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ZINC STIMULATION OF BONE PROTEIN SYNTHESIS IN TISSUE CULTURE

ACTIVATION OF AMINOACYL-tRNA SYNTHETASE

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Abstract—The present investigation was undertaken to clarify the effect of zinc on bone protein synthesis in tissue culture. Calvaria were removed from 3-week-old male rats and cultured for periods up to 96 hr in Dulbecco's Modified Eagle Medium (high glucose, 4500 mg/dl) supplemented with antibiotics and bovine serum albumin. The calvaria were incubated at 37° in 5% CO_2/95% air in the medium containing 10^{-6}–10^{-4} M zinc. Zinc content in bone cells was increased when the culture was treated with 10^{-5} and 10^{-4} M zinc for 48 hr. When calvaria cultured in the presence of 10^{-4} M zinc were pulsed with [^{14}C]uridine, the incorporation of [^{14}C]uridine into the bone RNA was not increased significantly. In the pulse with [^{3}H]leucine, the presence of 10^{-5} to 10^{-4} M zinc in the medium caused a significant increase in the incorporation of [^{3}H]leucine into the acid-insoluble residues of bone tissue. This increase was blocked completely by treatment with 10^{-7} M cycloheximide, an inhibitor of protein synthesis. When [^{3}H]leucine was added into the reaction mixture containing the 5500 *g* supernatant fraction of the homogenate prepared from calvaria cultured in the presence of 10^{-4} M zinc, the *in vitro* protein synthesis was increased about 2-fold. The activity of [^{3}H]leucyl-tRNA synthetase in the 105,000 *g* supernatant fraction (cytosol) of the bone homogenate was increased about 2-fold by the culture with 10^{-4} M zinc. The presence of 10^{-4} M dipicolinate, a specific chelator of zinc, in the culture medium negated the effect of zinc on [^{3}H]leucyl-tRNA synthetase activity. The addition of 10^{-7} to 10^{-6} M zinc into the reaction mixture containing enzyme extracts obtained from uncultured rat calvaria caused a 2-fold increase of [^{3}H]leucyl-tRNA synthetase activity. These results clearly indicate that zinc induces the stimulation of protein synthesis at the translational level in bone cells. The present study further supports the view that zinc increases protein synthesis in bone cells and that the metal induces bone formation.

Zinc has been demonstrated to have a wide variety of roles in the mammalian system, and this metal is essential for growth in humans and many animals [1]. Bone growth retardation is a common finding in various conditions associated with zinc deficiency [2, 3]. In recent years, it has been reported that zinc, of many essential trace metals, can effectively stimulate bone growth and bone mineralization in weanling rats [4–7]. These investigations suggest a physiologic role for zinc in the regulation of bone metabolism in weanling rats. The cellular mechanism of zinc action on bone metabolism, however, remains to be elucidated.

More recently, it has been demonstrated that zinc has a stimulatory effect on bone formation in tissue culture using the calvaria from weanling rats, and it has been suggested that bone protein synthesis is a necessary component of this response [8]. However, direct evidence of zinc action to stimulate bone protein synthesis has not been shown thus far. The present study was undertaken, therefore, to clarify the action of zinc on bone protein synthesis in tissue culture of calvaria from weanling rats. It was found that zinc stimulated bone protein synthesis *in vitro*.

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MATERIALS AND METHODS

Chemicals. Dulbecco's Modified Eagle Medium (high glucose) and a penicillin–streptomycin solution (5000 units/ml penicillin; 5000 μg/ml streptomycin) were obtained from Gibco Laboratories (Grand Island, NY). Bovine serum albumin (Fraction V), cycloheximide and transfer ribonucleic acid (tRNA; 50 units/mg from bovine liver) were obtained from the Sigma Chemical Co. (St. Louis, MO). L-[4,5-^{3}H]Leucine (60.0 μCi/mmol) and [2-^{14}C]uridine (55.9 μCi/mmol) were obtained from New England Nuclear (Boston, MA). Zinc sulfate and all other chemicals were reagent grade from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All water used was glass distilled.

