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THE CONTROL OF THE PLASMA CHOLINE CONCENTRATION IN THE CAT

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SUMMARY

1. Changes in choline concentration of the blood after injections or infusions of choline were studied in cats anaesthetized with chloralose.

2. Single I.V. injections of choline 10–100 μmole/kg produced arterial plasma levels 1 min later corresponding to an apparent volume of initial distribution of 430 ml./kg. The concentration then declined rapidly (half-time, 1–2 min), with a later slower decline after large doses.

3. Infusions of choline at a rate of 0·8 μmole/kg.min or greater produced steady rises in plasma level, corresponding to a clearance of 28·6 ml. plasma/kg.min. The half time of rate of approach to steady state was 7 min or less. Infusions at rates of 0·40 μmole/kg.min or less produced much smaller or negligible rises, suggesting mechanisms for disposal which were saturated at higher concentration. At low rates, little infused choline appeared in urine. At the end of an infusion, the plasma choline level usually fell without delay.

4. Portal blood contained about 50 % of the arterial level, renal venous blood 15–70 %, caval blood 30–60 %, and amniotic fluid 2·5 %. Occlusion of renal coeliac or mesenteric arteries raised plasma choline, but relatively rapid choline removal still occurred in the eviscerate animal.

5. After infusions of [methyl-^{14}C]choline, the level of radioactivity retained in the circulation amounted to only a few per cent of the total dose infused. At low rates of infusion (0·0125–0·1 μmole/kg.min) the radioactivity represented only a small fraction of bio-assayable choline; but at 0·40 μmole/kg.min it came to exceed the concentration of free choline, indicating metabolic conversion. Only traces of ^{14}C were found in expired air, and only 1–1·5 % of total infused radioactivity in the urine. After infusion of 150 μmole over 3 hr, high levels of radioactivity were found in liver, kidney, lung, brain and heart, but levels in muscle and spleen were comparable to that of blood.

6. It was concluded that choline is rapidly lost from the blood, that the abdominal viscera, liver, kidney and lung are important extraction sites, that some partial metabolism occurs, the metabolites also being rapidly lost from blood, and that it is probable that choline lost to the tissues becomes bound in some form.

INTRODUCTION

The rapid disappearance of choline injected into the circulation has long been known (Hunt, 1915; Kahane & Lévy, 1950; Bligh, 1953) although the mechanism of the process is still not clear. As part of a study into the mode of action of hemicholinium compounds (Schueler, 1955) which are thought to act by inhibiting the entry of choline into the tissues (MacIntosh, 1961; Gardiner, 1961) it was desirable to know more about the changes in plasma choline concentration following its administration by injection or infusion. The experiments reported here were conducted in cats.

METHODS

Adult cats of either sex were used. Anaesthesia was induced with ethyl chloride/ether and maintained with chloralose (I.V. 80 mg/kg). The following were routinely cannulated: the trachea, the right external jugular vein for injection or infusion of drugs, the right common carotid artery to record the blood pressure and the right femoral artery for blood sampling. Other vessels to be occluded or sampled from during the experiment were dissected clear and identified, either before any blood samples were taken, or during the control period before any choline was infused. The various abdominal vessels were prepared for occlusion by placing a loose ligature around them and leading both ends out through a short length of polyethylene tubing. After the ligature had been put in place the abdomen was closed and the cut edges held together with clips. The arterial blood pressure of the animals was recorded by means of a mercury manometer.

Samples of arterial blood 2, 1 or, usually, 0·5 ml., were collected in an all glass syringe fitted with a stop to ensure constancy of the volume taken. Before each sample the contents of the cannula and about 0·1 ml. of fresh blood was withdrawn and retained. The sample was taken and the cannula refilled with heparinized saline (10 i.u./ml. 0·9 %, w/v, NaCl). The original cannula contents, the 'flush-out' blood and saline equal in volume to the sample taken were returned to the animal intravenously. Blood samples were taken from various abdominal vessels towards the end of some experiments by means of a collection syringe fitted with a sharp short fine needle.

Urine samples were obtained by cannulating the bladder with a length of wide-bore polyethylene tubing; the cannula was arranged so that as the bladder contracted on to it there was only a small dead-space left where the ureters entered. The urine was collected in graduated glass test tubes.

When the respiratory excretion of $^{14}CO_2$ was measured the tracheal cannula was fitted with a double flap valve arrangement so as to separate the expired from the inspired air. The expired air was passed either directly, or after collection in a large polyethylene bag, through gas wash bottles containing NaOH solution (20 %, w/v) to trap the CO_2.

The blood samples on collection were put into centrifuge tubes containing 0·05 ml. heparinized saline (100 i.u./ml.). After mixing and removal of any required for the determination of radioactivity, the blood was centrifuged to separate the plasma. The choline content of the plasma was estimated by bio-assay after its conversion to acetylcholine. The method of extraction and acetylation was that of Bligh (1952) as modified by Gardiner & Domer (1968). The test tissue was a piece of guinea-pig ileum suspended in Krebs solution at 29–30° C, its contractions were recorded via a pendulum auxotonic lever (Paton, 1957) on a kymograph. The assay cycle, bath changes and the addition of constant fixed doses of acetylcholine were controlled by a device of the type described by Boura, Mongar & Schild (1954). Varying amounts of the unknown solution were added by hand alternately with the machine addition of the standard until the amounts of the unknown which most closely bracketed the standard had been determined; the equivalence point was estimated by linear interpolation between the two amounts.

