Effect of choline deficiency on *S*-adenosylmethionine and methionine concentrations in rat liver

Steven H. ZEISEL,* Todd ZOLA, Kerry-Ann DACOSTA and Elizabeth A. POMFRET

Nutrient Metabolism Laboratory, Departments of Pathology and Pediatrics, Boston University School of Medicine, 85 East Newton Street, Room M1002, Boston, MA 02118, U.S.A.

Choline and C_1 metabolism pathways intersect at the formation of methionine from homocysteine. Hepatic *S*-adenosylmethionine (AdoMet) concentrations are decreased in animals ingesting diets deficient in choline, and it has been suggested that this occurs because the availability of methionine limits AdoMet synthesis. If the above hypothesis is correct, changes in hepatic AdoMet concentrations should relate in some consistent manner to changes in hepatic methionine concentrations. Rats were fed on a choline-deficient or control diet for 1–42 days. Hepatic choline concentrations in control animals were 105 nmol/g, and decreased to 50 % of control after the first 7 days on the choline-deficient diet. Hepatic methionine concentrations decreased by less than 20 %, with most of this decrease occurring between days 3 and 7 of choline deficiency. Hepatic AdoMet concentrations decreased by 25 % during the first week, and continued to decrease (in total, by over 60 %) during each subsequent week during which animals consumed a choline-deficient diet. Hepatic *S*-adenosylhomocysteine (AdoHcy) concentrations increased by 50 % when animals consumed a choline-deficient diet. AdoHcy is formed when AdoMet is utilized as a methyl donor. In summary, choline deficiency can deplete hepatic stores of AdoMet under dietary conditions that only minimally decrease the availability of methionine within liver. Thus decreased availability of methionine may not have been the only mechanism whereby choline deficiency lowers hepatic AdoMet concentrations. We suggest that increased utilization of AdoMet might also have occurred.

INTRODUCTION

Choline is an essential nutrient for many mammals, and ingestion of a diet deficient in choline has major consequences, including fatty infiltration of the liver, renal dysfunction, and increased sensitivity to carcinogens [1–9]. Choline is a precursor for the biosynthesis of phosphatidylcholine, sphingomyelin and choline plasmalogens (all essential constituents of membranes) and for acetylcholine, an important neurotransmitter [1]. It is also a major dietary source of methyl groups.

Choline and C_1 metabolism pathways intersect at the formation of methionine from homocysteine ([10–12]; Fig. 1). Methionine is regenerated from homocysteine in a reaction catalysed by betaine–homocysteine methyltransferase (EC 2.1.1.5), in which betaine, a metabolite of choline, serves as the methyl donor [12,13]. The only alternative mechanism for regeneration of methionine is via a reaction catalysed by 5-methyltetrahydrofolate–homocysteine methyltransferase (EC 2.1.1.13), which uses a methyl group generated *de novo* from the C_1 pool [12–14]. Methionine is converted into *S*-adenosylmethionine (AdoMet) in a reaction catalysed by methionine adenosyltransferase (EC 2.5.1.6). AdoMet is the active methylating agent for many enzymic methylations [14].

Hepatic AdoMet concentrations are decreased in animals ingesting diets deficient in choline [15–19]. It has been suggested that AdoMet concentrations fall because methionine is not available [12,19]. Limited amounts of methionine are synthesized during choline deficiency

because the 5-methyltetrahydrofolate–homocysteine methyltransferase pathway cannot fulfil the total requirement for methionine when the betaine-dependent remethylation of homocysteine is limited by the availability of betaine [12,19]. Betaine concentrations in livers of choline-deficient rats are markedly diminished [11,12,20].

If the only reason why choline deficiency depletes hepatic AdoMet is that methionine is not available, methionine concentrations in liver should decrease before AdoMet concentrations. In addition, changes in the availability of methionine should relate in some consistent manner to changes in hepatic AdoMet. To determine whether this is the case, we characterized the time course of changes in hepatic methionine, AdoMet, *S*-adenosylhomocysteine (AdoHcy), and choline in rats fed on a choline-deficient or control diet for various intervals from 1 to 42 days.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (75–100 g body wt; Charles River Breeders, Wilmington, MA, U.S.A.) were housed in suspended cages in a climate-controlled room (24°C), and were exposed to light during 06:00–18:00 h daily. They were fed on a control diet (composition, by wt.: 10 % casein, 10 % soy α -protein, 20 % lard, 56 % sucrose, 4 % salt mix W, and AIN vitamin mix containing 0.2 % choline bitartrate by our assay; diet delivers 0.35 %

Abbreviations used: AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine.