Animals. Weanling male Wistar rats weighing 60–65 g (3-weeks-old) were obtained from the Nippon Bio Supply Center Co., Tokyo. The animals were fed commercial laboratory chow (solid) containing 1.1% calcium, 1.1% phosphorus and 0.012% zinc and were given distilled water freely until used.

Bone culture. Calvaria from 3-week-old male rats were removed aseptically and cut along the sagittal suture into left and right halves. One-half of each calvarium served as a control for its paired, treated half. Each half-calvarium (17–23 mg wet weight) was cultured in a 35-mm dish in 2.0 ml of medium consisting of Dulbecco's Modified Eagle Medium (high

glucose) supplemented with 0.25% bovine serum albumin (Fraction V) plus antibiotics, with either zinc (10^{-6}–10^{-4} M) or vehicle (zinc content was none). Cultures were maintained at 37° in a water-saturated atmosphere containing 5% CO_2 and 95% air for 96 hr. The respective media, containing either zinc or vehicle, were changed at 48 hr, and cultures were maintained for an additional 48 hr. In the separate experiments, the respective media contained cycloheximide or dipicolinate.

Bone zinc uptake. Calvaria were cultured in the medium containing zinc for 48 or 96 hr at 37°. After culture, the bone was removed and washed with ice-cold 0.25 M sucrose solution, blotted, and weighed. The bone tissues were minced in 2.0 ml of 0.1 N NaOH solution and shaken for 24 hr at 4° [9]. The bone tissues (matrix) remaining after alkaline extraction were separated by centrifugation at 600 *g* for 5 min, and the supernatant fractions (cellular components) were kept. Zinc content in the bone matrix and the cellular component was determined by atomic absorption spectrophotometry after digestion with nitric acid. Bone zinc content was expressed as micrograms of zinc per gram wet bone tissue. Zinc content in the complete control medium was none.

Bone RNA synthesis. The effect on newly synthesized bone RNA was determined by studying the incorporation of [^{14}C]uridine [10]. Labeled uridine was added to the culture of the time period indicated in the figures to a final concentration of 0.25 μCi/ml medium. At 2 hr after the pulse, the bones were removed and washed with ice-cold 5% trichloroacetic acid (TCA), acetone and ether, and then rinsed in ice-cold 0.25 M sucrose. The bones were dried and weighed. The dried bone residues were dissolved in 1.0 ml of 0.2 N NaOH for determination of the amount of [^{14}C]uridine incorporated into bone RNA. The radioactivity was counted with a scintillation spectrometer. Data are expressed as disintegrations per minute per milligram dry weight of acid-insoluble residues.

Bone protein synthesis. The effect on newly synthesized bone total protein was determined by studying the incorporation of [^{3}H]leucine [11]. The calvaria were pulsed with [^{3}H]leucine (5.0 μCi/ml medium) at the culture period indicated in the legends to the figures, and cultured for 2 hr. At the end of the culture, the calvaria were removed and washed with ice-cold 0.25 M sucrose. The calvaria were extracted with ice-cold 5% TCA, acetone and ether, and then rinsed in ice-cold 0.25 M sucrose. The bones were dried and weighed. For determination of the amount of [^{3}H]leucine incorporated into bone total protein, the dried bone residues were dissolved in 1.0 ml of 0.2 N NaOH and an aliquot was removed and placed in a vial for measurement of the disintegrations per minute by scintillation counting. Data are expressed as disintegrations per minute per milligram dry weight of acid-insoluble residues.

