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Modulation of Na^+-H^+ exchange by ethinyl estradiol in rat colonic brush-border membrane vesicles

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Prior studies by our laboratory have suggested that a relationship may exist between rat colonic brush-border membrane vesicular fluidity and Na^+-H^+ exchange. To further explore this possible relationship, in the present studies the effects of ethinyl estradiol (17α-ethinyl-1,3,5-estratriene-3,17-β-diol) administration subcutaneously (5 mg/kg body wt. per day) for 5 days, on rat colonic brush-border membrane fluidity and Na^+-H^+ exchange were examined. This treatment regimen has previously been shown to decrease the lipid fluidity of rat hepatic and rabbit small intestinal plasma membranes. In agreement with these prior studies, the present results demonstrate that this agent decreases the lipid fluidity of treated-rat colonic brush-border membranes compared to control membranes, as assessed by steady-state fluorescence polarization techniques using three different fluorophores. An increase in the cholesterol content and cholesterol/phospholipid molar ratio of treated-membranes appear to, at least partially, be responsible for the fluidity differences. Furthermore, examination of the kinetic parameters for amiloride-sensitive sodium-stimulated proton efflux in treated and control membrane vesicles, utilizing the pH-sensitive fluorescent dye, Acridine orange, revealed that ethinyl estradiol administration decreased the V_{max} for this exchange mechanism, expressed in arbitrary fluorescence units, by approx. 25% but did not influence its K_m for sodium. These data, therefore, lend further support to the contention that alterations in fluidity may modulate Na^+-H^+ exchange in rat colonic brush-border membrane vesicles.

Introduction

Prior studies have documented the existence of a Na^+-H^+ exchange process in the plasma membranes of several different cell types [1–4]. Moreover, these studies have suggested that this carrier-mediated transport system may be involved in a number of important physiological processes [3–6]. In the past few years the mechanism(s) involved in the regulation of Na^+-H^+ exchange has received considerable attention. In this regard, several studies have demonstrated increases in this plasma membrane exchanger activity after various in vivo perturbations [7–10].

Our laboratory [11–13] and others [14] have also shown that an amiloride-sensitive Na^+-H^+ exchange process is present in rat colonic brush-border membrane vesicles utilizing both ^{22}Na uptake [12,14] and pH-sensitive fluorescent dye tech-

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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niques [11–13]. This colonic transport system possesses a number of properties similar to Na^+-H^+ exchangers previously described in other plasma membranes [11–14]. Furthermore, recent experiments in our laboratory [12,13] have suggested that a relationship exists between rat colonic brush-border membrane lipid fluidity * and Na^+-H^+ exchange, i.e., increases in fluidity were correlated with increases in Na^+-H^+ exchange activity.

To further explore this possible relationship, in the present studies the effects of ethinyl estradiol (17 α-ethinyl-1,3,5-estratriene-3,17-β-diol) on rat colonic plasma membrane fluidity and Na^+-H^+ exchange were examined. Administration of this synthetic estrogen has previously been shown to decrease the lipid fluidity of rat hepatic [15–17] and rabbit small intestinal [18] plasma membranes. To date, similar experiments have not been performed in rat colonic brush-border membranes. The results of these studies indicate that this agent significantly decreases both the lipid fluidity and Na^+-H^+ exchange activity of rat colonic brush-border membrane vesicles.

Methods and Materials

Preparation of colonic brush-border membrane vesicles. Male albino rats of the Sherman strain weighing 250–300 g were injected subcutaneously with diluent or ethinyl estradiol dissolved in propylene glycol at a dose of 5 mg/kg body wt. per day for 5 days. The animals were then fasted overnight, with water ad libitum before being killed. The colons were excised, the cecum discarded and epithelial cells, relatively devoid of goblet cells, were obtained using a technique which combined chelation of divalent cations with mild mechanical dissociation as described [19].

Brush-border membrane vesicles from these cells were then prepared as described by Brasitus and Keresztes [19]. The purity of the membrane preparations and the degree of contamination by intracellular membranes were evaluated by marker enzymes. The specific activity ratios ((purified brush-border membranes)/(crude homogenate)) for the brush-border enzyme markers, total alkaline phosphatase (*p*-nitrophenylphosphatase) and cysteine-sensitive alkaline phosphatase were approximately 15–18 in all membrane preparations [19]. Furthermore, these preparations contained approx. 15–20% of the total activity of each of these enzymes. The corresponding specific activity ratios for succinic dehydrogenase, NADPH cytochrome *c* reductase and sodium potassium-dependent adenosine triphosphatase ((Na^+ + K^+)-ATPase) marker enzymes for mitochondrial, microsomal and basolateral membranes, respectively, ranged from 0.50 to 1.40 in all membrane preparations. Brush-border membrane vesicles were suspended in the appropriate buffer (see below) and used immediately.

