Tibia and intestine alkaline phosphatase activity and zinc status of the chick as related to dietary protein source and supplements of zinc and histidine
by Betty Engberg Finch

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Home Economics
Montana State University

Abstract:
Chicks were fed low-zinc rations containing each of four protein sources. Supplements were zinc and histidine. The objective was to measure the activity of the zinc-containing phosphatases in the tibia and intestine in order to relate these to the zinc status of the chick. Sesame or soybean oil meal, casein-gelatin or egg albumen were the protein sources. Criteria for the zinc status were weight gain, tibia zinc content and possible occurrence of the characteristic leg deformities.

The histidine supplement greatly decreased the severity of the leg deformities found in chicks given the sesame meal rations; a trend to an increase in tibia alkaline phosphatase activity accompanied this: other criteria for zinc status were not affected. The leg deformities occurring in chicks fed the casein-gelatin ration were little depressed by this supplement nor was tibia alkaline phosphatase activity increased. Leg deformities did not occur in chicks given soybean meal or egg albumen rations; the histidine supplement did not affect other criteria of zinc status nor was tibia alkaline phosphatase activity significantly affected.

When a full zinc supplement was fed, tibia alkaline phosphatase activity, tibia zinc content and body weight were increased with all proteins and few leg deformities occurred in chicks given the sesame meal or casein-gelatin rations.

Little relationship between intestinal alkaline phosphatase activity and the zinc status of the chick was found. Although the intestinal phosphatase contains zinc, its activity was little affected by dietary zinc or histidine and thus is not a good criterion for judging the various facets of zinc status of the chick.

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Date May 20, 1971

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ABSTRACT

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The histidine supplement greatly decreased the severity of the leg deformities found in chicks given the sesame meal rations; a trend to an increase in tibia alkaline phosphatase activity accompanied this: other criteria for zinc status were not affected. The leg deformities occurring in chicks fed the casein-gelatin ration were little depressed by this supplement nor was tibia alkaline phosphatase activity increased. Leg deformities did not occur in chicks given soybean meal or egg albumen rations; the histidine supplement did not affect other criteria of zinc status nor was tibia alkaline phosphatase activity significantly affected.

When a full zinc supplement was fed, tibia alkaline phosphatase activity, tibia zinc content and body weight were increased with all proteins and few leg deformities occurred in chicks given the sesame meal or casein-gelatin rations.

Little relationship between intestinal alkaline phosphatase activity and the zinc status of the chick was found. Although the intestinal phosphatase contains zinc, its activity was little affected by dietary zinc or histidine and thus is not a good criterion for judging the various facets of zinc status of the chick.

CHAPTER I

INTRODUCTION

Metal ions are essential constituents of many enzymes; zinc is the one necessary for the activity of alkaline phosphatase (AP). If the zinc concentration is low or lacking in the diet, the enzyme activity is limited or non-existent. A relationship between the effects of a low zinc diet and AP activity, therefore, may be postulated.

AP is found in many tissues. One of its functions is thought to be concerned with the formation of bone. When chicks are given certain rations low in available zinc, malformations of the long bones occur. These can be prevented by the addition of 1% of histidine to the ration, [1,2] but the other symptoms of the zinc-deficiency are not affected. Since AP, zinc and histidine all seem to be concerned with the formation of bone, it may be possible that a quantitative relationship exists among them.

As far as this investigator knows, no one has determined the AP activity when a variety of protein sources, low in available zinc, are fed with and without the addition of histidine. The object of the present study is to determine the effect of protein source and supplemental zinc and/or histidine on the AP activity of the bone and intestine of the zinc-deficient chick as related to the presence or absence of the leg deformity caused by the zinc-deficiency.

CHAPTER II

REVIEW OF LITERATURE

AP is a zinc-containing enzyme which occurs in many tissue in the body. One of its functions is thought to be related to formation of bone [3], although the details of its physiological role are not thoroughly understood. It has been observed that in all types of ossification there is an increase in AP preceding and accompanying the deposition of bone salts in developing bones [4]. It has been suggested that one function of AP is in the formation of collagen, the organic matrix of bone [5]. Vesicles rich in both hydroxyproline, a major component of collagen, and AP are located near fibrils of chick bone cells that presumably, when extruded, contribute to collagen fibers [5]. It has also been suggested that AP promotes ossification by producing a high phosphate ion concentration from the breakdown of organic phosphate compounds [6].

