Biochem. J. (1974) **140**, 503–508
Printed in Great Britain

The Mechanism of Intestinal Absorption of Phosphatidylcholine in Rats

By SAMPATH PARTHASARATHY, PAPASANI V. SUBBAIAH*
and
JAGANNATH GANGULY
Department of Biochemistry, Indian Institute of Science, Bangalore-560012, India

(*Received* 30 *January* 1974)

1. The mechanism of absorption of phosphatidylcholine was studied in rats by injecting into the intestine phosphatidylcholine specifically labelled either in the fatty acid or in the glycerol moiety or with ^{32}P, when considerable amounts of 1-acyl-lysophosphatidylcholine were found in the intestinal lumen. 2-([^{14}C]Acyl)phosphatidylcholine gave markedly more radioactive unesterified fatty acids in the lumen, compared with the 1-([^{14}C]acyl) derivative. Some of the radioactivity from either the fatty acid or the glycerol moiety of the injected phosphatidylcholine appeared in the mucosal triacylglycerols. 2. Injection of ^{32}P-labelled phosphatidylcholine or ^{32}P-labelled lysophosphatidylcholine led to the appearance of radioactive glycerylphosphorylcholine, glycerophosphate and P_i in the mucosa. 3. Rat mucosa was found to contain a highly active glycerylphosphorylcholine diesterase. 4. It was concluded that the dietary phosphatidylcholine is hydrolysed in the intestinal lumen by the pancreatic phospholipase A to 1-acylglycerylphosphorylcholine, which on entering the mucosal cell is partly reacylated to phosphatidylcholine, and the rest is further hydrolysed to glycerylphosphorylcholine, glycerophosphate, glycerol and P_i. The fatty acids and glycerophosphate are then reassembled to give triacylglycerols via the Kennedy (1961) pathway.

Phosphatidylcholine is one of the major constituents of dietary lipids, and it is also secreted into the intestine through the bile in considerable quantities. Although work on the absorption of triacylglycerols has been very extensive, only in recent years has information been available on the fate of phosphatidylcholine during absorption. By using everted intestinal sacs and slices of hamster intestine Nilsson & Borgstrom (1967) showed that lysophosphatidylcholine is absorbed more efficiently than phosphatidylcholine and is mainly reacylated to phosphatidylcholine. More extensive information was provided by Scow *et al.* (1967). By feeding phosphatidylcholine specifically labelled in the fatty acid at the 1- or 2-position, or the choline moiety or with ^{32}P to lymph-fistula rats they showed that, irrespective of whether the radioactive fatty acid was at the 1- or 2-position of the phosphatidylcholine molecule, about 80% of the original radioactivity appeared in the lymph triacylglycerols. They also observed that whereas the fatty acid at the 2-position of the phosphatidylcholine that is absorbed as phosphatidylcholine undergoes hydrolysis and re-esterification, that at the 1-position remains virtually unchanged. In similar experiments Nilsson (1968) arrived at almost identical conclusions.

By using phosphatidylcholine and lysophosphatidylcholine labelled in the fatty acid or glycerol moieties or with ^{32}P we show here that during absorption phosphatidylcholine is mainly hydrolysed in the intestinal lumen to 1-acylglycerylphosphorylcholine. On entering the mucosal cell this is partly reacylated to phosphatidylcholine and the remainder is further hydrolysed to glycerylphosphorylcholine and then to glycerophosphate. The glycerophosphate and unesterified fatty acids are reassembled to form triacylglycerols *via* the Kennedy (1961) pathway.

* Present address: Department of Botany, University of Texas, Austin, Tex. 78712, U.S.A.

Materials and Methods

Materials

$H_3{}^{32}PO_4$ and sodium [1-^{14}C]acetate were obtained from the Bhabha Atomic Research Centre, Bombay, India. [1-^{14}C]Linoleic acid and [1-^{14}C]glycerol were supplied by The Radiochemical Centre, Amersham, Bucks., U.K.

