Biochimica et Biophysica Acta, 600 (1980) 860—869

BBA 78877

DUAL EFFECT OF MEMBRANE CHOLESTEROL ON SIMPLE AND MEDIATED TRANSPORT PROCESSES IN HUMAN ERYTHROCYTES

M. GRUNZE *, B. FORST and B. DEUTICKE

Abteilung Physiologie, Medizinische Fakultät der Rheinisch-Westfälischen Technischen Hochschule, D-5100 Aachen (F.R.G.)

(Received December 5th, 1979)

Key words: Cholesterol; Permeability; Anion transport; Monosaccharide; Monocarboxylate (Erythrocyte membrane)

Summary

The influence of cholesterol on simple and facilitated transport processes across the membrane of intact human erythrocytes was studied after graded depletion or enrichment of membrane cholesterol by incubation of the cells in phospholipid or phospholipid/cholesterol suspensions.

1. The carrier-mediated transfer of L-lactate and of L-arabinose proved to be enhanced by cholesterol. In the case of L-lactate, a decrease in K_m seems to be involved in this effect. In contrast, the self-exchange of SO_4^{2-}, mediated by the inorganic anion-exchange system, and the simple diffusion of erythritol via the lipid phase of the membrane are inhibited by cholesterol.

2. Reversibility of these two opposite effects of cholesterol was demonstrated by measurements on cells depleted again after cholesterol enrichment and enriched again after previous depletion.

3. Certain phospholipids used for preparing the lipid dispersions that are required for cholesterol variation have effects on permeability of their own, due, for example, to traces of contaminants. A discrimination of such artifacts from the effects of cholesterol is only possible by demonstrating reversibility.

4. The opposite effects of cholesterol on various facilitated transfer processes, which have a correlation in the opposite effects of other modifications of the membrane lipid phase (Deuticke, B., Grunze, M. and Haest, C.W.M. (1979) Alfred Benzon Symposium 14, Munksgaard, Copenhagen, in the press), are indicative of different types of lipid-protein interaction in the erythrocyte membrane.

* Present address: Medizinische Klinik, Universität Heidelberg, D-6900 Heidelberg, F.R.G.

Introduction

The functional significance of cholesterol, a major constituent of the lipid domain of biomembranes, has aroused the interest of biologists for a long time. Numerous studies applying physical techniques have established that cholesterol modulates the structure and dynamic properties of the lipid part of membranes [1].

The relevance of cholesterol in terms of permeability became evident from studies on model systems and biological membranes, e.g., of micro-organisms and erythrocytes. In these systems cholesterol reduces the simple non-electrolyte permeability of the lipid domain at physiological temperatures [2—4]. This effect has been attributed either to a decrease in membrane fluidity or to a 'tightening' of the 'hydrogen belt' which has been claimed to form the rate-limiting barrier for solutes penetrating the membrane via the lipid part of the membrane [5]. These cholesterol-induced changes of non-electrolyte transfer are reversible, as could be demonstrated in studies using erythrocytes [6].

The permeation of lipophilic ions through model membranes is also affected by the presence of cholesterol: anion conductance is enhanced and cation conductance inhibited [7,8]. These opposite effects of cholesterol have been ascribed to cholesterol-induced changes of the dipole potential at the membrane interface.

In contrast to the fairly well characterized effects of cholesterol on 'translipid' diffusion of non-electrolytes, the influence of cholesterol on protein-mediated facilitated transport processes has only been studied to a limited extent. Certain facilitated diffusion processes across the erythrocyte membrane were reported to be enhanced by the removal of cholesterol [9], while others proved to be inhibited [9]. The ouabain-sensitive active transport of K^+ and Na^+ was shown to be enhanced by cholesterol depletion [10,11] while cholesterol enrichment had no effect on this process. On the other hand, the ouabain-insensitive, furosemide-sensitive cotransport of Na^+ and K^+ is inhibited by cholesterol enrichment [12].

In order to gain further insight into the influence of cholesterol on protein-mediated transport processes we have now studied the self-exchange of SO_4^{2-}, L-lactate and L-arabinose in erythrocytes after experimental variation of membrane cholesterol.

The two anions penetrate via two different transport systems [3,13,14] which might sense changes of membrane fluidity or of interfacial dipole potential induced by changes of cholesterol content. L-Arabinose transfer occurs via the common monosaccharide carrier [15] which has been claimed, on the basis of net flux measurements, to be inhibited after extensive cholesterol depletion [8,16] but to be enhanced by lower extents of depletion [16].