Types of AP

Distinct types of AP have been differentiated, by their resistance to denaturation by urea digestion, from bone, liver and intestine of the chick [7].

The AP of chick plasma is thought to be of intestinal origin [7], but it also has been suggested that this AP is derived mainly from bone with lesser amounts from the liver [8].

Factors Affecting AP Activity

A reduced AP activity has been found in bone, intestinal tissues and serum of zinc-deficient birds [9,10,11,12]. In the normal chick the epiphyseal plate cartilage is the area of growth or elongation of the long bones. In the zinc-deficient chick the epiphyseal cartilage was abnormal [13,14,15]. In the proliferating region only the cells nearest the blood vessels appeared normal, although there was a denser population of cells near the vessels [14]. The epiphyseal-diaphyseal region was changed from a lengthy strand of cartilage tunnels to a narrow zone where penetration of the cartilage by tunnels was markedly reduced [13,16]. In the normal chick the AP activity in this cartilage was found in the regions of the proliferating cells nearest the mature cells where calcification was taking place.

It has been suggested that the AP associated with calcification is little affected by a zinc deficiency, but the AP associated with the developing epiphyseal plate cartilage is markedly affected [5]. AP in non-calcifying epiphyseal plate tissue appears to be necessary for normal cell maturation and degeneration, processes which are defective in the zinc-deficient chicks [5].

It has been observed that during starvation the level of chick plasma AP dropped and the proportion of intestinal phosphatase was reduced [17]. AP activity seems to be raised by oral doses of calcium

and a deficiency of vitamin D_3 [8,18,19,20,21], and lowered in osteoporosis and deficiencies of manganese and magnesium [5,9,21,22,23].

In the rat, zinc-deficient animals were noted to have a reduction in intestinal AP activity which was not affected by the reduced food intake [24]. A reduced serum AP activity was found in both the zinc-deficient rat and the restricted-fed control animals. This reduction was thought to be due to inanition [24].

Species

A zinc-deficiency can be produced in other species of animals, e.g., the rat [24,25], but only the avian species develops the characteristic leg deformities.

Protein Source and Leg Deformities

Severe leg deformities occurred when chicks were fed zinc-deficient diets with sesame meal, isolated soy protein or casein-gelatin as the protein source [26,27,28,29]. The addition of 1% of histidine prevented the leg deformities which occurred with the sesame meal or isolated soy protein, but did not affect those which occurred on the casein-gelatin diets [1,2].

Leg deformities did not occur when egg albumen was the source of protein [30]. The addition of 2% of arginine, however, caused leg deformities which were prevented by an addition of 1% of histidine [31].

Zinc Requirements

Chicks fed a sesame meal diet needed the addition of 60 mg/kg of zinc for normal growth and the prevention of the leg deformities [29]. Casein-gelatin diets required 10 mg/kg of zinc for the prevention of the deficiency symptoms [29]. The addition of 10 mg/kg of zinc was sufficient to meet the chick's requirement when egg albumen diets were fed [32]. Isolated soy protein diets needed the addition of 30 mg/kg of zinc for the prevention of the deficiency symptoms [29].

CHAPTER III

METHODOLOGY

Sample

The chick was used for this investigation, because, although a zinc-deficiency can be produced in other species, the avian species is the only one which exhibits the characteristic bone deformities of a shortening and thickening of the long bones of the legs and an enlargement of the hock joint. Two factorial experiments were conducted using day-old White Rock (F) X Cornish (M) chicks,[1] without sex differentiation. The chicks were randomly distributed into duplicate groups of 10 for the first experiment and 10 or 13 for the second.

Chick Care

The chicks were housed in a stainless steel battery[2] to minimize contamination from environmental zinc. Food and deionized water were given ad libitum.

Each cage of chicks was weighed as a group for the first two weeks and individually the third week. The leg scores were determined and the chicks were sacrificed by decapitation at three weeks of age.

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[1] Obtained from Quality Hatchery, Billings, Montana.

[2] Petersime Incubator Company, Gettysburg, Ohio.

Criteria for Measurement

The criteria for determining the effect of feeding the various diets for three weeks were:

1. Body weight. A difference of 20 gms was considered significant.
2. Leg Score (Appendix A).
3. Tibia zinc as indicative of body zinc status (Appendix A).
4. AP activity of bone and intestine (Appendix A).

