Studies on the Origin of Choline in the Brain of the Rat

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1. Labelled precursors of choline, namely ethanolamine, dimethylaminoethanol and methionine and also labelled choline itself were injected intraperitoneally into the adult female rat and the incorporation into lipids and water-soluble fractions was traced in liver, blood and brain. 2. No significant free choline was detected and no labelling of the phosphorylcholine of blood. There was, however, considerable labelling of the phosphorylcholine of brain and liver. 3. After intracerebral injection, [1,2-¹⁴C]dimethylaminoethanol was rapidly phosphorylated and converted into phosphatidyl dimethylaminoethanol, presumably by the cytidine pathway. 4. In view of the pattern of labelling and the amount of phosphatidylcholine in the tissues examined, it seems highly likely that choline is transported to the brain by the blood in a lipid-bound form.

Previous experiments have demonstrated that, when [1,2-¹⁴C]ethanolamine was injected into the brain of the adult rat, the labelling of the lipids was largely confined to the ethanolamine moiety (Ansell & Spanner, 1967). This confirmed observations *in vitro* that brain tissue, unlike liver, cannot convert ethanolamine lipids into choline lipids by step-wise methylation of the base. Further, it was not possible to demonstrate the formation of free choline or any water-soluble choline derivative in the brain after the injection of labelled ethanolamine.

Subsequent studies with [*Me*-¹⁴C]choline showed that, when injected intracerebrally, this base rapidly labelled the choline of phosphatidylcholine in that 18% of the injected material was converted into this lipid-bound form in 1 h (Ansell & Spanner, 1968); this observation has been confirmed by Gomez, Domino & Sellinger (1970). If the brain, which contains in various forms approx. 20 μ mol of choline/g fresh weight, cannot synthesize the base from precursors, it must receive choline from the blood. At first sight, the most likely candidate would be the free base, although it is generally believed that water-soluble compounds containing a quaternary nitrogen atom are excluded by the blood-brain barrier (Brodie, 1964). However, Groth, Bain & Pfeiffer (1958) showed that [*Me*-¹⁴C]-choline or [1,2-¹⁴C]choline, when injected intraperitoneally, labelled the choline lipids of brain and liver; dimethylaminoethanol could also act as a precursor of choline. On the other hand, Bjørnstad & Bremer (1966) showed that, when labelled precursors of choline lipid, e.g. L-methionine, were given intraperitoneally, the form in which the radioactivity was carried in the blood could not be

'diluted out' with injected unlabelled choline. They concluded that choline was not carried to the tissues by the blood in its free form.

If it is accepted that, in the rat at least, most of the choline in the body is synthesized in the liver, it is important to know how it is transported to the brain. Since the formation of acetylcholine requires the presence of choline in a free form, the implications of the supply to the brain in a form other than its free state are of considerable biochemical and pharmacological interest. Experiments were therefore performed in which known precursors of choline were injected intraperitoneally and their incorporation into the choline-containing compounds of liver, blood and brain was traced.

MATERIALS AND METHODS

Materials. [*Me*-¹⁴C]Choline (5.4 μ Ci/ μ mol), [1,2-¹⁴C]-ethanolamine (4.85 μ Ci/ μ mol), L-[*Me*-¹⁴C]methionine (29.1 μ Ci/ μ mol) and [³²P]orthophosphate were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and the [1,2-¹⁴C]dimethylaminoethanol (1.3 μ Ci/ μ mol) from Tracerlab, Waltham, Mass., U.S.A. [³²P]-Phosphoryldimethylaminoethanol was kindly prepared by Dr R. F. Metcalfe by the method of Ansell & Chojnacki (1966). All chemicals were of A.R. quality whenever possible. The rats were albino females 12-14 weeks old (about 250 g body wt.).

Injections. Rats were injected either by the intraperitoneal route or intracerebrally by the method described by Ansell & Spanner (1967). The doses are given in the Results section.

