Incorporation of [2-^{3}H]glycerol into rat brain 1,2-diacyl-*sn*-glycero-3-phosphorylcholine and 1,2-diacyl-*sn*-glycerol molecular species in vivo

J. F. O'Brien and R. L. Geison

Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison, Wisconsin 53706

Abstract Rat brain 1,2-diacyl-*sn*-glycerols (diglycerides) and 1,2-diacyl-*sn*-glycerols obtained from 1,2-diacyl-*sn*-glycero-3-phosphorylcholine after treatment with phospholipase C differ markedly in carbon number distribution. 70% of the 1,2-diacyl-*sn*-glycerols had a total of 38 fatty acid carbon atoms, and there was no detectable change in the 1,2-diacyl-*sn*-glycerol mass pattern between 7 and 23 days of age. In contrast, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine contained at most 10% of this molecular species in the brains of rats of comparable age. A small increase in the C_{36} species of 1,2-diacyl-*sn*-glycero-3-phosphorylcholine, which is associated with myelination, was noted between 10 and 17 days. The incorporation of intracranially injected [2-^{3}H]glycerol into 1,2-diacyl-*sn*-glycero-3-phosphorylcholine species with polyunsaturated fatty acids containing 20 or 22 carbon atoms was greater than into the species containing only saturated and/or monoenoic fatty acids between 30 min and 24 hr. The 1,2-diacyl-*sn*-glycerol fractions containing polyunsaturated fatty acids had the lowest specific activity at 30 min. The specific activity of the particular 1,2-diacyl-*sn*-glycerol fraction containing the stearate–arachidonate pair is the lowest for 4 hr after intracranial injection of the isotope. Thus, molecular species of 1,2-diacyl-*sn*-glycerol and 1,2-diacyl-*sn*-glycero-3-phosphorylcholine differed considerably in their labeling patterns, and a direct precursor–product relationship could not be demonstrated during the time period studied.

Supplementary key words CDPcholine:1,2-diglyceride cholinephosphotransferase (EC 2.7.8.2) · diglyceride · lecithin · molecular species biosynthesis · preparative GLC

Investigations of the synthesis of various molecular species of CPG by rat liver from radioactive precursors have been conducted in a number of laboratories (1–5). These studies have shown that each of the three major pathways of biosynthesis has a different selectivity for specific fatty acid moieties. Precursors such as ^{14}C-labeled glycerol, [1,2-$^{14}C_2$]choline, L-[*Me*-^{14}C]methionine, $^{32}P_i$ and lyso-(1-acyl)-phosphatidyl-[1,2-$^{14}C_2$]choline have been used to study the biosynthesis of CPG (1, 3, 5). These studies have shown that CPG species containing monoenoic or dienoic acids are synthesized de novo at a faster rate than the polyenoic species. Species of CPG containing polyunsaturated fatty acids are synthesized by either acylation of lyso-CPG or methylation of phosphatidylethanolamine (1, 3, 5). Thus, the metabolism of CPG cannot be fully described without preliminary subfractionation of the molecular species.

To date there have been no reports on the relative rates of incorporation of precursors into the CPG species in rat brain. Since CPG is the major diacylglycerophosphatide of brain membranes, and the primary precursor is presumed to be DG, a study of the in vivo incorporation of [2-^{3}H]glycerol into CPG and DG of rat brain was undertaken. GLC was used for simultaneous separation and specific activity determination of molecular species fractions.

MATERIALS AND METHODS

Isolation of DG for determination of mass distribution

Sprague-Dawley rats (Holtzman Co., Madison, Wis.) 7, 12, 18, and 23 days old were decapitated and the brains removed and frozen. The lipids were extracted essentially as described in the procedure of Folch, Lees, and Sloane Stanley (6). Each brain was homogenized in 10 ml of chloroform–methanol 2:1 (v/v) using a Potter-Elvehjem homogenizer. The extracts were filtered through sintered

A preliminary report of the present work was given at the meeting of the Federation of American Societies for Experimental Biology, San Francisco, Calif., 13–18 June 1971.

