Effects of Zinc Deficiency on Protein Synthesis and Expression of Specific mRNAs in Rat Liver

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The effects of zinc deficiency on protein synthesis and expression of specific mRNAs were assessed in rat liver. Zinc deficiency had no apparent effect on liver weight, protein content, or RNA content when these properties were compared with values obtained using pair-fed rats. However, zinc deficiency resulted in a lower rate of hepatic protein synthesis. The decreased rate of protein synthesis was due to a decrease in the rate of synthesis of proteins retained in the liver, with no apparent change in the synthesis of secreted proteins. Analysis of expression of specific gene products, as assessed by in vitro translation of total RNA followed by two-dimensional gel analysis, showed that the expression of only a few mRNAs was altered by zinc deficiency. The patterns of change in gene expression resulting from zinc deficiency varied from almost complete repression to full expression. In additional studies, cDNA clones to serum retinol-binding protein and transthyretin were used to examine the effect of zinc deficiency on the relative abundance of mRNA for these two proteins. The relative abundance of mRNA for transthyretin was specifically elevated as a result of zinc deficiency. In contrast, the relative abundance of mRNA for hepatic serum retinol-binding protein was increased in both zinc-deficient and pair-fed rats. Therefore, the observed change in mRNA for serum retinol-binding protein was apparently at least in part due to the inanition that accompanies zinc deficiency. Overall, the results suggest that zinc can regulate the synthesis of specific proteins in rat liver through changes in the relative abundance of specific mRNAs.

ZINC IS AN ESSENTIAL trace element for normal growth and metabolism in both animals and humans.[1] The impaired rate of growth associated with zinc deficiency in animals is a consequence of suboptimal synthesis of DNA, RNA, and protein. Zinc deficiency causes a depression of protein synthesis as measured in vitro using isolated perfused rat liver[2] and in cell-free systems prepared from rat liver.[3] The impairment in protein synthesis caused by zinc deficiency is associated with a reduction in hepatic RNA content and abnormal polysome profiles in weanling rats.[4]

Zinc also plays an important role in the metabolism of vitamin A (reviewed in Smith[5] and Solomons and Russell[6]). In rats deprived of dietary zinc, plasma concentrations of retinol decrease,[7-12] whereas liver concentrations reportedly either increase[7] or are unaltered.[10] In contrast, a single intraperitoneal injection of zinc sulfate causes a rapid increase in plasma retinol with a concomitant decrease in hepatic retinol.[13] In addition, the plasma concentration of the specific vitamin A transport protein, serum retinol-binding protein, is depressed in zinc-deficient rats.[1] The basis for the decrease in plasma serum retinol-binding protein resulting from zinc deficiency is unknown. However, studies have shown the secretion of serum retinol-binding protein from liver to be regulated by the availability of retinol.[14] Thus, the possibility is presented that the secretion but not the synthesis of serum retinol-binding protein is impaired in livers from retinol-deficient rats.[15]

In the present study, we have examined the effect of prolonged zinc deficiency on hepatic protein synthesis in weanling rats. In addition, changes in gene expression resulting from zinc deficiency were examined using two approaches. First, isolated total RNA was translated in vitro followed by analysis of the radiolabeled translation products using two-dimensional gel electrophoresis and fluorography. Second, the effect of zinc deficiency on the relative abundance of mRNA for serum retinol-binding protein and transthyretin in rat liver was investigated using specific cDNAs.

MATERIALS AND METHODS

Animals and Experimental Design

Male weanling Long-Evans rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 50 to 60 g were randomly divided into one of three experimental groups and housed individually in stainless steel suspended cages in temperature- and humidity-controlled quarters. All rats were fed a purified diet (Modified AIN-76) containing 20% egg white solids with biotin enrichment.* Rats were randomly divided into groups and fed one of three diets for 6 weeks. The first group of animals received a zinc-deficient (< 1 ppm by analysis) diet (Teklad, Madison, WI) and deionized water; the second group (pair-fed) received zinc-supplemented water (25 mg zinc/L) ad libitum and an amount of zinc-deficient diet equivalent in weight to that consumed by a paired zinc-deficient rat the previous day; and the third group (ad libitum) were allowed free access to diet and zinc-supplemented water. All animals were housed

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*The purified diet was based on an AIN-76 formulation and contained the following in grams per kilogram: egg white solids, 200; cornstarch, 150; sucrose, 501.815; cellulose, 50; AIN-76 salt mix modified to delete zinc, 36.172; AIN-76 vitamin mixture, 10; chlorine bitartrate, 2; biotin, 0.0025; and ethoxyquin, 0.01. The zinc content was 0.8 μg/g diet by analysis. The diet was obtained from Teklad (Madison, WI).

individually in stainless steel, wire-bottomed cages and were isolated from animals used in other studies to avoid environmental trace metal contamination. The animals were maintained in an environment restricted for trace metals similar to that previously described.[16]

To assess zinc deficiency, the zinc content in the femur of experimental animals was determined by atomic absorption spectrophotometry after wet digestion in Ultrex nitric acid (J.T. Baker, Phillipsburg, NJ). After removal of marrow with stainless steel instruments, femurs were subjected to lipid extraction by refluxing in chloroform:methanol (2:1 vol/vol).[17] Bones were subsequently dried at 60°C overnight before weighing. Wet digestion was then performed according to the method of Clegg et al.[18] A Perkin-Elmer Model 403 atomic absorption spectrophotometer (Norwalk, CT) was used to determine absorbancies that were compared with standards (Alfa Products, Danvers, MS) in the range of 0 to 0.1 mg zinc/L.

