Studies on Phospholipids

9. THE COMPOSITION OF RAT-LIVER LECITHINS*

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It is well established that lipid fractions isolated from natural sources are not pure compounds even though they may be homogeneous with respect to the major analytical parameters. This is due to the presence of many molecular species differing only in the fatty acids esterified in the molecule. There is abundant evidence from experiments with [^{32}P]-phosphate that the several forms of, for example, lecithin are metabolically distinct (Rowe, 1959; Collins, 1960; Harris, Robinson & Getz, 1960). This has also been demonstrated by using [^{14}C]acetate, with respect to both phospholipids (Rowe, 1959) and triglycerides (Marinetti, Griffith & Smith, 1962). It is thus important that methods be developed for the complete determination of the composition of lipids, and the work presented below is a preliminary investigation of a possible procedure.

MATERIALS

Animals. Rats (Wistar strain) were given a commercial brand of dog cubes; the fatty acid composition of the lipids in the cubes is shown in Table 1. The [^{32}P]phosphate was injected intraperitoneally as 0.2 mc of $\text{H}_3^{32}\text{PO}_4$ in 0.2 ml. of 1 mM-phosphate buffer, pH 7.4, containing NaCl (0.1 M).

Solvents. All solvents were redistilled before use, and ethanol was the constant-boiling mixture (19:1, v/v) with water.

Chemicals. Diethylaminoethylcellulose (DEAE-cellulose) was obtained from Bio-Rad Laboratories, Calif., U.S.A. *n*-Propyl gallate was laboratory-reagent grade. L- α -Lecithin (dipalmitoyl) was obtained from the California Biochemical Corp., U.S.A.

METHODS

Extraction and dinitrophenylation of lipids. The extraction of lipids and their reaction with 1-fluoro-2,4-dinitrobenzene were as described by Wheeldon & Collins (1957).

Determination of lipid phosphorus. Lipid phosphorus was determined as described by Collins (1959b).

Determination of the purity of lecithin. The purity of the isolated lecithin was tested by using the paper-chromatographic technique of Marinetti (1962).

Measurement of radioactivity. Measurements of radioactivity (see Collins, 1959a) were carried out by using a 10 ml. liquid-counting tube with an efficiency of approx. 10%. Samples were counted either in water as inorganic

phosphate (see Collins, 1959b) or as phospholipids in the solvent mixture used for countercurrent distribution after the addition of ethanol so as to obtain one phase. The two procedures gave identical results. The counting rate varied from background to 1000 counts/min. and, where possible, at least 1000 counts were recorded. With samples with very low radioactivities (i.e. tubes 40–50 and 85–90 in Fig. 1), counting was continued for no more than 10 min. as a lower degree of accuracy was acceptable. All counts were corrected back to a standard time by assuming that the half-life of ^{32}P was 14.3 days. The carrier-free $\text{H}_3^{32}\text{PO}_4$ was diluted with NaH_2PO_4 before transfer from the bottle in which it was received.

Chromatography on diethylaminoethylcellulose. DEAE-cellulose was suspended in 2N-NaOH and poured on to a Buchner funnel, and most of the solution was removed by suction. It was essential that the DEAE-cellulose was kept covered with solution to prevent matting. Then successive amounts of distilled water were added and partially sucked off until the filtrate was neutral. The water was followed by acetone until it was judged that the water had been removed. The suspension was stored as a suspension in

Table 1. Fatty acid composition of the lipids in the rat-colony diet

The lipids of the rat-colony diet (a commercial brand of dog cubes) were subjected to methanolysis and the methyl esters of the fatty acids were isolated and analysed by gas-liquid chromatography. Experimental details are given in the text.

Fatty acid	Amount (moles/100 moles)
Caprylic acid*	0.2
Capric acid*	0.7
Lauric acid*	7.4
Isotetradecanoic acid	0.1
Myristic acid*	5.2
Myristoleic acid	0.4
ante-Isopentadecanoic acid	0.1
<i>n</i> -Pentadecanoic acid	0.3
Isohexadecanoic acid	0.1
Palmitic acid*	24.4
Palmitoleic acid*	1.4
Unknown	0.3
ante-Isoheptadecanoic acid	0.3
<i>n</i> -Heptadecanoic acid	0.6
Unknown	0.2
Stearic acid*	12.1
Oleic acid*	30.4
Linoleic acid*	14.0
Linolenic acid*	1.4

* Part 8: Collins & Shotlander (1961).