In separate experiments, *in vitro* protein synthesis was measured by using bone homogenate fraction. The bone tissues cultured were homogenized in 1.0 ml of ice-cold 0.25 M sucrose solution and centrifuged at 5500 *g* for 10 min, and the supernatant fraction was pooled. The incubation mixture to assay *in vitro* protein synthesis contained, in a total of 1.0 ml, 25 mM Tris–chloride (pH 7.5), 5 mM $MgCl_2$, 5 mM KCl, 2.5 mM potassium ATP, 1 mM [^{3}H]leucine (0.5 μCi/ml) and the 5500 *g* supernatant fraction (70–85 μg protein, ml) [12]. After incubation at 37° for 30 min, the reaction was stopped by addition of 10% TCA (1.0 ml), and an aliquot was withdrawn and applied to a Millipore filter disk (2.5 cm diameter); the latter was washed with an excess of cold aqueous 5% TCA. After three changes of 5% TCA solution, the disks were washed with an excess of cold ethanol–ether (1:1, v/v), and finally with cold ether. The disks were counted for radioactivity. Protein synthesis is expressed as disintegrations per minute per milligram of protein. Protein concentration was determined by the method of Lowry *et al.* [13].

Bone aminoacyl-tRNA synthetase. Cultured calvaria were homogenized in 1.0 ml of ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 105,000 *g* for 60 min, and the supernatant fraction was pooled to assay aminoacyl-tRNA synthetase activity. The enzyme activity was measured as described by Hoskinson and Khorana [12]. The incubation mixture contained, in a total of 1.0 ml, 25 mM Tris–chloride (pH 7.5), 5 mM $MgCl_2$, 5 mM KCl, 2.5 mM potassium ATP, 1.0 A_{260} units of tRNA, 1.0 mM [^{3}H]leucine (0.5 μCi) and the 105,000 *g* supernatant solution (60–80 μg protein). After incubation at 37° for 10 min, an aliquot was withdrawn and applied to a Millipore filter paper disk (2.5 cm diameter); the latter was washed with an excess of cold aqueous 5% TCA. After the washing, the disks were washed with an excess of cold ethanol–ether (1:1, v/v), and finally with cold ether. The disks were dried and counted for radioactivity with a scintillator. [^{3}H]Leucil-tRNA synthetase activity is expressed as disintegration per minute per milligram of protein.

Statistical analyses. Data are expressed as the mean ± SEM. Statistical differences were analyzed using Student's *t*-test. P values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Distribution of zinc in bone tissue. The uptake of zinc by rat calvaria cultured in medium containing 10^{-4} M zinc for 96 hr is shown in Fig. 1A. Alkaline extraction of bone tissue indicated the presence of zinc in the cellular components; zinc content in the cellular component of control calvaria was about 15% of that in the bone tissue. At 24 hr of culture with 10^{-4} M zinc, zinc content in the bone cellular components increased about 4-fold. This increase was enhanced slightly by the 96-hr culture. When calvaria were cultured for 48 hr with increasing concentrations of medium zinc (10^{-6}–10^{-4} M), a significant increase in the bone cellular zinc content was seen at concentrations greater than 10^{-5} M zinc (Fig. 1B). Zinc content in the bone matrix was remarkable in comparison with that in the bone cellular components. Meanwhile, medium zinc concentration was decreased by culture; when calvaria were cultured in medium containing 10^{-5} and 10^{-4} M zinc for 48 hr, the medium zinc concentration was 7.89×10^{-6} M and 7.52×10^{-5} M respectively.