Isolation of Total RNA

After being fed one of the three diets described above for 6 weeks, rats from each of the groups were anesthetized with sodium pentobarbital (10 mg/100 g body weight). Livers were quickly removed and frozen between aluminum blocks cooled to the temperature of liquid nitrogen. The livers were subsequently powdered under liquid nitrogen and stored in liquid nitrogen. Total RNA was then extracted essentially as described by Chirgwin et al.[19] Approximately 7 g tissue was homogenized in 100 mL of a solution consisting of 4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7.0, 16 mmol/L *N*-laurylsarcosine, 0.1 mol/L β-mercaptoethanol, and 0.1% (vol/vol) antifoam A. The homogenate was centrifuged at 5,800 × *g* for 15 minutes at 10°C. The supernatant was adjusted to pH 5.0 by the addition of 1 mol/L acetic acid, and RNA was precipitated by the addition of absolute ethanol (75 mL/100 mL supernatant) and incubation at −20°C overnight. The RNA was then pelleted by centrifugation at 5,800 × *g* for 10 minutes at −10°C and redissolved in 50 mL 7.5-mol/L guanidine hydrochloride containing 25 mmol/L sodium citrate, pH 7.0, and 5 mmol/L dithiothreitol. Following two additional ethanol precipitations, the residual guanidine hydrochloride was extracted with absolute ethanol. The RNA was then dissolved in sterile water (1 mL/g tissue), and insoluble material was removed by centrifugation. The solution was adjusted to 0.2 mol/L potassium acetate, and the RNA was precipitated by addition of 2 vol ethanol at −20°C. RNA was stored as a suspension in ethanol at −20°C.

Liver Perfusion

Livers were perfused in situ under conditions previously described to measure rates of synthesis of total protein retained in the liver and total protein secreted from the liver.[20] Briefly, livers were perfused at a flow rate of 1.25 mL/min/g liver. The livers were initially flushed with 50 mL perfusate, which was discarded. The remaining 100 mL perfusate was then recirculated through the liver for the remainder of the perfusion period (150 minutes). Following a 30-minute period of equilibration, [^{3}H]leucine (5 μCi/mL, Amersham, Arlington Heights, IL) was added to the perfusate and the perfusion was continued for an additional 2 hours. During the 2 hours of perfusion with [^{3}H]leucine, 3-mL aliquots of the perfusate were periodically removed and centrifuged to remove red blood cells, and the plasma fraction was frozen in liquid nitrogen. Following perfusion, livers were quickly weighed and then immediately frozen between aluminum blocks cooled to the temperature of liquid nitrogen. All samples were stored at −70°C until analyzed. Protein synthesis was determined as trichloroacetic acid–precipitable, protein-bound [^{3}H]leucine and corrected for the specific radioactivity of [^{3}H]leucine in the perfusate.[20] Protein content was determined by the method of Lowry et al.[21] RNA content was determined according to the method of Fleck and Monro.[22,23]

In Vitro Translation

RNA isolated as described above was used to program an mRNA-dependent rabbit reticulocyte lysate translation system (Gibco BRL, Gaithersburg, MD). The instructions included in the kit by the manufacturer were followed essentially without modification. Each assay included the following in a final volume of 60 μL: 20 μL rabbit reticulocyte lysate containing 3.5 mmol/L magnesium chloride, 0.05 mmol/L EDTA, 25 mmol/L KCl, 70 mmol/L NaCl, 0.5 mmol/L dithiothreitol, 25 μmol/L hemin, 50 μg/mL creatine kinase, 1 mmol/L calcium chloride, and 2 mmol/L EGTA; and 6 μL of a reaction mixture containing 250 mmol/L HEPES, pH 7.2, 400 mmol/L KCl, 100 mmol/L creatine phosphate, and 19 amino acids (minus methionine) at 500 μmol/L each. The remaining volume included 0.2 mmol/L spermidine, 180 mmol/L potassium acetate, 5 μCi [^{35}*S*]methionine (Amersham), and 6.4 μg RNA. Rabbit globin mRNA included in the kit was also translated as a control. Before translation, RNA samples were heated to 65°C for 10 minutes and then quick-chilled on ice to eliminate secondary structure. The reaction mixture was incubated at 30°C for 60 minutes. Incorporation of [^{35}*S*]methionine into protein products was measured for each assay by trichloroacetic acid precipitation of an aliquot of the translation mixture.

Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis was performed using a modification of the method described by O'Farrell.[24] Isoelectric focusing was performed in tube gels (4-mm diameter) consisting of 9.5 mol/L urea, 0.625 mol/L acrylamide, 0.75% (wt/vol) diallyltartardiamide, 8% (vol/vol) Nonidet P-40, and 2% ampholytes (Pharmacia Biotech, Piscataway, NJ; a mixture [2:1] of pH 5 to 7 and pH 3.5 to 10 was used). Before isoelectric focusing, the gels were allowed to polymerize for 3 hours. During the polymerization period, RNA was translated in the reticulocyte lysate as described above. The lysate was then diluted with an equal volume of a solution consisting of 139 mmol/L sodium dodecyl sulfate (SDS), 1 mmol/L magnesium chloride, and 100 mmol/L Tris hydrochloride, pH 6.8. Following a 15-minute incubation at room temperature with intermit-

tent mixing, the solution was centrifuged at 245,000 × *g* in a Beckman TL-100.2 rotor (Beckman Instruments, Palo Alto, CA) for 1 hour at room temperature. An aliquot of the supernatant (5 μL) was then precipitated with trichloroacetic acid as a measure of the amount of [^{35}S]methionine incorporated into protein in each assay. The remainder of the supernatant (95 μL) was mixed with 2 vol of a solution consisting of 9.5 mol/L urea, 8% (vol/vol) Nonidet P-40, 0.713 mol/L β-mercaptoethanol, and 2% ampholytes (LKB; a mixture (5.7:1) of pH 5 to 7 and pH 3.5 to 10 was used). Equal amounts of trichloroacetic acid–precipitable radioactivity (≈300,000 cpm) were then applied to the top of the tube gels and carefully overlayed with 1% ampholytes. The gels were then focused for 11,000 V·h using 0.05 mol/L NaOH as cathode buffer and 0.025 mol/L H_3PO_4 as anode buffer as recommended by Duncan and Hershey.[25]

In the second dimension, proteins were separated by molecular weight according to the method of O'Farrell[24] and Sinclair and Rickwood.[26] Following isoelectric focusing, the first-dimension gels were equilibrated for approximately 50 minutes in O'Farrell's[24] equilibration buffer. The tube gels were then placed on top of slab SDS-polyacrylamide gels and sealed in place with warm 1% agarose in equilibration buffer.[24] The slab gels were electrophoresed at 50 mA per gel for 3.5 hours and then stained with Coomassie R-250. Following destaining, the gels were treated with Enhance (DuPont–New England Nuclear, Boston, MA) according to the instructions provided by the manufacturer. The gels were then dried and exposed to Kodak XAR film (Eastman Kodak, Rochester, NY) at −70°C for approximately 10 days.

Fluorograms were scanned with a BioMolecular Dynamics laser scanner (Sunnyvale, CA) connected to a Sun 386i/150 workstation (Huntington, NY). The data were analyzed using PDQuest I and PDQuest II software (PPDI, Huntington Station, NY) for two-dimensional gel analysis. PDQuest II produces an output that includes a quantitation table listing the relative intensities of each spot from each fluorogram and the total quantitation of all the spots from each fluorogram. The value for total quantitation was used to normalize the intensity of individual spots on each fluorogram with respect to the ad libitum control (eg, if the total quantitation of all spots on a fluorogram was 10,000 and the control value was 8,000, then the individual spot intensities on the test fluorogram were multiplied by 0.8). Spot intensities were considered altered between the three groups only if the difference in values was at least twofold between the control and test groups.

Slot-Blot Hybridization Analysis

RNA was immobilized on nitrocellulose using a Minifold II slot-blot system (Schleicher and Schuell, Keene, NH). Before use, the nitrocellulose was wet in 10 × SSC buffer (SSC buffer consists of 0.3 mol/L NaCl and 0.03 mol/L sodium citrate). Note that all solutions were sterilized before use either by autoclaving or by filtration through a 0.22-μm filter. RNA (10 to 25 μg) was dissolved in 100 μL water and diluted with 300 μL of a solution of 6.15 mol/L formaldehyde in 10 × SSC. Before application onto the nitrocellulose membrane, the RNA solution was heated at 65°C for 15 minutes. After loading all the samples into slots in the Minifold II system, vacuum was applied and sample wells were each washed with 400 μL 10 × SSC. RNA was then covalently attached to the nitrocellulose by irradiation of the wet membrane in a UV Stratalinker 1800 (Stratagene, La Jolla, CA) as recommended by the manufacturer.

