A biosynthetic method for the preparation of high specific activity ^{32}P-labeled phospholipids

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Abstract ^{32}P-Labeled phospholipids with specific activities up to 400 mCi/mmole as well as [^{32}P]CDP-choline were prepared by cultivation of mouse fibroblasts or mouse Ehrlich ascites cells in the presence of [^{32}P]orthophosphate. The method was also used to prepare [methyl-^{3}H]choline-labeled glycerophospholipids from [^{3}H]choline. The yields and the specific activities of the phospholipids were significantly lower when preparations of ox white blood cells were used.

Supplementary key words suspension cultures · mammalian cells

Research on the structure and functions of biological membranes has progressively increased interest in phospholipid metabolism and has created a rising demand for radioactively labeled phospholipid substrates.

Besides chemical synthesis (1) another method of preparing labeled phospholipids is offered by using biosynthetic pathways. Thus, radioactive phospholipids have been prepared by incubating labeled glycerol or labeled fatty acids with cottonseed (2) or with tissue slices (3). In some cases, yields and specific activities have been sufficiently high to enable us to use these methods as standard preparations of radioactive lipids.

In this report a method for the preparation of highly labeled phospholipids and their precursors from various mammalian cells grown in suspension cultures is described. The labeled compounds can be isolated in a highly purified form. The method was used to prepare ^{32}P-labeled choline glycerophospholipids, ethanolamine glycerophospholipids, inositol glycerophospholipids, as well as [^{32}P]CDP-choline and [^{32}P]phosphatidic acid.

MATERIALS AND METHODS

Chemicals

HEPES, choline chloride, cytidine, serine, ethanolamine chloride and antibiotics were obtained from Serva (Heidelberg, Germany); sodium pyruvate, oxaloacetic acid, sodium fumarate, CDP-choline, CDP-ethanolamine, and cabbage phospholipase D were from Boehringer Biochemica (Mannheim, Germany); reference phospholipids were from Applied Science Laboratories (State College, Pa.); all other chemicals were from Merck (Darmstadt, Germany). The radiochemicals were purchased from The Radiochemical Center Amersham (UK).

Preparation of cells

SV-40 transformed mouse fibroblasts (STU 51A/232 B) (4) were taken from logarithmically growing suspension cultures. The cell sample was harvested by centrifugation (200 *g*, 5 min) and washed twice in ice-cold saline (20 ml per 0.1 ml of packed cells).

Ehrlich ascites cells were propagated in NMRI/HAN or STU-mice for 4–5 days (5); they were harvested by puncturing the peritoneum and then were suspended in 5 volumes of ice-cold saline. The washings were performed as described above.

Human blood cells were prepared by suspending 2 ml of fresh human blood in 8 ml of saline. Washings were performed as described above, except that centrifugations were carried out at 1000 *g* for 5 min.

White blood cells were separated from ox blood after osmotic disruption of the red blood cells. The following procedure was used. Two hundred ml of fresh ox blood containing 0.18% sodium citrate to prevent clotting was centrifugated at 1000 *g* for 10 min. One hundred ml of the sedimented cells were quickly suspended in 400 ml of ice-cold water. After 20 sec the isotonic conditions were restored by adding 60 ml of a 20% aqueous solution of NaCl.

Abbreviations: HEPES, *N*-2′-hydroxyethylpiperazine-2-ethanesulfonic acid; TRIS, tris(hydroxymethyl)aminomethane; CDP-choline, cytidine-5′-diphosphocholine; TLC, thin-layer chromatography.

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The cells were sedimented by centrifugation (200 *g*, 5 min) and washed with 60 ml of ice-cold saline. This preparation yielded 0.2–0.3 ml of packed white blood cells, corresponding to 1.5×10^8 cells. The cell sample obtained was free of red blood cells.

Incubation medium

Incubations were performed in a supplemented, balanced salt solution (**Table 1**). The incubation media used in Exp. 1, 2, and 3 are listed in Table 2. The HEPES-citrate-buffer pH 7.2 was prepared first, and the other constituents were then added. Finally the pH was readjusted and the medium was sterilized by filtration (Millipore, disposable filters, 0.22 μm). This medium could be stored at −20°C for 10 months. Fetal calf serum, dialyzed against saline for 72 hr (0.2 ml/4 ml medium), and penicillin G (100 U/ml), streptomycin sulfate (100 μg/ml) and nystatine (20 μg/ml) were added prior to use. When [^{32}P]orthophosphate or radioactive choline was used as precursor, the medium contained neither respective unlabeled precursor in order to prevent dilution of radioactivity.

