Plasma zinc as an indicator of zinc status in rats

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(*Received* 10 *March* 1971 – *Accepted* 20 *July* 1971)

1. Standard zinc solutions for the atomic absorption analysis of rat plasma were prepared to contain 14% (w/v) of sucrose. In this way the problems of sample nebulization were overcome with a minimum of manipulation before assay.

2. Plasma Zn concentrations in rats were found to fall by approximately 40% (from 1·2 to 0·7 μg/ml) after 1 d on a Zn-deficient (< 0·25 ppm) diet. Thereafter, the fall became less marked and after 5 d the concentrations usually varied between 0·4 and 0·6 μg/ml.

3. A single oral dose (20–200 μg) of Zn was reflected in high plasma Zn concentrations in the depleted rats 1·75 h after dosing, but to a much lesser extent in animals receiving 10–60 ppm Zn in their diet before dosing.

4. It is suggested that the plasma Zn response to a single oral dose of zinc sulphate may provide a useful method for the detection of a subnormal Zn status in individual farm animals without the necessity of determining breed norms.

The diagnosis of trace-element deficiencies in animals, especially at the marginal level, is notoriously difficult (Mills, Quarterman, Chesters, Williams & Dalgarno, 1969). At present most investigations of zinc status are based on plasma Zn concentrations, which have been shown to decline rapidly during states of dietary depletion (Mills, Dalgarno, Williams & Quarterman, 1967; Dreosti, Tao & Hurley, 1968). Unfortunately, individual analyses of plasma Zn are seldom unequivocal and studies of Zn status are usually complicated by the need to establish breed norms as well as the allowable variation between individuals.

In addition, although plasma appears to be ideally suited for analysis by atomic absorption spectroscopy, its viscosity renders it unsuitable for direct comparison with aqueous standards. Several procedures have been proposed whereby this problem may be overcome, but at present a simple and reliable method of analysis is not available.

Improved methods were therefore sought both for the assay of plasma Zn and for the early detection of Zn deficiency in animals. The studies reported in this paper were performed on rats, but it is hoped that these findings may prove to be of value with larger animals where the problems of Zn deficiency are of more economic importance.

EXPERIMENTAL AND RESULTS

Reagents

All chemicals used were of analytical reagent grade and water was distilled and deionized through a multibed ion-exchange resin (model B 125 Elgastat; Elga Products Ltd, Lane End, Buckinghamshire).

Animals and diets

Female rats of the Wistar strain (110–120 g) were housed individually in stainless-steel cages and given a diet consisting of sucrose 51%, soya-bean meal 38·5% (solvent-extracted, containing 44% protein, obtained from Hind Bros & Co. Ltd, Durban), maize oil 6·1%, salt mix (Hurley & Swenerton, 1966) 3·0%, cod-liver oil 0·7%, DL-methionine 0·5%, choline chloride 0·2%. The soya-bean meal was treated with ethylenediamine-tetraacetic acid (EDTA) to reduce its Zn content (Davis, Norris & Kratzer, 1962; Swenerton & Hurley, 1968) and the entire diet was found to contain less than 0·25 ppm of Zn. Control animals received the same diet supplemented with 60 ppm of Zn as zinc sulphate (Hurley, 1969). A mixture of crystalline vitamins was supplied separately in sucrose three times a week (Hurley & Swenerton, 1966).

Sampling techniques and analytical procedures

Blood samples (5 ml) for plasma Zn analyses were collected directly from the heart following ether anaesthesia. Clotting was avoided by the addition of 10 mg sodium citrate/ml blood.

Viscosity measurements of plasma and sucrose solutions were made with an Ostwald viscometer at 25°.

Zn determinations were made on an atomic absorption spectrophotometer (model Pm Q II Zeiss; Oberkochen, Baden-Württemberg, West Germany) operating with a detection limit of 0·1 ppm of Zn and a sensitivity of 0·2 ppm.

Plasma viscosity measurements

The viscosity of a pooled sample of plasma from ten rats was compared with the viscosity of a number of sucrose solutions (Table 1).

