Biosynthesis of Phosphatidylethanolamines and Phosphatidylcholines from Ethanolamine and Choline in Rat Liver

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1. The kinetics of phosphatidylcholine and phosphatidylethanolamine synthesis in rat liver were followed 5–60 min after the intraportal injection of [^{14}C]choline and [^3H]ethanolamine. 2. At all time-intervals the specific radioactivity of CDP-choline was only about half that of phosphorylcholine. This indicated that CDP-choline was formed at a similar rate from phosphorylcholine and phosphatidylcholines, the latter probably through the reverse reaction of cholinephosphotransferase (EC 2.7.8.2). In view of recent data obtained from experiments *in vitro* this implies a significant role for the cholinephosphotransferase reaction in the turnover of molecular species of phosphatidylcholine. 3. The specific radioactivity of CDP-ethanolamine was about twice that of phosphoryl-ethanolamine at all time-intervals studied. This supports a previous suggestion that the liver phosphorylethanolamine pool is subject to compartmentation and shows that there is no rapid equilibration between different pools. In contrast with a recent study, no evidence was found for any significant methylation of phosphoryl- or CDP-ethanolamine to the corresponding choline derivative. 4. Quantitative data on the biosynthesis of molecular species of phospholipids via CDP derivatives were calculated according to simple kinetic models. They were in the same range as those calculated from earlier data on precursors incorporated via diacylglycerols. 5. The proportion of radioactive phosphatidylethanolamines appearing in the plasma was approximately ten times lower than that for phosphatidylcholines. No selectivity was observed in the transfer into plasma of different molecular species of phosphatidylethanolamine.

Experiments *in vitro* have shown that phosphatidylcholines and phosphatidylethanolamines are synthesized by formation of the phosphorylated bases and their CDP derivatives (Kennedy & Weiss, 1956). Studies on intact rats showed that after the injection of [^{14}C]choline the specific radioactivity of CDP-choline was unexpectedly low compared with that of phosphorylcholine and approached that of phosphatidylcholines (Björnstad & Bremer, 1966; Bjerve & Bremer, 1969; Treble *et al.*, 1970). It was also observed that CDP-choline and phosphatidylcholines had equal specific radioactivities after the injection of methionine labelled in the methyl group (Bremer *et al.*, 1960; Björnstad & Bremer, 1966). These two observations suggested that CDP-choline was more rapidly formed from phosphatidylcholines than from phosphorylcholine. Short-term experiments, using a freeze-stop technique to prevent post-mortem changes in the liver, showed, however, that 2–5 min after the intraportal injection of [^{14}C]choline the specific radioactivity of CDP-choline approached that of phosphorylcholine. This suggests that CDP-choline formation from phosphatidylcholines was small compared with that from phosphorylcholine (Sundler *et al.*, 1972). Since the results of Bjerve & Bremer (1969) also threw doubt on the conclusions of Björnstad & Bremer (1966), the tracer

kinetics of CDP-choline and phosphorylcholine at longer time-intervals after [^{14}C]choline injection were reinvestigated. According to studies on rat liver microsomal fractions (Kano & Ohno, 1973*a,b*) and with isolated hepatocytes (Sundler *et al.*, 1974*a*) the molecular species of phosphatidylcholine that are degraded by the backward reaction of cholinephosphotransferase (EC 2.7.8.2) differ markedly from those formed by its forward action. The occurrence of reversibility at this step in the intact liver would thereby contribute to the turnover of individual species of phosphatidylcholine.

After the injection of radioactive ethanolamine into rats the specific radioactivity of liver CDP-ethanolamine is higher than that of phosphorylethanolamine (Björnstad & Bremer, 1966; Haines & Rose, 1970) and this relationship already holds before the specific radioactivity of phosphorylethanolamine has attained its maximum (Sundler, 1973). Since this is incompatible with a precursor-product relationship between the total liver pools of the two compounds, it was proposed that phosphorylethanolamine formed from exogenous ethanolamine is not mixed with the total liver pool before formation of the CDP derivative (Sundler, 1973). The kinetics of phosphatidylethanolamine synthesis from [1,1- $^3\text{H}_2$]sphingosine was compatible with this proposal (Offenbartl *et al.*,

1973). In the present report the kinetic studies with intraportally injected [^3H]ethanolamine were extended in time to see whether an equilibration between different liver pools of phosphorylethanolamine occurs with time. In addition the data have been used to calculate the rate of phospholipid synthesis, since widely varying estimates of such rates are encountered in the literature.