Prehybridization, hybridization, and washing of the nitrocellulose membrane were performed in a siliconized glass bottle (38 × 300 mm) in a Hybridization Incubator (Robbins Scientific, Sunnyvale, CA). The dried nitrocellulose was prehybridized for 2 hours at 68°C in 6 mL prehybridization buffer consisting of 6 × SSC, 2 × Denhardt solution (0.04% wt/vol Ficoll, 0.4% wt/vol polyvinylpyrrolidone), and 3.5 mmol/L SDS. The ^{32}P-labeled probe prepared as described below was denatured by heating at 100°C for 5 minutes and then added to the prehybridization buffer in the glass tube at a final concentration of more than 1×10^6 cpm/mL. The membrane was then hybridized at 68°C for 16 to 24 hours. Following hybridization, the nitrocellulose membrane was washed once in 100 mL 1 × SSC for 20 minutes at room temperature, followed by three 20-minute washes of 100 mL each in 0.2 × SSC at 68°C. The damp nitrocellulose membrane was wrapped in plastic wrap and exposed to Kodak XAR film at −70°C for 1 to 3 days.

Preparation of ^{32}P-Labeled cDNA Probes

The cDNAs for rat transthyretin (generously provided by Dr Robert H. Costa of the University of Illinois, Chicago, IL) and rat serum retinol-binding protein (generously provided by Dr James Chambers of The Scripps Clinic and Research Foundation, La Jolla, CA) were labeled by the random primer method using [^{32}P]dCTP and a kit purchased from Amersham. Before labeling, the cDNA was linearized using *PstI*. The linearized cDNA was then labeled exactly as described in the instructions provided by Amersham to a specific radioactivity of approximately 1.8×10^9 dpm/μg. The ^{32}P-labeled probe was then separated from unincorporated [^{32}P]dCTP by gel filtration through a Sephadex G-50 column (DNA grade) in buffer consisting of 10 mmol/L Tris hydrochloride, pH 7.5, and 1 mmol/L EDTA. The probe was then either used immediately or stored frozen at −20°C for no longer than 2 weeks.

Statistics

The experimental data for each condition are expressed as the mean ± SEM for (n) animals in each group. Statistical evaluation of the data was performed using ANOVA to test for overall differences among groups. Student's *t* test for unpaired comparisons was used to determine significance only when a significant difference was observed using ANOVA. Differences among means were considered significant when *P* was less than .05.

RESULTS

Animals that were deprived of zinc in this study showed signs of anorexia within 5 days and subsequently displayed a cyclic pattern of food intake. The extent of zinc deficiency was assessed by measurement of the zinc content in femurs

from animals in the three groups. As shown in Table 1, zinc content was significantly reduced in femurs of rats deprived of dietary zinc as compared with either pair-fed or ad libitum–fed animals. Growth rates of the zinc-deficient and pair-fed rats were impaired. Although initially similar in body weight, pair-fed rats were heavier than zinc-deficient rats from day 25 onward of the feeding regimen. Other symptoms observed in the zinc-deficient rats included alopecia, dermal lesions, diarrhea, and priapism. The decreased food intake was reflected in a significantly lower body weight in zinc-deficient animals as compared with animals allowed free access to food (Table 2). Animals fed an amount of food equivalent to that ingested by zinc-deficient animals (ie, pair-fed animals) also showed a reduced body weight as compared with the ad libitum group of rats. However, animals that were zinc-deficient exhibited an additional impairment to growth when compared with pair-fed animals.

The response of several compositional properties of liver to zinc deficiency was assessed (Table 2). The wet tissue weight of livers from zinc-deficient animals was 27% of the weight of livers from ad libitum animals. Thus, the decreased body mass associated with zinc deficiency was mirrored by a proportional decrease in liver mass. Depriving animals of zinc had no significant effect on either the protein or RNA content of liver when expressed per gram tissue. Likewise, no significant change in either the protein or RNA content was observed in pair-fed animals as compared with either zinc-deficient or ad libitum groups of animals.

Alterations in hepatic protein synthesis associated with zinc deficiency were quantified in isolated perfused liver preparations.[20] As seen in Table 2, the rate of synthesis of secreted proteins was not significantly different in livers from zinc-deficient animals as compared with either pair-fed or ad libitum controls. In contrast, the rate of synthesis of proteins retained in the liver was significantly reduced in livers from zinc-deprived rats as compared with rats fed ad libitum. This result suggests that the rate of protein degradation must have also decreased during zinc deprivation, because the protein content of the livers was unchanged by zinc deficiency (Table 2). The rate of retained protein synthesis was also lower in pair-fed animals than in ad libitum controls, but this difference was not significant.

The decrease in hepatic protein synthesis associated with zinc deficiency was further investigated by determining the translational activity of total RNA isolated from livers of zinc-deficient, pair-fed, and ad libitum animals. Equal amounts of total RNA from the three experimental groups were translated in an mRNA-dependent reticulocyte lysate system, and [^{35}S]methionine-labeled products were separated by two-dimensional gel electrophoresis. Translation products were visualized by fluorography, and individual protein spots were quantified by densitometry. Of the approximately 200 spots discernible on each fluorogram, only 12 appeared to be altered by zinc deficiency (Fig 1). The pattern of change in the intensity of the 12 spots could be classified into one of four categories: The first pattern, represented by spots 3 and 4, showed an increased intensity in samples from zinc-deficient animals as compared with ad libitum controls, with little or no change in samples from pair-fed animals (Fig 2). Four of the 12 spots exhibiting changes in response to zinc deficiency followed this pattern. The second pattern showed a decline in intensity in the zinc-deficient condition as compared with either pair-fed or ad libitum controls. Pattern two is represented by spots 1 and 2; three of the 12 spots followed this pattern. Spot 6 represents the third pattern observed and is the only spot to follow this pattern. Spot 6 was only observed in samples from zinc-deficient animals; no translation product was observed in the region of the gel corresponding to spot 6 in either pair-fed or ad libitum conditions. The fourth pattern, represented by spot 5, was characterized by an increase in both zinc-deficient and pair-fed samples as compared with ad libitum controls. Thus, changes observed in patterns one through three represent changes in mRNA associated specifically with zinc deficiency, whereas the changes observed in pattern four are most likely attributable to the