* Identified by comparison with authentic samples.

acetone until required for use. When the proper precautions were observed no aggregations were formed.

A column of DEAE-cellulose was prepared in acetone and cooled to -20° in a deep-freeze unit. The DNP-lipids were extracted with three successive amounts of 20 ml. of hot (50°) acetone, and the extract was cooled to -20° before being poured on to the column. On cooling, some material separated out and three acetone treatments were necessary to ensure complete extraction. Any acetone-insoluble material remaining in suspension was filtered off by the column. After all the soluble material had been added to the column, acetone was added until elution of the acetone-soluble material was complete. The acetone-insoluble material was then dissolved in CHCl_3 -ethanol (2:1, v/v) and poured on to the column, which had been removed from the deep-freeze unit and allowed to return to room temperature (18 – 20°). The CHCl_3 -ethanol was added until no more material was eluted. The material remaining on the column was then eluted with CHCl_3 -ethanol-water-triethylamine (4:10:4:1, by vol.). It was found that 0.1 g. of DEAE-cellulose/100 mg. of rat-liver DNP-lipids was a satisfactory proportion and in this case about 100 ml. of each solvent or solvent mixture was adequate for the elution of each fraction.

Countercurrent distribution. This was carried out in an all-glass machine capable of 120 transfers and with a capacity of 20 ml. of each phase. The solvent system used was CCl_4 -methanol-water (124:70:7, by vol.) containing 10 mg. of *n*-propyl gallate/l. as antioxidant. In experiments with and without antioxidant the arachidonic acid contents of a sample of lecithin were respectively 21.0% and 8.5%. The former value agreed with that obtained from lecithin that had not been subjected to countercurrent distribution.

Gas-liquid chromatography. A 6 ft. glass column (4 mm. diam.) was packed with 12% poly(ethylene glycol) adipate on 80–100 mesh Gas Chrom P, which was obtained already mixed from Applied Science Laboratory Inc., State College, Pa., U.S.A. An argon-ionization detector was used with a flow-rate of 40 ml./min., and 2500–3000 theoretical plates were obtained with methyl stearate at 173° . This equipment was standardized with the standard mixtures of methyl esters of fatty acids distributed by the National Institutes of Health, Bethesda, Md., U.S.A., and from purified samples obtained from California Biochemical Research Foundation. Other fatty acids were identified by their relative retention times and comparison with published values. For the fatty acids of longer chain length than arachidonic acid a relatively inefficient (600–700 plates for methyl arachidonate) 6 ft. column of 12% poly(ethyleneglycol) adipate on Gas Chrom P was used. This had been conditioned at 220° for 7 days and was used at 197° . The retention times were less than those obtained on the other column at this temperature. The fatty acids of lower molecular weight come off in the first 15 min. and the methyl arachidonate peak was used as a comparison to relate the chromatography to the results obtained on the more efficient column. In the calculation of the areas under the peaks the height was multiplied by the width of the curve at 0.324 times the height (Knox, 1961). For small peaks the width was calculated by interpolation by using a plot of retention times against the width calculated for the major peaks. This was a straight line (cf. Carroll, 1961), which did not pass through the origin.

Five of the C_{20-22} fatty acids with retention times between those of methyl arachidonate and methyl *n*-docosahexa-

enoate were not identified (see Table 4). Their retention times, relative to that of methyl stearate at 197° , were 3.44, 3.87, 4.63, 6.20 and 6.87.