The significance of these criteria was determined statistically by Duncan's Multiple Range Test.[3]

Diets

The diets were composed so as to contain 20% protein and 10% fat. Sesame meal, casein-gelatin, soybean meal or egg albumen were the basal protein sources (Table I). When additions of zinc[4] and/or histidine[5] were made, the sucrose was decreased accordingly.

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[3] Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics, 11:1.

[4] $ZnCO_3$ in sucrose; 1 gm = 10 mg/kg of zinc for 10 kg ration.

[5] 13.4 gm/kg L-histidine·HCL, monohydrate, Nutritional Biochemicals, Cleveland, Ohio, to give 1% of histidine.

TABLE I. Composition of Basal Diets.

	Texas 61 Sesame	Casein-Gelatin	Soybean Meal	Egg White
	g	g	g	g
Texas 61 sesame meal[1]	300			
Casein, vitamin free[2]		230		
Gelatin[3]		100		
Egg White[4]				200
Soybean meal[5]			400	
Vitamin mix[6]	5.0	5.0	5.0	5.0
MHA (Ca salt)[7]		3.3	3.3	
Choline chloride (70%)	3.0	3.0	3.0	3.0
Vitamin D[8]	1.5	1.5	1.5	1.5
Santoquin[9]	2.5	2.5	2.5	2.5
Corn oil[10]	94	96	96	96
Salts[11]	60.1	60.1	60.1	60.1
$MgCO_3$[12]		1.5		1.5
Biotin				10.0
L-lysine.HCL[13]	9.0			
Sucrose	524.9	497.1	428.6	620.4
	1,000.0	1,000.0	1,000.0	1,000.0

[1]Texas 61 sesame meal extracted twice with ethyl ether, 66% protein.

[2]Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3]Pharmaceutical grade gelatin, P. Leiner & Sons, America, Inc., St. Clair, Michigan.

[4]Egg white solids (albumen), Armour, Chicago.

[5]50% protein, commercial soybean meal, Farmer's Elevator, Bozeman, Montana.

[6]The vitamin mix contained: (in gm) vitamin B_{12} (as 0.1% mix), 4; menadione bisulfite sodium 0.9; biotin, 0.04; pyrodoxine · HCL, 1.0; folic acid, 1.0; niacin, 10.0; riboflavin, 2.0; D-Ca pnatothenate, 6.0; thiamin mononitrate, 2.0; vitamin A (250,000 IU/gm), 8; vitamin E (250 IU/gm), 40. The basic mix was made to 1,000 gm with sucrose.

[7]Calcium salt of methionine hydroxyanalogue.

[8]Vitamin D_3, 1,000 IU/ml.

[9]Santoquin, Monsanto Company, St. Louis, in corn oil, 0.05 gm/ml.

[10]Mazola.

(continued)

Footnotes Table I (continued)

[11]The salt mix contained: (in gm) $CaHPO_4 \cdot 2H_2O$, 5440; $CaCO_3$, 2984; K_2HPO_4, 2222; NaCL, 1200; $MgCO_3$, 25; Fe Citrate, 66.6; $MnSO_4 \cdot H_2O$, 66.6; KI, 0.52; $CuSO_4 \cdot 5H_2O$, 6.68.

[12]Biotin mix, 40 mg per 100 gm sucrose.

[13]L-lysine·HCL, Nutritional Biochemicals, Cleveland, Ohio, to give an added 0.72% lysine.

CHAPTER IV

RESULTS AND DISCUSSION

Tibia AP and Zinc Status

Source of Protein

Casein-Gelatin. Chicks developed symptoms of a zinc-deficiency when they were fed the basal diet (Table II). Growth was poor, AP activity and tibia zinc content were low and leg scores were high. The addition of 1% of histidine had little effect on these parameters, except the body weight, in either experiment 1 or 2.

Although the supplemental histidine affected the body weight, the change was not reflected in the tibia weight. In experiment 1 the supplemental histidine increased the chick body weight, but no significant effect was noted on the tibia weight or zinc content. In experiment 2 the histidine had the opposite effect on the body weight, but again, the change was not reflected in the tibia weight or zinc content.

A combined supplement of 5 mg/kg of zinc and 1% of histidine resulted in an increase in the AP activity, tibia weight and zinc content and body weight. The leg scores were markedly reduced.

The addition of 10 mg/kg of zinc prevented the symptoms of a zinc deficiency as shown by significant increases in AP activity, tibia weight and zinc content and body weight. There was also a noticeable reduction in the leg scores.