Incubation of cells

In all preparations after Exp. 4 the quantity of packed cells used was between 0.1 and 0.2 ml. The cells were incubated in glass-stoppered test tubes (2.5 × 8 cm) with 2.5 ml of the medium containing the radioactive precursors. The cell culture was incubated at 37°C with magnetic stirring for 17 hr. The pH of the incubation mixture was adjusted to neutrality every 3–5 hr with 0.16 N sodium hydroxide. Incubation was stopped by freezing the contents of the tube at −80°C.

Isolation of labeled compounds

The radioactive compounds were isolated using a modified form of the procedure of Folch, Lees, and Sloane Stanley (6). The frozen sample was placed in a Branson sonifier (Branson Instruments, Stamford, Conn.). A 12.5 ml portion of a cold mixture of chloroform–methanol 2:1 was added and ultrasonication was started. Extraction was finished as soon as the mixture reached a temperature of 30°C, normally after 1–2 min of sonication. The mixture was then centrifuged at 1000 *g* (5 min). A chloroform-rich lower phase, a small intermediate phase consisting of insoluble material, and a hydrophilic upper phase were obtained. Upper phase and lower phase were separated and dried at 30°C under reduced pressure in a N_2 atmosphere without further washings. The dry residue of the hydrophilic phase was stored at −20°C. The residue of the lipophilic phase, consisting mainly of neutral lipids and phospholipids, was dissolved in freshly distilled chloroform and stored in an atmosphere of N_2 at −80°C.

TABLE 1. Composition of the incubation medium[a] (pH 7.2)

	mg/100 ml		mg/100 ml
NaCl	410	Choline chloride[c]	2.0
KCl	26	Nicotinic acid	0.5
$CaCl_2$	11	Pantothenic acid	0.5
$MgSO_4 \cdot 7H_2O$	247	Pyridoxal	0.5
KH_2PO_4[b]	4	Riboflavine	0.05
HEPES	714	Thiamine	0.5
Citric acid	210	*i*-Inositol	1.0
Glucose	450	Folic acid	0.5
Na pyruvate	55	Cytidine	2.5
Oxaloacetic acid	13	Serine	10.0
Na fumarate	16	Ethanolamine	3.0
Phenol red	2.0		

[a] The medium was supplemented with dialyzed (saline) fetal calf serum (0.2 ml/4 ml) and antibiotics prior to use.
[b] Omitted when [^{32}P]orthophosphate was used as precursor.
[c] Omitted when [^{3}H]choline was used as precursor.

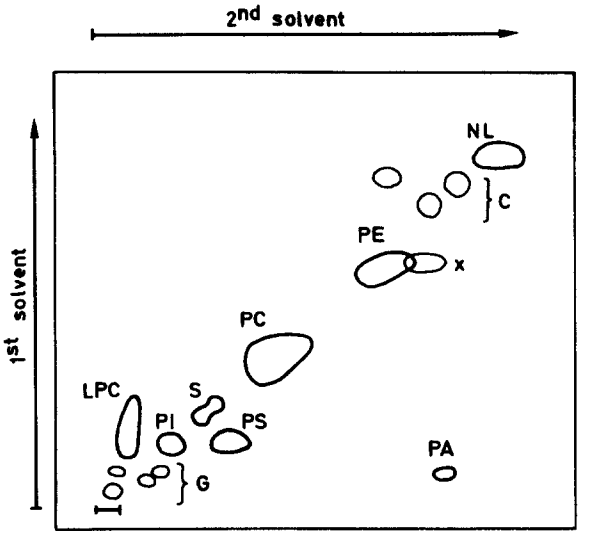

Fig. 1. Two-dimensional separation on silica gel 60 precoated TLC plates (Merck) of the lipids isolated from STU-232B cells. First solvent, chloroform–methanol–28% aqueous ammonia 13:7:1 (v/v); second solvent, chloroform–acetone–methanol–acetic acid–water 10:4:2:2:1 (v/v). Abbreviations: PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids; PI, inositol glycero phospholipids; PS, serine glycerophospholipids; S, sphingomyelin; PA, phosphatidic acid; LPC, choline lysoglycerophospholipids; C, cerebrosides; G, gangliosides; x, unidentified glycolipid.