Phosphatidylcholine, lysophosphatidylcholine, ^{32}P-labelled phosphatidylcholine, ^{32}P-labelled lysophosphatidylcholine and 1-([^{14}C]acyl)glycerylphosphorylcholine were prepared as described by Subbaiah & Ganguly (1971). 1-([^{14}C]Acyl)phosphatidylcholine and 2-[1-^{14}C]linoleoyl-phosphatidylcholine were prepared by acylating 1-([^{14}C]acyl)-glycerylphosphorylcholine with non-radioactive linoleic acid, and non-radioactive 1-acylglyceryl-phosphorylcholine with [1-^{14}C]linoleic acid respectively, by the method of Robertson & Lands (1962).

[^{14}C]Glycerol-labelled phosphatidylcholine was prepared as described by Parthasarathy & Ganguly (1973), and ^{32}P-labelled glycerylphosphorylcholine was obtained by mild alkaline hydrolysis of ^{32}P-labelled phosphatidylcholine.

When analysed by chromatography (as described below) all radioactive substances gave spots corresponding to those of authentic samples.

Methods

Treatment of animals and analysis of the lipids. Details of the preparation of lipid dispersions, the administration of the test materials and removal of the tissues were as described by Raghavan & Ganguly (1969).

The lipids from the intestinal contents and tissues were extracted by the method of Bligh & Dyer (1959). This method extracts at least 90% of ^{32}P-labelled lysophosphatides. The phospholipids and neutral lipids were separated on silica-gel plates as described by Subbaiah *et al.* (1968).

Separation and identification of the water-soluble compounds derived from phosphatidylcholine. After extraction of the lipids by the above method the upper aqueous phase was concentrated *in vacuo* and applied to a column (30cm×1cm) of Dowex 1 (formate form), after which the column was eluted with 100ml of 250mM-ammonium formate containing 5mM-sodium tetraborate. The eluate was passed through a column (5cm×1cm) of a mixture of Dowex resins (H^+ and Cl^- forms) which was then washed with 50ml of ion-free water. The eluate and the washings were pooled, concentrated *in vacuo* and chromatographed on acid-washed Whatman no. 1 paper as described by Dawson (1967).

Fractionation of mucosal cells and assays of glycerylphosphorylcholine diesterase (sn-glyceryl-3-phosphorylcholine glycerophosphohydrolase, EC 3.1.4.2). The intestinal mucosa was fractionated into the nuclear plus mitochondrial, microsomal and soluble fractions (Procedure I, Table 3) by the method of Schneider & Hogeboom (1950) as modified by Murthy *et al.* (1963). These crude preparations were not further characterized. The brush border, brush-border-free particles and particle-free supernatant fractions were prepared (Procedure II, Table 3) by the method of David *et al.* (1966) which was based on that of Miller & Crane (1961). However, it has been our experience that the EDTA used in the latter procedure must be removed by exhaustive dialysis, otherwise the assay of glycerylphosphorylcholine diesterase activity is unsatisfactory and unreliable.

The diesterase was assayed as described by Lloyd-Davies *et al.* (1972). The radioactivity of the products was determined by drying suitable portions of the eluate on strips of filter papers.

Determination of radioactivity. When singly labelled substances were used, the spots corresponding to those of the samples were scraped off the t.l.c. plates into vials and the radioactivity was determined with a Beckman LS-100 liquid-scintillation counter by using 10ml of toluene containing 0.5% (w/v) of 2,5-diphenyloxazole as the scintillation fluid. The spots from the paper chromatograms were cut into small pieces, placed in vials and the radioactivity was determined as described above. When doubly labelled compounds were used, the samples were eluted from the t.l.c. plates as described by Abramson & Blecher (1964), after which the eluates were dried in the vials in a stream of air. More than 95% of the phospholipids were recovered by this method. The radioactivity was then determined by using the appropriate channels in 10ml of the scintillation fluid and necessary corrections were applied for any shift of the ^{32}P radioactivity into the ^{14}C channel. This scintillation fluid gave up to 90% efficiency with both ^{32}P- and ^{14}C-labelled compounds in the scrapings from t.l.c. plates.

