The maximum capacity of the bovine liver to excrete manganese in bile, and the effects of a manganese load on the rate of excretion of copper, iron and zinc in bile

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1. The maximum capacity of the bovine liver to excrete manganese in bile was determined in three Friesian-cross steers surgically prepared to allow bile flow-rate to be measured and samples collected. Plasma Mn concentrations were increased by infusing manganese chloride solutions into a jugular vein and the biliary excretion rates of Mn, copper, zinc and iron were measured.

2. The maximum capacity of the liver to excrete Mn in bile was exceeded at an infusion rate of approximately 4000 μg Mn/min, and at this rate there was a significant decrease in the concentration of Cu, Fe and Zn in bile. The maximum concentration (mean $\pm$ SE) of Mn in bile was 193 ± 19 μg/ml, and the maximum excretion rate (mean $\pm$ SE) was 1210 ± 130 μg/min for the three animals. There was no reduction in bile flow or evidence of liver damage as a result of the infusions.

Manganese toxicity and deficiency occur naturally in ruminants and have been produced experimentally (Bentley & Phillips, 1951; Rojas *et al.* 1965; Cunningham *et al.* 1966; Bourne, 1967; Egan, 1972; Ford, 1972; Hidiroglou, 1979). The concentrations of Mn in blood and tissue are normally very low (0·02 μg/ml blood, 0·2 μg/g muscle), although dietary Mn concentrations may exceed 100 mg/kg. The homoeostatic mechanisms of the body therefore act principally to prevent excess Mn entering the systemic circulation and the major sites of control are the gut and liver. Over wide ranges of dietary Mn content only 0·5–1% of dietary Mn is absorbed from the small intestine (Sansom *et al.* 1978). The absorbed Mn reaches the liver through the portal vein, and is almost completely removed during the first passage of the blood through the liver (Gibbons *et al.* 1976). Mn is the only trace element known to be treated in this manner. Most of the Mn removed by the liver is excreted in bile (Carter *et al.* 1974).

There must be a limit to the capacity of the liver to remove Mn from the blood and excrete it in bile since abnormally high systemic blood Mn concentrations and Mn toxicity do occur. High Mn concentrations in the systemic circulation interfere with the metabolism of other minerals (eg. copper and zinc) (Gubler *et al.* 1954; Järvinen & Ahlström, 1975; Ivan & Grieve, 1975) and with the metabolism of monoamines in the brain causing severe neurological syptoms (Kimura *et al.* 1978; Leach & Lilburn, 1978). Excessive Mn uptake by the liver can cause liver damage and cholestasis particularly when plasma bilirubin concentrations are increased (Witzleben *et al.* 1968; Witzleben, 1971). The maximum capacity of the liver to extract Mn and prevent toxicity is unknown; a study was therefore undertaken to determine the maximum capacity of the bovine liver to excrete Mn in bile, and the effects of large quantities of Mn on bile flow and the biliary excretion of the trace elements Cu, iron and Zn. This information should indicate whether the liver's capacity to extract and excrete Mn is likely to be exceeded under normal conditions of husbandry.

MATERIALS AND METHODS

Animals

Three Friesian-cross steers (A, B and C) aged between 12 and 18 months were used. Each had its duodendum surgically altered to allow bile to be collected and its rate of flow measured. The procedure entailed isolating, from the rest of the duodenum, that section into which the common bile duct entered, and diverting the bile entering this isolated section back into the duodenum via a re-entrant cannula (Symonds, Mather & Hall, unpublished procedure). The animals remained healthy and grew normally, but to ensure that they had recovered completely they were not used until 4–6 weeks after surgery. The experiment took 4–5 months to complete on each animal. The weights of the steers A, B and C at the beginning of the experiment were 219, 279 and 240 kg, and at the end 355, 406 and 339 kg respectively. They were given a hay and dairy cake ration to give a gain of approximately 25 kg body-weight/month. The average concentrations of Mn, Cu, Fe and Zn in the hay were 127, 5·8, 182 and 21 μg/g DM, and in the dairy cake 107, 39, 247 and 67 μg/g DM respectively.

