The maximum capacity of the liver of the adult dairy cow to metabolize ammonia

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1. Three adult dairy cows were fitted with cannulas in a mesenteric, portal, hepatic and jugular vein and a carotid artery. They received infusions of step-wise increasing amounts of ammonia as ammonium acetate via a mesenteric vein until NH_3 intoxication occurred. Sodium acetate was used in control infusions. The maximum rate of uptake of NH_3 by the liver and the concentrations of glucose, urea, lactate, acetate and bilirubin in blood were measured.

2. During the infusions of ammonium acetate the liver extracted almost all the NH_3 present in the portal vein until an infusion rate of approximately 15·0 mmol/min was reached. The maximum capacity of the liver to remove NH_3 during its first pass was on average 1·84 mmol/min per kg wet weight. The cows became intoxicated when arterial plasma ammonia concentrations reached 0·8 mmol/l. Concentrations of NH_3 in jugular venous blood were between 66 and 74% of those in the carotid.

In ruminants much of the protein and non-protein nitrogen in the diet is normally metabolized to ammonia by rumenal micro-organisms. Some of this NH_3 is incorporated into microbial protein but a proportion of it (depending upon rumen pH) is absorbed through the rumen wall. If this NH_3 reaches the systemic circulation it is very toxic. In most physiological conditions it is converted in the liver to the less toxic urea thus keeping the NH_3 concentration in the systemic circulation low. However, under certain circumstances, for example when cattle are fed rations containing large amounts of either readily degradable protein or non-protein nitrogen the amount of NH_3 which enters the portal circulation may exceed the rate at which the liver can metabolize it. The concentration of NH_3 in the systemic circulation will then increase until, when arterial NH_3 concentrations are approximately 1·0 mmol/l, the cow shows clinical signs of NH_3 intoxication (Davidovitch *et al.* 1977).

One factor which will affect the susceptibility of the dairy cow to NH_3 intoxication is, therefore, the capacity of its liver to metabolize NH_3. When systemic NH_3 concentrations increase there is also an increase in blood concentrations of glucose, lactate, pyruvate and free fatty acids (Visek, 1972; Barej *et al.* 1974; Garwacki *et al.* 1979). The capacity of the bovine liver to metabolize NH_3 has not been measured in vivo.

This report describes an experiment in which NH_3, as ammonium acetate (NH_4Ac), was infused in increasing amounts into a mesenteric vein of three adult cows until they became intoxicated. The uptake of NH_3 by the liver was measured by determining the NH_3 concentration in portal and hepatic venous blood.

MATERIALS AND METHODS

Animals

Three mature, non-pregnant, non-lactating Friesian × Ayrshire cows weighing 450–500 kg were used. Silicone rubber cannulas were implanted surgically in a mesenteric, portal, hepatic and jugular vein and a carotid artery by a combination of the procedures described

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by Symonds & Baird (1973) and Baird *et al.* (1975). All cows received 4·5 kg hay and 3 kg dairy concentrate daily, fed in two equal portions at 07.30 and 14.30 hours.

Experimental procedure

To increase portal venous NH_3 concentrations a solution of NH_4Ac (approximately 2·0 mmol/ml) was infused into a mesenteric vein using a peristaltic pump (Watson–Marlow HR Flow Inducer, Watson Marlow Air Pump Co., Falmouth, Cornwall).

Each infusion was started at approximately 10.30 hours at a rate of 1·8 mmol NH_4Ac/min. At 15 min intervals the rate was increased by approximately 2 mmol/min, the actual amount infused being measured by weighing the reservoir containing the infusion fluid. Blood samples were collected simultaneously from the cannulas in portal, hepatic, jugular and carotid vessels 14 min after each increase in rate. The infusions were stopped when the cows became recumbent due to NH_3 intoxication (after 150–160 min of infusion). At least 7 d after the infusion of NH_4Ac, an infusion of sodium acetate (NaAc) was given to each cow using the same programme of increases in infusion rate.