The estimation of the choline content of urine samples was performed by treating them exactly as if they were plasma samples. The concentration of choline found, even in the control samples, was sufficient to ensure that any other substances present and active on the guinea-pig ileum did not interfere. Tests showed that the urine extracts before acetylation, when used in the amounts effective after acetylation, had no action upon the ileum and that the activity generated by acetylation was antagonized completely by presence of 0·3 μM hyoscine in the bath.

Estimation of the ^{14}C radioactivity present in samples was carried out by liquid scintillation counting techniques. Preparation of the samples was as follows: plasma, 0·1 or 0·2 ml. was warmed briefly with 0·1 or 0·2 ml. N-NaOH in a counting vial and then 10 or 15 ml. of scintillator solution in xylene-dioxan ethanol added (Lambie, 1964). Urine was treated similarly. Blood, 0·05 or 0·1 ml. together with an equal volume of water was digested overnight with 2 ml. KOH solution (0·5 M in ethanediol) at 60° C, the mixture decolorized as much as practicable with a few drops of 30 %, w/v, H_2O_2, cooled, diluted with 2 ml. methanol and finally mixed with 12 ml. scintillator solution. Tissues: sample pieces were squashed or chopped into a fine state of subdivision and a weighed amount (under 200 mg) put in a scintillation vial and treated as if it were blood. The period required for the tissue samples to dissolve was longer than for blood. To correct for possible destruction of choline and loss of ^{14}C during the digestion four portions of each tissue were treated, to two of them a known amount of labelled choline was added before digestion to act as an internal control. Respiratory CO_2: the final volumes of the NaOH solutions from the wash bottles were recorded and then 0·5 ml. samples were taken and added to 15 ml. portions of the scintillator solution. All samples were counted thrice for the time to record 10^5 counts or for 10 min and 0·1 ml. [carboxyl-^{14}C]benzoic acid solution in xylene (0·05 μc/ml.) then added and the sample recounted to determine the quenching correction to be applied.

All chemicals used, wherever practicable, were of A.R. quality. The acetylcholine perchlorate, or in the later part of the work, acetylcholine iodide, used as assay standards were obtained from B.D.H. Ltd. Poole, England. Their ester content was checked from time to time by chemical analysis. [Methyl-^{14}C]choline and [carboxyl-^{14}C]-benzoic acid were obtained from the Radiochemical Centre, Amersham, England. The composition of the Krebs solution used was (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, and glucose 5·6; before and during use the solution was bubbled with 95 % O_2 and 5 % CO_2.

RESULTS

Blood levels after single injections of choline

The first experiments consisted of measuring the concentration of choline in the blood before and after I.V. injections of choline 10 or 100 μmole/kg. Fig. 1 shows four such tests in two animals. In each test, the first sample after giving the choline injection was taken exactly 1 min later. In every experiment of this kind this sample contained the highest choline concentration. Thereafter the blood level declined rapidly. The decline was not exponential; the half-time of fall was initially of the order of 1–2 min, but lengthened to 10 min or more, especially with large doses.

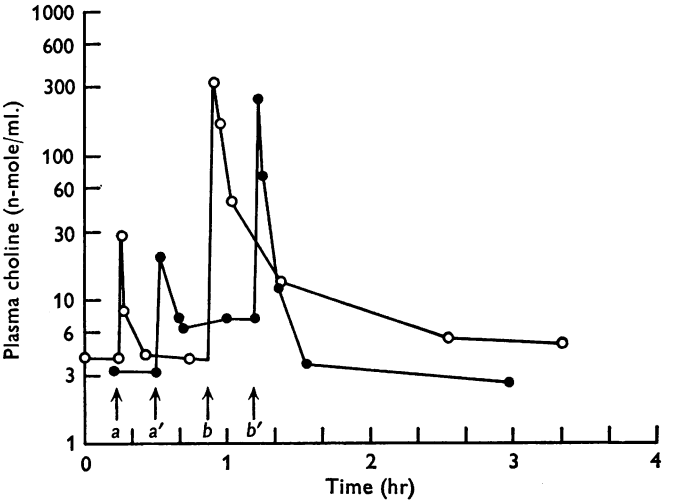

Fig. 1. Plasma choline concentration following injection of choline. The plasma choline concentrations in two cats are shown ○ and ●. Choline was injected I.V. at arrows *a* and *a'* (10 μmole/kg) and at *b* and *b'* (100 μmole/kg). Semi-logarithmic scales.

Only with the higher doses of choline were the blood pressure and respiration markedly affected. Following the lower doses there were brief falls (30–40 mm Hg) in the blood pressure lasting no more than 30 sec. There was no apparent effect on respiration. The high doses caused similar falls of blood pressure but these were superseded by a sharp pressor response which reached its maximum 1–1½ min after the injection. Associated with these changes there was an initial pause in the respiration and then a period of rapid shallow breathing until the blood pressure returned to normal. The circulatory and respiratory effects ceased, within 10 min, while the plasma choline level was still considerably raised.

A volume of apparent initial distribution can be estimated from the peak concentration; it had a mean value of 430 ml./kg body wt. This is surprisingly high. From the study of the circulation time in the cat by Gray & Paton (1949) one could expect that in 1 min, i.e. about three circulation times, the injected material would be mixed almost completely with circulating blood: but this accounts for not more than 70 ml./kg. Dilution of other cations in extracellular or intracellular water is known to be a much slower process. The values for the peak concentration indicate, therefore, that within a minute of injection, about 85 % of the choline is being disposed of in some way other than by dilution in body fluids.

