NUCLEAR MEMBRANES FROM MAMMALIAN LIVER

II. Lipid Composition

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ABSTRACT

The qualitative and quantitative lipid composition of nuclei and nuclear membranes from pig and rat liver were determined. These determinations were compared with the corresponding data obtained for microsomes from the same material after similar treatments. The results indicate that, at least, by far the major part of the nuclear lipids is located in the membranes of the nuclear envelope. The phospholipid pattern of the nuclear membranes and the endoplasmic reticulum (ER) membranes in general is widely identical in both species. As a striking difference in the lipid composition, however, a fourfold increase of esterified cholesterol in the nuclear membranes was found. In a quantitative approach the ratio of total surface area of the nuclear lipids to the total surface area of the nuclear envelope membranes was calculated as being 3.6, a value which fairly approximates the requirements of a bimolecular lipid leaflet model.

INTRODUCTION

From numerous studies on biomembranes the diversity of the different kinds of cellular membranes with respect to their structure, function, and chemical composition is evident. In comparison to other membrane systems, such as plasma membranes, endoplasmic reticulum, mitochondrial membranes, etc., little is so far known about the chemical constituents of the nuclear membrane. Only Gurr et al. (30) investigated the phospholipid composition of isolated rat liver nuclei, and Rouser et al. (45) presented some data on the phospholipids of the nuclei of some bovine organs. Since the comparison of the chemical composition of different intracellular membrane systems requires the preparation of highly pure fractions of these membranes in a structurally intact state, special care has to be taken to provide membrane fractions with an extremely low degree of contamination by other membranous material.

In the accompanying paper (26) we reported the isolation and characterization of pure fractions of nuclei, nuclear membranes, and microsomes from rat and pig liver. The present paper deals with the qualitative and quantitative lipid composition of these fractions and, in particular, the lipids of the nuclear membrane are compared with those of the microsomal fractions of the same material.

MATERIALS AND METHODS

Isolation Procedures

Nuclei, nuclear membranes, and microsomes from rat and pig liver were prepared as described in the foregoing paper (26). For control, microsomes were also isolated according to the modified Dallner et al. (13) procedure (26). It should be noted that these microsomes had not been exposed to high salt concentrations.

Extraction of Lipids

The nuclear, nuclear membrane, and microsomal pellets were thoroughly extracted three times in the cold (2°–4°C) with chloroform–methanol (2:1 v/v) under nitrogen according to Folch et al. (24). The combined lipid extracts were washed two times with 1% NaCl solution, and the water phase was reextracted with chloroform-methanol. The lipid extract then was evaporated to dryness under nitrogen and redissolved in chloroform.

Separation of Neutral Lipids and Phospholipids

The chloroform extract was applied to a column containing silica gel H (Merck, Darmstadt, Germany). Neutral lipids were eluted with chloroform and phospholipids with methanol.

Separation and Estimation of Neutral Lipids

Neutral lipids were applied to 20 × 20 cm plates coated with silica gel H (Merck)–cellulose (Macherey Nagel and Co., Düren, Germany) 2:1 (v/v). Before application the plates were preactivated for 1 hr at 110°C. The chromatograms were developed in petroleum ether–chloroform–acetone 50:20:3 (v/v/v), dried, sprayed with 0.05% Rhodamine B in ethanol supplemented with 5% Tinopal (Geigy A. G., Frankfurt, Germany) according to Popov and Stefanov (44), and photographed in UV light when desired. The individual lipids were identified by cochromatography with standards. Cholesterol esters were also identified by saponification and rechromatography, resulting in cholesterol and free fatty acids. The neutral lipids were also separated into three fractions containing cholesterol esters, triglycerides, and cholesterol plus free fatty acids on a silica gel H column with petroleum ether plus increasing amounts of ether. Cholesterol and cholesterol esters were determined with $FeCl_3$ (9), and the amount of free fatty acids was evaluated by microtitration (47) and by the colorimetric method of Duncombe (21). Triglycerides were measured by quantitative infrared spectrophotometry at approximately 5.67 μm (wavelength for carbonyl stretching vibration) as described by Skipski et al. (47) using a Perkin-Elmer double-beam infrared spectrophotometer (Perkin-Elmer Corp., Wilton, Conn.) in NaCl cells with a light path of 0.2 mm (solvent CCl_4).