TABLE II. Tibia Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Casein-Gelatin Diets

Variations	Alkaline Phosphatase[2] Total	Alkaline Phosphatase[2] Per gm	Tibia Weight	Tibia Zinc	Chick Weight	Leg Score
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 1			
0 Zn	13.65 c	14.63 b	0.88 e	38	85	2.7
0 Zn + Hist	14.51 c	15.50 b	0.96 e	53	106	3.0
5 Zn + Hist	47.71 b	33.00 a	1.46 d	118	194	1.6
10 Zn	109.52 a	33.82 a	3.31 a	308	287	1.1
			Experiment 2			
0 Zn	48.59 a	28.30 c,d	1.82 a	243	197	2.0
0 Zn + Hist	53.66 a	33.84 b,c	1.62 a	213	161	1.9

[1]Mean of six chicks; 20 per ration originally.

[2]p-nitrophenol liberated at 38°C in 30 minutes.

[3]P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

If AP is concerned with bone formation, the prevention of leg deformities in chicks given zinc supplements is consistent with the increase in AP found, and with reports of other workers. Similarly, the histidine supplement neither prevented the leg deformities nor increased tibia AP. This experiment at least established the fact that histidine per se does not increase AP activity since no effect was found with the basal ration, nor an increase beyond what might be expected when half the requirement, 5 mg/kg of zinc was supplied.

In experiment 2 there was a change in the source of the casein. It contained more zinc that was available to the chick, as is shown by the higher tibia zinc content on the basal diet (Table II). The AP activity obtained with this casein was about the same as that obtained from the combined supplement of 5 mg/kg of zinc and 1% histidine used in experiment 1. It was postulated that the casein used in experiment 2 contained approximately 5 mg/kg of zinc and, therefore, the results obtained on this ration are not typical. Even at this level, however, with a lower leg score and better growth, presumably due to zinc, histidine did not increase AP activity nor decrease leg scores. Thus the results of both experiments 1 and 2 confirm the general conclusion that histidine per se has little effect on zinc-deficiency symptoms, nor AP activity, when casein-gelatin is the protein source.

Egg Albumen. Leg deformities are not found when chicks are fed egg albumen diets [30]. Chicks fed any variation of this diet did not

grow well (Table III). Total growth is a determining factor in the development of the leg deformities, therefore, in our experiment, they would not be expected to develop.

When the basal ration was fed, the zinc status of the chicks was poor, as shown by the low tibia zinc content, tibia weight and very low AP activity. The addition of 1% of histidine had little effect on these parameters.

A further decrease in the low leg score was found with the full zinc supplement, accompanied by an increased AP activity. The addition of histidine to the zinc supplement did not affect either parameter.

Sesame Meal. Severe zinc-deficiency symptoms were found when chicks were fed the basal diet (Table IV). Growth was poor, AP activity, tibia weight and zinc content were low, and leg scores were high.

In experiment 1 the addition of 1% of histidine markedly decreased the leg score and there was a trend for an increase in AP activity, without a marked increase in the tibia zinc content. Further work in this laboratory has shown that histidine supplements do increase AP activity and significantly reduce the leg scores without a change in tibia zinc content [33].

The supplement of 60 mg/kg of zinc caused a significant increase in AP activity, body weight and tibia zinc content and decreased the leg scores.

TABLE III. Tibia Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Egg Albumen Diets

Variations	Alkaline Phosphatase[2]		Tibia		Chick Weight	Leg Score
	Total	Per gm	Weight	Zinc		
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 2			
0 Zn	5.09 c	8.42 d	0.52 b	35	58	1.4
0 Zn + Hist	14.46 c	24.00 c,d	0.56 b	47	68	0.9
20 Zn	50.80 a	50.44 b	1.00 b	230	122	0.3
20 Zn + Hist	56.02 a	76.03 a	0.85 b	232	131	0.3

[1]Mean of six chicks; 20 per ration originally.

[2]p-nitrophenol liberated at 38°C in 30 minutes.

[3]P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

TABLE IV. Tibia Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Sesame Meal Diets

Variations	Alkaline Phosphatase[2]		Tibia		Chick Weight	Leg Score
	Total	Per gm	Weight	Zinc		
	mg^3	mg^3	gm^3	mg/kg	gm	
			Experiment 1			
0 Zn	37.38 b,c	19.99 b	1.89 c,d	103	198	3.6
0 Zn + Hist	50.62 b	22.85 a,b	2.23 b,c	139	237	1.2
60 Zn	86.04 a	31.92 a	2.59 b	376	291	1.0
			Experiment 2			
0 Zn	27.40 b,c	17.70 c,d	1.76 a	64	173	3.2
0 Zn + Hist	40.36 a,b	25.65 c,d	1.63	81	190	2.4

[1] Mean of six chicks; 20 per ration originally.