Infusion procedure

The systemic plasma concentration of Mn was increased by infusing manganese chloride solution at a constant rate into a jugular vein for 6 h using a Harvard Syringe Pump (Harvard Apparatus, Massachusetts, USA). The initial infusion rate of Mn was 30 μg/min for animal C and 70 μg/min for animals A and B. The rate of infusion was increased in succesive experiments at intervals of at least 2 weeks until there was no further increase in biliary Mn concentration, when it was assumed that the capacity of the liver to excrete Mn had been exceeded. Saline (9 g sodium chloride/l) was infused before the first infusion of $MnCl_2$ to each steer to determine daily variations in bile flow and biliary Mn, Cu, Fe and Zn concentrations.

Bile and plasma collection

During experiments bile was collected into a bag attached to a harness on the animal. The volume was measured every 30 min to determine flow-rate and a small sample taken for analysis. The remainder of the bile was returned to the duodenum to ensure that the enterohepatic circulation of bile salts was maintained. The removal of the small volume of bile for analysis had a negligible effect on bile flow. Bile flow-rate was measured during each 6 h infusion, for 1 h before and after the infusion and for 3 h during the day before and the day after each infusion of Mn. After 6 h infusions of 73·2 μg Mn/min and 1760 μg Mn/min in animals B and C respectively bile flow and Mn excretion were measured for a further 9 h.

During each infusion three 20 ml blood samples were collected at 2 h intervals by venepuncture into bottles containing 100 i.u. heparin, and the plasma was removed after centrifugation.

Preparation of solutions for infusion

Pyrogen-free solutions were prepared as follows. The $MnCl_2$ was first dried at 200° for 24 h to remove the water of crystallization and then sealed into glass vials in an atmosphere of nitrogen and heated to 400° overnight. It was then dissolved in an isotonic saline solution made up in double-glass distilled water. The solution was finally passed under pressure through a 45 μm pore filter held in an autoclaved millipore sterilizing filter holder (Millipore (UK) Ltd, London) into a sterile bag.

Analyses

Trace elements. Mn, Fe, Cu and Zn concentrations in bile and blood plasma were determined directly by flame atomic absorption using a 751 or 107 Perkin-Elmer Atomic

Absorption Spectrophotometer (Perkin Elmer Ltd, Beaconsfield, Bucks). Food samples were first dry ashed, and digested in 2 M-hydrochloric acid before the concentrations of trace elements were determined by atomic absorption spectrophotometry.

Bilirubin. Total plasma bilirubin concentrations were measured by an automated procedure based on the method of Jendrassik & Grof (1938) as adapted by Gambino & Schreiber (1964).

Enzymes. The activities of sorbitol dehydrogenase (SHD; *EC* 1.1.1.4), glutamate dehydrogenase (GDH; *EC* 1.4.1.3), γ-glutamyl transpeptidase (γGT; *EC* 2.3.2.2) and creatine phosphokinase (CK; *EC* 2.7.3.2) were measured using test kits (Boehringer Corporation, London) on a Vitatron Automatic Kinetic Enzyme and Substrate Analyser (MSE Scientific Instruments, Crawley, Sussex).

Statistical analysis

Bile flow-rates and concentrations of Cu, Fe and Zn in bile during the infusions were analysed by split-plot analysis of variance to determine differences due to animal, treatment and time.

When calculating the excretion of infused Mn, the basal Mn excretion measured in the hour before each infusion was subtracted from the Mn excreted during the infusion. Any variation in Cu, Fe and Zn concentrations in the bile, which may have been due to variations in the trace element concentrations in the diet, were eliminated for statistical analysis of the results by expressing values during the infusions as percentages of the control concentrations measured before the start of each infusion.