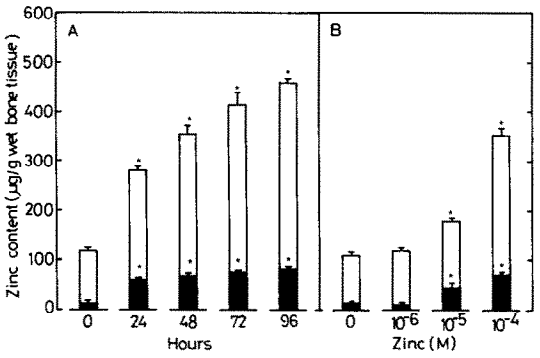

Fig. 1. Distribution of zinc taken up by rat calvaria *in vitro*. (A) Calvaria were cultured for 96 hr in medium containing 10^{-4} M zinc. Zinc concentrations in calvaria cultured for 0, 48 and 96 hr in the absence of zinc were 117.5 ± 5.5, 113.0 ± 6.0 and 118.1 ± 7.2 (μg/g wet bone) respectively. (B) Calvaria were cultured for 48 hr in medium containing 10^{-6} M to 10^{-4} M zinc. The bone zinc concentration at 0 hr of culture was 113.0 ± 6.0 μg/g wet bone. Each bar is the mean of five calvaria per group. The vertical lines represent the SEM. Key: (*) $P < 0.01$, compared with control group; (□) bone matrix: and (■) bone cellular component.

There was no zinc in the control medium. Also, calvaria did not release zinc into the medium with the 48-hr culture.

Effect of zinc on bone RNA synthesis. The effect of zinc on the incorporation of [^{14}C]uridine into the acid-insoluble residues of rat calvaria is shown in Fig. 2. When the calvaria were cultured for 24–96 hr in the presence of 10^{-4} M zinc and, at each time point of the culture, pulsed with [^{14}C]uridine, the acid-insoluble residues of bone were not enhanced significantly by the presence of 10^{-4} M zinc. In addition, the bones cultured for 48 hr were pulsed with [^{14}C]uridine, and 30 or 60 min later the bones were removed from the medium (Fig. 3). [^{14}C]Uridine incorporation was not increased significantly by the presence of 10^{-4} M zinc. These results indicate that zinc did not stimulate RNA synthesis significantly in cultured bone.

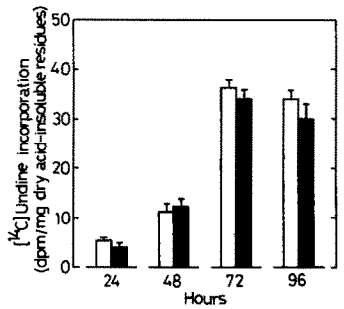

Fig. 2. Effect of zinc on the incorporation of [^{3}C]uridine into the acid-insoluble residues of rat calvaria *in vitro*. Calvaria were cultured for 96 hr in the presence of 10^{-4} M zinc. At each time point of culture, the bones were pulsed with [^{14}C]uridine (0.25 μCi/ml medium), and 2 hr later the bones were removed from the medium. Each bar represents the mean of five calvaria. The vertical lines give the SEM. Data were not significant. Key: (□) control; and (■) 10^{-4} M zinc.

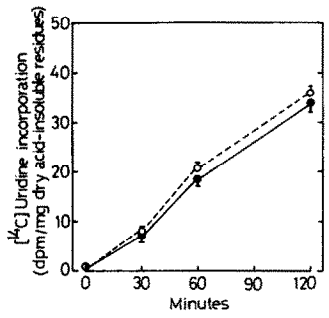

Fig. 3. Effect of zinc on the time course of the incorporation of [^{14}C]uridine into the acid-insoluble residues of rat calvaria *in vitro*. Calvaria were cultured for 48 hr in the presence of 10^{-4} M zinc, and then pulsed with [^{14}C]uridine (0.25 μCi/ml medium); 30, 60 and 120 min later the bones were removed from the medium. Each point represents the mean of five calvaria. The vertical lines give the SEM. Data were not significant. Key: (○) control; and (●) 10^{-4} M zinc.

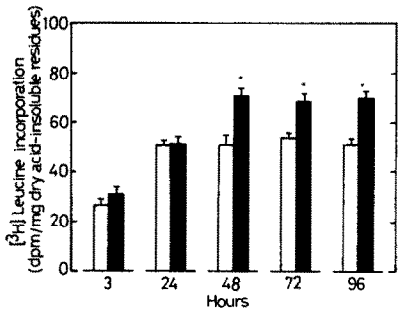

Fig. 4. Effect of zinc on the incorporation of [^{3}H]leucine into the acid-insoluble residues of rat calvaria *in vitro*. Calvaria were cultured for 96 hr in the presence of 10^{-4} M zinc. At each time point of culture, the bones were pulsed with [^{3}H]leucine (5.0 μCi/ml medium), and 2 hr later the bones were removed from the medium. Each bar represents the mean of five calvaria. Vertical lines give the SEM. Key: (*) $P < 0.01$, compared with the control group; (□) control; and (■) 10^{-4} M zinc.