Isolation of the individual lipids

The crude lipid fraction (1.5–2.2 μmole of lipid-P) was applied to three chromatoplates (precoated TLC plates, 0.25 mm, silica gel 60, obtained from Merck) and separated by two-dimensional chromatography (**Fig. 1**) using the systems of Siakotos and Rouser (7). Spots of phospholipids were visualized by radioautography. The exposure times ranged from a few seconds to several minutes depending on the amount

of radioactivity to be detected. The areas of silica gel containing the phospholipids were collected and the phospholipids were eluted with 10 ml of methanol that had been saturated with N_2. The eluate was immediately dried and dissolved in chloroform to avoid transacylation reactions with methanol. The phospholipid solutions were stored at −80°C or at the temperature of liquid N_2. (Storing for more than three days should be avoided.) Since the pure phospholipids are highly susceptible to autoxidation, all manipulations after the thin-layer chromatography were performed in a N_2 atmosphere at a temperature of not more than 30°C.

In order to test the purity of the isolated phospholipids, samples containing 1 μCi were co-chromatographed with 200 μg of the pure carrier phospholipid in the system given above (7). The lipid spot was visualized by short exposure to iodine vapor, scraped off the thin-layer plate, and the radioactivity was determined by liquid scintillation counting as described previously (8). Counting efficiency was 17% for ^3H, and 80% for ^{32}P. The radiochemical purity is given as the percentage of radioactivity recovered from the chromatoplate together with the reference phospholipid.

In order to reveal possible contaminations of the labeled phospholipids by nonradioactive material, e.g., glycolipids that may overlap with the ^{32}P-labeled phospholipids, complex lipids deriving from STU 51A/232B cells that had been grown in the presence of [^{32}P]orthophosphate were separated and visualized as given above. One of the chromatoplates (Fig. 1) was stained by H_2SO_4 charring. The phospholipids appeared as intense spots, identical to those on the radioautographs. Additionally, eight very faint spots were observed on the chromatogram, which could be identified as cerebrosides and gangliosides by their R_f-values (9) and by the use of different spray reagents specific for the carbohydrate moiety of glycolipids (orcinol, α-naphthol, resorcinol) (9). Neither of the glycolipid spots overlapped with any of the phospholipid spots, except for ethanolamine glycerophospholipids, which exhibited a partial overlap with traces of an unidentified glycolipid. If cell lines other than STU 51A/232B are used for the preparation of labeled phospholipids, the analysis may possibly reveal a different contamination pattern.

Preparation of [^{32}P]phosphatidic acid

The crude lipid extract obtained from the partition procedure (6) was dried in a stream of N_2, dissolved in 1 ml of chloroform, and chromatographed on a column (diameter, 0.7 cm) packed with 0.2 g of silica gel (Kieselgel H, Merck). Neutral lipids were eluted with 5 ml of chloroform. The phospholipids were then eluted with 75 ml of methanol. The methanol was evaporated and the phospholipid residue was suspended in 5 ml of N_2-saturated water. The suspension was treated with cabbage phospholipase D (10) to convert the choline-, ethanolamine-, and serine-glycerophospholipids to phosphatidic acid. Incubation was stopped after 15 min by adding 5 volumes of chloroform–methanol 2:1 (v/v) followed by ultrasonication. Phase partition and isolation of the [^{32}P]phosphatidic acid were performed as described above.