Abbreviations: DG, 1,2-diacyl-*sn*-glycerols; CPG, 1,2-diacyl-*sn*-glycero-3-phosphorylcholines; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; cholinephosphotransferase, CDPcholine:1,2-diglyceride cholinephosphotransferase (EC 2.7.8.2); TMS-DG, trimethylsilyl-1,2-diacyl-*sn*-glycerols.

glass funnels and partitioned by adding 2 ml of 0.73% NaCl. The lower phase was removed, taken to dryness under N_2, and redissolved in 1 ml of chloroform–methanol 2:1 (v/v). Approximately 500 μl of the lipid extract obtained from each brain was applied in a streak to two TLC plates coated with 500-μm layers of silica gel G (Brinkmann, Great Neck, N.Y.). The plates were developed twice as described by Freeman and West (7). The plates were then sprayed with a 0.02% solution of 2,7-dichlorofluorescein in methanol and the lipid bands were visualized under UV light. The lipid area having the same R_F as 1,2-diolein (Applied Science Laboratories, Inc., State College, Pa.) was scraped off the plates into 15-ml conical glass tubes, and 5 ml of diethyl ether–methanol 9:1 (v/v) was added. The suspension was thoroughly agitated on a Vortex mixer, and the liquid phase containing the DG was separated from the silica gel by centrifugation of the slurry for 10 min at 2500 rpm. After repeating the elution the combined eluates representing approximately one-half of the diglycerides from individual brain extracts were dried in aliquots in small conical ampoules made by drawing out pasteur pipettes. The residue was silylated by warming at approximately 60°C with 10 μl of *N,O*-bis-(trimethylsilyl)-acetamide (Pierce Chemical Co., Rockford, Ill.) for 10 min.

Isolation of [2-^{3}H]glycerol-labeled CPG and DG

Glycerol lipids of 10-day-old rat brain were labeled by slowly infusing 20 μl of a solution of [2-^{3}H]glycerol in sterile water (1 mCi/ml) (New England Nuclear, Boston, Mass.) over a 30-sec period into the parietal–occipital cortex of rats that had been lightly anesthetized with ether. The rats were decapitated at various time intervals and the brains were rapidly removed and frozen between blocks of CO_2; they were stored frozen until they were analyzed. The lipids were extracted (6), and one-half of the lipid extract was applied to preparative TLC plates (500 μm thick layers of silica gel G) that were then developed in chloroform–methanol–water 65:25:4 (v/v/v). The lipids were visualized after the plates were sprayed with a solution of 2,7-dichlorofluorescein, and the CPG band was scraped into a scintillation vial containing 4 ml of diethyl ether. 2 ml of a solution of phospholipase C (1 mg/ml) (*Clostridium perfringens*; Schwarz BioResearch, Inc., Orangeburg, N.Y.) in 0.1 M Tris (pH 7.35) was added and, after mixing, the biphasic solution was allowed to stand for 4 hr at room temperature. The ether layer was then removed and dried over anhydrous Na_2SO_4. The ether solution was transferred to 3-ml conical centrifuge tubes and the ether was removed with a stream of N_2. The DG were silylated with 10–50 μl of *N,O*-bis-(trimethylsilyl)-acetamide.

There was considerable variability from animal to animal in the degree of incorporation of the labeled glycerol into CPG and DG. Consequently, in the experiment in which ratios of specific activities of DG species versus CPG species were determined after GLC fractionation, both species of lipid were isolated from the same brains. In these experiments CPG was recovered from TLC plates as described above. The area of the plate corresponding to the neutral lipids was extracted with diethyl ether–methanol 9:1 (v/v), and the lipids were rechromatographed on a TLC plate coated with silica gel H (500 μm thick) that was developed in heptane–diethyl ether–formic acid 60:50:1 (v/v/v). Thus, after the neutral lipids were obtained from the TLC run in the polar system, a single development was used to isolate DG, thereby avoiding the added delay of double development, which was used to isolate DG from total brain lipids. The band that migrated with 1,2-diolein was scraped off and eluted as before. Some cholesterol was removed with the DG because it migrates only slightly slower than DG in this system. However, it had been reduced to a manageable concentration and was useful as a marker by providing a GLC peak prior to the DG peaks that were collected.

