investigate whether changes occur in plasma membrane fluidity during the earliest cell cycle of liver regeneration following partial hepatectomy. For this purpose, the correlations existing among lipid components have been investigated in rat liver plasma membranes prepared at different times after partial hepatectomy, i.e.: phospholipid composition, total phospholipid/protein ratio, free cholesterol/phospholipid ratio and fatty acid composition of membrane phospholipids. The modifications of the physical state of the membrane have been studied both directly by electron paramagnetic resonance (EPR) and indirectly by investigating both temperature dependence and cooperativity of some membrane enzymes. In fact, those two parameters can be correlated with the membrane fluidity state [17—20].

Materials and Methods

Male Sprague Dawley rats (average body weight 150 g) fed ad libitum before and after surgery (either sham operation or partial hepatectomy) were used. Partial hepatectomy was performed according to the procedure of Higgins and Anderson [21] under light ether anaesthesia. Animals were killed 4, 15 and 24 h after surgery. Autoradiographic analysis of [^{3}H]thymidine loaded cells indicated that these times corresponded to G_1 phase, beginning and end of S phase, respectively. Membranes from partially hepatectomized, sham operated, anaesthetized, and control animals were prepared in parallel, immediately after liver removal, by the method of Ray et al. [22]. Membrane proteins were estimated using the method of Lowry et al. [23], with bovine serum albumin as a standard. Membrane purity was checked as described by Luly et al. [24].

Cholesterol/phospholipid ratio

Total membrane lipid extraction and purification were performed following the procedure of van Hoeven and Emmelot [25]. Single lipid classes were separated by silica gel thin-layer chromatography using petroleum ether/diethylether/acetic acid, (75 : 25 : 1) as the solvent. Zones containing free cholesterol and phospholipids were scraped off and eluted according to the method of Skipski and Barclay [26]. Cholesterol was measured in the eluates using Liebermann-Burchard reagent (acetic anhydride/sulfuric acid, 19 : 1). Membrane and eluate lipid P_i was determined according to the method of Morrison [27]. The P_i contents were multiplied by 25 to calculate the amount of phospholipids.

Phospholipid composition

Phospholipid fractions were separated from the whole lipid extract by silica gel thin-layer chromatography using chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2) as the solvent system, according to Ray et al. [22]. The identification of separated phospholipids was carried out by comparing them with simultaneously chromatographed standards (supplied by Supelchem, Milano, Italy) and by specific staining reactions.

Zones containing single phospholipids were scraped off and processed for the determination of P_i, according to the method of Morrison [27]. The total P_i recovery of all phospholipid classes was in the range of 95%.

Fatty acid composition of phospholipids

Phospholipids were separated from the total lipid extract by silica gel TLC following the procedure of van Hoeven and Emmelot [25]. The zone corresponding to the phospholipid band was scraped off and suspended in 1.15 N H_2SO_4 in methanol for hydrolysis and methylation of fatty acids according to the method of Stancliff [28]. Methyl esters were extracted with petroleum ether and subjected to gas liquid chromatography (GLC) analysis on a Perkin-Elmer F 30 equipped with a flame ionization detector.

A glass column 11.8 ft long was used, packed with 15% diethyleneglycol succinate (DEGS) on 80—100 mesh chromosorb with a 25 ml/min nitrogen flow rate under isothermal conditions at 200°C. The temperature of both the injector and the detector was kept at 300°C.

Fatty acids with 16—22 carbon atoms were considered. The individual fatty acid percentage was calculated on the basis of the elution area. The saturated/unsaturated fatty acid ratio and unsaturation index (average number of double bonds/fatty acid) were also calculated.

Spin label studies

The spin labels used were either 5- or 16-*N*-oxyl-4′,4′-dimethyloxazolidine ('doxyl') derivatives of stearic acid (termed 5NS and 16NS, respectively) obtained from Synvar Co., Palo Alto, CA.

The labels were dissolved in absolute ethanol at a concentration of 10 mM, and added to membranes, taking care that the label to phospholipid molar ratio was in the range of 1 : 100.

Previous experiments have shown that all the label is incorporated into the membranes under such conditions and no free label is detectable either in the supernatant after centrifugation or by examination of the typical signal of unincorporated label.

The freedom of motion of spin labels in the membranes was calculated by measuring the following parameters.

