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PHOSPHOLIPID PATTERNS IN SUBCELLULAR FRACTIONS OF ADULT- AND IMMATURE-RAT ORGANS

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SUMMARY

The following were determined: (a) phospholipid content of whole tissue, mitochondria, microsomes, final cytoplasmic supernatant and nuclear fractions of immature-rat livers, kidneys, uteri and ovaries. (b) Distribution of phospholipids in whole cytoplasmic fractions. (c) Amount and percentage distribution of lipid serine, ethanolamine, choline, nitrogen and sphingomyelin in lipid extracts of all fractions of the four organs of both adult and immature rats.

Many specific variations between individual phospholipid levels of organs and subfractions were detected *e.g.* low liver and high kidney sphingomyelin or high nuclear lipid serine and "other" phospholipids (obtained by difference of sum of lipid choline, serine and ethanolamine from total).

One broad common pattern characterized by high lipid choline followed by ethanolamine with little serine, sphingomyelin or "other" was presented by the phospholipid-rich mitochondria and microsomes. Another general pattern in which choline phospholipids, "cephalins" and "other" were approximately evenly divided was detected in the phospholipid-poor nuclei and supernatant.

Comparison between adult- and immature-rat organs and fractions showed that with age there occurs an overall decrease in the "cephalins" and "other" phospholipids with a concomitant increase in the lecithins without alteration in the total phospholipid content.

INTRODUCTION

Metabolism of a growing animal differs considerably from that of its adult counterpart. Exigencies of growth imply heightened anabolic processes as well as a generous supply of building material, and specific structural demands are imposed. These considerations have long been evident to both the histologist and the biochemist, but not until the recent introduction of the ultracentrifuge has it been possible to isolate cellular organelles and study functional and structural characteristics of organs at the subcellular level. Of the many lipid groups present in tissues, phospholipids,

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a major lipid class[1], have always been refractory to detailed study due partly to lack of reliable quantitative techniques and partly to chemical similarity. As a result, comparatively few detailed investigations concerning the distribution of individual phospholipids in subcellular fractions are found in the literature[2-8]; these are mainly related to the liver. Even fewer reports present comparative phospholipid studies on the growing and adult animal[9,10]. In an earlier study[11] it was shown that subcellular fractions of adult rat liver, kidney, uterus, and ovary generally presented a characteristic amount of phosphorus-containing lipids and that mitochondria as well as microsomes were richest in those lipids. This communication presents more detailed quantitative data on the distribution of major individual phospholipids in subcellular fractions of these organs in the immature as well as in the adult rat.

METHODS

The animals used were virgin female adult and immature (about 80 g) Wistar rats. The preparation of subcellular fractions of pooled livers, kidneys, uteri and ovaries, and the extraction of lipids were carried out as described previously[11]. The non-lipid contaminants were removed from lipid extracts by a technique reported separately[12]. The extracts concentrated in chloroform were applied in a linear fashion to silicic acid impregnated Whatman No. 40 paper strips and eluted with methanol–chloroform (20:80) in an atmosphere as anhydrous as possible. The impurities remained on the paper and pure lipids were collected in a beaker. The lipid eluate showed absence of free amino acids and sugar as determined by paper chromatography, and a marked reduction in inorganic salts. The recovery was close to 100% without selective loss. Avoidance of contact with water reduced the possibility of degradation or loss of more polar lipid compounds.

Chromatographic or chemical methods are used for the determination of amounts of individual phospholipids. The chief advantage of column chromatography lies in its simultaneous use as a preparatory procedure particularly if repeated fractionation is employed. On the other hand the almost invariable overlap of compounds in the peaks introduces a difficult to estimate error in the quantitative determination of individual phospholipids. Chemical methods are based on partial hydrolysis followed by quantitative assays of the phospholipid "base". Consequently it is impossible to differentiate between fully esterified and lyso-compounds or to determine compounds such as polyglycerol phosphatides or phosphatidic acids which have no "base". The methods chosen showed a high degree of reproducibility with a scatter of no more than 10% within the wide concentration margins used. The determinations carried out consisted of lipid P (see ref. 13), total nitrogen[14], lipid ethanolamine and serine[15], total lipid choline[16] which included sphingomyelin[17]. No attempt at separate assessments of lysophospholipids or plasmalogen forms was made; nor were determinations of phospholipids other than choline-containing and "cephalins" carried out. Thus, compounds such as polyglycerolphosphatides, phosphatidic acids and inositol phospholipids were grouped together under "other" phospholipids and obtained by difference from the total. The possibility that the residues assessed might have derived from complex phospholipids[18] was not considered. With few exceptions, imposed by scarcity of material, determinations were carried out at least in duplicate.

