Serum alkaline phosphatase activity during zinc deficiency and long-term inflammatory stress

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Abstract

A decrease in serum zinc can be caused by a real zinc deficiency but can also be caused by an apparent zinc deficiency, e.g. in inflammatory stress. The aim of this study was to evaluate the diagnostic power of serum alkaline phosphatase (AP) activity in the discrimination between pathophysiologic states of “real” and “apparent” zinc deficiency. A decrease in serum zinc was induced in growing and adult rats, by providing a diet low in zinc and by causing inflammatory stress. AP activity was determined using reagents low or enriched in zinc. Serum AP was decreased in zinc-deficient adult rats (P < 0.01). In zinc-deficient growing rats AP activity was not different from normal rats but AP activity decreased rapidly. In the same growing rats a significant difference was found in AP activities determined using buffers low and enriched in zinc (P < 0.001). After inducing inflammatory stress a decrease in AP activity (P < 0.01) and serum zinc (P < 0.001) was seen during the first few days. After the initial phase of inflammation AP activity normalized, serum zinc showed a rise which after correction for the decrease in serum albumin reached the level of the control rats. A difference in AP activity in buffers low and enriched in zinc was observed only during the first few days after induction of inflammatory stress (P < 0.001). Probably the method of measurement of the difference in

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enzyme activity, using buffers low and enriched in zinc, can be used as an indication for zinc deficiency in situations with changing AP enzyme concentrations. AP activity is decreased during the initial phase of inflammatory stress due to a decrease in serum zinc.

**Keywords:** Zinc; Inflammatory stress; Rats; Serum alkaline phosphatase activity; Zinc deficiency; Serum albumin

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### 1. Introduction

Zinc deficiency is a serious disorder expressed by a variety of symptoms such as delayed wound healing, dysfunction of the immune system, anorexia, decreased protein synthesis, night blindness, skin diseases, hair loss and growth retardation [1].

Usually the serum zinc concentration is used to determine zinc status [2], but the serum zinc concentration is not only decreased in real zinc deficiency, but also in stress [3]. During stress serum zinc is redistributed from the serum into the liver. In addition, the serum albumin concentration will decrease and since about 75% of serum zinc is bound to albumin [4], a decrease of serum albumin will result in a decrease of serum zinc. Because the decrease of serum zinc during stressful events does not result in symptoms characteristic of real zinc deficiency, this situation is called apparent zinc deficiency. Thus, serum zinc is not the perfect parameter for zinc status. Several other methods are used to determine zinc status, e.g. determination of zinc concentrations in urine [5], in hair [6], and in blood cells [7]. All of these methods have disadvantages.

Zinc is a component of more than 200 enzymes [8]. In case of zinc deficiency, zinc-containing metallo-enzymes often do not function optimally. Alkaline phosphatase (AP) is one of these zinc-dependent metallo-enzymes. Serum AP activity may be used to determine zinc status [9], but AP activity is also influenced by other factors, for instance growth. The aim of this study was to investigate the influence of a physiologically induced decrease in the serum zinc concentration, as in dietary-induced zinc deficiency and inflammatory stress, on serum AP activity in growing and adult rats. The concentration of zinc in the reagents influences the determination of AP activity [10–12]. In general for complete expression of serum AP activity a reagent supplemented with zinc is used. We made the hypothesis that, in case of real zinc deficiency, the difference in AP activity using buffers low and enriched in zinc is greater than in normal situations.
2. Materials and methods

2.1 Animals

In all experiments male Wistar rats were used. The rats were housed separately in stainless steel metabolic cages. Before the adaptation period the rats were fed commercial chow. The diet on which the rats were raised contained variable amounts of zinc (<2, 12 or 92 mg/kg; IRI-OB [13]). In Experiments 1, 2, and 3, a diet containing 12 mg/kg zinc was given from 2 weeks before the start of the experiments, to adapt the animals to the diet and the metabolic cages. The zinc concentration of 12 mg/kg is considered sufficient to maintain a normal zinc status [14]. All blood samples were taken under ether anaesthesia by heart puncture. The guidelines for the care and use of laboratory animals in The Netherlands were followed.

