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# Arterio-Venous Differences of Choline and Choline Lipids across the Brain of Rat and Rabbit

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The concentration of unesterified choline in the plasma in the jugular vein of the rat (0.85nmol/ml) was found to be three times that of the arterial supply to the brain (0.25nmol/ml), indicating a higher efflux than uptake of unesterified choline by the brain. No such difference was found for the rabbit and no arterio-venous difference for phosphatidylcholine or lysophosphatidylcholine was observed in either species. No arterio-venous difference was found for choline in blood cells. The infusion of [*Me*-$^3$H]choline into the circulation of the rat or rabbit indicated an uptake of radioactive choline by the brain and an efflux of non-radioactive choline. In the rabbit such an infusion produced a steady rise in the labelling of phosphatidylcholine and lysophosphatidylcholine in the plasma. When [$^{14}C_2$]ethanolamine was injected intraperitoneally into the rat there was a labelling of phosphatidylcholine, lysophosphatidylcholine and sphingomyelin in the plasma and cells of blood from the jugular vein and the arterial supply, as well as in the brain tissue. However, no labelling of unesterified choline in these tissues could be detected. Unesterified choline was shown to be liberated into the plasma when whole blood from the rat or man, but not the rabbit, was incubated for short periods at 30°C.

It was shown by Schuberth *et al.* (1969) that when [*Me*-$^3$H]choline was injected into the tail vein of the mouse there was a rapid uptake of radioactive choline into the brain. Within 15s of the injection, about 0.5% (2.25nmol) of the injected dose had entered the brain and one-third of this had been converted into acetylcholine. Subsequent observations by Diamond (1971) confirmed this and showed that the choline taken up was also phosphorylated and converted into a lipid-bound form. Phosphorylation and conversion into a lipid-bound form as a consequence of intracerebral injection of radioactive choline was shown by Ansell & Spanner (1968). Thus it might be concluded that the unesterified choline in the blood is the major source of the choline used by the brain. There is, however, very good evidence that choline can be transported to the brain as a lipid-bound form, probably as lysophosphatidylcholine (Illingworth & Portman, 1972) and this evidence has been discussed in some detail by Ansell & Spanner (1975). Briefly, the argument is that, when choline is synthesized 'naturally' *in vivo* in a lipid-bound form from a precursor such as ethanolamine or methionine, no radioactive unesterified choline can be detected in the blood (Ansell & Spanner, 1971). Further, the transport of radioactive choline synthesized in this way to various organs cannot be 'diluted out' by the injection of non-radioactive choline (Bjørnstad & Bremer, 1966). Sparf (1973) has criticized this argument because of the short half-life (5min) of free choline in the blood. However, in the experiments described by Ansell & Spanner (1971) and Bjørnstad & Bremer (1966) the short half-life of radioactive unesterified choline would not have prevented its detection, because its production in the liver and transfer to the blood would have been continuous.

The relationship between blood choline and brain choline is further complicated by the finding of Dross & Kewitz (1972) of an efflux of unesterified choline from the brain of the rat at a rate of 7.2nmol/min per g wet wt. of brain. This result has been confirmed by ourselves (Spanner *et al.*, 1975) and by Choi *et al.* (1975). The latter workers concluded that some of this unesterified choline in the venous output from the brain is generated from sources within the brain, presumably from lipid-bound choline. The experiments reported in the present paper extend these observations by an examination, in the rat and the rabbit, of the concentrations of unesterified choline and lipid-bound choline across the brain and the labelling of unesterified and lipid-bound choline after the infusion of [*Me*-$^3$H]choline or the injection of [$^{14}C_2$]ethanolamine. A preliminary account of some of these results has appeared (Spanner *et al.*, 1975).

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## Materials and Methods

### *Materials*

[*Me*-$^3$H]Choline, [$^{14}C_2$]ethanolamine and [$\gamma$-$^{32}$P]-ATP were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The [*Me*-$^3$H]choline chloride was diluted with non-radioactive choline chloride to give a specific radioactivity of 2$\mu$Ci/$\mu$mol. The [$^{14}C_2$]ethanolamine had a specific radioactivity of 30$\mu$Ci/$\mu$mol. Choline kinase (EC 2.7.1.32), prepared from baker's yeast, was supplied by Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. (1unit converts 1$\mu$mol of choline and ATP into phosphorylcholine and ADP in 1min at 25°C). All other chemicals were of AR quality whenever possible. Urethane (BDH Chemicals Ltd., Poole, Dorset, U.K.) was prepared as a 25% (w/v) solution in 0.15M-NaCl. Heparin (Boots Pure Drug Co., Nottingham, Notts., U.K.) was a preparation containing 1000i.u./ml. The animals used were adult male Wistar rats of approx. 300g, or adult male rabbits of approx. 2kg body weight. The rabbits were starved for 16h before the experiments.

