THE BIOLOGICAL ROLE OF ZINC, COPPER AND MAGNESIUM DURING THE GROWTH OF EMBRYONIC CHICK LIMB BUDS

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Abstract

Background: The limbs bud development is induced by the apical ectodermal ridge. The trace elements were found to have important role in biological growth of tissues such as tumor tissues.

Objectives: This study was done to reveal the possible biological role of trace elements as zinc, copper, and magnesium in the limb development induced by the apical ectodermal ridge.

Methods: Chick embryos of Hamburger and Hamilton stages 20-26 were used. Trace elements were measured in limb buds with and without apical ectodermal thickening.

Results and discussion: The decrease in zinc concentration found in limb buds tissues having no apical ridge suggested a relationship between this trace element and the inductive effect produced by the apical ectoderm for limb buds formation. The detectable concentration of copper only in buds having no apical ridge was correlated with the embryonic organization of the limb buds. The lower magnesium concentration in the limb buds was considered as a criterion for the metabolism accompanies the functioning apical ectoderm during the embryogenesis of the limb buds.

Conclusions: Trace elements may have a significant role in the embryonic process of budding.

Keywords: Limb buds, trace elements, zinc, copper, magnesium, development.

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Introduction

Embryonic development of the limb bud is induced by thickening of the ectoderm at the apex of the bud[1,2]. The limb primordium starts to develop at stage 16 of Hamburger and Hamilton (H&H) corresponding to about 50-60 hours of incubation. It appears as inconspicuous condensation of the mesoderm at the wing level of the chick embryo, followed by formation of the primordia of the leg bud. The toe plate becomes distinct at stage 24 H&H (at about 4.5th day of incubation) prior to the digital plate that develops a stage later. Faint grooves appear in the toe and the digital plates during the 5th day of development (stages 25 &26 H&H). The differentiation of the wing and leg buds exhibit many resemblances, both have apical ectodermal thickened at earlier embryonic periods[3,4]. The centers of codifications appear in the condensed mesenchyme of limb bud at about the end of sixth day of incubation[5].

The biochemical aspects of induction in embryonic development were not investigated meticulously. In this study, a trial has been carried out to reveal the possible biological role of trace elements as zinc, copper, and magnesium in early embryonic development of limb buds induced by the apical ectodermal ridge. Influence of trace elements in body metabolism and their physiological importance have motivated their accurate quantitative

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determination in biological tissues[6]. In chick embryos growth of the limb bud and its parts (including the apical ectodermal ridge) were investigated during the period between the onset of its formation (Hamburger and Hamilton's stage 17) and the stage of nerve fiber in growth (H&H stage 22)[7].

Materials & methods

Fertilized chick eggs of Euribrid Lohman and Faobro type were incubated at 38°C in a humid environment. Embryos of Hamburger and Hamilton stages 20-26 were used corresponding to the incubation period of 3-5 days[3]. Sixty embryos were used in this study. Forty five embryos of stages 20-22 having the apical ectodermal thickening were classified as group (A), fifteen embryos of stages 23-26 with no apical ectodermal thickening were classified as group (B). Limb buds of embryos group (A) were excised and collected in a container with 0.5 ml of normal saline in three sets each set containing the buds of fifteen embryos. Similar procedure was done for the limb buds of group (B) embryos, in each of the three sets of containers limb buds of five embryos were collected.

At first the container were weighed empty, then they were weighed with 0.5 normal saline and lastly we weighed the container and normal saline plus the tissue added. The final weight of the tissue was obtained by abstracting the weight of container plus normal saline from the total weight (Table 1).