[2] p-nitrophenol liberated at 38°C in 30 minutes.

[3] P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

The results obtained with histidine are not as great as those obtained with the zinc supplement, but the AP activity is about halfway between the basal diet and the zinc-supplemented diet.

In experiment 2 the histidine supplement had the same effects as in experiment 1, except the leg score was not as markedly reduced.

Soybean Meal. Chicks given soybean meal rations do not exhibit severe leg deformities (Table V), although the zinc content of the ration was not high, 32 mg/kg [34], as compared to the suggested 35 mg/kg with vegetable proteins [35]. This is thought to be due to better utilization of zinc by a factor inherent in the soybean meal [36].

A histidine supplement was used with this vegetable protein ration to determine its effect on AP activity when leg deformities were not involved. The trend was toward a reduction in the AP activity in contrast to a trend with the sesame meal toward an increase. As with the casein-gelatin rations, addition of histidine did not cause an increase in AP *per se*.

Intestinal AP and Zinc Status

It was realized that the sample segment arbitrarily taken comprised a greater portion of the total intestine in the smaller chicks than in the larger ones. Possible variation in the AP activity from the duodenum to the caecum has not been investigated in the chick. With the rat and mouse [24,37], however, the highest AP activity was found to be in the first 1 to 2 gram segment of the duodenum proximal to the

TABLE V. Tibia Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Soybean Meal Diets

Variations	Alkaline Phosphatase[2]		Tibia		Chick Weight	Leg Score
	Total	Per gm	Weight	Zinc		
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 2			
0 Zn	43.29 a,b	26.80 c,d	1.64 a	89	177	0.7
0 Zn + Hist	31.28 b	20.71 c,d	1.66 a	86	184	0.2

[1]Mean of six chicks; 20 per ration originally.

[2]p-nitrophenol liberated at 38°C in 30 minutes.

[3]P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

pyloric valve and fell sharply to a much lower level in the jejunum. In these experiments approximately 1 gram of the pyloric valve end of the intestine was taken and homogenized for the AP determinations, so the area of highest enzyme activity was taken.

Source of Protein

Casein-Gelatin. Although severe zinc-deficiency symptoms were found in chicks given the basal diet (Table VI, Exp. 1), the intestinal AP activity was high. It was significantly decreased by the addition of histidine although the tibia zinc content was not affected. The full zinc supplement not only alleviated the overt deficiency symptoms, but further increased the enzyme activity. Half the zinc supplement, 5 mg/kg, tended to counteract the depressing effect of histidine.

The intestinal enzyme probably has first demand on limited supplies of zinc to enable the chick to utilize food to stay alive but not necessarily grow. Increased growth would imply tibia growth and the AP to support this. This could explain the high intestinal enzyme activity in the chicks given the basal ration even though the tibia AP was low (Table II) and other zinc-deficiency symptoms were severe.

There is not obvious explanation of why histidine depressed the intestinal enzyme activity but had no effect on the tibia enzyme. Nor does it appear that histidine facilitated the passing of available zinc through the intestine to be deposited in the bone, as shown by the lack of large increases in the tibia zinc content. Since growth,

TABLE VI. Intestine Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Casein-Gelatin Diets

Variations	Alkaline Phosphatase[2] Total	Alkaline Phosphatase[2] Per gm	Tibia Weight	Tibia Zinc	Chick Weight	Leg Score
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 1			
0 Zn	1,695.13 b	374.29 a,b	0.88 e	38	85	2.7
0 Zn + Hist	834.05 c	212.73 c	0.96 e	53	106	3.0
5 Zn + Hist	1,356.72 b,c	299.08 b,c	1.46 d	118	194	1.6
10 Zn	2,580.90 a	508.29 a	3.31 a	308	287	1.1
			Experiment 2			
0 Zn	617.28 b,c	178.23 a,b,c	1.82 a	243	197	2.0
0 Zn + Hist	1,034.56 a	247.98 a	1.62 a	213	161	1.9

[1] Mean of six chicks; 20 per ration originally.

[2] p-nitrophenol liberated at 38°C in 15 minutes.

