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The assessment of zinc status of an animal from the uptake of ^{65}Zn by the cells of whole blood in vitro

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1. ^{65}Zn uptake by blood cells in vitro has been compared with plasma Zn concentration and plasma alkaline phosphatase (*EC* 3.1.3.1) activity as indicators of an animal's Zn status.

2. Dietary Zn deficiency, low food intake, reduced dietary protein content and endotoxin administration all reduced plasma Zn concentration in the rat. In each case there was a parallel reduction in plasma alkaline phosphatase activity and an increase in ^{65}Zn uptake in vitro by cells of whole blood.

3. A similar relationship between the three measurements existed in sheep with lowered plasma Zn concentrations whether these were caused by dietary deficiency or by post-surgical stress.

4. ^{65}Zn uptake by cells of whole blood did not differentiate dietary Zn deficiency from the other factors which reduce plasma Zn under 'field' conditions.

5. ^{65}Zn uptake by the cells in whole blood in vitro was three to five times less rapid in blood of ruminant origin than in that from non-ruminants. This difference related to the erythrocytes rather than to the leukocytes or the plasma.

Experimentally-induced zinc deficiency is associated with marked reduction in growth and food intake and with a lowering of plasma Zn concentration (Mills, Quarterman, Chesters, Williams & Dalgarno, 1969). Attempts to assess Zn status under practical conditions by measurements of plasma Zn concentration have been frustrated, however, by wide variations between individuals which were not readily associated with differences in Zn intake. Many factors other than dietary supply are now known to affect plasma Zn concentration and may be partially responsible for its variability under 'field' conditions. Low values are found in animals subject to infection (Vikbladh, 1951; Pekarek, Burghen, Bartelloni, Calia, Bostian & Beisel, 1970), trauma (Lindeman, Bottomley, Cornelison & Jacobs, 1972), low protein intake (Van Campen & House, 1974) and during pregnancy (Hambidge & Droegemueller, 1974). Assessment of the Zn status of animals would be greatly aided if a diagnostic measurement could be found which varied with the Zn intake of the animal but was not affected by the other conditions listed previously.

The activity of plasma alkaline phosphatase (*EC* 3.1.3.1) decreases when dietary Zn intake is inadequate (Miller, Luecke, Ullrey, Baltzer, Bradley & Hoeffer, 1968; Miller, Pitts, Clifton & Morton, 1969). It may also be reduced, however, by low food intake (Miller *et al.* 1969), by the nature of the protein source (Miller *et al.* 1968) and by manganese or magnesium deficiency (Lassiter & Morton, 1968; Loveless & Heaton, 1976). Alkaline phosphatase concentrations in plasma are also influenced by growth rate (Healy & McInnes, 1975) and tend to decrease, at least in ruminants, as the animal ages (Allcroft & Folley, 1941).

Berry, Bell & Wright (1966) investigated the uptake of ^{65}Zn in vitro by the cells of whole blood and demonstrated an inverse relationship between dietary Zn intake and ^{65}Zn uptake. They suggested that ^{65}Zn uptake provided a sensitive indicator of Zn status and might prove useful as a diagnostic test. Its usefulness under practical conditions would depend, however, on the range of factors which influence it and an investigation of these will be reported here.

Table 1. *Semi-synthetic diet for sheep*

Component	Concentration (g/kg)	Component	Concentration (mg/kg)
Maize starch	400	$FeSO_4.7H_2O$	199
Glucose	356	$ZnSO_4.7H_2O$	176
Cellulose (cotton linters)*	90	$MnSO_4.4H_2O$	162
Egg albumen (spray-dried)	60	$CuSO_4.5H_2O$	20
Urea	30	KIO_3	9·6
Arachis oil	10	$CoCl_2.6H_2O$	4·0
$CaHPO_4.2H_2O$	22		(μg/kg)
$MgSO_4.7H_2O$	12·4	Retinol	300
NaCl	9·2	Cholecalciferol	9
$KHCO_3$	7·6	α-Tocopherol	6700
$CaCO_3$	2·5		

* Hercules Powder Co., London W1H 8AL.