In our investigation the reversibility of the cholesterol-induced changes of transport processes served as a criterion for the causal involvement of cholesterol in the effects observed.

Materials and Methods

Materials

Human blood was obtained from the local blood bank and anticoagulated by heparin. Lipids used for cholesterol depletion and cholesterol enrichment were: cholesterol (Sigma, Artic. No. CH-S, or Merck, Artic. No. 24622), dipalmitoyl phosphatidylcholine (DPPC) (Sigma, Artic. No. P 6138), purified egg lecithin (Sigma, Artic. No. P 5763) and commercial grade egg lecithin (Sigma, Artic. No. P 8640). All other chemicals used were of analytical grade.

Incubation procedures

Erythrocytes were washed three times in 154 mM NaCl prior to incubation. Variation of membrane cholesterol was achieved, following a procedure described before [4] by incubating red cells at 37° C (hematocrit 10%) in suspensions of sonicated lipids in a saline medium of the following composition: KCl 100 mM, NaCl 50 mM, sucrose 40 mM, NaH_2PO_4/Na_2HPO_4 12.5 mM, glucose 5 mM (medium A). The standard phospholipid concentration was 2 mg/ml, cholesterol concentration was varied from 0 to 3 mg/ml depending on the extent of cholesterol depletion or enrichment intended. Incubation times varied from 18 to 60 h. To prevent bacterial growth, penicillin (8 mg/100 ml) and streptomycin (30 mg/100 ml) were added. Subsequently, various transport processes were studied under equilibrium conditions by measuring tracer back-exchange from preloaded cells as described previously [12]. The media used had the following composition: KCl 100 mM, NaCl 50 mM, Na_2HPO_4/NaH_2PO_4 6 mM, glucose 5 mM, and test substance as given in the figure legends (Medium B). For experiments on L-arabinose transfer, glucose-free medium B was used. Cells pre-incubated in saline media for corresponding time periods served as controls.

Lipid determinations

Washed erythrocytes were hemolysed with 2 vols. of water, the hemoglobin content of the hemolysate determined and 2 ml of the hemolysate extracted with chloroform/isopropanol [17]. A precisely measured amount of *cis*-androstane (Sigma, Artic. No. A-9755), which served as internal standard for cholesterol determinations, was added to the extract. Lipid phosphorus was determined [18] in an aliquot of the extract after removal of non-lipid phosphorus from the extract [19].

Cholesterol was determined by gas-liquid chromatography using a Packard Becker 419 chromatograph equipped with a 1.5 m, 2 mm inner diameter glass column filled with 2% QF1 (WGA, Düsseldorf) on Gas Chrom Q (oven temperature 225° C; carrier gas, N_2; flame ionization detector). Calculations were carried out on the basis of an internal standard procedure, using an automatic integrator for peak area measurements (Minigrator, Spectra Physics).

The cholesterol-to-phospholipid ratio of the normal erythrocyte membrane determined by this procedure was 0.7 ± 0.07 ($n = 6$). A set of determinations was also carried out by using the Lieberman-Burchardt method [20]. Values obtained with this colorimetric assay were approx. 10% higher than those obtained by gas-liquid chromatography due to a nonspecific reaction between

the extract and the colour reagent. This finding and our cholesterol-to-phospholipid ratio are in agreement with recent observations of Johnson [21]. The cholesterol-to-phospholipid ratio of the erythrocyte membrane is thus apparently lower than claimed previously.

Results

Studies with dipalmitoyl phosphatidylcholine dispersions

In a first series of experiments the simple, non-mediated permeation of erythritol was studied as an indicator of permeability changes of the lipid part of the membrane after cholesterol variation. This process can be investigated by measuring erythritol exchange in the presence of cytochalasin B or other inhibitors of monosaccharide transfer, which completely block the mediated component of erythritol transfer. For pertinent evidence concerning this notion see Ref. 3. Non-mediated non-electrolyte permeability served as a reference for the subsequent study of the influence of cholesterol on protein-mediated transfer processes.