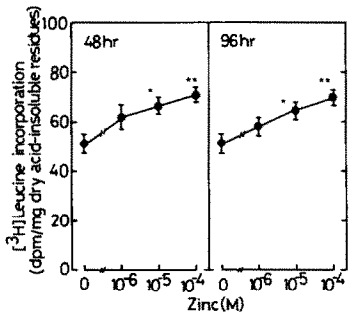

Fig. 5. Effect of increasing concentrations of zinc on the incorporation of [^{3}H]leucine into the acid-insoluble residues of rat calvaria *in vitro*. Calvaria were cultured for 48 and 96 hr in the presence of 10^{-6}–10^{-4} M zinc. The bones were pulsed with [^{3}H]leucine (5.0 μCi/ml medium), and 2 hr later the bones were removed from the medium. Each point represents the mean of five calvaria. Vertical lines give the SEM. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with the control group.

Effect of zinc on bone protein synthesis. The effect of zinc on the incorporation of [^{3}H]leucine into the acid-insoluble residues of rat calvaria is shown in Fig. 4. Calvaria were incubated for up to 96 hr in the presence of 10^{-4} M zinc. When the bones were pulsed with [^{3}H]leucine (5.0 μCi/ml medium) at 3 and 24 hr of the culture, the incorporation of [^{3}H]leucine into the acid-insoluble residues of bones was not enhanced significantly by the presence of zinc. At 48, 72 and 96 hr of culture, [^{3}H]leucine incorporation was increased significantly by the presence of zinc.

The effect of increasing concentrations of zinc (10^{-6}–10^{-4} M) on the incorporation of [^{3}H]leucine into the acid-insoluble residues of bone is shown in Fig. 5. The bones were incubated for 48 and 96 hr in the presence of zinc and, at each time point of the culture, the bones were pulsed with [^{3}H]leucine (5.0 μCi/ml medium). In the presence of 10^{-6} M zinc, the incorporation of [^{3}H]leucine into the acid-insoluble residues was not enhanced significantly. The presence of 10^{-5} M and 10^{-4} M zinc produced a significant increase of [^{3}H]leucine incorporation.

The effect of cycloheximide, an inhibitor of protein synthesis, on zinc-stimulated [^{3}H]leucine incorporation into the acid-insoluble residues of bone is shown in Fig. 6. Calvaria were incubated for 48 hr in the presence of either 10^{-4} M zinc or 10^{-7} M cycloheximide plus 10^{-4} M zinc. The increase of [^{3}H]leucine incorporation into the acid-insoluble residues of bone caused by the presence of 10^{-4} M zinc was blocked completely by the presence of 10^{-7} M cycloheximide. In the absence of zinc, the inhibitor had an appreciable effect on [^{3}H]leucine incorporation into the acid-insoluble residues of bone.

The radioactivity of [^{3}H]leucine in the acid (10% TCA)-insoluble residues of culture medium was not significantly different between the control group and the 10^{-4} M zinc-treated group (data not shown). This indicates that the release of newly synthesized protein from the cultured bone to the medium was not prevented by the presence of zinc.

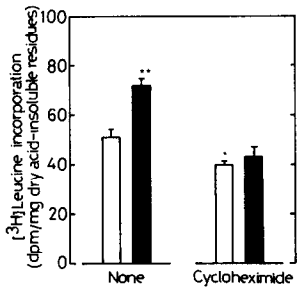

Fig. 6. Effect of cycloheximide on zinc-stimulated [^{3}H]leucine incorporation into the acid-insoluble residues of rat calvaria *in vitro*. Calvaria were cultured for 48 hr in medium containing either: vehicle alone; 10^{-4} M zinc; 10^{-7} M cycloheximide; or 10^{-4} M zinc plus 10^{-7} M cycloheximide. The bones were pulsed with [^{3}H]leucine (5.0 μCi/ml medium), and 2 hr later the bones were removed from the medium. Each bar represents the mean of five calvaria. The vertical lines give the SEM. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with the control group; (□) control; and (■) 10^{-4} M zinc.