2.2 Design of the experiments

Five experiments were performed to study the influence of physiologically induced variations in zinc concentration on the AP activity. In the first 2 experiments zinc deficiency was induced in adult and, respectively, growing rats. The AP activity was studied. In the first experiment also the $^{65}$Zn retention was determined as a gold standard for zinc status. In the third experiment adult rats were made zinc deficient and the AP activity was determined in buffers low and enriched in zinc. Because in the first experiment the effect of zinc supplementation in zinc deficiency was investigated, the fourth experiment was designed to study the influence of zinc supplementation in rats on a diet with a normal or a high zinc content. In the fifth experiment inflammatory stress was induced.

2.2.1 Experiment 1: zinc deficiency in adult rats, AP activity and the effects of zinc supplementation

After adaptation (see above) 16 male rats (300 g) were injected i.p. with 74 kBq (2 μCi) $^{65}$Zn (day 0). Eight rats received a diet with an adequate amount of zinc (IRI-OB; 12 mg/kg Zn), 8 others were fed a zinc-deficient diet (<2 mg/kg Zn). After 1 month all rats received a diet with an adequate amount of zinc (12 mg/kg). The control group received pair feeding throughout the experiment. Blood (3 ml) was taken under ether anaesthesia by heart puncture on days 0, 7, 14, 21, 28 and 36. The serum was analyzed for zinc and magnesium concentration, albumin and for AP activity, in a buffer enriched in zinc. The total body retention of i.p. administered $^{65}$Zn served as reference for the degree of zinc deficiency.
Total body counting was performed after 4 h and on days 0, 1, 2, 5, 9, 14, 21, 28 and 35. The total body retention at day 1 was taken as 100%.

2.2.2. Experiment 2: zinc deficiency in growing rats, AP activity using buffers low and enriched in zinc

This experiment was identical to Experiment 1, but it was performed in young rats with a body weight of 100 g at the start of the adaptation period. Blood samples were taken at days 0, 17, 24, 37, 51, 65 for serum zinc and albumin assay, and for AP activity determination with reagents low or enriched in zinc.

2.2.3. Experiment 3: zinc deficiency in adult rats, AP activity using buffers low and enriched in zinc

The difference was that serum AP activity was determined with reagents low or enriched in zinc content. Blood was withdrawn on days 0, 3, 7, 14, 21, and 28.

2.2.4. Experiment 4: zinc supplementation in normal rats, AP activity

After an adaptation period of 2 weeks, 32 Wistar rats (250 g) were divided into 2 groups. Sixteen rats received a diet with an adequate amount of zinc (IRI-OB; 12 mg/kg Zn). Sixteen others received a diet enriched in zinc (92 mg/kg Zn). After the first day 8 rats in both groups received a supplementation of 10 mg/kg of zinc (as zinc acetate) in the diet. Blood (3ml) was taken under anaesthesia by heart puncture on days 0, 7, and 14. The serum was analyzed for zinc, magnesium and albumin concentration and for AP activity determined with reagents low or enriched in zinc.

2.2.5. Experiment 5: inflammatory stress, AP activity using buffers low and enriched in zinc

After an adaptation period of 7 days in which 64 rats, with a mean body weight of 200 g, were housed separately in stainless steel metabolic cages and raised on a standard IRI-OB diet, rats were divided into 2 groups of 32 animals of equal mean body weight. To induce stress in one of these groups turpentine was injected intramuscularly in a dose of 0.25 ml into both hind legs to induce a long-standing inflammation. Before turpentine was injected, blood was collected from 4 rats in the inflammation group and 4 rats in the control group. Four hours, 1, 2, 3, 4, 7 and 14 days after the turpentine injection 2 ml blood were taken under ether anaesthesia by heart puncture from 4 rats in the inflammation group and from 4 rats in the control group, for determination of serum zinc, magnesium and...
albumin concentrations and determination of AP activity with reagents low or enriched in zinc. The control group was pair fed throughout the experiment. The total body retention of i.p. injected $^{65}$Zn served as a control to determine if zinc deficiency occurred. All the rats were injected i.p. with 74 kBq (2 µCi) $^{65}$Zn 7 days before the turpentine injection. The total body activity at the time of the turpentine injection was taken as 100%. Total body counting was performed after 4 h and on days 1, 2, 3, 4, 7, and 14.