Lipid phosphorus was measured by the method of Bartlett (1959) as modified by Marinetti (1962). The method of Lowry *et al.* (1951) was used to determine proteins with crystalline bovine serum albumin as the standard.

Results

Experiments with 1-([^{14}C]*acyl*)- *or* 2-([^{14}C]*acyl*)-^{32}*P*]-*phosphatidylcholine and* 1-([^{14}C]*acyl*)*glyceryl*[^{32}*P*]-*phosphorylcholine*

Table 1 shows that at 60min after an injection of phosphatidylcholine with radioactive fatty acid labelled at either position into the intestine there was considerable hydrolysis of the injected material, leading to the appearance of radioactive lysophosphatidylcholine and radioactive unesterified fatty acids in the lumen as well as in the mucosa. It should, however, be noted that the 2-([^{14}C]acyl)-labelled compound gave more of the radioactive unesterified fatty acids and correspondingly less of the radioactive lysophosphatidylcholine in the lumen as well as in the mucosa, as compared with the 1-([^{14}C]acyl)-labelled derivative. Both compounds also led to the appearance of significant amounts of radioactive triacylglycerols in the mucosa, with the 2-([^{14}C]acyl) derivative giving markedly more of radioactive triacylglycerols. When similar experiments were attempted with 1-([^{14}C]acyl)glyceryl-[^{32}P]phosphorylcholine, very small amounts of the injected material could be detected in the intestine at 60min after the injection (see also Table 2). Therefore these experiments were carried out at 30min, and Table 1 shows that even at this time-interval the amounts of radioactive unesterified fatty

Table 1. *Fate of the radioactive fatty acid of* 1- *or* 2-([^{14}C]*acyl*)[^{32}P]*phosphatidylcholine or of* 1-([^{14}C]*acyl*)*glyceryl*[^{32}P]*phosphorylcholine when introduced into the duodenum of rats*

A portion (20μmol) of the radioactive compound with a ^{14}C/^{32}P ratio of 1.00 and specific radioactivity (of ^{14}C or ^{32}P) of 6000c.p.m./μmol was injected into the rats in each experiment. The animals receiving phosphatidylcholine were killed at 60min after the injection, and those given lysophosphatidylcholine were killed at 30min. The values are averages obtained with six or more separate animals and are expressed as μmol of the ^{14}C-labelled compound. The values in parentheses indicate the ^{14}C/^{32}P ratios.

	Intestinal lumen				Intestinal mucosa			
Compound administered	Phosphatidylcholine	Lysophosphatidylcholine	Unesterified fatty acids	Triacylglycerols	Phosphatidylcholine	Lysophosphatidylcholine	Unesterified fatty acids	Triacylglycerols
2-([^{14}C]Acyl)-[^{32}P]phosphatidylcholine	5.8 (1.1)	0.23 (0.17)	0.71	0.02	2.1 (1.05)	0.32 (0.17)	0.5	2.7
1-([^{14}C]Acyl)-[^{32}P]phosphatidylcholine	5.75 (1.00)	2.35 (1.1)	0.13	—	1.70 (0.94)	0.18 (1.05)	0.15	0.95
1-([^{14}C]Acyl)glyceryl[^{32}P]-phosphorylcholine	—	4.5 (1.05)	2.10	0.05	1.55 (1.00)	0.9 (1.20)	0.7	2.55

Table 2. *Distribution of* ^{32}*P radioactivity at* 60*min after intraduodenal injection of* 20μ*mol of* [^{32}P]*phosphatidylcholine* (*sp. radioactivity* 15000*c.p.m./*μ*mol of P*) *or lyso*[^{32}P]*phosphatidylcholine* (*sp. radioactivity* 16000*c.p.m./*μ*mol of P*)

The methods of separation of the water-soluble compounds are described in the text. Values are expressed as percentages of the administered radioactivity (c.p.m.) and are means of results from four experiments with four animals.