Blood levels after infusion of choline

The disposal of choline after a single injection was too rapid to attempt to study the effect of various procedures on the choline blood level; I.V. infusions of choline were therefore set up, so as to produce an approximately steady state in which supply and disposal of choline are balanced.

To produce a significant rise in plasma choline, infusions faster than 0·10 μmole/kg.min were required, and with 0·80 μmole/kg.min, increases in plasma choline levels of between 10 and 27 nmole/ml. were obtained. The rate of approach to the steady state was not analysed in detail; but it is clear that this was relatively rapid. Fig. 2 shows the responses to infusions at 0·8, 1·0, 1·2 and 5·0 μmole/kg.min. Where the first sample was taken within 15 min of beginning the infusion, it was at least 80 % of that taken 15 min later, and sometimes was already at peak level. If the approach to equilibrium is regarded as approximately exponential, this implies that the half-time of disposal of choline is of the order of 7 min or less. Except at the highest rates employed (3·2 and 5·0 μmole/kg.min) the infusion of choline did not greatly affect the blood pressure or the respiration even when continued for up to 4 hr. The usual response was a slow fall in blood pressure as the infusion continued although in some animals pressure was well maintained even after 0·80 μmole/kg.min had been infused for 3 hr and various surgical and other manipulations carried out. With the lower rates of infusion no distinct effects were seen on the respiration and in almost all experiments the animals breathed spontaneously throughout. When choline was infused at 3·2 or 5·0 μmol/kg.min the respiratory and circulatory state of the animals remained good for about 30–45 min, thereafter the blood pressure fell progressively and the breathing faltered so that artificial respiration had to be started.

Fig. 3 shows the relationship between the rate of infusion and the plasma level achieved (taken 30–40 min after the start of the infusion). This has some interest for the question as to how far the changes in choline level can be described by a simple exponential equation. For an infusion rate s,

and a volume of distribution V, the rate of change of concentration of choline in the blood should be given by the following expression, on the assumption that the rate of loss is proportional to the blood concentration, x:

$$\frac{dx}{dt} = \frac{s}{V} - kx$$

for $x = 0$ at zero time $\quad x = \frac{1}{k} \cdot \frac{s}{V} \cdot (1 - e^{-kt})$.

For equilibrium conditions $\quad \frac{s}{x} = kV.$

Thus the concentration achieved should vary linearly with infusion rate, and the reciprocal of the slope in Fig. 3*a* should give the product of volume of distribution and rate constant of disposal. The results for the higher con-

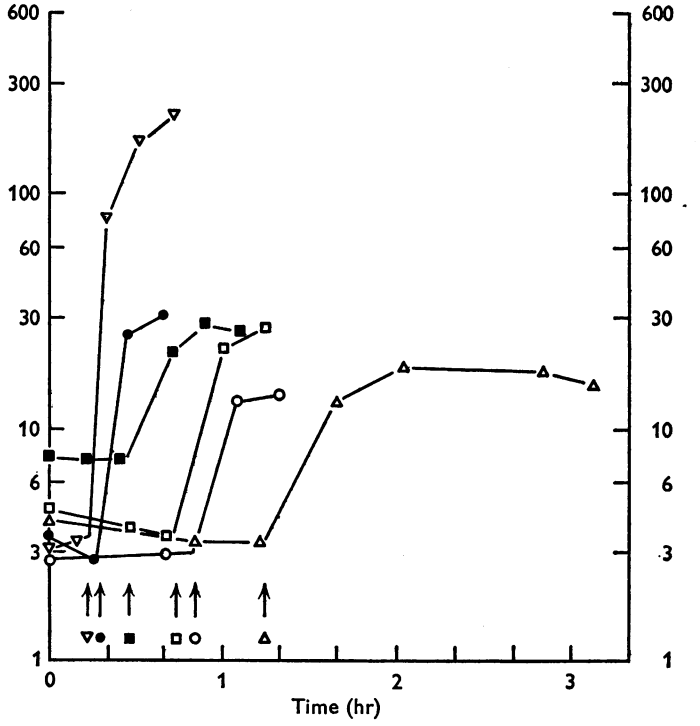

Fig. 2. Plasma choline concentration during a continuous infusion of choline. The plasma choline concentrations of six cats given intravenous infusions of choline at the following rates are shown thus: 5·0 μmole/kg.min ▽, 1·2 μmole/kg.min ●, 1·0 μmole/kg.min ■, 0·8 μmole/kg.min □, ○ and △. The infusions commenced at the times indicated by the arrows.

centrations of choline are compatible with this interpretation. The reciprocal slope also represents the volume, per kg body weight, which is cleared of choline in 1 min; it has a value of 28·6 ml. For a half-time of disposal of 5 min, this would give a volume of distribution of about 210 ml.