[3] P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

tibia AP and zinc content were increased and leg score reduced by the addition of 5 mg/kg of zinc plus histidine, regardless of the intestinal enzyme activity, the ready utilization of this zinc in correcting the above parameters of zinc-deficiency suggests that the effect of histidine occurs at the intestinal level and that this enzyme activity is not a measure of zinc status.

In experiment 2, even though more zinc was available, as shown by better growth, tibia zinc content and lower leg scores, the intestinal enzyme activity was low in chicks given the basal ration. In this experiment histidine increased rather than depressed the enzyme activity, and is comparable to experiment 1 with the supplement of 5 mg/kg of zinc. It is possible that there was variation in the chicks or in the analyses. This again suggests that the intestinal AP activity is not a measure of zinc status.

Egg Albumen. The AP activity of the chicks given the basal diet was low (Table VII). Histidine caused a non-significant trend to decrease the AP activity, but had little effect on the other parameters measured in this experiment. The full zinc supplement tended to increase the AP activity. The zinc was utilized by the body as can be seen in the tibia zinc content.

Food intake for chicks fed any variation of this diet was not markedly different from that of the other diets. Bide [17] found that starvation causes a drop in the intestinal AP of birds. These chicks

TABLE VII. Intestine Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Egg Albumen Diets

Variations	Alkaline Phosphatase[2] Total	Alkaline Phosphatase[2] Per gm	Tibia Weight	Tibia Zinc	Chick Weight	Leg Score
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 2			
0 Zn	189.78 c,d	136.37 b,c	0.52 b	35	58	1.4
0 Zn + Hist	149.17 d	114.32 b,c	0.56 b	47	68	0.9
20 Zn	351.61 b,c,d	145.79 a,b,c	1.00 b	230	122	0.3
20 Zn + Hist	203.65 c,d	87.21 c	0.85 b	232	131	0.3

[1]Mean of six chicks; 20 per ration originally.

[2]p-nitrophenol liberated at 38°C in 15 minutes.

[3]P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

were not starved, but seemed to be unable to utilize the food they ate. It was thought that starvation was not a factor in the low AP activity found in these chicks. The zinc supplements had a tendency to increase the food intake but had only a small effect on the AP activity.

Sesame Meal. In spite of the severe zinc-deficiency symptoms found in chicks given the basal diet (Table VIII), the intestinal AP activity was high.

The addition of histidine or the full zinc supplement made no significant change in the enzyme activity although the tibia zinc content and body weight were increased with both supplements. As with the animal proteins, AP activity was not correlated with zinc status. Sufficient zinc to make more was apparently available when 60 mg/kg was fed, as shown by the large increase in the tibia zinc, but this did not occur to any significant extent. Again it appears that the intestinal AP has priority on supplies of zinc (p.18) and when the desideratum is met, more is not made simply because more zinc is available. Thus correction of overt signs of zinc-deficiency by additional zinc, or of leg deformities by histidine seemed to have no relation to intestinal AP activity, and vice versa.

Soybean Meal. As with the tibia enzyme, histidine had no significant effect on AP activity.

TABLE VIII. Intestine Alkaline Phosphatase Activity and Zinc Status of Chicks[1] Fed Sesame Meal Diets

Variations	Alkaline Phosphatase[2]		Tibia		Chick Weight	Leg Score
	Total	Per gm	Weight	Zinc		
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 1			
0 Zn	1,636.47 b	361.96 b,c	1.89 c,d	103	198	3.6
0 Zn + Hist	1,762.32 b	312.43 b,c	2.23 b,c	139	237	1.2
60 Zn	2,080.54 a,b	356.92 b,c	2.59 b	376	291	1.0
			Experiment 2			
0 Zn	693.82 b,c	218.75 a,b	1.76 a	64	173	3.2
0 Zn + Hist	778.20 a,b	198.63 a,b	1.63 a	81	190	2.4

[1] Mean of six chicks; 20 per ration originally.

[2] p-nitrophenol liberated at 38°C in 15 minutes.

[3] P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

TABLE IX. Intestine Alkaline Phosphatase Activity and Zinc Status in Chicks[1] Fed Soybean Meal Diets

Variations	Alkaline Phosphatase[2]		Tibia		Chick Weight	Leg Score
	Total	Per gm	Weight	Zinc		
	mg[3]	mg[3]	gm[3]	mg/kg	gm	
			Experiment 2			
0 Zn	513.29 b,c,d	183.79 a,b,c	1.64 a	89	177	0.7
0 Zn + Hist	518.89 b,c	156.15 a,b,c	1.66 a	86	184	0.2

[1]Mean of six chicks; 20 per ration originally.