Materials and Methods

Animal experiment

[1,2- $^{14}\text{C}_2$]Choline (19mCi/mmol) was obtained from Calatomic, Los Angeles, Calif., U.S.A. and [2- ^3H]ethanolamine (320mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. They were purified as described earlier (Sundler *et al.*, 1972; Sundler, 1973). Each rat was injected with 0.5ml of a solution containing 0.50 μmol of [^{14}C]choline and 0.08 μmol of [^3H]ethanolamine in 0.9% NaCl. Male Sprague-Dawley rats weighing 210–235 g, which were fed on a balanced diet, were used. The experimental procedure was the same as described by Sundler *et al.* (1972) and Sundler (1973) with the following additions. The abdominal wall was sewn up after the intraportal injection of radioactive solution and the rats were allowed to awaken from the diethyl ether anaesthesia. They were then anaesthetized again with diethyl ether and the abdominal wall was reopened. Blood was aspirated from the aorta and transferred to tubes containing sodium citrate and the liver was freeze-clamped and extracted as described earlier (Sundler, 1973).

Analytical procedures

Plasma was aspirated after centrifugation of the blood samples (1000g for 10min) and was extracted

with 6ml of chloroform-methanol (1:1, v/v) per ml of plasma. After 1 h at room temperature the samples were centrifuged and the supernatant was decanted. After several rinsings of the sediment the combined extracts were equilibrated against 0.5 vol. of NaCl (10mg/ml) and the lower phase was aspirated after centrifugation. Phosphatidylethanolamines and phosphatidylcholines were isolated by t.l.c. on silica gel H with chloroform-methanol-water (14:6:1, by vol.) as the developing solvent, which clearly separated phosphatidylethanolamines from other phospholipids. Phosphatidylcholines were rechromatographed, after elution from the gel, with chloroform-methanol-aq. 4M-NH $_3$ (13:6:1, by vol.) as developing solvent. In this system they were clearly separated from lysophosphatidylethanolamines which contained small amounts of ^3H . A standard sample of lysophosphatidylethanolamine was prepared by phospholipase A $_2$ digestion (Lands & Hart, 1964). The phosphoryl and CDP derivatives of choline and ethanolamine were isolated and determined as described earlier (Sundler, 1973).

Calculations

The rate of phosphatidylethanolamine and phosphatidylcholine synthesis was calculated by the method of Zilversmit *et al.* (1943). The increment in the total radioactivity of the product for different time-intervals was divided by the corresponding area between the specific-radioactivity curves of the precursor and the product to give the rate of synthesis expressed as $\mu\text{mol}/\text{min}$. Before the measurement of areas (by weighing) the curves were smoothed out. Only radioactive isotope transport in the direction from ethanolamine to phosphatidylethanolamines or from choline to phosphatidylcholines was considered. The small amounts of bases incorporated by base ex-

Table 1. Calculation of the rate of phospholipid synthesis

The calculation is described in the Materials and Methods section. Roman numerals indicate different ways of calculation and the values within parentheses indicate the factor by which the specific radioactivity of the phospholipid was multiplied. Data are expressed as $\mu\text{mol}/\text{min}$ per whole liver. The data at each time-interval are the means for two rats.

Reaction	Time-interval (min) ...	Rate of phospholipid synthesis					
		5–10	10–20	20–30	30–60	5–30	5–60
CDP-ethanolamine $\rightarrow$ phosphatidylethanolamines	I (1)	0.34	0.26	0.22	—	0.27	0.19
	II (4.5)	0.38	0.31	0.35	0.36	0.34	0.35
CDP-choline $\rightarrow$ phosphatidylcholines	I (1)	0.96	0.43		—	0.57	0.29
	II (5)	1.08	0.57		—	0.70	0.43
	III (10)	1.29	0.94		—	1.07	1.07
Phosphorylcholine $\rightarrow$ CDP-choline + phosphatidylcholines	I (1)	0.43	0.17		—	0.23	0.14
	II (10)	0.48	0.20		—	0.27	0.20

