Regulation of Phospholipid Biosynthesis in Isolated Rat Hepatocytes

EFFECT OF DIFFERENT SUBSTRATES*

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SUMMARY
The effects of choline, ethanolamine and its N-methyl analogs, different fatty acids, and l-methionine on phospholipid biosynthesis via the CDP-ester pathways and the methylation pathway were studied in rat hepatocytes. Phosphatidylethanolamine synthesis was stimulated severalfold by 0.02 to 0.1 mM ethanolamine, especially in the presence of long chain unsaturated fatty acids. At higher concentrations of ethanolamine, phosphorylethanolamine accumulated but the level of CDP-ethanolamine and the rate of phosphatidylethanolamine synthesis did not increase further. The rate of phosphatidylcholine synthesis via the CDP-ester pathway responded in a way analogous to that of phosphatidylethanolamine synthesis upon the addition of choline and fatty acid, except that a 10- to 20-fold higher concentration of choline was required for maximal stimulation, probably due to the rapid oxidation of choline to betaine. Phospholipids containing N-monomethyl- or N,N-dimethylethanolamine were efficiently formed from the corresponding free bases in the absence of ethanolamine and choline. Ethanolamine, but not other bases, inhibited completely phospholipid formation from N-monomethylthanolamine, probably as a result of competition at the level of CDP-ester formation. The cytidylyltransferase reactions are rate-limiting steps in the synthesis of phosphatidylethanolamine and probably also phosphatidylcholine. In addition, the availability of diacylglycerol and its fatty acid composition may significantly affect the rate of phospholipid synthesis.

The rate of phosphatidylethanolamine formation via phospholipid N-methylation approximately doubled when l-methionine was added at concentrations similar to that in rat plasma. Under these conditions the rate of phosphatidylethanolamine synthesis via this pathway was 20 to 40% of that via diacylglycerols and CDP-choline. The methylation of phosphatidylethanolamine to phosphatidylcholine remained essentially constant when the rate of phosphatidylethanolamine synthesis was varied 8-fold, but was significantly reduced when the formation of N-monomethyl- or N,N-dimethylphospholipid was stimulated by addition of the corresponding base. These phospholipids not only replaced phosphatidylethanolamine as the substrate for methylation but also increased the rate of phosphatidylcholine formation via this pathway.

A method for the determination of nanomole amounts of different ethanolamine compounds is described.

Phospholipids synthesized in liver participate in membrane formation within the organ or are transferred into bile or the lipoproteins of blood plasma (1). Although little is known about physiological variation in the rates of these processes certain experimental situations, such as the induction of microsomal enzymes by phenobarbital, seem to be accompanied by an increased rate of phospholipid biosynthesis (2, 3). The mechanisms behind such changes, and even the means by which the biosynthesis of phospholipids is controlled under normal conditions, are, however, largely unknown.

We have therefore studied the effects of different substrates on the rate of phosphatidylethanolamine and phosphatidylcholine synthesis via the Kennedy pathway (4), on phosphatidylcholine synthesis via phospholipid N-methylation (5), and on the level of some intermediary metabolites in rat hepatocytes in order to identify critical steps in the regulation of these pathways.

MATERIALS AND METHODS
Radioactively labeled compounds were purchased as follows: [1(3)-3H]glycerol, [2-3H]ethanolamine, l-[methyl-14C]methionine, and [methyl-3H]choline from the Radiochemical Centre, Amersham, and [3P]phosphate from AB Atomenergi, Studsvik, Sweden. Phosphoryl-3H]ethanolamine (0.36 mCi/μmol) and CDP-[3H]ethanolamine (1.08 mCi/μmol) were prepared enzymatically (6) from [2-3H]ethanolamine. They were purified by ion exchange chromatography (7) and determined as Dnsl-ethanolamine (see below).

Fatty acids (Hormel Institute, Austin) were complexed to delipidated (8) bovine serum albumin (Serva, Heidelberg) by the method of Spector and Hoak (9). Protein was determined (10) with bovine serum albumin as a standard.