### *Treatment of animals*

The rats and rabbits were anaesthetized with a single intraperitoneal injection of urethane (150mg/kg body wt.) and subjected to tracheotomy. An external jugular vein was cannulated for infusion of drugs, saline or dextran, and a femoral or carotid artery cannulated for blood sampling. When a carotid artery was cannulated it was always on the same side as the cannulated jugular vein. The second jugular vein was prepared for cannulation, but was not cannulated until immediately before sampling so that minimal disturbance to cerebral venous drainage would occur. In the rabbits from which multiple samples were taken, a side-branch of the external jugular vein was cannulated, and the tip of the cannula passed into the jugular vein, with the tip directed towards the brain. A loose ligature was around the cardiac side of the cannula, and when samples were required, the loose ligature was tightened. After completion of surgery all animals were treated with heparin (1000i.u./kg).

Infusions of choline in 0.15M-NaCl were given at 0.2ml/min per kg body wt in all animals for 30min. The concentration of choline was adjusted so that the amount administered at this flow rate was 1$\mu$mol/min per kg. In control animals the choline diluent, 0.15M-NaCl, was infused at the same rate for the same period of time.

After completion of surgery at least 20min (and usually 50min) elapsed before any samples were taken or infusions commenced.

Human blood (male) was obtained from the forearm by venipuncture and the samples transferred immediately to tubes containing heparin.

In the experiments with [$^{14}C_2$]ethanolamine, the radioisotope was injected intraperitoneally and in one rat the blood sample was taken after 60min and in the other after 140min.

### *Treatment of blood samples*

Blood was withdrawn simultaneously from arterial and venous cannulae and immediately transferred to cold centrifuge tubes which were centrifuged at 4°C for 15min at 2250$g_{av.}$. This brought down the blood cells and the platelets, leaving a clear supernatant of plasma which was removed for analysis. It was necessary to carry out this centrifugation in the cold under controlled conditions, as there was a rapid rise in unesterified choline concentrations in the plasma if the blood was allowed to remain at room temperature and centrifuged in a conventional bench centrifuge. This rise of unesterified choline was particularly marked with blood from the rat and man but only slight for the rabbit (see the Results section).

### *Brain*

At the end of the experiment the rats were killed by decapitation and the heads dropped immediately into liquid $N_2$. The heads were split in half longitudinally and the brain tissue was chiselled out. Each half brain was crushed separately to a fine powder and the tissue extracted for phospholipid analysis and free choline determination.

### *Liver*

The livers were removed at death and extracted for phospholipid analysis.

### *Assay of free choline*

This is a modification of the enzymic radiochemical method of Goldberg & McCaman (1973).

(*a*) *Preparation of blood.* A sample of plasma or packed cells re-suspended in 0.15M-NaCl was homogenized for 1min with acetone/1M-formic acid (17:3, v/v) in the proportion of 1ml of acetone/formic acid to 0.15ml of plasma or suspended cells. After cooling for 10min in ice, the tubes were centrifuged for 10min at 1000$g_{av.}$. A measured sample of the supernatant was evaporated to dryness in a rotary evaporator at a temperature below 50°C and the residue was suspended in 1.5ml of 0.05M-sodium phosphate buffer, pH6.6.

(*b*) *Preparation of brain.* The brain was extracted with 10ml of 10% (w/v) trichloroacetic acid and a sample of the extract washed four times with an equal

volume of diethyl ether to remove most of the trichloroacetic acid. After the pH had been adjusted to 8 with an 'ammonia wick' the sample was applied to a column (0.8cm×6.0cm) of Dowex 50 (X8; $H^+$ form; 100–200 mesh) and washed through with 10ml of water. The column was then washed through with 40ml of 0.1M-HCl and the unesterified choline eluted with 10ml of 3M-HCl. The sample was taken to dryness in a rotary evaporator and the last traces of acid were removed by leaving the flask over KOH pellets in a vacuum desiccator for at least 2h. The residue was dissolved in 1.5ml of 0.05M-sodium phosphate buffer, pH6.6. From this stage the treatment of blood and brain samples was identical.