Extraction and determination of water-soluble components of blood, brain and liver. The tissues were quickly removed from the animal and homogenized in ice-cold water. Blood was collected immediately after the decapitation of the

animal. The volumes were adjusted to 10 ml for the brain and blood and to 25 ml for the liver. A sample (5 ml) was removed to a centrifuge tube, 1 ml of 50% (w/v) trichloroacetic acid added and the tube left on ice for 15 min. The tube was centrifuged and the supernatant poured off through a Whatman no. 40 filter paper. The precipitate was washed twice with water (1 ml) and these washings were added to the supernatant. The combined acid-soluble fraction was washed with ether until the pH was about 5 and then adjusted to pH 8 with a trace of ammonia vapour. Samples (0.5 ml) were taken for radioactivity counting and for chromatography on Dowex 50 (X8; H⁺ form).

Separation and identification of the water-soluble labelled components. The slightly alkaline water-soluble extract (equivalent to 1.0–1.5 g of brain or liver and 2.5 g of blood) was applied to a small column (10 mm × 80 mm) of Dowex 50 (X8; H⁺ form). The column was washed with a further 15 ml of water. The effluent contained the glycerophosphoryl esters of ethanolamine and choline. The phosphate monoesters were eluted with 30 ml of 0.1 M-HCl and the free bases with 20 ml of 3 M-HCl. This is essentially the method of Webster & Cooper (1968). Each fraction was evaporated to dryness under reduced pressure, then left for several hours over KOH pellets in a vacuum desiccator. The residue was then dissolved in water and samples were taken for radioactivity counting, chromatography and, in the case of the 3 M-HCl fraction, for the determination of free choline.

Chromatography of the fractions. The components of the 0.1 M-HCl fraction, the phosphate monoesters of choline, ethanolamine and dimethylaminoethanol, were separated by one-dimensional paper chromatography on Whatman no. 1 paper in ethanol–18 M-NH₃–water (6:3:1, by vol.); it took about 9 h for the solvent to descend 36 cm. The *R_F* values were: phosphorylethanolamine, 0.53; phosphoryldimethylaminoethanol, 0.72; phosphorylcholine, 0.68. The spots were located by radioautography on Kodak Royal Blue X-ray film and eluted with water, and a sample of each was taken for radioactivity counting and for phosphorus determination.

The bases in the 3 M-HCl fraction were separated by t.l.c. on silica gel G (E. Merck A.-G., Darmstadt, Germany). In view of the rather unexpected results, several systems were investigated, including a two-dimensional system in methanol–18 M-NH₃ (3:1, v/v) followed by methanol–acetone–11 M-HCl (45:5:2, by vol.) (Speed & Richardson, 1968). One-dimensional t.l.c. was also carried out with methanol–11 M-HCl (19:1, v/v), methanol–acetone–water (9:1:1, by vol.) and methanol–18 M-NH₃–water (7:1:2, by vol.). Choline was determined by the method of Long, Odavic & Sargent (1967) and the radioactivity determined in the choline iodide after decolorizing with a few mg of Na₂S₂O₃.

Extraction and determination of phospholipids. The remainder of the aqueous homogenate of brain, blood and liver was shaken with 4 vol. of chloroform–methanol (2:1, v/v) and centrifuged. The upper aqueous phase was removed and the chloroform-soluble phase evaporated to dryness. This residue was taken up in chloroform–methanol (2:1, v/v) and washed (Folch, Lees & Sloane-Stanley, 1957). The washed extract was evaporated to dryness and the residue dissolved in chloroform and filtered before being made up to 10 ml for brain and blood

and 25 ml for liver. Samples (0.5 ml) were taken for radioactivity counting. The remaining 9.5 ml of the extract (sample of 7.5 ml for liver) was then applied to a column of alumina (2.5 g) and the lipids were eluted by the method of Ansell & Chojnacki (1966). The lipids were checked by chromatography of the products of mild alkaline hydrolysis (Dawson, Hemington & Davenport, 1962). The solvents used were water-saturated phenol–ethanol–acetic acid (50:6:5, by vol.), which separates glycerophosphorylcholine from glycerophosphorylethanolamine and propan-2-ol–18 M-NH₃–water (7:1:2, by vol.) which separates glycerophosphorylcholine, glycerophosphorylethanolamine and glycerophosphoryldimethylaminoethanol (Letters, 1966). The spots were again located by radioautography, eluted and samples were taken for radioactivity counting and phosphorus determination.

