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The Low Availability of Dietary Choline for the Nutrition of the Sheep

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1. Choline, which is present in the diet of the sheep either in the non-esterified form or combined in phospholipids, is rapidly degraded in the rumen. The ultimate product formed from the *N*-methyl groups is methane. 2. Analysis of the non-esterified choline and the phosphatidylcholine in ruminal and abomasal digesta indicate that the phospholipid is the main vehicle for the passage of choline to the lower digestive tract. 3. The concentration of phosphatidylcholine in abomasal digesta is lower than that of ruminal digesta, which is in line with a selective retention of protozoa in the rumen as observed by others. 4. On defaunation of the rumen to remove ciliated protozoa the concentration of phosphatidylcholine in ruminal digesta falls markedly and becomes lower than that in abomasal digesta. 5. Calculation shows that the adult sheep obtains at most only about 20–25mg of effective choline per day from its diet (0.002–0.0025% of dietary total dry-weight intake). This is some fifty times less than the minimum required to avoid pathological lesions and death in other species investigated (0.1%+ of dietary dry-weight intake). 6. Sheep liver can synthesize choline from [^{14}C]ethanolamine both *in vitro* and *in vivo*, but the synthesis of choline per kg body weight is many times less than it is in the rat. 7. The intact sheep oxidizes an injected dose of [1,2-^{14}C]choline to CO_2 at a rate that is several times less than that observed for the rat. This could help to explain the apparent minimal requirement of sheep for dietary choline.

Choline present in the diet of a sheep largely as the phosphatidylcholine of higher plant membranes is rapidly liberated and degraded in the rumen by microbes. The *N*-methyl groups form trimethylamine and ultimately methane (Neill *et al.*, 1978). The only choline escaping such degradation appears to be a small percentage that is incorporated into the membranes of ciliated protozoa as phosphatidylcholine (Broad & Dawson, 1976). There is evidence that protozoa are selectively retained on the passage of digesta out of the rumen (Weller & Pilgrim, 1974; Bauchop & Clarke, 1976; Harrison, 1979). Moreover, sheep whose rumens have been defaunated to remove ciliated protozoa remain, in our experience, completely healthy for 6 months or longer and show no signs of choline deficiency. Thus it is important to know how much choline is passing from the rumen in both normal and defaunated sheep. Analysis of abomasal digesta indicates that the dietary choline made available for absorption in the lower digestive tract is minimal. In other species a dietary intake at an equivalent low concentration would cause pathological lesions and death.

Experiments have been carried out to try to ascertain the reason for the lack of sensitivity of the sheep to supply of exogenous choline. The main synthetic organ in mammals, the liver, appears to be less effective at synthesizing choline via phosphatidylethanolamine than does rat liver. On the other hand, whole-animal experiments show that choline appears to be oxidized to CO_2 less readily in the sheep than in the rat.

Experimental

Collection of digesta samples and preparation for analysis

Unless otherwise indicated, Clun Forest wether sheep (*Ovis aries*) were used. They were housed indoors and fed once daily (09.00h) a ration of 1kg of hay/chaff and 100g of oats. Defaunation of the rumen was carried out as described by Dawson & Kemp (1969), and checked periodically for the absence of ciliate protozoa by microscopic examination of the rumen fluid.

Animals were fitted with a standard cannula (Jarrett, 1948) in a rumen fistula. Abomasal cannulae were constructed of polythene tubing (15cm × 1cm)

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to which a polythene flange was heat-sealed about 2cm from the end. The cannula was inserted near the pylorus and exteriorized about 10–12cm dorsal to the midline.

Fluid was collected through the rumen fistulae by gentle suction through a wide-bore tube; fluid from the abomasum cannulae ran out under gravity. Representative portions (5ml) were dried to constant weight at 110°C. A further portion (20ml) from the same sample of digesta was mixed with 50ml of methanol and then with 25ml of chloroform and homogenized by blending. A further 25ml of chloroform and 25ml of water was then added and the mixture reblended. The upper aqueous layer was removed and was used to determine the concentration of non-esterified choline. The lower chloroform layer was filtered through a small glass-wool pad and the filtrate evaporated to dryness *in vacuo* at 50°C. The residue was taken up in chloroform (5ml).

Determination of choline

In the initial stages of the present investigation, non-esterified choline was determined in ethanolic extracts of digesta by the frog-rectus-muscle assay of Eadie *et al.* (1973).