Table 1. Effect of Zinc Deprivation on Femur Zinc Content

Group	Zinc Content (μg/g fat-free bone)
Zinc-deficient	109.1 ± 8.6 (7)
Pair-fed	279.1 ± 6.9 (7)
Ad libitum	314.1 ± 16.7 (6)

NOTE. Values are the mean ± SEM for (n) observations. Zinc content was measured in the right femur as described. Results among the three groups were significantly ($P < .001$) different by ANOVA.

*$P < .001$ *v* pair-fed or ad libitum–fed animals.

Table 2. Comparison of Properties Related to Control of Protein Metabolism During Zinc Deficiency

	Zinc-Deficient	Pair-Fed	Ad Libitum
Body weight (g)§	55.4 ± 1.8 (7)*†	75.1 ± 2.0 (7)*	218.6 ± 2.0 (7)
Liver weight (g)§	2.6 ± 0.2 (6)*	2.7 ± 0.2 (7)*	9.7 ± 0.3 (7)
Liver protein (mg/g liver)	233.6 ± 6.7 (3)	247.7 ± 17.9 (3)	244.8 ± 12.9 (3)
Liver RNA (mg/g liver)	8.6 ± 0.5 (3)	8.7 ± 0.9 (3)	7.7 ± 0.4 (3)
Rate of synthesis of secreted protein (mg/g liver/h)	1.3 ± 0.2 (4)	1.8 ± 0.4 (4)	1.3 ± 0.2 (4)
Rate of synthesis of retained protein (mg/g liver/h)	2.4 ± 0.3 (4)‡	2.7 ± 0.1 (3)	3.5 ± 0.4 (4)
Plasma protein (mg/mL plasma)	57.8 ± 5.1 (3)	63.2 ± 0.5 (3)	70.5 ± 5.4 (3)

NOTE. Values are the mean ± SEM for (n) observations. Rates of synthesis of protein secreted from and retained in the liver were determined as described. Results among the three groups for the rate of protein synthesis were marginally significant ($P = .08$) by ANOVA. Therefore, individual pairs of data were compared using Student's *t* test.

*$P < .005$ *v* ad libitum.

†$P < .005$ *v* pair-fed.

‡$P < .05$ *v* ad libitum.

§Results among the three groups were significantly ($P < .001$) different by ANOVA.