For 5NS, which probes the outer region of the bilayer, the spectra exhibit probe ordering proportional to the separation of the two hyperfine extrema and the order parameter S may be calculated according to Seelig [29] by the following equation:

$$S = \frac{T_{\parallel} - T_{\perp}}{T_{zz} - T_{xx}} \cdot \frac{a_n}{a_n'}$$

where $T_{/\!/}$, $T_{\perp}$ are the hyperfine splitting parameters, T_{zz} and T_{xx} represent intrinsic hyperfine splitting in rigid matrix, and a is the anisotropic constant in rigid matrix (a' being the value obtained in this work).

For the 16NS, the pseudoisotropic rotational correlation times, τ_c, were calculated by using the following equation according to Eletr and Inesi [30]:

$$\tau_c = 6.5 \cdot 10^{-10} w_0 (\sqrt{h_0/h_{-1}} - 1)$$

where ω_0 is the width of the medium-field line and h_0, h_{-1} refer to the heights of the medium- and upper-field lines, respectively.

Enzyme analysis

$(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase were assayed using the method of Bonring [31] in an incubation medium containing 92 mM Tris-HCl (pH 7.5)/15 mM $MgSO_4$/60 mM NaCl/0.1 mM EDTA/4 mM ATP and about 100 μg membrane protein either in the presence or in the absence of 5 mM KCl.

5′-nucleotidase was assayed by the method of Aronson and Touster [32] in an incubation medium (0.5 ml final volume) containing 0.1 M glycine-NaOH (pH 9.0) buffer; 0.01 M $MgCl_2$; 5 mM 5′-AMP; about 100 μg membrane protein in the presence of various concanavalin A concentrations (μM) (0.00; 0.025; 0.05; 0.125; 0.25; 0.5; 1).

The reactions were stopped by addition of 10% trichloroacetic acid after 15 min incubation and the P_i content was measured in the supernatant as reported by Bonting [31].

Results

Lipid composition of isolated plasma membranes

Fig. 1A shows the cholesterol/phospholipid ratio in liver membranes from control, anaesthetized, sham operated, and partially hepatectomized animals, at 4, 15 and 24 h. The parameter studied appears to be already equally altered in sham operated and in anaesthetized animals with respect to controls.

At the earliest time after operation (4 h) a marked decrease is evident in the

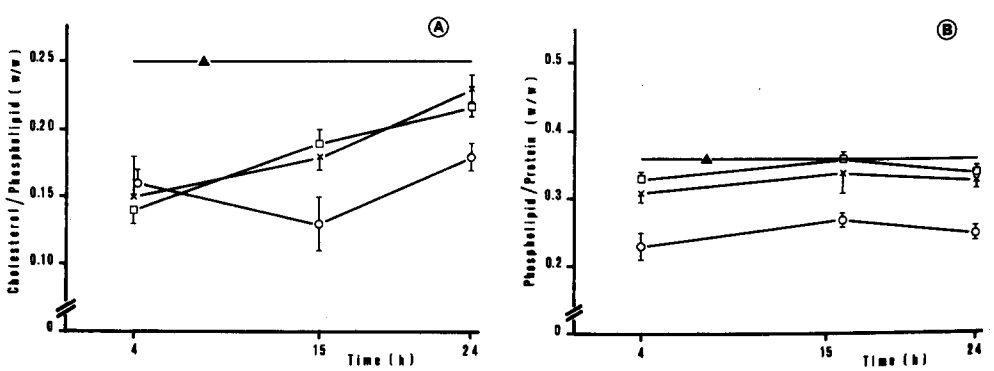

Fig. 1. Cholesterol/phospholipid (A) and phospholipid/protein (B) ratios (w/w) in isolated liver plasma membranes from control (▲———▲), anaesthetized (□———□), sham operated (X———X), and partially hepatectomized (○———○) rats. Values are the mean of three experiments ±S.D.

TABLE I

PHOSPHOLIPID COMPOSITION OF LIVER PLASMA MEMBRANES FROM CONTROL (C), AND 15 AND 24 h SHAM OPERATED (SO) AND PARTIALLY HEPATECTOMIZED (PH) RATS

Each value is the average of n determinations, expressed as a percentage of total phospholipid P_i. Error was less than 5%.