Choline was later determined by using a choline acetyltransferase method (Eadie *et al.*, 1973), either directly for non-esterified choline in the aqueous layer (see above) or after hydrolysis of the phospholipids in the solvent layer. The specificity of the method was improved by using controls with choline esterase (EC 3.1.1.8) and acetyldephospho-CoA added (S. P. Mann & A. M. Snoswell, unpublished work). To liberate choline from the phospholipids, a suitable portion of the chloroform solution of the phospholipids was evaporated to dryness at 50°C, and treated with 0.08ml of carbon tetrachloride, 0.75ml of ethanol, 0.07ml of water and 0.025ml of 1M-NaOH. After incubating the mixture for 20min at 37°C to deacylate the phosphatidylcholine and its lyso analogue (Dawson *et al.*, 1962), 0.23ml of 5M-HCl was added and the unstoppered tubes heated at 100°C for 15min to release choline from glycerophosphocholine.

Determination of phosphatidylcholine

The lipid extracts of digesta samples were highly coloured with partially degraded plant pigments and rich in neutral lipids, mainly fatty acids. Consequently a second run of solvent on t.l.c. was necessary to obtain satisfactory resolution of the phosphatidylcholine spot. A portion of the chloroform solution of digesta lipids (0.5–1.0ml) was evaporated to near-dryness under N_2 at 50°C and quantitatively applied to a silica-gel t.l.c. plate (Merck 60F 254) that had previously been chromatographically washed with diethyl ether. The plate was first developed to the end in chloroform/methanol (9.1, v/v). After drying it was again developed with chloroform/methanol/water/acetic acid solvent (60:60:5:1, by vol.) in the same direction by using a saturation chamber for both separations. The dried plate was sprayed to detect phospholipids (Vaskovsky & Kostetsky, 1968), and the phosphatidylcholine spot located with the help of markers, scraped off and its phosphorus content determined (Bartlett, 1959). On some occasions the lipids were run as a strip, the phosphatidylcholine was located with iodine vapour, and the appropriate strip eluted and analysed for phosphorus and choline.

Respiratory-gas analysis

The head of the animal was enclosed in a chamber connected to a respiratory-pattern analyser developed in this Department by A. Northrop and D. B. Lindsay and similar to that described by Young *et al.* (1975). Respiratory gases were assayed continuously by using i.r. analysers for CO_2 and methane [Hartmann and Braun (U.K.) Ltd., Moulton Park, Northampton, U.K.], and the total radioactivity monitored with a Cary–Tolbert ionization chamber and Cary 401 amplifier (Cary Instruments, Monrovia, CA, U.S.A.). Radioactivity in CO_2 was determined by passing the expired air through barium hydroxide.

Studies using [^{14}C]ethanolamine as substrate

After overnight food deprivation for 18h, sheep were maintained under anaesthesia by continual injections of pentobarbitone sodium via a catheterized jugular vein. The mesenteric region was exposed surgically and a catheter inserted in the portal vein. [2-^{14}C]Ethanolamine hydrochloride in 1.25ml of sterile 0.9% NaCl was injected slowly via this catheter followed by the injection of a further 1.5ml of 0.9% NaCl. The liver was biopsied hourly over a period of 4h. Excessive bleeding was prevented by clamping the lesions. Liver samples were homogenized and extracted as described above.

In a separate experiment, sheep liver obtained at slaughter was immediately perfused with Krebs–Ringer bicarbonate buffer, pH7.4, at 4°C and kept on ice while preparing tissue slices (0.5mm thick). Tissue samples (0.5g wet wt.) were incubated at 37°C under O_2/CO_2 (19:1) in 2.5ml of Krebs–Ringer bicarbonate buffer, pH7.4, containing [2-^{14}C]-ethanolamine hydrochloride, 2mM-L-asparagine, 2mM-L-glutamine and 2mM-propionic acid with or without 0.67M-L-methionine. After 3h the incubation flasks were immersed in boiling water for 7min. Lipids were extracted with chloroform/methanol (2.1, v/v), washed and analysed as described below.

Lipid extracts were analysed by t.l.c. on activated silica gel. The plates were developed in diethyl ether to remove neutral lipids, dried and redeveloped in

chloroform/methanol/aq. NH_3 (sp.gr. 0.88)/water (110:50:3:5, by vol.). Individual components were located by using markers and radioautography. Areas of silica gel containing radioactivity were scraped into vials for liquid-scintillation counting. Lipid phosphorus in the total extracts and in the separated phospholipids was determined by the method of Bartlett (1959).