Preparation of Hepatocytes—Adult male Sprague-Dawley rats

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The abbreviation used is Dnsl-, 5-dimethylamino-naphthalene-1-sulfonyl-.
(Anticimex AR, Stockholm) weighing 200 to 300 g were fed a balanced diet ad libitum. Hepatocytes were prepared by the method of Berry and Friend (11) as described elsewhere (12). In later experiments a modification similar to that of Ingebritsen and Wagle (13) was used. Then hyaluronidase was omitted, the amount of collagenase (type 1, Sigma, St. Louis) was lowered (0.2% w/v) and 1% (w/v) delipidated bovine serum albumin was included in the perfusate. Vital staining of the cells with trypan blue showed 80 to 95% of them to be intact.

**Incubation Conditions**—Hepatocytes were incubated in 25-ml Erlenmeyer flasks in a total volume of 0.5 ml of Hanks' solution (14) buffered with 10 mm phosphate (pH 7.4) and containing 2% (w/v) delipidated bovine serum albumin. After shaking on a Vortex mixer for 1 min, the supernatant was aspirated off and the sediment was washed twice with 1 ml of 3% trichloroacetic acid. The sediment was then neutralized with 1 drop of 4 M NaOH and diluted with 1 ml of 0.1 M NaCl, 1.5 mM EDTA (pH 7.4). After 1 hour at room temperature, 0.4 ml of 0.01 M NaOH was added. The samples were read in a Jobin Yvon spectrofluorimeter at an excitation wavelength of 355 nm and an emission wavelength of 474 nm. Trioleglycerol (Hormel Institute, Austin) was used as a standard. The fluorescence of blank incubations corresponded to 0.1 to 0.5 nmol of diacylglycerol per mg of protein in different experiments. The amount of cell protein ranged between 1.5 and 4.0 mg per incubation in different experiments. In experiments where metabolites were determined hepatocytes (20 to 107 mg of protein) were incubated in 250-ml flasks in a final volume of 8 ml. Different substrates and radioactive precursors were added as indicated.

**Extraction and Separation of Labeled Products**—Lipids were extracted with chloroform-methanol (1:1), washed free of labeled precursors, and separated by thin layer chromatography on Silica Gel H (15). For the isolation of phosphatidyl diglycerol, thin layer plates were developed in chloroform-methanol-concentrated NH\_4\_OH (60:30:5 by volume) (16). A reference sample of this compound was prepared by reductive methylation of phosphatidylglycerol with formaldehyde and formic acid (17). For the isolation of phosphatidyl ethanolamine, the developing system was the same procedure. Dns-ethanolamine was then isolated by thin layer chromatography on Silica Gel H (15). For the isolation of phosphatidyl ethanolamine, the same procedure was used.

**Determination of Diacylglycerol**—Diacylglycerol was isolated from the lipid extract of incubations prepared as described above. Blank incubations lacking hepatocytes were carried through the same procedure. After thin layer chromatography on Silica Gel H (developing solvent, toluene-chloroform-methanol (85:12:3 by volume)) only reference substances running beside the sample were sprayed with 0.2% dichlorofluorescein in ethanol. The diacylglycerol zone was eluted with 12 ml of chloroform-methanol-acid-water (50:39:1:10 by volume) and the extract was washed once with 4 ml NaOH and twice with methanol-water (1:1). Aliquots (2 to 20 nmol) were taken for quantitative determination by a modification of Laurell's procedure (20). The samples were taken to dryness and 100 µl of 0.015 M KOH in ethanol were added. After 15 min at 65° the samples were again taken to dryness and 100 µl of the following solution were added: 1 mm ATP, 1.5 mm NAD, 10 mm cysteine (pH 9), 0.5 mm MgCl\_2, 0.2 mm hydrazine-HCl (pH 9.4), crystalline-defatted bovine serum albumin (5 mg/ml), glycerokinase from Candida mycoderma (Boehringer Mannheim) (0.5 µg/ml), and glycerol-3-phosphate dehydrogenase (Boehringer Mannheim) (25 µg/ml). After 1 hour at room temperature, 0.5 ml of 0.01 M NaOH containing EDTA (0.4 µl/g liter) was added. The samples were read in a Jobin Yvon spectrofluorimeter at an exciting wavelength of 355 nm and an emission wavelength of 474 nm. Trioleglycerol (Hormel Institute, Austin) was used as a standard. The fluorescence of blank incubations corresponded to 0.1 to 0.5 nmol of diacylglycerol per mg of protein in different experiments. The determined values of diacylglycerol were corrected for losses during isolation from the recovery of iso.

