THE TRANSFER OF ZINC FROM THE BLOOD TO THE RUMEN IN SHEEP

By R. H. Weston*† and J. Kastelic*

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Summary

^{65}Zn was found to be present in rumen digesta after a single dose of $^{65}ZnCl_2$ was administered intravenously; the maximum level was reached approximately 3 days after the dose was given. After a single dose of $^{65}ZnCl_2$ was administered intraruminally, ^{65}Zn disappeared exponentially from the rumen, the time taken for the quantity present in the rumen to halve being 0·6–0·7 days. It was calculated that in 7 days following intravenous injections of $^{65}ZnCl_2$, 1–2% of the injected dose was transferred to the rumen; transfer appeared to take place through the rumen epithelium as well as via saliva.

I. Introduction

The transfer of substances from the blood to the rumen either via saliva or through the rumen wall may enable the ruminant host to maintain conditions in the rumen favourable for its rumen microflora. Although the transfer of some substances, e.g. urea has been studied in some detail, few corresponding studies have been conducted with trace elements. There appear to have been no studies of the transfer of zinc, although the requirement of microorganisms for zinc has long been recognized and it has been shown that the rate of cellulose digestion by rumen microorganisms is retarded under conditions of both a deficiency and an excess of zinc (Little, Cheng, and Burroughs 1958; Hubbert, Cheng, and Burroughs 1958; Uesaka, Kawashima, and Zembayashi 1965). The essential role of zinc in the intermediary metabolism of ruminants has been demonstrated experimentally (Miller and Miller 1960; Miller *et al.* 1964; Ott *et al.* 1964) and a zinc deficiency syndrome has been described in grazing cattle (Legg and Sears 1960). It would appear then that the extent of recycling of zinc to the rumen is possibly of physiological significance with respect to both the ruminant and its rumen microflora.

The studies reported here were conducted to determine the extent of transfer from the blood to the rumen. To calculate the extent of this transfer it was necessary to measure both the levels of radioactive zinc present in rumen digesta following the administration of ^{65}Zn intravenously and the rate of removal of ^{65}Zn from the rumen after its administration intraruminally.

II. Materials and Methods

(*a*) *Animals and Experimental Procedures*

Two adult castrate male sheep weighing approximately 45 kg were prepared with rumen fistulae, kept in metabolism cages, and given 700 g of coarsely ground hay each day in two equal parts spaced 12 hr apart.

* Department of Animal Science, University of Illinois, Urbana, Illinois.

† Present address: Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

The radioactive zinc, administered in the form of $^{65}ZnCl_2$, had a specific activity of 117 μc/mg. Intravenous doses of ^{65}Zn were given into the jugular vein via a polythene catheter and intraruminal doses were administered through the rumen fistula; each dose contained less than 1·7 mg of zinc.

Samples (500–800 g) of rumen digesta were withdrawn immediately before feeding by suction applied to tubing 1·6 cm in internal diameter; a 100-g subsample was then taken and the remainder returned to the rumen. At the end of the experiments, the sheep were killed 12 hr after feeding and the quantity of digesta in the rumen was measured.

Saliva was collected as it dripped by gravity from the tongues of the sheep anaesthetized with sodium pentobarbitone given intravenously. Collections of saliva were commenced approximately 2 hr before the sheep were offered feed. The induction and maintenance of the anaesthesia and other associated manipulations were as described by Somers (1961).

(*b*) *Determination of the* ^{65}*Zn Content of Samples*

Samples of rumen contents and faeces containing 2–5 g of dry matter were dried to constant weight at 105°C and ignited at 430°C for 16 hr. The resulting ash was dissolved in 6N HCl and this solution was evaporated to dryness; the residue was dissolved in water, transferred quantitatively to a separating funnel, and extracted in the form of zinc dithizonate by a method similar to that described by Vallee and Gibson (1948). The zinc dithizonate was shaken with 3 ml of 0·3N HCl; 0·2 ml of the aqueous solution containing the ^{65}Zn was then plated on a circular copper planchet and assayed for radioactivity using a gas flow counter. Samples of saliva and urine were evaporated to dryness and digested successively with nitric, perchloric and hydrochloric acids; after the residue was evaporated to dryness, ^{65}Zn was extracted and assayed by the technique described above. The efficiency of assaying ^{65}Zn was determined by extracting and counting samples containing known amounts of the injected solution of $^{65}ZnCl_2$.