A sample (1.0ml) was shaken with 1.0ml of heptanone (ethyl butyl ketone, available from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) containing 20mg of sodium tetraphenylboron. After centrifuging, the heptanone layer was transferred to a stoppered centrifuge tube and the buffer phase re-shaken with a further 1ml of heptanone/tetraphenylboron. The two heptanone phases (2ml) were combined, shaken with 2ml of 0.4M-HCl and centrifuged. The HCl phase was evaporated to dryness in a rotary evaporator and the residue taken up in 150$\mu$l of a mixture containing 100mM-sodium phosphate buffer, pH8.0, 2mM-[$\gamma$-$^{32}$P]ATP (approx. 350c.p.m./nmol) and 10mM-$MgCl_2$. To 50$\mu$l was added 50$\mu$l of a choline kinase preparation containing 0.0094unit of the enzyme. Duplicate samples were incubated for 30min at 37°C and the reaction was stopped by the addition of 100$\mu$l of 0.3M-barium acetate. Excess of [$\gamma$-$^{32}$P]ATP was largely precipitated and, after 15min at 0°C, the tubes were centrifuged for 10min. A sample of the clear supernatant (50–100$\mu$l) was applied to a column (5mm×40mm) of Dowex 1 (X8; formate form). The samples were washed through with 800$\mu$l of water followed by 4×800$\mu$l of 75mM-ammonium formate. These effluents containing [$^{32}$P]phosphorylcholine were collected through a Whatman no. 41 filter paper, diluted to 10ml and the $^{32}$P radioactivity was counted in a M6 liquid counting tube connected to conventional ancillary equipment. The amount of [$^{32}$P]phosphorylcholine formed, equivalent to the choline originally present, was calculated by knowing the specific radioactivity of the [$\gamma$-$^{32}$P]ATP. The columns of Dowex (formate form) were regenerated by passing through them 5vol. of a solution containing 5M-formic acid and 2M-ammonium formate followed by water until the effluent was pH5.0.

*Phospholipid analysis*

A sample of plasma or resuspended cells was homogenized with 20vol. of chloroform/methanol (2:1, v/v). Brains and livers were treated similarly and the filtered extract was evaporated to dryness and washed by the method of Folch *et al.* (1957). The phospholipid extract was dissolved in chloroform. Choline phospholipids of plasma and blood-cell samples could be separated by t.l.c. on silica gel H without prior fractionation. For brain and liver the chloroform solution was subjected to chromatography on a column (0.8cm×3cm) of aluminium oxide (BDH chromatographic grade) and the choline lipids were eluted with chloroform/methanol (1:1, v/v) (Davison & Wajda, 1959). These lipids were then separated by t.l.c. on silica gel H in chloroform/methanol/water (14:6:1, by vol.). Areas corresponding to sphingomyelin, phosphatidylcholine and lysophosphatidylcholine were detected by exposing the plates to iodine vapour and scraped off into tubes. Subsequent treatment of the spots depended on whether or not the animals had been infused or injected with radioactive compounds.

In the non-radioactive experiments, phosphatidylcholine and lysophosphatidylcholine spots were scraped into stoppered test tubes (18mm×150mm in length) containing 1ml of 10M-$H_2SO_4$/0.1M-periodic acid/95% (v/v) ethanol (3:1:6, by vol.) and the tubes placed in a boiling-water bath for 45min (Rosenthal & Han, 1969). The phosphorus released from glycerophospholipids under these conditions was then determined by a modification of the method of Ernster *et al.* (1950). In our hands, the original method for phosphorus determination by Rosenthal & Han (1969) gave very inconsistent results.