**RESULTS**

**Phosphatidylethanolamine Synthesis**—The addition of ethanolamine to rat hepatocytes incubated with [H]glycerol increased the incorporation of iso into phosphatidylethanolamine approximately 2-fold at 0.02 to 0.04 mm concentration (Fig. 1), which is only slightly higher than the concentration of ethanolamine in rat blood plasma (Table 1). The increased labeling of phosphatidylethanolamine was not accompanied by any significant change in total [H]glycerol incorporation into lipids but was compensated for by a decreased incorporation into phosphatidylcholine and, above all, the neutral glycerides (Fig. 1). Since this indicates that the enzymes catalyzing triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine synthesis may compete for diacylglycerol we also studied the effect of ethanolamine after stimula-

* Diacylglycerol denotes in this paper 1,2-diacyl-sn-glycerol.
Fig. 1. Effect of ethanolamine on the incorporation of [3H]-glycerol (2 mM) into total lipids and individual lipid classes. Incubation time, 60 min. Each point represents the mean value from 2 to 4 incubations. Individual values deviate less than 5% from the means.

Table I

| Sample       | Ethanolamine concentration* |
|--------------|----------------------------|
|              | Rat 1 | Rat 2 | Rat 3 |
| Portal       | 20.6  | 23.1  | 18.9  |
| Arterial     | 19.5  | 23.2  | 17.4  |

* In arterial samples from 11 rats, the mean ± S.D. was 17.1 ± 4.0 μM in a range of 12.6 to 23.3.

Fig. 2. Effects of fatty acids and ethanolamine on phosphatidylethanolamine (PE) synthesis from [3H]glycerol (2 mM). Incubation time, 60 min. The points represent mean values from duplicate incubations.

Fig. 3. Effect of different fatty acids on phosphatidylethanolamine (PE) synthesis from [3H]glycerol (2 mM). In b, the incubations contained 0.4 mM ethanolamine. Incubation time was 60 min. The points represent mean values from duplicate incubations.

The addition of long chain fatty acids to hepatocytes incubated with [3H]glycerol results in a severalfold increase in the isotope content of diacylglycerol (15). However, this accumulation was reduced by the simultaneous presence of ethanolamine or choline (Table II). The chemically determined diacylglycerol level in the
Fig. 4. Effect of ethanolamine and fatty acids on the level of phosphoryl- and CDP-ethanolamine in rat hepatocytes. Incubation time was 30 min. Each incubation contained 29 mg of cell protein. The data are representative of three separate experiments.

Table II
Effect of phospholipid substrates on the content of \(^[\text{H}]\)glycerol in diacylglycerol

Hepatocytes were incubated for the time indicated with \(^[\text{H}]\)glycerol (2 mM) and oleic acid (1 mM), lauric acid (1 mM), ethanolamine (0.4 mM), or choline (2.0 mM). Data are means \(\pm\) S.E. from 4 experiments (left part) or mean values from duplicate incubations. Values within parentheses are given as per cent of the control in each experiment.

| Additions          | Diacylglycerol | 60 min | 15 min | 30 min | 60 min |
|--------------------|----------------|--------|--------|--------|--------|
|                    | % of lipid \(^{\text{H}}\) | % of added \(^{\text{H}}\)glycerol |        |        |        |
| Oleic acid         | 7.7 \(\pm\) 1.1 (100) | 0.020 | 0.029 | 0.053 |
| Oleic acid + ethanolamine | 4.7 \(\pm\) 0.8 (60 \(\pm\) 1.7) | 0.118 | 0.185 | 0.257 |
| Oleic acid + choline | 5.8 \(\pm\) 1.4 (73 \(\pm\) 0.8) | 0.084 | 0.081 | 0.073 |
| None               |                |        |        |        |