RESULTS

Adult rats

It can be seen from Tables I and II that amounts of the various phospholipids in subcellular fractions present considerable differences. It appears that each fraction possesses a characteristic quantitative distribution which varies from tissue to tissue as well as from fraction to fraction of the same tissue. Nevertheless, two broad patterns of distribution, apparently related to the total phospholipid content of the fractions, are discernible. Subcellular fractions can be divided into the phospholipid-rich mitochondria and microsomes, and the phospholipid-poor final cytoplasmic supernatant and nuclei. Most cytoplasmic phospholipids are situated in mitochondria and microsomes. This is particularly obvious in liver and kidney where those particles are most abundant (Table III). Nuclei constitute a variable proportion of the cell mass, depending on the type of tissue, but because of their uniformly low phospholipid content[11] (Table IV) they do not contain more than 5 % of total cell phospholipid[19]. Thus most cell phospholipids are situated in mitochondria and microsomes which explains why phospholipid distribution of whole tissue extracts follows the pattern

TABLE I

CHEMICAL ANALYSIS OF PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF ADULT-RAT ORGANS

Results expressed in mg/g dry fraction. Values for whole tissue and final cytoplasmic supernatant fractions originally calculated on wet tissue basis were multiplied by 5.

Organ	*Fraction*	*Total* phospho-lipids*	*Serine phospho-lipids*	*Ethanol-amine phospho-lipids*	*Total** choline phospho-lipids*	*Other*** phospho-lipids*	*Sphingo-myelin*
Liver	Whole tissue	134.5	8.0	33.5	72.5	20.5	12.1
Liver	Mitochondria	130.5	9.8	19.6	101.1	0.0	6.5
Liver	Microsomes	73.5	8.1	11.8	33.1	20.5	5.9
Liver	Final cytoplasmic supernatant	4.0	0.5	0.4	2.8	0.3	0.1
Liver	Nuclei	23.4	7.0	1.8	3.4	11.2	1.2
Kidney	Whole tissue	79.5	5.0	16.5	47.5	10.5	13.1
Kidney	Mitochondria	217.0	21.7	41.3	126.8	27.2	30.4
Kidney	Microsomes	141.7	16.3	19.8	89.2	16.4	26.9
Kidney	Final cytoplasmic supernatant	2.7	0.5	0.5	1.0	0.7	0.2
Kidney	Nuclei	30.2	4.5	5.7	9.1	10.9	3.3
Uterus	Whole tissue	21.5	2.2	4.3	11.0	4.0	2.4
Uterus	Mitochondria	60.2	3.9	6.6	43.3	6.4	9.0
Uterus	Microsomes	48.4	9.9	11.4	22.8	4.3	8.5
Uterus	Final cytoplasmic supernatant	2.2	0.3	0.4	0.7	0.8	0.5
Uterus	Nuclei	20.4	4.1	1.6	8.6	6.1	0.8
Ovary	Whole tissue	68.0	7.5	13.5	35.7	11.3	8.1
Ovary	Mitochondria	94.9	10.4	16.6	56.0	11.9	6.2
Ovary	Microsomes	51.0	4.1	5.6	33.2	8.1	6.1
Ovary	Final cytoplasmic supernatant	5.4	0.5	0.8	2.8	1.3	0.6
Ovary	Nuclei	30.2	4.9	2.7	11.0	11.6	—§

* Details described previously[11].
** Includes sphingomyelin.
*** Obtained by subtraction of sum of serine, ethanolamine and total choline values from total.
§ Material insufficient or lost.

TABLE II

CHEMICAL ANALYSIS OF PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF ADULT-RAT ORGANS

Results expressed in moles/100 moles lipid P.