Where the animals had been infused or injected with radioactive compounds, the spots were scraped into stoppered centrifuge tubes, extracted with 4×2ml of chloroform/methanol/water/formic acid (97:97:4:2, by vol.) (Abramson & Blecher, 1964) and the combined extracts made up to 10ml with the same mixture. A sample was transferred to a counting vial, the solvent removed under a stream of air and 10ml of scintillator (Ansell & Spanner, 1971) added and the $^{14}$C or $^{3}$H radioactivity counted in a Philips liquid-scintillation counter. A further sample was transferred to a stoppered test tube, the solvent removed under a stream of air and the residue wet-ashed with $HClO_4$ (60%, v/v). The phosphorus content was determined by the modification of the method of Ernster *et al.* (1950). Sphingomyelin could be assayed for phosphorus by this method.

## Results and Discussion

*Rat*

*Arterio-venous differences of unesterified choline.* In Table 1 are given the values for unesterified choline in the femoral artery and jugular vein of the anaesthetized rat. Although the value for the plasma of the femoral artery was 0.25±0.03nmol/ml, that

Table 1. *Concentration of unesterified choline, phosphatidylcholine, lysophosphatidylcholine and sphingomyelin in the blood of rat, rabbit and man*

Values given are means ± S.D., with the numbers of animals in parentheses.

| Species | Vessel | | Plasma | Cells |
|---|---|---|---|---|
| Rat | Femoral artery | Choline (nmol/ml) | 0.25 ± 0.03 (12) | 2.57 ± 1.67 (8) |
| | Jugular vein | Choline (nmol/ml) | 0.85 ± 0.12 (12) | 3.18 ± 1.47 (8) |
| | Artery or vein | Phosphatidylcholine (μmol/ml) | 0.75 ± 0.18 (24) | 1.32 ± 0.25 (4) |
| | Artery or vein | Lysophosphatidylcholine (μmol/ml) | 0.23 ± 0.07 (24) | 0.162 ± 0.022 (4) |
| | Artery or vein | Sphingomyelin (μmol/ml) | 0.16 ± 0.03 (4) | 0.22 ± 0.05 (4) |
| Rabbit | Carotid artery | Choline (nmol/ml) | 5.18 ± 1.11 (7) | 7.93 ± 0.57 (3) |
| | Jugular vein | Choline (nmol/ml) | 4.22 ± 1.91 (6) | — |
| | Artery or vein | Phosphatidylcholine (μmol/ml) | 0.78 ± 0.08 (6) | — |
| | Artery or vein | Lysophosphatidylcholine (μmol/ml) | 0.14 ± 0.05 (6) | — |
| Man | Vein | Choline (nmol/ml) | 0.81 (2) (0.72–0.90) | 1.36 (2) (1.33–1.39) |

of the jugular vein was 0.85 ± 0.12 nmol/ml, i.e. over three times as high as that of the artery. There was no significant difference in the unesterified choline concentrations of the cells of these vessels, which in both instances was about 3.0 nmol/ml of blood. Dross & Kewitz (1972) were the first to demonstrate an arterio-venous difference across the brain of the rat, but their values, which are apparently for whole blood, were higher than those in Table 1 by a factor of 4. Choi *et al.* (1975) have also demonstrated this arterio-venous difference in the plasma of rats, but their values are also higher than those quoted in Table 1. This discrepancy may be due to several factors. The concentration of unesterified choline in the plasma of the rat rises rapidly if the blood is allowed to remain at room temperature (approx. 22°C) for any length of time and, in preliminary work, very variable values were obtained when a bench centrifuge was used for separating the plasma. However, if the blood was maintained and centrifuged at 4°C after sampling, no such rise occurred and consistent values for unesterified choline were obtained. In Fig. 1 the effect of incubating rat, rabbit and human blood at 30°C on the concentration of unesterified choline in plasma is shown. Wang & Haubrich (1975) have shown that when guinea-pig plasma is incubated at 22.5°C there is a release of unesterified choline. Also, we have found, as did Wang & Haubrich (1975), that the unesterified choline concentrations in rat plasma can rise dramatically when the animals are maintained on a high-choline diet. We therefore suggest that the values reported in the present paper are minimal values and that absolute concentrations are to some extent determined by dietary choline intake.