EXPERIMENTAL

Animals and diets

Rats. Male hooded Lister rats were offered the semi-synthetic diets described by Williams & Mills (1970). Control rats received the Zn-supplemented diet *ad lib.* for at least 7 d and Zn-deficient rats the Zn-deficient diet *ad lib.* for at least 14 d before investigations started. The Zn-deficient rats showed the reduced growth rate and cyclic pattern of food intake characteristic of the deficiency (Williams & Mills, 1970). A third group of rats were offered the Zn-supplemented diet but were individually pair-fed with rats of the Zn-deficient group. For studies of the effects of low protein intake the egg albumen content of the diets was reduced from 200 to 50 g/kg by replacement of albumen with sucrose. Endotoxin-treated rats were injected intraperitoneally with lipopolysaccharide B (2 mg/kg body-weight, *Escherichia coli* 055:B5; Difco Laboratories, Detroit, USA) dissolved in 0·154 M-sodium chloride. At the end of each treatment, the rats, which all weighed between 120 and 140 g, were anaesthetized with pentobarbitone sodium and blood samples were taken by heart puncture for analysis.

Sheep. A group of three Cheviot wethers weighing approximately 20 kg were offered *ad lib.* the semi-synthetic diet described in Table 1.

After 4 d on the diet, blood samples were taken for analysis from the jugular vein. Thereafter the sheep were offered *ad lib.* the same diet but with Zn omitted from the mineral supplement. Blood samples were taken at weekly intervals to monitor the onset of Zn deficiency.

Samples of blood were also obtained both before and after operations for insertion of vascular catheters (F. White, G. Wenham, I. Grant & L. E. Vowles, personal communication) from a group of 18-month-old maiden, grey-faced ewes which had been established on various conventional feeds.

Other species. For inter-species comparisons, blood samples were obtained from normal individuals of various species as follows. Jugular venepuncture was used to collect blood from young Friesian steers, adult British Saanen nanny goats and captive male red deer (*Cervus elaphus*). Blood was obtained from mice and guinea-pigs by heart puncture under anaesthesia with pentobarbitone sodium and rabbit blood was obtained by jugular incission. Peripheral venepuncture was used in obtaining blood samples from adult male human volunteers.

Methods

^{65}Zn uptake by blood cells in vitro. Portions of whole blood (0·5 ml) were incubated with 0·2 μCi ^{65}Zn ($^{65}ZnCl_2$, approximately 270 Ci/g; Radiochemical Centre, Amersham, Bucks.) under an atmosphere of oxygen–carbon dioxide (95:5, v/v) for 2 h at 37°. The cells were then washed twice with 20 vol. 0·154 M-NaCl, resuspended in 4 ml of the same solution and radioactivity measured using a scintillation counter (Tracerlab Gamma Guard 400 scintillation counter; ICN-Tracerlab, Hersham, Surrey). Initial experiments showed that ^{65}Zn uptake in various mixtures of cells and plasma from the same individual was directly proportional to the packed cell volume over the range 0·2–0·5 and results, expressed as fractional uptakes of ^{65}Zn/2 h, were therefore corrected to a standard packed cell volume of 0·40.

For one investigation preparations enriched in erythrocytes or leukocytes were required. With pig blood, enrichment occurred naturally when the blood was left to sediment under unit gravity, the value for erythrocytes:leukocytes of the lower 'cell' layer being 1580:1 and that of the 'plasma' layer 6:1. Cells from these layers were therefore suspended in 1·5 times their volume of homologous plasma for ^{65}Zn uptake studies. With cattle blood, natural sedimentation was too slow and the blood was centrifuged at 500 *g* for 10 min. Plasma from the same steer was used to wash the cells of the 'buffy layer' to provide a leukocyte-rich fraction while the cells beneath the 'buffy layer' were sufficiently enriched in erythrocytes to be used without further treatment.

Plasma alkaline phosphatase activity. Plasma alkaline phosphatase was assayed by the hydrolysis of *p*-nitrophenylphosphate in the presence of a 2-amino-2-methyl-1-propanol buffer (Sigma Chemical Co., 1974). Activities were determined after 15 min incubation at 37° and were then expressed as μmol/h per ml plasma.

Plasma Zn concentration. Samples of plasma were treated with an equal volume of trichloroacetic acid (100 g/l), centrifuged and the Zn content of the supernatant fluid estimated using an atomic absorption spectrometer (Techtron AA5 spectrophotometer; Varian Instruments, Walton-on-Thames, Surrey).

Blood cell concentrations. Concentrations of erythrocytes and leukocytes were determined using a Coulter counter (Coulter Electronic Ltd, Dunstable, Beds.).