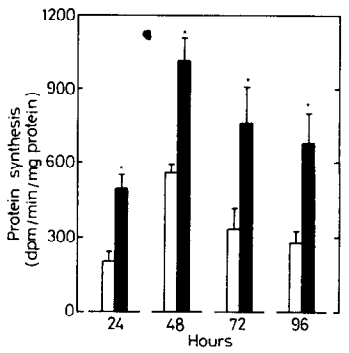

Fig. 7. Effect of zinc on *in vitro* protein synthesis in rat calvaria. Calvaria were cultured for 96 hr in medium containing either vehicle or 10^{-4} M zinc. The bone tissue homogenate was prepared from the cultured calvaria. [^{3}H]Leucine was added to the incubation mixture containing the 5500 *g* supernatant fraction of the bone homogenate. Each bar is the mean of five calvaria per group. The vertical lines represent the SEM. Key: (*) $P < 0.01$, compared with control group; (□) control; and (■) zinc.

The effect of zinc on *in vitro* protein synthesis is shown in Fig. 7. Cultured calvaria were homogenized, the homogenate was centrifuged at 5500 *g*, and the supernatant (cytosol and microsomal fraction) was used to assay protein synthesis. When [^{3}H]leucine was added into the assay mixture containing the 5500 *g* supernatant fraction of the homogenate of calvaria cultured in the presence of 10^{-4} M zinc for 24 hr, the incorporation of [^{3}H]leucine into the TCA-insoluble fraction increased about 2-fold in comparison with the value obtained from the control bone tissue. Such an increase was also seen by using the bone tissues cultured for 48–96 hr. Thus, zinc taken by bone cells stimulated protein synthesis.

Effect of zinc on aminoacyl-tRNA synthetase activity in bone. The effect of zinc on [^{3}H]leucyl-tRNA synthetase activity in bone tissue is shown in

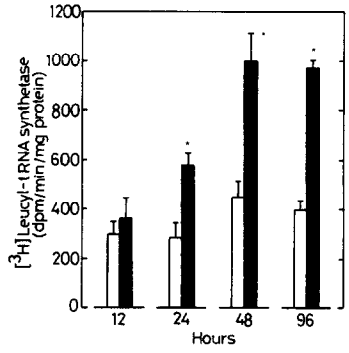

Fig. 8. Effect of zinc on [^{3}H]leucyl-tRNA synthetase activity in rat calvaria *in vitro*. Calvaria were cultured for 96 hr in medium containing either vehicle or 10^{-4} M zinc. The 105,000 *g* supernatant fraction (cytosol) was prepared from the bone homogenate. [^{3}H]Leucine was added to the incubation mixture containing the cytosol. Each bar is the mean of five calvaria per group. The vertical lines represent the SEM. The bone enzyme activity at 0 hr of culture was 62.0 ± 13.0 (dpm/min/mg protein). Key: (*) $P < 0.01$, compared with control group; (□) control; and (■) zinc.

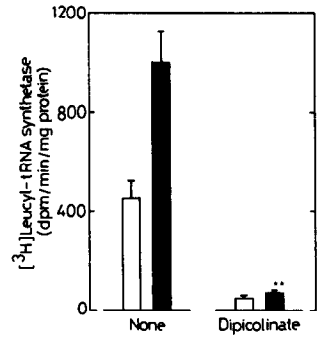

Fig. 9. Effect of dipicolinate, a chelator of zinc, on [^{3}H]leucyl-tRNA synthetase activity in rat calvaria *in vitro*. Calvaria were cultured for 48 hr in medium containing: vehicle alone; 10^{-4} M zinc; 10^{-4} M dipicolinate; or 10^{-4} M zinc plus 10^{-4} M dipicolinate. Each bar is the mean of five calvaria per group. The vertical lines represent the SEM. Key: (*) $P < 0.01$, and (**) $P < 0.05$, compared with control group; (□) control; and (■) zinc.