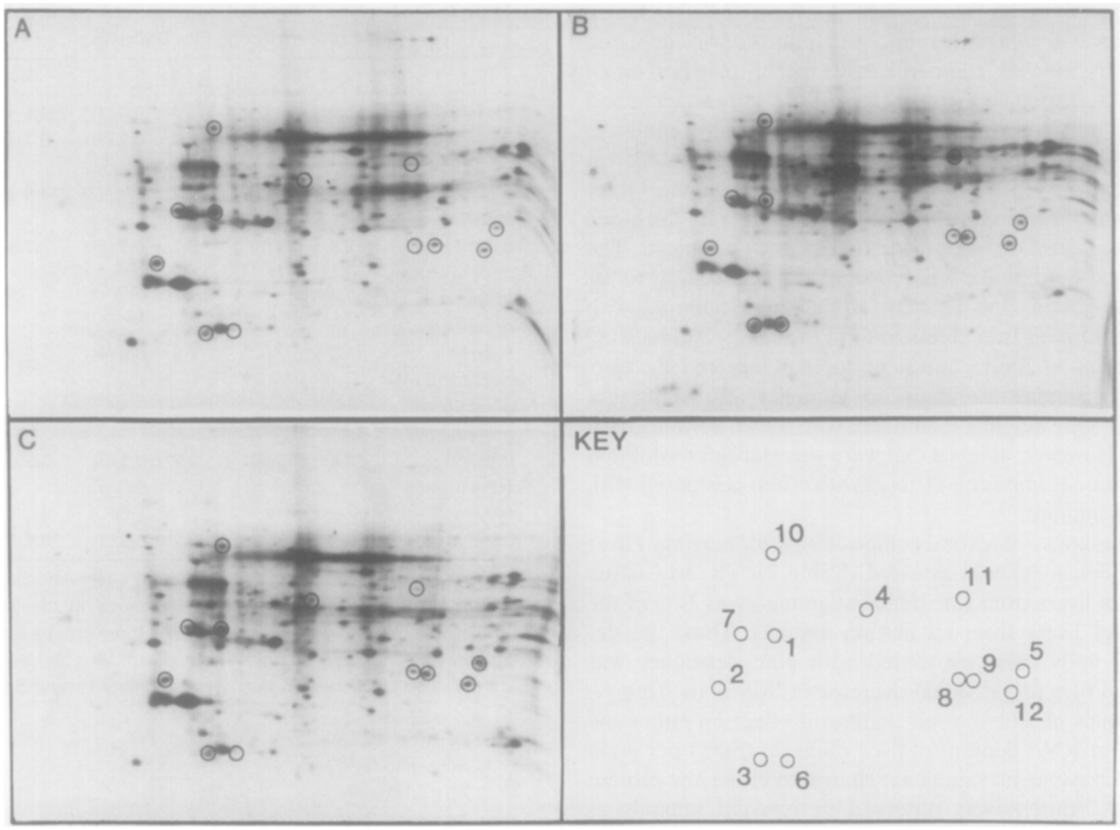

Fig 1. In vitro translation of mRNA isolated from livers of (A) ad libitum, (B) zinc-deficient, and (C) pair-fed rats. Total RNA was isolated from liver and translated in a messenger-dependent in vitro translation system. Translation products were analyzed by two-dimensional gel electrophoresis. Gels were treated for fluorography, dried, and exposed to x-ray film for approximately 10 days as described. Fluorograms are shown. Translation products demonstrating major changes between the conditions are circled in each panel and identified by number in the key.

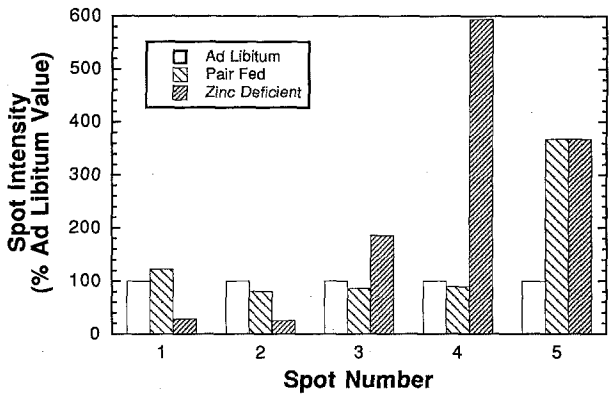

Fig 2. Densitometric determination of changes in specific mRNAs in livers from ad libitum, pair-fed, and zinc-deficient rats. Fluorograms in Fig 1 were scanned and analyzed as described. Changes in intensity of each spot are presented as a percentage of the value for that spot for rats fed ad libitum. In these experiments, two individual RNA preparations were analyzed for each condition, and each RNA sample was analyzed in three separate experiments. To minimize differences between animals, three livers were pooled for each condition before isolation of RNA.

inanition characteristic of zinc deficiency. It should be noted that the 200 spots discernible on the fluorogram (Fig 1) represent only a fraction of the total number of proteins present in liver. Proteins with isoelectric points outside the range of the pH gradient or molecular weights larger or smaller than resolved by the gels used in these experiments would not have been detected. Furthermore, proteins with low-abundance mRNAs or mRNAs that are inefficiently translated in reticulocyte lysate would not have been detected in these experiments. Therefore, the 12 spots that were observed to change in response to zinc deficiency in these experiments probably represent only a portion of the total number of proteins that actually respond to zinc deficiency.

Zinc deficiency is associated with specific decreases in transthyretin[27] and serum retinol-binding protein concentrations in rat serum.[11] Since both transthyretin and serum retinol-binding protein are synthesized primarily in the liver, the amounts of transthyretin and serum retinol-binding protein mRNAs were determined in livers from pair-fed, zinc-deficient, and ad libitum control rats by slot-blot analysis (Fig 3). As a control, the RNA was also analyzed for β-actin mRNA content and the data for transthyretin and serum retinol-binding protein mRNAs