Fluorescence polarization studies. Three fluorophores were used for these studies: 1,6-diphenyl-1,3,5-hexatriene, DL-2-(9-anthroyl)stearic acid and DL-12-(9-anthroyl)stearic acid. These compounds were obtained from Aldrich Chemical Co. or Molecular Probes Inc. Steady-state fluorescence polarization experiments were performed with a Perkin-Elmer 650-40 spectrofluorometer adapted for fluorescence polarization [20]. The techniques used to load the membranes and liposomes as well as the quantification of polarization of fluorescence have been described [20–22]. The content of each fluorophore in the preparations was estimated fluorometrically as described [23]. Final molar ratios of probe/lipid ranged from 0.002 to 0.003, and the anisotropy variations seen in the present experiments could not be ascribed to differences in probe concentrations in the preparations. Correction for light scattering was also routinely performed as described [20–22].

Fluorescence polarization was expressed as the fluorescence anisotropy, r. The results were assessed according to the modified Perrin relationship [24,25]: $r = r_\infty + (r_0 - r_\infty)\ [T_c/(T_c + T_f)]$,

* In this paper the term 'lipid fluidity' is used to denote the relative motional freedom of the lipid molecules or substituents thereof in anisotropic bilayer membranes. A more detailed description has been previously published [27]. Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, 'fluidity' is assessed by the parameters of the modified Perrin equation described under Methods and Materials. An increase in fluidity corresponds to a decrease in either the correlation time, T_c, or the hindered anisotropy, r_∞, of the fluorophore, thereby combining the concepts of the dynamic and static (lipid order) components of fluidity.

where r is the fluorescence anisotropy, r_0 is the maximal limiting anisotropy, taken as 0.365 for diphenylhexatriene [26] and 0.285 for DL-2- and DL-12-(9-anthroyl)stearic acid [27], r_∞ is the limiting hindered anisotropy, T_c is the correlation time and T_f is the mean lifetime of the excited state. T_f was estimated by the total fluorescence intensity as previously described [20]. No changes in the excited-state lifetimes were noted using each probe in each preparation. Values for r_∞ for diphenylhexatriene were calculated from r values as previously decsribed [28].

Compositional studies. Lipids were extracted from the plasma membranes by the method of Folch et al. [29]. Cholesterol [30] and phospholipids [31] were measured as previously described. The neutral lipid and phospholipid compositions of the extracts were analyzed further by thin-layer chromatography according to the method of Katz et al. [32]. Fatty acids of the total lipid extracts were derivatized and the fatty acid methyl esters analyzed on a Hewlett-Packard 5790A gas-liquid chromatograph equipped with a flame ionization detector as previously described by Gartner and Vahouny [33]. Protein was estimated by the method of Lowry et al. [34].

Na^+-H^+ exchange assay. Sodium-stimulated proton efflux was assessed in colonic brush-border membrane vesicles, using the pH-sensitive fluorescent dye, Acridine orange, as previously described by our laboratory [11–13] and others [1,35]. The fluorescence of Acridine orange was measured at 26 °C using a Perkin-Elmer 650-40 spectrofluorometer (Perkin-Elmer Corp., Norwalk, CT) (excitation 493 nm, emission 530 nm) equipped with a thermostated cuvette stirring system and adding port. The assay solution (2 ml) contained 6 μM Acridine orange, 100 mM *N*-methylglucamine gluconate/250 mM sucrose/10 mM Tris-Hepes (pH 7.5). After this solution reached steady-state fluorescence (approx. 90 s), 50 μl of membrane vesicles (100–150 μg protein) preloaded with 100 mM *N*-methylglucamine gluconate/250 mM sucrose/10 mM Tris-Hepes (pH 6.0) were added. As previously described [13,36], there was a 30–40% quenching in the Acridine orange fluorescence signal which reached equilibrium within 2 min. Sufficient quantities of sodium gluconate were then added with constant stirring to achieve a final concentration of 2.5–50 mM in the external buffer. The addition of sodium resulted in a collapse of the outwardly directed proton gradient and a reappearance of Acridine orange fluorescence [37]. The increase in fluorescence was linear for greater than 2 s, and the initial rate of fluorescence recovery was measured as the initial slope [11–13]. After 300 s the pH gradient was dissipated with 150 mM potassium gluconate and 10 μg nigericin as described [10]. The small fluorescence quenching still remaining after nigericin addition was due to binding of the dye to the membranes [38]. Appropriate corrections were made for this binding as described [39]. Care was exercised to maintain a constant temperature and pH, factors which are known to influence the reproducibility of these assay results [37].