Presence of oleic acid deviated less than 20% from that calculated from \(^{[\text{H}]}\)glycerol incorporation. It increased with time (Fig. 5) in agreement with the isotope data (Table II) and this increase was counteracted by the presence of ethanolamine. Lauric acid affected the level of diacylglycerol in a curious way. After a transient increase, both the content of \(^{[\text{H}]}\)glycerol (Table II) and the level determined chemically (Fig. 5) declined. It is therefore probable that the inhibition of phosphatidylethanolamine synthesis by lauric acid (Figs. 2 and 3) results from a shortage of diacylglycerols suitable for phospholipid formation, since the decline in total diacylglycerol is accompanied by the formation of an increasing proportion of dilauryglycerol, which is insubstantially utilized for phosphatidylethanolamine synthesis (15).

Phosphatidylcholine Synthesis via CDP-choline and Diacylglycerol—The addition of increasing concentrations of choline to hepatocytes incubated with \(^{[\text{H}]}\)glycerol increased the incorporation of isotope into phosphatidylcholine (Fig. 6). Compared to the corresponding effect of ethanolamine on phosphatidylethanolamine synthesis (Fig. 1) the effect of choline was smaller and occurred only at a 10- to 20-fold higher concentration. The presence of oleic acid enhanced the stimulatory effect of choline on the incorporation of both \(^{[\text{H}]}\)glycerol and \(^{[\text{H}]P}\)phosphate (Table III) but the effect on \(^{[\text{H}]}\)P was always more pronounced. Although other factors might give rise to such a difference it is noticeable that since the cholinephosphotransferase (EC 2.7.8.2.) reaction is reversible (25-28), the rate of diacylglycerol incorporation into phosphatidylcholine would be higher than the net synthesis of phosphatidylcholine from phosphorylcholine. One would therefore expect a greater relative increase of the latter reaction (and...
anesulfonic acid (Hepes) as a buffer; i.e. [3H]glycerol (2 mM) was added after 15 min of incubation with all other components including [*PIphosphate (25 μCi) present and the incubations were continued for 60 min. PC, phosphatidylcholine.

Additions | [3H]Glycerol in PC | [*P]Phosphate in PC
---|---|---
Fatty acid (mm) | Choline | Percent of added isotope | cpm | Per cent of control | cpm | Per cent of control
None | 0.280 | 100 | 5,655 | 100
None | 0.4 | 0.311 | 111 | 6,467 | 114
None | 1.0 | 0.358 | 127 | 8,142 | 143
None | 2.0 | 0.389 | 139 | 8,902 | 156
Oleic acid (1.0) | 0.301 | 100 | 8,309 | 100
Oleic acid (1.0) | 0.4 | 0.727 | 201 | 21,490 | 262
Oleic acid (1.0) | 1.0 | 0.767 | 213 | 25,388 | 310
Oleic acid (1.0) | 2.0 | 0.751 | 233 | 27,331 | 297

Fig. 7. The metabolism of [3H]choline in isolated hepatocytes. Incubation time was 60 min. The points represent mean values from duplicate incubations.

of the incorporation of [*P] when phosphatidylycerol synthesis is stimulated by the addition of choline.

A reason for the high concentration of choline required for stimulation of phosphatidylycerol synthesis (Fig. 6) was found by studying the metabolism of [3H]choline (Fig. 7). The oxidation of choline to betaine under the present conditions exceeded glycerol addition was 60 min. The incubations shown in the lower part were preincubated for 60 min with the bases prior to addition of [3H]glycerol. The concentration of ethanolamine, N-methyl ethanolamine (MME), and N,N-dimethylethanolamine (DME) was 0.4 mM. The concentration of choline was 2.0 mM. The bars represent triacylglycerols + diacylglycerols (A), phosphatidyl ethanolamine (B), phosphatidyl methyl ethanolamine (C), phosphatidyl dimethylethanolamine (D), phosphatidylcholine (E), and lysophospholipids (F).