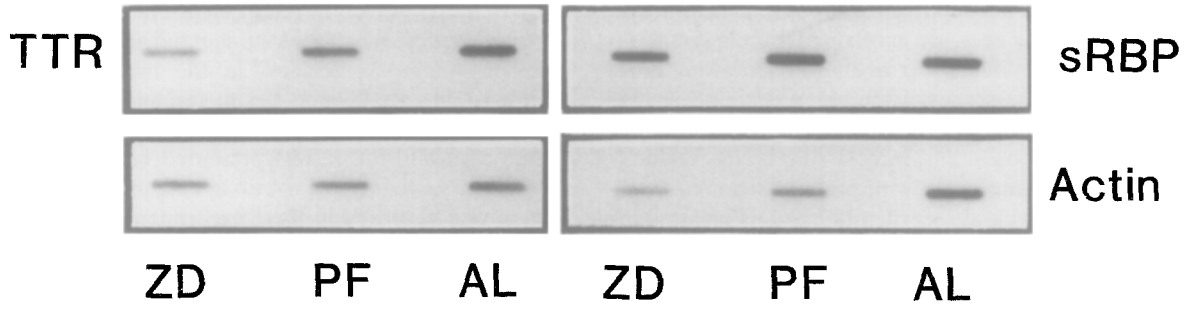

Fig 3. Quantitation of transthyretin (TTR) and serum retinol-binding protein (sRBP) mRNA in liver. Total RNA was extracted from livers of zinc-deficient (ZD), pair-fed (PF), and ad libitum (AL) rats as described. RNA (2 μg) was immobilized on a nitrocellulose membrane, and the blot was probed with ^{32}P-labeled cDNA to TTR, sRBP, or β-actin as described. RNA samples from two different groups of animals for each condition were used; each RNA sample was analyzed in two separate experiments.

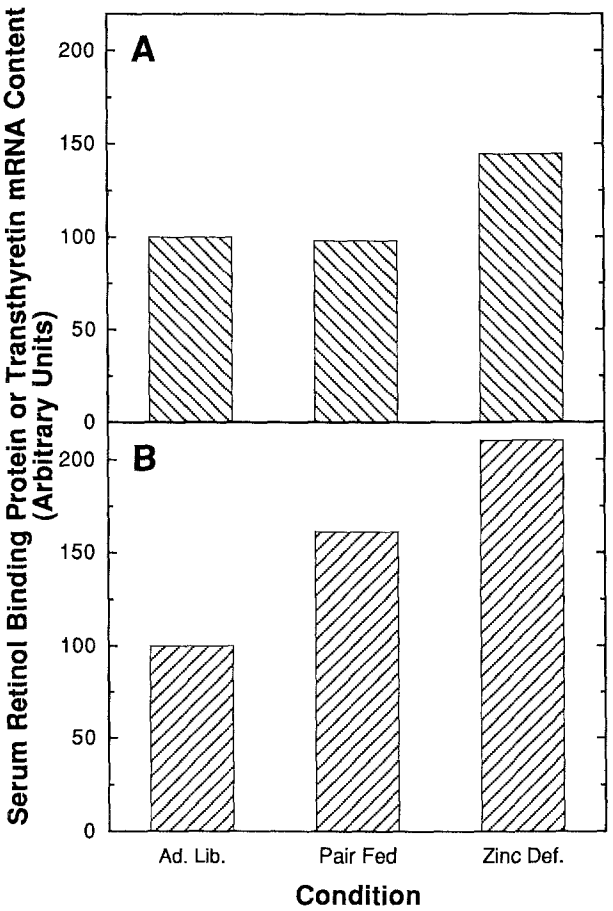

Fig 4. Densitometric determination of changes in (A) transthyretin and (B) serum retinol-binding protein mRNA in liver. Fluorograms in Fig 3 and similar fluorograms were scanned using a Beckman DU-8B spectrophotometer equipped with a gel-scanning package. Results represent the mean of values obtained for two individual RNA preparations for each condition; each sample was analyzed in two separate experiments. To minimize differences between animals, three livers were pooled for each condition before isolation of RNA. In addition, for each sample a series of 1:2 dilutions were prepared and applied to nitrocellulose to ensure that the response was linear. Results obtained for β-actin were used to normalize the data for transthyretin and serum retinol-binding protein.

were normalized using the β-actin mRNA values (Fig 4). The relative abundance of transthyretin mRNA from livers of zinc-deficient rats was elevated to 145% of the value observed in ad libitum controls. In contrast, the relative abundance of liver transthyretin mRNA was unchanged in pair-fed as compared with ad libitum–fed rats. The relative abundance of serum retinol-binding protein mRNA was increased in livers of both zinc-deficient and pair-fed rats as compared with the ad libitum controls. These results suggest that in liver the amount of transthyretin mRNA is specifically regulated in response to deprivation of dietary zinc, whereas the increase in the amount of serum retinol-binding protein mRNA may be due to a decrease in food intake rather than to zinc deficiency per se.

DISCUSSION

It has been reported previously that the rate of protein synthesis in rat liver is reduced during zinc deficiency.[2-4] In the present study, the synthesis of total liver protein (secreted + retained) was depressed by 23% in zinc-deficient as compared with ad libitum–fed animals. The effect appears to be specific for zinc deficiency as opposed to a nonspecific effect of the inanition characteristic of zinc deficiency, since pair-fed rats exhibited only a 6% reduction in total liver protein synthesis as compared with ad libitum controls. The impairment to protein synthesis associated with zinc deficiency was limited to the synthesis of proteins retained in the liver, since the rate of synthesis of proteins secreted from the liver was unchanged by zinc deprivation. The decrease in the rate of protein synthesis was apparently not a result of a change in the overall capacity for protein synthesis, since the total RNA content of the liver was unaffected by deprivation of dietary zinc. In contrast, the efficiency of hepatic protein synthesis (milligrams protein synthesized per milligram RNA per hour) was reduced to 69% and 83% of the ad libitum control value in zinc-deficient and pair-fed animals, respectively. A decrease in the efficiency of protein synthesis is generally assumed to be a reflection of a decrease in the activity of one or more of the components of the protein synthetic apparatus. Thus, Hicks and Wallwork[3] have shown that aminoacyl-tRNA synthetase activity was significantly lower in extracts of livers from zinc-deficient rats as compared with controls.