change (Sundler *et al.*, 1972; Sundler, 1973; Sundler *et al.*, 1974b) were disregarded. If the true rates can be calculated by this procedure the rates obtained for different time-intervals should be equal. Since this was not the case (Table 1), an attempt was made to find a better mode of calculation. Several studies have shown that after the injection of radioactive precursors the specific radioactivity of phosphatidylethanolamines in the microsomal fraction is up to five times that in the mitochondrial fraction (Balint *et al.*, 1967; Stein & Stein, 1969; Parkes & Thompson, 1973) and some molecular species have up to 2.5 times the specific radioactivity of total phosphatidylethanolamine (Sundler, 1973). Similar results have been found for phosphatidylcholines (Björnstad & Bremer, 1966; Balint *et al.*, 1967; Stein & Stein, 1969; Parkes & Thompson, 1973; Sundler *et al.*, 1972). Therefore the observed specific radioactivity of the total pool of the phospholipid was multiplied by factors found to give a similar rate of synthesis over the whole time-interval. This empirical mode of calculation is somewhat speculative since the metabolic relationship between different phospholipid pools is not known in detail, but it was found to be justified in view of the observations cited above.

Results and Discussion

Metabolism of ethanolamine-containing compounds

At 5 min after the injection of radioactive isotope most of the ^3H in the liver was recovered in phosphorylethanolamine, whereafter it appeared in the phosphatidylethanolamines. Most of the lipid ^3H was in the phosphatidylethanolamines at all times. The proportion recovered in phosphatidylcholines increased from 1.2 to 7.3% of lipid ^3H during the experimental period. Any preferential utilization of different phosphatidylethanolamines for the methylation (Arvidson, 1968) would thus have a negligible influence on the analyses made in this paper. The specific radioactivity of CDP-ethanolamine was significantly higher than that of phosphorylethanolamine (Fig. 1) and the specific radioactivity ratio of CDP-ethanolamine/phosphorylethanolamine showed no decrease with time. This indicates that if the high specific radioactivity ratio is due to the existence of different phosphorylethanolamine pools (Sundler, 1973), then the rate of a possible equilibration between such pools must be low.

The appearance of radioactivity in different ethanolamine-containing compounds was similar to that reported by Salerno & Beeler (1973). These workers suggested that phosphorylethanolamine and CDP-ethanolamine are methylated to the corresponding choline-containing compounds. The specific radioactivity of ^3H in phosphorylcholine expressed as a percentage of that in phosphorylethanolamine was

0.1–1.5% in our experiments and did not change with time, in contrast with the values of 0.4–1215% found by Salerno & Beeler (1973). The specific radioactivity of ^3H in CDP-choline compared with that in CDP-ethanolamine was also very low in our experiment which agrees with the other study. So far, most available evidence indicates that any methylation of water-soluble ethanolamine compounds is of small quantitative importance. As noted by Salerno & Beeler (1973) the separation of metabolites is critical for the interpretation of the data. In addition we have found that unless the [^3H]ethanolamine is purified just before use, spurious radioactive compounds may be found when the phosphorylated metabolites are analysed.

Rate of phosphatidylethanolamine synthesis

The rate of phosphatidylethanolamine synthesis varied from 0.34 to 0.22 $\mu\text{mol}/\text{min}$ for different time-intervals. It was more constant at about 0.35 $\mu\text{mol}/\text{min}$ when the specific radioactivity of the product pool of phosphatidylethanolamines was assumed to be 4.5 times higher than that observed for total phosphatidylethanolamines (Table 1). This rate is in the same range as that calculated from [^3H]glycerol incorporation and is somewhat higher than the rates calculated from the incorporation of radioactive ethanolamine over the initial 5 min and of [1,1- $^3\text{H}_2$]sphingosine (Table 2). The synthetic rate for individual molecular species also agrees well with rates calculated from [^3H]glycerol incorporation (Table 3). The distribution of

Fig. 1. Specific radioactivity in liver ethanolamine-containing compounds after the injection of [^3H]ethanolamine

■, Phosphorylethanolamine; ●, CDP-ethanolamine; ▲, phosphatidylethanolamines. Each point represents one animal except that data on phosphatidylethanolamines are means from two animals. For comparison data obtained 0.33–5 min after the injection of labelled ethanolamine from an earlier study (Sundler, 1973) are included. The two upper curves at 0.33–5 min represent CDP-ethanolamine and the two lower curves phosphorylethanolamine.