(*c*) *Calculation of the Rate of Disappearance of* ^{65}*Zn from the Rumen*

In these experiments it was shown that ^{65}Zn disappeared exponentially from the rumen. The fraction of the ^{65}Zn that disappeared per unit time (λ) was calculated from the time (T) required for the quantity of ^{65}Zn present to halve using the equation $\lambda = 0 \cdot 693/T$ (Comar 1955).

III. Results

The dry matter content of the digesta present in the rumen after the sheep were killed was 10·8% for sheep 1 and 13·1% for sheep 2; the total amounts of dry matter were 0·36 kg and 0·30 kg respectively. The mean dry matter contents of samples of rumen digesta obtained during the experiments were 10·4 and 12·6%. Preliminary experiments showed that ^{65}Zn in rumen digesta was chiefly associated with particulate material. Accordingly, the concentration of ^{65}Zn in samples of rumen contents has been expressed in relation to dry matter, and in calculating the amount of ^{65}Zn present in the rumen it has been assumed that the amount of dry matter present at each sampling time was equal to that present when the animals were killed.

(*a*) *Experiment* 1

The quantity of ^{65}Zn present in the rumen after the administration of a single dose of $^{65}ZnCl_2$ intravenously is shown in Figure 1. The quantities were always small,

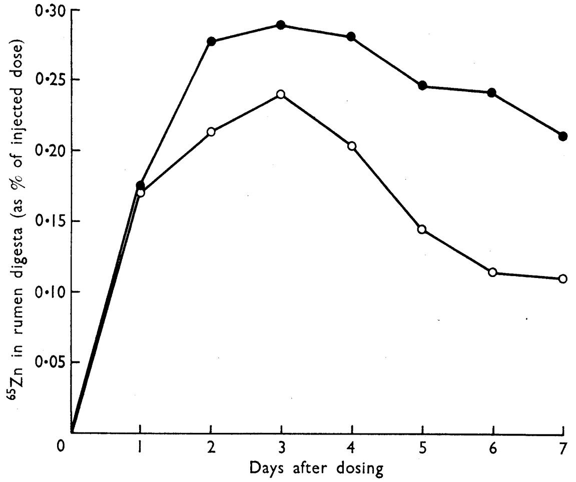

Fig. 1.—Quantity of ^{65}Zn present in rumen digesta after administration of a single dose of $^{65}ZnCl_2$ intravenously. ● Sheep 1. ○ Sheep 2.

rising to maxima of 0·29 and 0·24% of the injected dose for individual sheep, about 3 days after dosing, and decreasing slowly thereafter.

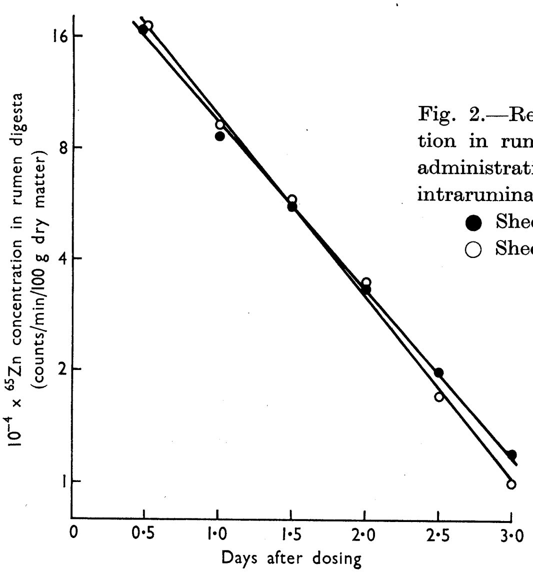

Fig. 2.—Relationship between ^{65}Zn concentration in rumen digesta (y) and time after the administration of a single dose of $^{65}ZnCl_2$ intraruminally (x).
● Sheep 1: $\log y = 5 \cdot 47 - 0 \cdot 45x$.
○ Sheep 2: $\log y = 5 \cdot 42 - 0 \cdot 49x$.