Preparation of [^{32}P]CDP-choline

CDP-Choline was isolated from the aqueous phase of the partition procedure, which contained the main part of the cellular CDP-choline. (The interphase, though containing considerable amounts of CDP-choline, was not extracted in order to shorten handling of the highly radioactive sample.) The method of Sundler, Arvidson, and Akesson (11) was applied. The dry residue of the upper phase was dissolved in 4 ml of water and adjusted to pH 8 with 0.1 N KOH. Carrier CDP-choline (100 μg) and CDP-ethanolamine (500 μg) were added and the solution was then passed through a column (1 × 12 cm) containing AG 1 × 2 (formate). The column was washed with 40 ml of CO_2—free water to eliminate cationic and neutral components. The [^{32}P]CDP-choline was eluted with a linear gradient of 0–0.05 M formic acid in a total volume of 600 ml and detected by ultraviolet absorption at 260/280 nm. The fractions of the eluate containing the [^{32}P]CDP-choline were combined and dried. The crude [^{32}P]CDP-choline was further purified by two-dimensional thin-layer chromatography on silica gel in propanol–water–28% ammonia 6:3:1 (v/v) and propanol–28% ammonia 1:1. CDP-Choline was detected by UV light and by radioautography. The silica gel containing the spot was collected and [^{32}P]CDP-choline was eluted with water.

Cell countings were performed in a hemocytometer. Phosphorus was determined according to the method of Bartlett (12).

RESULTS AND DISCUSSION

Earlier studies on the phospholipid metabolism of logarithmically-grown suspension cultures of SV40 transformed mouse fibroblasts had shown that [^{32}P]orthophosphate added to the growth medium can easily be incorporated into the cellular phospho-

TABLE 2. ^{32}P-Labeled choline glycerophospholipids isolated from STU 51A/232B cells grown in different media containing 10 mCi of [^{32}P]orthophosphate[a]

Experiment	Composition of Incubation Mixture	[^{32}P]Orthophosphate	Total Number of Cells	Population Density	Isolated ^{32}P-Labeled Choline Glycerophospholipids	
					Amount	Specific Activity
		mCi/mmole		*cells/ml*	*μCi*	*mCi/mmole*
1	500 ml of Eagle's Minimum Essential Medium plus Earle's Spinner Balanced Salt Solution supplemented with 10% fetal calf serum	2.6	10^9	2×10^6	0.35	2×10^{-2}
2	100 ml of medium (Exp. 1) but containing only 0.1 of the nonlabeled orthophosphate	130	2×10^8	2×10^6	15	2.5
3	50 ml of medium as in Exp. 4, but TRIS-buffer instead of HEPES, pyruvate, serine, ethanolamine omitted	4.5×10^6	10^8	2×10^6	90	45
4	2.5 ml of a HEPES-citrate buffered balanced salt solution (Table 1) supplemented with 5% fetal calf serum and other cofactors	4.5×10^6	3×10^7	8×10^6	180	180

[a] Cells were incubated for 17 hr in all experiments.

lipids (8). This paper deals with experiments undertaken to improve both the yield of ^{32}P-labeled phospholipids and the specific activities.

Factors influencing the yields and specific activities of the labeled phospholipids

Increases of the amounts as well as of the specific activities of the [^{32}P]phospholipids were accomplished by reducing the volume of the incubation mixture, by decreasing its content of carrier inorganic phosphate, and by raising the population density of the cells. (**Table 2**, Exp. 1, 2, 3)

Increasing the specific activity of the [^{32}P]orthophosphate in the incubation medium from 2.6 to 130 and from 130 to 4.5×10^6 mCi/mmole yielded an increase in the amount of radioactivity of ^{32}P-labeled choline glycerophospholipids isolated from the STU-232B cells from 0.35 to 15 and from 15 to 90 μCi, respectively. The simultaneous increase in the specific activity was much higher (0.02 to 2.5, and 2.5 to 90 mCi/mmole) since the total number of incubated cells was decreased (10^9, 2×10^8, 10^8 cells), thus diminishing the amount of nonlabeled cellular phospholipids initially brought into the incubation mixture. Whereas the media used in Experiments 1 and 2 supported logarithmic growth of the STU-232B cells for the whole incubation period (17 hr), the medium used in Experiment 3 did not show this property any more since it contained neither carrier inorganic phosphate nor amino acids.