Fig. 8. Effect of phospholipid bases on the incorporation of 2 mM [3H]glycerol into lipid classes. Incubation time after [3H]glycerol addition was 60 min. The incubations shown in the lower part were preincubated for 60 min with the bases prior to addition of [3H]glycerol. The concentration of ethanolamine, N-methyl ethanolamine (MME), and N,N-dimethylethanolamine (DME) was 0.4 mM. The concentration of choline was 2.0 mM. The bars represent triacylglycerols + diacylglycerols (A), phosphatidyl ethanolamine (B), phosphatidyl methyl ethanolamine (C), phosphatidyl dimethylethanolamine (D), phosphatidylcholine (E), and lysophospholipids (F).

Biosynthesis of Phosphatidyl Mono- and Dimethylethanolamine via CDP-ester Pathways—Exposure of the hepatocytes to N-methyl ethanolamine or N,N-dimethylethanolamine resulted in considerable formation of phosphatidyl methyl ethanolamine or phosphatidyl dimethylethanolamine from labeled glycerol, although the formation of the dimethyl phospholipid started more slowly and required preincubation with the corresponding base to become prominent (Fig. 8). Like the addition of ethanolamine or choline, N-methyl ethanolamine or N,N-dimethylethanolamine did not significantly affect total incorporation of [3H]glycerol into lipids but only its distribution among lipid classes. Incorporation of labeled N,N-dimethylethanolamine into liver phospholipids has been demonstrated in vivo (32, 33), and in vitro the CDP-esters of the N-mono- and di-methylated bases are utilized for phospholipid synthesis to about the same extent as CDP-choline and CDP-ethanolamine (34). The formation of the CDP-esters of N-methyl ethanolamine and N,N-dimethylethanolamine from their phosphate esters and CTP is, however, less efficient than the corresponding formation of CDP-choline and CDP-ethanolamine (34). Data showing that CDP-N-methyl ethanolamine and CDP-N,N-dimethylethanolamine are formed by different enzymes, probably phosphoryl ethanolamine cytidylyltransferase (EC 2.7.7.14.) and phosphorylcholine cytidylyltransferase (EC 2.7.7.15.), respectively, have also been presented (35). We therefore studied the competition between pairs of such bases, since competition between ethanolamine and N-methyl ethanolamine and between choline and N,N-dimethylethanolamine would be expected to occur at the steps catalyzed by the cytidylyltransferases. All different combinations of bases inhibited the incorporation of [3H]glycerol into the corresponding phospholipid classes in a mutual way but only the addition of ethanolamine resulted in complete inhibition of phospholipid formation from N-methyl ethanolamine (Table IV). This is further evidence, although indirect, for a limited capacity in the step catalyzed by phosphoryl ethanolamine cytidylyltransferase. The less pronounced inhibition of phosphatidyl methyl ethanolamine formation by other bases most probably results from competition for a common substrate, such as diacylglycerol. A corresponding competition between choline and N,N-dimethylethanolamine at the phosphorylcholine cytidylyltransferase level could not be demonstrated. The formation of phosphatidyl dimethylethanolamine fell to 40, 62, and 72% of the initial value (mean from four experiments) when ethanolamine, N-methyl ethanolamine, and choline, respectively, were included in addition to N,N-dimethyl ethanolamine.

Phosphatidylylycerol Synthesis via Phospholipid N-methylamidation—The relative rate of phosphatidyl ethanolamine methylation was determined by following the conversion of phosphatidyl [3H] ethanolamine into N-methylated phospholipids during incubation with [3H]ethanolamine, while the total rate of phospholipid N-
TABLE IV

Effect of phospholipid bases on formation of phosphatidyl methylethanolamine

Hepatocytes were preincubated for 30 min without base or with ethanolamine, N-methylethanolamine (MME), N,N-dimethylethanolamine (DME) (0.4 mM) and/or choline (2.0 mM) as indicated; then [3H]glycerol (2.0 mM) was added and incubation continued for another 60 min. PMME, phosphatidyl methylethanolamine.

| Added base(s)          | PMME* % of lipid *H |
|------------------------|----------------------|
| None                   | 2.9                  |
| MME                    | 18.9                 |
| MME + ethanolamine     | 2.6                  |
| MME + DME              | 14.2                 |
| MME + choline          | 13.2                 |

* Including phosphatidyleserine and phosphatidylinositol.

methylation was assessed with L-[methyl-14C]methionine under the assumption that L-methionine via S-adenosylmethionine is the only donor of methyl groups in this pathway (5).