A 14% (w/v) sucrose solution was found to have a viscosity at 25° similar to that of rat plasma; standard Zn solutions (0·1, 0·4, 1·0 ppm) were accordingly prepared to contain 14% sucrose.

Table 1. *Viscosity of pooled rat plasma and sucrose solutions expressed as flow times*

(Mean values with their standard errors for five determinations on each sample performed at 25° in an Ostwald viscometer)

Solution	Flow time (s)
Plasma	340 ± 0·316
Sucrose	
11%	323 ± 0·806
12%	327 ± 0·316
13%	333 ± 0·707
14%	338 ± 0·552
15%	346 ± 0·387
16%	356 ± 0·316

Comparison of Zn assay methods

Pooled rat plasma was assayed by three methods. First, by the method of Prasad, Oberleas & Halsted (1965) involving the precipitation of plasma proteins with trichloroacetic acid before assay. Secondly, by a procedure involving preliminary dilution (1:4) of the sample with distilled water (Dreosti *et al.* 1968) and, thirdly, by a method using untreated plasma and standard Zn solutions containing 14% sucrose. The findings presented in Table 2 indicate close agreement between the last two methods; the protein precipitation technique, however, yielded somewhat higher results.

Table 2. *Comparison of methods for the estimation of zinc in plasma*

(Mean values with their standard errors for five determinations by each method)

Method	Estimated Zn concentration in pooled plasma sample (μg/ml)
Protein precipitation	1·62 ± 0·024
Aqueous dilution (1:4)	1·25 ± 0·024
Sucrose standard	1·30 ± 0·025

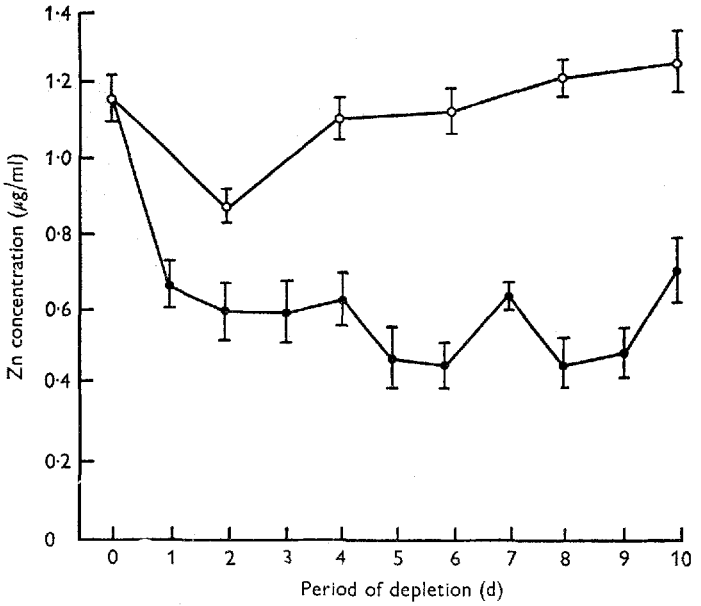

Fig. 1. Concentration of plasma zinc in groups of rats after increasing periods on a Zn-deficient (< 0·25 ppm) or a Zn-supplemented (60 ppm) diet. ○—○, controls; ●—●, Zn-depleted. The vertical bars represent the standard errors.

Plasma Zn during progressive Zn depletion

Plasma Zn concentrations were determined in groups of five to nine rats which were killed on successive days of dietary Zn depletion. Similar determinations were performed on control animals every 2nd day. The results are presented in Fig. 1 and they indicate that the concentration of plasma Zn fell by approximately 40% within

the 1st day of dietary restriction. Thereafter, the fall became less marked, and after 5 d the concentrations stabilized at about 50% of the control values. Plasma Zn concentrations in the Zn-supplemented animals also fell by about 23% after 2 d on the experimental diet but returned to original levels by day 4.