RESULTS

The effects of dietary Zn deficiency on ^{65}Zn uptake by blood cells in vitro, plasma Zn concentration and plasma alkaline phosphatase activity were investigated with rats offered Zn-adequate or Zn-deficient diets *ad lib.* and with rats offered the Zn-adequate diet but pair-fed with the Zn-deficient animals (Table 2). Since Zn deficiency in rats results in a cyclical pattern of voluntary food intake and in similar but inverse changes in plasma Zn concentration (Chesters & Will, 1973), the Zn-deficient and pair-fed rats were studied after days of both high and low food intake. All three measurements were influenced both by dietary Zn concentration and by food intake. The increases in ^{65}Zn uptake by blood cells resulting from dietary Zn deficiency were, however, proportionately less than the corresponding reductions in plasma Zn concentration, regardless of whether the Zn-deficient animals were compared with rats fed *ad lib.* or with the pair-fed animals. Although plasma alkaline phosphatase activity was reduced by Zn deficiency, the effects of food intake were such that the values for the Zn-deficient rats after high food intake overlapped those of the pair-fed controls after a period of low intake.

Changes in plasma Zn concentration similar to those occurring during the early phase of an acute infection have been found after administration of endotoxin, maximal reduction

Table 2. *Effects of dietary Zn content and food intake on plasma Zn concentration, ^{65}Zn uptake by blood cells and plasma alkaline phosphatase* (EC 3.1.3.1) *activity of rats*

Dietary treatment*	Dietary Zn concentration (mg/kg)	No. of rats	Food intake in previous 24 h (g)		Plasma Zn concentration (mg/l)		Fractional ^{65}Zn uptake by blood cells		Plasma alkaline phosphatase activity (μmol/h per ml)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-supplemented, *ad lib.*	40	8	12·7[a]	0·5	1·81[a]	0·04	0·147[a]	0·005	9·82[a]	0·34
Zn-deficient and pair-fed after high food intake	< 1	4	12·2[a]	0·5	0·47[b]	0·02	0·331[b]	0·021	7·14[bd]	0·66
	40	4	12·2[a]	0·5	1·83[a]	0·08	0·152[a]	0·008	9·04[ab]	0·66
Zn-deficient and pair-fed after low food intake	< 1	4	0·4[b]	0·3	1·15[c]	0·02	0·186[c]	0·005	4·70[c]	0·20
	40	4	0·4[b]	0·3	1·59[a]	0·08	0·160[a]	0·004	6·70[d]	0·36

* For details, see p. 298.

Within a column, values without common superscripts are significantly different ($P < 0·05$).

Table 3. *Effects of endotoxin administration (2 mg lipopolysaccharide B/kg body-weight) on plasma Zn concentration, ^{65}Zn uptake by blood cells and plasma alkaline phosphatase* (EC 3.1.3.1) activity in rats

(Mean values with their standard errors for groups of four rats offered Zn-supplemented, semi-synthetic diets *ad lib.** The control rats were not injected with endotoxin)

Time elapsed after endotoxin administration (h)	Food intake in previous 24 h (g)		Plasma Zn concentration (mg/l)		Fractional ^{65}Zn uptake by blood cells		Plasma alkaline phosphatase activity (μmol/h per ml)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10	$8{\cdot}9^{a}$	0·5	$0{\cdot}50^{a}$	0·03	$0{\cdot}234^{a}$	0·013	$5{\cdot}90^{a}$	0·34
24	$3{\cdot}0^{b}$	0·7	$1{\cdot}06^{b}$	0·11	$0{\cdot}197^{ab}$	0·012	$7{\cdot}98^{b}$	0·26
48	$5{\cdot}4^{c}$	0·6	$1{\cdot}42^{ce}$	0·08	$0{\cdot}178^{b}$	0·007	$7{\cdot}94^{bd}$	0·54
72	$10{\cdot}9^{d}$	0·4	$1{\cdot}33^{bc}$	0·06	$0{\cdot}179^{b}$	0·007	$8{\cdot}78^{bcd}$	0·30
Control rats:								
ad lib.-fed	$12{\cdot}7^{f}$	0·5	$1{\cdot}81^{d}$	0·04	$0{\cdot}147^{c}$	0·005	$9{\cdot}82^{cd}$	0·34
Starved for final 10 h	$9{\cdot}8^{adf}$	1·1	$1{\cdot}59^{e}$	0·04	$0{\cdot}189^{b}$	0·006	$9{\cdot}88^{d}$	0·76

* For details of dietary treatment, see p. 298.