Phospholipids	C (n = 11)	15 h		24 h	
		SO (n = 4)	PH (n = 5)	SO (n = 7)	PH (n = 8)
Lysophosphatidylcholine	1.53	1.88	2.73	2.37	2.00
Sphingomyelin	23.86	21.31	21.45	23.80	21.74
Phosphatidylcholine	39.18	35.55	37.50	37.68	38.87
Phosphatidylserine + phosphatidylinositol	13.26	18.93	16.52	12.78	15.87
Phosphatidylethanolamine	22.65	22.30	21.70	23.30	21.31

cholesterol/phospholipid ratio, both in sham operated and in hepatectomized animals, whereas at later times the ratio appears to increase again towards normal values in sham operated animals and to remain constantly lower in hepatectomized ones.

The phospholipid/protein ratio (Fig. 1B) is lower in partially hepatectomized with respect to sham operated animals at all times considered.

Table I shows the phospholipid composition of rat liver plasma membranes of controls and 15 and 24 h sham operated and partially hepatectomized animals. The phospholipid content of each fraction is given as total phospholipid

TABLE II

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS FROM LIVER PLASMA MEMBRANES OF CONTROL (C) AND 4, 15 AND 24 h SHAM OPERATED (SO) AND PARTIALLY HEPATECTOMIZED (PH) RATS

Each value is the average of three determinations expressed as percentage of total content. Error was less than 5%.

Acyl groups	C	4 h		15 h		24 h	
		SO	PH	SO	PH	SO	PH
16:0	25.64	23.11	23.90	20.59	21.07	23.80	21.36
16:1	2.90	1.48	1.68	1.09	1.37	0.99	1.18
18:0	24.90	28.56	30.07	28.16	30.88	31.50	30.31
18:1	9.34	9.39	8.89	8.81	9.41	7.89	9.62
18:2	16.78	14.59	17.80	16.94	15.93	15.05	15.46
20:1	0.10	0.14	0.00	0.09	0.09	0.16	0.12
20:3	0.67	0.65	0.36	0.83	0.37	0.52	0.64
20:4	18.46	18.68	14.44	20.85	18.25	18.74	18.65
20:5	0.38	0.73	0.66	0.45	0.51	0.24	0.56
22:4	0.79	2.61	2.16	2.15	2.10	1.07	2.03
$\frac{\text{saturated}}{\text{unsaturated}}$ ratio	1.02	1.07	1.17	0.95	1.08	1.23	1.06
unsaturation index	1.27	1.31	1.16	1.40	1.27	1.21	1.29

percentage. The percent amount of phosphatidylserine plus phosphatidylinositol is given as a single value.

The values obtained for normal liver membranes are in the range of those already reported [25]. No significant variation is observed in the proportions of the single phospholipid classes after partial hepatectomy, results being similar to those obtained for membranes from sham operated animals.

The percent fatty acid compositions of phospholipids from liver membranes of control, sham operated, and partially hepatectomized rats 4, 15 and 24 h after operation are reported in Table II.

The percent composition of the single fatty acids does not appear to be significantly affected either by sham operation or by partial hepatectomy. The variations observed appear to be within physiological range, except for a slight decrease in arachidonic acid 4 h after hepatectomy. The saturated/unsaturated fatty acid ratio, and the unsaturation index show some small differences in hepatectomized with respect to sham operated animals. Fatty acids of membranes from hepatectomized animals appear to be less unsaturated at 4 and 15 h after operation, and more unsaturated after 24 h than those from sham operated animals.

Membrane fluidity

Spin label studies. The order parameter of 5NS inserted in the membrane reflects the extent of mobility of the surrounding lipid alkyl chains in the surface region of the bilayer. It can be seen in Fig. 2 that at the initial times a decrease of order occurs in all groups, although this is greater in the hepatectomized rats than in anaesthetized or sham operated animals. There is a marked increase in fluidity in hepatectomized rats 15 h after operation.