Table 2. *Different estimates of the rate of phosphatidylethanolamine synthesis via the Kennedy pathway in rat liver*

Data are expressed as $\mu\text{mol}/\text{min}$ per 10g wet wt. of liver or as $\mu\text{mol}/\text{min}$ per whole liver (usually 200g body weight). The values in parentheses were calculated by assuming that the formation of diacylglycerols from sources other than phosphatidic acids is zero.

Injected precursor	CDP-ethanolamine $\rightarrow$ phosphatidylethanolamines	Reference
[3- ^{14}C]Serine	0.55	Wise & Elwyn (1965)
[^3H]Glycerol	(0.18)	Åkesson (1969)
[^3H]Glycerol	0.36* (0.22)*	Åkesson <i>et al.</i> (1970)
[^3H]- and [^{14}C]-Ethanolamine	0.082; 0.056	Sundler (1973)
[^3H]Ethanolamine	0.27†; 0.35‡	Present study
[1,1- $^3\text{H}_2$]Sphingosine	0.10	Offenbartl <i>et al.</i> (1973)

* The calculations were made from the pool sizes and rate constants for individual molecular species of phosphatidic acids (within parentheses) and diacylglycerols.

† Calculated for the period 5–30min after the injection of radioactive isotope (Table 1).

‡ Calculated for the period 5–60min after the injection by assuming that the specific radioactivity of the actual product pool of phosphatidylethanolamines was 4.5 times higher than that observed (Table 1).

Table 3. *Biosynthesis of individual rat liver ethanolamine phosphoglycerides*

Liver ethanolamine phosphoglycerides from a rat killed 60min after injection were converted into *N*-benzoyl-*O*-methyl derivatives (Sundler & Åkesson, 1973), which were separated into alkenyl-acyl-*sn*-glycero-3-phosphorylethanolamine (1.4% of total radioactivity) and diacyl-*sn*-glycero-3-phosphorylethanolamine plus alkyl-acyl-*sn*-glycero-3-phosphorylethanolamine (98.6%). The latter fraction and also the total plasma ethanolamine phosphoglycerides (pooled sample from four rats) were resolved by argentation t.l.c. The dioenoic, tetraenoic and hexaenoic phosphatidylethanolamines were also resolved according to fatty acid chain length as *N*-acetyl-*O*-methyl derivatives (Sundler & Åkesson, 1973). From these data and the rate of phosphatidylethanolamine synthesis (0.35 $\mu\text{mol}/\text{min}$, Table 2) the synthetic rate for individual species were calculated (column I). Column II shows data calculated from the rate constants for the conversion of diacylglycerols into phosphatidylethanolamines and the corresponding pool sizes (Åkesson *et al.*, 1970). The turnover time was calculated for the major species.

Molecular species	% of total phosphatidyl- ethanolamine radioactivity		Synthetic rate ($\mu\text{mol}/\text{min}$ per liver)		Turnover time (h)
	Liver	Plasma	I	II	
Saturated	0.0	1.4	0.000		
Monoenoic	5.8	9.4	0.020	0.025	1.4
Monoenoic-monoenoic	1.4		0.005		
Palmitoyl-linoleoyl	10.4	15.3	0.035	0.028	2.4
Stearoyl-linoleoyl	3.1		0.010		7.8
Monoenoic-dienoic	2.0		0.007		
Dienoic-dienoic	3.1	6.3	0.010		
Palmitoyl-arachidonoyl	7.0		0.023	0.077	7.4
Stearoyl-arachidonoyl	14.4	22.7	0.049		9.3
Pentaenoic	5.9	3.0	0.020		
Palmitoyl-docosahexaenoyl	40.2		0.134	0.203	2.0
Stearoyl-docosahexaenoyl	6.7	41.8	0.023		6.4

[^3H]ethanolamine among different liver phosphatidylethanolamines was analysed by argentation t.l.c. and reversed-phase partition chromatography at each time-interval. Since no change with time was observed, the data in Table 3 should be representative for the phosphatidylethanolamines formed via the CDP-ethanolamine pathway.