Statistical methods. All results are expressed as mean ± S.E. Paired or unpaired Student's *t*-tests were used for all statistical analysis. A P value of < 0.05 was considered significant.

Materials. Unless otherwise indicated, all materials were obtained from Fisher Chemical Co. (Fairlawn, NJ) or Sigma Chemical Co. (St. Louis, MO).

Results

Fluorescence polarization studies

As shown in Table I, the lipid fluidity of colonic brush-border membranes of rats treated with ethinyl estradiol for 5 days was significantly lower than control membranes, as assessed by steady-state fluorescence polarization techniques using all three fluorophores. As discussed previously [20], these probes differ in a number of respects. In this regard, it is important to note that the structural organization of the lipid bilayer of membranes appears to limit the extent of rotation of diphenylhexatriene. Therefore, r_∞ values for this probe are high and largely determine r [28]. In contrast, other probes such as DL-2- and DL-12-(9-anthroyl)stearic acid yield relatively low values of r_∞ in bilayer membranes and their r values reflect mainly T_c, i.e., the speed of rotation [27,40].

In the present experiments, both the 'static' and 'dynamic' components of membrane fluidity, as assessed by r_∞ values of diphenylhexatriene and r values of DL-2- and DL-12-(9-anthroyl)

TABLE I

FLUORESCENCE POLARIZATION STUDIES OF RAT COLONIC BRUSH-BORDER MEMBRANES AND THEIR LIPOSOMES AFTER ADMINISTRATION OF ETHINYL ESTRADIOL OR DILUENT

Values represent means ± S.E. of eight different preparations of each membrane and three different preparations of each liposome sample measured at 25 °C. DPH, diphenylhexatriene; 2-AS, DL-2-(9-anthroyl)stearic acid; 12-AS, DL-12-(9-anthroyl)stearic acid.

Probe	Preparations	Control		Treated	
		r	r_∞	r	r_∞
DPH	Intact membranes	0.221 ± 0.004	0.194 ± 0.006	0.239 ± 0.002 *	0.219 ± 0.004 *
	Liposomes	0.208 ± 0.004	0.177 ± 0.005	0.221 ± 0.004 *	0.195 ± 0.005 *
2-AS	Intact membranes	0.149 ± 0.003	–	0.161 ± 0.003 *	–
12-AS	Intact membranes	0.096 ± 0.005	–	0.107 ± 0.003 *	–

* $P < 0.05$ or less compared to control values.

stearic acid respectively, were found to be decreased in membranes prepared from treated animals. Differences were also detected in liposomes of treated membranes when compared to liposomes prepared from control membranes using diphenylhexatriene (Table I).

Lipid compositional studies

Earlier studies in model bilayers and natural membranes have correlated a low lipid fluidity with high molar ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine [41,42]. More saturated or longer fatty acyl chains in phospholipids [43] have also been associated with a lower membrane lipid fluidity [43]. It was, therefore, of interest to examine and compare these compositional parameters in control and estrogen-treated membranes (Table II).

In agreement with the results of earlier studies performed in rat hepatic plasma membranes [15–16], membranes prepared from estrogen-treated animals possessed a greater cholesterol content as well as a higher cholesterol/phospholipid ratio (mol/mol) than control membranes (Table II). The esterified cholesterol and phospholipid contents (Table II), however, as well as the relative percentages of the individual phospholipids (not shown), were similar in treated and control membranes. Additionally, the saturation indices of these membranes (Table II), their individual fatty acids (not shown) and their sphingomyelin/phosphatidylcholine molar ratios (Table II) were not found to be significantly different.