* To whom correspondence and reprint requests should be addressed.

Fig. 1. Interrelationships between choline and methionine metabolism

Abbreviations: THF, tetrahydrofolate; Hcy, homocysteine.

methionine; ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.) and water *ad libitum* for 1 week. Groups of 35 rats then were randomly assigned to receive the control diet or a choline-deficient diet (deficient diet prepared as above, but with AIN vitamin mix without choline: contained 0.02% choline by our assay) and water *ad libitum*. Each control animal was paired with a deficient animal, and both were offered the amount of food consumed by the deficient animal on the previous day. Animals were weighed at 3-day intervals. On days 1, 3, 7, 14, 21 and 42, five animals from each group were anaesthetized with diethyl ether, and samples of liver were rapidly collected by clamping between stainless-steel tongs precooled in liquid N₂. Animals were then killed by exsanguination. Liver was pulverized with a mortar and pestle under liquid N₂ and stored at -95°C until assayed.

Determination of hepatic methionine

Methionine concentrations were determined by the method of Bidlingmeyer *et al.* [21] for extraction from liver, and a modification of the h.p.l.c. technique described by Scholze [22] for quantification. The phenylisothiocyanate derivative of methionine was formed and applied to a reverse-phase h.p.l.c. column (Zorbax C-8: 5 µm, 4.6 mm × 250 mm; Dupont, Wilmington, DE, U.S.A.). We used a gradient controller and data-management system (ISCO, Lincoln, NE, U.S.A.) with two pumps (ISCO model 2350) and an on-line spectrophotometric detector (model LC-85B; Perkin-Elmer, Norwalk, CT, U.S.A.). Column temperature was maintained at 55°C with a heater (Eldex Laboratories, Menlo Park, CA, U.S.A.). Methionine was eluted with a stepped-linear binary gradient consisting of buffer A [5%

(v/v) acetonitrile in 10 mM-potassium phosphate (pH 6.5)] and solution B (100% acetonitrile) (0–3 min at 5% B; 3–10 min changed from 5% to 15% B; 10–12 min changed from 15% to 25% B; 12–21 min changed from 25% to 30% B; 21–23 min changed from 30% to 100% B; 23–26 min held at 100% B; 26–28 min changed from 100% to 5% B; and 28–30 min held at 5% B). Flow rate was 2 ml/min. An internal standard of norleucine (a synthetic amino acid; Sigma Chemical Co., St. Louis, MO, U.S.A.) was used to correct for recovery. Absorbance was monitored at 254 nm.

Determination of hepatic AdoMet and AdoHcy

This was done by a modification of the h.p.l.c. technique of Shivapurker & Poirier [15]. Protein in liver homogenates was precipitated with trichloroacetic acid, and the supernatant was washed three times with peroxide-free ether and filtered (0.45 µm-pore microfilter; Schleicher and Schuell). A portion was applied to a reverse-phase h.p.l.c. column (Supelguard LC-18 guard column: 5 µm, 4.6 mm × 20 mm; Supelco, Bellefonte, PA, U.S.A.) in series with a Microsorb C-18 column (5 µm, 4.6 mm × 250 mm; Rainin Instruments, Woburn, MA, U.S.A.) by using the h.p.l.c. system described above. Column temperature was maintained at 55°C with a heater (Eldex Laboratories). AdoHcy and AdoMet were eluted with a stepped-linear binary gradient consisting of buffer A (5 mM-heptanesulphonic acid, adjusted to pH 3.5 with acetic acid) and solution B (100% h.p.l.c.-grade methanol) (0–3 min at 10% B; 3–13 min changed from 10% to 50% B; 13–13.5 min changed from 50% to 100% B; 13.5–16 min held at 100% B; 16–16.5 min changed from 100% to 10% B; and 16.5–20 min held at 10% B). Flow rate was 2 ml/min. Absorbance was monitored at 254 nm. Peak areas were calculated by using authentic external standards (Sigma), with recovery corrected by using an internal standard of *S*-adenosyl-ethionine (a compound not normally found in liver; Sigma).