Fatty acid determination. The diacyl phospholipids of brain were prepared as described by Ansell & Spanner (1971). A sample of the lipid (about 2.5 μmol) was evaporated to dryness in a 10 ml round-bottomed flask. The residue was dissolved in 0.5 ml of benzene. After the addition of 2 ml of methanol and 0.04 ml of conc. H₂SO₄, the mixture was refluxed at 60–70°C for 4 h. The mixture was cooled, shaken with 2 ml of light petroleum (b.p. 40–60°C) and after centrifuging, the petroleum phase was removed. This was washed twice with 1 ml of water, then dried with anhydrous Na₂SO₄ and a 10 μl sample was injected on to a column (5 ft × 0.25 in) for g.l.c. (Ways, Reed & Hanahan, 1963). The column was filled with 10% SE 30 on 100/120 mesh Diatomite C and run at 230°C in a Pye 104 gas chromatograph.

Phosphorus was determined by the method of Ernster, Zetterström & Lindberg (1950).

Determination of radioactivity. The radioactivities of ¹⁴C-labelled samples were counted in a Packard liquid-scintillation counter and corrected for 100% efficiency by using an external standard. The scintillator was made by dissolving 6.0 g of 2,5-diphenyloxazole and 0.12 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of xylene and mixing it with Triton X-100 in a ratio of 73:27 (v/v).

'No labelling' was defined as less than 0.05 d.p.s./sample.

RESULTS

Intraperitoneal injection of [1,2-¹⁴C]dimethylaminoethanol. When labelled dimethylaminoethanol was injected intraperitoneally into rats, radioactivity was detected in the lipids and acid-soluble fractions of liver, blood and brain. After 1 h, 75% of the radioactivity in the total lipid of liver was in the phosphatidylcholine and this rose to 84% after 6 h; the remaining activity was in the phosphatidyltrimethylaminoethanol (Table 1). No labelling (see the Materials and Methods section) of the free choline was detected in any of the tissues examined. No phosphorylcholine, labelled or unlabelled, was detected in the blood (Table 2), which would agree with the observation of Dawson (1955). In brain and liver, the specific radioactivity (d.p.s./μmol) of the phosphorylcholine shows a possible precursor-product relationship with phosphatidylcholine (Table 2) though the very low total radio-

Table 1. *Distribution of radioactivity between the total ethanolamine and choline lipids in rat liver after the injection of ^{14}C -labelled bases*

The results are expressed as a percentage of the radioactivity found in the total lipids.

Base injected	Time of exchange (h)	% Radioactivity in total lipid as	
		Ethanolamine lipid	Choline lipid
[1,2- ^{14}C]Ethanolamine	1	84	16
	6	58.5	41.5
[1,2- ^{14}C]Dimethylaminoethanol		Dimethylamino-ethanol lipid	
	1	25	75
	6	16	84
[Me- ^{14}C]Choline	1		90
	6		88

activity in the phosphorylcholine compared with that of the phosphatidylcholine makes it unlikely. If the specific-radioactivity values for brain, blood and liver phosphatidylcholine are compared over 24 h (Fig. 1), the value for liver is always greater than that for blood, which in turn is greater than for the brain. That the phosphatidylcholine in the brain comes unchanged from the liver by way of the blood is therefore a possibility. The results could suggest that in 1 h, $2.5\text{ }\mu\text{mol}$ of liver phosphatidylcholine passes into the blood and $1.2\text{ }\mu\text{mol}$ from the blood to the brain.