	Intestinal lumen					Intestinal mucosa				
Compound administered	Phosphatidylcholine	Lysophosphatidylcholine	Glyceryl-phosphorylcholine	Glycerophosphate	P_i	Phosphatidylcholine	Lysophosphatidylcholine	Glyceryl-phosphorylcholine	Glycerophosphate	P_i
[^{32}P]Phosphatidylcholine	22.5	16.5	7.0	0.2	0.5	9.2	0.85	4.1	2.6	5.1
Lyso[^{32}P]phosphatidylcholine	0.6	5.7	3.3	0.44	0.6	13.4	2.8	7.4	2.6	8.1

acids were markedly higher in the intestinal lumen, and significant amounts of radioactive unesterified fatty acids and triacylglycerols were found in the mucosa.

The values for the ^{14}C/^{32}P ratio given in Table 1 show that when 2-([^{14}C]acyl)[^{32}P]phosphatidylcholine was used, although the ratio for the phosphatidylcholine of both lumen and mucosa was virtually similar, that of the lysophosphatidylcholine was markedly less in both lumen and mucosa. In sharp contrast the ratio did not change much when the radioactive fatty acid was labelled at the 1-position of either phosphatidylcholine or lysophosphatidylcholine.

Table 2 shows that, in both cases, although glycerylphosphorylcholine was the major radioactive water-soluble species in the lumen, in the mucosa the radioactivity was more evenly distributed between glycerylphosphorylcholine, glycerophosphate and P_i. Table 2 further shows that compared with the radioactivity of phosphatidylcholine, that of lysophosphatidylcholine virtually disappeared from the lumen, and that, although phosphatidylcholine gave rise to more radioactive glycerylphosphorylcholine in the lumen, more radioactive glycerylphosphorylcholine and P_i were formed in the mucosa from lysophosphatidylcholine. It is thus clear that lysophosphatidylcholine is more rapidly absorbed and hydrolysed to glycerylphosphorylcholine in the mucosal cell.

Glycerylphosphorylcholine diesterase activity in rat intestinal mucosa

An enzyme system that can hydrolyse glycerylphosphorylcholine to glycerophosphate and choline has been shown in several animal tissues. In their work on the distribution of this enzyme system in various tissues of the rat, Baldwin & Cornatzer (1968) found the intestine to contain least of this

activity. Since in our present work considerable amounts of ^{32}P radioactivity appeared in the intestine in the form of glycerophosphate and P_i after administration of ^{32}P-labelled phosphatidylcholine or lysophosphatidylcholine, attempts were made to determine the activity of this enzyme in the intestine. Table 3 shows that it is highly active in the mucosa and is mostly concentrated in the microsomal fractions or in the brush-border-free particulate materials of the mucosal cell.

Table 3. *Intracellular distribution of glycerylphosphorylcholine diesterase in rat intestinal mucosa*

The preparation of the crude subcellular fractions of the mucosa and assay of the glycerylphosphorylcholine diesterase activity are described in the text. Details of procedures I and II are given in the Materials and Methods section.

Fraction	Total activity (% recovered)	Sp. activity (μmol of glycerylphosphorylcholine hydrolysed/h per mg of protein)
Procedure I		
Whole homogenate	100	1.75
Nuclear + mitochondrial fractions	14	0.8
Microsomal fraction	71	9.2
Soluble	13.5	1.1
Procedure II		
Whole homogenate	100	1.8
Brush-border fraction	11	1.1
Brush-border-free particles	69	4.8
Particle-free supernatant	15	0.4

Fate of [^{14}C]glycerol-labelled phosphatidylcholine and triacylglycerols in the intestine

Table 4 shows that at 60min after the [^{14}C]glycerol-labelled phosphatidylcholine was administered, of 52% of the original radioactivity recovered only a very small proportion was incorporated into the triacylglycerols of the mucosa, whereas the rest was present mostly as phosphatidylcholine and lysophosphatidylcholine in the lumen as well as in the mucosa. However, at 180min, although there was a decrease in the total recovery to 26–33% of the injected radioactivity, the incorporation of the [^{14}C]-glycerol into the triacylglycerol fraction of the mucosa was considerably higher. This amount of radioactivity in the neutral-lipids fraction must be considered significant, because under these experimental conditions the synthesized triacylglycerols are not allowed to accumulate in the mucosa, but are constantly removed through the lymph. By contrast, when comparable amounts of the glycerol-labelled triacylglycerols were given, even at 60min markedly higher amounts of the radioactivity appeared in the mucosal triacylglycerols.