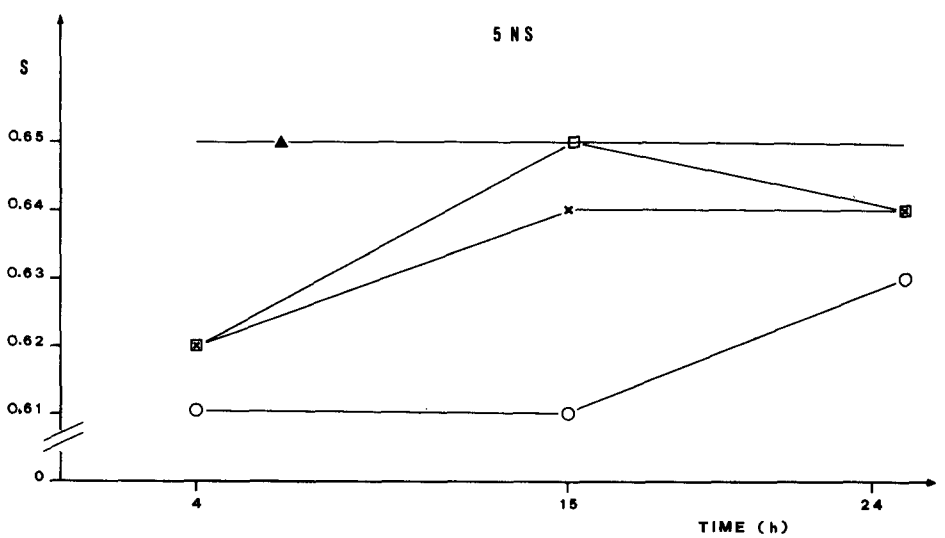

Fig. 2. Mobility of 5-doxyl stearic acid (5SN) in isolated liver plasma membranes from control (▲———▲), anaesthetized (□———□), sham operated (X———X), and partially hepatectomized (○———○) rats. Data are expressed as order parameter values (*S*).

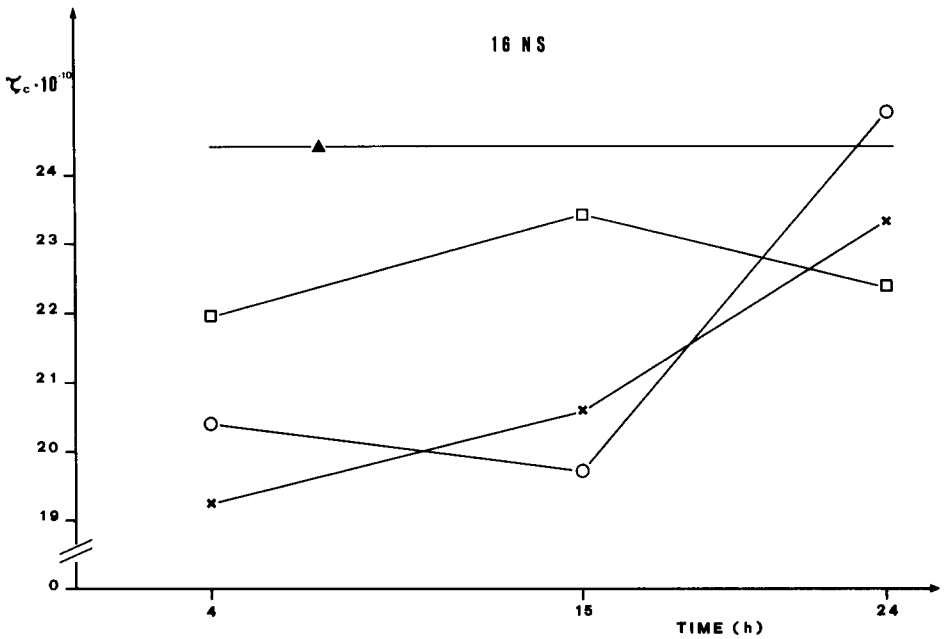

Fig. 3. Mobility of 16-doxyl stearic acid (16NS) in isolated liver plasma membranes from control (▲———▲), anaesthetized (□———□), sham operated (X———X), and partially hepatectomized (○———○) rats. Data are expressed as pseudoisotropic rotational correlation time values (τ_c).