Simple erythritol permeability proved to be enhanced by cholesterol depletion and slightly reduced by cholesterol enrichment, in agreement with previous findings in porcine and bovine erythrocytes [4,6]. As is evident from Fig. 1A, cholesterol depletion to 0.5 (arbitrary units relative to control) results in an enhancement of transfer to approx. 1.4 (arbitrary units relative to control), whereas cholesterol enrichment to 1.9 only causes a diminution of the transfer rate to 0.75. These changes are not due to an alteration of the affinity of the monosaccharide carrier for cytochalasin B, since they were also observed at much higher concentrations of the inhibitor (data not shown).

The cholesterol-induced changes of non-electrolyte transfer via the lipid phase are reversible. If, for instance, cells were first enriched with cholesterol to 1.75 and then depleted again to 1.15 in a subsequent incubation, the rate

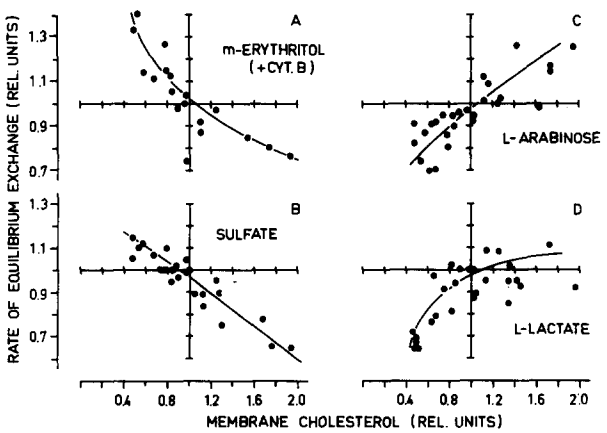

Fig. 1. Cholesterol dependence of membrane transport processes in human erythrocytes. Experimental variation of cholesterol and measurements of equilibrium exchange were carried out as described in Materials and Methods. Data are given in arbitrary units, relative to the controls. (A) *m*-Erythritol 5 mM, 35°C, pH 7.35 + cytochalasin B $1 \cdot 10^{-5}$ M; (B) sulfate 5 mM, 35°C, pH 7.35; (C) L-arabinose 5 mM, 10°C, pH 7.35; (D) L-lactate 5mM, 10°C, pH 7.35.

of transfer increased from 0.8 to 0.91. On the other hand, cells first depleted to 0.45 had an erythritol transfer rate of 1.4, which after subsequent enrichment of cholesterol to 1.54 shifted to a transfer rate of 0.85 (see Fig. 2A).

The major set of experiments dealt with facilitated transport processes. SO_4^{2-} was studied as a probe for the inorganic anion-exchange system which is supposed to be localized in the major intrinsic membrane-spanning protein, band 3, according to the nomenclature of Steck [22]. SO_4^{2-} self-exchange depends on membrane cholesterol level as shown in Fig. 1B. Cholesterol enrichment has an inhibitory effect, cholesterol depletion enhances SO_4^{2-} transfer. In the range between cholesterol depletion to 0.5 and cholesterol enrichment to 2.0, a linear relationship is evident between the rate of SO_4^{2-} exchange and the cholesterol content of the membrane. Fluxes are reduced to 0.65 at a cholesterol level of 2.0, while flux acceleration reaches a value of 1.15—1.2 at a cholesterol level of 0.5. Qualitatively, these results agree with those observed for the simple, non-mediated transfer of erythritol. Quantitatively, however, there is a difference between the two, since SO_4^{2-} exchange depends on membrane cholesterol in a linear fashion while erythritol permeability changes non-linearly with membrane cholesterol. Cholesterol-induced changes of SO_4^{2-} transfer are reversible like those of erythritol transfer (Fig. 2B).

Essentially similar results, namely enhancement by cholesterol depletion and inhibition by cholesterol enrichment, were obtained for the transfer of oxalate, a divalent organic anion penetrating the human erythrocyte membrane via the anion-exchange system [23]. Increasing the cholesterol content from 0.45 to 1.8 decreases the rate of equilibrium exchange by a factor of about 2 (mean value from four experiments). The transfer of oxalate was measured at 10°C to allow comparison with the solutes studied in the following experiments.