Intraperitoneal injection of [1,2- ^{14}C]ethanolamine. When labelled ethanolamine was injected intraperitoneally and the choline-containing components of brain, blood and liver were examined, a similar result was found to that obtained after the injection of labelled dimethylaminoethanol. There was no labelling of the choline or phosphorylcholine of blood and no labelling of the free choline of liver or brain, but some labelling of the phosphorylcholine. However, the phosphatidylcholine in all tissues was significantly labelled (Tables 1 and 3). The distribution of radioactivity between ethanolamine and choline phospholipids in liver after 1 h is strictly comparable with that found by Arvidson (1968) and Tinoco, Sheehan, Hopkins & Lyman (1970). The value for the specific radioactivity of the phosphorylcholine compared with that of the phosphatidylcholine in liver again makes a precursor-product relationship extremely unlikely (Table 3).

Studies on the phosphate monoesters of ethanolamine, dimethylaminoethanol and choline after the injection of labelled precursors. After the intraperitoneal injection of [1,2- ^{14}C]ethanolamine and [1,2- ^{14}C]dimethylaminoethanol, there was, in the liver, a marked labelling of the phosphorylcholine (Tables 2 and 3) but no labelling of the choline. Any formation of labelled phosphorylcholine by choline kinase (EC 2.7.1.32) is dependent on the presence of

labelled choline. In these experiments, however, no labelling of the free choline was detected, so the possibility of the methylation of the water-soluble phosphate monoesters to their choline analogue was considered. Gibson, Wilson & Udenfriend (1961) demonstrated the labelling of phosphorylcholine without the previous formation of choline and Bjørnstad & Bremer (1966) demonstrated a considerable labelling of the phosphorylcholine from [Me- ^{14}C]methionine in the liver after intraperitoneal injection. These results would suggest a probable direct synthesis of phosphorylcholine from phosphorylethanolamine by methylation although we were unable to demonstrate this *in vitro* with [^{32}P]phosphoryldimethylaminoethanol and liver microsomes in the presence of *S*-adenosylmethionine.

In some of the early studies, the choline was determined by using the reineckate method and the radioactivity determined on the reineckate salt decolorized by the method of Bremer & Greenberg (1961). It was found that this method is very unspecific, since compounds other than choline were co-precipitated. When trace amounts of radioactive dimethylaminoethanol were added to an unlabelled brain extract and choline was determined, there was a high percentage of the label still in the reineckate. Some of the values for the specific radioactivity of choline after the injection of labelled precursors reported by Ansell & Spanner (1970) are therefore too high. No radioactive choline could, in fact, be detected in blood, brain or liver by t.l.c. after the injection of either labelled ethanolamine or dimethylaminoethanol, nor in brain and blood after the injection of choline.

Intraperitoneal injection of [Me- ^{14}C]choline. When labelled choline was injected intraperitoneally, it was lost very rapidly. The specific radioactivity of the free choline in liver was only $87\text{ d.p.s./}\mu\text{mol}$ when measured 1 h after the injection of $20\text{ }\mu\text{Ci}$ (i.e.

Table 2. Radioactivity in choline, phosphorylcholine and phosphatidylcholine of rat liver, blood and brain after the intraperitoneal injection of 20 μ Ci of [1,2- 14 C]dimethylaminoethanol

N.D., not detected.

Time (h)	Radioactivity (d.p.s./whole tissue)								
	Liver			Blood			Brain		
	Choline	Phosphoryl- choline	Phosphatidyl- choline	Choline	Phosphoryl- choline	Phosphatidyl- choline	Choline	Phosphoryl- choline	Phosphatidyl- choline
1	N.D.	254	3320	N.D.	N.D.	84	N.D.	1.7	5
2	N.D.	308	—	N.D.	N.D.	—	N.D.	3.2	—
4	N.D.	334	—	N.D.	N.D.	—	N.D.	7.4	—
6	N.D.	570	7450	N.D.	N.D.	118	N.D.	15.5	102
7	N.D.	710	15310	N.D.	N.D.	1050	N.D.	40.0	460
17	N.D.	1240	21270	N.D.	N.D.	2050	N.D.	47.0	690
24	N.D.	—	27450	N.D.	N.D.	1450	N.D.	—	615