Radioactivity determinations

Aqueous samples (0.1–1.0ml) were mixed with 10ml of Unisolve (Koch–Light, Colnbrook, Bucks., U.K.) for liquid-scintillation counting. The solvent was removed from the samples of the lipid extract before adding the Unisolve. $^{14}CO_2$ was trapped as insoluble barium carbonate, dried and suspended in Cab-O-Sil (Koch–Light) for liquid-scintillation counting. Lipid bound to silica gel was counted similarly.

Materials

[*Me*-^{14}C]Choline and [2-^{14}C]ethanolamine were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and [1,2-^{14}C]choline from New England Nuclear, Dreieichenheim, Germany. U-^{14}C-labelled grass was prepared in the chamber described by Dawson & Hemington (1974).

Results and Specific Discussion

Free choline in digesta

The hay used as fodder contained 524nmol of non-esterified choline/g wet wt., whereas the concentration of lipid choline was only 64nmol/g. (Similar results have been obtained by Snoswell *et al.*, 1978.) By contrast, the oats that were used as a minor component of the diet contained 467nmol of lipid choline/g, whereas the non-esterified choline concentration was only 53nmol/g. Calculation from these values shows that the experimental sheep on the standard diet (1000g of hay plus 100g of oats per day) would be receiving approx. 529μmol of non-esterified choline per day plus any liberated from the dietary phosphatidylcholine by the combined action of the phospholipases and phosphodiesterases of rumen micro-organisms (Dawson, 1959; Hazlewood & Dawson, 1975). In spite of this, the concentration of non-esterified choline in the digesta passing into the abomasum is minimal. Nine determinations on such digesta samples removed from three different sheep gave a mean value of 1.07 ± 0.19 (S.E.M.) nmol of choline/ml, with no apparent variation with the time after feeding. Assuming a maximum daily flow rate of the digesta of 9 litres (Faichney & Weston, 1971; Faichney, 1972), the maximum amount of non-esterified choline passing through the abomasum (9.6μmol/day) is only a few per cent of the total choline in the diet.

The explanation for this loss is presumably the active microbial destruction of choline in the rumen to produce trimethylamine and eventually methane (Neill *et al.*, 1978). To investigate this further we injected non-esterified [*Me*-^{14}C]choline into the rumen of sheep and observed the liberation of radioactivity in the expired respiratory gases. Radioactivity rapidly appeared in the expired gases, and Fig. 1 shows that there was a close relationship between this radioactivity and the methane released. This methane was released in bursts, presumably appearing each time the sheep eructated (belched). The CO_2 expired was collected in alkali and contained negligible quantities of $^{14}CO_2$.

In a further experiment of this type, an animal was fed before and during the experimental procedure to slow the formation of methane from the trimethylamine formed from choline (Neill *et al.*, 1978). The animal expired 76% of the [*Me*-^{14}C]choline radioactivity injected into the rumen as methane over 6h. The animal was then killed and the radioactivity in the liver, rumen wall and blood plasma assessed. Of

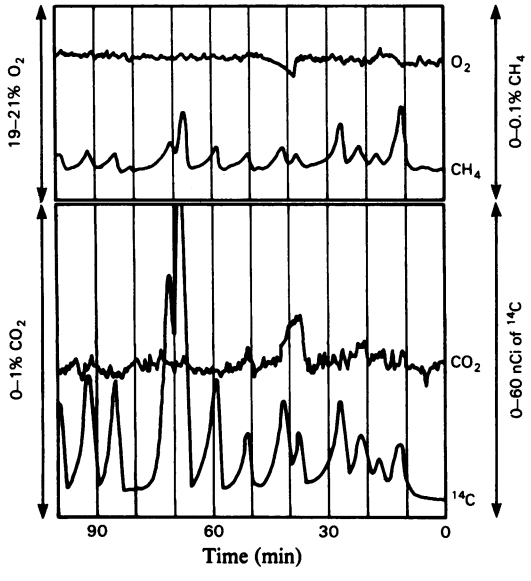

Fig. 1. *Recorder traces of the concentrations of respiratory gases and gaseous radioactivity in expired air from a sheep after injection into the rumen of* 20μCi *of* [*Me*-^{14}C]*choline*
[*Me*-^{14}C]Choline was injected through a rumen cannula in a sheep deprived of food for 24h. The sheep's head and neck were confined in a chamber through which outside air was drawn at about 30 litres/min. Part of the outflow passed to a gas-analysis system that continuously monitored the gas concentrations by means of two twin-channel recorders. The results were reproducible in two additional experiments of this type.