For low rates of infusion, however, the choline levels obtained are much lower than would be expected on this basis. Fig. 3*b* illustrates this point. In one experiment quadrupling the rate of infusion from 0·10 to 0·40 μmole/kg.min produced only a small rise in blood choline, but the further

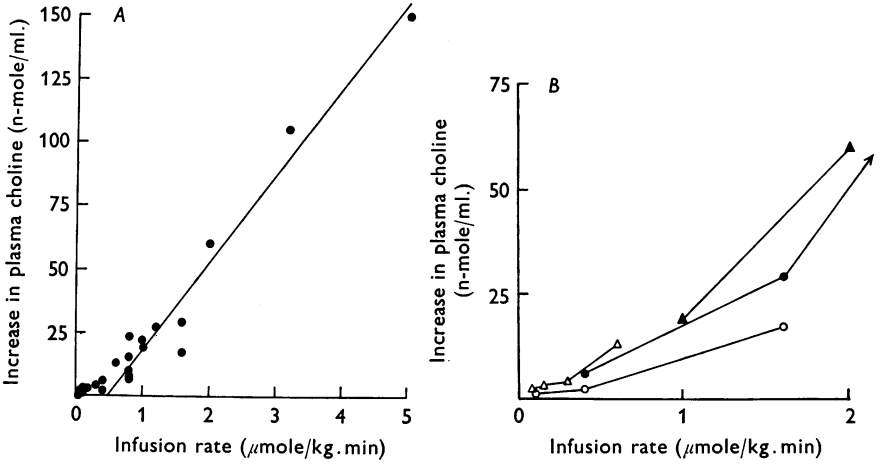

Fig. 3. Increases in plasma choline level of cats given continuous intravenous infusion of choline.

A, each point is the increase in plasma choline concentration observed after 30–40 min infusion at the rate indicated on the ordinate. Also shown is the regression line calculated from the results for infusions of 0·8 μmole/kg.min or greater. Combined results from thirteen animals.

B, data from four animals (included in Fig. 3*A*) plotted to show response to various infusion rates in the same animal. The arrow indicates the direction of the point for the highest infusion rate in one animal, 105 n-mole/ml. at 3·2 μmole/kg.min.

quadrupling from 0·40 to 1·60 μmole/kg.min had a very substantial effect. This suggests that at low concentrations, mechanisms for choline disposal exists which are not effective at higher concentrations, perhaps because they are saturated. At low rates of infusion little of the infused choline appeared in the urine (see Fig. 7).

In most experiments in which blood samples were taken after the infusion had stopped the plasma choline concentration fell quite sharply (see, for example, Fig. 5*A* and *C*). Sometimes after low rates of infusion the level attained was below that of the initial control period.

Arteriovenous differences

If choline is being extracted from the blood by particular organs, the venous blood from such organs should be relatively poor in choline. Table 1 shows the results of assays on venous blood samples in animals with sustained arterial choline levels. Portal blood contains about 50% of the arterial level; renal venous blood 15–70%; caval blood about 30–60%. In one experiment on a pregnant animal, amniotic fluid contained only 2·5%

TABLE 1. Choline content of venous plasma samples during infusions of choline

Choline concentration in n-mole/ml.; figures in parentheses are values expressed as % of that in arterial blood during sampling period.

A. Cat ♀ 2·8 kg, infusion rate 0·8 μmole/kg.min, samples taken between 110–116th min of infusion

Arterial	14
Abdominal vena cava	8 (57)
Right renal vein	10 (70)
Portal vein	7 (50)

B. Cat ♂ 3·2 kg, infusion rate 1·2 μmole/kg.min, samples taken between 80 and 87th min of infusion

Arterial	52
Portal vein	23 (50)
Right renal vein	10 (21)

C. Cat ♂ 4·1 kg, of infusion rate 1·0 μmole/kg.min, samples taken between 90–95th min of infusion

Arterial	47
Abdominal vena cava	33 (60)
Right jugular vein	23 (44)
Portal vein	35 (67)

D. Cat ♀ 3·2 kg, infusion rate 5·0 μmole/kg.min, samples taken between 45 and 50th min of infusion

Arterial	510
Abdominal vena cava	149 (30)
Left renal vein	75 (15)
Portal vein	137 (27)
Amniotic fluid	13 (2·5)

of the estimated arterial content. The fact that caval blood, sampled below the renal vein, had a substantially lower content than arterial blood shows that choline disposal is not restricted to organs such as liver and kidney.

The effects of occlusion of vascular beds

If a particular organ is concerned in choline disposal, its removal from the circulation, during an infusion of choline, should cause a rise in choline blood level. Such an effect is complicated, however, by two factors, one,

illustrated in the analysis above, is that the blood level depends on volume of distribution as well as on disposal rate; with a large organ, the reduction in total body mass alone will have a substantial effect. The other, relevant also when venous samples are taken, is that the surgery and handling required to locate the vessels might reduce the functioning of the organ before the vessels were intentionally occluded.

Removal of the abdominal viscera from the circulation during infusions of choline in the range 0·8–1·2 μmole/kg.min showed that much of the

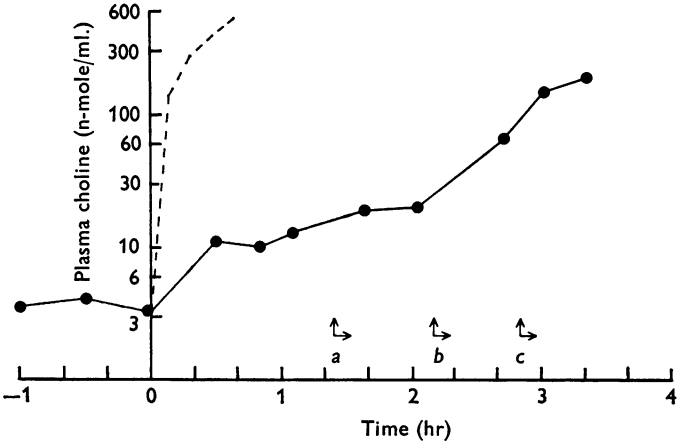

Fig. 4. Effect of evisceration during a continuous I.V. infusion of choline. The infusion of choline 0·8 μmole/kg.min started at zero time. The assayed plasma choline concentration shown by ● and continuous line, the calculated plasma choline concentration, assuming none lost from the circulation, shown by the interrupted line.