Contrary to what is observed with 5NS, which probes the membrane surface, the 'deep' probe 16NS does not show appreciable differences in the pseudoisotropic rotational correlation times (τ_c) among the different groups (Fig. 3). In all instances we observed a decrease in τ_c, indicative of increased fluidity, sug-

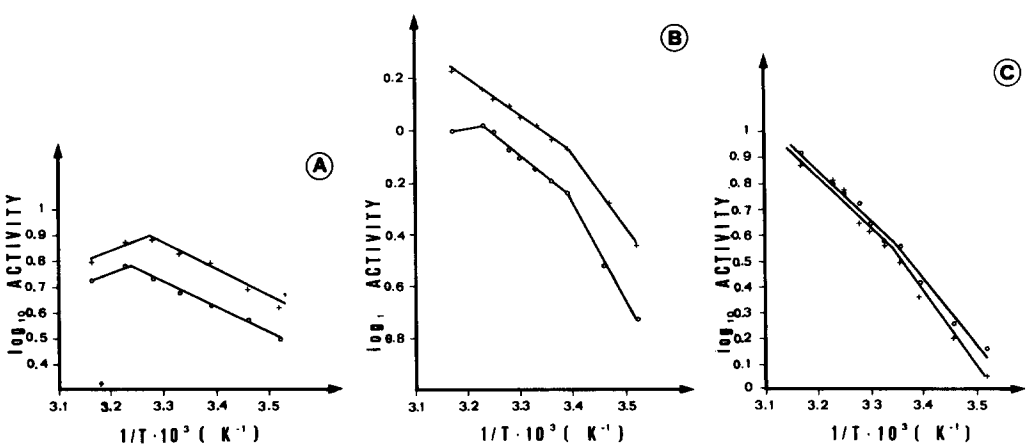

Fig. 4. Arrhenius plots of Mg^{2+}-ATPase (A); ($Na^+ + K^+$)-ATPase (B), and 5′-nucleotidase (C) activity in isolated liver plasma membranes from 15 h sham operated (+———+) and partially hepatectomized (○———○) rats. The activity has been measured as μmol P_i/mg protein per h. Values are the mean of five experiments.

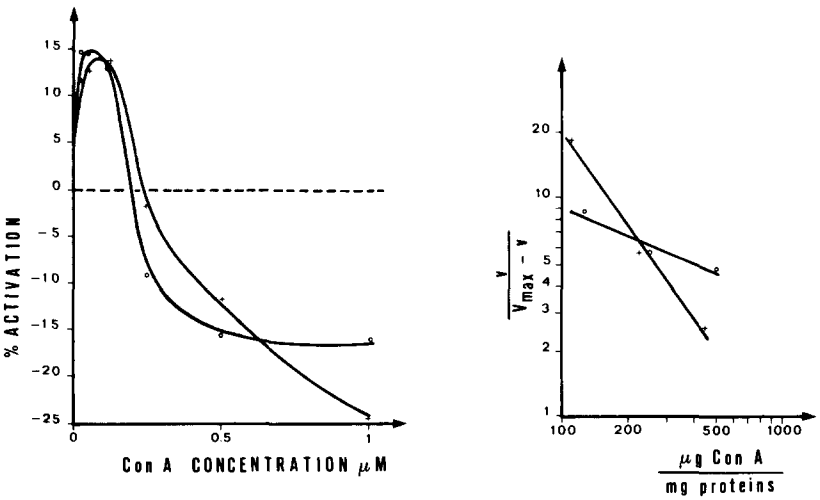

Fig. 5. Effect of concanavalin A on 5′-nucleotidase activity of isolated liver plasma membranes from 15 h sham operated (+———+), and partially hepatectomized (○———○) rats. The enzymatic activity has been measured as μmol P_i/mg protein per h. Values, average of five experiments, are expressed as percentage of total enzyme activity. Standard deviations do not exceed 5% of the mean.

Fig. 6. Hill's plot of concanavalin A (Con A) inhibition on 5′-nucleotidase activity of isolated liver plasma membranes from 15 h sham operated (+———+) and partially hepatectomized (○———○) rats. The enzyme activity has been measured as μmol P_i/mg protein per h. Values are the mean of five experiments and standard deviations do not exceed 5% of the mean. For (○———○) $n = 0.68 \pm 0.12$ and for (+———+) $n = 1.4 \pm 0.2$ ($P < 0.001$).

gesting that fluidization in the membrane core is not to be ascribed to regeneration.