the 0.07% of the administered ^{14}C dose found in the whole liver, the major portion was present as lipid; the other tissues examined contained only traces of radioactivity. It can be calculated from the results of Neill *et al.* (1978) that under these conditions a minimum of 15% of the ^{14}C administered would have remained in the rumen as trimethylamine. Clearly therefore only a very small percentage of the non-esterified choline that enters the rumen, or is produced in this organ, is absorbed as such and becomes incorporated into the tissue lipid.

Lipid choline in digesta

Choline analysis of the abomasum contents showed that, although these contained negligible amounts of non-esterified choline, there were appreciable quantities of lipid choline. Samples taken at random showed 11–44nmol of lipid choline/g wet wt., and this amounted to 81–96% of the total choline in the sample. T.l.c. revealed that this lipid choline was almost exclusively phosphatidylcholine, although traces of lysophosphatidylcholine were occasionally observed. A sample of the phospholipid that chromatographed as phosphatidylcholine was isolated and identified by (*a*) co-chromatography with authentic phosphatidylcholine, (*b*) its chemical degradation to glycerophosphocholine (Dawson *et al.*, 1962), and (*c*) its analysis giving a choline/P molar ratio of 0.95:1 (authentic phosphatidylcholine gave a molar ratio of 0.94:1 by the same methods of analysis).

The question therefore arose as to whether this phosphatidylcholine present in the abomasum was of dietary origin, and if so, whether it contributed substantially to the nutritional choline requirements of the sheep. When U-^{14}C-labelled grass was introduced into the rumen of sheep, the [^{14}C]phosphatidylcholine contained in it was initially rapidly broken down, but eventually the radioactivity in the rumen phosphatidylcholine remained constant (Fig. 2). This can possibly be attributed to the ruminal protozoa requiring choline for growth, so that some of the dietary choline of the sheep is incorporated into protozoal phosphatidylcholine and thus spared from bacterial degradation (Broad & Dawson, 1976). This ruminal preservation of phosphatidylcholine is virtually eliminated when the rumen is free of ciliated protozoa (Broad & Dawson, 1976).

Studies have suggested that ruminal protozoa are selectively retained in the rumen (Weller & Pilgrim, 1974; Bauchop & Clarke, 1976). To investigate the throughput of phosphatidylcholine to the abomasum we measured the concentration of this phospholipid in the rumen and in the abomasum at various times after feeding. The rumen concentration of phosphatidylcholine proved to be very variable. This can possibly be attributed, at least in part, to the difficulty of obtaining a representative sample of fluid from the organ through a narrow fistula. Even samples removed in rapid succession tended to show considerable variation in dry weight and in phosphatidylcholine concentration. Nevertheless, even with this analytical limitation, at all times after feeding the concentration of phosphatidylcholine in the rumen was appreciably higher than that in the abomasum on a digesta-dry-weight basis (Fig. 3). The suggestion of an increase in the ruminal concentration some hours after feeding could reflect the conversion of the non-esterified choline of the diet into protozoal phosphatidylcholine, also perhaps because these same protozoa are much easier to remove from the rumen during sampling than the recently ingested large pieces of leaf material. The mean concentration of phosphatidylcholine in rumen contents was $28.4 \pm 2.46 \mu g$ of P/g dry wt. of digesta for 49 observations made at all times after