Preparation of methyl esters. Samples of lipids (corresponding to not less than $0.5 \mu\text{mole}$ of fatty acids) were refluxed in 1.5M-hydrogen chloride in methanol in a stream of oxygen-free nitrogen. At the end of 3 hr. the mixture was evaporated to dryness *in vacuo* and the methyl esters were distilled on to a 'cold finger' at 0.01 mm. Hg. The methyl esters were washed off the 'cold finger' with light petroleum into a 3 ml. conical tube, from which the light petroleum was removed with a stream of nitrogen and the methyl esters were concentrated in the tip of the tube. This procedure is based on that of Stoffel, Chu & Ahrens (1959) and might have yielded dimethylacetals if plasmalogens had been present.

RESULTS

Separation of lecithin. The procedure described in the Methods section was devised to isolate the choline-containing phospholipids rapidly and uncontaminated by other lipids. At first, alumina chromatography (Rhodes & Lea, 1957) was used, but on countercurrent distribution of the material so obtained there was often a small inflexion or peak on the leading edge of the lecithin peak. In samples of lecithin that had been fractionated on DEAE-cellulose this peak was absent (see Fig. 1).

Table 2 shows the analytical results with the DNP-lipids that were used in the present investigation; similar results were obtained in other experiments. In each instance the neutral-fat fraction contained a small amount of phosphorus (about 0.6%) which in the present experiment had a specific radioactivity of 197 as compared with 360 for the lecithin fraction. The purity of the lecithin fraction was judged from its countercurrent distribution curve in carbon tetrachloride-methanol-water. The results are shown in Table 3. The two small peaks with maxima at tubes 11 and 110 account for 2.3% of the phosphorus and these are completely separated from the lecithin. Paper chromatography of all fractions coming within the lecithin peak showed only one spot, corresponding to that of an authentic sample of lecithin, except in tubes 78–98 (Fig. 1) which contained in addition a little sphingomyelin (R_f 0.30; lecithin has R_f 0.36). Lysolecithin has a partition coefficient of 3.14 in this solvent system (Collins, 1963); this corresponds to a maximum at tube 91.

Analysis of lecithin. The contents of every third tube from 45 to 84 (Fig. 1) were analysed for phosphorus and radioactivity, and the contents of every third tube from 47 to 80 were subjected to methanolysis and the methyl esters of the fatty acids analysed by gas-liquid chromatography. The analyses of these fractions are shown in Table 4. Fig. 1 shows the distribution of phosphorus and radioactivity, and Fig. 2 shows the distribution of

Table 2. *Separation of lipid fractions on diethylaminoethylcellulose*

The DNP-lipids were fractionated by the use of a DEAE-cellulose column at -20° from which the neutral fat only was eluted with acetone. The temperature was then raised to 20° and the neutral phospholipids were eluted with CHCl_3 -ethanol. The remainder of the phospholipids were eluted with CHCl_3 -ethanol-water-triethylamine. Experimental details are given in the text.

Fraction	Eluting solvent	Amount (mg.)	P content ($\mu\text{g. atoms}$)	DNP content (μmoles)	Total radio-activity (counts/min.)	Specific radio-activity (counts/min./ $\mu\text{g. atom of P}$)
Neutral fat	Acetone at -20°	118.3	2.4	—	472	197
Lecithin	CHCl_3 -ethanol at 20°	170.6	190.4	—	74 600	392
Remainder of phospholipids	CHCl_3 -ethanol-water-triethylamine	187.8	170.0	120.5	486 000	2 860
		476.7	362.8	120.5	561 100	1 550

Table 3. *Countercurrent distribution of the lecithin fraction in carbon tetrachloride-methanol-water*

The neutral phospholipids (see Table 2) were distributed in CCl_4 -methanol-water, and P and radioactivity were determined at intervals and the totals shown below were computed from the areas under their respective graphs. Experimental details are given in the text.

Fraction	Partition coefficient	P content ($\mu\text{g. atoms}$)	Total radioactivity (counts/min.)	Specific radioactivity (counts/min./ $\mu\text{g. atom of P}$)
1	0.09	0.8	5 700	7 100
2	1.18	186.0	67 100	360
3	8.2	3.6	1 800	500
		190.4	74 600	392

Table 4. *Fatty acid composition of lecithin distributed in carbon tetrachloride-methanol-water*

The methyl esters of the fatty acids were prepared from the lipids in the tubes shown and analysed by gas-liquid chromatography. Experimental details are given in the text. N.D., Not determined; —, not detected.