Blood sampling and analytical procedures

Samples of blood were taken into perchloric acid (60 g/l); into bottles containing 10 u/ml of heparin; and into bottles containing an ammonium fluoride/oxalate mixture (Anderson, 1969) for glucose estimation by an automated glucose-oxidase method (Sigma, 1970). Plasma was separated from the heparinized blood after centrifugation at 2000 rev/min. The concentrations of NH_3 in plasma were determined by a procedure based on the enzymic conversion of α-ketoglutarate into glutamate (Sigma, 1978) using a manual Vitatron Universal Photometer UFD. Plasma urea was analysed using the automated method described by Rowlands *et al.* (1974). Plasma total bilirubin concentrations were estimated by the Technicon method AAII-18 (Technicon Instruments). Acetate concentration in the neutralized $HClO_4$ extracts of whole blood was determined by the method of Snoswell *et al.* (1978) and lactate by the method of Bergmeyer (1963).

RESULTS

The changes which occurred in the mean plasma NH_3 and whole blood glucose concentrations during the infusion of NH_4Ac and NaAc into the three cows are shown in Fig. 1. Table 1 records the mean ($\pm$ SE) concentrations of NH_3, glucose, urea, lactate, acetate and bilirubin in portal and hepatic venous blood and carotid arterial blood before the infusion of either salt and in the last samples taken when the cows were intoxicated or at a comparable point during the NaAc infusion.

Clinical changes

The infusions of NaAc produced no adverse clinical signs. The animals drank more and passed more urine as the amount of acetate infused increased. When NH_4Ac was infused there was an increase in the frequency of urination and small quantities of looser than normal faeces were passed. When the infusion rate was increased to 18·0 mmol/min (arterial plasma concentration 0·12–0·26 mmol/l) the cows became apprehensive and restless and their respiratory movements were exaggerated. At an infusion rate of between 21·0 and 24·0 mmol/min (arterial concentration 0·66–0·76 mmol/l) the cows became unsteady, with increased muscular fasciculation over the hind-quarters, shoulders and flank. Very fluid faeces were passed. When the infusion rate was increased to 27·0 mmol/min in cows A and B and 31 mmol/min in cow C (arterial concentration 0·79–0·95 mmol/l) the cows became recumbent and the infusion was stopped. The cows usually stood up within 20 min of stopping the infusion.

Table 1. *The means (± SE) concentrations of ammonia, glucose, urea, lactate, acetate and bilirubin in the portal, hepatic and carotid blood of three cows before infusion of either ammonium acetate (NH₄Ac) or sodium acetate (NaAc) and at the end of the infusions*

Metabolite		Portal vein				Hepatic vein				Carotid artery			
		NH₄Ac		NaAc		NH₄Ac		NaAc		NH₄Ac		NaAc	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ammonia	P	191·6	16·17	265·2	39·01	40·7	13·9	39·9	4·83	37·5	1·84	40·7	8·26
(μmol/l plasma)	F	2382·5	129·51	141·2	38·33	1634·6	106·3	51·1	19·59	870·9	47·25	31·2	11·74
Glucose (mmol/l	P	3·3	0·12	3·3	0·23	3·4	0·12	3·5	0·22	3·0	0·06	3·0	0·07
whole blood)	F	5·0	0·87	3·5	0·21	6·1	1·05	3·8	0·20	5·3	1·09	3·6	0·23
Urea (mmol/l	P	4·7	0·15	4·9	0·24	4·9	0·15	5·0	0·27	5·1	0·12	5·5	0·43
plasma)	F	6·4*	—	4·3	0·38	7·0*	—	4·4	0·35	6·6*	—	4·3	0·29
Lactate (mmol/l	P	0·7	0·23	0·6	0·15	0·6	0·20	0·5	0·17	0·7	0·25	0·5	0·12
whole blood)	F	2·5	0·86	0·8	0·17	2·1	0·72	0·6	0·03	1·6	0·32	0·5	0·03
Acetate (mmol/l	P	1·5	0·33	1·8	0·44	1·5	0·29	1·5	0·32	0·9	0·15	0·8	0·17
whole blood)	F	5·0	1·07	4·2	0·67	4·6	0·46	4·0	0·62	3·0	0·45	2·7	0·38
Bilirubin	P	2·8	0·25	2·9	0·92	1·9	0·47	2·2	0·58	1·9	0·24	2·0	0·46
(μmol/l plasma)	F	4·0	0·57	3·3	0·78	4·0	0·43	3·2	0·92	3·5	0·62	3·1	1·07