Metabolism of choline-containing compounds

The incorporation of [^{14}C]choline into phosphorylcholine and CDP-choline was maximal at 10min after injection, whereas the radioactivity in liver phosphatidylcholines increased during the whole experimental period (Fig. 2). Plasma phosphatidylcholines exhibited a similar time-course, although

Fig. 2. Specific radioactivity in liver choline-containing compounds after the injection of [^{14}C]choline

■, Phosphorylcholine; ●, CDP-choline; ▲, phosphatidylcholines. Each point represents one animal. For comparison data obtained 0.17–5 min after the injection of [^{14}C]choline (Sundler *et al.*, 1972) are included. Upper curve, phosphorylcholine; lower curve, CDP-choline.

no ^{14}C could be detected earlier than 20 min after injection. They contained 0.2–2.6% of the amount of both ^3H and ^{14}C radioactivity in liver phosphatidylcholines, whereas the labelling of plasma phosphatidylethanolamines was only 0.01–0.14% of that in liver phosphatidylethanolamines. This probably reflects a preferential utilization of phosphatidylcholines for plasma lipoprotein synthesis or a slower rate of exchange of phosphatidylethanolamines than of phosphatidylcholines between liver and plasma (Zilversmit, 1971) or both. No selectivity in the transfer of different phosphatidylethanolamines to plasma was observed (Table 3).

Since the hepatic CDP-choline pool is considerably smaller than the phosphorylcholine pool (Sundler *et al.*, 1972) one would expect the specific radioactivity ratio of phosphorylcholine/CDP-choline to be close to unity if CDP-choline is formed only from phosphorylcholine. In the present study a ratio of 2.4 ± 0.6 (mean $\pm$ S.D., $n = 10$; range, 1.6–3.4) was found with no consistent change with time. This is probably caused by the formation of CDP-choline of low specific radioactivity from phosphatidylcholines by a reversal of the cholinephosphotransferase reaction (Björnstad & Bremer, 1966; Kanoh & Ohno, 1973a). The rate of CDP-choline formation from phosphatidylcholines would then be of the same order as that from phosphorylcholine, although a heterogeneity in the liver pools of phosphorylcholine and CDP-choline may complicate such calculations. Earlier studies have given conflicting results as to the specific radioactivity ratio between the two compounds after the injection of radioactive choline (Björnstad & Bremer, 1966; Bjerve & Bremer, 1969; Sundler *et al.*, 1972; Salerno & Beeler, 1973).

This may in part be due to differences in methodology since lower amounts of CDP-choline were observed in freeze-clamped livers (Sundler *et al.*, 1972) than in livers extracted directly with ethanol–water. A post-mortem formation of CDP-choline from phosphatidylcholines in livers not freeze-clamped would lead to an overestimation of the formation of CDP-choline from phosphatidylcholines. The rate of this reaction has also been estimated by using liver microsomal fractions (Weiss *et al.*, 1958; Kanoh & Ohno, 1973a,b) and isolated hepatocytes (Sundler *et al.*, 1974a). In the latter system 26 and 13% of the diacylglycerols incorporated into phosphatidylethanolamines and phosphatidylcholines respectively originated from sources other than phosphatidic acids, presumably phosphatidylcholines (Sundler *et al.*, 1974a). Bar-On *et al.* (1973) claim that a few per cent of the diacylglycerols incorporated in secreted triacylglycerols originate from phosphatidylcholines. It is noticeable that the composition of the diacylglycerol units transferred in either direction of the cholinephosphotransferase reaction is not the same. Mainly monoenoic and dienoic molecules take part in the forward reaction (see, e.g., Table 5), whereas dienoic and tetraenoic phosphatidylcholines predominate in the backward reaction (Kanoh & Ohno, 1973a; Sundler *et al.*, 1974a) owing to the relative absence of selectivity of the cholinephosphotransferase.

Rate of phosphatidylcholine synthesis

The rate of phosphatidylcholine synthesis was calculated in two ways. When CDP-choline was the precursor the rate was lower at the later time-intervals (Table 1). Again a constant rate was obtained when the specific radioactivity of the product pool of phospholipid was assumed to be higher than that observed for the total phosphatidylcholines. These data include the rates both of net synthesis of phosphatidylcholines and of an exchange reaction between CDP-choline and phosphatidylcholines. They are in the same range as the rate calculated from the rate constants for diacylglycerol metabolism after [^3H]glycerol injection and are somewhat larger than rates calculated from [^{14}C]choline incorporation over the initial 5 min (Table 4). The synthetic rate for the different major species of phosphatidylcholine calculated from the present data are in the same range as those calculated from an experiment where [^3H]glycerol was injected, except for the tetraenoic fraction (Table 5). The rate of phosphatidylcholine synthesis was also calculated from the assumption that phosphorylcholine was the precursor and CDP-choline plus phosphatidylcholines was the product with the specific radioactivity of the latter. Then the rate reflects only net synthesis of phosphatidylcholines without influence from an exchange