In contrast to the effects of cholesterol on SO_4^{2-} and oxalate exchange, the mediated transfer of L-arabinose is inhibited reversibly by cholesterol depletion

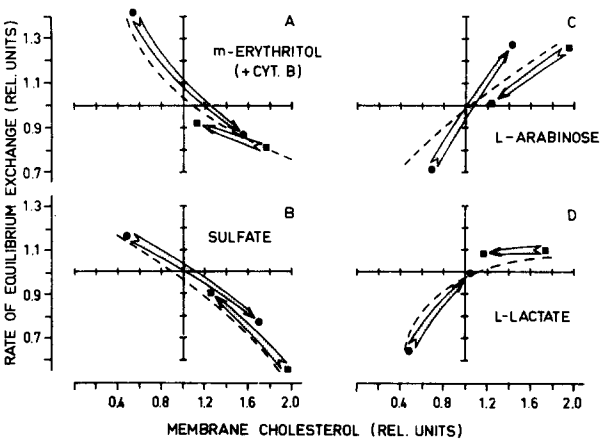

Fig. 2. Reversal of cholesterol-induced changes of permeability. Fluxes were measured on the same batch of cells after enrichment (or depletion) and subsequent depletion (or enrichment) of cholesterol. Further experimental details as in Fig. 1; data from one representative experiment out of a series of four. The dashed line corresponds to the line drawn in Fig. 1.

and enhanced by cholesterol enrichment (Figs. 1C and 2C). At a cholesterol level of 0.5, fluxes are decreased to 0.7—0.8, while cholesterol enrichment to 2.0 enhances the transfer to 1.2—1.3.

The monovalent organic anion, L-lactate, has been shown to penetrate the erythrocyte membrane predominantly via a specialized, SH-dependent monocarboxylate carrier which exhibits Michaelis-Menten kinetics. Only some 5% of the transfer involves the inorganic anion-exchange system, while approx. 2—3% penetrates by simple non-ionic diffusion via the lipid phase of the membrane under the prevailing experimental conditions [14]. According to the data shown in Fig. 1D, cholesterol depletion inhibits L-lactate transfer, while cholesterol enrichment has essentially no effect. Cholesterol depletion to 0.5 results in a reduction of lactate transfer to approx. 0.65. The results of a typical experiment carried out to demonstrate the reversibility of this phenomenon are shown in Fig. 2D.

In order to decide whether changes of affinity or changes of maximal transport rates (V) are responsible for the alteration of L-lactate transfer after cholesterol depletion, the concentration dependence of L-lactate equilibrium exchange was studied in a series of experiments on cells of different cholesterol content. The data obtained were evaluated according to the procedures of Eadie and Hofstee [24] and of Eisenthal and Cornish-Bowden [25]. In spite of a considerable scatter of the data, which is probably due to the prolonged incubation of the cells necessary for cholesterol depletion, it seems likely (Fig. 3) that the K_m value increases with decreasing cholesterol content and is therefore responsible for the observed inhibition of transfer. Changes of V observed were smaller and of varying extent. No consistent evidence for an involvement of this parameter could be obtained.

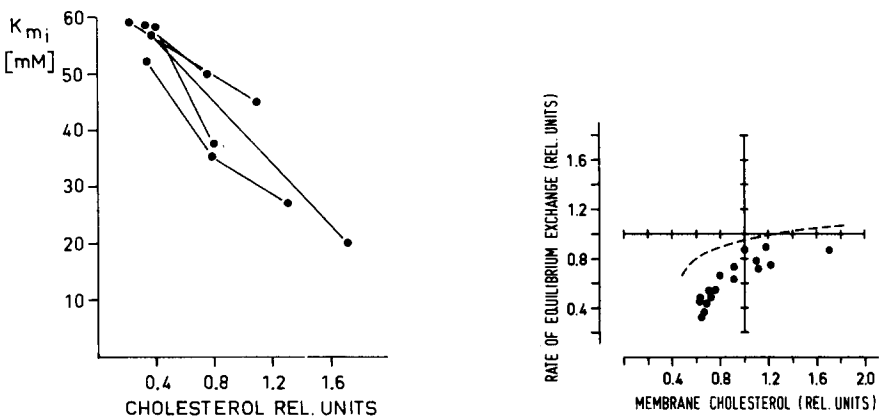

Fig. 3. Influence of variation of membrane cholesterol on the K_m value of L-lactate equilibrium exchange. Cholesterol variation and measurements of equilibrium exchange as described in Materials and Methods. Lactate concentrations: 5, 10, 20, 36, 70 and 140 mM; 10°C, pH 7.35. 150 μM 4,4'-dinitrostilbene-2,2'-disulfonate were added in order to inhibit lactate exchange via the inorganic anion-exchange system. Data were evaluated according to the method of Eisenthal and Cornish-Bowden [25] by computer analysis. Experiments on erythrocytes from the same donor are connected by the lines.