P, prior to infusion; F, at intoxication for NH₄Ac or final sample for NaAc.
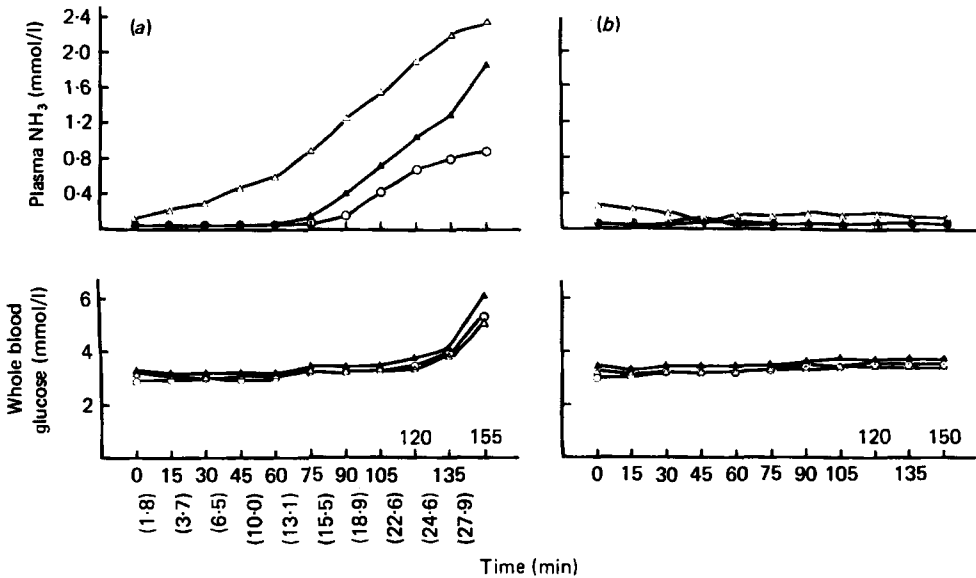• Value is mean for cows A and B only.

Fig. 1. The changes in portal (△—△), hepatic (▲—▲) and carotid (○—○) plasma NH₃ concentration (mmol/l) and whole blood glucose concentration (mmol/l) during (a) an infusion of ammonium acetate and (b) sodium acetate. Values in brackets denote NH₃ infusion rate (mmol/min).

Effect upon NH₃ metabolism

Before infusion the concentrations of NH_3 in the arterial plasma were 0·040, 0·034 and 0·039 mmol/l and there were no significant increases in these concentrations until the infusion rate reached 15, 12 and 17 mmol/min for cows A, B and C respectively. Thereafter, arterial plasma concentrations of NH_3 increased indicating that the liver was no longer able to remove all the NH_3 present in portal blood. When the cows became recumbent their arterial NH_3 concentrations were 0·95, 0·79 and 0·87 mmol/l and their portal plasma NH_3 concentrations were 2·1, 2·6 and 2·4 mmol/l in cows A, B and C respectively.

The liver weights of the cows were: cow A 7·1 kg, cow B 9 kg and cow C 8·2 kg and the mean ($\pm$ SE) maximum capacity of the livers to remove NH_3 at its first pass was 1·84 ± 0·25 mmol/min per kg liver.

The NaAc infusion had no effect upon arterial plasma NH_3 concentrations. NH_3 concentrations were higher in the portal than in the hepatic vein indicating that NH_3 was being absorbed from the gut.

Effect upon glucose metabolism

Before infusion the normal concentration of glucose in arterial whole blood was approximately 3 mmol/l (Table 1). A small increase in concentration occurred only during the latter stages of the infusion of NH_4Ac (Fig. 1, Table 1) in cows A and B, and a larger increase in cow C which began when the infusion rate was 16 mmol NH_3/min.