Table 4. *Different estimates of the rate of phosphatidylcholine synthesis via the Kennedy pathway in rat liver*

Data are expressed as $\mu\text{mol}/\text{min}$ per 10 g wet wt. of liver or as $\mu\text{mol}/\text{min}$ per whole liver (usually 200 g body weight). The values in parentheses were calculated by assuming that the formation of diacylglycerols from sources other than phosphatidic acids is zero.

Injected precursor	Phosphorylcholine $\rightarrow$ phosphatidylcholines	CDP-choline $\rightarrow$ phosphatidylcholines	Reference
[^{14}C]Serine	2.6		Wise & Elwyn (1965)
[^3H]Glycerol	(0.45)		Åkesson, (1969)
[^3H]Glycerol	(0.54)*	0.89*	Åkesson <i>et al.</i> (1970)
[1,2- $^{14}\text{C}_2$]Choline	0.20–0.25		Björnstad & Bremer (1966)
[1,2- $^{14}\text{C}_2$]Choline	0.20	0.25	Sundler <i>et al.</i> (1972)
[1,2- $^{14}\text{C}_2$]Choline	0.23†; 0.27‡	0.57†; 1.07§	Present study

* The calculations were made from the pool sizes and rate constants for individual molecular species of phosphatidic acids (within parentheses) and diacylglycerols.

† Calculated for the period 5–30 min after injection of radioactive isotope.

‡ Calculated for the period 5–30 min by assuming that the specific radioactivity of phosphatidylcholines was 10 times that observed.

§ Calculated for the period 5–60 min by assuming that the specific radioactivity of phosphatidylcholines was 10 times that observed.

Table 5. *Rate of biosynthesis of individual rat liver phosphatidylcholines*

The synthetic rate for molecular species was calculated from the rates of phosphatidylcholine synthesis from CDP-choline given in Table 4, 1.07 (column I) and 0.57 column II), and the distribution of [^{14}C]choline among different molecular species 5 min after its injection (Sundler *et al.*, 1972). The distribution of [^{14}C]choline among phosphatidylcholines of different degrees of saturation at each time-interval was analysed by argentation t.l.c. It was very similar to the earlier more detailed analysis (Sundler *et al.*, 1972) and did not change significantly with time. Column III shows data calculated from the rate constants for the conversion of diacylglycerols into phosphatidylcholines and the corresponding pool sizes (Åkesson *et al.*, 1970).

Molecular species	Synthetic rate ($\mu\text{mol}/\text{min}$ per whole liver)			Turnover time (h)	
	I	II	III	I	II
Palmitoyl-oleoyl	0.26	0.14	0.13	0.92	1.73
Stearoyl-oleoyl			0.004		
Palmitoyl-linoleoyl	0.47	0.25	0.22	0.87	1.64
Stearoyl-linoleoyl	0.027	0.014	0.006	6.5	12.2
Palmitoyl-arachidonoyl	0.13	0.067	0.31	3.9	7.3
Stearoyl-arachidonoyl	0.037			11.3	21.0
Hexaenoic	0.071	0.037	0.053	4.8	9.0

reaction between the phospholipid and CDP-choline. The values calculated were similar to those obtained in other experiments on [^{14}C]choline metabolism, whereas studies with other labelled precursors gave higher values (Table 4).

The range of synthetic rates from different phospholipid precursors given in this report is sufficiently close to warrant the conclusion that they represent valid estimates of the actual rates of synthesis, although it must be kept in mind that several simplifying assumptions have been made. The half-lives of different molecular species were 0.6–14 h which are clearly lower than those calculated from the elimination of radioactive isotope from phospholipids (Omura *et al.*, 1967; Lee *et al.*, 1973). The latter data are probably influenced by recirculation of radioactive

isotope and therefore calculations involving the specific radioactivity of the precursor must be more reliable.

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