Determination of hepatic choline

Liver was extracted with 15% (v/v) 1 M-formic acid in acetone, and choline was isolated from the resulting supernatant by ion-pair extraction with dipicrylamine into dichloromethane [23]. Choline was then converted into the propionyl ester, and was demethylated with sodium benzenethiolate [24]. This volatile derivative was then isolated by g.l.c. (1.83 m × 2 mm i.d. glass column packed with 3% OV-17 on GC22 precoated with 1% 4-dodecyl-diethylenetriamine succinamide) and measured with a mass-selective detector (MSD; Hewlett Packard 5970 GC/MSD equipped with a model 7673A auto-sampler) [24]. An internal standard of [³H]choline was used to correct for variations in recovery.

Determination of hepatic betaine

Approx. 4000 d.p.m. of [¹⁴C]betaine (New England Nuclear, Boston, MA, U.S.A.) was added as an internal standard to a sample of tissue (100 mg), and the liver was extracted by the method of Bligh & Dyer [25]. The entire aqueous phase of tissue extract was used to separate the hydrophilic metabolites of choline in accordance with a modification of the method of Liscovitch *et al.* [26]. Samples were dried by vacuum centrifugation (SpeedVac, Savant Instruments, Farmingdale, NY, U.S.A.) and were resuspended in 100 µl of methanol/water (10:1, v/v) for

Fig. 2. Hepatic choline concentration in choline-deficient rats

Rats were fed on a control or a choline-deficient diet for up to 42 days. At the times noted, animals were killed and liver was quick-frozen in liquid N₂. Choline was measured by a g.l.c./mass-spectrometry technique as described in the Materials and methods section. Data are expressed as percentages of the control value on the same day (means $\pm$ S.E.M.; $n = 5$ per point): ** $P < 0.01$ for difference from control by one-way ANOVA and Scheffe's test.

injection on to the h.p.l.c. We used a microprocessor-controlled solvent delivery system (Series 4, Perkin-Elmer) with a silica guard column (Supelguard LCSi Column: 5 μ m, 4.6 mm $\times$ 20 mm; Supelco) and a silica column (Pecosphere-3CSi: 5 μ m, 4.6 mm $\times$ 83 mm; Perkin-Elmer). The column was kept at room temperature and the flow rate was 1.5 ml/min. Buffer A contained acetonitrile / ethanol / acetic acid / 1.0 M-ammonium acetate / water / 0.1 M-sodium phosphate (800:68:2:3:127:10, by vol.), and buffer B contained the same constituents but in different proportions (200:34:22:44:200:5, by vol.). The column was equilibrated for 6 min at a flow rate of 1.5 ml/min in 100% A. After the injection, buffer A was delivered for 6 min, which eluted betaine from the column. Over a period of 10 min, solvents were switched to 100% B by using a concave gradient (Perkin-Elmer Series 4, curve 3). This solvent mixture was maintained for 10 min. The initial conditions were restored (100% A) over 1 min by using a linear gradient. Fractions were collected which corresponded to the peak of radioactivity detected with an on-line radioactivity monitor (LB506C; Berthold, Nashua, NH, U.S.A.). These were dried by vacuum centrifugation (SpeedVac). Betaine was assayed by the method of Barak & Tuma [20]. Betaine periodide was precipitated and then resuspended in ethylene dichloride. Absorbance was measured at 365 nm.

Protein assay

Protein was measured by the colorimetric assay of Bradford [27].

Statistics

Data were analysed by Student's *t* test, and one-way analysis of variance and Scheffe's or Dunnett's test [28]. Data are expressed as means $\pm$ S.E.M.

RESULTS

Choline-deficient and control animals gained identical amounts of weight during the study. Liver protein was

Fig. 3. Hepatic methionine, AdoMet and AdoHcy concentrations in choline-deficient rats

Rats were fed on a control or a choline-deficient diet for up to 42 days, and liver was collected as described in Fig. 2. Methionine, AdoMet and AdoHcy concentrations were determined by h.p.l.c. as described in the Materials and methods section. Total available methionine (Σ methionine) was calculated as the sum of methionine, AdoMet and AdoHcy concentrations. Data are expressed in nmol/g wet wt., as means $\pm$ S.E.M. ($n = 5$ per point); * $P < 0.05$, ** $P < 0.01$ for difference from control by one-way ANOVA and Dunnett's test.

slightly lower in the deficient animals than in the controls (0.142 versus 0.149 g of protein/g wet wt; $P < 0.01$ by *t* test). When data were expressed in terms of protein content, the relationships between control and deficient-animal values were not significantly different from those observed when the data were expressed per g wet wt. of tissue. Hepatic choline concentration in control animals was 105 ± 8.2 nmol/g throughout the 42 days of the study. Hepatic choline concentrations in deficient animals were approx. 50% of those in control animals after the first 7 days on the choline-deficient diet (Fig. 2).