Enzyme analysis. The Arrhenius plots of Mg^{2+}-ATPase, $(Na^+ + K^+)$-ATPase and 5′-nucleotidase of liver membranes from 15 h sham operated and partially hepatectomized rats are given in Fig. 4. Both in sham operated and hepatectomized animals — according to previous observations in normal rat liver membranes [33] — the Arrhenius plots of $(Na^+ + K^+)$-ATPase and 5′-nucleotidase show a break at 22—24°C, while those of Mg^{2+}-ATPase are linear. Analogous experiments performed on liver membranes from 24 h partially hepatectomized animals do not show any difference (data not reported) from those from sham operated animals. Partial hepatectomy does not appear to modify the temperature dependence of these enzymes.

Fig. 5 shows the effect of different concanavalin A concentrations on 5′-nucleotidase activity of 15 h sham operated and partially hepatectomized rat liver membranes. It can be seen that in both 15 h partially hepatectomized and sham operated animals, low concentrations of the lectin induce an activation of the enzyme followed by a clear-cut inhibition, in accordance with data reported by other authors for liver membranes of normal rats [34]. Several authors have reported that both in liver [34] and in other systems [35,36] concanavalin A is an allosteric effector of 5′-nucleotidase. The values of concanavalin A-induced inhibition presented as Hill's plots (Fig. 6) give an n value for the membrane enzyme from sham operated animals of 1.4 ± 0.2, thus showing

the presence of cooperativity, and an n value for liver membranes from 15 h partially hepatectomized rats of 0.68 ± 0.12, which suggests a loss of cooperativity of the enzyme. 24 h after partial hepatectomy no difference is detected with respect to sham operated animals (data not shown).

Discussion

Some modifications have been observed in the plasma membrane in the course of liver regeneration during the earliest cell cycle after partial hepatectomy.

An increase in fluidity has been detected in the surface of the membrane probed with the 5NS. This modification, which is at first similar to that observed in sham operated and anaesthetized rats, is greater in 15 h hepatectomized animals than in controls. An increase in fluidity is also observed in the inner part of the bilayer, probed using 16NS, with respect to control animals, but such a modification cannot be correlated with the regeneration process, since no difference has been observed between hepatectomized and sham operated animals.

Phospholipid composition does not appear to be modified at the times considered. The fatty acid composition of phospholipids shows only a slight trend towards a lower unsaturation at 4 and 15 h and a trend towards a higher unsaturation at 24 h. These modifications, however, are not such as to either induce changes in membrane fluidity or counterbalance the effects induced by the decrease in the cholesterol/phospholipid ratio. The phospholipid/protein ratio appears to be lower both in sham operated and in hepatectomized animals at all times considered.

The increase in surface fluidity detected by EPR analysis can be correlated with the change in cholesterol/phospholipid ratio. It is well known that cholesterol exerts a condensing effect on fluid membrane lipids and is involved in physiological regulation of membrane fluidity [37]. The only exception to the parallel behaviour of cholesterol/phospholipid ratio and fluidity occurs 24 h after hepatectomy, at which time the cholesterol/phospholipid ratio is lower in hepatectomized animals, and fluidity is not appreciably different in hepatectomized and sham operated animals. At that time other membrane components may counterbalance the effect exerted by the decreased cholesterol/phospholipid ratio. Membrane proteins have, for instance, been shown to exert a rigidizing effect on the lipids throughout the bilayer [38].

Results obtained in enzyme assays performed at the times when either a composition or a fluidity modification occurred (15 h and 24 h) show that, whereas Arrhenius plots of the membrane enzymes examined ($(Na^+ + K^+)$-ATPase, Mg^{2+}-ATPase and 5′-nucleotidase) do not change, the 15 h Hill's plots of 5′-nucleotidase — in the presence of concanavalin A — show a change in the Hill's coefficient from 1.4 ± 0.2 to 0.68 ± 0.12, which suggests a loss of cooperativity in the enzyme [35,36]. Since it has already been reported [17] that variations in Hill's coefficient in cooperative membrane enzymes depend on their relationship to lipids and on the fluidity condition of the latter, we may suggest that the different behaviour of 5′-nucleotidase 15 h after partial hepatectomy is related to the fluidity enhancement observed with the EPR

spectrum of the 5NS probe. This seems to be supported by the fact that 5′-nucleotidase cooperativity returns to the values observed in the sham operated animals 24 h after partial hepatectomy, i.e. the time at which the EPR data of the 5NS probe reveal only a very slight fluidity modification in hepatectomized with respect to sham operated animals. The fact that no variation has been detected in the Arrhenius plots could be ascribed to the lower sensitivity of this parameter to membrane fluidity [17].