RESULTS

At each infusion rate the maximum concentration in bile was reached after approximately 3 h, and remained stable at this concentration for the next 3 h of infusion. The rates at which Mn was infused, the mean concentration and excretion rates of Mn in bile during the last 3 h of the infusions, and the plasma Mn concentrations during the last 3 h of the infusions are shown in Table 1. For each infusion rate, except the highest in each animal, the mean ($\pm$ SE) percentage of Mn excreted in bile was $67\cdot5 \pm 2\cdot5$ of that infused/min. At the highest infusion rates a lower percentage of the infused Mn (22–$31\cdot5\%$/min) was excreted in bile, indicating that the maximum excretion rate of Mn had been attained. The mean ($\pm$ SE) maximum concentration of Mn in bile and the mean ($\pm$ SE) maximum excretion rate, calculated from the two highest infusion rates into each steer, were 193 ± 19 μg/ml and 1210 ± 130 μg/min respectively.

The mean ($\pm$ SE) percentage of the total infused Mn recovered in bile during the 6 h infusion plus 1 h post-infusion collection was $61\cdot2 \pm 2\cdot9$ when the maximum capacity of the liver to excrete Mn in bile had not been exceeded. When the maximum biliary excretion of Mn had been exceeded $34\cdot5$, $29\cdot4$ and $32\cdot3\%$ of the infused Mn was recovered in bile from animals A, B and C respectively. During the 6 h infusion plus 9 h collection after the end of the infusions at $73\cdot2$ and 1760 μg Mn/min the percentage of Mn recovered was $62\cdot4$ and $79\cdot7$ respectively of that infused, leaving $9\cdot9$ mg and $128\cdot7$ mg of Mn unaccounted for. The patterns of excretion of Mn during these two infusions and for 9 h post infusion are shown in Fig. 1. After the Mn infusion was stopped the changes in the rate of excretion of Mn in bile could be described in terms of two exponentials with half-lives of 46 and 248 min after infusion of 73 μg Mn/min and 32 and 196 min after infusion 1760 μg Mn/min.

Trace element excretion

For statistical analysis the trace element excretion values were considered under four ranges of infusion rates: 30–260, 960–1100, 1570–1700 and 3470–4320 μg Mn/min. During the

Table 1. *The rate of excretion and concentration of manganese in bile during the last 3 h of each 6 h infusion of manganese chloride into the jugular vein and the plasma Mn concentration after 3 h of infusion*

Animal	Infusion rate (μg/min)	Mean Mn concentration in bile (μg/ml)	Mean Mn excretion rate (μg/min)	Percentage Mn excreted /min	Plasma Mn concentration (ng/ml)
A	30	2·7	20	66·7	*
	60	6·4	40	66·7	*
	130	13·3	90	69·2	*
	240	25·5	160	66·7	*
	1100	89·9	730	66·4	*
	1700	164·0	1131	66·5	401
	3900	187·7	1229	31·5	2060
B	70	4·9	40	57·1	29
	260	23·9	150	57·7	49
	960	112·9	660	68·8	176
	1570	197·5	1240	79·0	346
	3500	285·3	1820	52·0	1744
	4320	166·7	989	22·0	1586
C	73	5·6	56	71·0	24
	92	12·3	86	93·5	30
	950	113·3	650	68·3	160
	1656	177·9	1184	71·5	310
	3470	177·6	916	26·4	2380

* No values available.

Table 2. *Changes in the concentrations of copper, iron and zinc in bile during infusions of 3470–4320 μg Mn/min into the jugular vein*

(Values expressed as percentage of the original concentration)

Time after start of infusion (h)	Trace element		
	Cu	Fe	Zn
−1	100·0	100·0	100·0
0	98·3	102·0	126·7
1	60·3*	96·0	91·3
2	60·0*	83·7*	121·3
3	51·7*	69·0*	85·3*
4	36·7*	56·3*	67·3*
5	30·3*	62·0*	70·0*
6	36·4*	64·7*	76·7
7	29·6*	80·1	106·7

* Concentration significantly lower than the concentration at the same time during infusion at 1570–1700, 960–1100 or 30–260 μg Mn/min; $P \leqslant 0.05$.