Effect upon urea metabolism

The concentration of urea in the plasma from all three vessels of cows A and B increased during the NH_4Ac infusion and decreased during the NaAc infusion (Table 1). In cow C, urea concentrations decreased during both infusions. No reason for this decrease in urea

concentration in cow C during the NH₄Ac infusion could be identified but its data were not included in the calculation of the mean urea concentrations for this infusion given in Table 1. The final concentrations in cow C were 3·9, 4·3 and 4·7 mmol/l of portal, hepatic and carotid plasma respectively.

Effect upon lactate metabolism

During the infusion of NH₄Ac blood lactate concentrations increased. In cow A this increase was observed only in the final sample but in cows B and C the concentration of lactate increased when the infusion rate reached 18 mmol/min. During the NaAc infusion there was little change in lactate concentrations in cows A and B but a 220% increase in cow C.

Effect upon acetate metabolism

There was an uptake of acetate by the head, demonstrated by the fact that the concentration in jugular blood was lower than that in carotid blood. In cows A, B and C arterial acetate concentrations were 37·5, 33·7 and 49·8% of portal acetate concentrations respectively, whether or not NH₃ was being infused. This difference demonstrates that the uptake of acetate by peripheral tissues reduced the amount returning in vena caval blood and that NH₃ had little effect upon the uptake.

Effect upon bilirubin

There was an increase in bilirubin concentration in all vessels during both infusions.

DISCUSSION

The results presented show that until its concentration exceeded approximately 1·0 mmol/l most of the NH₃ in the portal blood was extracted during its first passage through the liver. The maximum capacity of the liver to extract NH₃ was approximately 1·84 mmol/min per kg liver weight.

The relevance of these results is principally to those animals whose rumen micro-organisms and hepatic enzyme systems have not been adapted to high protein intakes. At the point at which the hepatic capacity was exceeded (after 90 min of infusion) approximately 13 g of NH₃ had been infused but acute intoxication occurred after approximately 39 g NH₃ had been infused. Therefore, it appears that the rate of input was more important than the total input.

Cattle grazing pasture which has a high soluble protein content may well be exposed to amounts of NH₃ similar to those used in these infusions. A grazing cow may consume up to 500 g/d of N (assuming an 18 kg DM/day intake, of 18% protein content). In calves fed fresh pasture, up to 30–40% of the protein ingested may disappear anterior to the duodenum, and is presumably converted to NH₃ in the rumen (Beever *et al.* 1980). If a similar percentage was lost in adult cattle it would represent up to 200 g N ($\sim$ 14 mol NH₃ or 25 mmol/min) during a grazing period of approximately 10 h. The amount of NH₃ reaching the liver therefore may be greater than the liver's capacity to metabolize it and result in an increase in systemic NH₃ concentration. Although the conditions in the rumen, such as pH, will influence the rate of production and absorption of NH₃, it is possible that a subclinical NH₃ intoxication could occur, one manifestation of which could be the frequent passage of loose faeces.

Severe signs of intoxication occurred when the arterial concentration exceeded 0·8 mmol NH₃/l. The concentrations in jugular plasma were 66–74% lower than the arterial plasma concentrations. This reduction and the nervous signs exhibited were presumably due to the brain taking up NH₃. Davidovitch *et al.* (1977) observed values of 1·05 and 0·68 mmol of

NH_3, in the carotid and jugular blood respectively, of cows showing signs of intoxication after receiving urea intraruminally.

Blood glucose concentrations increased only when the cows were severely intoxicated. The hyperglycaemia associated with NH_3 intoxication has been attributed to the combined effects of reduced utilization of glucose by peripheral tissues and increased hepatic glycogenolysis caused by release of adrenalin. Adrenalin would also reduce or inhibit insulin secretion and thereby prolong the hyperglycaemia. Ammonia may, however, also have a direct action. Barej & Harmeyer (1979) observed that after an infusion of NH_4Cl, NH_3 concentrations in plasma returned to normal much more rapidly than concentrations of glucose and considered that the ammonium ion had an inhibitory action on the pancreatic β cells. Prior et al. (1971) showed that, in the rat, hyperglycaemia induced by NH_3 was refractory to insulin. It is likely that in the experiments described here the hyperglycaemia arose at least in part from an increase in hepatic glucose production which occurred only when the cows were severely intoxicated.

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