Organ	*Fraction*	*Serine phospho-lipids*	*Ethanol-amine phospho-lipids*	*Total* choline phospho-lipids*	*Other** phospho-lipids*	*Sphingo-myelin*	*Lipid nitrogen*
Liver	Whole tissue	6.0	24.5	54.0	15.5	9.0	95
Liver	Mitochondria	7.5	15.0	77.5	0.0	5.0	107
Liver	Microsomes	11.0	16.0	45.0	28.0	8.0	103
Liver	Final cytoplasmic supernatant	12.0	10.0	70.0	8.0	2.0	95
Liver	Nuclei	30.0	7.5	14.5	48.0	5.0	—***
Kidney	Whole tissue	6.0	20.5	60.0	13.5	16.5	111
Kidney	Mitochondria	10.0	19.0	58.5	12.5	14.0	135
Kidney	Microsomes	11.5	14.0	63.0	11.5	19.0	95
Kidney	Final cytoplasmic supernatant	17.0	17.5	40.0	25.5	8.0	92
Kidney	Nuclei	15.0	19.0	30.0	36.0	11.0	—
Uterus	Whole tissue	10.0	20.0	52 0	18.0	11.0	—
Uterus	Mitochondria	6.5	11.0	72.0	10.5	15.0	113
Uterus	Microsomes	20.5	23.5	47.0	9.0	17.5	113
Uterus	Final cytoplasmic supernatant	13.0	15.0	32.0	40.0	22.0	—
Uterus	Nuclei	20.0	8.0	42.0	30.0	4.0	—
Ovary	Whole tissue	11.0	19.5	52.5	17.0	12.0	91
Ovary	Mitochondria	11.0	17.5	59.0	12.5	6.5	—
Ovary	Microsomes	8.0	11.0	65.0	16.0	12.0	88
Ovary	Final cytoplasmic supernatant	9.5	13.5	52.0	25.0	11.0	87
Ovary	Nuclei	16.5	9.0	36.5	38.0	—	—

* See Table I.
** Obtained by subtraction of sum of serine, ethanolamine and total choline from 100.
*** See Table I.

of those two fractions. The phospholipid-rich fractions are characterized by the prevalence of choline-containing phospholipids, followed by ethanolamine phospholipids, whereas lipid serine, sphingomyelin and "other" are least abundant. This is particularly obvious in mitochondria which display the highest percentage of choline lipids of all fractions (Table II). The supernatant and nuclei, on the other hand, present a picture which is to a large extent a reversal of the previous pattern. The "other" phospholipids and the "cephalins" predominate whereas choline lipids are relatively reduced. The transition is especially evident in nuclei where the three major phospholipid groups are of approximately even proportions. Moreover, the nuclei are characterized by a higher lipid serine than ethanolamine content which is not encountered in any other fraction.

Marked differences in phospholipid distribution in the various fractions of the same organs are not observed but of interest is a comparatively low sphingomyelin level in liver fractions and a high lipid ethanolamine in the kidney.

Immature rats

Amounts of individual phospholipids are shown in Table IV. It can be seen that liver and kidney are the organs richest in phospholipids. It is also evident that mito-

TABLE III

THE DISTRIBUTION OF PHOSPHOLIPIDS WITHIN THE CYTOPLASM OF IMMATURE-RAT POOLED ORGANS

Fractions were prepared as described previously[11] but care was exercised to obtain quantitative recovery. The total cytoplasmic phospholipids recovered amounted to about half of whole tissue phospholipids. Values expressed as percentages of total cytoplasmic phospholipids.