The addition of methionine stimulated the conversion of phosphatidyl[3H]ethanolamine into phosphatidylethanolamine about 2-fold over at least 2 hours (Fig. 9a), but no further increase occurred when the concentration of added methionine was raised above 0.1 mM (Fig. 9b). In accordance, the incorporation of [methyl-14C]-methionine was linear for about 2 hours and leveled off at about 0.1 mM concentration (Fig. 9c). The values reported for the concentration of L-methionine in rat plasma (55 to 90 µM, Refs. 36, 37) are only slightly lower than the concentration which gave maximal stimulation of phosphatidylcholine synthesis via N-methylation.

The addition of N-methylethanolamine or N,N-dimethylethanolamine, which results in appreciable synthesis of the corresponding phospholipid (Fig. 8) increased the incorporation of labeled methyl groups at a saturating concentration of L-[methyl-14C]methionine (Table V). In contrast, the conversion of phosphatidyl[3H]ethanolamine into phosphatidylethanolamine was inhibited by these bases. This was not due to trapping of isotope in partially methylated phospholipids, as a result of their formation from the added N-methylethanolamine or N,N-dimethylethanolamine. Instead the rate of phosphatidylethanolamine methylation decreased. Since also the total incorporation of [3H]ethanolamine into phosphatidylethanolamine was depressed by the addition of N-methyl- or N,N-dimethylethanolamine, we thought that inhibition of phosphatidylethanolamine synthesis by the added bases might, secondarily, result in a lowered rate of phosphatidylethanolamine methylation. However, the addition of lauric acid, which lowers the rate of phosphatidylethanolamine synthesis (Figs. 2 and 3), did not inhibit its conversion into N-methylated phospholipids; neither was it stimulated by the addition of ethanolamine, alone or together with oleic acid (Table V). Thus, we found no evidence for an immediate co-regulation of the rates of phosphatidylethanolamine synthesis and N-methylation, but the latter is apparently inhibited by an increased formation and methylation of phosphatidyl methylethanolamine or phosphatidyl dimethylethanolamine.

The total rate of phosphatidylethanolamine formation via phospholipid N-methylation at a saturating concentration of methionine was found to be 20 to 40% of the rate of phosphatidylethanolamine synthesis from labeled glycerol, depending on the concentration of fatty acid and choline present (Table VI).

DISCUSSION

Albumin-bound long chain fatty acids stimulate glycerolipid synthesis over a wide concentration range in liver slices (38) and in isolated hepatocytes (15, 39). Characteristically the formation of triglycerides accounts for most of this increase while the synthesis of phosphatidylethanolamine, and especially phosphatidylcholine, is much less affected. Thus the rate of phosphatidylethanolamine and phosphatidylcholine synthesis appears to be limited at the steps catalyzed by cholinephosphotransferase and ethanolaminephosphotransferase (EC 2.7.8.1.) converting diacylglycerols into phosphatidylethanolamine and phosphatidylcholine, respectively. However, this does not necessarily mean that the capacity of these enzymes is the limiting factor. The present study demonstrates that the rate of phosphatidylcholine synthesis and probably also phosphatidylethanolamine synthesis is
Effect of phospholipid substrates on the methylation pathway of phosphatidylcholine synthesis

Hepatocytes were incubated for 120 min with [3H]ethanolamine (0.01 mM) and l-methionine (0.4 mM), or with [3H]-methionine (0.4 mM). Data are means ± S.E. (n = 4) or means from three experiments. The conversion of phosphatidyl[3H]ethanolamine (P^3HIE) into phosphatidylcholine (PC) in the controls was 9.6 ± 1.0 (M ± S.E., n = 4) % of lipid ^3H. Phosphatidyl mono- and dimethylethanolamine together contained less than 2% of lipid ^3H, regardless of additions. When N,N-dimethylethanolamine (MME) was added, ^14C in phosphatidyl dimethylethanolamine rose from 0.5 to 0.9 up to 2.5 to 4.1% of lipid radioactivity. DME, N,N-di-
methylethanolamine.