2.3. Materials
The diet (IRI-OB) was produced by Hope Farms (Woerden, The Netherlands) using components low in zinc (<2 mg/kg Zn, [13]). In the zinc-sufficient diet zinc was added as sulphate to a final zinc concentration of 12 mg/kg. Sufficient quantities of other trace elements were also added. The Cu concentration in both versions of the diet was 9 mg/kg.

Stainless steel needles and plastic syringes (Monoject, Sherwood, USA) were used. All solutions were prepared with deionized distilled water (resistance >15 MΩ), processed through a Millipore system (Bedford, MA).

Only polypropylene pipette tips, plastic tubes (15 ml polystyrene, Greiner, Solingen, Germany and 50 ml polypropylene, Falcon, Becton Dickinson and Company, Lincoln Park, NJ) and bottles (0.8 l, NUNC, Roskilde, Denmark) were used. All these materials were free of zinc contamination. All chemicals were of analytical grade (Merck, Darmstadt, Germany).

2.4. Determination of zinc
All the determinations of zinc and AP activity were performed in a blind fashion. Zinc was determined by flame atomic absorption spectrophotometry (AAS; Perkin Elmer 5000; Norwalk, CT). The lower limit of detection was 0.28 µmol/l and the response was linear till at least 15 µmol/l. Standard reference containing ZnCl$_2$ (Tritisol, Merck, Darmstadt, Germany) was used in the concentrations expected to be found in the samples. No detectable zinc could be found in solutions and in deionized water used for the analytical experiments.

2.5. Determination of AP activity
AP activity was determined at 30°C according to recommendations of the IFCC [15], except for the modification of the zinc concentration. A Multistat III centrifugal analyzer (Instrumentation Laboratory, IJsselstein, The Netherlands) was used. 4-Nitrophenylphosphate and 2-amino-2-methyl-1-propanol (AMP) were provided by Instruchemie (Hilversum,
The Netherlands). The AMP contained a relatively low concentration of zinc (1.6 \mu mol/l) and is used in clinical practice. Appropriate zinc supplementation for the zinc-enriched modification of the AP activity determination resulted in a zinc concentration of 1.0 mmol/l in the test assay mixture.

AMP buffers contain a chelator [16], which inhibits the activity of AP by removing zinc. By addition of zinc, this inactivation can be reversed. If high concentrations of the chelator are present in the buffer, more zinc should be added to compensate for the zinc-sequestering properties of the chelator. Thus the increase in activity of AP by zinc is in part due to the counteraction of the chelator and in part because zinc is a structural component in the enzyme. In the buffer used in the present experiments only a slight contamination was found using the method described by Derks et al. [17], implying that the low zinc concentration of the AMP buffer was valid. A buffer with a low zinc concentration was necessary to be able to detect small variations in serum zinc concentration by variations in the activity of the enzyme. If high concentrations of zinc were present in the buffer, small variations in the zinc concentration of the serum would not result in a variation in enzyme activity.

Final concentrations of the other components in the test mixture were: AMP 350 mmol/l, 4-nitrophenylphosphate 16 mmol/l, Na 140 mmol/l, Cl 160 mmol/l, phosphate 0.4 mmol/l, pH 10.3, magnesium as magnesium acetate 2.0 mmol/l.

2.6. Total body counting

The radio tracer \(^{65}\text{Zn}\) was supplied by Amersham International (Amersham, Buckinghamshire, UK, code ZAS 2, 3.7-37 GBq per mg Zn as chloride in 0.1 mol/l HCl). Total body radioactivity of the rats was counted with a shadow shield counter consisting of 2 TI-activated NaI crystals (4 x 6") connected to a Nuclear Data multi-channel analyzer N.D. 60 A (Nuclear Data; Schaumburg, IL).

2.7. Calculations

2.7.1. Biological half-life of the \(^{65}\text{Zn}\) total body retention

The biological half-life (T\(_{1/2}\)) of \(^{65}\text{Zn}\) was calculated from the total body retention in per cent measured as a function of time.