control periods the mean ($\pm$ SE) concentrations of Cu, Fe, Zn and Mn in bile were 0·43 $\pm$ 0·02, 0·28 $\pm$ 0·01, 0·13 $\pm$ 0·04 and 1·04 $\pm$ 0·05 μg/ml respectively. No significant changes in the excretion of Cu, Fe or Zn occurred during infusion at the three lowest rates, but at 3470–4320 μg Mn/min there was a significant decrease in the concentrations of all three trace elements in bile. Cu excretion was affected most while the excretion of zinc was depressed for only 2 h (Table 2).
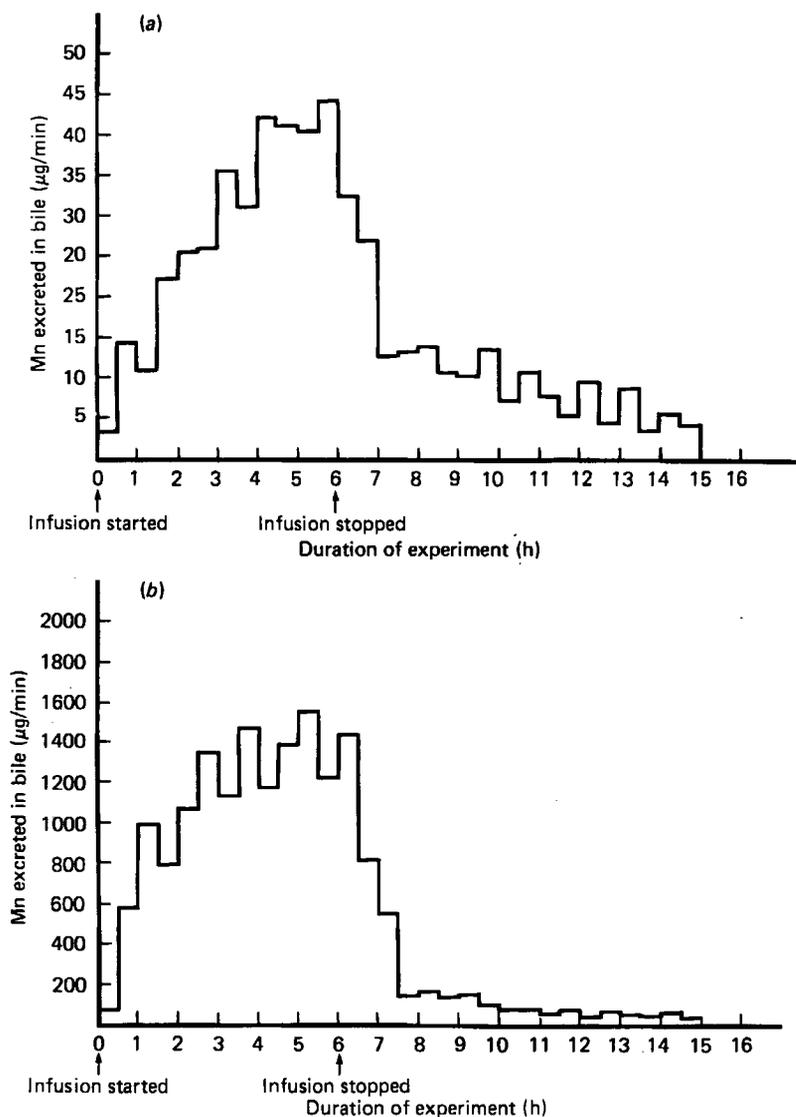
Fig. 1. Manganese excreted in bile (μg/min) during 6 h of infusion and 9 h after infusion of (a) 73·2 and (b) 1760 μg Mn as manganese chloride into jugular veins of steers.

Plasma enzyme and bilirubin concentrations

There were no consistent changes in plasma enzyme activities or bilirubin concentrations as a result of the Mn infusions. The concentrations found in samples taken during infusions and control periods have therefore been combined and the mean ($\pm$SE) plasma activities of SDH, GDH, γGT and CK were 18 ± 1, 16 ± 2, $12\pm<1$ and 45 ± 3 IU respectively, and the mean ($\pm$SE) plasma bilirubin concentration was $1·62\pm0·04$ μmol/l.