Whether a decrease in aminoacyl-tRNA synthetase activity is responsible for the decrease in retained protein synthesis in the present experiments is unknown. However, it is difficult to imagine a scenario whereby a decrease in the activity of the aminoacyl-tRNA synthetases could specifically cause a reduction in the synthesis of proteins retained within the liver as compared with proteins secreted from the liver, unless a separate set of synthetases is involved in the synthesis of the two populations of proteins.

The results of the present study suggest that zinc may have a vital role in regulating gene expression, because the abundances of a limited number of mRNAs in rat liver are altered by zinc deprivation. There are several possible mechanisms by which zinc might affect gene expression. For example, several of the enzymes involved in nucleic acid metabolism bind zinc. Specifically, both DNA polymerase and RNA polymerase are zinc metalloenzymes.[28] In addition, DNA polymerase[29] and RNA polymerase[30] activities are significantly lower in tissues from zinc-deficient rats than from ad libitum or pair-fed controls. Zinc has also been implicated in the determination and stabilization of nucleic acid conformation.[31,32] Changes in tissue zinc content could therefore structurally alter template availability for transcription or translation. Finally, zinc-dependent changes in the cellular concentration of other ions, possibly mediated by plasma membrane alterations, might also affect nucleic acid structure and/or the activity of enzymes involved in nucleic acid metabolism. For example, zinc-deficient *Euglena gracilis* accumulates manganese, iron, and copper.[33] Changes in manganese concentration have been shown to affect the template specificity and products generated by RNA polymerase in *E gracilis*.[33]

Plasma concentrations of both serum retinol-binding protein[11] and transthyretin[27] are reportedly decreased during zinc deficiency. Since both of these proteins are synthesized in the liver, we examined the liver content of serum retinol-binding protein and transthyretin mRNAs in zinc-deficient, pair-fed, and ad libitum–fed rats. The relative abundance of transthyretin mRNA was specifically elevated in livers from zinc-deficient rats. Possible explanations for the elevation include an increase in the rate of transcription of the transthyretin gene, a change in the stability of the mRNA, or an alteration in mRNA processing. A recent study has shown that the relative abundance of transthyretin mRNA in liver is decreased in rats fed a protein-free diet, whereas serum retinol-binding protein mRNA is unaltered.[34] The fact that the relative abundance of transthyretin mRNA did not change in livers from pair-fed rats as compared with ad libitum–fed rats suggests that in the present study both the pair-fed and zinc-deficient rats were receiving an adequate amount of dietary protein to prevent the changes in transthyretin mRNA content observed in rats fed a protein-free diet.[34]

The relative abundance of serum retinol-binding protein mRNA increased in both zinc-deficient and pair-fed animals. In a related study, the liver content of serum retinol-binding protein mRNA was unchanged in rats deprived of retinol[35] even though, as is the case for zinc deficiency, the concentration of serum retinol-binding protein in blood decreased.[36] However, in retinol-deficient rats, serum retinol-binding protein synthesis is unaltered and serum retinol-binding protein accumulates in the liver[15] whereas in zinc-deficient rats hepatic serum retinol-binding protein content is diminished.[5]

One caveat to the results presented herein is that the synthesis of neither transthyretin nor serum retinol-binding protein was measured in these experiments. The reported decrease in liver content of serum retinol-binding protein[5] and transthyretin[27] in zinc-deficient rats could be due to either a decrease in the rate of synthesis, an increase in the rate of degradation, or both. If the rate of synthesis of serum retinol-binding protein or transthyretin is decreased during zinc deficiency, then the effect must occur at the translational level since the relative amount of serum retinol-binding protein mRNA increases in livers from zinc-deficient rats. Also, it is important to note that the increase in the amount of serum retinol-binding protein mRNA is not zinc-specific, since pair-fed rats demonstrated the same response as zinc-deficient animals.

In summary, we have shown that protein synthesis is inhibited in livers from rats deprived of zinc. The inhibition of protein synthesis can be accounted for by a decrease in the rate of synthesis of proteins retained in the liver, with no apparent change in the overall rate of synthesis of protein secreted from the liver. Furthermore, the liver exhibits zinc-dependent alterations in the expression of a few specific genes. Finally, the relative abundances of both hepatic transthyretin and serum retinol-binding protein mRNAs are altered during zinc deficiency. The increase in transthyretin mRNA content is observed only in zinc-deficient and not pair-fed rats, indicating that the effect is zinc-dependent. However, the changes in serum retinol-binding protein mRNA are observed in both zinc-deficient and pair-fed animals, and are therefore likely due to the inanition characteristic of zinc deficiency rather than to zinc deficiency per se.

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