From the slope (\(\lambda\)) of the linear part of the line, which is calculated by the least square method from the natural logarithm of the total body data, the biological half-life (T\(_{1/2}\)) of the \(^{65}\text{Zn}\) was calculated using \(T_{1/2} = \ln 2/\lambda\).
2.7.2. Estimation of the corrected serum zinc concentration in serum during stress

In case of inflammation the serum zinc concentration can be corrected for the decrease in serum albumin by increasing that part of the serum zinc concentration that is bound to albumin, to the same extent as the concentration of albumin in the inflammation group has to be increased to reach the level of the control group. That part of the total serum zinc, that is transported by albumin, should be increased by the factor Alb control/Alb inflammation. The corrected serum zinc concentration can be calculated by the equation:

\[
\text{Corr Zn tot (inflam)} = 0.25 \times \text{Zn tot (control)} + \frac{\text{Alb control}}{\text{Alb inflam}} \times ((\text{Zn tot (inflam)}) - (0.25 \times \text{Zn tot (control)}))
\]

where Zn tot (control) = total serum zinc concentration of the control group; Corr Zn tot (inflam) = corrected serum zinc concentration of the inflammation group; Alb control = serum albumin of the control group; Alb inflam = serum albumin of the inflammation group; Zn tot (inflam) = total serum zinc concentration of the inflammation group.

The factor 0.25 represents the fraction of the serum zinc concentration that is mainly bound to the stable fraction of alpha macroglobulin, in rat \( \alpha-1 \)-macroglobulin [18] and in human \( \alpha-2 \)-macroglobulin. The concentration of this protein is not significantly changed by stress [18,19] and affinity of zinc to this protein is high [20], so that the number of zinc molecules transported by \( \alpha-1 \)-macroglobulin in the inflammation group will be equal to that of the control group. Using this equation we postulate that the binding of zinc to the binding proteins (the number of zinc molecules transported by one protein molecule), in the normal situation and in inflammation, will be similar, and that zinc bound to \( \alpha-1 \)-macroglobulin is not affected by inflammation. If a difference still remains between the corrected serum zinc concentration and the serum zinc concentration of the control group this will be due to other causes, e.g. redistribution of serum zinc into the liver.

2.7.3. Correction of AP activity

A difference was seen between AP activity in the serum of rats determined with buffers low and enriched in zinc content. The results are provided without correction unless stated otherwise. To calculate the difference between both methods the assumption was made that the AP activities should be identical on day 0. The AP activity determined using
a buffer low in zinc was corrected by using the following equation for each serum sample:

\[
\text{Corr AP} = \frac{\text{mean AP high Zn at day 0}}{\text{mean AP low Zn at day 0}} \times \text{AP low Zn}
\]

2.7.4. Statistics

The Wilcoxon rank sum test was used to compare the results in both groups of rats during a period of the experiment. The Student \( t \)-test was performed on the data collected at one time-point; differences between 2 groups were considered significant if \( P < 0.05 \) (two tails).

3. Results

3.1. Experiment 1

Normally in adult rats the total body retention of \( ^{65}\text{Zn} \) is calculated as a percentage of injected dose immediately after administration. Because 2 rats lost a significantly larger quantity of \( ^{65}\text{Zn} \) (52 and 36%) compared to the other rats (5.55% S.D. 4.42%) during the first 24 h, the total body retention at day 1 is taken as 100%. Probably this greater loss can be explained by a partial injection of the \( ^{65}\text{Zn} \) into the gut. When plotting the natural logarithm of the total body data of Experiment 1 vs. time, a linear relation was found from day 7 onwards. Zinc-deficient rats retained more \( ^{65}\text{Zn} \) compared to control rats (T\( _{1/2} \) = 176 days vs. T\( _{1/2} \) = 51 days; Fig. 1a), confirming that these animals indeed had become zinc deficient.