Radioactivity (d.p.s./ μ mol)				
Liver		Brain		
Time (h)	Phosphorylcholine	Phosphatidylcholine	Phosphorylcholine	Phosphatidylcholine
1	54.5	33	2.5	0.2
1½	121	—	—	1.6
2	143	—	5.8	—
4	120	—	11.0	—
6	123	65	23.0	4.0
7	153	100	81.0	25.0
17	266	100	94.0	21.0

Fig. 1. Specific radioactivity of the phosphatidylcholine of liver (●), blood (▲) and brain (■) at different times after the intraperitoneal injection of 20 μ Ci of [1,2- 14 C]-dimethylaminoethanol into the adult female rat.

2.0×10^6 d.p.s./ μ mol). No labelled choline was detected in the brain or blood and no phosphorylcholine in the blood. This tremendous fall in specific radioactivity could not be due to a dilution from the free unlabelled choline present in the tissues since, as shown in Table 4, the concentration of free choline in the tissues is extremely low. However, the specific radioactivity of the phosphorylcholine in liver was high and the lipid choline in brain, blood and liver was significantly labelled (Table 5).

Although the labelling of the free choline of liver was extremely low, there was a very high count in the so-called base fraction of the acid-soluble extract (see the Materials and Methods section). Over 98% of the radioactivity in this fraction was in a compound tentatively identified as betaine after co-chromatography of the liver extract with carrier betaine on t.l.c. by the two-dimensional chromatography method of Speed & Richardson (1968). The absence of radioactive choline was further demonstrated in blood and brain by counting the radioactivity of a choline iodide derivative after addition of unlabelled choline to the extract.

Intracerebral injection of [1,2- 14 C]dimethylaminoethanol and L-[Me- 14 C]methionine. When labelled dimethylaminoethanol (1.06 μ Ci, 0.8 μ mol) was injected into the brain of the rat, there was a rapid loss of the labelled compound. However, a measurable percentage was incorporated into the lipid and by 7 h this was equivalent to 7% of the injected dose (Fig. 2). [After a similar injection of labelled choline, the lipid labelling reached nearly 30% in this time

(Ansell & Spanner, 1968).] The phosphatidylcholine was very poorly labelled and amounted to only 3% of the total labelled lipid at 7 h; the remaining 97% could be accounted for in the phosphatidyl-dimethylaminoethanol.

When L-[Me- 14 C]methionine was injected intracerebrally no radioactivity could be detected in the total lipid of the brain. This indicated that there was no measurable transfer of methyl groups from methionine through the *S*-adenosylmethionine pathway.

Fatty acid composition of brain and liver phosphatidylcholine and phosphatidylethanolamine. It is interesting to compare the fatty acid pattern of phosphatidylcholine with that of phosphatidylethanolamine in brain and liver (Table 6). As can be seen, in liver where most of the phosphatidylcholine derives from phosphatidylethanolamine by stepwise methylation, their fatty acid patterns are similar. In brain, however, the fatty acid patterns of the two lipids are very different, particularly with respect to $C_{16:0}$ and $C_{18:0}$ fatty acids. This further supports other evidence that, in brain, methylation of phosphatidylethanolamine to phosphatidylcholine almost certainly does not occur.

DISCUSSION

The present investigation developed from the following observations. Choline is normally synthesized in mammalian tissues by the stepwise methylation of phosphatidylethanolamine, a pathway absent in brain. Labelled choline, injected intracerebrally, is rapidly incorporated into a lipid-bound form (Ansell & Spanner, 1968), and into acetylcholine (Chakrin & Whittaker, 1969). Therefore either choline is transported to the brain in a free form or it is supplied in a bound form and at some stage liberated as the free base before being utilized. It is notable that intracerebrally injected phosphorylcholine and CDP-choline are rapidly hydrolysed before the utilization of the choline (Ansell & Spanner, 1968).