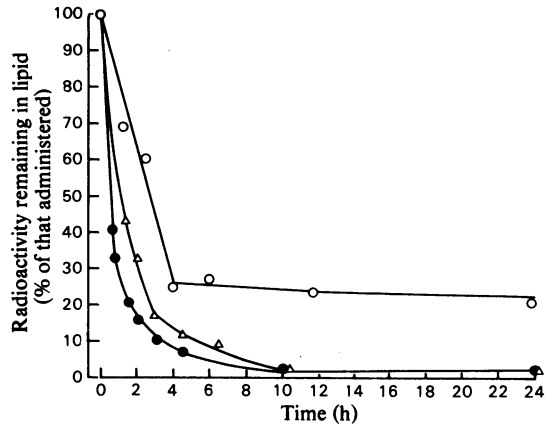

Fig. 2. *Degradation of grass phosphatidylcholine in the rumen*

U-^{14}C-labelled grass was introduced into the rumen via a fistula in a pasture-fed animal at zero time. The animal was then allowed to continue eating pasture. Rumen contents were removed at various times, the lipids extracted, separated on columns and by t.l.c. as described by Dawson & Hemington (1974). The activity in the phosphatidylcholine obtained from each sample was compared with that of the non-metabolized β-carotene, as described by Dawson *et al.* (1977). The change in the ratio of activities of these two lipids represents the proportion of the phosphatidylcholine radioactivity lost in a given time. For comparison, included on the same graph is the disappearance of radioactivity from two grass galactolipids, which are totally metabolized and where little recycling of the radioactivity into microbial galactolipids occurs. Essentially similar results were obtained in three additional experiments using two experimental animals. ○, Phosphatidylcholine; ●, monogalactosyldiacylglycerol; △, digalactosyldiacylglycerol.

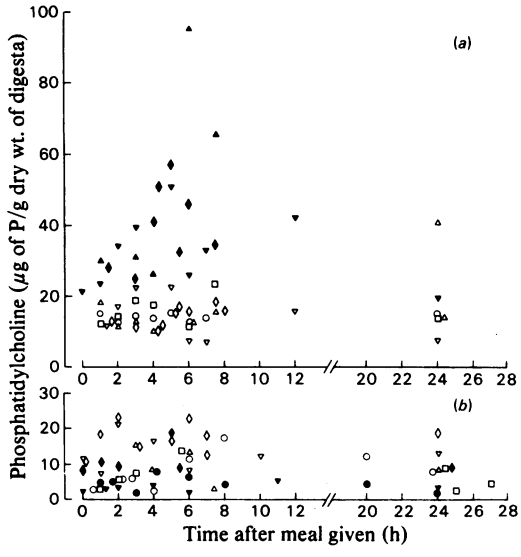

Fig. 3. *Phosphatidylcholine content of digesta removed from the rumens and abomasums of normal and rumen-defaunated sheep*

The digesta were removed through fistulae in the rumen and abomasum at various times after the presentation of a meal of 1000g of hay/chaff and 100g of oats. This was usually consumed within 2h, but in some defaunated animals the food ingestion was over a longer period. Each symbol represents a different experiment on an individual sheep. Open symbols, abomasal digesta; closed symbols, rumen digesta; (*a*) normal sheep; (*b*) rumen-defaunated sheep.

feeding, whereas the corresponding value for abomasal digesta was $14.3 \pm 0.69 \mu g$ of P/g dry wt. (68 observations), the difference being significant. [$P = 0.0005$ (Student's t test)]. No evidence was observed of any change in abosmasal phosphatidylcholine concentration at various times after feeding (Fig. 3).

When the rumens of sheep were defaunated, the amount of ruminal phosphatidylcholine decreased dramatically (Fig. 3), 23 observations showing a mean value of $6.4 \pm 0.90 \mu g$ of P/g dry wt. of digesta, which was significantly lower than that in the abomasal contents [$11.4 \pm 1.11 \mu g$ of P/dry wt., 37 observations; $P = 0.0022$ (Student's t test)]. This marked decrease in ruminal phosphatidylcholine on defaunation is to be expected, since the ciliated protozoa that contain phosphatidylcholine as the major component of their membrane phospholipids (Dawson & Kemp, 1967) are eliminated. By contrast, the bacterial membranes contain little phosphatidylcholine (Dawson & Kemp, 1969). The origin of the ruminal phosphatidylcholine in the defaunated rumen is not obvious. It could represent non-hydrolysed plant phospholipid, it could arise from flagellated protozoa, which are known to proliferate after defaunation, or it could come from sources within the sheep tissues, e.g. secretions or sloughed-off rumen epithelial cells. The higher concentration of phosphatidylcholine in the abomasal digesta compared with that in the rumen might be due to a number of possibilities, e.g. a selected throughput of material that contains phosphatidylcholine from the rumen digesta, a selective digestion and absorption of materials that do not contain phosphatidylcholine, alternatively an enrichment of the abomasal digesta with phosphatidylcholine-containing material from the secretions into it or by regurgitation from the lower digestive tract, e.g. bile.