Serum zinc and AP activity decreased gradually in the zinc-deficient rats (zinc: 11.7 vs. 29.3 \( \mu \text{mol/l} \), \( P < 0.001 \) on day 21, Fig. 1b; AP: 60 vs. 77 U/l, \( P < 0.01 \), Fig. 1c). On day 28 the serum zinc concentration was 40% of that of control rats. No change was seen in the serum albumin concentration in the zinc-deficient rats. After 1 month zinc was added to the diet. Serum zinc and AP activity were normalized after 1 week.

3.2. Experiment 2

In growing rats on a zinc-deficient diet, serum zinc gradually decreased to 8.0 \( \mu \text{mol/l} \) after 37 days (control group 24.7 \( \mu \text{mol/l} \), \( P < 0.001 \); Fig. 2a). No significant difference was observed in the activity of AP between normal and zinc-deficient rats (Fig. 2b), but the activity decreased rapidly in both groups. In the zinc-deficient rats the lowest value was reached later.
Fig. 1. Zinc deficiency experiment in adult rats. (a) Total body retention of $^{65}\text{Zn}$ in zinc-deficient adult rats (diet <2 mg/kg; •) and control rats (12 mg/kg; O). The total body retention of $^{65}\text{Zn}$ 1 day after i.p. injection was taken as 100%. The error bars indicate standard deviations (**$P = <0.01$; ***$P = <0.001$). (b) Serum zinc in zinc-deficient adult rats (diet <2 mg/kg; •) and control rats (12 mg/kg; O; **$P = <0.01$; ***$P = <0.001$). (c) Serum AP activity in zinc-deficient adult rats (diet <2 mg/kg; •) and control rats (12 mg/kg; O; **$P = <0.01$; ***$P = <0.001$).
The activity was also determined using buffers low and enriched in zinc. At the first day (day 0) the difference between the AP activity using buffers with a low and an enriched zinc content was, in the zinc-deficient group, 21 U/l, and in the control group, 24 U/l. Serum AP activity using both methods should by definition be identical at day 0. Using the equation mentioned in Section 2.7, the AP activity using a buffer low in zinc was corrected.

The AP activity in zinc-deficient rats, determined using a buffer low in zinc and a buffer enriched in zinc is shown (Fig. 3a). The reagents low in
Fig. 3. Zinc deficiency experiment in growing rats. (a) Serum AP activity in zinc-deficient rats, using buffers low (●) and enriched (○) in zinc. The error bars indicate standard deviations (*P = <0.05). (b) Serum AP activity in control rats, using buffers low (●) and enriched (○) in zinc. (c) Difference in AP activity by using buffers low and enriched in zinc, between zinc-deficient rats (●) and control rats (○) (*P = <0.05, **P = <0.01; ***P = <0.001).
zinc result in a lower AP activity during the study. In Fig. 3b the same is shown for the control rats. Comparing Figs. 3a and b the difference between both methods is greater in zinc-deficient rats than control rats. In Fig. 3c this difference is shown. This difference was highly significant at individual points after inducing zinc deficiency and for the total period using the Wilcoxon rank sum test ($P < 0.001$).

3.3. Experiment 3

Because of the results in Experiment 2 the difference in AP activity using buffers low and enriched in zinc was also studied in adult rats. The results of this experiment confirmed the results in Experiment 1, especially the serum zinc and AP activity.

After correction for the difference in AP activity with the reagents low and enriched in zinc at day 0, a significant difference was observed in AP activity for the total period using the Wilcoxon rank sum test ($P = 0.01$) and for individual sample times on day 20 (Fig. 4).

3.4. Experiment 4

In this experiment the results of zinc supplementation in normal quantities was studied in rats raised on a diet with sufficient zinc and on a diet rich in zinc. No change in time or difference between the group supplemented by extra zinc and the control group was observed in serum zinc concentration and in AP activity.