At *a* the renal and suprarenal arteries were tied off, at *b* the coeliac axis, inferior and superior mesenteric arteries tied off and at *c* removal of the abdominal organs completed.

choline was normally removed by them (Figs. 4, 5). However an experiment in which complete evisceration was progressively carried out (Fig. 4) indicated that other parts of the body could also effectively dispose of choline at high plasma levels.

None of the individual abdominal regions tested was of overriding importance. Occlusion of the renal arteries usually doubled the plasma choline level; occlusion of the coeliac axis or of the superior mesenteric artery also had a distinct effect of raising the level by about 50 % while hepatic artery occlusion produced little change.

Infusion of [methyl-^{14}C]choline

In the above experiments the choline disposed of may have been metabolized and the metabolites retained within the circulation or it (and they)

may have passed into the tissues. To test these possibilities four experiments were carried out in which [methyl ^{14}C]choline was infused. The results are shown in Figs. 6, 7 and Table 2.

In one experiment very low infusion rates were used so as to cause minimal disturbance to the plasma choline concentration. After 33 min of an infusion of 12·5 n-mole/kg.min the plasma radioactivity was equivalent to 2% of bio-assayed level of choline, after a further 45 min at

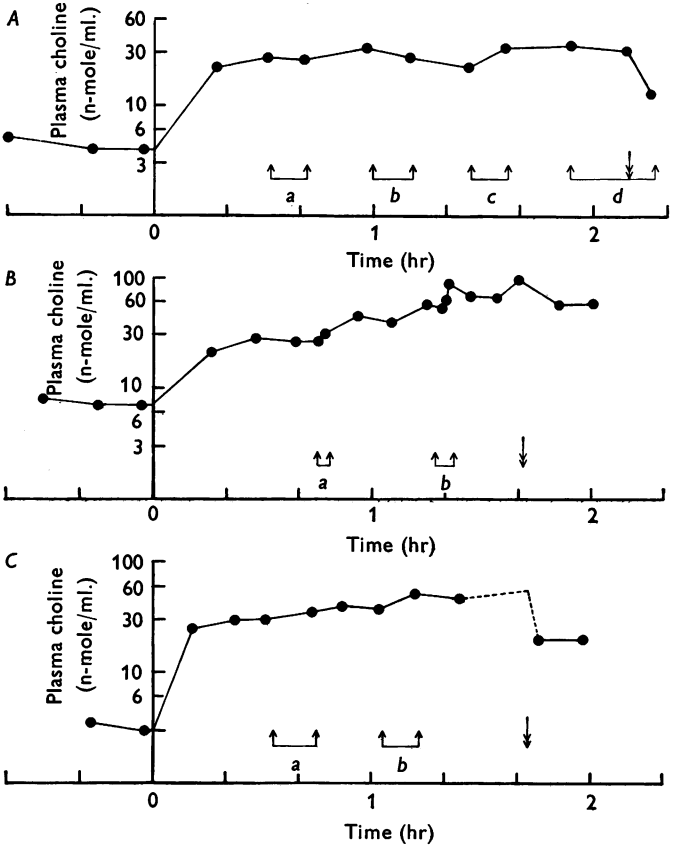

Fig. 5. Effect of temporary occlusion of various abdominal vessels during a continuous intravenous infusion of choline. In each experiment the infusion commenced at zero time and continued until the time shown by the downward double arrow. The plasma choline concentration shown by ●.

A, infusion rate 0·8 μmole/kg.min. The vessels occluded were during periods *a* and *d* the hepatic artery, *b* the superior mesenteric artery and *c* the coeliac axis.

B, infusion rate 1·0 μmole/kg.min. The vessels occluded were during period *a* the hepatic artery and *b* the renal arteries.

C, infusion rate 1·2 μmole/kg.min. The vessels occluded were during period *a* the hepatic artery and *b* the coeliac axis.

0·10 μmole/kg.min the level of ^{14}C had risen to be equivalent to 30 % of the choline present. This level of radioactivity, however, represented a retention in the circulation of only about 4 % of the total amount infused.

The rate of infusion in the other three experiments was 0·40 μmole/kg.min. In these animals as the infusion proceeded the concentration of radioactive material expressed as 'choline' exceeded the concentration of free choline showing that some metabolic conversion was occurring. However, the amount of radioactive material retained within the circulation at the end of the infusion was again only a few per cent of the total dose infused. In three experiments the amounts of ^{14}C in whole blood as well as in the plasma was measured. The whole blood choline concentration was the same as or lower than that in the plasma. This result has also been observed in other experiments (Gardiner & Domer, 1968) and indicates that the blood cells are not a major factor in the loss of choline from the plasma.

In two experiments in which the respiratory and urinary loss of ^{14}C was followed trace amounts appeared in the exhaled gases and only 1–1·5 % in the urine. At least 30 % of the activity in the urine must have been due to metabolites of choline at the end of one experiment since the radioactive 'choline' exceeded that found by bio-assay.