(*b*) *Experiment* 2

The disappearance of ^{65}Zn from the rumen following the administration of a single dose of $^{65}ZnCl_2$ intraruminally is shown in Figure 2; this experiment commenced

9 days after the beginning of experiment 1. The concentration of ^{65}Zn in rumen digesta decreased exponentially with time, the half-time values being 0·67 and 0·62 days for the individual sheep; the corresponding rates of removal of ^{65}Zn from the rumen, calculated as described earlier, were 103 and 113% per day of the quantity present in the rumen.

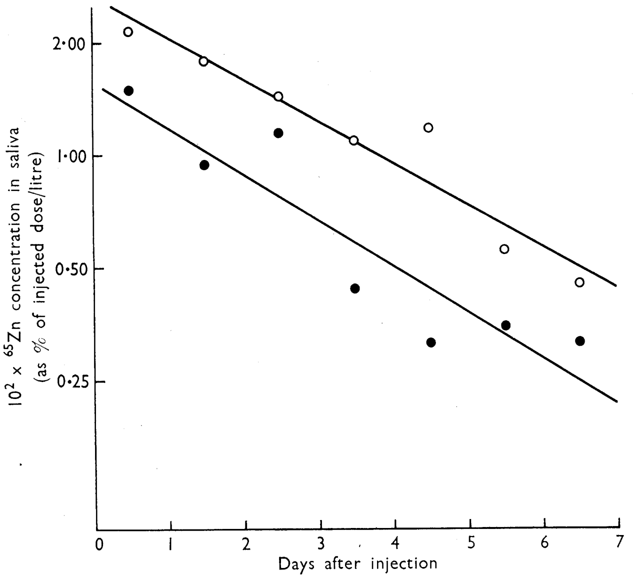

Fig. 3.—Relationship between ^{65}Zn concentration in the saliva (Y) and the time after the administration of a single dose of $^{65}ZnCl_2$ intravenously (X); saliva was collected for 1 hr on each occasion.

● Sheep 1: $\log Y = 0·197 - 0·124X$.
○ Sheep 2: $\log Y = 0·462 - 0·114X$.

(c) *Experiment* 3

The concentration of ^{65}Zn in saliva collected for 1 hr on each of 7 successive days that followed the administration of a single dose of $^{65}ZnCl_2$ intravenously is shown in Figure 3. The concentration decreased exponentially with time after dosing, the rate of decline being similar in both sheep although there were differences in concentration between sheep. The concentrations were low at all times, maximum values being approximately 0·02% of the injected dose per litre of saliva.

The major route of excretion of the ^{65}Zn injected was via the faeces. Over the period of 7 days after administration of the ^{65}Zn both sheep excreted 0·18% of the injected dose in the urine and the quantity excreted in the faeces was 14·2% from one sheep and 18·6% for the other.

(*d*) *Transfer of Radioactive Zinc to the Rumen*

The quantity of ^{65}Zn transferred to the rumen in a 24-hr period following intravenous injection has been calculated as the sum of (1) the change in the quantity of ^{65}Zn in the rumen between the end and the start of the period, and (2) the ^{65}Zn leaving the rumen during the period. The change in the quantity of ^{65}Zn in the rumen during each period was estimated from the data obtained in experiment 1 as presented in Figure 1. The quantity removed from the rumen in each period was estimated by multiplying the mean of the amounts present at the beginning and the end of each period by the average daily rate of removal obtained in experiment 2, viz. 103% per day for sheep 1 and 113% per day for sheep 2.

TABLE 1

TRANSFER TO THE RUMEN OF ^{65}Zn FOLLOWING THE ADMINISTRATION OF A SINGLE DOSE OF $^{65}ZnCl_2$ INTRAVENOUSLY

Time Interval after Injection (days)	(A) Change in Quantity of ^{65}Zn in the Rumen (as % of injected dose)		(B) Quantity of ^{65}Zn Leaving the Rumen (as % of injected dose)		(A+B) Quantity of ^{65}Zn Entering the Rumen (as % of injected dose)	
	Sheep 1	Sheep 2	Sheep 1	Sheep 2	Sheep 1	Sheep 2
0–1	0·18	0·17	0·09	0·10	0·27	0·27
1–2	0·10	0·05	0·24	0·23	0·34	0·28
2–3	0·01	0·02	0·29	0·26	0·30	0·28
3–4	−0·01	−0·04	0·30	0·25	0·29	0·21
4–5	−0·04	−0·06	0·28	0·20	0·24	0·14
5–6	−0·01	−0·03	0·26	0·15	0·25	0·12
6–7	−0·03	−0·01	0·23	0·13	0·20	0·12