Within a column, values without a common superscript are significantly different ($P < 0{\cdot}05$).

of Zn in plasma occurring 8–10 h after endotoxin injection (Pekarek & Beisel, 1969). In the present experiments, endotoxin caused a reduction in plasma Zn concentration and alkaline phosphatase activity of rats within 10 h of administration. However, ^{65}Zn uptake also significantly increased even though the animals were always offered diets adequate in Zn (Table 3). With each of the three measurements of Zn status the changes found 10 h after endotoxin injection were comparable with those produced by extreme dietary deficiency of Zn (Table 2). These effects were not observed in starved but non-injected rats and were therefore unrelated to the reduction in food intake immediately after endotoxin administration.

Although low protein intake reduces the plasma Zn concentration of a rat offered a diet adequate in Zn, the low plasma Zn of a Zn-deficient rat is increased by reduction of the dietary content of protein (Mills, 1973; Van Campen & House, 1974). In the present experiments, these opposing changes abolished the differences in plasma Zn concentration between rats offered Zn-adequate and Zn-deficient diets when these contained only 50 g albumen/kg (Table 4). It is apparent, however, that in the absence of adequate protein intake ^{65}Zn uptake and plasma alkaline phosphatase activity were also unaffected by changes in dietary Zn intake.

Further studies were conducted with sheep given Zn-deficient and Zn-adequate semi-synthetic diets and with sheep offered practical diets after surgery (Table 5). When the diets were deficient in Zn, changes in plasma Zn concentration were associated with analogous alterations in the other two measurements of Zn status. However, similar changes occurred when low plasma Zn concentrations were induced by surgical stress. The relationship between ^{65}Zn uptake and plasma Zn concentration is shown in Fig. 1. Reductions in plasma Zn concentration after surgery on animals given practical diets were associated with alterations in ^{65}Zn uptake comparable with those produced by dietary Zn deficiency.

The investigations described here revealed a two- to threefold difference in the rates of ^{65}Zn uptake by blood cells from rats and sheep and a brief survey of other species indicated that those from the ruminants were consistently lower than the uptakes found with blood from the non-ruminants (Table 6). The concentrations of Zn in plasma were, however, species dependent. Certain of the differences in ^{65}Zn uptake by blood cells in vitro could have

Table 4. *Effects of dietary protein content on plasma Zn concentration, ^{65}Zn uptake by blood cells and plasma alkaline phosphatase* (EC 3.1.3.1) activity in rats

(Mean values with their standard errors for groups of four rats. Unless indicated all rats were offered the diets *ad lib.*)

Dietary treatment*	Dietary protein content (g/kg)	Food intake in previous 24 h (g)		Plasma Zn concentration (mg/l)		Fractional ^{65}Zn uptake by blood cells		Plasma alkaline phosphatase activity (μmol/h per ml)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Zn-supplemented	50	$8{\cdot}9^{a}$	1·0	$1{\cdot}03^{a}$	0·05	$0{\cdot}194^{a}$	0·011	$8{\cdot}00^{a}$	0·16
	200†	$8{\cdot}9^{a}$	1·0	$2{\cdot}05^{b}$	0·09	$0{\cdot}147^{bc}$	0·013	$9{\cdot}38^{b}$	0·48
	200	$12{\cdot}7^{b}$	0·5	$1{\cdot}81^{b}$	0·04	$0{\cdot}147^{b}$	0·005	$9{\cdot}82^{b}$	0·34
Zn-deficient	50	$6{\cdot}7^{a}$	0·6	$1{\cdot}07^{a}$	0·09	$0{\cdot}184^{ac}$	0·012	$7{\cdot}40^{a}$	0·56

* For details, see p. 298.

† Rats pair-fed with those receiving the Zn-supplemented, low protein diet.

Within a column, values without a common superscript are significantly different ($P < 0{\cdot}05$).

Table 5. *Effects of dietary Zn deficiency and surgical stress on plasma Zn concentration, ^{65}Zn uptake by blood cells and plasma alkaline phosphatase* (EC 3.1.3.1) *activity in sheep*

(Mean values before and after treatment and the significances of the differences were calculated by a paired *t* test; no. of animals/treatment is given in parentheses)

Group	Treatment	Plasma Zn concentration (mg/l)	Fractional ^{65}Zn uptake by blood cells	Plasma alkaline phosphatase activity (μmol/h per ml)
Cheviot wethers (3)				
	Zn-adequate	0·81	0·064	12·26
	Zn-deficient*	0·18	0·155	1·60
	Difference between treatments	0·63	0·091	10·66
	SE of difference	0·09	0·019	1·84
	Statistical significance of difference	$P < 0·05$	$P < 0·05$	$P < 0·05$
Grey-face ewes (6)				
	Before operation	0·71	0·058	3·81
	24 h after operation	0·46	0·085	2·80
	Difference between treatments	0·25	0·027	1·01
	SE of difference	0·05	0·005	0·39
	Statistical significance of difference	$P < 0·01$	$P < 0·01$	$P < 0·01$

* 28 d on Zn-deficient diet.