Calculations based on the phosphatidylcholine concentration of abomasal digesta and the known throughput of 7.5–10 litres per day (Faichney & Weston, 1971; Faichney, 1972) indicate that at maximum the sheep can receive only 15–20mg of dietary choline per day through the passage of phosphatidylcholine and 2–3mg through the non-esterified base (a maximum of 0.25mg/kg body wt.). Since the sheep receives only these limited amounts of choline from its diet, experiments were initiated to see whether this species avoided the pathological results of choline deficiencies (so well documented in other species) either by a greatly increased synthesis in the liver by *N*-methylation of phosphatidylethanolamine or alternatively by a decreased oxidation through the choline oxidase system.

Choline synthesis in sheep liver

It is to be expected, by analogy with other mammals, that maximum synthesis of choline would occur in the liver (Bremer & Greenberg, 1961). In preliminary experiments it has been found that, when [2-^{14}C]ethanolamine was injected intravenously into a sheep, there was a definite incorporation of label into the phosphatidylcholine pool of the liver in biopsy samples taken at times up to 4h after isotope administration (Table 1). Assuming a liver-to-body-weight ratio of 1.9:100, it can be calculated from the results that the sheep's liver would be able to synthesize approx. 9mg of choline/day per kg body wt. This is very considerably more than that available through dietary sources. When sheep liver slices were incubated with [^{14}C]ethanolamine in the absence of methionine, an equivalent calculation gave a maximum of 11.3mg of choline formed per kg body wt., which agreed well with the results obtained *in vivo*. As is to be expected, the addition of methionine to the incubation medium stimulated the synthesis of choline (Table 1).

The rate of conversion of [^{14}C]ethanolamine into choline in the sheep appears to be much lower than that reported from similar experiments with [^{14}C]-ethanolamine in the intact rat (Bjornstad &

Table 1. *Incorporation of* [2-^{14}C]*ethanolamine into sheep liver lipids*

In Expt. (*a*) a Soay ram (19.5 kg body wt.) deprived of food overnight was anaesthetized with pentobarbitone sodium. [2-^{14}C]Ethanolamine hydrochloride (250 μCi; 55 mCi/mmol) was injected in the portal vein and the liver biopsied hourly for 4 h. In Expt. (*b*) sheep liver slices (500 mg wet wt.) were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, at 37°C under O_2/CO_2 (19:1) with 1.6 μCi of [2-^{14}C]ethanolamine hydrochloride, L-asparagine (2 mM), L-glutamine (2 mM) and sodium propionate (2 mM) with or without L-methionine (0.67 mM). Total medium volume was 2.5 ml. Incubations were stopped after 3 h. The lipids were extracted from tissues and analysed as described in text. The results are the specific radioactivities (nCi/μmol of P) in total phospholipid (TP), phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

Expt. (*a*)

Sampling time (h)	TP	PC	PE
1	3.6	0.2	10.7
2	3.8	0.4	10.9
3	4.2	0.3	12.3
4	4.4	0.5	12.8

Expt. (*b*)

Incubation conditions	TP	PC	PE
−Methionine	76.6	9.8	214.0
−Methionine	71.2	7.7	199.8
+Methionine	67.3	14.5	175.7
+Methionine	83.8	23.8	214.4

Table 2. *Production of* $^{14}CO_2$ *from* [1,2-^{14}C]*choline in the intact sheep and rat*

Rats were injected with 5–10 μCi of [1,2-^{14}C]choline and sheep with 40–50 μCi, together with choline carrier in proportion to the body weight of sheep or rat. The output of $^{14}CO_2$ was monitored and integrated over a 5 h period with the animal's head in a chamber connected to a respiratory-pattern analyser.

Animal	Injection route	Percentage of [^{14}C]choline oxidized to $^{14}CO_2$ in 5 h
Sheep	Intraperitoneal	1.6
	Intravenous	2.1
	Intravenous	1.5
		1.73 ± 0.19 (mean ± S.E.M.)
Rat	Intraperitoneal	7.5
	Intra-arterial	2.7
	Intravenous	5.5
	Intravenous	5.3
	Intravenous	5.1
		5.22 ± 0.76 (mean ± S.E.M.)

Bremer, 1966). From these results it can be calculated that male rat liver can synthesize about 100 mg/day per kg body wt., and the value is about 25% higher for female animals. Bremer & Greenberg (1961) found that isolated microsomal fractions from sheep liver were less effective at synthesizing choline when compared with those from species known to be susceptible to choline deficiency, e.g. rat, mouse and chicken.