Fig. 4. Comparison between the cholesterol dependence of L-lactate transfer after cholesterol variation by treatment with dipalmitoyl phosphatidylcholine (dashed lines) and egg lecithin dispersions (data points). For details see text.

Studies with egg lecithin dispersions

In a number of experiments egg lecithin was used instead of DPPC for cholesterol depletion and enrichment. All other conditions were as in the experiments in which DPPC was used for preparing the lipid dispersions. In the case of arabinose transfer, the results obtained with egg lecithin dispersions were not different from those obtained with DPPC. In the case of SO_4^{2-} and lactate transfer, however, differences were observed. SO_4^{2-} equilibrium exchange lost its cholesterol dependence when cholesterol was varied by treatment with egg lecithin. L-Lactate exchange, on the other hand, seemed to be more sensitive to changes of cholesterol under these conditions (Fig. 4). Other than in the case of changes produced by DPPC suspensions, these alterations of lactate transfer were not reversible upon enrichment again with cholesterol and are therefore obviously not due to changes of cholesterol levels.

Discussion

The data reported in this paper clearly show that variation of membrane cholesterol reversibly alters simple and facilitated diffusion processes in human erythrocytes. The findings for simple diffusion via the lipid phase are in line with data for model systems [1,2], micro-organisms [2,3] and porcine and bovine erythrocytes [4,6]. Concerning protein-mediated, facilitated transport processes, effects of cholesterol have previously been reported only for certain non-electrolytes [9] and for cations [9—12]. Our study adds anion transfer to the list of cholesterol-sensitive transport phenomena and demonstrates selectivity in the influence of cholesterol, since even the two different anion-transfer systems of the erythrocyte membrane respond in opposite directions to the variation of cholesterol.

The reversibility of the transfer changes unequivocally relates the effects observed to an influence of cholesterol and excludes an involvement of membrane alterations caused by the procedure required for changing membrane cholesterol, but independent of cholesterol variation per se. Demonstration of reversibility is mandatory in experiments on the effects of cholesterol, since it has been demonstrated that incubation of erythrocytes in phospholipid dispersions (liposomes), the most common method to achieve cholesterol variation, may cause extraction of other membrane constituents, e.g., proteins, and thereby interfere with membrane permeability [26,27]. Furthermore, any constituent of the liposomes which is exchangeable between cells and liposomes may be delivered to the cells and have effects of its own. This phenomenon has been used to alter the sterol pattern of erythrocytes [28] or to insert long chain fatty acids and long chain alcohols into cells (Grunze, M. and Deuticke, B., unpublished results). In analogy, contaminants of phospholipid liposomes, such as traces of fatty acids or lysolecithin, known to inhibit L-lactate transfer [14], may contribute to the lack of reversibility of the transfer changes in egg lecithin-treated cells. This interpretation is supported by our observation that treatment of such cells with albumin, known to extract fatty acids and lysolecithin from the membrane [14,29], partially restored L-lactate transfer (data not shown).

Before discussing molecular events which might underlie the effects of

cholesterol, it should be emphasized that the transport changes induced by cholesterol in the erythrocyte membrane are small in relation to the pronounced changes of the composition of the lipid phase of the membrane. A 4-fold change of the cholesterol level from 45 to 190% of the normal value only alters the permeability of the lipid phase by a factor of 2, effects on protein-mediated transport processes are even smaller. Nevertheless, transport properties may be a very sensitive indicator for direct or indirect interactions between cholesterol and erythrocyte membrane proteins that were not detected by other experimental approaches [30—32].

A number of phenomena may underlie the influence of cholesterol on transport functions. In the case of 'trans-lipid' permeation, for which passage through the interface or through the core of the membrane is rate limiting, cholesterol could act, in principle, via a decrease in the fluidity of the hydrophobic core [33], or an increase in rigidity of the interfacial 'hydrogen belt' [5]. Membrane 'microviscosity', which is a measure of the fluidity of the core as well as of the rigidity of the hydrogen belt [34], increases by a factor of 2 when cholesterol is raised from 50 to 150% of its normal value [33]. This change correlates well with that of erythritol permeability. Moreover, from the inverse Stokes-Einstein relationship between viscosity and diffusion coefficient, a non-linear dependence of trans-lipid permeation on cholesterol content is predicted, in agreement with the data in Fig. 1A.