	Mitochondria	*Microsomes*	*Final cytoplasmic supernatant*
Liver	80.0	17.6	2.4
Kidney	70.3	23.4	6.3
Uterus	46.8	35.9	17.3
Ovary	61.8	18.2	20.0

TABLE IV

TOTAL PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF IMMATURE-RAT POOLED ORGANS

Organ	*Whole tissue*			*Mitochondria*			*Microsomes*			*Final cytoplasmic supernatant*			*Nuclei*		
	Wet wt. (g)	*Phospholipids*		*Dry wt. (mg)*	*Phospholipids*		*Dry wt. (mg)*	*Phospholipids*		*Wet wt. (g)*	*Phospholipids*		*Dry wt. (mg)*	*Phospholipids*	
		(mg)	*% of wet wt.*		*(mg)*	*% of dry wt.*		*(mg)*	*% of dry wt.*		*(mg)*	*% of wet wt. of organ*		*(mg)*	*% of dry wt.*
Liver	49	1200	2.45	554	94.89	17.15	70	6.48	9.25	7.0	3.42	0.049	65	1.10	1.70
Kidney	11	220	2.00	1257	156.10	12.42	188	19.83	10.54	10.0	8.03	0.080	150	4.80	3.20
Uterus	6	38	0.63	443	21.90	4.94	72	5.91	8.20	11.0	5.74	0.052	70	1.10	1.57
Ovary	1	11	1.10	144	12.20	8.47	40	2.79	6.97	4.0	3.60	0.090	9	0.25	2.78

chondria and microsomes display a much higher phospholipid content than final cytoplasmic supernatant and nuclei. The various fractions present considerable quantitative discrepancies with the exception of nuclei, whose phospholipid content is practically the same in all tissues. All these features are similar to those found previously in adults[11]. The only exception is a somewhat higher microsomal content of the immature animals, at the expense of mitochondria. This is particularly obvious in uterine microsomes which actually contain a higher phospholipid percentage than uterine mitochondria.

Amounts of individual phospholipids in the immature animals (Tables V and VI) reveal a specificity of phospholipid distribution noted above for the adults. Here also, two broad patterns associated with the phospholipid-rich and phospholipid-poor fractions can be observed. However, comparisons of cumulative average values (Figs. 1, 2) underline one outstanding difference: immature animals contain considerably more "cephalins" and less choline phospholipids than adults. This is evident in nearly all fractions as well as tissues. The young animals are also characterized by an overall higher "other" phospholipid content, although this finding is less universal.

TABLE V

CHEMICAL ANALYSIS OF PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF IMMATURE-RAT ORGANS

Results expressed in mg/g dry fraction. Values for whole tissue and final cytoplasmic supernatant fractions originally calculated on wet tissue basis were multiplied by 5.

Organ	*Fraction*	*Total* phospho-lipids*	*Serine phospho-lipids*	*Ethanol-amine phospho lipids*	*Total** choline phospho-lipids*	*Other*** phospho-lipids*	*Sphingo myelin*
Liver	Whole tissue	122.5	12.3	24.5	67.5	18.2	7.0
Liver	Mitochondria	171.5	17.2	31.7	88.4	34.2	6.0
Liver	Microsomes	92.5	13.9	17.1	29.1	32.4	12.0
Liver	Final cytoplasmic supernatant	2.5	0.5	0.7	1.1	0.2	0.2
Liver	Nuclei	17.0	2.6	3.7	4.8	5.9	1.0
Kidney	Whole tissue	100.0	8.0	18.0	50.0	24.0	17.5
Kidney	Mitochondria	124.2	9.3	24.2	67.1	23.8	24.8
Kidney	Microsomes	105.4	13.2	26.4	52.2	13.6	18.4
Kidney	Final cytoplasmic supernatant	4.0	0.7	0.9	1.7	0.7	0.8
Kidney	Nuclei	32.0	4.0	2.7	17.3	8.0	2.2
Uterus	Whole tissue	31.5	5.5	8.5	16.0	1.5	3.2
Uterus	Mitochondria	49.4	4.5	9.9	23.2	11.8	4.7
Uterus	Microsomes	82.0	11.5	14.8	—§	—	—
Uterus	Final cytoplasmic supernatant	2.6	0.4	0.5	1.0	0.7	0.3
Uterus	Nuclei	15.7	2.3	2.0	6.0	5.4	—
Ovary	Whole tissue	55.0	13.0	9.0	31.0	2.0	7.5
Ovary	Mitochondria	84.7	12.3	13.5	51.2	7.7	5.9
Ovary	Microsomes	69.7	18.8	9.1	32.4	9.4	—
Ovary	Final cytoplasmic supernatant	4.5	1.0	1.0	2.0	0.5	—
Ovary	Nuclei	27.8	10.0	9.5	—	—	—

* For details see Table IV.
** See Table I.
*** See Table I.
§ See Table I.