Determination of specific activities of DG and CPG molecular mass subgroups

A Barber-Colman 5000 gas chromatograph was used for the GLC of TMS-DG. The determination of mass percentages of DG by GLC has been adequately described previously (8, 9). For preparative GLC of DG, a stream splitter delivered 85% of the column effluent to the collection port. The stainless steel tubing from splitter to collection port was maintained at 350°C. The samples were collected in pasteur pipettes fitted with "through-hole" type 6 × 9 mm rubber septums (Applied Science Laboratories, Inc.). After removal of the septum, the top of the pipette was placed in liquid scintillation vials, and scintillation fluid consisting of 0.5% PPO (2,5-diphenyloxazole) (w/v) and 0.01% POPOP (*p*-bis-[2-(5-phenyloxazolyl)]-benzene) (w/v) in toluene was drawn up into the pipette three times to dissolve and rinse out all collected DG. Radioactivity was measured using a Packard counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The mass of each peak was obtained by integration using a Disc integrator (Finnigan Instruments Corp., Santa Anna, Calif.). The recovery of radioactivity from the column was determined by dividing the cpm of a collected sample by the cpm of a sample of equal volume that had not been subjected to GLC. The inclusion of a glass wool plug 2 cm from the outlet of the collection port and the coating of the inside of the collector with silicone stopcock grease (Dow Corning Corp., Midland, Mich.) was found to increase the efficiency of collection from 40–50% to an apparent 100%.

No effort was made to determine the yield of either CPG or DG from each brain. Administration of 20 μCi/

TABLE 1. Distribution of molecular species of 1,2-diacyl-*sn*-glycerols from brains of rats of different ages

Carbon Number[a]	Age (days)			
	7	12	18	23
32	4.35[b]	6.19	5.42	4.20
34	9.68	13.10	13.25	11.65
36a[c]	10.55	11.45	10.70	12.35
36b[c]	1.43	1.81	1.76	2.25
38	73.70	66.15	68.30	69.75

[a] The sum of the fatty acid carbon atoms; 30- and 40-carbon DG were also detected (<1% of total).

[b] Values are means of determinations done on two animals.

[c] 36a has been shown to contain arachidonic and palmitic acids; 36b contains combinations of saturated and monoenoic C_{16}, C_{18}, and C_{20} fatty acids (9).

animal resulted in CPG with an average specific activity of 3.3×10^4 cpm/μmole after 30 min. This was determined in the first experiment in which the incorporation of [2-^{3}H]glycerol into CPG was studied (Table 3). For this determination, purified CPG which was recovered from TLC plates was redissolved in chloroform–methanol 2:1 (v/v). One aliquot was dried down with a stream of N_2 and the ^{3}H radioactivity was measured. A second aliquot was used to determine phosphorus by the method of Bartlett (10). The cpm found in the CPG were then divided by the μmoles of phosphorus present in an equal sample.

The determinations of the incorporation of labeled glycerol in CPG species (Fig. 1) and DG species (Fig. 2) as a function of time were done in separate experiments. The data were normalized by calculating relative specific activities. This was done by determining the specific activity for each peak (cpm divided by the peak area), which was in turn divided by the total DG specific activity determined by summation of the individual peak areas and counts. For determination of incorporation into CPG, a total of between 500 and 5000 cpm was recovered from the GLC column. This represented between 40 and 50 μg of DG derived from CPG. A considerably smaller amount was available for DG analysis owing to the low concentration of this lipid in brain (11). Since the final amount of DG isolated from each animal was variable, the amount of a 10-μl silylation reaction mixture injected into the GLC column was necessarily varied from 1 μl to 5 μl. The usual recovery of radioactivity was between 500 and 1500 cpm in the total DG.