As a further alternative, a cholesterol-induced increase in the thickness of the phospholipid bilayer [35] could affect permeability.

Protein-mediated, facilitated transport systems are influenced by cholesterol in a dual way. The carrier systems for monocarboxylates (L-lactate) and monosaccharides (L-arabinose) are activated by cholesterol and thus respond in a direction opposite to that observed for the inorganic anion-exchange system (SO_4^{2-}, oxalate) which is inhibited by cholesterol. From these results and the data already available on effects of cholesterol on mediated transport systems in the erythrocyte membrane, the scheme given in Table I emerges. No correlation can be established between the direction of the cholesterol dependence of a transport process and any other of its properties, or the chemical nature of its substrate. The opposite action of cholesterol on different protein-mediated transport systems has a correlation in its effects on membrane enzymes, which can be inhibited [36—38] or activated [39,40] by the sterol.

In terms of enzyme kinetics, the influence of cholesterol on transport

TABLE I

INFLUENCE OF CHOLESTEROL ON PROTEIN-MEDIATED TRANSPORT PROCESSES IN THE ERYTHROCYTE MEMBRANE

Inhibited	Ref.	Activated	Ref.
Inorganic anion exchange	This work	Monosaccharide transfer	(9,16; and this work)
Cation leak fluxes	(10)	Monocarboxylate transfer	This work
Ouabain-insensitive, furosemide-sensitive (Na^+, K^+)-cotransport	(12)	Choline transport	(9)
		Thiourea transfer	(9)
Ouabain-sensitive Na^+ transport	(11)		
Leucine transfer	(9)		

systems may result from changes of affinity, of maximal transport rates, or both, which could even lead to a compensation. In the case of L-lactate, cholesterol depletion seems to lower the affinity of the carrier for its substrate (Fig. 3). For the transport systems studied by other authors (see Table I), changes of affinity as well as of maximal transport rates were reported without any relationship to the direction of cholesterol dependence.

The cholesterol sensitivity of protein-mediated transport systems can be caused by a number of mechanisms. Besides direct cholesterol-protein interactions [41—43], cholesterol-induced changes of the physical state of lipids which are sensed by the protein have to be considered. The following observations and hypotheses support the possibility of such an indirect action. (1) Changes of membrane 'microviscosity' apparently influence the conformation of membrane proteins [44,45] and may account for effects of cholesterol on transport ATPases [36—38]. (2) Kinetic constants of enzymes are influenced by the viscosity and the polarity of their environment [46,47]. (3) The dipole potential at a phospholipid interface, which increases in the presence of cholesterol [8], has been shown to influence carrier-mediated transport through artificial lipid bilayers [48]. (4) Proteins have been claimed [5] to sense changes in the 'hydrogen belt' at the membrane interface.

Studies using other techniques of modifying the membrane lipid phase, such as phospholipid degradation by phospholipase A_2 [53], as well as experimental insertion of the split products of this treatment into the membrane [14], have also revealed differences in the extent and the direction of the response of the transport systems studied here. A different reaction of protein-mediated transport functions to modification of one particular membrane lipid constituent may thus be a more general phenomenon.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 160). The secretarial help of Mrs. H. Thomas in preparing the manuscript is gratefully appreciated.

References

1 Lee, A.G. (1975) Prog. Biophys. Mol. Biol. 29, 5—56
2 McElhaney, R.N., de Gier, J. and van der Neut-Kok, E.C.M. (1973) Biochim. Biophys. Acta 298, 500—512
3 Deuticke, B. (1977) Rev. Physiol. Biochem. Pharmacol. 78, 1—97
4 Grunze, M. and Deuticke, B. (1974) Biochim. Biophys. Acta 356, 125—130
5 Brockerhoff, H. (1974) Lipids 9, 645—650
6 Deuticke, B. and Ruska, C. (1976) Biochim. Biophys. Acta 433, 638—653
7 Szabo, G. (1974) Nature 252, 47—49
8 Andersen, O.S. (1978) in Membrane Transport in Biology (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), Vol. I, pp. 369—446, Springer-Verlag, Berlin
9 Wilbrandt, W. and Becker, C. (1978) in Transport by Proteins (Blauer, G. and Sund, H., eds.), pp. 215—220, Walter de Gruyter, Berlin
10 Cooper, R.A., Arner, E.C., Wiley, J.S. and Shattil, S.J. (1975) J. Clin. Invest. 55, 115—126
11 Claret, M., Garay, R. and Giraud, F. (1978) J. Physiol. 274, 247—263
12 Wiley, J.S. and Cooper, R.A. (1975) Biochim. Biophys. Acta 413, 425—431
13 Deuticke, B., Rickert, I. and Beyer, E. (1978) Biochim. Biophys. Acta 507, 137—155
14 Deuticke, B., Grunze, M. and Haest, C.W.M. (1979) in Membrane Transport in Erythrocytes (Lassen,