Hepatic methionine concentrations decreased by less than 20%, with most of this decrease occurring between days 3 and 7 of choline deficiency (from 52.1 ± 2.3 nmol/g on day 0, to 49.4 ± 1.5 nmol/g on day 3, to

41.9 ± 1.9 nmol/g on day 7, to 43.4 ± 1.7 nmol/g on day 42; Fig. 3). Animals fed on the control diet had constant hepatic methionine concentrations. Plasma methionine concentrations also decreased by approx. 20% in the deficient animals ((from 48.9 ± 5.6 nmol/g on day 0, to 43.1 ± 3.0 nmol/g on day 7, to 40.0 ± 1.1 nmol/g on day 42; results not shown).

Hepatic AdoMet concentrations decreased by over 60% when animals consumed a choline-deficient diet (from 74.7 ± 2.4 nmol/g on day 0, to 60.1 ± 2.8 nmol/g on day 3, to 49.3 ± 2.7 nmol/g on day 7, to 29.4 ± 1.6 nmol/g on day 42; Fig. 3). Animals fed on the control diet had constant hepatic AdoMet concentrations.

Hepatic AdoHcy concentrations increased by 50% when animals consumed a choline-deficient diet, (from 29.8 ± 1.3 nmol/g on day 0, to 39.1 ± 1.5 nmol/g on day 7, to 45.3 ± 1.6 nmol/g on day 42; Fig. 3). Animals fed on the control diet had relatively constant hepatic AdoHcy concentrations.

Hepatic betaine concentrations were measured on day 14. They were 0.9 ± 0.2 µmol/g in animals which consumed a choline-deficient diet, and 2.7 ± 0.7 µmol/g in animals fed on the control diet ($P < 0.05$ by *t* test).

DISCUSSION

We have characterized changes in the liver over a 42-day period of choline deficiency. Our choline-deficient diet contained sufficient methionine (0.35%) so as to avoid the severe toxicity that has been reported in rats deficient in both choline and methionine. Our diet did deplete choline stores, as hepatic choline concentrations decrease to 50% of normal within 1 week on a choline-deficient diet (Fig. 2). Though there are some data available about methyl pools during choline deficiency [11,12,15,16,19,29], this is the first study making measurements of methionine and AdoMet at many time points. For this reason our data make it easier to appreciate the development of changes in these pools as choline deficiency progresses. We found that, during ingestion of a choline-deficient diet, hepatic AdoMet concentrations decreased far more than did hepatic free methionine concentrations (Fig. 3). The fall in AdoMet concentrations occurred before any changes in free methionine concentrations. Even if it is argued that the amount of methionine available for AdoMet synthesis would have been more accurately reflected by the sum of methionine, AdoMet and AdoHcy concentrations (Σ methionine; Fig. 3), we calculate that choline deficiency resulted in only a 25% decrease in available methionine. For these reasons we suggest that choline deficiency did not result in a 60% decrease in AdoMet simply because methionine availability had become limiting, so there must be additional mechanisms contributing to this decrease.

Finkelstein *et al.* [12], studying rats that were fed on a 0.3%-methionine diet for 4 days, observed that choline deficiency was associated with a 27–33% fall in both methionine and AdoMet concentrations, as well as with a marked diminution in hepatic betaine concentrations. From this observation, it was concluded that the availability of methionine limited AdoMet synthesis during choline deficiency because the 5-methyltetrahydrofolate–homocysteine methyltransferase reaction alone could not meet the total requirement for methionine, and the betaine-dependent remethylation of homocysteine was limited by the availability of betaine [12]. If this hypothesis

is correct, choline-deficiency-mediated methionine depletion should always precede and be greater than AdoMet depletion.

We supplied more methionine than did Finkelstein *et al.* [12] (0.35% versus 0.30%), and we studied the effects of choline deficiency over a much longer period. Hepatic methionine in our choline-deficient rats did not decrease as much as it did in their experiments. This difference allows us to distinguish changes in methionine concentrations from those in AdoMet concentrations. We found that hepatic methionine concentrations were difficult to change by limiting choline availability; they appear to be relatively well regulated. It is likely that methionine concentrations were maintained by catabolism of proteins as well as by remethylation of homocysteine. Hepatic methionine concentrations are also hard to increase by dietary manipulations. Finkelstein & Martin [29] varied dietary methionine content over a 10-fold range (0.3–3%) without changing methionine concentrations in liver.