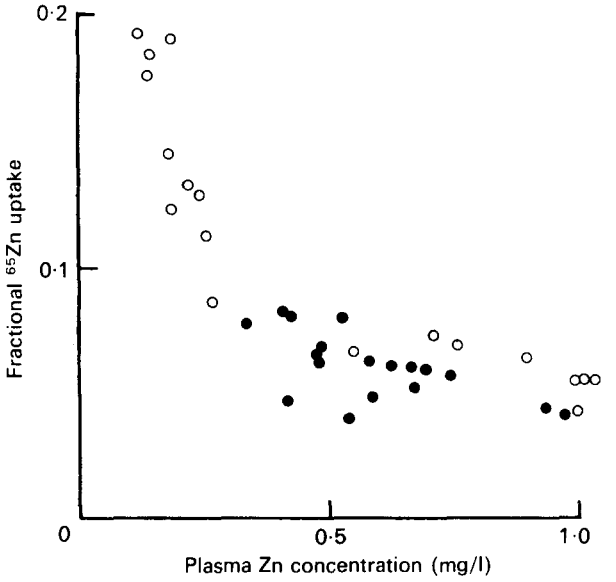

Fig. 1. Relationship of ^{65}Zn uptake by blood cells in vitro to plasma Zn concentration in sheep. (○—○), Wethers given semi-synthetic diets with and without Zn supplementation; (●—●), ewes given conventional diets before and after surgery. For details of sheep, see p. 298.

Table 6. *Plasma Zn concentration and ^{65}Zn uptake by blood cells of various animal species**

	No. of individuals	Plasma Zn concentration (mg/l)		Fractional ^{65}Zn uptake by blood cells		Zn uptake by blood cells (ng/2 h per ml packed cells)	
		Mean	SE	Mean	SE	Mean	SE
Non-ruminants							
Guinea-pig	3	2·00	0·46	0·103	0·015	289	28
Man	8	0·83	0·03	0·136	0·003	170	9
Mouse	6	1·64	0·15	0·174	0·014	417	28
Pig	4	1·16	0·08	0·188	0·006	326	17
Rabbit	5	1·69	0·20	0·091	0·010	209	15
Rat	8	1·81	0·04	0·147	0·005	399	14
Ruminants							
Cattle	6	0·99	0·05	0·051	0·003	77	6
Goat†	2	0·88, 0·73		0·050, 0·067		66, 73	
Red deer	4	0·78	0·07	0·035	0·004	45	2
Sheep	7	0·74	0·05	0·060	0·005	65	5

* For details, see p. 298.
† Individual values for each animal.

resulted, therefore, from variations in the specific activity of Zn in the plasma after the addition of a constant, tracer dose of ^{65}Zn rather than from differences in the rates of Zn uptake. When, however, the quantities of Zn taken up in 2 h were estimated from the product of the total Zn in the plasma and the fractional uptake of ^{65}Zn, the marked differences in Zn uptake between the ruminants and the non-ruminants were still apparent. Investigations of the time-course of ^{65}Zn uptake in the two groups showed that the differences in uptake at 2 h were equally apparent at all time intervals between 15 min and 2 h. When a 'cross-over' experiment was performed in which cells obtained from either pig or sheep blood were incubated either in their own plasma or in that from the other species, the ^{65}Zn uptakes depended entirely on the origin of the cells rather than on that of the plasma.

In all the experiments described whole blood was used and the cells therefore consisted of a mixture of erythrocytes and leukocytes. In order to determine the origin of the species differences in ^{65}Zn uptake, preparations enriched in either erythrocytes or leukocytes were obtained from both cattle and pigs and were incubated with ^{65}Zn in their own plasma. The concentrations of both erythrocytes and leukocytes were determined for each preparation and the differential contributions of the two types of cells to the over-all uptakes of ^{65}Zn were then calculated. Uptakes (ng Zn/10^6 cells per 2 h) were: cattle, leukocytes 600, erythrocytes 2·1; pigs, leukocytes 500, erythrocytes 14·9. Thus the rates of uptake of ^{65}Zn by leukocytes were similar for both species but the pig's erythrocytes incorporated Zn seven times more rapidly than those of the cattle. In whole blood the differences in normal cell concentrations are such that erythrocyte ^{65}Zn uptake would account for approximately 75% of the total uptake in pigs and over 90% in cattle.