Discussion

In a review on the intestinal absorption of lipids Ganguly *et al.* (1972) have pointed out that, to be able to enter the mucosal cell, lipids must be brought into water solution in the form of micellar aggregates and that this is accomplished by their partial or complete hydrolysis; after being absorbed into the mucosal cell the hydrolysed lipids are re-esterified to their parent compounds.

Hoffmann & Borgstrom (1962) have suggested that such a mechanism operates during the absorption

Table 4. *Effect of intraduodenal administration of 20 μmol of [^{14}C]glycerol-labelled phosphatidylcholine (sp. radioactivity 6000 c.p.m./μmol of P), and 20 μmol of [^{14}C]glycerol-labelled triacylglycerols on the appearance of the radioactivity in various lipid fractions of the lumen and mucosa*

Values are expressed as c.p.m. per whole sample. The values in parentheses represent percentages of the administered radioactivity.

Compound administered	Time (min)	Intestinal lumen: Phosphatidylcholine	Intestinal lumen: Lysophosphatidylcholine	Intestinal lumen: Triacylglycerol	Intestinal mucosa: Phosphatidylcholine	Intestinal mucosa: Lysophosphatidylcholine	Intestinal mucosa: Triacylglycerol	Total recovery of administered radioactivity (%)
[^{14}C]Glycerol-labelled phosphatidylcholine	60	39500 (31.6)		—	23500 (18.8)	1395 (1.1)	900 (0.78)	52
	180	16000 (10.7)	7040 (4.7)	860 (0.6)	10600 (7.1)	640 (0.41)	3400 (2.2)	26
	180	20800 (13.8)	9200 (6.1)	—	13320 (8.9)	2600 (1.7)	3550 (2.3)	33
[^{14}C]Glycerol-labelled triacylglycerol	60	—	—	55000 (45.8)	—	—	24500 (20.4)	66.2

of triacylglycerols, and their views have been confirmed by Porter & Saunders (1971). On the other hand Ganguly *et al.* (1972) have shown that cholesteryl esters and retinyl esters are also absorbed by a similar mechanism. However, no such attempts appear to have been made with regard to absorption of phosphatidylcholine. In the present work we attempted to investigate the mechanism by which phosphatidylcholine is absorbed, and also the mechanism by which it is converted into triacylglycerols of the lymph.

We have shown here that injection into the intestine of phosphatidylcholine containing a radioactive fatty acid labelled at the 2-position is followed by extensive hydrolysis of the injected compound, giving rise to considerable amounts of radioactive unesterified fatty acids and unlabelled lysophosphatidylcholine. By contrast, when the radioactive fatty acid was labelled at the 1-position of the injected phosphatidylcholine less of the radioactive unesterified fatty acids and comparatively more of the radioactive lysophosphatidylcholine appeared in the lumen of the intestine. These results therefore agree with the well-established concepts as to the action of the pancreatic phospholipase A on phosphatidylcholine.

The present results also show that irrespective of the position occupied by the radioactive fatty acid in the administered phosphatidylcholine most of the radioactivity appears in the triacylglycerols of the mucosal cell, which agrees with the observations of Scow *et al.* (1967) and of Nilsson (1968). Ganguly *et al.* (1972) have shown that lysophosphatidylcholine can be metabolized in the mucosal cell by three enzymes, namely lysophosphatidylcholine acyltransferase (EC 2.3.1.23), lysophospholipase (EC 3.1.1.5) and lysophosphatidylcholine dismutase. Clearly, the acyltransferase catalyses the re-acylation of lysophosphatidylcholine inside the mucosal cell and thus accounts for the phosphatidylcholine found in the lymph. But the lysophospholipase has a very high specific activity, and therefore can readily hydrolyse lysophosphatidylcholine to unesterified fatty acids and glycerylphosphorylcholine. This has been fully borne out by the experiments with 1-([^{14}C]-acyl)glycerylphosphorylcholine, because not only did the injected lysophosphatidylcholine disappear very rapidly, but even at 30min after the injection more of the radioactive triacylglycerols were found in the mucosa.