Determination of the extent of [2-^{3}H]glycerol incorporation into fatty acids

To determine whether significant incorporation of ^{3}H into fatty acids had occurred, the radioactivity of CPG fatty acids was determined. BF_3–methanol reagent (Applied Science Laboratories, Inc.) was added to purified whole brain CPG that had been labeled in vivo for various times. After 4 hr at 60°C, the reaction mixture was partitioned in a hexane–water system. The methyl esters in the hexane layer were purified by TLC using hexane–diethyl ether–formic acid 60:50:1 (v/v/v). The radioactivity was determined in both total esterified fatty acids and individual fatty acid esters fractionated by preparative GLC using an 8 ft × 5 mm column packed with 15% (w/w) stabilized diethylene glycol succinate coated on 80–90 mesh ABS support (Analabs, Inc., North Haven, Conn.). The peaks were monitored and collected on silicone grease-coated glass wool plugs in tubes using a model 852 fraction collector (Packard Instrument Co.). The tubes and plugs with adsorbed fatty acid methyl esters were placed in scintillation vials and counted.

RESULTS

Mass distribution of CPG and DG of whole brain

The determination of mass composition of DG, isolated from the brains of rats of different ages, was done to see if significant changes occurred during a period in which rapid phospholipid synthesis is necessary for membrane formation. This then might be reflected in changes of the composition of precursor DG, particularly since DG may be incorporated more rapidly into the phospholipids of myelin during this stage of development. The carbon number distribution of DG isolated from whole brains of rats of four different ages is given in Table 1. There were

TABLE 2. Distribution of molecular species of 1,2-diacyl-*sn*-glycero-3-phosphorylcholine from brains of rats of different ages

Carbon Number	Age (days)				
	10(6)[a]	11(4)	13(4)	15(4)	17(2)
32	32.77 ± 1.68[b]	37.50 ± 3.15	34.68 ± 2.97	33.63 ± 2.41	27.95
34	39.63 ± 1.23	39.23 ± 3.32	45.33 ± 2.25	43.28 ± 3.35	45.40
36a[c]	6.98 ± 0.77	4.65 ± 1.93	2.25 ± 1.34	1.75 ± 0.77	3.55
36b[c]	6.00 ± 0.60	5.55 ± 1.55	9.25 ± 1.52	11.60 ± 1.54	11.70
38	9.95 ± 1.26	8.60 ± 3.46	5.63 ± 3.98	7.35 ± 1.16	8.50

[a] The numbers in parentheses indicate the number of animals used per age group.

[b] Percentages of total CPG ± SD.

[c] 36a has been shown to contain arachidonic and palmitic acids; 36b contains combinations of saturated and monoenoic C_{16}, C_{18}, and C_{20} fatty acids (9).

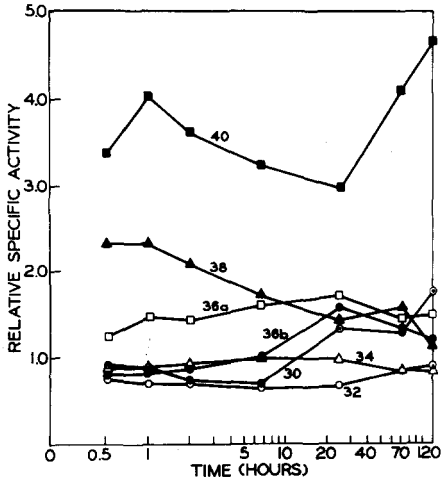

Fig. 1. Relative specific activity (radioactivity/mass of individual carbon number species divided by total radioactivity/mass) of DG derived from CPG of whole brain at 30 min to 5 days after intracranial injection of [2-^{3}H]glycerol in 10-day-old rats. Symbols for the various DG with even numbers of fatty acid carbon atoms between 30 and 40 are as follows: 30; ⊙; 32, ○; 34, △; 36a composed mainly of palmitic and arachidonic acids (9), □; 36b, containing mainly saturated and/or monoenoic fatty acids, ●; 38, ▲; and 40, ■.