DISCUSSION

The results show that the bovine liver can excrete large quantities of Mn in bile. Klaassen (1974) found that the maximum excretion rate of Mn into the bile of rats was 8·5 μg/min per kg body-weight. In the three steers the mean ($\pm$SE) maximum excretion rate of Mn

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was $3 \cdot 7 \pm 0 \cdot 5$ μg/min per kg body-weight at the time of the infusion. The values are in reasonable agreement, since the liver constitutes approximately 4·0% of the body-weight of the rat, and 1·2% of the body-weight of the cow (Spector, 1956).

Biliary excretion alone would not account for the removal of all infused Mn. When biliary clearance of Mn was followed for 9 h after the infusion of 26·2 and 633·6 mg Mn in 6 h, 37·6 and 20·3% respectively had not been excreted in bile by the end of the period. In the rat 35% of Mn in bile is reabsorbed (Cikrt, 1973). If this percentage reabsorption occurred in cattle and were independent of the concentration of Mn in bile then the amount of Mn cleared from the body by excretion would be over-estimated in these experiments. The enterohepatic circulation of Mn would delay the excretion of Mn from the body.

Although bile is the major route of excretion of Mn, some loss occurs across the intestinal wall or via pancreatic secretions (Bertinchamps *et al.* 1966). If the liver was the only route by which Mn was excreted, then at equilibrium the rate of removal by the liver should equal the rate at which Mn was infused. Since only approximately 67% of the infused Mn was excreted in bile, 33% was either retained in the tissues of the body or lost by other routes. The kidney was not an important route of loss. During infusion of approximately 4000 μm Mn/min urinary Mn did not increase above 0·6 μg/ml. Assuming the steer produced 2 1 urine during the 6 h infusion, the maximum loss of Mn would be only 1·2 mg (0·08% of the total infused).

When Mn enters the visceral circulation from the gut or by infusion into a mesenteric vein it is normally removed by the liver during the first passage of the blood through the liver (Gibbons *et al.* 1976; Sansom *et al.* 1978). It has been shown that the liver of the adult cow can remove up to 3260 μg Mn/min as a first pass effect before Mn concentration in hepatic venous plasma increases (Hall & Symonds, unpublished observations). The livers of the steers should therefore have been capable of extracting all the Mn presented to them, particularly at the lower infusion rates. However, when $MnCl_2$ is infused into the jugular vein it is most likely that some Mn is in circulation long enough to be temporarily bound to a plasma protein such as α-macroglobulin (Gibbons *et al.* 1976) and is not immediately removed in passage through the liver. Therefore the excretion rate for Mn of only 67%/min of that infused via the jugular vein may reflect the ability of the liver to extract the unbound Mn from plasma.

Liver damage and cholestasis which had been reported to occur as a result of Mn infusions in rats (Witzleben *et al.* 1968; Witzleben, 1969), did not occur in the steers. In preliminary studies (Symonds *et al.* 1979) it was observed that intramesenteric infusions of $MnCl_2$ solution at 69 μg Mn/min into a steer reduced bile flow-rate, and increased plasma bilirubin and plasma enzyme activities 24 h later. These observations suggested that the bovine liver was extremely sensitive to Mn given through the mesenteric vein. The present findings suggest that other factors must have been involved to produce such acute effects and it is evident that the bovine liver is not as susceptible to damage by Mn as was previously thought.

In summary, the biliary excretion of Mn in the steer may be increased 200-fold for a few hours with no apparent toxic effects. This facility for increased excretion could cope with an increase in the Mn concentration in food of 200-fold (i.e. from approximately 60 to 12000 mg/kg) if the percentage absorption of Mn from the gut remained unchanged. It is very unlikely that ruminants would be subjected to such high concentrations of Mn in food under normal husbandry conditions. However, the infusions were continued for only 6 h, and it is possible that exposure to a diet containing high concentrations of Mn over a period of several months could exceed the liver's excretory capacity, particularly if during the same period bilirubin concentrations in plasma became increased of liver damage occurred.

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