Fig. 8. Calvaria were cultured in the presence of 10^{-4} M zinc for 96 hr. The 105,000 *g* supernatant fraction (cytosol) was obtained from the homogenate of cultured calvaria. [^{3}H]Leucyl-tRNA synthetase activity in the cytosol was increased about 2-fold by the bone tissue cultured for 24-hr, although the 12-hr culture did not have a significant effect. The increase in the enzyme activity was also seen in the case of the 96-hr culture.

The effect of dipicolinate, a chelator of zinc, on [^{3}H]leucyl-tRNA synthetase activity in calvaria is shown in Fig. 9. Calvaria were cultured in the presence of 10^{-4} M dipicolinate for 48 hr. In the absence of 10^{-4} M zinc, the culture with 10^{-4} M dipicolinate caused a remarkable decrease of [^{3}H]leucyl-tRNA synthetase activity in calvaria. In the presence of 10^{-4} M dipicolinate, the effect of 10^{-4} M zinc on the enzyme activity was negligible. Thus, leucyl-tRNA synthetase might be a zinc-containing enzyme.

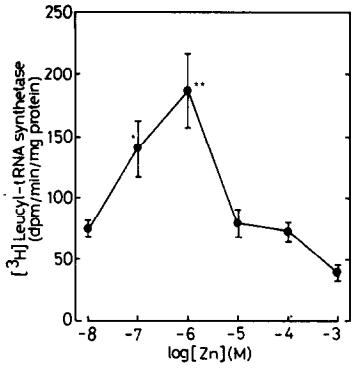

Fig. 10. Effect of zinc addition on [^{3}H]leucyl-tRNA synthetase activity of enzyme extracts from rat calvaria. Zinc chloride solution was added to the incubation mixture containing the cytosol to final concentrations of 10^{-8} to 10^{-3} M zinc. The cytosol was prepared from the homogenate of normal rat calvaria. The enzyme control value was 62.0 ± 13.0 (dpm/min/mg protein). Each point is the mean of five calvaria. Vertical lines represent the SEM. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with no addition values.

The effect of the addition of zinc on leucyl-tRNA synthetase activity in enzyme extracts obtained from uncultured rat calvaria was examined; the result is shown in Fig. 10. Zinc was added in the range of 10^{-8} to 10^{-3} M as a final concentration in the reaction mixture. [^{3}H]Leucyl-tRNA synthetase activity was not altered in the presence of 10^{-8} M zinc. At zinc concentrations of 10^{-7} and 10^{-6} M, the enzyme activity increased markedly; the maximal effect was seen at 10^{-6} M zinc and the activity increased about 2-fold. With zinc concentrations greater than 10^{-5} M, the enzyme activity was clearly reduced; zinc had an inhibitory effect on the enzyme. Thus, a physiological level of zinc in bone cells could activate leucyl-tRNA synthetase.

DISCUSSION

In recent years, it has been demonstrated that zinc, an essential trace metal, induces stimulation of bone growth and bone mineralization in weanling rats [4–7]. More recently, it has been found that zinc can stimulate bone mineralization in tissue culture *in vitro*, and that bone protein synthesis may be a necessary component of this response; the metal produces increases of calcium content, alkaline phosphatase activity and collagen content in tissue culture of the calvaria obtained from weanling rats [8].