Several possible mechanisms could explain markedly decreased AdoMet concentrations in the face of almost normal methionine concentrations. Two isoenzymes of methionine adenosyltransferase have been identified in liver [30,31]. The α form has a low K_m for methionine (40 µM), whereas that for the β form is higher (200 µM) [31]. Our observed hepatic methionine concentrations varied from 50 µM on day 0 to 40 µM on days 14–42. Thus, for both isoenzymes, endogenous methionine concentrations are such that small changes in the availability of substrate might result in large changes in the formation of AdoMet. This might explain how a 20% decrease in methionine concentration could result in a 60% decrease in AdoMet concentration. However, it does not explain why AdoMet concentrations decreased at a time before any observed decrease in methionine (days 0–3), or why AdoMet continued to decrease during a period when methionine increased slightly (days 7–42). It is possible that changes in the relative proportions of α and β isoenzymes of methionine adenosyltransferase might have occurred such that the lower affinity form predominated, resulting in decreased AdoMet synthesis despite constant availability of methionine. Excess dietary methionine intake is associated with an increase in α isoenzyme activity, and does not change the β isoenzyme activity [32]. No measurements of isoenzymes of methionine adenosyltransferase have been made during choline deficiency.

We suggest an alternative hypothesis, that choline deficiency resulted in an increased utilization of AdoMet. Choline deficiency increases the requirement for phosphatidylcholine synthesis via the sequential methylation of phosphatidylethanolamine [33–35]. The enzyme catalysing these reactions, phosphatidylethanolamine *N*-methyltransferase (EC 2.1.1.17), uses AdoMet as the methyl donor [35,36], and choline deficiency does increase the utilization of AdoMet for phosphatidylcholine synthesis [33,37,38]. This depletes limited stores of AdoMet in the liver, which in turn acts to limit the amount of phosphatidylcholine that can be formed by this pathway during choline deficiency [39,40]. We observed accumulation of AdoHcy, suggesting that the rate of AdoHcy formation (AdoMet utilization) was increased relative to the rate of AdoHcy hydrolysis. This is consistent with an increased rate of AdoMet utilization during choline deficiency. AdoHcy is normally hydrolysed to form homocysteine [41]. As AdoHcy hydrolase catalyses a

reversible reaction, it is possible that the increase in hepatic AdoHcy concentrations that we observed occurred secondary to an increase in the availability of homocysteine. Homocysteine might accumulate if betaine were not available as a substrate for betaine-homocysteine methyltransferase. We observed that betaine concentrations decreased in livers from animals fed on choline-deficient diet for 14 days. However, Svardal and colleagues [19] observed that homocysteine concentrations did not increase in the livers of rats fed on choline-deficient diet for 10 days, while during the same period AdoHcy concentrations did rise. Thus we suggest that increased formation of AdoHcy from homocysteine probably did not occur in our experiment, and that AdoHcy concentrations can be used as an indirect marker of AdoMet utilization.

In summary, we found that choline deficiency can deplete hepatic stores of AdoMet under dietary conditions which only minimally decrease the availability of methionine within liver. Thus decreased availability of methionine could not have been the only mechanism whereby choline deficiency lowered hepatic AdoMet concentrations. We suggest that increased utilization of AdoMet must also have occurred.

This work was supported by a grant from the National Institutes of Health (HD16727). E.A.P. was a Predoctoral Fellow of the American Liver Foundation and the American Gastroenterological Association.