[2]p-nitrophenol liberated at 38°C in 15 minutes.

[3]P(<0.5) with Duncan's Multiple Range Test. Values followed by the same letters within the same column are not significantly different.

Comparison of Proteins

Tibia AP Activity. With the basal rations, a higher AP activity and tibia zinc content were found with vegetable proteins than with animal proteins (Tables II-V). These parameters seemed to have no direct relation to the leg deformities, severe with sesame meal or casein-gelatin but absent with soybean meal or egg albumen. It appears that AP activity *per se* will vary with the protein source of the ration. A base line cannot arbitrarily be set in relation to zinc-deficiency symptoms. Each protein source must be considered individually.

With all the proteins, supplemental zinc increased AP activity, tibia zinc content and body weight. Where leg deformities were a factor, these were markedly reduced. The AP activity and tibia zinc content of chicks given either sesame meal or casein-gelatin rations were comparable, regardless of the differences found with the basal ration. This suggests that in relating AP activity to zinc-deficiency symptoms, following changes in this parameter is a better criterion than absolute numerical values.

As far as the histidine supplement is concerned, only the chicks given the sesame meal rations showed a trend to an increase in AP activity. Only with the sesame meal did the histidine have a preventive effect on the leg deformities. This suggests that with this protein the partial correction of the leg deformities by histidine is

related to an increase in AP activity. With the other proteins, histidine had no consistent effect on the parameters measured.

Intestine AP Activity. With the exception of the egg albumen rations, AP activity did not seem to be related to any of the facets of the zinc-deficiency or with the source of protein and supplements of zinc and/or histidine (Tables VI-IX). The AP activity of the chicks given the basal egg albumen ration was very low. It was not significantly affected by either zinc or histidine supplements. This was thought to be due primarily to the poor growth.

While supplements of zinc and/or histidine to the various protein sources could influence the tibia AP activity and zinc status of the chick, intestinal AP activity did not seem to be influenced by these factors or related to the zinc status of the chick.

Although a zinc-deficiency can be produced in other species of animals, only the avian species develop the characteristic leg deformities. The data obtained in this experiment showed that changes in tibia AP activity is related to supplements of zinc and/or histidine to the diet. It appears that the determination of tibia AP activity is a more desirable criteria for investigation of a zinc-deficiency in the fowl than the intestinal AP activity.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Chicks were fed low-zinc rations containing each of four protein sources. Supplements were zinc and histidine. The objective was to measure the activity of the zinc-containing phosphatases in the tibia and intestine in order to relate these to the zinc status of the chick. Sesame or soybean oil meal, casein-gelatin or egg albumen were the protein sources. Criteria for the zinc status were weight gain, tibia zinc content and possible occurrence of leg deformities.

The histidine supplement greatly decreased the severity of the leg deformities found in chicks given the sesame meal ration; a trend to an increase in tibia AP activity accompanied this; other criteria of zinc status were not affected. The leg deformities occurring in chicks fed the casein-gelatin ration were little decreased by this supplement nor was tibia AP activity increased. Leg deformities did not occur in chicks given soybean meal or egg albumen; the histidine supplement did not affect other criteria of zinc status nor was tibia AP activity significantly affected.

When a full zinc supplement was fed, tibia AP activity, tibia zinc content and body weight were increased with all proteins and few leg deformities occurred in chicks given the sesame meal or casein-gelatin rations.

Little relationship between intestinal AP activity and the zinc status of the chick was found. Although the intestinal enzyme contains zinc, its activity was little affected by dietary zinc or histidine and thus is not a good criterion for judging the various facets of zinc status of the chick.

Conclusions

1. Tibia AP activity of chicks fed the basal low-zinc rations varied with the source of the protein.

2. Zinc supplements increased the tibia AP activity and corrected the overt signs of the zinc-deficiency regardless of the protein source.

3. The histidine supplement corrected the leg deformities that occurred with the sesame meal rations and a trend to an increase in the tibia AP activity was found. With the casein-gelation rations, histidine did not correct the leg deformities nor increase the tibia AP activity. With the soybean meal or egg albumen rations, histidine had little consistent effect on the parameters for determining zinc status.

4. Intestinal AP activity was not consistently affected by either the zinc or histidine supplements. It appears that this parameter is not a desirable criterion for the assessment of the zinc status of the chick.