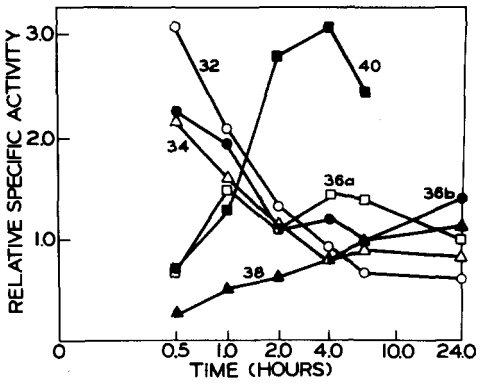

Fig. 2. Relative specific activity (radioactivity/mass of individual carbon number species divided by total radioactivity/mass) of DG of whole brain at 30 min to 1 day after intracranial injection of [2-^{3}H]glycerol in 10-day-old rats. Symbols for the various DG with even numbers of fatty acid carbon atoms between 32 and 40 are as follows: 32, ○; 34, △; 36a, composed mainly of palmitic and arachidonic acids (9), □; 36b, containing mainly saturated and/or monoenoic fatty acids, ●; 38, ▲; and 40, ■.

no striking variations in the DG pattern between 7 and 23 days of age. The significant feature of the DG distribution was that approximately 70% of the DG had 38 fatty acid carbon atoms, which is similar to the findings for adult rat brain (11).

The mass distribution of DG species derived from the CPG is presented in Table 2. A comparison of these data with those in Table 1 clearly indicates a difference between the DG derived from the phospholipid and the DG present as such in brain. The species with 32 and 34 carbons comprise 70–80% of the total CPG. The polyene species are represented by 36a and 38, and although they have been revised to a higher percentage than previously reported (9), they never constitute more than 15% of the DG derived from CPG. The C_{38} species range between 5 and 10% of the total CPG species. CPG with the carbon number 36b have been shown to be most abundant in myelin (9), and this species increased during the myelinating period between 10 and 17 days of age.

Incorporation of [2-^{3}H]glycerol into CPG and DG species in vivo

The incorporation of [2-^{3}H]glycerol into various CPG species in brains from 10-day-old rats was determined from 30 min to 5 days after intracranial injection (Fig. 1). The data for the first three time periods were from analyses of 10 or more individual animals in six separate experiments. The differences in the initial (30 min) relative specific activities between each of the CPG species containing 34, 36a, 38, and 40 fatty acid carbon atoms were statistically significant ($P < 0.01$). This was true for the 40-carbon species in spite of 100% variations in relative specific activities due to its small peak heights on chromatograms, which made its quantitation more subject to error. The highest relative specific activities were in the carbon number fractions 36a, 38, and 40, which were previously found to contain arachidonic or docosahexaenoic acids in combination with palmitic and stearic acids (9). The percentage of the total CPG radioactivity found in fatty acids was 5% after 30 min and 10% after 5 days. At all times studied, palmitic acid contained most of the label and would therefore contribute more activity to the lower carbon number DG (32 and 34). Thus, corrections would tend to increase the disparity between the labeling of polyene-containing fractions and those containing a higher content of palmitic acid.

The labeling pattern of DG from 30 min to 24 hr was determined in an attempt to elucidate the specificity of cholinephosphotransferase for particular DG species (Fig. 2). After 30 min the DG containing saturated fatty acids designated 32, 34, and 36b had higher specific activities than the DG species 36a, 38, and 40, which contained polyenoic fatty acids. Moreover, after 4 hr the C_{38} DG still had the lowest specific activity. The DG labeling pattern was therefore profoundly different from that of CPG.

The absolute specific activity of total CPG was compared with that of total DG at several time intervals after administration of 20 μCi of [2-^{3}H]glycerol (Table 3). The specific activity of DG was 20 times higher than that of CPG at 0.5 hr and 3 times higher at 6.5 hr. The ratio of specific activities of fractionated species of CPG to DG were compared in the same animals in a single experiment. For C_{34} this ratio increased from 0.14 to 0.35 between 1 and 6.5 hr, while the activity ratio for the species with 38 carbons decreased from 0.82 to 0.53 during this time period. At periods longer than 24 hr the amount of label remaining in the DG became exceedingly small, and

TABLE 3. Specific activity[a] of total DG and CPG isolated from rat brains at various times after intracranial injection of [2-^{3}H]glycerol

	Time (min)				
	30(3)[b]	60(2)	120(3)	240(3)	390(4)
DG	760[c]	660	180	130	110
CPG	33	44	44	48	33

[a] The specific activity of CPG at 30 min was found by determining phosphorus (10) and counting equal aliquots of CPG as described in Materials and Methods. The specific activity for CPG at 30 min was also calculated by dividing the sum of the ^{3}H counts collected during a GLC run by the sum of the peak areas. Thus, by knowing the ratio of the specific activities for CPG at 30 min found by both methods, the cpm/mass values for the other CPG and DG were converted to cpm/nmole. No correction was made for the slight average molecular weight shift found between CPG and DG when the cpm/nmole were calculated for DG.