REFERENCES

- Zeisel, S. H. (1981) *Annu. Rev. Nutr.* **1**, 95–121
- Atsushi, I., Hellerstein, E. E. & Hegsted, D. M. (1963) *J. Nutr.* **79**, 488–492
- Best, C. H. & Huntsman, M. E. (1932) *J. Physiol. (London)* **75**, 405–412
- Lombardi, B. (1971) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 139–142
- Haines, M. (1966) *Can. J. Biochem.* **44**, 45–57
- Best, C. H., Hershey, J. M. & Huntsman, M. E. (1932) *J. Physiol. (London)* **75**, 56–66
- Bruni, C. & Hegsted, D. M. (1970) *Am. J. Pathol.* **61**, 413–436
- Degertekin, H., Akdamar, K., Yates, R., Chen, I. I., Ertan, A. & Vaupel, R. (1986) *Acta Anat.* **125**, 174–179
- Chen, S. H., Estes, L. W. & Lombardi, B. (1972) *Exp. Mol. Pathol.* **17**, 176–186
- Mudd, S. H. & Poole, J. R. (1975) *Metab. Clin. Exp.* **24**, 721–735
- Wong, E. R. & Thompson, W. (1972) *Biochim. Biophys. Acta* **260**, 259–271
- Finkelstein, J. D., Martin, J. J., Harris, B. J. & Kyle, W. E. (1982) *Arch. Biochem. Biophys.* **218**, 169–173
- Finkelstein, J. D., Kyle, W. E. & Harris, B. J. (1974) *Arch. Biochem. Biophys.* **165**, 774–779
- Cantoni, G. L. (1975) *Annu. Rev. Biochem.* **44**, 435–451
- Shivapurkar, N. & Poirier, L. A. (1983) *Carcinogenesis* **8**, 1051–1057
- Poirier, L. A., Grantham, P. H. & Rogers, A. E. (1977) *Cancer Res.* **37**, 744–748
- Shivapurkar, N., Wilson, M. J., Hoover, K. L., Mikol, Y. B., Creasia, D. & Poirier, L. A. (1986) *J. Natl. Cancer Inst.* **77**, 213–217
- Barak, A. J., Beckenhauer, H. C. & Tuma, D. J. (1982) *Anal. Biochem.* **127**, 372–375
- Svardal, A. M., Ueland, P. M., Berge, R. K., Aarsland, A., Aarsaether, N., Lonning, P. E. & Refsum, H. (1988) *Cancer Chemother. Pharmacol.* **21**, 313–318
- Barak, A. J. & Tuma, D. J. (1979) *Lipids* **14**, 860–863
- Bidlingmeyer, B. A., Cohen, S. A. & Tarvin, T. L. (1984) *J. Chromatogr.* **336**, 93–104
- Scholze, H. (1985) *J. Chromatogr.* **350**, 453–460
- Freeman, J. J., Choi, R. L. & Jenden, D. J. (1975) *J. Neurochem.* **24**, 729–734
- Jenden, D. J., Roch, M., Booth, R. & Duffield, A. M. (1973) *Anal. Biochem.* **55**, 438–448
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Liscovitch, M., Freese, A., Blusztajn, J. K. & Wurtman, R. J. (1985) *Anal. Biochem.* **151**, 182–187
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Bruning, J. L. & Kintz, B. L. (1977) *Computational Handbook of Statistics*, pp. 125–128, Scott, Foresman and Co., Glenview, IL
- Finkelstein, J. D. & Martin, J. J. (1986) *J. Biol. Chem.* **261**, 1582–1587
- Cabrero, C., Puerta, J. & Alemany, S. (1987) *Eur. J. Biochem.* **170**, 299–304
- Suma, Y., Shimizu, K. & Tsukada, K. (1986) *J. Biochem. (Tokyo)* **100**, 67–75
- Matsumoto, C., Suma, Y. & Tsukada, K. (1984) *J. Biochem. (Tokyo)* **95**, 287–290
- Vance, D. E. & Schneider, W. J. (1981) *Methods Enzymol.* **71**, 581–588
- Hoffman, D. R., Haning, J. A. & Cornatzer, W. E. (1981) *Can. J. Biochem.* **59**, 543–550
- Pascale, R., Pirisi, L., Daino, L., Zanetti, S., Satta, A., Bartoli, E. & Feo, F. (1982) *FEBS Lett.* **145**, 293–297
- Ridgeway, N. D. & Vance, D. E. (1987) *J. Biol. Chem.* **262**, 17231–17239
- Schneider, W. J. & Vance, D. E. (1978) *Eur. J. Biochem.* **85**, 181–187
- Glenn, J. L. & Austin, W. (1971) *Biochim. Biophys. Acta* **231**, 153–160
- Lyman, R. L., Sheehan, G. & Tinoco, J. (1973) *Lipids* **8**, 71–79
- Skurdal, D. N. & Cornatzer, W. E. (1974) *Proc. Soc. Exp. Biol. Med.* **145**, 992–995
- Finkelstein, J. D. (1979) in *Transmethylation* (Usdin, E., Borchardt, R. T. & Creveling, C. R., eds.), pp. 49–58, Elsevier/North-Holland, New York

Received 24 October 1988/10 January 1989; accepted 10 February 1989