U., Ussing, H.H. and Wieth, J.O., eds.), Alfred Benzon Symposium 14, Munksgaard, Copenhagen , in the press
15 Jung, C.Y. (1975) in The Red Blood Cell (Surgenor, M.D., ed.), Vol. II, pp. 706—751, Academic Press, New York
16 Masiak, S.J. and LeFevre, P.G. (1974) Arch. Biochem. Biophys. 162, 442—447
17 Rose, H.G. and Oklander, M. (1965) J. Lipid Res. 6, 447—479
18 Gerlach, E. and Deuticke, B. (1963) Biochem. Z. 337, 447—479
19 Folch, J., Lees, M. and Stanley, G.H.S. (1957) J. Biol. Chem. 226, 497—509
20 Richterich, R. and Lauber, K. (1962) Klin. Wochenschr. 40, 1552—1554
21 Johnson, S.M. (1979) Anal. Biochem. 95, 344—350
22 Weinstein, R.S., Khodadad, J.K. and Steck, T.L. (1978) J. Supramol. Struct. 8, 325—335
23 Deuticke, B., van Bentheim, M., Beyer, E. and Kamp, D. (1978) J. Membrane Biol. 44, 135—158
24 Segel, I.H. (1975) Enzyme Kinetics, J. Wiley, and Sons, New York
25 Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715—720
26 Bouma, S.R., Drislane, F.W. and Huestis, W.H. (1977) J. Biol. Chem. 252, 6759—6763
27 Huestis, W.H. (1977) J. Biol. Chem. 252, 6764—6768
28 Bruckdorfer, K.R., Demel, R.A., de Gier, J. and van Deenen, L.L.M. (1969) Biochim. Biophys. Acta 183, 334—335
29 Gul, S. and Smith, A.D. (1974) Biochim. Biophys. Acta 367, 271—281
30 Nigg, E.A. and Cherry, R.J. (1979) Biochemistry 18, 3457—3465
31 Bieri, V.G. and Hoelzl Wallach, D.R. (1975) Biochim. Biophys. Acta 406, 415—423
32 Cullis, P.R. and Grathwohl, C. (1977) Biochim. Biophys. Acta 471, 213—226
33 Cooper, R.A., Leslie, M.H., Fischkoff, St., Shinitzky, M. and Shattil, S.J. (1978) Biochemistry 17, 327—331
34 Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133—149
35 Johnson, S.M. (1973) Biochim. Biophys. Acta 307, 27—41
36 Madden, T.D., Chapman, D. and Quinn, P.J. (1979) Nature 279, 538—541
37 Papahadjopoulos, D., Cowden, M. and Kimelberg, H. (1973) Biochim. Biophys. Acta 330, 8—26
38 Sinensky, M., Pinkerton, F., Sutherland, E. and Simon, F.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4893—4897
39 Brown, M.S., Dana, S.E. and Goldstein, J.L. (1975) J. Biol. Chem. 250, 4025—4027
40 Graham, J.M. and Green, C. (1970) Eur. J. Biochem. 12, 58—66
41 Klappauf, E. and Schubert, D. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1225—1236
42 London, Y., Demel, R.A., Geurts van Kessel, W.S.M., Zahler, P. and van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 332, 69—84
43 Westphal, R. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 431—447
44 Borochov, H. and Shinitzky, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4526—4530
45 Shinitzky, M. and Rivnay, B. (1977) Biochemistry 16, 982—986
46 Gavish, B. and Werber, M.M. (1979) Biochemistry 18, 1269—1275
47 Maurel, P. (1978), J. Biol. Chem. 253, 1677—1683
48 Benz, R. and Cros, D. (1978) Biochim. Biophys. Acta 506, 265—280
49 Wilbers, K.H., Haest, C.W.M., van Bentheim, M. and Deuticke, B. (1979) Biochim. Biophys. Acta 554, 400—409