In the present study, the presence of zinc in the culture medium produced a significant increase in the incorporation of [^{3}H]leucine by acid-insoluble residues of rat calvaria after a pulse of labeled leucine was added to the culture medium. The increase did not result from the inhibition of the release of [^{3}H]leucine-labeled proteins from the bone tissue into the culture medium caused by the presence of zinc. The presence of cycloheximide, an inhibitor of protein, in the culture medium completely blocked the stimulatory effect of zinc on the incorporation of [^{3}H]leucine into the acid-insoluble residues of bone. These results suggest that zinc stimulates a synthesis of proteins in cultured bone. On the other hand, the incorporation of [^{14}C]uridine, a precursor in the synthesis of RNA, into the acid-insoluble residues of calvaria increased linearly as a function of time (min) after the pulse of labeled uridine. This increase, however, was not enhanced significantly by the presence of 10^{-4} M zinc in the culture medium. This indicates that zinc did not stimulate the synthesis of RNA in the bone cells, although it is known that RNA polymerase is a zinc-containing enzyme [14]. However, since zinc can increase bone mRNA synthesis, which is only a small percentage of total RNA synthesis, one cannot exclude the possibility that zinc-stimulated protein synthesis in bone cells is partly based on the enhancement of mRNA synthesis.

Zinc could stimulate *in vitro* protein synthesis in bone cells. When [^{3}H]leucine was added to the reaction mixture containing the 5500 *g* supernatant fraction (cytosol and microsomes) of the homogenate prepared from calvaria cultured in the presence of 10^{-4} M zinc, *in vitro* protein synthesis increased about 2-fold. Such an increase in protein synthesis was also seen in the 24-hr culture. Zinc uptake by bone cells clearly occurred in the 24-hr culture in

the presence of 10^{-4} M zinc. These results further support the view that the cellular zinc in the bone tissue directly stimulates protein synthesis.

The first steps in the biosynthesis of proteins involve the enzymatic activation of the amino acids with adenosine triphosphate followed by the transfer of the amino acids to amino acid-specific ribonucleic acids (RNA) [15]. Aminoacyl-tRNA synthetase is an enzyme which synthesizes aminoacyl-tRNA [12]. Leucyl-tRNA synthetase activity in the cytosol fraction of calvaria was increased about 2-fold by 24 hr culture in the presence of 10^{-4} M zinc. This increase was prevented completely by the presence of 10^{-4} M dipicolinate, a specific chelator of zinc [16]. These results clearly indicate that zinc acts during the first steps in the biosynthesis of proteins. Furthermore, addition of zinc ion (10^{-7} and 10^{-6} M) into the enzyme reaction mixture containing the cytosolic enzyme extract prepared from uncultured calvaria produced a remarkable elevation of leucyl-tRNA synthetase activity. With concentrations of zinc greater than 10^{-5} M, the enzyme activity was not enhanced. Thus, the activation of leucyl-tRNA synthetase by zinc was revealed in a narrow range of concentration of the metal. This suggests a physiologic role of cellular zinc in the stimulation of protein synthesis in bone cells. The present finding, that zinc can directly activate leucyl-tRNA synthetase, suggests that the enzyme is a zinc-containing protein. Thus far, it has not been reported that leucyl-tRNA synthetase is activated by zinc. Zinc may be an essential factor in protein synthesis.

In a previous investigation [8], it was demonstrated that zinc increases alkaline phosphatase activity in cultured rat calvaria, while acid phosphatase and β-*N*-acetylglucosaminidase activities are not elevated. Also, zinc increases collagen content in cultured rat calvaria [8]. Those increases were blocked completely by treatment with cycloheximide, an inhibitor of protein synthesis [8]. Presently, we do not know whether zinc stimulates the induction of other proteins besides alkaline phosphatase and collagen, which are involved in bone formation. This, however, may be possible.

It is known that bone growth retardation is a common finding in various conditions associated with zinc deficiency [2, 3]. There is an appreciable amount of zinc in bone cells. Zinc deficiency may cause the decrease of zinc content in bone cells. Presumably, this induces the retardation of the development of bone growth and bone formation, because zinc plays a physiologic role as an activator of protein synthesis in bone cells.

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