Effects of ethinyl estradiol treatment on Na^+-H^+ exchange

Kinetics of the Na^+-H^+ exchange process were evaluated in control and treated colonic brush-border membrane vesicle preparations by determining the effect of increasing sodium concentrations (2.5 to 50 mM) on sodium-stimulated proton efflux with an Acridine orange fluorescence technique. The exchange process in both

TABLE II

EFFECTS OF ETHINYL ESTRADIOL ADMINISTRATION ON THE COMPOSITIONAL PARAMETERS OF RAT COLONIC BRUSH-BORDER MEMBRANES

Values represent means ± S.E. of six different preparations of each membrane.

Parameters	Control membranes	Treated membranes
Total cholesterol (μg/mg protein)	134.8 ± 6.4	202.3 ± 11.8 *
Esterified cholesterol (μg/mg protein)	4.3 ± 0.6	5.1 ± 1.2
Phospholipid (μg/mg protein)	488.0 ± 22.2	518.3 ± 15.1
Cholesterol/phospholipid (mol/mol)	0.53 ± 0.05	0.73 ± 0.06 *
Sphingomyelin/PC (mol/mol)	0.48 ± 0.03	0.45 ± 0.02
Saturation index [a]	0.34 ± 0.03	0.34 ± 0.02

[a] Calculated by dividing the total saturated acyl chains by the sum of each unsaturated chain multiplied by the number of double bonds.

* $P < 0.05$ or less compared to control values.

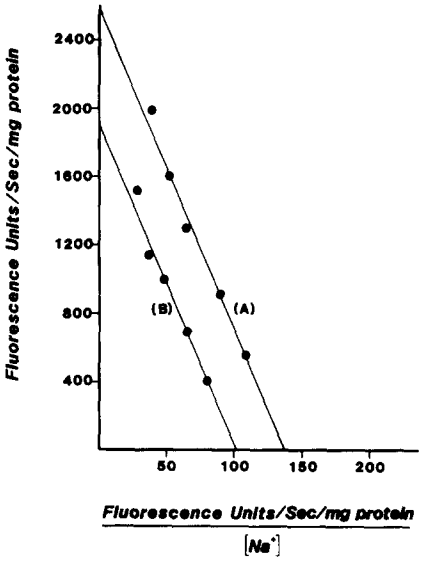

Fig. 1. Sodium-stimulated proton efflux was measured in rat colonic brush-border membrane vesicles, using the pH-sensitive fluorescent dye, Acridine orange, as described under Methods and Materials. Representative Hofstee plots of four separate experiments, using membrane vesicles prepared from the colon of control (A) and ethinyl estradiol-treated (B) animals, are shown (see Table III for further details).

control and treated preparations demonstrated saturation kinetics, and at a 50 mM concentration of sodium was inhibited by approximately 85% by amiloride (1 mM) (not shown). As assessed by alterations in the initial slope of fluorescence, estrogen treatment significantly decreased sodium-stimulated proton efflux at all concentrations tested (2.5–50 mM) (not shown). The values for V_{max}, expressed in arbitrary fluorescence units, and the K_m for sodium were then obtained from Hofstee plots [44] (Fig. 1), and these data are summarized in Table III. Estrogen treatment was found to decrease the V_{max} of this exchange process by approx. 25%. This treatment, however, was not found to significantly alter the K_m for sodium of this exchange process (Table III).

TABLE III

EFFECTS OF ETHINYL ESTRADIOL ADMINISTRATION ON THE KINETIC PARAMETERS OF SODIUM-STIMULATED PROTON EFFLUX IN RAT COLONIC BRUSH-BORDER MEMBRANE VESICLES

Values represent means ± S.E. of six separate preparations of control and eight separate preparations of estrogen-treated membrane vesicles.

Preparation	V_{max} (arbitrary fluorescence units)	K_m of sodium (mM)
Control membranes	2600 ± 66	18.8 ± 1.7
Treated membranes	1920 ± 97 *	18.7 ± 1.5

* $P < 0.05$ compared to control values.

Discussion

Ethinyl estradiol administration has previously been shown to decrease the lipid fluidity as well as increase the cholesterol/phospholipid molar ratio of rat hepatocyte plasma membranes by increasing the content of free [15,16] and/or esterified cholesterol [15,17]. Furthermore, these membrane changes in lipid composition and fluidity have been correlated with a decrease in $(Na^+ + K^+)$-ATPase activity [15–17] and may account, at least partially, for the inhibition of bile salt-dependent and -independent bile flow seen with this agent [15–17]. Ethinyl estradiol-induced cholestasis has, moreover, been shown to be reversible in the rat by treatment regimens that increase the fluidity of hepatic plasma membranes such as administration of Triton WR-1399 [17], *S*-adenosyl-L-methionine [45] as well as by a starve-refeed diet [46].