Incubation system used for preparations of labeled phospholipids

From the results discussed above it was evident that cell growth was not a condition for effective utilization of [^{32}P]phosphate in the biosynthesis of choline glycerophospholipids. In order to further promote the utilization of the carrier-free [^{32}P]orthophosphate, its concentration was increased 20-fold by reducing the volume of the incubation mixture from 50 ml to 2.5 ml. Similarly the number of cells/ml was increased four-fold to 8×10^6/ml (Experiment 4). The medium (Table 1) consisted of a HEPES-citrate buffered balanced salt solution that was supplemented with cofactors and dialyzed fetal calf serum. Due to the higher population density of the cells, the buffering capacity of the medium was too low to maintain a constant pH during the whole period of incubation (17 hr). It was therefore necessary to adjust the pH of the incubation mixture to the physiological value every 3–7 hr with 0.16 N NaOH. As can be seen from Experiment 4 (Table 2) the yield of cpm of ^{32}P isolated as choline glycerophospholipids increased by a factor of 2 and its specific radioactivity by a factor of 4 compared with Experiment 3.

The data show that under the conditions used in Experiment 4 there was a 400-fold overall increase of [^{32}P]orthophosphate incorporated into choline glycerophospholipids, compared to the first experiment. The increase of the specific activity was 10^4-fold. Thus the conditions described in Experiment 4 were used for all subsequent preparations of labeled phospholipids. This system is hereafter referred to as the standard incubation system.

Kinetics of ^{32}P incorporation into phospholipids

In order to determine the optimal time of incubation, the kinetics of ^{32}P incorporation were studied using 0.1 ml aliquots of the incubation mixture taken 3.5, 9, 16, and 22 hr after the addition of [^{32}P]orthophosphate. At the same time the number of intact cells still present in the incubation mixture was determined. The amount of ^{32}P incorporated into

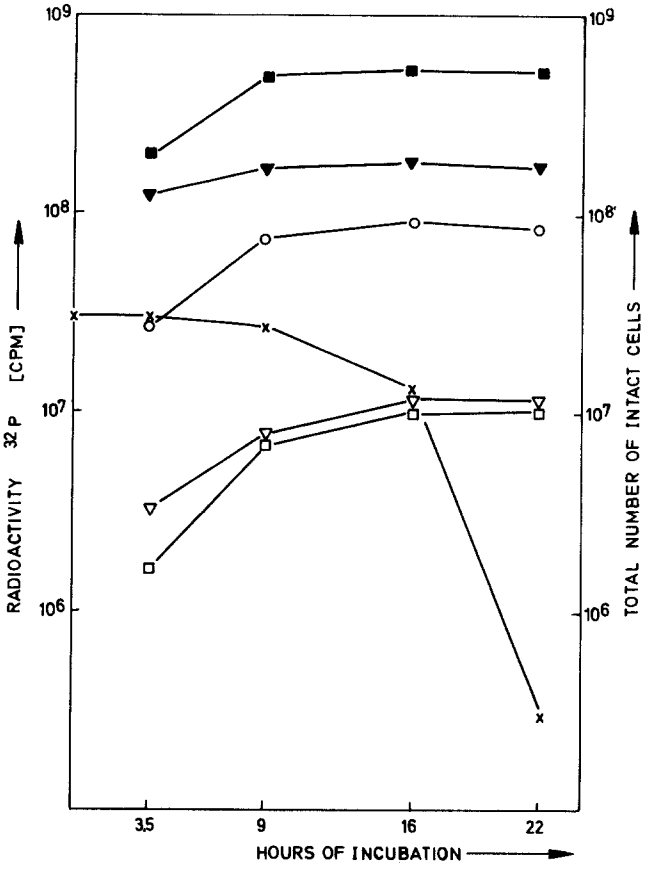

Fig. 2. The rate of incorporation of ^{32}P radioactivity into individual phospholipids under standard conditions. (■) Choline glycerophospholipids, (○) ethanolamine glycerophospholipids, (▽) serine glycerophospholipids, (□) sphingomyelin, (▼) inositol glycerophospholipids, (×) total number of intact cells present.

choline, ethanolamine, inositol, and serine glycerophospholipids and sphingomyelin was determined. Incubation was stopped after 22 hr, when the number of cells had decreased to 10% of the initial value. The kinetic data in **Fig. 2** show that high amounts of [^{32}P]orthophosphate were incorporated into the cellular phospholipids of a nongrowing culture of STU 51A/232 B cells under the given conditions. The incorporation of radioactivity into the individual phospholipids was most rapid during the first 9 hr, when the cell number remained constant. During the next 7 hr, the incorporation increased only slightly, whereas the number of intact cells decreased rapidly (autolysis).