In those experiments where ^{32}P-labelled phosphatidylcholine was given, considerable amounts of ^{32}P-labelled water-soluble products appeared in the lumen as well as in the mucosa. By contrast, when comparable amounts of lyso[^{32}P]phosphatidylcholine were given not only did it disappear much faster than phosphatidylcholine, but the amounts of the radioactive water-soluble compounds were significantly higher in the mucosa than were obtained from comparable amounts of [^{32}P]phosphatidylcholine. It can therefore be concluded that the lysophosphatidylcholine formed in the lumen is hydrolysed in the cell, which would mean that lysophosphatidylcholine constitutes a major source of the glycerylphosphorylcholine of the cell. No doubt the very active lysophospholipase of the mucosa is obviously responsible for the appearance of glycerylphosphorylcholine there, but we have shown here that the same tissue contains an active glycerylphosphorylcholine diesterase that can readily hydrolyse glycerylphosphorylcholine to glycerophosphate and choline.

The results presented here have further shown that irrespective of whether glycerol-labelled triacylglycerol or glycerol-labelled phosphatidylcholine was given to the rats the radioactive glycerol appeared in the triacylglycerols of the mucosa. According to our current concepts, the dietary triacylglycerols during absorption are hydrolysed in the lumen of the small intestine to 2-monoacylglycerol, which is directly acylated to triacylglycerols in the mucosa (Ganguly *et al.*, 1972). Triacylglycerols can also be synthesized *via* the Kennedy (1961) pathway, and the enzymes involved have been shown to be present in the intestine of several species of animals (Hübscher, 1970). But here the starting material is *sn*-glycerol 3-phosphate, which is usually formed in many tissues, including the intestine, from endogenous dihydroxyacetone phosphate (Hübscher, 1970) or by the action of glycerokinase on free glycerol (Clark & Hübscher, 1962; Haessler & Isselbacher, 1963). It has been shown here that *sn*-glycerol 3-phosphate can also be formed in rat intestine from glycerylphosphorylcholine. It is therefore clear from the present work that the [^{14}C]glycerol of the glycerol-labelled phosphatidylcholine was incorporated into the triacylglycerols of the mucosa *via* the Kennedy (1961) pathway.

In our experiments more of the radioactive glycerol was incorporated into the mucosal triacylglycerols from the glycerol-labelled triacylglycerols than from comparable amounts of glycerol-labelled phosphatidylcholine. This difference can readily be explained because, whereas in the former case the steps involved are hydrolysis to 2-monoacylglycerol followed by direct acylation, in the latter case there are considerably more steps leading to triacylglycerol synthesis. Moreover, Raghavan & Ganguly (1969) have shown that in the absence of an exogenous glyceride backbone acceptor the unesterified fatty acids are absorbed as triacylglycerols through the *sn*-glycerol 3-phosphate pathway, where the glycerol is derived from endogenous sources. It is quite possible that in the present experiments some of the exogenous glycerylphosphorylcholine, glycerophosphate and glycerol were lost through portal absorption; in such a situation the excess of unesterified fatty acids should be absorbed by the mechanism shown by Raghavan &

Ganguly (1969), which would obviously lead to considerable dilution of the radioactive glycerol of the glycerol-labelled phosphatidylcholine.