With the possible exception of skeletal muscle, the liver contains the largest pool of choline in the body of the adult rat (about 150 μ mol/liver). It is the only organ with a significant capacity to transfer methyl groups to phosphatidylethanolamine (Bremer & Greenberg, 1961) and it is believed that this route, the stepwise methylation of phosphatidylethanolamine via phosphatidylmonomethylaminoethanol and phosphatidyl-dimethylaminoethanol, is the major pathway to the synthesis of phosphatidylcholine in the liver of the adult female rat (Lyman *et al.* 1967). The first step has not yet been demonstrated *in vitro* in liver tissue (cf. Rehnbinder & Greenberg, 1965), but Morgan (1969)

Table 3. Radioactivity in choline, phosphorylcholine and phosphatidylcholine of rat liver, blood and brain after the intraperitoneal injection of $18 \mu\text{Ci}$ of $[1,2-^{14}\text{C}]\text{ethanolamine}$

N.D., not detected.

Time (h)	Radioactivity (d.p.s./whole tissue)								
	Liver			Blood			Brain		
	Choline	Phosphoryl- choline	Phosphatidyl- choline	Choline	Phosphoryl- choline	Phosphatidyl- choline	Choline	Phosphoryl- choline	Phosphatidyl- choline
1	N.D.	14	22 000	N.D.	N.D.	17	N.D.	N.D.	0.6
2	N.D.	200	26 050	N.D.	N.D.	78	N.D.	N.D.	7.0
4	N.D.	564	40 600	N.D.	N.D.	212	N.D.	9.6	15.0
7	N.D.	567	54 700	N.D.	N.D.	415	N.D.	20.0	70.0

Radioactivity (d.p.s./ μmol)					
Liver			Brain		
Time (h)	Phosphorylcholine	Phosphatidylcholine	Phosphorylcholine	Phosphatidylcholine	
1	98	172	N.D.	0.1	
2	111	188	N.D.	0.2	
4	94	300	24.6	0.7	
6	—	407	26.0	0.8	
7	85	500	51.0	2.5	
9	71	258	71.2	2.3	

Table 4. *Concentrations of some choline compounds in female rat brain, blood and liver*Results are expressed as $\mu\text{mol/g}$ of tissue ($\pm$ s.d.). Numbers of animals are given in parentheses.

	Concentration		
	Brain	Blood	Liver
Choline	0.17 ± 0.01 (5)	Trace	0.35 ± 0.05 (6)*
Phosphorylcholine	0.38 ± 0.05 (10)	Trace	1.91 ± 0.69 (15)
Phosphatidylcholine	14.70 ± 1.50 (39)	1.41 ± 0.26 (21)	16.2 ± 2.1 (19)

* This value agrees with that of Smith & Saelens (1967). It is much lower than the value reported by Wells & Remy (1965) which seems to include all water-soluble choline compounds in 'free' choline.

Table 5. *Specific radioactivities of choline, phosphorylcholine and phosphatidylcholine after the intraperitoneal injection of $20 \mu\text{Ci}$ of $[\text{Me-}^{14}\text{C}]$ choline*

N.D., not detectable.

Organ	Lipid	Time ...	Sp. radioactivity (d.p.s./ μmol)	
			1 h	6 h
Liver	Choline		87	20
	Phosphorylcholine		4040	1390
	Phosphatidylcholine		243	374
Blood	Choline		N.D.	N.D.
	Phosphorylcholine		N.D.	N.D.
	Phosphatidylcholine		24.5	188
Brain	Choline		N.D.	N.D.
	Phosphorylcholine		118	70
	Phosphatidylcholine		3.3	5.3

has demonstrated the reaction with lung microsomes. Although apparently a minor route, phosphatidylcholine is also synthesized in the liver via the cytidine pathway (Kennedy, 1957) and choline can be incorporated into phosphatidylcholine by a base-exchange reaction (Dils & Hübscher, 1961). The liver has been shown to be the source of plasma phosphatidylcholine by Fishler, Entenman, Montgomery & Chaikoff (1943).