At the end of another experiment, in which 150 μmole of labelled choline had been infused over 3 hr, samples of various tissues were taken and analysed for their ^{14}C contents. The results are shown in Table 2. They are reported as μmole choline/g wet weights of tissue although the proportions present as choline and as metabolites were not determined. In addition to the expected high levels in the liver and kidney the lung, brain and heart also contained amounts well above the terminal whole blood level.

DISCUSSION

The cat has a large and varied capability to dispose of choline introduced into its circulation. Intravenous infusions at rates less than a threshold value of about 0·4 μmole/kg.min are effectively removed and little change in the general plasma concentration results. At greater rates the capacity of this removal process is exceeded and the resulting rise in plasma concentration becomes proportional to the infusion rate. With a high plasma concentration such as those which follow immediately after intravenous injection of 10–100 μmole choline/kg the rate of loss is very rapid and approximately 85 % of the dose disappears within 1 min. A similar estimate of the loss under these conditions was noted by Hunt (1915) for his experiment.

The experiments on factors affecting the plasma choline level during

infusions in the range 0·4–1·2 μmole/kg.min indicated that the abdominal viscera are important in restricting the rise in plasma concentration and hence are probably the sites of the complete removal of choline at lower rates of infusion.

Although the results show that much choline was being removed by the

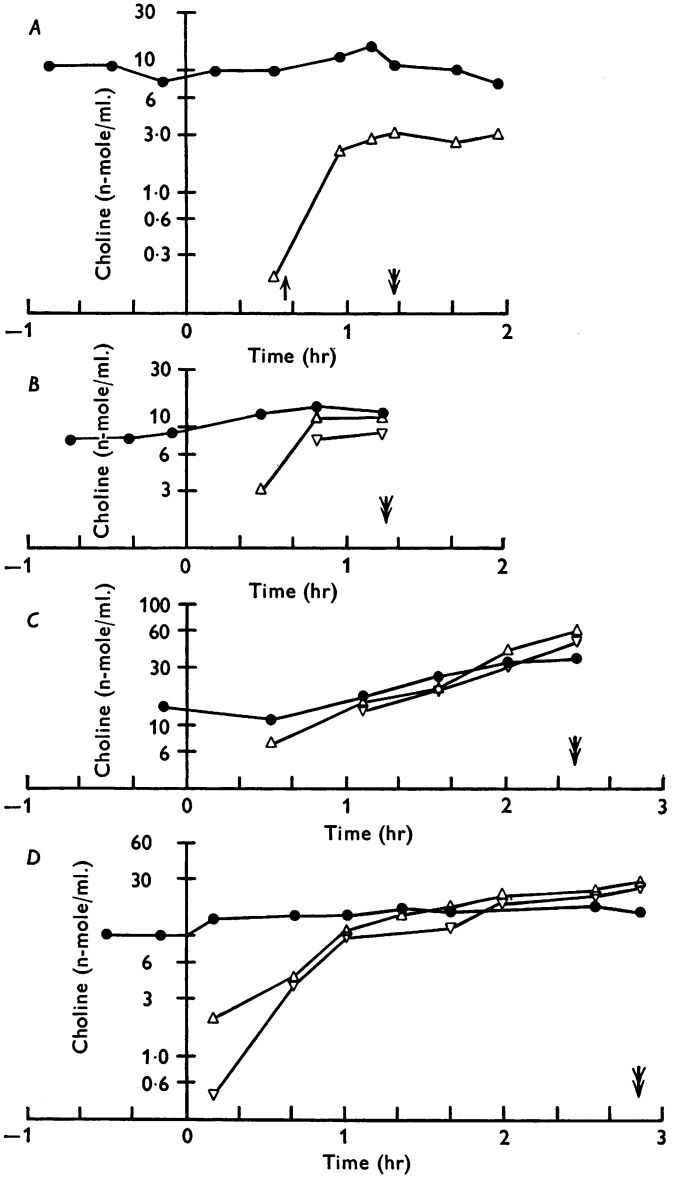

Fig. 6. For legend see opposite page.

kidneys at these threshold rates of infusion it was not being excreted in the urine other than in trace amounts either unchanged or as a metabolite. Urinary excretion of choline following infusion at higher infusion rates was not investigated but it is likely (cf. Fig. 7*A*) that it would then become significant since such is known to be the case with other species (Vander, 1962).

As has been noted by Bligh (1953) in the dog the abdominal organs are not the only parts of the body able to reduce a raised plasma choline level. Complete evisceration of a cat did not remove its ability to maintain a reasonably constant although raised concentration of choline while an infusion of 1·2 μmole/kg.min continued. The site(s) where the choline was removed from the circulation was not investigated but other experiments in the cat and in the rabbit (Gardiner, 1969; D. Chan, J. E. Gardiner, M. C. E. Gwee & H. S. Lim, unpublished) have indicated that most tissues can rapidly remove choline from the blood if the level in the arterial supply is raised five- to tenfold. This however, does not appear to happen with the erythrocytes; experiments in which labelled choline was used indicated that little of the infused choline was being retained in the blood either in the formed elements or as pharmacologically inert metabolites. Such a result is also to be expected from the work of Martin who has extensively studied the entry of choline into human erythrocytes (Martin, 1968). The rates of entry of choline that he observed, if applicable to cat erythrocytes, could account for the loss of only a small amount of the choline injected or infused in these present experiments.

Only after labelled choline had been infused for some time did the level of radioactivity in the plasma (or blood) rise above that of free choline

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Legend to Fig. 6.