An alternative mechanism for triacylglycerol synthesis from phosphatidylcholine could conceivably be hydrolysis of phosphatidylcholine to 1,2-diacylglycerol and phosphorylcholine, followed by acylation of the diacylglycerol to triacylglycerol. Phosphatidylcholine could also be hydrolysed to phosphatidate and choline; the phosphatidate could then be hydrolysed to 1,2-diacylglycerol, which could similarly be acylated to triacylglycerol. But the phospholipase C (EC 3.1.4.3) and D (EC 3.1.4.4) types of enzymes have not been demonstrated in animal intestine, though they are normally found in lower organisms and plants. Moreover, we found no significant amounts of radioactive diacylglycerols after giving glycerol-labelled phosphatidylcholine.

Our present results can therefore be interpreted as follows. The phosphatidylcholine is hydrolysed in the lumen of the small intestine by the pancreatic phospholipase A (EC 3.1.1.4) to lysophosphatidylcholine. Small portions of the lysophosphatidylcholine are further hydrolysed in the lumen to glycerylphosphorylcholine, whereas most of it is admitted into the cell. Inside the cell some of the lysophosphatidylcholine is reacylated to phosphatidylcholine and the rest is further hydrolysed to glycerylphosphorylcholine, glycerophosphate, glycerol and P_i. The glycerophosphate and the unesterified fatty acids are then reassembled in the cell to triacylglycerols via the Kennedy (1961) pathway.

This work was supported by the Indian Council of Medical Research, New Delhi. We thank the Wellcome Trust, London, for a grant for equipment.

References

Abramson, D. & Blecher, M. (1964) *J. Lipid Res.* **5**, 628–631

Baldwin, J. J. & Cornatzer, W. E. (1968) *Biochim. Biophys. Acta* **164**, 195–204

Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468

Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917

Clark, B. & Hübscher, G. (1962) *Nature (London)* **195**, 599–600

David, J. S. K., Malathi, P. & Ganguly, J. (1966) *Biochem. J.* **98**, 662–668

Dawson, R. M. C. (1967) in *Lipid Chromatographic Analysis* (Marinetti, G. V., ed.), vol. 1, pp. 163–189, Marcel Dekker Inc., New York

Ganguly, J., Subbaiah, P. V. & Parthasarathy, S. (1972) *Biochem. Soc. Symp.* **35**, 67–80

Haessler, H. A. & Isselbacher, K. J. (1963) *Biochim. Biophys. Acta* **73**, 427–436

Hoffmann, A. F. & Borgstrom, B. (1962) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **21**, 43–49

Hübscher, G. (1970) in *Lipid Metabolism* (Wakil, S. J., ed.), pp. 279–370, Academic Press, London and New York

Kennedy, E. P. (1961) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **20**, 934–940

Lloyd-Davies, K. A., Michell, R. H. & Coleman, R. (1972) *Biochem. J.* **127**, 357–368

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Marinetti, G. V. (1962) *J. Lipid Res.* **3**, 1–20

Miller, D. & Crane, R. K. (1961) *Biochim. Biophys. Acta* **52**, 293–298

Murthy, S. K., David, J. S. K. & Ganguly, J. (1963) *Biochem. Biophys. Acta* **70**, 490–492

Nilsson, A. (1968) *Biochim. Biophys. Acta* **152**, 379–390

Nilsson, A. & Borgstrom, B. (1967) *Biochim. Biophys. Acta* **137**, 240–254

Parthasarathy, S. & Ganguly, J. (1973) *Biochim. Biophys. Acta* **296**, 62–64

Porter, H. P. & Saunders, D. R. (1971) *Gastroenterology* **60**, 997–1007

Raghavan, S. S. & Ganguly, J. (1969) *Biochem. J.* **113**, 81–87

Robertson, A. F. & Lands, W. E. M. (1962) *Biochemistry* **1**, 804–810

Schneider, W. C. & Hogeboom, G. H. (1950) *J. Biol. Chem.* **183**, 123–128

Scow, R. O., Stein, Y. & Stein, O. (1967) *J. Biol. Chem.* **242**, 4919–4924

Subbaiah, P. V. & Ganguly, J. (1971) *Indian J. Biochem. Biophys.* **8**, 197–203

Subbaiah, P. V., Raghavan, S. S. & Ganguly, J. (1968) *Indian J. Biochem.* **5**, 147–152