On the basis of the data obtained we may conclude that liver plasma membrane fluidity is affected by several factors. Surgical operation, or anaesthesia alone, causes this parameter to increase soon after hepatectomy. Such an effect appears to be markedly reduced at later times. From this observation emerges the need to use membranes from sham operated animals as controls, since they allow for effects caused by surgical operation (anaesthesia, surgical stress, altered food intake) which, especially at an early stage of liver regeneration, complicate the detection of fluidity and composition modifications in the membrane. The probable occurrence of these modifications is supported by the fact that in early liver regeneration there is a variation in the blood content of some hormones [11,39], and ions [40] which are known to affect the activity of some membrane enzymes by changing the physical state of lipids [18, 41].

The effect of regeneration on plasma membrane fluidity observed 15 h after partial hepatectomy appears to be of particular interest. At this time our proliferation model is at the onset of the S phase of the cell cycle as shown by autoradiography.

A fluidity increase, ascribed to neutral lipid contamination, has been detected by other investigators [42] in regenerating liver membranes 24 h after hepatectomy (S phase of their system). We believe that the correlation observed between surface fluidity, cholesterol/phospholipid ratio, and the 5′-nucleotidase Hill's coefficient in the presence of concanavalin A, suggests that the fluidity increase might be better correlated with the proliferative process. This hypothesis is in agreement with previous data from our laboratory showing an increased surface mobility of concanavalin A receptors in hepatocytes isolated from 15 h hepatectomized rats (unpublished results).

The increased activity of adenylate cyclase, which we have observed in previous research, in regenerating liver at 15 h, the time at which fluidity increases, suggests that a causal ratio may be established for regenerating liver between the increase in fluidity (and cholesterol/phospholipid ratio decrease) and the increase in adenylate cyclase activity. This is supported by the correlation between decreased adenylate cyclase activity and radioactive cholesterol uptake observed in kidney fibroblasts by Klein et al. [43].

In our system the assumption [44], that the membrane exerts its influence on the regulation of the cell cycle by modulating the activity of some enzymes through lipid fluidity changes, seems valid.

Acknowledgments

Thanks are due to Mr. Maurizio Di Felice (Laboratorio di Biochimica, Instituto Nazionale della Nutrizione, Rome, Italy) for skilful technical assistance in fatty acid analysis.