Tube no. ...	Amount (moles/100 moles)											
	47	50	53	56	59	62	65	68	71	74	77	80
Fatty acid												
Myristic acid*	—	—	—	—	—	—	0.2	0.2	0.2	0.4	0.6	1.3
<i>n</i> -Pentadecanoic acid	—	—	—	—	—	—	0.2	0.3	0.3	0.7	0.6	0.9
Palmitic acid*	28.5	11.1	5.0	5.9	8.3	12.8	17.8	25.5	35.0	40.4	38.5	41.6
Palmitoleic acid*	—	—	—	—	—	—	0.8	0.9	1.4	1.8	2.2	4.1
<i>n</i> -Heptadecanoic acid	—	—	—	0.4	0.5	0.7	1.0	0.7	0.5	0.5	1.1	0.6
Stearic acid*	35.8	51.2	50.9	45.3	42.8	34.1	26.0	20.8	13.5	8.3	9.2	13.5
Oleic acid*	18.9	10.3	6.3	6.9	7.8	7.5	9.1	9.0	8.8	8.1	8.3	10.0
Linoleic acid*	8.4	7.2	6.3	9.5	9.8	14.3	16.1	18.5	18.5	21.3	21.7	18.2
<i>n</i> -Eicosatrienoic acid	—	0.2	1.7	1.4	1.1	1.1	0.9	1.0	0.2	0.4	0.4	—
Arachidonic acid*	8.4	20.0	23.2	25.7	24.9	26.1	23.9	19.8	15.7	12.8	11.7	9.9
<i>n</i> -Eicosapentaenoic acid	N.D.	N.D.	—	—	—	—	—	—	0.5	0.9	1.0	N.D.
Unidentified	N.D.	N.D.	0.4	—	0.7	0.9	0.6	1.0	1.1	1.1	0.7	N.D.
Unidentified	N.D.	N.D.	—	—	—	—	—	0.2	0.6	0.8	1.1	N.D.
Unidentified	N.D.	N.D.	0.5	0.3	0.4	—	0.8	0.3	0.9	1.1	0.9	N.D.
Unidentified	N.D.	N.D.	0.6	0.3	0.5	—	—	0.3	0.8	0.4	1.3	N.D.
<i>n</i> -Docosahexaenoic acid	N.D.	N.D.	4.5	4.5	3.2	2.6	2.4	1.5	1.5	1.2	0.6	N.D.
Total saturated acids	64.3	62.3	55.9	51.6	51.6	47.6	50.5	47.5	49.5	50.3	50.0	57.9

* Identified by comparison with authentic samples.

fatty acids. The distribution of lecithins containing stearic acid (partition coefficient = 1.08) is different from those containing palmitic acid (partition coefficient = 1.38). However, both these curves are broader than required by theory, implying that they still represent the sum of several curves. This conclusion is also true of the distributions of the unsaturated fatty acids with the exception of palmitoleic acid. Comparison of Figs. 1 and 2 also shows that the curve for radioactivity (partition coefficient = 1.31) follows closely the distribution of palmitic acid and indicates that it is the sum

Fig. 1. Countercurrent distribution of rat-liver lecithins in carbon tetrachloride-methanol-water. Every third tube was analysed for phosphorus (continuous line) and radioactivity (broken line). Experimental details are given in the text.

Fig. 2. Countercurrent distribution of rat-liver lecithins in carbon tetrachloride-methanol-water showing the distribution of the individual fatty acids. Every third tube was analysed for palmitic acid (· · · · ·), stearic acid (—), palmitoleic acid (— · — · —), oleic acid (— — — — —), linoleic acid (— — — — —), arachidonic acid (— · · · · —) and docosahexaenoic acid (— · — · — · —). Experimental details are given in the text.

of several curves. To a first approximation it is possible to say that most of the radioactivity is associated with lecithins containing palmitic acid. Stearic acid, arachidonic acid, eicosatrienoic acid and docosahexaenoic acid are present in their maximal proportions in the region of tubes 53–56; palmitic acid, palmitoleic acid, linoleic acid and the minor C_{20-22} acids occur maximally in tubes 74–77; oleic acid is nearly equally divided.