The results in Tables 1, 2 and 3 confirm that labelled ethanolamine and dimethylaminoethanol are rapidly incorporated into the choline moiety of liver phosphatidylcholine presumably by reactions outlined in Scheme 1, which is based on observations by Kennedy & Weiss (1956), Ansell & Chojnacki (1966), Bjørnstad & Bremer (1966), Groth *et al.* (1958) and Lombardi, Pani, Schlunk & Shi-Hua (1969).

Tables 2 and 3 show that at no time was there any significant labelling of free choline in the liver, nor could any be detected in the blood. This confirms other studies that methylation of free ethanolamine or dimethylaminoethanol does not occur (Gibson *et al.* 1961). It also indicates that *in vivo* very little choline is liberated via phospholipases and glycerophosphorylcholine diesterase (EC 3.1.4.2); any

Fig. 2. Radioactivity in the acid-soluble fraction (●) and total lipid fraction (▲) of rat brain after the intracerebral injection of $1.06 \mu\text{Ci}$ of $[\text{1,2-}^{14}\text{C}]$ dimethylaminoethanol. The results are expressed as a percentage of the injected dose.

choline that is liberated must be rapidly oxidized by choline dehydrogenase (EC 1.1.99.1). This was confirmed by the intraperitoneal injection of $[\text{Me-}^{14}\text{C}]$ choline which, although incorporated either by the cytidine pathway or by the exchange reaction into phosphatidylcholine (Table 5), was barely

Table 6. *Fatty acid composition (as a percentage) of the phosphatidylcholine and phosphatidylethanolamine of whole rat brain and liver*

The values for brain agree with others in the literature, e.g. O'Brien, Fillerup & Mead (1964), Svennerholm (1968), Debuch (1956). Values for liver are taken from Wood & Harlow (1969) and Spitzer, Norman & Morrison (1969).

Fatty acid	Brain		Liver	
	Phosphatidylcholine	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylethanolamine
C _{16:0}	43	6	15	17
C _{16:1}	0	0	1	0
C _{18:0}	16	40	34	37
C _{18:1}	30	37	10	9
C _{18:2}	0.3	3	12	8
C _{20:n}	7.7	9	22	22
C _{22:n}	3.0	5	6	6

Scheme 1. Pathways for the formation of phosphatidylcholine in liver from ethanolamine and dimethylaminoethanol based on references given in the text.

detectable in the free state in the liver. The major water-soluble radioactive compounds found in liver after the injection of labelled choline were phosphorylcholine and a compound provisionally identified as betaine.

From the experiments summarized in Tables 2, 3 and 5 it seems highly unlikely that the free base is the form in which choline is carried in the blood to the brain, a conclusion supplementing that of Bjørnstad & Bremer (1966). It is true that human plasma contains about $0.01 \mu\text{mol}$ of free choline/ml (Bligh, 1952) and our values would suggest that in rat whole blood the concentration is of the same order. If this was supplying the brain it would have to be very significantly labelled to account for the labelling of the phosphatidylcholine of that tissue.

Thus, even if the active transport system for free choline that has been demonstrated with isolated brain tissue (Schuberth, Sundwall, Sörbo & Lindell, 1966) could operate in the intact animal, such a mechanism could not account for the labelling observed.

It was observed that there was significant labelling of phosphorylcholine in the liver after the injection of either ethanolamine or dimethylaminoethanol and this is difficult to explain in the absence of labelled choline of higher specific radioactivity. It is also contrary to the findings of Bremer, Figard & Greenberg (1960), who found no labelled phosphorylcholine in liver after the injection of labelled ethanolamine. One possible pathway is the methylation of phosphorylethanolamine and phosphoryldi-

Table 7. *Radioactivity of the phosphate monoesters of choline, ethanolamine and dimethylaminoethanol in liver after the injection of [1,2-¹⁴C]ethanolamine and [1,2-¹⁴C]dimethylaminoethanol*