The arterio-venous difference shown in Table 1 was found only if the venous measurement was carried out on blood from the jugular vein. The concentration of plasma choline in the hepatic vein was the same as that in the femoral artery, indicating

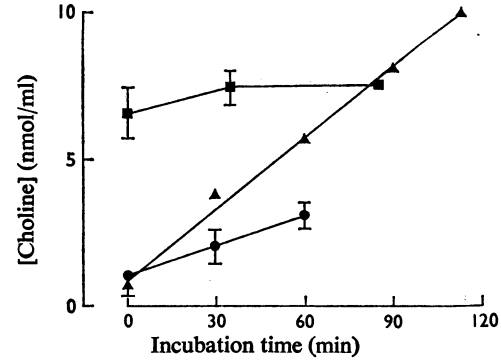


Fig. 1. *Concentration of unesterified choline in plasma after incubating whole blood at* 30°C

Rabbit (■), rat (●), man (▲). Where there are more than four determinations for a given time, the S.D. values are indicated.

that the efflux of choline into the jugular vein was a characteristic of brain metabolism in the rat.

Although there was a large increase in the unesterified choline of plasma as the blood flowed across the brain, the values for choline-containing glycerophospholipid were the same, i.e. 0.75 ± 0.18 (S.D.) μmol/ml for phosphatidylcholine and 0.23 ± 0.07 (S.D.) μmol/ml for lysophosphatidylcholine. These values are not dissimilar to those of Nelson (1967) for total blood plasma. If the release of free choline into the venous return was balanced by a decreased concentration of either of these glycerophospholipids it would not, however, be possible to detect this because of the vast difference between the lipid concentration and that of the unesterified choline in the plasma, a factor of about 1000. An infusion of choline had no effect on the choline glycerophospholipid concentration nor on the arterio-venous ratio, although, in contrast with the

results of Freeman *et al.* (1975), we found a rise in the absolute concentration of unesterified choline.

*Infusion of [Me-$^3$H]choline.* When [*Me*-$^3$H]choline was infused into rats via the jugular vein, we found the same rise in the unesterified choline concentration in plasma that we observed with infusions of unlabelled choline, i.e. 13.8nmol/ml in the artery and 24.9nmol/ml in the jugular vein. The converse was true with radioactivity, i.e. 1317d.p.s./ml (3) in the artery and 671d.p.s./ml (3) in the jugular vein, giving specific radioactivities of 102.6d.p.s./nmol and only 28.5d.p.s./nmol respectively after an infusion time of 30min (Table 2). Assuming that the blood flow through the brain of the rat is 1ml/min per g of tissue (Goldman & Sapirstein, 1973), it can be calculated that in these experiments there was an uptake of 114nmol/30min per g, which agrees very well with the findings of Choi *et al.* (1975) of 118nmol/32min infusion per g. The high venous–arterial difference and the considerable fall in the specific radioactivity of the venous plasma choline indicates a considerable efflux of unlabelled choline from the brain, a finding also described by Choi *et al.* (1975) and Freeman *et al.* (1975).

*Intraperitoneal injection of [$^{14}C_2$]ethanolamine.* Rats were injected intraperitoneally with [$^{14}C_2$]-ethanolamine and blood samples from the jugular vein and femoral artery were taken either 60min or 140min after the injection. In these experiments, the brains and livers were also analysed. There was no detectable radioactivity in the free choline of the blood cells or plasma nor in that of the brain, which confirms our earlier observations (Ansell & Spanner, 1971). The phosphatidylcholine and sphingomyelin in the brain and in the blood were, however, significantly labelled (Table 3). In addition, lysophosphatidylcholine, present in the blood cells and plasma but undetectable in the brain, was highly labelled.

In earlier work (Ansell & Spanner, 1971) we failed to demonstrate any radioactivity in the free choline of the rat blood 1–7h after the intraperitoneal injection of either [$^{14}C_2$]ethanolamine or dimethylamino-[1,2-$^{14}$C]ethanol (Tracerlab, Waltham, Mass., U.S.A.), although the choline-containing glycerophospholipids of liver, blood, and to a lesser extent brain, were markedly labelled. At that time we postulated that brain probably received its choline in a lipid-bound form. Illingworth & Portman (1972), using squirrel monkeys, demonstrated that doubly labelled lysophosphatidylcholine given intravenously could cross the blood/brain barrier. This could then be converted into phosphatidylcholine by lysophosphatidylcholine acyltransferase (EC 2.3.1.23) or by the reaction between two lysophosphatidylcholine molecules to give phosphatidylcholine and glycero-3-phosphorylcholine. This last-named reaction has