Mathematical analysis of the distribution of the lecithins. Attention has been concentrated on fractions 53–77 because (1) the percentage of saturated fatty acids is approximately constant (50.5 ± 0.84) within this range and (2) the size of the sample is smaller outside this range (less than $4 \mu\text{moles}$ of fatty acids), which makes the determination of the higher-molecular-weight polyene acids difficult. A number of assumptions have been made (the validity of which is considered in the Discussion section) and on this basis an attempt has been made to calculate the proportions of the lecithins and their specific radioactivities. Because the proportion of saturated fatty acids is so close to 50% it can be assumed that the findings of Tattre (1959) and Hanahan, Brockerhoff & Barron (1960) are true for rat-liver lecithin. Thus it is assumed that in each molecule of lecithin the fatty acid on C-3 of the glycerol residue is saturated and that on C-2 is unsaturated. The proportions of the saturated fatty acids other than palmitic acid and stearic acid amount to no more than 1.0% and these acids will be neglected, as also will be the minor unsaturated fatty acids which account for 2.8%. It follows that because there are only two saturated fatty acids the distribution curves of each of the unsaturated fatty acids must be the sum of only two component distributions, that of the stearyl ester and that of the palmitoyl ester. As a distribution curve depends on only two parameters, the amount of the substance and its partition coefficient, in principle it should be possible to find the best fit for each parameter that satisfies the results. In practice the equations are non-linear and are very difficult to solve. In order to obtain some approximation it has been assumed (on the basis of Fig. 2) that the partition coefficients of the stearyl lecithins are 1.08 and those of the palmitoyl lecithins are 1.38. By using the method of least squares (Levi, 1958) the proportions of each unsaturated fatty acid occurring as the stearyl lecithin and as the palmitoyl lecithin were calculated, with the results shown in Table 5. Also in Table 5 is a comparison with the proportions of the fatty acids in the total mixture of lecithins. Although the agreement with the unsaturated fatty acids is good (as is to be expected from the method of calculation) the discrepancies with stearic acid and palmitic acid are greater. However, allowance has to be made for the effects

Table 5. *Calculated composition of rat-liver lecithin*

The composition of the lecithins was calculated on the assumption that (1) each lecithin molecule had one saturated and one unsaturated fatty acid, (2) that the only saturated fatty acids present were stearic acid and palmitic acid, and (3) that the partition coefficients were either 1.08 for the stearyl lecithins or 1.38 for the palmitoyl lecithins. Experimental details are given in the text.

	Amount (moles/100 moles)			
	Stearyl	Palmitoyl	Total unsaturated acids	Found
Docosaheptaenoyl	3.7 ± 0.25	0.4 ± 0.24	2.05 ± 0.17	2.2
Arachidonoyl	32.3 ± 1.90	9.6 ± 1.84	21.05 ± 1.32	21.0
Linoleoyl	13.2 ± 0.52	18.4 ± 0.51	15.8 ± 0.34	15.6
Oleoyl	9.8 ± 0.77	7.4 ± 0.74	8.6 ± 0.53	8.4
Palmitoleoyl	0.0	1.4 ± 0.16	0.7 ± 0.08	0.7
Total saturated acids:	29.0 ± 0.93	18.5 ± 1.02		
Found:	27.0	21.3		
	Minor saturated fatty acids: 1.0			
	Minor unsaturated fatty acids: 2.8			

of the minor acids as well as for the overall accuracy of the gas-liquid-chromatographic analyses. Within these limits the agreement is satisfactory.

By making the assumption that all the stearyl lecithins have the same partition coefficient, as also the palmitoyl lecithins, it can be calculated, again by the method of least squares, that the average specific radioactivity of the two groups of lecithins are respectively 81 ± 32 and 756 ± 24 counts/min./ $\mu\text{g.}$ atom of phosphorus.

DISCUSSION

The diet on which the rats were fed was low in linolenic acid, and thus the proportions of C_{20} and C_{22} acids derived from linolenic acid were also low (Beare, 1961; Klenk & Mohrhauser, 1960). This circumstance ensured that five fatty acids made up 93.5% of the liver-lecithin fatty acids and this simplified the present investigation.