Response of plasma Zn to the oral administration of zinc sulphate

Groups of six rats were maintained on the Zn-deficient diet for 2 d and 8 d periods. Thereafter, the animals were dosed by mouth with 100 μg Zn as zinc sulphate in 1 ml water. Plasma Zn concentrations were determined 1·75 h later. Control animals were treated in the same way. Plasma Zn concentrations rose markedly in the depleted animals after dosing and showed increases of 292% in the 2 d group and 426% in the 8 d group (Table 3). Plasma Zn concentrations in control animals rose by about 30% following dosing.

Table 3. *Concentration of plasma zinc 1·75 h after a single oral dose of 100 μg zinc sulphate in rats given a Zn-deficient or a Zn-supplemented diet*

(Mean values with their standard errors for six animals in each group)

Zn status	Period on diet (d)	Dose (μg Zn)	Plasma Zn (μg/ml)
Control	2	—	0·91 ± 0·059
	8	—	1·11 ± 0·075
	2	100	1·24 ± 0·164
	8	100	1·50 ± 0·137
Deficient	2	—	0·61 ± 0·050
	8	—	0·45 ± 0·064
	2	100	2·41 ± 0·114
	8	100	2·37 ± 0·513

Time-course of the plasma Zn response following oral dosing

Groups of two to three rats were deprived of Zn for 2 d before receiving a single dose of 100 μg Zn by mouth. Thereafter, plasma Zn concentrations were determined on different groups at increasing time intervals from 30 min. The results are presented in Fig. 2 and they indicate that plasma Zn concentrations reached a maximum in both the deficient and the control animals 1·75 h after dosing, although in the deficient group the magnitude of the response was far greater (228%) than in the controls (40%).

Response of plasma Zn to the oral administration of varying concentrations of zinc sulphate

Groups of two to three rats were deprived of Zn for 2 d before receiving single oral doses of Zn in varying concentrations. Control animals were dosed similarly and plasma Zn concentrations of all groups were determined 1·75 h after dosing. The plasma Zn concentration did not vary significantly with the oral dose of Zn, and in most instances was higher in the deficient animals than in the controls (Table 4).

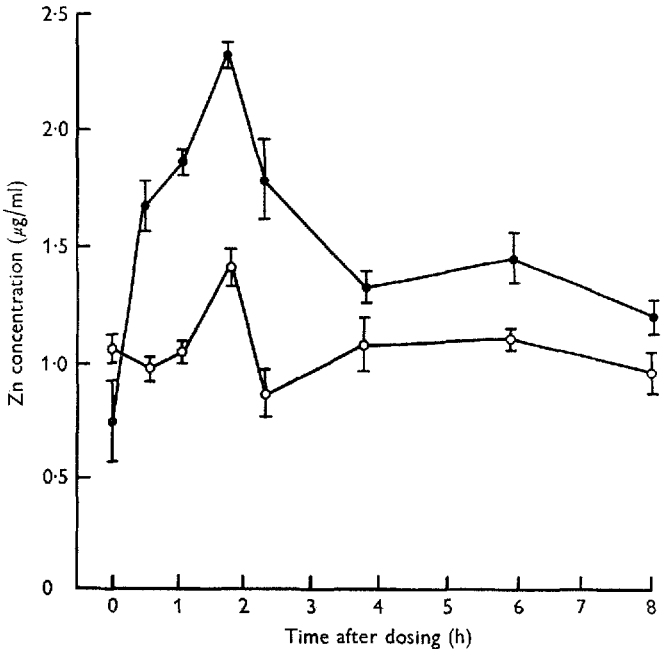

Fig. 2. Time-course of plasma zinc response in groups of rats after oral dosing with 100 μg Zn. ○—○, controls; ●—●, Zn-depleted. The vertical bars represent the standard errors.