![Fig. 4. Zinc deficiency experiment in adult rats. Difference in AP activity by using buffers low and enriched in zinc, between zinc-deficient adult rats (●) and control rats (○). A significant difference was observed using a Wilcoxon rank sum test, and on day 20 using a Student $t$-test. The error bars indicate standard deviations (***$P = <0.01$).](image)
3.5. Experiment 5

The total body retention of $^{65}$Zn (Fig. 5a) showed no difference between the groups, indicating that zinc deficiency did not occur during inflammatory stress (inflammation group $T_{1/2} = 34$ days, control group $T_{1/2} = 31.5$ days). None of the rats showed symptoms of zinc deficiency, such as loss of hair. The serum albumin concentration decreased during the first day. Thereafter a constant difference persisted ($23.8$ vs. $31.3$ g/l, $P < 0.0005$; Fig. 5b). Using a Wilcoxon rank sum test a highly significant difference was calculated if all the data of both groups were compared ($P < 0.0001$). Simultaneously during the first day a considerable decrease in serum zinc concentration was seen in the rats with stress (day 1: inflammation group $10.3 \, \mu$mol/l and control group $23.0 \, \mu$mol/l, $P = 0.002$; Fig. 5c). After the first few days a smaller difference persisted. A Wilcoxon rank sum test was performed for comparison of the independent results of both groups of rats on all the data collected during the experiment. A highly significant difference was calculated ($P < 0.0001$). When the serum zinc concentration of the rats with an inflammation was corrected for the decrease in serum albumin using the equation described in Materials and methods, the differences between the groups largely disappeared, especially after the third day (Fig. 5c). A decrease in AP activity was only seen on the first 2 days ($71$ vs. $115$ U/l, $P < 0.05$; Fig. 6a). Using the same method described in Section 2.7 and applied in Experiments 2 and 3, a significant difference was also observed in AP activity determined using buffers low and enriched in zinc. This difference largely disappeared after the fourth day (Fig. 6b). During these experiments no changes in serum magnesium concentrations were demonstrated.

4. Discussion

The total body retention of $^{65}$Zn as a function of time served as an indication of zinc status. In Experiment 1, in which adult rats were made zinc deficient, the $^{65}$Zn total body retention confirmed that the animals had become zinc deficient. To detect eventual changes in zinc status during the first hours and days of the inflammatory stress experiment (Experiment 5), we preferred a linear decrease in the total body retention, so $^{65}$Zn was injected 7 days before the turpentine injection. During the inflammatory stress experiment no difference in $^{65}$Zn retention was observed between the rats with inflammatory stress and the control rats, indicating that no zinc deficiency occurred.

After introduction of a zinc-deficient diet the serum zinc concentration fell in adult, and more impressively in growing, rats. Normalization of the diet resulted in an increase in serum zinc concentration. Supplementation
Fig. 5. Inflammatory stress experiment. (a) Total body retention of $^{65}$Zn in rats with an inflammation (●) and control rats (○) during the experiment. (b) Serum albumin concentrations in rats with an inflammation (●) and control rats (○) during the experiment ($***P < 0.001$). (c) Serum zinc concentrations in rats with an inflammation (●) and control rats (○) and serum zinc concentrations corrected for the decrease in serum albumin (▲) during the experiment. The error bars indicate standard deviations ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$, for serum zinc in rats with an inflammation compared with control rats, and $*P < 0.05$; $**P < 0.01$ for corrected serum zinc in rats with an inflammation compared with control rats).
with the same quantity of zinc in the diet of control rats did not influence the serum zinc concentrations.

After induction of inflammatory stress the serum zinc concentration decreased rapidly. This decrease continued during the first 3 days, after which a smaller difference persisted. The serum albumin concentration decreased within 1 day to a level which remained stable throughout the
experiment. After correction of the serum zinc concentration for the decrease in serum albumin, no difference in serum zinc concentration was observed after the second day. This indicates that after the initial phase of inflammatory stress the decrease in serum zinc is mainly due to a decrease in serum albumin. Probably the initial rapid decrease in serum zinc is caused by an increase in zinc uptake by the liver, an observation that has been demonstrated by Solomons et al. [21].