| Addition | Fatty acid (mm) | Base (mm) | PE, % of control | PC, % of control |
|----------|----------------|-----------|------------------|------------------|
| None     | None           | Ethanolamine (0.4) | 99 ± 8.8         | 107 ± 3.2        |
| None     | None           | Ethanolamine (0.4) | 90 ± 8.8         | 100 ± 0.9        |
| None     | MME (2.0)      | Ethanolamine (0.4) | 44 ± 4.8         | 140 ± 7.8        |
| None     | DME (2.0)      | Ethanolamine (0.4) | 64 ± 2.5         | 139 ± 6.5        |
| None     | Choline (2.0)  | Ethanolamine (0.4) | 99 ± 3.1         | 97 ± 5.4         |

* Absolute values are given in Table VI.

Table VI

Rate of phospholipid synthesis in rat hepatocytes

Incubation time was 60 min ([3H]glycerol) or 120 min (methyl-
[^14C]methionine). The data are means ± S.E. PE, phosphatidyl-
ethanolamine; PC, phosphatidylcholine.

| Labeled substrate (mm) | Additions (mm) | PE | PC | No. of experiments |
|------------------------|----------------|----|----|-------------------|
| [1(3)H]glycerol (2.0)  | None           | 12 ± 1.1 | 31 ± 2.4 | (17) |
| [1(3)H]glycerol (2.0)  | Oleic acid (1.0) | 16 ± 1.0 | 43 ± 3.2 | (16) |
| [3H]glycerol (2.0)     | Ethanolamine (0.4) | 25 ± 3.3 | 37 ± 5.4 | (9) |
| [3H]glycerol (2.0)     | Choline (2.0)   | 49 ± 5.0 | 64 ± 4.5 | (5) |
| [3H]glycerol (2.0)     | Oleic acid (1.0) + ethanolamine (0.4) | 64 ± 4.5 | 64 ± 4.5 | (5) |
| L-[methyl-[^14C]]-
methionine (0.4)        | None           | 13 ± 1.4 | 13 ± 1.4 | (7) |

* Picomoles of[^14C]methionine × 14.

mainly determined by the availability of CDP-ester and diacyl-
glycerol, substrates for the phosphotransferase enzymes.

The formation of CDP-ethanolamine is limited by the supply of ethanolamine, unless exogenous ethanolamine is present at a concentration of about 0.05 mM. At higher concentrations phosphatidylethanolamine accumulates but the rate of phosphatidylethanolamine synthesis and the level of CDP-ethanolamine re-
ment in rats, show a high degree of parallelism between the activity of phosphorylcholine cytidylyltransferase and the rate of [3H]choline incorporation. To what extent the activity of cholinephosphotransferase in vitro (43) and factors affecting this activity (44) are relevant to the overall rate of the CDP-choline pathway may therefore be questioned.

The methylation pathway of phosphatidylcholine synthesis has been studied in rat liver in vivo by measuring either the transfer of isotope from phosphorylcholine to ethanolamine (7, 21, 22, 46) and an uneven intracellular distribution of newly synthesized phosphatidylethanolamine (50), has indicated that the rate of phosphatidylcholine formation via methylation is of the order 3.5 to 10% of that via the CDP-choline pathway.

The methylation pathway of phosphatidylcholine synthesis has been studied in rat liver in vivo by measuring either the transfer of isotope from phosphorylcholine to ethanolamine, prelabeled in the ethanolamine portion (5, 26, 48, 49). The former type of experiment, although complicated by metabolic heterogeneity among molecular species of phosphatidylethanolamine (7, 21, 22, 46) and an uneven intracellular distribution of newly synthesized phosphatidylethanolamine (50), has indicated that the rate of phosphatidylcholine formation via methylation is of the order 3.5 to 10% of that via the CDP-choline pathway. The efficient formation of partially methylated phospholipids from N-methylethanolamine and N,N-dimethylethanolamine (Fig. 8) provided means for interference with the methylation pathway. These bases increased the utilization of methyl-labeled methionine for phospholipid N-methylation, in accordance with the findings in guinea pig liver (45).

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