Fig, 6. Plasma choline and radioactivity concentrations following infusion of [methyl-^{14}C]choline.

In each experiment the I.V. infusion started at zero time and finished at time shown by downward double arrow, the assayed plasma choline concentrations are shown by ●, the plasma radioactivity (expressed as choline) by △ and the whole blood radioactivity (expressed as choline) by ▽.

A, cat 2·8 kg ♀ [^{14}C]choline (5·7 μc/μmole) was infused at a rate of 12·5 n-mole/kg.min until the time shown by the single arrow when the rate was increased to 0·1 μmole/kg.min.

B, cat 2·4 kg ♂ [^{14}C]choline 0·11 μc/μmole infusion rate 0·4 μmole/kg.min.

C, cat 2·3 kg ♂ [^{14}C]choline 0·11 μc/μmole infusion rate 0·4 μmole/kg.min.

D, cat 2·1 kg ♀ [^{14}C]choline 0·25 μc/μmole infusion rate 0·4 μmole/kg.min.

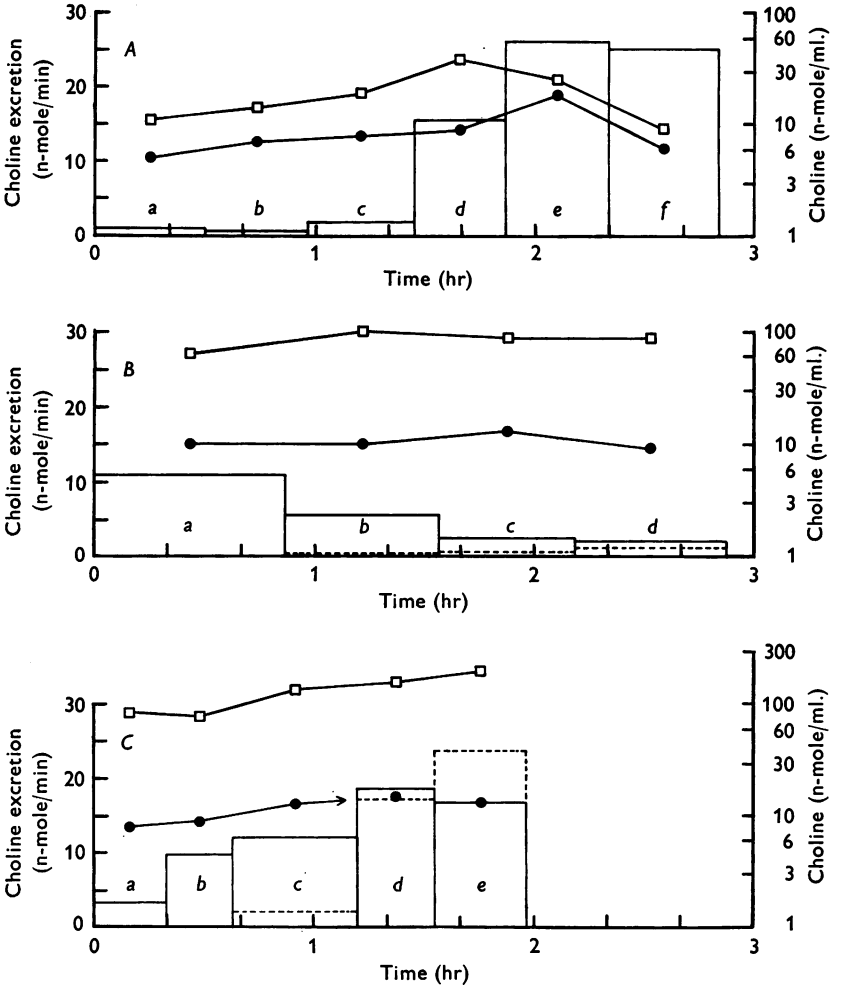

Fig. 7. Urinary excretion of choline infused I.V. In each experiment the amount of choline in the urine for the various collection periods is represented by the area within columns outlined by a continuous line (note linear scale on left) the urine choline concentration by □ and in the mid period plasma concentration by ● (log scale on the right). In the two experiments in which [methyl-^{14}C]choline was infused the radioactivity (expressed as choline) in the urine is represented by the areas beneath the interrupted lines.

A, cat 2·6 kg ♀ choline infused as follows. Periods *a* and *f* no infusion. During period *d* infusion rate 75 n-mole/kg.min, *c* infusion rate 0·15 μmole/kg.min, *d* 0·3 μmole/kg.min, and *e* 0·6 μmole/kg.min.

B, cat 2·8 kg ♀ [methyl-^{14}C]choline (5·7 μc/μmole) infused as follows. Periods *a*, *d* no infusion, during period *b* infusion rate 12·5 n-mole/kg.min and *c* 0·1 μmole/kg.min. Total radioactivity excreted via respiration during periods *b*, *c* and *d* equivalent to 10·1 n-mole choline.

C, cat 2·4 kg ♂ [methyl-^{14}C]choline (0·11 μc/μmole) infused as follows. Periods *a*, *b* no infusion, during periods *c*, *d* and *e* infusion rate 0·4 μmole/kg.min. Total radioactivity excreted via respiration during periods *b*, *d* and *e* equivalent to 0·3 n-mole choline.

itself. This shows that if the loss of choline observed is the result of very rapid metabolism then the metabolites were equally quickly lost from the circulation. Apart from these observations the removal of choline by rapid metabolism within the blood would seem unlikely since free choline is a normal blood constituent; this is unlike the situation for acetylcholine where hydrolytic esterases ensure a negligibly small level of the ester in the circulation. Such metabolism of choline as does occur during an infusion does not appear to involve the complete destruction of the molecule since little of the ^{14}C label appeared in the respiration or the urine.