DISCUSSION

Under 'field' conditions, the problem of diagnosing Zn deficiency arises not from any lack of Zn-responsive measurements but from a plethora of other conditions which influence these indices of Zn status. In the present experiments each of the factors which altered plasma Zn concentration produced corresponding changes in ^{65}Zn uptake by erythrocytes

in vitro. Furthermore, since the latter was inversely correlated with plasma Zn concentration, alterations in uptake were most marked when the concentrations of Zn in plasma were relatively low (Fig. 1). On the other hand, sensitivity of response of the diagnostic measurement would be most desirable at intermediate concentrations of Zn since these are probably more likely to be found under practical conditions and would be hardest to diagnose. There appeared therefore to be little advantage to be gained by using ^{65}Zn uptake by erythrocytes in whole blood rather than plasma Zn concentration as an index of Zn status. Since plasma alkaline phosphatase activity was also altered by each of the factors which reduced plasma Zn concentration it appeared that only considerations of methodology could favour replacement of the latter as the prime index of Zn status. The consistent responses of each of the three measurements to a range of imposed stresses did however emphasize the alterations of Zn metabolism which occur and the need for a better understanding of their significance in the physiology of stress.

In contrast to the findings described here, Sasser, Bell & Jarboe (1975) working with sheep between 1 and 10 months after β-irradiation of their skin showed an increase in ^{65}Zn uptake by erythrocytes in vitro without a corresponding change in plasma Zn concentration. The increase was associated equally with differences in both plasma and erythrocytes but its relevance to the Zn status of the animal was uncertain.

In calculating the uptakes of Zn by blood cells of different species it was assumed that the differences did not stem from variations in the proportion of the total Zn in plasma which exchanged with added ^{65}Zn. Since the mean plasma Zn concentration for the non-ruminant species was nearly twice that for the ruminants, the two- to threefold lower uptake of ^{65}Zn by cells from the latter would have required a four- to sixfold higher proportion of exchangeable Zn in the ruminants' than in the non-ruminants' plasma to explain the differences in uptake entirely on the basis of the specific activity of the exchangeable Zn. This did not seem likely and furthermore the species differences were found to be associated with the cells rather than with the plasma. Sasser *et al.* (1975) similarly found that differences in erythrocyte ^{65}Zn uptake between sheep and pigs related to the origin of the cells rather than that of plasma. The similarity of the calculated uptakes of Zn by leukocytes from both cattle and pigs further suggests that the marked differences in the rates of Zn uptake by erythrocytes of the ruminant and non-ruminant species were not artefacts of the method of calculation. Estimation of the total Zn present in the erythrocytes gave mean ($\pm$ SE) values of $6{\cdot}7 \pm 0{\cdot}4$ μg/ml packed cells for sheep and $9{\cdot}5 \pm 0{\cdot}4$ μg/ml packed cells for rats. Although these values might be thought to reflect the differences in the rates of ^{65}Zn uptake by the erythrocytes, most of the Zn in these cells is present in carbonic anhydrase (*EC* 4.2.1.1) and does not exchange with ^{65}Zn added in vivo (Hove, Elvehjem & Hart, 1940; Tupper, Watts & Wormall, 1952). Since the rates of uptake of ^{65}Zn/2 h could, at most, represent uptakes of 0·04 and 0·01 of the total Zn of rat and sheep erythrocytes respectively, it is likely that the uptakes depend on an exchange reaction with a quantitatively minor component of the cellular Zn either on or within the cells and do not relate to the total Zn of the cells.

The fundamental cause of the difference in Zn uptake between the ruminant and non-ruminant erythrocytes is unknown but Zwaal, Roelofsen & Calley (1973) have suggested that the outer layer of the plasma membrane of erythrocytes is largely composed of choline phospholipids and Nelson (1967) found almost total replacement of phosphatidylcholine by sphingomyelin in ruminant erythrocytes. It is possible that the differences in Zn uptake may be related to the proportions of sphingomyelin and lecithin in the erythrocytes' membrane but the relative rates of Zn uptake by erythrocytes of non-ruminant species did not correlate well with the values for sphingomyelin concentration quoted by Nelson (1967).

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