Recently, Schwarz et al. [18] have demonstrated that ethinyl estradiol treatment also decreased the lipid fluidity of rabbit ileal brush-border membranes by increasing their cholesterol/phospholipid molar ratio. In contrast to the results obtained in rat hepatic plasma membranes [15–17], however, these investigators found that the increased molar ratio of cholesterol/phospholipid in treated membrane was secondary to a decrease in their phospholipid content without a significant alteration in their free or esterified cholesterol content compared to control plasma membranes [18].

The present results demonstrate for the first time that both the 'static' component of membrane fluidity, i.e., the degree of order of the lipids, as well as the 'dynamic' component of fluidity were decreased in colonic brush-border membranes prepared from ethinyl estradiol-treated

rats. The relatively low fluidity of these membranes appeared, at least partially, to be explained by a higher cholesterol/phospholipid molar ratio in the treated animals' membranes compared to their control counterparts. In agreement with the prior studies performed in rat hepatocytes [15,16] and in contrast to those performed in rabbit enterocytes [18], this increased ratio was due to a higher cholesterol content with no significant change in phospholipid content in treated membranes. Unlike certain earlier studies in rat hepatocyte membranes [15,17], however, the content of esterified cholesterol in both treated and control membranes was low (approx. 3% of the total cholesterol) and was not found to be significantly different in these colonic membranes.

In the present experiments ethinyl estradiol administration was also found to decrease the V_{max} of Na^+-H^+ exchange in rat colonic brush-border membrane vesicles by approximately 25% compared to control membrane vesicles without affecting the K_m of sodium for this process. The exact mechanism(s) involved in this phenomenon remains unclear at the present time. Previous studies by our laboratory have suggested that a relationship exists between rat colonic brush-border membrane vesicular fluidity and Na^+-H^+ exchange [12,13]. Increases in fluidity secondary to benzyl alcohol [12] and transmethylation reactions [13] were correlated with increases in the V_{max} without affecting the K_m for sodium of this process. The present results, taken together with these previous observations [12,13], suggest that estrogen administration decreases membrane fluidity which, in turn, may lead to a decrease in Na^+-H^+ exchange. It should be emphasized, however, that the present experiments do not preclude other possibilities. Ethinyl estradiol could have a direct effect on the Na^+-H^+ exchanger, or may indirectly influence the rate of the exchange process by an alteration of sodium/proton permeability. Alternatively, estradiol might influence Na^+-H^+ exchange in rat colonic brush-border membrane vesicles by altering the sensitivity of a possible nontransporting H^+ site. Such a modifier H^+ site has previously been shown to be present in the Na^+-H^+ exchanger of renal brush-border membrane vesicles [47], although its existence in the Na^+-H^+ exchanger of colonic apical membranes has not been established. Further studies will, therefore, be necessary to elucidate the exact mechanism(s) whereby ethinyl estradiol modulates this exchange process. Regardless of the mechanism(s) involved, however, it does appear that ethinyl estradiol can influence Na^+-H^+ exchange in rat colonic brush-border membranes.

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References

1 Knickelbein, R., Aronson, P.S., Atherton, W. and Dobbins, J.W. (1983) Am. J. Physiol. 245, G504–G510
2 Kinsella, J.L. and Aronson, P.S. (1981) Am. J. Physiol. 241, F374–F379
3 Arias, I.M. and Forgac, M. (1984) J. Biol. Chem. 259, 5406–5408
4 Moolenaar, W.H., Boonstra, J., Vander Saag, P.T. and De Laat, S.W. (1981) J. Biol. Chem. 256, 12883–12887
5 Aickin, C.C. and Thomas, R.C. (1977) J. Physiol. (Lond.) 273, 295–316
6 Johnson, J.D., Epel, D. and Paul, M. (1976) Nature (Lond.) 262, 661–664
7 Harris, R.C., Brenner, B.M. and Seifter, J.L. (1986) J. Clin. Invest. 77, 724–733
8 Cohn, D.E., Klahr, S. and Hammerman, M.R. (1983) Am. J. Physiol. 245, F217–222
9 Cohn, D.E., Hruska, K.A., Klahr, S. and Hammerman, M.R. (1982) Am. J. Physiol. 245, F293–F299
10 Harris, R.C., Seifter, J.L. and Brenner, B.M. (1984) J. Clin. Invest. 74, 1979–1987
11 Foster, E.S., Dudeja, P.K. and Brasitus, T.A. (1986) Am. J. Physiol. 250, G781–G787
12 Brasitus, T.A., Dudeja, P.K., Worman, H.J. and Foster, E.S. (1986) Biochim. Biophys. Acta 855, 16–24
13 Dudeja, P.K., Foster, E.S. and Brasitus, T.A. (1986) Biochim. Biophys. Acta 859, 61–68
14 Binder, H.J., Stange, G., Murer, H., Stieger, B. and Hauri, H.-P. (1986) Am. J. Physiol. 251, G382–G392
15 Davis, R.A., Kern, F. Jr., Showalter, R., Sutherland, E., Sinensky, M. and Simon, F. (1978) Proc. Natl. Acad. Sci. USA 75, 4130–4134