References

1 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367—394
2 Horowitz, A.F. (1977) in Growth, Nutrition and Metabolism of Cells in Culture (Rothbles, G.H. and Cristofalo, V.Y., eds.), Vol. 3, pp. 109—148, Academic Press, New York
3 Chapman, D. (1975) in Biomembranes (Eisenberg, H., Katchalski-Katzir, E., Manson, L.A., eds.), Vol. 7, pp. 1—9, Plenum Press, New York
4 Yau, T.M., Buckman, T., Hale, A.M. and Michael, J.W. (1976) Biochemistry 15, 3212—3219
5 Collard, J.G., de Wildt, A., Oomen-Meulemans, E.P.M., Smeekens, J., Emmelot, P. and Inbar, M. (1977) FEBS Lett. 77, 173—178
6 Hulbert, A.J., Augee, M.L. and Raison, J.K. (1976) Biochim. Biophys. Acta 455, 597—601
7 Van Deenen, L.L.M., de Gier, J. and Demel, R.A. (1972) in Current Trends in the Biochemistry of Lipids (Ganguly, J. and Smellie, R.M.S., eds.), pp. 377—382, Academic Press, New York
8 Nicolson, J.L. (1977) Biochim. Biophys. Acta 457, 57—108
9 Houslay, M.D., Hesketh, T.R., Smith, G.A., Warren, G.B. and Metcalfe, J.C. (1976) Biochim. Biophys. Acta 436, 495—504
10 Bales, B.L., Lesin, E.S. and Oppenheimer, S.B. (1977) Biochim. Biophys. Acta 465, 400—407
11 Leffert, H., Alexander, M.N., Faloona, G., Rubalcava, B. and Unger, R. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4033—4036
12 Akasaki, M., Kawasaki, T. and Yamashina, I. (1975) FEBS Lett. 59, 100—104
13 Dyatlovitskaya, E.L., Novikov, A., Gorkova, N.P. and Bergelson, L.D. (1976) Eur. J. Biochem. 63, 357—364
14 Koizumi, K., Ito, Y., Kojima, K. and Fujii, T. (1976) J. Biochemistry 79, 739—748
15 Wright, G.H. (1977) Biochim. Biophys. Acta 470, 368—381
16 Leoni, S., Luly, P., Mangiantini, M.T., Spagnuolo, S., Trentalance, A. and Verna, R. (1975) Biochim. Biophys. Acta 394, 317—322
17 Farias, R.N., Bloj, B., Morero, R.D., Sineriz, F. and Trucco, R.E. (1975) Biochim. Biophys. Acta 415, 231—251
18 Massa, E.N., Morero, R.D., Bloj, B. and Farias, R.N. (1975) Biochem. Biophys. Res. Commun. 66, 115—122
19 Kimelberg, H.K. (1977) in Dynamic Aspects of Cell Surface Organization (Poste, G. and Nicolson, G.L., eds.), pp. 205—291, Elsevier/North-Holland Biomedical Press, Amsterdam
20 Lenaz, G. (1978) in Molecular Biology of Membranes (Flejscher, S., ed.), pp. 137—162, Plenum Press, New York
21 Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. 12, 186—202
22 Ray, T.K., Skipski, V.P., Barclay, M., Essner, E. and Archibald, F.M. (1969) J. Biol. Chem. 244, 5528—5536
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
24 Luly, P., Barnabei, O. and Tria, E. (1972) Biochim. Biophys. Acta 282, 447—452
25 Van Hoeven, R.P. and Emmelot, P. (1972) J. Membrane Biol. 9, 105—126
26 Skipski, V.P. and Barclay, M. (1969) Methods Enzymol. 14, 530—598
27 Morrison, W.R. (1964) Anal. Biochem. 7, 218—224
28 Stancliff, R.C., Williams, M.A., Utsumi, K. and Packer, L. (1969) Arch. Biochem. Biophys. 131, 629—642
29 Seelig, J. (1976) in Spin Labeling (Berliner, L.J., ed.), pp. 373—409, Academic Press, New York
30 Eletr, S. and Inesi, G. (1972) Biochim. Biophys. Acta 290, 178—185
31 Bonting, S.L. (1970) in Membranes and Ion Transport (Bittar, E.E., ed.), Vol. 1, pp. 257—363, Wiley Interscience, London
32 Aronson, N.N. and Touster, O. (1974) Methods Enzymol. 31, 90—102
33 Kimelberg, H.K. and Papahadjopoulos, D. (1974) J. Biol. Chem. 249, 1071—1080
34 Riordan, J.R. and Slavik, M. (1974) Biochim. Biophys. Acta 373, 356—360
35 Dornand, J., Reminiac, C. and Mani, J.C. (1977) Biochimie 59, 425—432
36 Dornand, J., Bonnafous, J.C. and Mani, J.C. (1978) Biochem. Biophys. Res. Commun. 82, 685—692
37 Chapman, D. (1973) in Form and Function of Phospholipids, 2nd edn. (Ansell, G.B., Dawson, R.M.C. and Hawthorne, J., eds.), pp. 117—142, Elsevier, Amsterdam
38 Rottem, S. and Samuni, H. (1973) Biochim. Biophys. Acta 298, 32—38
39 Morley, C.G.D., Kuku, S., Robenstein, A.H. and Boyer, J.L. (1975) Biochem. Biophys. Res. Commun. 67, 653—661
40 Mangiantini, M.T., Leoni, S., Spagnuolo, S. and Trentalance, A. (1972) Soc. It. Biol. Sper. 48, 642—645
41 Haller, I. and Freiser, M.J. (1976) Biochim. Biophys. Acta 455, 739—748
42 Van Hoeven, R.P., van Blitterswijk, W.J. and Emmelot, P. (1979) Biochim. Biophys. Acta 551, 44—54
43 Klein, I., Moore, L. and Pastan, I. (1978) Biochim. Biophys. Acta 506, 42—53
44 De Laat, S.W., van der Saag, P.T. and Shinitzky, M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4458—4461