Table 4. *Concentration of plasma zinc 1·75 h after varying doses of zinc sulphate in rats given a Zn-deficient or a Zn-supplemented diet for 2 d before dosing*

(Values given for individual animals)

Dose (μg Zn)	Control	Zn-deficient
20	0·63, 1·63, 1·75	1·40, 2·25, 3·00
50	1·71, 1·71	1·78, 3·25
75	1·00, 1·17, 1·17	1·25, 1·37, 1·65
100	1·56, 1·60, 1·60	2·13, 2·20, 2·25
150	1·17, 1·25, 1·43	1·13, 1·90, 2·08
200	1·08, 1·16, 1·38	2·00, 2·34

Table 5. *Concentration of plasma zinc 1·75 h after a single oral dose of 100 μg zinc sulphate in rats given diets containing various levels of Zn for 2 d before dosing*

(Mean values with their standard errors for six animals in each group)

Zn content of diet (μg/g)	Plasma Zn (μg/ml)
0·25	2·12 ± 0·065
1·0	2·11 ± 0·110
3·0	2·25 ± 0·014
5·0	1·91 ± 0·113
7·0	1·77 ± 0·117
10·0	1·07 ± 0·078
25·0	1·10 ± 0·100
60·0	1·10 ± 0·071

Response of plasma Zn to oral dosing in rats at various stages of Zn depletion

Groups of six rats were maintained for 2 d on the basal Zn-deficient diet supplemented with increasing amounts of Zn as zinc sulphate. Thereafter, all animals received 100 μg Zn in 1 ml water by mouth and plasma Zn concentrations were determined 1·75 h later (Table 5). The characteristic response associated with Zn deficiency was observed only in those animals receiving less than 10 ppm of dietary Zn.

DISCUSSION

Viscosity and sucrose standards

Although for many reasons plasma provides the tissue of choice for Zn analyses, its viscosity renders it unsuitable for direct comparison with aqueous standards. Deproteinization of plasma samples (Prasad *et al.* 1965) or their dilution with distilled water (Dreosti *et al.* 1968) and dilute hydrochloric acid (Girard, 1968; Dawson & Walker, 1969) have been used with some success to overcome the effect of protein on sample nebulization. However, deproteinization is time-consuming and increases the risk of contamination, while the dilution techniques reduce the amount of Zn in plasma, especially in samples taken from Zn-deficient animals, to concentrations ranging from 0·1 to 0·3 ppm which are not conveniently measured by most atomic absorption spectrophotometers.

In the present study, attention was accordingly focused on the use of undiluted plasma together with standard Zn solutions of similar viscosity. Dextran has previously been suggested (Hackley, Smith & Halsted, 1968) to raise the viscosity of the standard solutions, although with this material the problems of contamination and polymer heterogeneity should not be underemphasized. We consider sucrose to be a better choice of solute as it is readily obtained in highly purified form and it is completely homogeneous. Viscosity measurements at 25° showed that a 14% sucrose solution had a viscosity similar to that of pooled rat plasma. A comparison of the deproteinization, dilution and sucrose standard methods for plasma Zn measurements indicates a close agreement between the dilution and the sucrose standard methods, whereas results from the protein precipitation technique appeared to be somewhat higher (24–29%). Possibly, the disparity reflects some degree of contamination in the latter samples, which highlights the need for a minimum of sample manipulation before assay.

Plasma Zn during progressive Zn depletion

It has previously been reported that in calves and lambs (Mills *et al.* 1967) and in rats (Dreosti *et al.* 1968) plasma Zn concentration falls rapidly (40–60%) following dietary restriction. The present findings confirm these reports and indicate that with Zn, unlike with other elements, a low plasma concentration rapidly follows a reduced dietary intake. The recent findings of Parisi & Vallee (1970) have shown that 30–40% of the plasma Zn is firmly bound to an α_2-macroglobulin, whereas 60–70% is loosely bound to the albumin. It is probably the loosely bound fraction that accounts for the

initial rapid fall in Zn concentration, whereas the firmly bound portion persists even during periods of Zn depletion.

In the present studies (Fig. 1, Table 3) plasma Zn concentrations in the control rats were found to fall temporarily by about 23% after 2 d. Possibly, the decline reflects an initial effect of the soya-bean meal on the availability of Zn from the control ration. The use of soya-bean meal and not isolated soya-bean protein in the present diets would have introduced high levels of plant material and possibly phytates into the diet.