Separation and Estimation of Phospholipids

Phospholipids were applied to the same plates as described above, and the chromatograms were developed with chloroform–methanol–water 50:25:1 (v/v/v). For visualization the same Rhodamine–Tinopal spray was used as reported above. Phosphatidylethanolamine and phosphatidylserine were also detected with ninhydrin reagent (50). The phospholipids were also separated and analyzed by two-dimensional thin-layer chromatography as described by Abramson and Blecher (1) on silica gel G plates (Merck) with the solvent system chloroform–methanol–acetic acid–water 40:12:3:1 (v/v/v/v) in the first direction, and chloroform–methanol–7 M NH_4OH 50:15:3 (v/v/v) in the second direction. The individual phospholipids were identified by cochromatography with reference substances and by mild alkaline and acid hydrolysis and subsequent separation of water-soluble phosphates (15, 16). The hydrolysates were chromatographed on cellulose layers with water-saturated phenol–acetic acid–ethanol 50:5:6 (v/v/v). The chromatograms were sprayed with molybdate reagent (Dawson's modification of the reagent of Hanes and Isherwood (31)). With this method the amount of plasmalogens can also be calculated.

Phosphorus-containing areas were scraped from the plates and analyzed quantitatively (28).

The absence of glycolipids in the lipid extract was shown by the negative test with diphenylamine reagent (50) on the thin-layer chromatograms.

Reference Substances

Lecithin, lysolecithin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, diphosphatidylglycerol (cardiolipin), and sphingomyelin were purchased from Applied Science Laboratories, Inc., State College, Pa.; tripalmitate from Serva (Serva Feinbiochemica GMBH and Co., Heidelberg, Germany); cholesterol from Nutritional Biochemicals Corporation, Cleveland, Ohio; oleic acid from Roth OHG, Karlsruhl, Germany. The myristic acid esters of cholesterol and methanol were synthesized with myristic acid chloride and free cholesterol and methanol, respectively, in dry pyridine.

RESULTS

The data for pig and rat liver nuclear and microsomal phospholipids are summarized in Tables I and II. Nuclei, nuclear membranes, and microsomes in general contain the same phospholipids in almost identical relative concentrations. It should be noted that only traces of lysolecithin were found. Other lysoderivatives as well as cardiolipin were not detected. This extraordinarily low level of lysoderivatives demonstrates that no remarkable lipid degradation takes place during the particular isolation procedures used. About 1% of lecithin of both nuclear membranes and microsomes was determined as alk-1-enyl-acyl lecithin (plasmalogen). Plasmalogens from other phospholipids could not be detected.

For pig liver no corresponding reference data

TABLE I

Phospholipid Composition of Pig Liver Nuclei, Nuclear Membranes, and Microsomes in Per Cent of Total Lipid Phosphorus with Standard Deviations of Single Determinations

Lipid	Pig liver nuclei	Pig liver nuclear membranes	Pig liver microsomes
Sphingomyelin	2.0 ± 0.3	2.4 ± 0.4	2.9 ± 0.5
Lecithin	58.2 ± 2.1	58.2 ± 2.4	59.9 ± 1.4
Phosphatidylethanolamine	25.0 ± 1.2	25.9 ± 1.8	27.4 ± 0.9
Phosphatidylserine	4.8 ± 1.0	4.4 ± 0.6	3.2 ± 0.8
Phosphatidylinositol	8.8 ± 0.7	8.9 ± 0.9	6.5 ± 1.0
Phosphatidic acid	<1.0	<1.0	<1.0
Lysolecithin	1.0 ± 0.4	<1.0	<1.0
Other lysoderivatives	ND*	ND	ND
Cardiolipin	ND	ND	ND
Molar ratio cholesterol to phospholipid	0.102	0.104	0.092

* ND: not detected.