The method adopted for the separation of the lecithin was based on the application of two procedures: chromatography with cold acetone for the separation of acetone-insoluble and acetone-soluble lipids (Collins & Shotlander, 1960) and the use of DEAE-cellulose (Rousser, Bauman, Kritchevsky, Heller & O'Brien, 1961). Three modifications of the method described by Rousser *et al.* (1961) were introduced: (1) the use of DNP-lipids, (2) the use of ethanol instead of methanol, and (3) the use of the basic form of DEAE-cellulose instead of the acetate form. DNP-lipids are acids with no basic groups and this permits the use of shorter columns. The use of ethanol [ethanol-water (19:1, v/v)] in the place of methanol ensured that the ionic exchange with the column is quicker because of the presence of water. The separation described above is merely the separation of the neutral compounds (and in theory also basic compounds) from acids. The

chromatography with cold acetone separates the neutral fat from the acetone-insoluble lipids.

The portion of the countercurrent distribution that was used in the calculation was shown to contain only lecithin by paper chromatography, and it is known that lecithin plasmalogens are low in rat liver (Getz, Bartley, Stirpe, Notton & Renshaw, 1961). There is direct evidence for all the assumptions made in calculating the results in Table 5 except for the assumption that the lecithins present had partition coefficients of either 1.08 or 1.38. This is certainly not true, as is shown by the broadness of the palmitic acid and stearic acid distributions, but the fair agreement obtained between the calculated and the experimental values for total stearic acid and palmitic acid indicates that the partition coefficients cannot be greatly different from either 1.08 or 1.38. However, it is difficult to predict how great is the resulting error in the proportions of the individual lecithins, and the results in Table 5 must be regarded as a first approximation.

The fact that stearyl arachidonoyl lecithin comprises nearly a third of the total lecithin and more than half of the stearyl lecithins seems clearly established. It is also clear that docosaheptaenoic acid occurs mainly as the stearyl lecithin but that palmitoleic acid is present exclusively as the palmitoyl lecithin. Oleic acid and linoleic acid are more nearly equally divided. The significance of this distribution cannot be discussed without considering the specific radioactivities.

Evidence that the different lecithins exchange at different rates with inorganic [^{32}P]phosphate and [^{14}C]acetate has been presented by Rowe (1959), Collins (1960) and Harris *et al.* (1960). Marinetti *et al.* (1962) have also shown that the specific radioactivities of the triglycerides labelled by exchange with [^{14}C]acetate cover a range of values. The assumption that the partition coefficients of the lecithins

thins can have only two values precludes a calculation of their specific radioactivities by the method of least squares. The fact that the stearoyl lecithins average 81 ± 32 counts/min./ $\mu\text{g.}$ atom of phosphorus as compared with 756 ± 24 counts/min./ $\mu\text{g.}$ atom of phosphorus for the palmitoyl lecithins shows that there are marked differences in the rates of exchange with inorganic phosphate.

It is possible to speculate on the radioactivity experiments by combining the present result with those obtained by Collins (1962), who compared liver lecithins obtained from a normal rat and from a rat deficient in essential fatty acids. The rats were injected with inorganic [^{32}P]phosphate an hour before death and the specific radioactivities and fatty acid composition of the lecithins compared. It was found that the differences in specific radioactivities could be explained if the arachidonoyl lecithins had a mean specific radioactivity 0.0655 that of the remainder of the lecithins. If this result of Collins (1962) were true of the present sample of lecithin then it can be calculated that respective specific radioactivities of stearoyl arachidonoyl lecithin, palmitoyl arachidonoyl lecithin, the remainder of the stearoyl lecithins and the remainder of the palmitoyl lecithins are approximately 20 ± 6 , 80 ± 30 , 160 ± 70 and 1000 ± 120 counts/min./ $\mu\text{g.}$ atom of phosphorus. These values, if true, are so striking that it is clearly of importance to devise methods for the accurate determination of the specific radioactivities of all the individual lecithins and other phospholipids in order to obtain a better understanding of phospholipid metabolism.