The determination of activity of zinc-dependent enzymes has been used as a method to determine zinc status, e.g. serum AP activity [22]. The AP activity is limited by the influence of liver function and bone turnover, factors that influence the concentration of AP enzyme. The advantage of using AP activity as a method to determine zinc status is that the determination of AP activity is possible in every clinical laboratory. Serum AP activity is the result of 2 factors: the protein concentration of the enzyme in the serum and the activity of the enzymatic molecules. The former factor is influenced by release of the enzyme by AP-containing tissues, mainly liver and bone. The latter factor is influenced by the number of zinc and magnesium atoms in the AP enzyme molecule [10-12]. During the experiments no changes in magnesium concentration were observed. The zinc deficiency experiments in adult rats confirmed that serum AP activity is decreased in zinc deficiency. Sandstead et al. [23] demonstrated an increase in serum zinc concentration and AP activity during zinc deprivation in marginally zinc-deficient men. This was not confirmed in rats in the present experiments. The increase in AP activity after zinc supplementation may be an indication of a preceding zinc deficiency (Experiment 1). Our experiments confirmed studies in animals [24-26] and in patients [27,28]. No increase was demonstrated in case of zinc sufficiency (Experiment 4). Kasarkis and Shuna [25] proposed the AP ratio (the magnitude of the increase in AP activity after zinc supplementation) as an indicator of zinc status. Some criticism can be addressed to this method. The results of Experiment 4 demonstrated that this method, the AP ratio, cannot be used to differentiate between rats receiving food with a marginal but sufficient quantity of zinc and rats receiving a diet rich in zinc. In growing rats (Experiment 2), no difference was observed between normal and zinc-deficient rats. In a study in infants the same problem was observed; no correlation was demonstrated between serum zinc and AP activity [29]. During growth the AP activity declines because of a decrease in concentration of the enzyme. In zinc deficiency growth is retarded. Perhaps the concentration of the AP enzyme in the serum remains high in the zinc-deficient rats, which counteracts the decrease in activity of the enzyme due to a decrease in zinc concentration. In the zinc-deficient rats the lowest AP activity value was reached later.
In the inflammatory stress experiment AP activity was decreased during the initial phase of inflammation, showing that AP activity is influenced by inflammatory stress. This is not known from clinical practice [30], although in physiological stress experiments published in veterinary journals a decrease in AP activity has been observed [31] (without explanation). After the initial phase no difference in AP activity was observed in the present experiment, although serum zinc was still decreased. The normalisation of the serum zinc concentration after correction for the decrease in serum albumin indicates that, after the second day, the decrease in serum zinc was mainly due to a decrease in serum albumin. Probably AP activity is influenced by the shift of serum zinc into the liver during the first days after inducing inflammatory stress, but not by an apparent decrease of serum zinc due to a decrease of serum albumin in long-lasting inflammation. AP activity probably reflects the serum concentration of free zinc.

We postulated that in case of zinc deficiency the difference of AP activity, determined in a buffer low and a buffer enriched in zinc, would be greater than in zinc sufficiency. This method would largely bypass the influence of the concentration of the enzyme on the assessment of AP activity and the influence of other minerals such as magnesium, and offers the opportunity of having a instantaneous monitor of zinc status. In our experiments the concentrations in the buffer were chosen within the range of zinc concentrations used in laboratory practice because it is well known that very low (see Materials and methods) and high concentrations in the AMP buffer (several times higher than the concentrations used in clinical practice) added in vitro, will decrease serum AP activity [11,26,32]. The hypothesis was confirmed in Experiment 2 in zinc-deficient growing rats, in Experiment 5 in rats with inflammatory stress, and in Experiment 3 in zinc-deficient adult rats. At individual sampling times this was highly significant in growing rats and rats with inflammatory stress. In zinc-deficient adult rats this difference was smaller due to rather large standard deviations caused by physiological differences and the limited number of animals. The method could be improved by increasing the difference of the AP activity. Essential for this method is the use of an AMP buffer with a low concentration of chelator and consequently low zinc concentration. To our knowledge only one study has suggested that manipulation of the method of determination of AP activity could be used for the diagnosis of zinc deficiency [33]. In this study a method was described in which inactivated AP was reactivated by serum. Also in this study a greater difference was observed in zinc deficiency.

We may conclude that the experiments demonstrate that, in case of a low serum zinc concentration, the difference in AP activity probably can
differentiate between a true zinc deficiency and an apparent zinc deficiency due to a decrease in serum albumin, even if AP enzyme levels are changing. This method should be confirmed in humans.

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