Table 2. *Radioactivity and concentration of unesterified choline in the femoral artery and jugular vein of the rat after 30min infusion of [Me-$^3$H]choline into the jugular vein*

Each value is the mean of two independent determinations which agreed within 2.5%. (The [*Me*-$^3$H]choline had a specific radioactivity of 2$\mu$Ci/$\mu$mol and was infused at a rate of 0.25$\mu$mol/min.)

| Rat | Vessel | [Choline] (nmol/ml) | $^3$H radioactivity (d.p.s./ml) | $^3$H radioactivity (d.p.s./nmol) |
|---|---|---|---|---|
| 1 | Artery | 10.43 | 780 | 74.7 |
| | Vein | 27.80 | 630 | 22.7 |
| 2 | Artery | 12.50 | 1650 | 132.0 |
| | Vein | 23.25 | 600 | 25.8 |
| 3 | Artery | 18.60 | 1520 | 81.9 |
| | Vein | 23.60 | 782 | 33.0 |

Table 3. *Labelling of unesterified choline and choline glycerophospholipids in the blood, brain and liver of the rat after the intraperitoneal injection of [$^{14}C_2$]ethanolamine*

Each value is a mean of two independent determinations. N.D., not detectable.

| Time after injection (min) | | Specific radioactivity (d.p.s./$\mu$mol) Phosphatidylcholine Plasma | Phosphatidylcholine Cells | Lysophosphatidylcholine Plasma | Lysophosphatidylcholine Cells | Sphingomyelin Plasma | Sphingomyelin Cells | Choline Plasma | Choline Cells |
|---|---|---|---|---|---|---|---|---|---|
| 60 | Artery | 63.0 | 9.2 | 174.0 | 255.0 | 121.0 | 79.0 | <1 | |
| | Jugular vein | 65.5 | 16.4 | 114.0 | 340.0 | 118.0 | 110.0 | <1 | |
| | Brain | 22.5 | | N.D. | | 56.0 | | <1 | |
| | Liver | 167.0 | | — | | — | | — | |
| 140 | Artery | 232.5 | 5.8 | 210.0 | 108.0 | 134.5 | 51.2 | <1 | |
| | Jugular vein | 232.0 | 5.4 | 192.0 | 528.0 | 192.5 | 87.5 | <1 | |
| | Brain | 33.0 | | N.D. | | 97.5 | | <1 | |
| | Liver | 262.0 | | — | | — | | — | |

only been described for liver (Erbland & Marinetti, 1965) and for lung (Abe *et al.*, 1972). In the experiments of Illingworth & Portman (1972) there was a significant labelling of the phosphatidylcholine in brain and in the water-soluble breakdown products of choline lipid metabolism within 10min of injection. Among these metabolites the highest percentage of radioactivity was isolated as unesterified choline, but the labelling was also detected in acetylcholine, phosphorylcholine, glycero-3-phosphorylcholine and betaine. In our earlier experiments (Ansell & Spanner, 1971) we detected labelled phosphorylcholine in the brain, but not unesterified choline.

Table 3 shows that, 140min after injection, the specific radioactivity of the plasma phosphatidylcholine and that of the liver were the same. This agrees with the findings of Bjørnstad & Bremer (1966), who showed that some hours after a single injection of L-[*Me*-$^{14}$C]methionine, the specific radioactivity of liver and plasma phosphatidylcholine was the same. The specific radioactivities of the phosphatidylcholine and lysophosphatidylcholine were also very similar. Stein & Stein (1966) postulated that lysophosphatidylcholine may be produced either in the liver by the deacylation of phosphatidylcholine and released into the plasma, or in the plasma by the action of lecithin acyltransferase (EC 2.3.1.43), a reaction since described in detail by Glomset (1968). Either reaction would lead to an equilibration of the specific radioactivities of the phosphatidylcholine and the lysophosphatidylcholine.