Table 1: Method of weighing limb buds with AER

Wt. for empty container (gm)	Wt. of the container with normal saline (gm)	Wt. of the container with normal saline + the tissue (gm)
0.8962	1.4182	1.4533
0.8913	1.4171	1.4529
0.8906	1.4167	1.4599

Table 2: Method of weighing limb buds with AER

Wt. for empty container (gm)	Wt. of the container with normal saline (gm)	Wt. of the container with normal saline + the tissue (gm)
0.7404	1.2586	1.2780
0.7511	1.2776	1.2974
0.7415	1.2685	1.2877

Each set of the specimens of limb buds in each groups (group A and B) were mixed with 0.5 ml of normal saline, homogenized by homogenizer for few minutes, and centrifuged. Then after, a known amount of segmentate was treated as following:

1. determination of Zinc and Copper:

One hundred *u*g\ml stock solution was diluted with de-ionized water to give the following concentrations of the working standard (0.0, 0.4, 0.8, 1.2, 1.6, and 2 One hundred *u*g\ml) of Zinc and Copper. Samples were diluted 1:10 with 6% butanol as diluents. This method achieved 30% increase in sensitivity compared with the used of water only[8]. This effect is due to a decrease in viscosity and difference in droplet formation. This technique is more widely used[9].

2. determination of Magnesium:

The samples used for determination of magnesium were diluted 1:50 with 1% lanthanum chloride solution to exclude the effect of segmentate phosphorous[10]. Standard solution of 1000 ppm was diluted with de-ionized water. Working standards of Magnesium were (0, 5, 10, 15, 20, 25, and 30 One hundred *u*g\ml).

Table 3: Mean concentrations of the trace elements

Trace element	Group (A) mean concentration (mg/gm)	Group (B) mean concentration (mg/gm)
Magnesium	0.002	0.037
Zinc	0.007	0.005
Copper	0	0.006

Results and Discussion

Embryonic stages included in-group (A) embryos have well developed ectodermal thickening at the apices of their limb buds[7]. Embryos of group (B) have toe and digital plates in their limb buds[4]. The mean values of the trace elements concentrations measured in this study are shown in table 1.

Embryonic tissue of the limb bud of group (B) shows higher magnesium and lower zinc concentration compared to that of group (A). Buds of group (B) only demonstrate detectable tissue concentration of copper.

It was concluded that many trace elements in tumor tissues exert direct or indirect action that accelerate growth of tumors[11]. Trace elements measured in this study may have a role to accelerate the growth of the limb buds.

Experimental studies showed evidence for special function of the ectodermal apical ridge in limb morphogenesis indicating chemical messengers. Striking and distinctive characteristics of the mesoderm close to the apical ectodermal ridge were found providing possibilities for the understanding of the function of the apical ridge in limb morphogenesis[12].

The higher concentration of the zinc was linked with rapidly growing tumor tissues in many studies; such elevated concentration was suggested to be a sign of increased cellular activities[13,14]. The higher concentration of zinc in limb bud tissues of group (A) having the apical ridge may signify the biological role of this element in motivation of limb development. The decrease of zinc concentration in limb buds of group (B) having no apical ridge may suggest a relationship between this trace element and the inductive effect produced by the apical ectoderm for limb bud formation. Lower zinc concentration of group (B) bud tissues may be a sign of a reduced inductive influence.

The detectable concentration of copper in limb buds of group (B) may indicate the beginning of embryonic organization. It was suggested that concentration of tissue copper is lowered during the metabolic changes of biological stress[15]. Accordingly, the low and undetectable concentration of copper in buds of group (A) may be correlated with the metabolic stressful events accompany the induction of the apical ectoderm for the early rapid growth and budding of limbs. Extirpation studies of the apical ectodermal ridge reveal its essential task during the critical early stages of limb bud formation; no limb was formed if the apical ectoderm is removed[16].

The above interpretation of the copper concentration in limb tissue may be applied to explain the higher magnesium concentration measured in limb buds of group (B) compared to that of group (A). Lower magnesium concentration in buds of group (A) may be a criterion for the metabolism accompanies the functioning apical ectoderm during the embryogenesis of the limb buds.

Budding is an embryological phenomenon observed in many embryonic regions. The concentration of trace elements should be more thoroughly investigated to establish a judgment for the significance of the biological role of trace elements in this embryological process.

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