TABLE II

Phospholipid Pattern of Different Membranes of Rat Liver

Lipid	Rat liver nuclei (30)	Rat liver nuclei (this study)	Rat liver nuclear membranes (this study)	Rat liver micro-somes*	Rat liver microsomes (this study)	Rat liver Golgi mem-branes*	Rat liver plasma mem-branes*
SPH‡	6.3	2.1 ± 0.3	3.2 ± 1.2	3.7	4.0 ± 1.3	12.3	18.9
PC	52.2	61.1 ± 2.1	61.4 ± 1.5	60.9	60.0 ± 1.6	45.3	39.9
PE	25.1	22.0 ± 1.8	22.7 ± 1.4	18.6	23.0 ± 1.9	17.0	17.8
PS	5.6	4.0 ± 1.1	3.6 ± 1.0	3.3	3.5 ± 1.1	4.2	3.5
PI	4.1	8.4 ± 1.0	8.6 ± 0.8	8.9	8.1 ± 0.7	8.7	7.5
PA		<1.0	<1.0		<1.0		
LPC		1.2 ± 0.5	1.5 ± 0.6	4.7	1.3 ± 0.5	5.9	6.7
LPE		ND	ND		ND	6.3	5.7
Molar ratio cholesterol to phospholipid		0.108	0.104		0.110		

* D. J. Morré, personal communication. Compare also, e.g., the data by Pfleger et al. (43).
‡ SPH, sphingomyelin; PC, lecithin; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; LPC, lysolecithin; LPE, lysophosphatidylethanolamine; ND, not detected.

could be found in the literature. The phospholipid values for bovine liver nuclei and microsomes as given by Rouser et al. (45), however, are in the same range as our values, with only one serious exception, namely that these authors found considerably more lysoderivatives (8.2%).

In Table II some phospholipid data for different kinds of rat liver membranes are listed together with those of the present study. As can be seen, our data are in good agreement with the data of D. J. Morré (personal communication) for microsomes, whereas the values of Gurr et al. (30) for nuclei differ slightly. It should be noted, however, that these nuclei were isolated by using the citrate method and contain almost exclusively only the inner sheet of the nuclear envelope. The comparatively higher content of sphingomyelin within plasma membranes (Table II) seems to be well established (see also references 18, 27, 39, 43).

In Table III the neutral lipid content of pig liver

TABLE III

Nonpolar Lipids of Pig Liver Nuclei, Nuclear Membranes, and Microsomes

Lipids	Pig liver nuclei	Pig liver nuclear membranes	Pig liver microsomes
		μmoles/μmoles phospholipid	
Cholesterol	0.102 ± 0.005	0.104 ± 0.005	0.092 ± 0.005
Cholesterol ester	0.0077 ± 0.0007	0.0085 ± 0.0010	0.0018 ± 0.0007
Triglycerides	0.050 ± 0.009	0.051 ± 0.010	0.037 ± 0.009
Diglycerides	traces	traces	traces
Fatty acids	0.143 ± 0.027	0.163 ± 0.031	0.115 ± 0.030
Molar ratio cholesterol to cholesterol ester	13.2	12.2	51.1

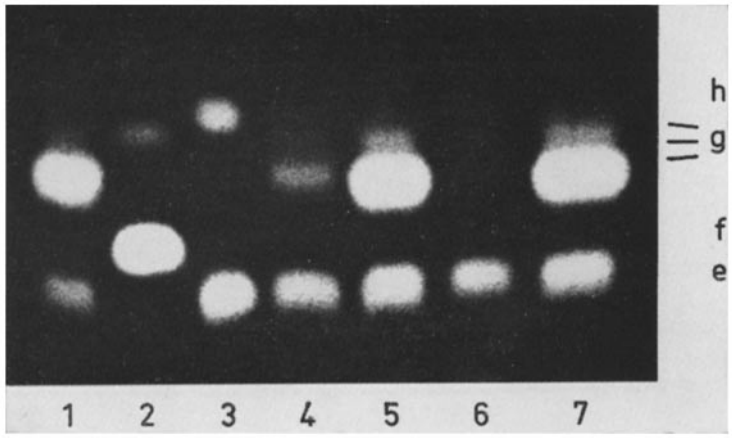

FIGURE 1 Thin-layer chromatogram of nonpolar lipids. 1. Pig liver nuclei. 2. Reference substances. 3. Reference substances. 4. Pig liver. 5. Pig liver nuclear membranes. 6. Pig liver microsomes. 7. Pig liver nuclear membranes. *e*. Triglycerides. *f*. Myristic acid ester (with methanol). *g*. Cholesterol esters. *h*. Alkanes.