TABLE VI

CHEMICAL ANALYSIS OF PHOSPHOLIPIDS IN SUBCELLULAR FRACTIONS OF IMMATURE-RAT ORGANS

Results expressed in moles/100 moles lipid P.

Organ	*Fraction*	*Serine phospho-lipids*	*Ethanol-amine phospho-lipids*	*Total* choline phospho-lipids*	*Other** phospho-lipids*	*Sphingo-myelin*	*Lipid nitrogen*
Liver	Whole tissue	10.0	20.0	54.0	16.0	5.5	90
Liver	Mitochondria	10.0	18.5	51.5	20.0	3.5	103
Liver	Microsomes	15.0	18.5	31.5	35.0	13.0	114
Liver	Final cytoplasmic supernatant	19.0	21.0	46.5	13.5	5.0	115
Liver	Nuclei	15.0	22.0	28.5	34.5	6.0	72
Kidney	Whole tissue	8.0	18.0	50.0	24.0	17.5	121
Kidney	Mitochondria	7.5	19.5	54.0	20.0	20.0	129
Kidney	Microsomes	12.5	24.5	49.5	13.5	17.5	102
Kidney	Final cytoplasmic supernatant	16.5	23.0	42.5	18.0	20.0	—
Kidney	Nuclei	12.5	8.5	54.0	25.0	7.0	73
Uterus	Whole tissue	18.0	27.0	50.0	5.0	10.0	111
Uterus	Mitochondria	9.0	20.0	47.0	24.0	9.5	106
Uterus	Microsomes	14.0	18.0	—***	—	—	—
Uterus	Final cytoplasmic supernatant	15.0	17.5	39.0	20.5	12.0	105
Uterus	Nuclei	14.5	12.5	38.5	34.5	—	—
Ovary	Whole tissue	24.0	16.0	56.0	4.0	14.0	—
Ovary	Mitochondria	14.5	16.0	60.5	9.0	7.0	129
Ovary	Microsomes	27.0	13.0	46.5	13.5	—	—
Ovary	Final cytoplasmic supernatant	22.0	22.5	44.5	11.0	—	—
Ovary	Nuclei	36.0	34.0	—	—	—	—

* See Table I.
** See Table II.
*** See Table I.

DISCUSSION

The results obtained are subject to the methodological variations discussed above. Amino-compounds released by hydrolysis are known for their instability whereas unavoidably small yields of nuclear phospholipids might affect the accuracy of determinations. These factors may have contributed to the possibly high percentage of "other" phospholipids in some fractions (*e.g.* nuclei) and to the occasional discrepancy between total lipid nitrogen and sum of nitrogenous bases. In most instances, however, "other" phospholipids representing, as they do, inositol phosphatides, polyglycerol phosphatides, and phosphatidic acids agree well with the sum of these compounds as reported individually by various authors[3,4,6,7,20,21].

The results on liver fractions agree with some of those reported in the literature[3,8,20] but are at variance with others[4]. No detailed data on kidney, uterus and ovary fractions are available for comparison, but amounts of individual phospholipids in whole organs of adult rats reported[21] are mostly in agreement with ours. On the other hand we were not able to confirm the results of WILLIAMS *et al.*[10] who found

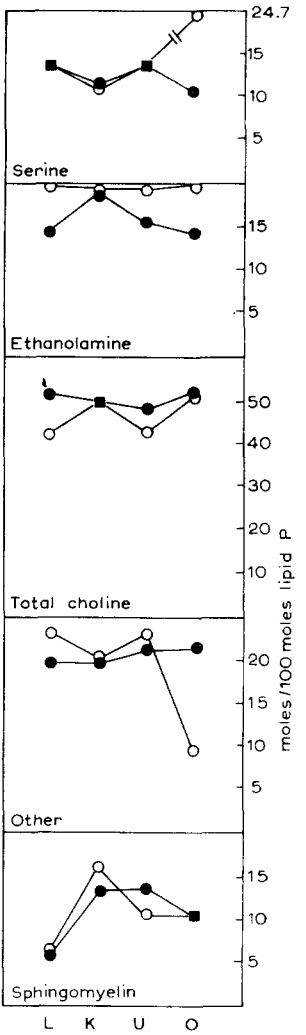

Fig. 1. Comparison of distribution of phospholipids in adult and immature rat organs. The points represent average values for all fractions in each organ. ●—●, adult; ○—○, immature; ■—■, values coincide. L, liver; K, kidney; U, uterus; O, ovary.