The estimates of transfer to the rumen are shown in Table 1. During the 7 days following intravenous injection of ^{65}Zn, transfer to the rumen was equal to 1·9 and 1·4% of the injected dose for the respective sheep; these values were equal to 13 and 8% of the excretion in faeces over the same period. The rate of transfer did not reach a maximum until the second or third day after injection although the contribution of ^{65}Zn in saliva to the total transfer was highest on the first day (Fig. 3). Hence, ^{65}Zn transfer via the rumen wall must have taken place, its contribution becoming maximal later than that of ^{65}Zn from saliva. On the basis of the relationship between ^{65}Zn concentration in saliva and time after dosing (expt. 3) it may be calculated that a salivary secretion rate of 39 litres per day for sheep 1 and 17 litres per day for sheep 2 would be required to account for the total transfer of ^{65}Zn to the rumen over the 7 days. The rate of secretion required to account for the total transfer of ^{65}Zn in sheep 1 is much higher than the normal range of 6–16 litres per day reported for sheep by Kay (1960). This again suggests that part of the transfer to the rumen occurred through the rumen epithelium.

IV. Discussion

It was calculated that a small proportion of the ^{65}Zn injected intravenously was transferred to the rumen. The main sources of error in estimating this transfer appear to be first, the use of rates of removal obtained in experiment 2 to calculate transfer rates in experiment 1 and second, the use of a value for the quantity of dry matter present in the rumen that was obtained from a single determination on each animal. The errors involved are probably small. Thus experiment 2 was performed shortly after experiment 1 without any change in experimental conditions and the data were collected over a period of 3 days. Further, reasonable agreement was found between sheep in the quantity of dry matter present in the rumen and this was measured at the same time relative to feeding as samples were taken. The mean dry matter content of digesta samples taken during the experiments agreed well with the dry matter content of the entire digesta, thus suggesting that the samples were representative of the digesta as a whole. In estimating the rate of removal of ^{65}Zn from the rumen (expt. 2), the transfer to the rumen during the period of measurement of ^{65}Zn originating from the dose given intravenously in experiment 1 was not taken into account; accordingly, the true rate of removal of ^{65}Zn from the rumen could be faster than was calculated. The discrepancy is probably negligible as the quantity of ^{65}Zn transferred to the rumen during the experiment would be expected to be small relative to the quantity of ^{65}Zn in the rumen at that time. Thus the transfer to the rumen of ^{65}Zn originating from the dose given in experiment 1 would be expected to be less than 0·20% of this dose per day, the value calculated for the seventh day of measurement during experiment 1 (Table 1); this quantity is equivalent to less than 0·5% of the dose introduced intraruminally in experiment 2.

The transfer of 1·4 and 1·9% of the intravenously injected ^{65}Zn to the rumen over a period of 7 days may be regarded as indicative of the fate of zinc atoms that are absorbed from the alimentary tract. However, the net gain of zinc by the rumen may be less than this since a fraction of the ^{65}Zn entering the rumen via the rumen wall may be the result of an exchange of zinc ions between the blood and the rumen digesta. The entry of a small quantity of zinc into the rumen of cattle is suggested by the data of Miller and Cragle (1965) who employed a technique showing the net result of the absorption of zinc and its entry into various sections of the alimentary tract. Their studies also indicated that the chief site of zinc secretion into the alimentary tract was the proximal small intestine; the relatively small contribution of ^{65}Zn that entered the rumen to the total excretion of ^{65}Zn in faeces in the present study is consistent with this observation.

Maximum values for ^{65}Zn transfer to the rumen via the rumen wall on the one hand, and via saliva on the other, were not reached at the same time. Hence the extent of transfer by both pathways at any time was not related to the concentration of a specific form of zinc in the blood. It is possible that while the rate of entry of ^{65}Zn into the rumen epithelium may parallel that of entry into saliva, part of the transfer of ^{65}Zn from the epithelium to the rumen may depend on epithelial cells being sloughed off.

V. Acknowledgment

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