membranes is shown. As can be seen from this Table, the contents of triglycerides and fatty acids fluctuate over a comparatively wide range. On the other hand, the amounts of cholesterol and cholesterol esters are very constant. Moreover, there exists a significant difference with respect to the cholesterol esters in that nuclei and nuclear membranes contain four times as much esterified cholesterol (in relation to μmoles phospholipid) than microsomes. These differences are illustrated in more detail by the thin-layer chromatogram of Fig. 1. It should be emphasized that about the same cholesterol:cholesterol ester ratio has been found for rat liver nuclear membranes and microsomes, respectively.

On the assumption that the nuclear lipids are nearly exclusively located in the nuclear envelope (see Discussion), two isolations of pig liver nuclei were performed as quantitative experiments with 7×10^{10} and 4.4×10^{10} nuclei, respectively, to determine the lipid content and the lipid-covered surface area per nucleus. The data are given in Table IV. The nuclei were found to have a mean diameter of 5.7 μm, i.e. a surface area of about 102 μm^2 per nucleus. From this total nuclear surface area, about 20%, which is occupied by nuclear pores (Franke, personal communication), is thus to be subtracted. The result is a value of about 80 μm^2 for the membrane surface area per nucleus. It should be kept in mind that this refers to the entire nuclear envelope, which includes both the inner and the outer membrane of the nucleus. On the basis of the assumptions and results of Bar et al. (4), molecular cross-sectional areas of

TABLE IV

Phospholipids and Cholesterol of Pig Liver Nucleus

Lipid	Molecules per nucleus ($\times 10^7$)	μg per nucleus ($\times 10^{-10}$)
Sphingomyelin	0.6	74
Lecithin	18.1	2370
Phosphatidylethanolamine	7.8	962
Phosphatidylserine	1.5	196
Phosphatidylinositol	2.7	380
Lysolecithin	0.3	21
Total phospholipid	31.0	4208
Cholesterol	3.2	205

Phospholipids estimated as 1-stearoyl-2-oleoyl-phospholipids.

87.5 A^2 for phospholipids and 38 A^2 for cholesterol (49) were chosen. With these values, the phospholipids and cholesterols of one nucleus can be calculated to occupy an area of 284 μm^2 as a monolayer. Thus the ratio of lipid surface area to the surface area of double membrane of the nuclear envelope (pore area subtracted) is about 3.6. The ratio might be somewhat underestimated since free fatty acids and triglycerides were not taken into account. The value is, however, in fair approximation to the value of 4.0 which would be required for example by a bimolecular lipid leaflet model (14, for recent discussion see reference 48).

DISCUSSION

Although some sporadic comments exist on the occurrence of membranous structures within the nucleus (e.g. Golgi-like structures in neoplastic cells of intracranial chicken sarcoma [6], nucleolus-associated membranes in young crayfish oocytes [36], and in rat and mouse chorio-allantoic placenta [7], intranuclear annulate lamellae in ascidian oocytes [23, 35]), the general assumption seems to be justified, in particular for liver cells, that most if not all nuclear lipids are located within the nuclear membranes. This is all the more likely, since intranuclear membranes have so far not been reported for mammalian liver although this cell type is probably the best investigated one in electron microscopy. Furthermore, the nearly identical lipid pattern in whole nuclei and isolated nuclear membranes supports this assumption. It is therefore reasonable to conclude that qualitatively as well as quantitatively the nuclear membrane lipids are representative for the total nuclear lipids. This does not exclude, of course, the possibility that a small part of the lipoprotein of the nuclear envelope can be present in close association with the chromatin, since such interactions might be prerequisites for DNA synthesis as is suggested by the work of Comings (10) and Comings and Kakefuda (11). In preliminary experiments in our laboratory, however, only small amounts of phospholipid could be detected in isolated chromatin which had been prepared from nuclei of pig liver using a combination of the methods of Marushige and Bonner (38) and Atchley and Bhagavan (3) including a final 1 hr centrifugation through 1.7 M sucrose (ρ = 1.22). Jackson et al. (32) isolated an insoluble residue from calf thymus chromatin which contained lipoprotein and approximately 1% of total DNA. Since the over-all ratio of total phospholipid to DNA in whole nuclei generally does not exceed 0.2 (w/w), the ratio of phospholipid to DNA included in such residues should be very low in quantitative experiments. Thus the phospholipid to DNA ratios of Dolbeare and Koenig (20), who reported values as high as 0.22 (w/w) for dense chromatin and 0.44 (w/w) for diffuse chromatin prepared from rat liver nuclei, can hardly be explained.