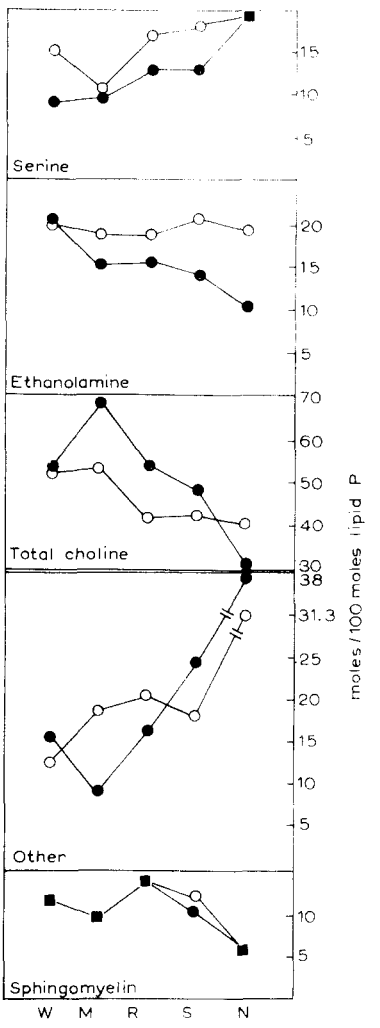

Fig. 2. Comparison of distribution of phospholipids in adult- and immature-rat subcellular fractions. The points represent average values for all fractions in organ. ●—●, adult; ○—○, immature; ■—■, values coincide. W, whole tissue; M, mitochondria; R, microsomes; S, final cytoplasmic supernatant; N, nuclei.

that with age there occurs an increase in the total phospholipid content of different organs, apparently due mainly to an increase in the "cephalins".

Our previous observation[11] on the specificity of phospholipid content in tissuse and subcellular fractions has now been confirmed and extended to the distribution of individual members of the phospholipid group. The results indicate that one formula of distribution cannot be applied either to the various fractions of a particular organ or to individual subfractions of several organs. Even the generally seen prevalence of choline phospholipid over the "cephalins" presents a notable exception in most

nuclear preparations. We were nevertheless able to detect a broad but distinct pattern of distribution associated with the phospholipid-rich mitochondria and microsomes and a different pattern encountered in the phospholipid-poor nuclei and supernatant. However, the significance of even such clear-cut findings as high mitochondrial lipid choline or high nuclear lipid serine is far from obvious. It is accepted that lipids, and particularly phospholipids, constitute an important structural component of limiting cellular and subcellular membranes[22]; this may be the reason for the differences in fraction patterns. On the other hand phospholipids present in cell wall might also perform functions associated with the cell metabolic activity. Such inference may be drawn from the work of SOLOMON *et al.*[23] and KIRSCHNER[24] who suggested that phospholipids were concerned with transcellular cation transport. Recent studies by KARNOVSKY AND WALLACH[25] on the incorporation of ^{32}P into phospholipids of leucocyte membranes during phagocytosis indicate that phospholipids also may be involved in transfer of larger particles into cells.

Quantitative differences alone do not explain the nature of function or its biological importance. In this respect more information can be obtained from turnover studies or from assessments of quantitative changes under specifically altered circumstances. The growth of the animal represents one such circumstance. Our results indicate that as the animal reaches maturity (*i.e.*, its anabolic activity achieves balance) a proportion of "cephalins" and "other" is replaced by lecithins without alteration of total phospholipid content. It is of interest that induced heightened glandular activity results in increased ^{32}P incorporation into inositol phosphatide and phosphatidic acid. This was demonstrated in the pancreas, salt gland, adrenal and hypophysis by HOKINS *et al.*[26–28] who concluded that those phospholipids were involved in the production of protein secretions and in the transcellular sodium transport.