*Rabbit*

*Unesterified choline and glycerophospholipid choline in the arteries and veins.* A species difference in choline metabolism across the brain became apparent when the experiments described for the rat were carried out in the rabbit, where no apparent arterio-venous difference was demonstrated (Table 1). The carotid artery and the jugular vein had concentrations of unesterified choline in the plasma which were very similar (Table 1). As had been found with the rat, the blood-cell value was the same for both vessels (7.93nmol/ml) and no difference in choline glycerophospholipid could be detected, i.e. $0.78\pm0.08\,\mu$mol/ml for phosphatidylcholine and $0.14\pm0.05\,\mu$mol/ml for lysophosphatidylcholine (Table 1). Another difference between the rat and the rabbit was found when blood was incubated at room temperature for up to 90min. There was no rise in the concentration of unesterified choline in rabbit blood plasma; on the other hand, there was a rise in the concentration in human plasma (Fig. 1).

An infusion of choline caused a rise in the concentration of unesterified choline in plasma in both the carotid artery and in the jugular vein, followed by a subsequent fall when the infusion was stopped

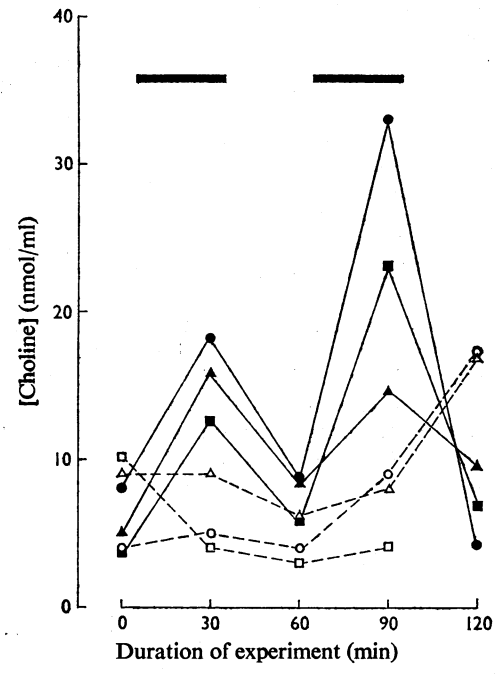


Fig. 2. *Effect of choline infusions on the concentration of unesterified choline in the plasma and cells of the rabbit carotid artery*

Rabbit 1 (●, ○), rabbit 2 (▲, △), rabbit 3 (■, □). Solid symbols and continuous lines, plasma; open symbols and broken lines, cells. The reproducibility of duplicate estimations was within 3%. Horizontal bars indicate periods of choline infusion.

(Fig. 2). This agrees with the results of experiments with cats described by Gardiner & Paton (1972). There was a considerable time-lag before any detectable rise in the unesterified choline concentration in the cells could be found. This would also agree with the work on choline uptake by human erythrocytes described by Martin (1968). In all experiments with the rabbit, there was a steady fall in the concentration of choline glycerophospholipid in plasma (Fig. 3), a phenomenon not observed with the rat. It may be that this reflected a state of shock or an effect of prolonged anaesthesia on the liver.

*Infusion of* [*Me*-$^{3}$*H*]*choline.* Radioactive choline was infused into rabbits during two 30min periods with an intervening 30min rest period. After the second choline infusion, samples were taken of arterial and venous blood to determine the rate of return to the resting concentration. Unesterified choline concentration rose during the infusion (Table 4) and, as had been found with infusions of non-radioactive choline (Fig. 2), the concentration remained high even 30min after the infusions had

ceased. The specific radioactivity of unesterified choline in the jugular vein rose very slowly and never reached that of the artery. This indicated an efflux of non-radioactive choline and an uptake of the [*Me*-$^3$H]choline by the brain of 390nmol/30min per brain, though no direct measurement on brain tissue was made (Table 4). Meanwhile the specific radioactivity of the phosphatidylcholine and the lysophosphatidylcholine rose steadily, apparently unaffected by intervals between choline infusions (Table 4). This phospholipid labelling in the plasma was almost certainly a reflection of the labelling of choline lipid in the liver. It is noteworthy that Bjørnstad & Bremer (1966) showed that, after a single injection of [1,2-$^{14}$C]choline, the labelling of the choline lipids in the extrahepatic tissues rose steadily for 8h after the injection. They also showed that the specific radioactivity of phosphatidylcholine in the plasma rose slowly until, at about 8h after the injection, it was the same as that of the liver.