Any differences in the lipid composition between nuclei and microsomes in liver would not have been expected from the outset, since the outer nuclear membrane is continuous with the ER cisternal system at many sites. Nevertheless, a striking difference in the lipid composition of the nuclei and the nuclear membranes on the one hand, and the microsomes on the other hand, is the constantly four times higher content of esterified cholesterol in the nuclear membranes (Fig. 1, Table III). These data are consistent with the findings of Kemp and Mercer (34) for maize shoot tissue. These authors report that the sterol ester content in the nuclear fraction is about eight times higher than in the microsomal fraction.

It has been shown by Nelson (40) that cholesterol esters are absent from well-washed erythrocyte membranes. The rat intestinal microvillus membrane also lacks esterified cholesterol (25), as does a fraction of the brush border membranes from the kidney tubules (5). However, in other plasma membranes, from rat liver, for example, cholesterol esters seem to exist (22, 43). The "free" liver lipids, i.e. those of the lipid droplets, contain much more

esterified cholesterol than the cytomembranes (molar ratio cholesterol to cholesterol esters 0.79 [Kleinig, unpublished observations]). A selective contamination of the nuclear membrane with cholesterol esters is unlikely since both microsomes and nuclear membranes were treated in the same way. Two cholesterol esterifying systems are known from rat liver, one located in the "cell sap" (2) and the other bound to particulate material (microsomes, 29). This latter finding, then, might be seen as a further indication that cholesterol esters can exist as natural constituents of intracellular membranes. In a recent investigation, Kashnig and Kasper (33) also reported the occurrence of esterified cholesterol in nuclear membranes. In Fig. 1, at least three different cholesterol esters can be recognized in the nonpolar nuclear lipids. The fatty acid composition of these esters as well as the fatty acid moieties of the other lipid fractions will be presented in a forthcoming paper (H. Kleinig and J. Stadler, manuscript in preparation).

The finding of similarity of phospholipid composition of nuclear membranes and microsomes is somewhat contradictory to the data of Rouser et al. (45) who reported that the phospholipids of microsomes are different from those of whole nuclei for bovine liver and heart tissue. In this context, it is noteworthy that in these authors' bovine liver nuclear preparations 8.2% of total phospholipids were lysoderivatives and that the phospholipid differences they pointed out lie chiefly in the relative high amount of such lysoderivatives.

The molar ratio of cholesterol to phospholipid, which can be regarded as an indicator for the tightness of packing of the lipid molecules within a membrane (41), is found in pig liver to be 0.102 for nuclei, 0.104 for nuclear membranes, and 0.092 for microsomes. The corresponding values for rat liver are 0.108, 0.104, and 0.110, respectively. This agrees nicely with the 0.122 ratio found by Dallner et al. (12, cf. also reference 42) for rat liver microsomes. It is in contrast, however, to Kashnig and Kasper (33) who reported a high ratio of 0.300 and 0.410 for two nuclear membrane fractions from rat liver. Such cholesterol to phospholipid molar ratios are markedly higher than the corresponding values for, e.g., the outer mitochondrial membrane (0.068 [42]), but are lower than the ratios reported for plasma membranes of rat liver (0.38 [22]; 0.74 [43]), rat intestinal microvilli (1.26 [25]), and erythrocytes of various species (0.74–1.04 [40]). For pig erythrocytes a value of 0.66 was determined in our laboratory (Kleinig and Nöller, unpublished observations).