TABLE 2. Tissue radioactivity after infusion of [methyl-^{14}C]choline. Cat; 2·1 kg female, infusion rate 0·40 μmole/kg.min, sp. activity of choline 0·25 $\mu c/\mu$mole. Total infused 151·2 μmole.

The radioactivity of tissues is expressed as μmole choline/g wet wt. of tissue; each value is the mean derived from two samples prepared with and two samples without 0·1 μmole [^{14}C]choline as internal control

Tissue	Liver	Kidney	Brain (cortex)	Lung	Heart	Muscle	Spleen	Blood
'Choline'	0·90	0·82	0·34	0·26	0·12	0·030	0·023	0·028

The distribution of the radioactive label in the tissues of an animal after choline had been infused for 3 hr confirm that the kidneys and the liver are the major sites where the choline is sequestered. The finding of a high concentration in the kidneys complements the earlier observation that little of the choline removed by them entered the urine. The amounts of ^{14}C found in the brain, lungs and heart indicate some of the extra-abdominal sites able to abstract excess choline from the circulation. The brain and lungs are of especial interest since they may be sites in addition to the neuromuscular junction where the unusual respiratory depressant effects of the hemicholiniums are brought about (Schueler, 1960; Gardiner & Lee, 1969).

The contribution of free [^{14}C]choline and of its metabolites to the radioactivity found in the tissues was not determined so that it is not possible to state how the choline was retained within the tissue. In those experiments in which blood samples were taken after an infusion was ended the plasma choline level usually fell quickly suggesting that the uptake process was not readily reversible. Related experiments being conducted in the rabbit (J. E. Gardiner & M. C. E. Gwee, unpublished) indicate that immediately after an injection much of the additional choline in the tissues is present in the free form although after an infusion this is not the case. It would seem likely that shortly after entering into the tissue the choline becomes bound. For both the brain and the lung conversion of circulating choline into phospholipid has been demonstrated (Spitzer, Morrison & Norman, 1968;

Diamond, 1971). To what extent this can occur in other tissues remains to be determined.

These experiments were begun in the Department of Pharmacology, Royal College of Surgeons, London.

REFERENCES

BLIGH, J. (1952). The level of free choline in plasma. *J. Physiol.* **117**, 234–240.

BLIGH, J. (1953). The role of the liver and the kidneys in the maintenance of the level of free choline in plasma. *J. Physiol.* **120**, 53–62.

BOURA, A., MONGAR, J. L. & SCHILD, H. O. (1954). Improved automatic apparatus for pharmacological assays on isolated preparations. *Br. J. Pharmac.* **9**, 24–30.

DIAMOND, I. (1971). Choline metabolism in brain. The role of choline transport and the effects of phenobarbital. *Archs Neurol. Psychiat., Chicago* **24**, 333–339.

GARDINER, J. E. (1961). The inhibition of acetylcholine synthesis in brain by a hemicholinium. *Biochem. J.* **81**, 297–303.

GARDINER, J. E. (1969). The effect of hemicholinium HC-3 on the uptake of choline by the head. In *Abstracts 4th Int. Pharmacol. Congress, Basle*, p. 69.

GARDINER, J. E. & DOMER, F. R. (1968). Movement of choline between the blood and cerebrospinal fluid in the cat. *Archs int. Pharmacodyn. Thér.* **175**, 482–496.

GARDINER, J. E. & LEE, H. S. (1969). A *p*-terphenyl hemicholinium compound. *Br. J. Pharmac.* **36**, 171*P*.

GRAY, J. A. B. & PATON, W. D. M. (1949). The circulation time in the cat studied by a conductivity method. *J. Physiol.* **110**, 173–193.

HUNT, R. (1915). A physiological test for choline and some of its applications. *J. Pharmac. exp. Thér.* **7**, 301–337.

KAHANE, E. & LÉVY, J. (1950). Sort de la choline administration au rat et a la souris. *Archs Sci. physiol.* **4**, 173–183.

LAMBIE, D. A. (1964). *Techniques for the Use of Radioisotopes in Analysis: a Laboratory Manual.* London: E. and F. N. Spon.

MACINTOSH, F. C. (1961). Effect of HC-3 on acetylcholine turnover. *Fedn Proc.* **20**, 562–568.

MARTIN, K. (1968). Concentrative accumulation of choline by human erythrocytes. *J. gen. Physiol.* **51**, 497–516.

PATON, W. D. M. (1957). A pendulum auxotonic lever. *J. Physiol.* **137**, 35–36*P*.

SCHUELER, F. W. (1955). A new group of respiratory paralysants I. The 'Hemicholiniums'. *J. Pharmac. exp. Ther.* **115**, 127–143.

SCHUELER, F. W. (1960). The mechanism of action of the hemicholiniums. *Int. Rev. Neurobiol.* **2**, 77–97.

SPITZER, H. L., MORRISON, K. & NORMAN, J. R. (1968). The incorporation of L-[Me-^{14}C]methionine and [Me-^{3}H]choline into lung phosphatides. *Biochim. biophys. Acta* **152**, 552–558.

VANDER, A. J. (1962). Renal excretion of choline in the dog. *Am. J. Physiol.* **202**, 319–324.