From the results described, it is obvious that there is a species difference between the rat and the rabbit in the metabolism of choline across the brain. In both species there is an efflux of choline which, in the rat, appears to be much greater than the uptake, thus giving a high vein/artery ratio. When unesterified choline in the arterial blood was increased by intravenous infusion of [*Me*-$^3$H]choline, there was apparently an uptake of the radioactive unesterified choline into the brain by both species. This was also demonstrated by Choi *et al.* (1975) and Freeman *et al.* (1975), who used [$^2H_4$]choline. There is, in fact, remarkable agreement between their results and those described for the rat in the present paper. However, it has been reported (Gardiner & Paton, 1972) that most tissues can rapidly remove unesterified choline from the blood if the concentration in the arterial supply is raised five- to ten-fold.

This raises the doubt as to whether this choline uptake is the method whereby choline enters the brain when the arterial concentration of unesterified choline is not artificially raised, albeit temporarily. In the experiments of Schuberth *et al.* (1969) mice were given a single dose of 500nmol of choline, which, if the concentration of unesterified choline in mouse blood compares with that of rat and rabbit, would raise the concentration 50-fold. In the experiments of Diamond (1971) the concentration would have been raised fivefold. In our experiments with

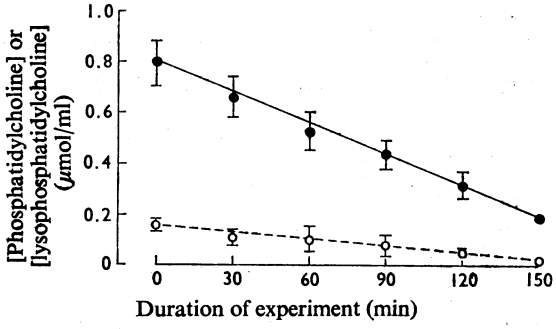


Fig. 3. *Fall in concentration of phosphatidylcholine* (●) *and lysophosphatidylcholine* (○) *in rabbit plasma during the course of all the experiments described in the text*

The S.D. for each value, as indicated by the bars, was determined for results from four to ten animals.

Table 4. *Radioactive labelling of unesterified choline and choline glycerophospholipids in the blood of the rabbit after intermittent intravenous infusions of* [*Me*-$^3$*H*]*choline into the femoral vein*

Samples were taken at the end of each 30min period. Each value is an average of two determinations. Unesterified choline concentrations before the infusion were 5.7nmol/ml in the carotid artery and 4.3nmol/ml in the jugular vein.

| | [*Me*-$^3$H]Choline infusion (30min) | Dextran infusion (5min), then 25min rest | [*Me*-$^3$H]Choline infusion (30min) | 30min rest |
|---|---|---|---|---|
| Unesterified choline | | | | |
| Carotid artery (nmol/ml) | 24.6 | 18.8 | 22.3 | 26.7 |
| Jugular vein (nmol/ml) | 24.3 | 21.3 | 21.5 | 29.3 |
| Artery (d.p.s./ml) | 2090 | 375 | 1990 | 350 |
| Vein (d.p.s./ml) | 1118 | 251 | 1320 | 377 |
| Artery (d.p.s./μmol) | 85000* | 20000 | 89200 | 13100 |
| Vein (d.p.s./μmol) | 46000 | 11820 | 62860 | 12900 |
| Phosphatidylcholine | | | | |
| Artery (d.p.s./μmol) | 43.2 | 81.2 | 158.0 | 255.0 |
| Vein (d.p.s./μmol) | 56.0 | 95.0 | 180.0 | 256.0 |
| Lysophosphatidylcholine | | | | |
| Artery (d.p.s./μmol) | 109.0 | 146.0 | 152.0 | 328.0 |
| Vein (d.p.s./μmol) | 108.0 | 171.0 | 181.0 | 227.0 |

* A second rabbit gave values of 98000 and 40250 for artery and vein respectively.

[*Me*-$^3$H]choline infusions, the choline concentration in the plasma of the rat was raised to about 10nmol/ml and in the rabbit to 24.5nmol/ml. However, when [$^{14}$C]ethanolamine was injected intraperitoneally and the choline glycerophospholipids and sphingomyelin in liver became radioactively labelled, the plasma lipids and the brain lipids also became labelled, but no label in the unesterified choline of plasma could be detected (cf. Ansell & Spanner, 1971). From these results it appears that the greater part of choline is carried to the brain in a lipid-bound form, possibly as lysophosphatidylcholine, as proposed by Illingworth & Portman (1972).

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