The over-all effect of free cholesterol on phospholipids as a reduction of their molecular area is well documented by Van Deenen and his collaborators (discussion see 17). Their data were obtained using artificial liposomes with relatively high cholesterol to phospholipid molar ratios (0.4 and more), thus simulating myelin and plasma membranes. In view of the comparatively low cholesterol content of the nuclear envelope, any condensing effect of cholesterol on the packing of lipid molecules should be negligible for the calculation of the surface area of nuclear lipids given above. In this calculation the value of 87.5 A^2 (4) for phospholipid cross-sectional area was used; this has been derived from a plot of the ratio of lipid film area to erythrocyte area against the molecular area of phospholipids of erythrocytes under varying surface pressure (4). Values for the ratio of surface area of the membrane extracted lipid to the total surface area of membrane in question were hitherto only available for erythrocyte membranes. These values approximate the required value of 2.0 for a bilayer model (for discussion see references 4, 37, 41). Although there is only limited direct proof for the unit membrane concept (37) and alternative models exist (e.g. 8, 46), it is interesting that in a gross calculation, using the same molecular surface areas as Bar et al. (4), the lipid content of the mammalian liver nuclear envelope appears roughly to comply with the requirement of a bilayer configuration of the lipids of each of the two membranes.

Glycolipids could not be detected in either rat or pig liver intracellular membrane preparations. This finding is consistent with the results obtained with bovine liver nuclei and microsomes (45) and rat liver cells (19). In contrast to the intracellular cytomembranes the plasma membranes contain various amounts of glycolipids (18, 25, 39).

From a comparison of the liver nuclear membrane lipids of the two studied mammalian species, it is apparent that there exist no significant interspecific differences in the lipid compositions.