16 Keeffe, E.B., Scharschmidt, B.F., Blankenship, N.M. and Ockner, R.K. (1979) J. Clin. Invest. 64, 1590–1598
17 Simon, F.R., Gonzalez, M., Sutherland, E., Accatino, L. and Davis, R.A. (1980) J. Clin. Invest. 65, 851–860
18 Schwarz, S.M., Watkins, J.B., Ling, S.C., Fayer, J.C. and Mone, M. (1986) Biochim. Biophys. Acta 860, 411–419
19 Brasitus, T.A. and Keresztes, R.S. (1984) Biochim. Biophys. Acta 773, 290–300
20 Brasitus, T.A. and Dudeja, P.K. (1985) J. Biol. Chem. 260, 12405–12409
21 Brasitus, T.A., Schachter, D. and Mamouneas, T.G. (1979) Biochemistry 18, 4136–4144
22 Schachter, D. and Shinitzky, M. (1977) J. Clin. Invest. 59, 536–548
23 Cogan, U. and Schachter, D. (1981) Biochemistry 20, 6396–6406
24 Heyn, M.P. (1979) FEBS Lett. 108, 359–364
25 Jahnig, F. (1970) Proc. Natl. Acad. Sci. USA 76, 6361–6365
26 Shinitzky, M. and Berenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657
27 Schachter, D., Cogan, U. and Abbott, R.E. (1982) Biochemistry 21, 2146–2150
28 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332
29 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
30 Zlatkis, A., Jak, B. and Boyle, A.J. (1953) J. Lab. Clin. Med. 41, 486–492
31 Ames, B.N. and Dubin, D.T. (1960) J. Biol. Chem. 235, 769–775
32 Katz, S.S., Shipley, G.G. and Small, D.M. (1976) J. Clin. Invest. 58, 200–211
33 Gartner, S.L. and Vahouny, G.V. (1972) Am. J. Physiol. 222, 769–775
34 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
35 Cassano, C.B., Stieger, B. and Murer, H. (1984) Pfluegers Arch. 400, 309–317
36 Reenstra, W.W., Warnock, D.G., Yee, V.J. and Forte, J.G. (1981) J. Biol. Chem. 256, 11664–11666
37 Ives, H.E., Yee, V.J. and Warnock, D.G. (1983) J. Biol. Chem. 258, 9710–9716
38 Warnock, D.G., Reenstra, W.W. and Yee, V.J. (1982) Am. J. Physiol. 242, F729–733
39 Burnham, C., Munzeheimer, C., Rabon, E. and Sachs, G. (1982) Biochim. Biophys. Acta 685, 260–272
40 Vincent, M., DeForesta, B., Gallay, J. and Alfsen, A. (1982) Biochem. Biophys. Res. Commun. 107, 914–921
41 Shinitzky, M. and Inbar, Y. (1978) Biochim. Biophys. Acta 433, 133–149
42 Chapman, D. and Penkett, S.A. (1966) Nature 211, 1304–1305
43 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394
44 Hofstee, B.M.J. (1959) Nature (Lond.) 184, 1296
45 Boelsterli, U.A., Rakhit, G. and Balasz, T. (1983) Hepatology 3, 12–17
46 Storch, J. and Schachter, D. (1984) Biochim. Biophys. Acta 798, 137–140
47 Aronson, P.S., Nee, J. and Suhm, M.A. (1982) Nature (Lond.) 299, 161–163