After 16 hr of incubation further incorporation of ^{32}P into the phospholipids was not observed when extensive lysis of cells occurred. From these results an incubation period of about 17 hr seems optimal to obtain high yields of ^{32}P-labeled phospholipids in the given system.

The observed correlation between the number of intact cells present in the incubation mixture and the amount of radioactivity incorporated shows that the structure of the cells must be intact for effective utilization of [^{32}P]orthophosphate in phospholipid biosynthesis. This interpretation is supported by the observation that only negligible amounts of ^{32}P-labeled phospholipids were obtained when a "broken cell" preparation (derived from the same quantity of cells) is incubated under essentially the same conditions.

Preparation of [^{32}P]phosphatidic acid

Since phosphatidic acid is only a minor component of the cellular phospholipids, it was prepared from the crude ^{32}P-labeled phospholipids by treatment with cabbage phospholipase D (10). From the digestion mixture containing 400 μCi of crude ^{32}P-labeled phospholipids (2 μmoles) up to 200 μCi of ^{32}P-labeled phosphatidic acid with a specific activity ranging from 150 to 200 mCi/mmole could be isolated.

Preparations including labeled precursors other than [^{32}P]orthophosphate

To investigate whether the standard incubation conditions also favor the incorporation of other labeled precursors of phospholipids, experiments were performed with [^{3}H]choline and [^{3}H]glycerol. When [^{3}H]choline was used, nonlabeled choline was omitted from the incubation medium. Results of the formation of radioactive phospholipids (**Table 3**) show large differences in the yield of the labeled compounds, depending on the precursor used and the product isolated. Best results were obtained when choline glycerophospholipids were labeled with [^{32}P]orthophosphate or with [^{3}H]choline. Up to 2% of the added ^{32}P-labeled precursor could then be isolated as choline glycerophospholipids (mean of five preparations). However, only 0.25–0.54% of the ^{32}P radioactivity was isolated in inositol or ethanolamine glycerophospholipids. [^{3}H]Glycerol was not very effective as a radioactive precursor of choline glycerophospholipids, presumably due to a low capacity of the cells to phosphorylate free glycerol.

Characterization of the isolates and reproducibility of yields

The radiochemical purity of the different labeled phospholipids, determined as given in the methods section, was in all cases better than 97%.

The distribution of radioactivity between the diacyl types and the alkyl-acyl types of the different labeled phospholipids was determined according to the method of Renkonen (13). The following data (given as ratio of radioactivity present in the diacyl fraction/radioactivity present in the alkenyl-acyl plus alkyl-acyl fraction) represent the distribution of the

TABLE 3. Labeled compounds isolated from 3×10^7 STU 51A/232B cells grown in 2.5 ml of medium (Table 1) containing the radioactive precursors

Radioactivity	Compound Isolated	Radioactivity Isolated		Yield of Radioactivity[a]
amount and specific activity		*μCi*	*mCi/mmole*	%
10 mCi ^{32}Pi[b], 4500 Ci/mmole P	choline glycerophospholipids	200[d]	180	2.0
5 mCi [1-^{3}H]glycerol, 1 Ci/mmole	choline glycerophospholipids	5	5	0.1
5 mCi [methyl-^{3}H]choline, 2 Ci/mmole	choline glycerophospholipids	40	100	0.8
10 mCi ^{32}Pi, 4500 Ci/mmole P	ethanolamine glycerophospholipids	20	70	0.2
10 mCi ^{32}Pi, 4500 Ci/mmole P	inositol glycerophospholipids	50	400	0.5
10 mCi ^{32}Pi, 4500 Ci/mmole P	CDP-choline[c]	20	250	0.2

[a] Calculated as the percentage isolated radioactivity/radioactivity present in the incubation mixture.
[b] ^{32}Pi = [^{32}P]orthophosphate.
[c] In this experiment 4×10^7 cells per ml were incubated for 9 hr instead of 17 hr.
[d] Mean of five preparations.

different glycerophospholipid samples: ^{32}P- and [^{3}H]-choline-labeled glycerophospholipids, 94:6; ^{32}P-labeled ethanolamine glycerophospholipids, 84:16; and [^{32}P]phosphatidic acid 92:8.