[b] Number of animals.

[c] Mean cpm/nmole.

activity could be determined accurately only in the 34 and 38 carbon number species.

DISCUSSION

Diglycerides are precursors of the major glycerophospholipids in brain (12). The determination of the mass distribution of the diglyceride moieties of CPG and a specific association of certain CPG species with various brain membranes have previously been reported (9). There has been no documentation of the mass distribution of DG or CPG as a function of early postnatal development. These determinations were done to see if either DG or CPG mass distributions reflect rapid synthesis of particular CPG species involved in membranes being elaborated during this period.

The results of mass determinations of DG and CPG indicate that except for an increase in saturated and/or monoenoic CPG species containing 36 carbon atoms during this period of myelination, there was little effect of maturation on DG or CPG mass distribution. The most remarkable finding is that DG and CPG mass distributions were very different. Of particular interest is the extremely high (70%) proportion of DG with fatty acids totaling 38 carbon atoms. It has previously been shown by fatty acid analysis that stearic and arachidonic acids each make up approximately 30% of the fatty acids of mouse and rat brain DG (11, 13). The only glycerophospholipid that shows a similarity in fatty acid content to DG is phosphatidylinositol, approximately 60% of which is 1-stearoyl-2-arachidonyl-*sn*-glycero-3-phosphorylinositol (11).

The labeling of CPG species using [2-^{3}H]glycerol is the first study to determine if in vivo selectivity of incorporation was present in brain as it has been shown to be in liver (1–4). Although the site of synthesis of phospholipid in brain is still in question (14), glycerol can be incorporated into phospholipids by brain enzymes (15). The experiments presented here, which describe the incorporation of [2-^{3}H]glycerol into CPG, lead to the conclusion that CPG species with long-chain polyunsaturated fatty acids are synthesized more rapidly than the species with combinations of palmitic, stearic, and oleic acids.

There are a number of possibilities as to the nature of the specificity of in vivo labeling of CPG in brain. The CPG species produced by de novo synthesis may be diluted to varying extents by alternate synthetic pathways involving DG or other single reactions that yield CPG, such as base exchange (16) or acylation of endogenously derived monoacyl-*sn*-glycero-3-phosphorylcholine (17). Since [2-^{3}H]glycerol was used as the radioactive precursor of CPG, the mitochondrial dihydroxyacetone phosphate pathway, if present in brain, could not contribute to the labeled product (18). Another possibility is the compartmentalization among cell types or within the cell depending on the constituent fatty acids since membranes have a particular complement of CPG species (9). Preliminary experiments in our laboratory suggest that there are variations in labeling patterns of both DG and CPG depending on the subcellular localization. [1]

The most obvious possibility is that cholinephosphotransferase may show specificity toward DG species. The specificity of this enzyme for DG containing unsaturated fatty acids was previously implied by the experiments of McCaman and Cook (19). The specific activities of DG species were determined in an attempt to isolate and therefore evaluate the possible specificity of cholinephosphotransferase. The striking dissimilarity between DG labeling patterns and those of CPG make it obvious that a precursor–product relationship between DG and CPG derived from total brain extracts is difficult to establish. If the DG specific activities measured were truly those of DG that are available for CPG synthesis, an extremely rapid labeling of the C_{36a}, C_{38}, and C_{40} DG, causing their activities to peak much earlier than the activities of the C_{32}, C_{34}, and C_{36b} DG, and most probably much before 30 min, must occur. There is some question as to the validity of in vivo labeling patterns at short time intervals in anesthetized rats if diglyceride labeling is extremely rapid. Consequently, definitive results on DG labeling at short time periods will be the subject of future in vitro experiments.