The sensitivity of plasma Zn concentration to dietary intake is of considerable value as a diagnostic tool. However, the inherent variability between individuals usually renders a diagnosis extremely difficult – especially at the incipient or marginal level. A method was therefore sought whereby the Zn status of a single animal could be determined without recourse to extensive preliminary sampling.

Response of plasma Zn to the oral administration of zinc sulphate

Earlier work on the diagnosis of copper deficiency in rats (Dreosti & Quicke, 1968) has pointed to the practicality of a technique based on the response of plasma concentrations to a single oral dose of copper. In the present studies a similar response was observed with Zn-depleted animals. Large rises in plasma Zn concentrations occurred following dosing of the deficient animals but a considerably smaller response was noted with the controls.

The response extended over a wide range of dose levels and notwithstanding the present uncertainty (Sandstead, 1968) may support the view that enhanced absorption of Zn occurs in depleted animals (Pate, Miller, Blackmon & Gentry, 1970). The magnitude of the response, however, was not increased by increasing the dose, which may suggest that, if absorption was increased, the mechanism of uptake may have become limiting at the lower dose levels. Also, the response did not occur in rats fed on a diet containing 10 ppm or more of Zn before dosing, although diets providing less than this amount elicited the response. The findings are of interest in view of the dietary requirements of rats suggested by other workers which range from 8 to 18 ppm (Underwood, 1962).

The present findings suggest that the difference in plasma Zn concentrations before and after dosing with Zn could provide a useful method for the detection of a subnormal Zn status in a particular individual, without the need for extensive determinations of species or breed norms and the natural variation between individuals within the group. The applicability of these findings to other species, especially to ruminants, must await further investigation, but it is hoped that these findings with rats will pave the way for the development of a more effective method for the detection of a marginal Zn depletion in farm animals.

The authors wish to express their thanks to Libra Chemicals Pty Ltd and to the Mooi River Farmers' Association for the research bursaries awarded to P.J.W. and P.C.G. for 1970–1.

REFERENCES

Davis, P. N., Norris, L. C. & Kratzer, F. H. (1962). *J. Nutr.* **78**, 445.
Dawson, J. B. & Walker, B. E. (1969). *Clinica chim. Acta* **26**, 465.
Dreosti, I. E. & Quicke, G. V. (1968). *Br. J. Nutr.* **22**, 1.
Dreosti, I. E., Tao, S. & Hurley, L. S. (1968). *Proc. Soc. exp. Biol. Med.* **128**, 169.
Girard, M. L. (1968). *Clinica chim. Acta* **20**, 243.
Hackley, M. B., Smith, J. C. & Halsted, J. A. (1968). *Clin. Chem.* **14**, 1.
Hurley, L. S. (1969). *Am. J. clin. Nutr.* **22**, 1332.
Hurley, L. S. & Swenerton, H. (1966). *Proc. Soc. exp. Biol. Med.* **123**, 692.
Mills, C. F., Dalgarno, A. C., Williams, R. B. & Quarterman, J. (1967). *Br. J. Nutr.* **21**, 751.
Mills, C. F., Quarterman, J., Chesters, J. K., Williams, R. B. & Dalgarno, A. C. (1969). *Am. J. clin. Nutr.* **22**, 1240.
Parisi, A. F. & Vallee, B. L. (1970). *Biochemistry, Easton* **9**, 2421.
Pate, F. M., Miller, W. J., Blackmon, D. M. & Gentry, R. P. (1970). *J. Nutr.* **100**, 1259.
Prasad, A. S., Oberleas, D. & Halsted, J. A. (1965). *J. Lab. clin. Med.* **66**, 508.
Sandstead, H. H. (1968). *Nutrition Today* **3**, 12.
Swenerton, H. & Hurley, L. S. (1968). *J. Nutr.* **95**, 8.
Underwood, E. J. (1962). *Trace Elements in Human and Animal Nutrition* 2nd ed. New York: Academic Press Inc.

Printed in Great Britain