Oxidation of choline by the intact sheep

To obtain a measure of the rate of oxidation of choline in the whole sheep, [1,2-^{14}C]choline was injected into the animal and the output of $^{14}CO_2$ monitored and integrated by using the respiratory-pattern analyser over a 5 h period. This showed that 1.5–2% of the injected radioactivity was expired as $^{14}CO_2$ during this time (Table 2). Corresponding values for the rat were on average three times higher (P=0.012; Student's t test), suggesting that the rate of oxidation of choline was much more rapid in this species. This assumes that the values obtained are not distorted by any large differences in the rate at which the non-esterified choline of tissues (particularly liver, where choline oxidase is concentrated) comes into equilibrium with the body pool.

Discussion

The present measurements clearly show that, in a sheep fed a diet of hay/oats, the extensive microbial hydrolysis of phospholipids, and the degradation of the *N*-methyl groups of any non-esterified or released choline into trimethylamine and eventually methane in the rumen, leads to only minimal passage of choline, mainly as phosphatidylcholine, to the lower digestive tract. Calculation based on the assumption that all the choline present in the abomasum is of dietary origin suggests that no more than 20–25 mg/day survives destruction in the rumen, and it is very possible that this is an overestimate. This represents an amount of choline equivalent to about 0.002–0.0025% of the total dietary intake of dry matter, and is a concentration that would certainly produce severe pathological lesions (e.g. fatty liver, haemorrhagic kidney) and death in many non-ruminant species. For example, the choline requirements for mice, rats, guinea pigs, pigs and poultry have been found to be 0.1% or more of the dry-matter intake (Reid, 1955; Lucas & Ridout, 1967).

It would appear, therefore, that the adult sheep is less sensitive to a low choline supply from the diet. Few studies have been carried out on the choline requirements of ruminant animals, although Johnson *et al.* (1951) showed that the neonatal calf rapidly deteriorated if fed an artifical diet milk replacer devoid of choline. However, two animals given the same diet when a few weeks of age developed no sign of choline deficiency. This could indicate that sensitivity to choline deficiency in ruminants decreases with age.

Reasons for the apparent normal health of sheep receiving such limited amounts of dietary choline are not fully established. Clearly choline can be synthesized by the animals, presumably by using methionine as methyl donor for the methylation of phosphatidylethanolamine. Calculation based on our limited observations indicates, however, that this formation of choline is many times less on a body-weight basis than is the choline synthesis observed in rat liver (Bjornstad & Bremer, 1966). This would therefore in no way help to explain the resistance of the sheep to low choline intake compared with the rat. Furthermore, since methionine is the first limiting amino acid in the nutrition of sheep (Chalupa, 1972; Reis *et al.*, 1973), its supplies for transmethylation reactions are likely to be limited.

Within the limitations of the experimental technique, the demonstration of a severalfold decrease in the oxidation of choline in the intact sheep compared with the rat could help to explain the decreased requirement of the ruminant for choline. However, although rat liver is known to be very rich in choline oxidase, there appears to be no direct relationship between the activity of hepatic choline oxidase and susceptibility to choline deficiency throughout a variety of species (Lucas & Ridout, 1967).

For economic reasons it was not possible to determine the rates of oxidation of choline after the total body pool of lipid choline had equilibrated with the injected [^{14}C]choline. If such an exchange is slower in the sheep than in the rat (which is likely in a situation where conservation of a vital nutrient is essential), then it could be that the real difference in the rate of oxidation of choline between the two species is even greater than that measured. In this respect, Henderson (1978) found that, in sheep hepatocyte preparations, the ratio of incorporation of label from [1,2-^{14}C]choline into betaine as compared with phosphatidylcholine for a 1h incubation was 0.84:1. A similar ratio for rat hepatocytes calculated from the data published by Sundler & Akesson (1975) is 26:1. If these values can be directly compared, they indicate that sheep liver cells can use available choline more effectively for membrane maintenance than can the cells of rat liver. Also, once encaptured in the pool of phosphatidylcholine in the liver, the very low concentrations of glycerophosphinicocholine diesterase (EC 3.1.4.2) in sheep liver (Dawson, 1956) may prevent the ready release of non-esterified choline as a substrate for choline oxidase.

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