The need for such methods has been stressed by Dittmer & Hanahan (1959), who showed that [^{14}C]palmitic acid was more rapidly incorporated into the lipids of rat liver than was [^{14}C]stearic acid. Other workers have reported that palmitic acid (as palmitoyl-coenzyme A) is generally more reactive in the biosynthesis of triglycerides from diglycerides (Goldman & Vagelos, 1961), and this may reflect the general metabolic role of palmitic acid that is implied by the present results.

Whether the arachidonic acid acts as a modulator of phospholipid turnover as suggested by Collins (1962) cannot be settled at present, but the relative rates of turnover of the individual phospholipids may indicate that their roles in metabolism and cell structure can vary widely according to their fatty acid composition.

SUMMARY

1. Lecithin was isolated from dinitrophenylated rat-liver lipids by chromatography on diethylaminoethylcellulose, with cold acetone as solvent.

2. The lecithin fraction was purified by counter-current distribution in carbon tetrachloride-methanol-water and analysed for phosphorus and fatty acid.

3. A mathematical analysis enabled an estimate of the probable complete composition to be made. Stearoyl arachidonoyl lecithin was the major component.

4. After exchange of inorganic [^{32}P]phosphate for 1 hr., the stearoyl lecithins were calculated to have an average specific radioactivity of 81 ± 32 counts/min./ $\mu\text{g.}$ atom of phosphorus as compared with 756 ± 24 counts/min./ $\mu\text{g.}$ atom of phosphorus for the palmitoyl lecithins.

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REFERENCES

- Beare, J. L. (1961). *Canad. J. Biochem.* **39**, 1855.
- Carroll, K. K. (1961). *Nature, Lond.*, **191**, 377.
- Collins, F. D. (1959a). *Biochem. J.* **72**, 281.
- Collins, F. D. (1959b). *Biochem. J.* **72**, 532.
- Collins, F. D. (1960). *Nature, Lond.*, **186**, 366.
- Collins, F. D. (1962). *Biochem. biophys. Res. Commun.* **9**, 289.
- Collins, F. D. (1963). *Biochem. J.* (in the Press).
- Collins, F. D. & Shotlander, V. L. (1960). *J. Lipid Res.* **1**, 352.
- Collins, F. D. & Shotlander, V. L. (1961). *Biochem. J.* **79**, 321.
- Dittmer, J. C. & Hanahan, D. J. (1959). *J. biol. Chem.* **234**, 1983.
- Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M. & Renshaw, A. (1961). *Biochem. J.* **80**, 176.
- Goldman, P. & Vagelos, P. R. (1961). *J. biol. Chem.* **236**, 2620.
- Hanahan, D. J., Brookerhoff, H. & Barron, E. J. (1960). *J. biol. Chem.* **235**, 1917.
- Harris, P. M., Robinson, D. S. & Getz, G. (1960). *Nature, Lond.*, **188**, 742.
- Klenk, E. & Mohrhauer, H. (1960). *Hoppe-Seyl. Z.* **320**, 218.
- Knox, J. H. (1961). *J. chem. Soc.* p. 423.
- Levi, A. A. (1958). *Biochem. J.* **69**, 516.
- Marinetti, G. V. (1962). *J. Lipid Res.* **3**, 1.
- Marinetti, G. V., Griffith, M. & Smith, J. (1962). *Biochim. biophys. Acta*, **57**, 543.
- Rhodes, D. N. & Lea, C. H. (1957). *Biochem. J.* **65**, 526.
- Roussier, G., Bauman, A. J., Kritchevsky, G., Heller, D. & O'Brien, J. S. (1961). *J. Amer. Oil Chem. Soc.* **38**, 544.
- Rowe, C. F. (1959). *Biochem. J.* **73**, 438.
- Stoffel, W., Chu, F. & Ahrens, E. H. (1959). *Analyt. Chem.* **31**, 307.
- Tattree, N. H. (1959). *J. Lipid Res.* **1**, 60.
- Wheeldon, L. W. & Collins, F. D. (1957). *Biochem. J.* **66**, 435.