Our studies of the four morphologically and functionally different organs added to the data obtained on heart[2] and brain[6] provide a spectrum of mammalian tissue phospholipid distribution. No definite inferences can be drawn from the above as to the functions of individual phospholipids but the specific quantitative characteristics should be of value as a guide to future investigations.

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REFERENCES

1 H. WITTCOFF, *The Phosphatides*, Reinhold, New York, 1951, p. 256.
2 G. V. MARINETTI, J. ERBLAND AND E. STOTZ, *J. Biol. Chem.*, 233 (1958) 562.
3 M. G. MACFARLANE, G. M. GRAY AND L. W. WHEELDON, *Biochem. J.*, 77 (1960) 626.
4 E. H. STRICKLAND AND A. A. BENSON, *Arch. Biochem. Biophys.*, 88 (1960) 344.
5 J. SPIRO AND J. M. MCKIBBIN, *J. Biol. Chem.*, 219 (1956) 643.
6 L. A. BIRAN AND W. BARTLEY, *Biochem. J.*, 79 (1961) 159.
7 F. D. COLLINS AND V. L. SHOTLANDER, *Biochem. J.*, 79 (1961) 321.
8 M. A. SWANSON AND C. ARTOM, *J. Biol. Chem.*, 187 (1950) 281.
9 H. H. WILLIAMS, H. GALBRAITH, M. KAUCHER AND I. G. MACY, *J. Biol. Chem.*, 161 (1945) 463.
10 H. H. WILLIAMS, H. GALBRAITH, M. KAUCHER, E. Z. MOYER, J. RICHARDS AND I. G. MACY, *J. Biol. Chem.*, 161 (1945) 475.

[11] J. J. BIEZENSKI AND T. H. SPAET, *Biochim. Biophys. Acta*,,51 (1961) 221.
[12] J. J. BIEZENSKI, *J. Lipid Res.*, 3 (1962) 120.
[13] E. J. KING, *Biochem. J.*, 26 (1932) 292.
[14] F. J. CONWAY, *Microdiffusion Analysis and Volumetric Error*, 4th Edition, Crosby Lockwood and Son, Ltd., London, 1957.
[15] S. NOJIMA AND N. UTSUGI, *J. Biochem. (Tokyo)*, 44 (1957) 565.
[16] A. D. APPLETON, B. N. LADU, Jr., B. B. LEVY, J. M. STEELE AND B. B. BRODIE, *J. Biol. Chem.*, 205 (1953) 803.
[17] G. SCHMIDT, J. BENOTTI, B. HERSHMAN AND S. J. THANNHAUSER, *J. Biol. Chem.*, 166 (1946) 505.
[18] F. D. COLLINS AND V. L. SHOTLANDER, *Biochem. J.*, 79 (1961) 316.
[19] G. CLÈMENT, J. CLÈMENT AND E. LEBRETON, in G. POPJÀK AND E. LEBRETON, *Biochemical Problems of Lipids*, Interscience, New York, 1956, p. 385.
[20] G. S. GETZ AND W. BARTLEY, *Nature*, 184 (1959) 1229.
[21] G. V. MARINETTI, J. ERBLAND AND E. STOTZ, *Biochim. Biophys. Acta*, 30 (1958) 642.
[22] R. M. C. DAWSON, *Biol. Rev. Cambridge Phil. Soc.*, 32 (1957) 188.
[23] A. SOLOMON, F. LIONETTI AND P. CURRAN, *Nature*, 178 (1956) 582.
[24] L. B. KIRSCHNER, *J. Gen. Physiol.*, 42 (1958) 231.
[25] M. L. KARNOVSKY AND P. F. H. WALLACH, *J. Biol. Chem.*, 236 (1961) 1895.
[26] L. E. HOKIN AND M. R. HOKIN, *Nature*, 184 (1959) 1068.
[27] M. R. HOKIN, L. E. HOKIN, M. SAFFRAN, A. V. SCHALLY AND B. V. ZIMMERMAN, *J. Biol. Chem.*, 233 (1958) 811.
[28] C. M. REDMAN AND L. E. HOKIN, *J. Biophys. Biochem. Cytol.*, 6 (1959) 207.