Label	Time (h)	Sp. radioactivity (d.p.s./ μ mol)		
		Phosphoryl-ethanolamine	Phosphoryldimethyl-aminoethanol	Phosphorylcholine
[1,2- ¹⁴ C]Ethanolamine	1	421		98
	2	528		110
	4	1200		94
	5.5	1236		96
	7	945		95
[1,2- ¹⁴ C]Dimethylaminoethanol	1		11 460	545
	2		9940	143
	4		7 660	120
	6		6360	123
	7		4400	133

methylaminoethanol, since their specific radioactivities were at all times much higher than that of the phosphorylcholine (Table 7). Gibson *et al.* (1961) gave indirect evidence for such a pathway although it would seem to be eliminated, at least for male hamsters, by the experiments by Beeler, Treble, Kyriakides & Balint (1970). We were unable to demonstrate the pathway *in vitro*.

In any event Riley (1944) demonstrated that after intravenous injection, [³²P]phosphorylcholine is rapidly hydrolysed by the blood and Dawson (1955) found blood to contain no free phosphorylcholine. As is shown in the Tables, the blood contained only a trace of labelled phosphorylcholine after the injection of choline, ethanolamine or dimethylaminoethanol. It therefore follows that phosphorylcholine cannot be the mode of choline transport in the blood.

In terms of both labelling and amount, lipid-bound choline would appear to be the most likely form in which the base is supplied to the brain though the evidence presented in this paper is indirect. Stein & Stein (1966) showed that doubly labelled lysophosphatidylcholine was carried intact to the various organs of the rat though they did not study the uptake into the brain. Hoelzl & Franck (1969) showed that when phosphatidylcholine doubly labelled with ³²P and ¹⁴C was injected intravenously into rats, the specific radioactivity of the phosphatidylcholine in the brain had the same isotopic ratio as the injected material and concluded that there was no barrier to the phospholipid. This strongly suggests that lipid-bound choline can pass through the blood-brain barrier. In our experiments the specific radioactivity of phosphatidylcholine included that of the lyso-derivative. It was, however, demonstrated by radioautography, after t.l.c. of the choline lipids, that lysophosphatidylcholine was significantly labelled. The concept of lipid transport to the brain is not novel since

Davison, Dobbing, Morgan & Payling-Wright (1958, 1959) showed that [4-¹⁴C]cholesterol was incorporated intact into chick and rabbit brain.

The fatty acid composition of liver phosphatidylcholine is very different from that of brain (Table 6) but it must be remembered that within the brain the lipids of the various subcellular compartments have very different fatty acid patterns. For example, the fatty acid pattern of the phosphatidylcholine of myelin is very different from that of the nerve endings (Kishimoto, Agranoff, Radin & Burton, 1969). Further, reacylation of lysophosphatidylcholine readily occurs with brain preparations (Webster, 1965). Because of this the 1.0 μ mol of phosphatidylcholine/h brought to the brain would not make a significant difference to the fatty acid pattern as a whole.

It is known that the phosphatidylcholine of brain turns over more rapidly than any other major phospholipid (Ansell & Dohmen, 1957). It is possible that this is reflected in the rapid incorporation of free choline when injected intracerebrally (Ansell & Spanner, 1968) and is conceivably connected with the supply of choline for acetylcholine synthesis. It is also possible that the rapid phosphorylation of intracerebrally injected choline and its incorporation via the cytidine pathway is a mechanism by which choline is conserved in the brain and not a synthesis *de novo* of phosphatidylcholine. In this connexion it is notable that choline not so 'trapped' was rapidly lost by the brain (Ansell & Spanner, 1968) whereas injected ethanolamine was lost to a much smaller extent (Ansell & Spanner, 1967).

From the results described in this paper it can be seen that, although the brain is capable of converting choline into a lipid-bound form via the cytidine pathway, it is unlikely that it is as the free base that choline is supplied to the brain in the adult rat. It seems almost certain that choline is supplied to the

brain by the blood in a lipid-bound form as either phosphatidylcholine or as its lyso-derivative. This would be in agreement with the findings of Davson (1967) that lipid solubility is an important criterion for compounds crossing the blood-brain barrier.

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