In a series of five preparations of ^{32}P-labeled choline glycerophospholipids the yields varied from 170 to 230 μCi. These variations were not due to differences in the amounts of cells used but rather due to variances in the metabolic activity of the cell preparations. On the other hand variability in the recovery of lipids could not be avoided since the time of handling the highly radioactive samples was kept at a minimum.

Preparation of [^{32}P]CDP-choline

In two experiments [^{32}P]CDP-choline was isolated from the upper phase of the partition procedure (6). When the conditions of the standard incubation were used, only 0.02% of the [^{32}P]orthophosphate could be isolated as [^{32}P]CDP-choline. This yield could be improved 10-fold when four times as many cells (1.2×10^8) were incubated for 9 hr in the same volume of medium as before. These alterations, however, caused a 40% decrease in the yield of ^{32}P-labeled choline phosphoglycerides. The labeled CDP-choline had a specific activity of 250 mCi/mmole and gave a single spot after radioautography.

The relatively low specific radioactivity (250 mCi/mmole) of the compound isolated is a result of the nonlabeled CDP-choline added before isolation in order to facilitate optical detection during the isolation procedure.

Incubations using other cell types

To examine whether the method described for the preparation of labeled phospholipids was applicable to other types of mammalian cells, similar volumes (0.1–0.2 ml) of packed Ehrlich ascites cells, human red blood cells, and white blood cells from ox blood were incubated with [^{32}P]orthophosphate using standard incubation conditions. The amount of [^{32}P]orthophosphate incorporated into the phospholipids was determined. The results are listed together with those from incubations of mouse fibroblasts in order to compare the biosynthetic capacities of the different cell lines under the given conditions (**Table 4**).

Depending on the type of cells used and the compound isolated, large differences in the amounts of

TABLE 4. ^{32}P-Labeled phospholipids isolated from various types of cells grown in 2.5 ml of medium (Table 1) containing 10 mCi of [^{32}P]orthophosphate

Type of Cells Used	STU 51A/232B (Mouse)	Ehrlich Ascites Cells (Mouse)	Human Blood Cells	White Blood Cells (Bovine)
Total Number of Cells Used for Incubation[a]	3×10^7	2×10^7	4×10^8	10^8
^{32}P isolated with:		*μCi ^{32}P isolated*		
Sphingomyelin	4	12	0.04	2
Serine glycerophospholipids	4	4	0.04	1.2
Inositol glycerophospholipids	50	25	0.5	18
Choline glycerophospholipids	200	220	2.5	110
Ethanolamine glycerophospholipids	20	40	0.2	6
Phosphatidic acid	4	2	16	16

[a] Amounts correspond to 0.15 ± 0.05 ml of packed cells. The cells were incubated for 17 hr in all experiments.

incorporated ^{32}P were noted. STU 51A/232B cells, Ehrlich ascites cells, and white blood cells exhibited a very high incorporation of [^{32}P]orthophosphate into choline, inositol, and ethanolamine glycerophospholipids but a relatively low incorporation into sphingomyelin, serine glycerophospholipids, and phosphatidic acid.

The amounts of ^{32}P incorporated into the phospholipids of human blood cells are very low compared with those found for the other cells, except for the relatively high incorporation into phosphatidic acid. This behavior may be explained by the metabolic state of the red blood cells.

These results show that, under the given standard conditions, STU 51A/232B cells, ascites cells, and white blood cells are suitable to prepare highly labeled phospholipids.

In summary the method has been shown to be useful for the preparation of highly labeled phospholipids from cellular phospholipids of three different mammalian cell lines that had been incubated with an appropriate radioactive precursor. Since the conditions of the standard incubation were designed for optimal yields of [^{32}P]phosphatidylcholine, the yields of the other phospholipids may still be subject to improvement by appropriate alterations of the incubation system, especially in the case of using cell lines other than STU 51A/232B cells.

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