Another explanation for the difference in DG and CPG species labeling patterns is the existence of a large pool of DG containing polyunsaturated fatty acids and particularly of 1-stearoyl-2-arachidonyl-*sn*-glycerol, which does not freely exchange with a DG pool used to synthesize CPG. The apparent inertness of the 38-carbon

[1] O'Brien, J. F. Unpublished data.

DG species at short labeling periods points to the possibility of a closed cycle involving DG with 38 fatty acid carbons, such as the cycle involving phosphatidylinositol that has been suggested by Durell, Garland, and Friedel (20).

We wish to thank Mr. Larry Kneeland for technical assistance in some of the experiments.

This investigation was supported by research grants HD-00341, HD-131, and HD-5342 from the National Institute of Child Health and Human Development.

Manuscript received 19 October 1972 and in revised form 28 March 1973; accepted 7 September 1973.

REFERENCES

1. Arvidson, G. A. E. 1968. Biosynthesis of phosphatidylcholines in rat liver. *Eur. J. Biochem.* **5:** 415–421.
2. Van Golde, L. M. G., G. L. Scherphof, and L. L. M. Van Deenen. 1969. Biosynthetic pathways in the formation of individual molecular species of rat liver phospholipids. *Biochim. Biophys. Acta.* **176:** 635–637.
3. Kanoh, H. 1969. Biosynthesis of molecular species of phosphatidylcholines and phosphatidyl ethanolamines from radioactive precursors in rat liver slices. *Biochim. Biophys. Acta.* **176:** 756–763.
4. Åkesson, B., J. Elovson, and G. Arvidson. 1970. Initial incorporation into rat liver glycerolipids of intraportally injected [^{3}H]glycerol. *Biochim. Biophys. Acta.* **210:** 15–27.
5. Holub, B. J., and A. Kuksis. 1971. Structural and metabolic interrelationships among glycerophosphatides of rat liver in vivo. *Can. J. Biochem.* **49:** 1347–1356.
6. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
7. Freeman, C. P., and D. West. 1966. Complete separation of lipid classes on a single thin-layer plate. *J. Lipid Res.* **7:** 324–327.
8. O'Brien, J. F., and W. E. Klopfenstein. 1971. Gas–liquid chromatographic analysis of diglycerides. *Chem. Phys. Lipids.* **6:** 1–7.
9. O'Brien, J. F., and R. L. Geison. 1971. The mass distribution of the phosphatidylcholines in subcellular fractions of rat brain. *J. Neurochem.* **18:** 1615–1623.
10. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
11. Keough, K. M. W., G. MacDonald, and W. Thompson. 1972. A possible relation between phosphoinositides and the diglyceride pool in rat brain. *Biochim. Biophys. Acta.* **270:** 337–347.
12. Ansell, G. B. 1972. The metabolism of phosphatidylcholine in brain tissue. *Biochem. J.* **128:** 6P–8P.
13. Sun, G. Y. 1970. Composition of acyl groups in the neutral glycerides from mouse brain. *J. Neurochem.* **17:** 445–446.
14. Miller, E. K., and R. M. C. Dawson. 1972. Can mitochondria and synaptosomes of guinea-pig brain synthesize phospholipids? *Biochem. J.* **126:** 805–821.
15. Lunt, G. G., and E. G. Lapetina. 1970. Phospholipid metabolism of isolated nerve endings. *Brain Res.* **17:** 164–167.
16. Kanfer, J. N. 1972. Base exchange reactions of the phospholipids in rat brain particles. *J. Lipid Res.* **13:** 468–476.
17. Webster, G. R., and R. J. Alpern. 1964. Studies on the acylation of lysolecithin by rat brain. *Biochem. J.* **90:** 35–42.
18. Okuyama, H., and W. E. M. Lands. 1970. A test for the dihydroxyacetone phosphate pathway. *Biochim. Biophys. Acta.* **218:** 376–377.
19. McCaman, R. E., and K. Cook. 1966. Intermediary metabolism of phospholipids in brain tissue. III. Phosphocholine–glyceride transferase. *J. Biol. Chem.* **241:** 3390–3394.
20. Durell, J., J. T. Garland, and R. O. Friedel. 1969. Acetylcholine action: biochemical aspects. *Science.* **165:** 862–866.