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REFERENCES

1. ABRAMSON, D. and M. BLECHER. 1964. *J. Lipid Res.* **5**:628.
2. AKIYAMA, M., O. MINARI, and T. SAKAGAMI. 1967. *Biochim. Biophys. Acta.* **137**:525.
3. ATCHLEY, W. A., and N. V. BHAGAVAN. 1964. *Arch. Biochem. Biophys.* **106**:505.
4. BAR, R. S., D. W. DEAMER, and D. G. CORNWELL. 1966. *Science (Washington).* **153**:1010.
5. BINKLEY, F., N. KING, E. MILIKIN, R. K. WRIGHT, C. H. O'NEAL, and I. J. WUNDRAM. 1968. *Science (Washington).* **162**:1009.
6. BUCCIARELLI, E. 1966. *J. Cell Biol.* **30**:664.
7. CARLSON, E. L., and A. D. OLLERICH. 1968. *J. Cell Biol.* **39**:22a.
8. CHAPMAN, D., and D. F. H. WALLACH. 1968. *In* Biological Membranes. D. Chapman, editor. Academic Press Inc., New York. 125.
9. CLARK, B. R., R. T. RUBIN, and R. G. ARTHUR. 1968. *Anal. Biochem.* **24**:27.
10. COMINGS, D. E. 1968. *Amer. J. Hum. Genet.* **20**:440.
11. COMINGS, D. E., and T. J. KAKEFUDA. 1968. *J. Mol. Biol.* **33**:225.
12. DALLNER, G., P. SIEKEVITZ, and G. E. PALADE. 1966. *J. Cell Biol.* **30**:73.
13. DALLNER, G., A. BERGSTRAND, and R. NILSSON. 1968. *J. Cell Biol.* **38**:257.
14. DANIELLI, J. F., and H. A. DAVSON. 1935. *J. Cell Comp. Physiol.* **5**:495.
15. DAWSON, R. M. C. 1960. *Biochem. J.* **75**:45.
16. DAWSON, R. M. C., N. HEMINGTON, and J. B. DAVENPORT. 1962. *Biochem. J.* **84**:497.
17. DE GIER, J., J. G. MANDERSLOOT, and L. L. M. VAN DEENEN. 1969. *Biochim. Biophys. Acta.* **173**:143.
18. DOD, B. J., and G. M. GRAY. 1968. *Biochim. Biophys. Acta.* **150**:397.
19. DOD, B. J., and G. M. GRAY. 1968. *Biochem. J.* **110**:50p.
20. DOLBEARE, F., and H. KOENIG. 1968. *J. Cell Biol.* **39**:35a.
21. DUNCOMBE, W. G. 1963. *Biochem. J.* **88**:7.
22. EMMELOT, P., C. J. BOS, E. L. BENEDETTI, and P. RÜMKE. 1964. *Biochim. Biophys. Acta.* **90**:126.
23. EVERINGHAM, J. W. 1968. *J. Cell Biol.* **37**:540.
24. FOLCH, J., M. LEES, and G. H. SLOAN STANLEY. 1957. *J. Biol. Chem.* **226**:497.
25. FORSTNER, G. G., K. TANAKA, and K. J. ISSELBACHER. 1968. *Biochem. J.* **109**:51.
26. FRANKE, W. W., B. DEUMLING, B. ERMEN, E. D. JARASCH, and H. KLEINIG. 1970. *J. Cell Biol.* **46**:379.
27. GERCKEN, G., and U. BROCKMANN. 1969. *Eur. J. Biochem.* **8**:489.
28. GERLACH, E., and B. DEUTICKE. 1963. *Biochem. Z.* **337**:477.
29. GOODMAN, D. S., D. DEYKIN, and T. SHIRATORI. 1964. *J. Biol. Chem.* **239**:1335.
30. GURR, M. I., J. B. FINEAN, and J. N. HAWTHORNE. 1963. *Biochim. Biophys. Acta.* **70**:406.
31. HANES, C. S., and F. A. ISHERWOOD. 1949. *Nature (London).* **164**:1107.
32. JACKSON, V., J. EARNHARDT, and R. CHALKLEY. 1968. *Biochem. Biophys. Res. Commun.* **33**:253.
33. KASHNIG, D. M., and C. B. KASPER. 1969. *J. Biol. Chem.* **244**:3786.
34. KEMP, R. J., and E. I. MERCER. 1968. *Biochem. J.* **110**:119.
35. KESSEL, R. G. 1968. *J. Ultrastruct. Res.* **10** (Suppl.).
36. KESSEL, R. G., and H. W. BEAMS. 1968. *J. Cell Biol.* **39**:735.
37. KORN, E. D. 1966. *Scierce (Washington).* **153**:1491.
38. MARUSHIGE, K., and J. BONNER. 1966. *J. Mol. Biol.* **15**:160.
39. NELSON, G. J. 1967. *Biochim. Biophys. Acta.* **144**:221.
40. NELSON, G. J. 1967. *J. Lipid Res.* **8**:374.
41. O'BRIEN, J. S. 1967. *J. Theor. Biol.* **15**:307.
42. PARSONS, D. F., and Y. YANO. 1967. *Biochim. Biophys. Acta.* **135**:362.
43. PFLEGER, R. C., N. G. ANDERSON, and F. SNYDER. 1968. *Biochemistry.* **7**:2826.
44. POPOV, A. D., and K. L. STEFANOV. 1968. *J. Chromatogr.* **37**:533.
45. ROUSER, G., G. J. NELSON, S. FLEISCHER, and G. SIMON. 1968. *In* Biological Membranes. D. Chapman, editor. Academic Press Inc., New York. 5.
46. SJÖSTRAND, F. S. 1968. *In* The Membranes. A. J. Dalton and F. Haguenau, editors. Academic Press Inc., New York. 151.
47. SKIPSKI, V. P., J. J. GOOD, M. BARCLAY, and and R. B. REGGIO. 1968. *Biochim. Biophys. Acta.* **152**:10.
48. STOECKENIUS, W., and D. M. ENGELMAN. 1969. *J. Cell Biol.* **42**:613.
49. VANDENHEUVEL, F. A. 1963. *J. Amer. Oil Chem. Soc.* **40**:455.
50. WAGNER, H., L. HÖRHAMMER, and P. WOLFF. 1961. *Biochem. Z.* **334**:175.