Recommendations

Research. Further research should be done with the egg albumen rations. It is suggested that 2% arginine be added in an attempt to obtain leg deformities. The effect of histidine supplements on the tibia AP activity and possible leg deformities obtained in chicks given this animal protein could possibly confirm or disprove the present results obtained with the animal proteins, casein plus gelatin.

Technique. The intestine sample segment removed from the chick should be standardized in some way. Possibly a certain proportion of the total intestine, or a certain length of the intestine be taken.

The tibia removed for AP activity analysis should be labeled individually rather than all three from one cage put in the same sack with no individual differentiation.

LITERATURE CITED

1. Nielsen, F. H., M. L. Sunde and W. G. Hoekstra. 1967. Effect of Histamine, Histidine and Some Related Compounds on the Zinc Deficient Chick. Proc. Soc. Exp. Biol. Med., 124:1106.

2. Lease, J. G. 1968. Effect of Histidine or Soybean Meal Additions on Zinc Deficiency Symptoms. Fed. Proc., 27:483.

3. Bloom, William and Don W. Fawcett. 1964. A Textbook of Histology. Philadelphia: W. B. Saunders Company, p. 154.

4. Moog, Florence and Eleanor Lerner Wenger. 1952. The Occurrence of a Neutral Mucopolysaccharide at Sites of High Alkaline Phosphatase Activity. Amer. J. Anat., 90:339.

5. Westmoreland, Nelson and W. G. Hoekstra. 1969. Histochemical Studies of Alkaline Phosphatase in Epiphyseal Cartilage of Normal and Zinc-deficient Chicks. J. Nutr., 98:83.

6. Comar, C. L. and Felix Bronner. 1961. Mineral Metabolism. New York: Academic Press, p. 610.

7. Bide, R. W. 1969. Plasma Alkaline Phosphatase in the Fowl: Differentiation of Tissue Isoenzymes by Urea. Technicon Symposia.

8. Kuan, S. S., W. G. Martin and H. Patrick. 1966. Alkaline Phosphatases of the Chick. Partial Characterization of the Tissue Isozymes. Proc. Soc. Exp. Biol. Med., 122:172.

9. Starcher, Barry and F. H. Kratzer. 1963. Effect of Zinc on Bone Alkaline Phosphatase in Turkey Poults. J. Nutr., 79:18.

10. Britton, W. M. and C. H. Hill. 1967. The Influence of Cadmium and Copper on Zinc Deficiency. Fed. Proc., 26:523.

11. Spivey Fox, M. R. and Richard M. Jacobs. 1967. Zinc Requirement of the Young Japanese Quail. Fed. Proc., 26:524.

12. Harrison, B. N. and M. R. Spivey Fox. 1968. Effect of Zinc Deficiency on Alkaline Phosphatase in the Liver, Plasma and Tibia of Japanese Quail. Fed. Proc., 27:483.

13. Young, R. J., H. M. Edwards, Jr. and M. B. Gillis. 1958. Studies on Zinc in Poultry Nutrition. Poul. Sci., 37:1100.

14. Westmoreland, Nelson and W. G. Hoekstra. 1969. Pathological Defects in the Epiphyseal Cartilage of Zinc-deficient Chicks. J. Nutr., 98:76.

15. O'Dell, B. L., P. M. Newberne and J. E. Savage. 1958. Significance of Dietary Zinc for the Growing Chick. J. Nutr., 65:503.

16. Nielsen, Forrest H., Richard P. Dowdy and Zigmund Z. Ziporin. 1970. Effect of Zinc Deficiency on Sulfur-35 and Hexosamine Metabolism in the Epiphyseal Plate and Primary Spongiosa of the Chick. J. Nutr., 100:903.

17. Bide, R. W. and W. J. Dorward. 1970. Plasma Alkaline Phosphatase in the Fowl: Changes with Starvation. Poul. Sci., 49:708.

18. Martin, W. G. and H. Patrick. 1962. The Relationship of Serum Alkaline Phosphatase to Ca^{45} Metabolism in the Chick as Influenced by Age, Vitamin D_3 and Treatment. Poul. Sci., 41:916.

19. Martin, W. G. and H. Patrick. 1961. The Effect of Oral Doses of Ca^{45} to Chicks on Changes in Serum Alkaline Phosphatase. Poul. Sci., 40:1360.

20. Waibel, Paul E. and Frank R. Mraz. 1964. Calcium, Strontium and Phosphorus Utilization by Chicks as Influenced by Nutritional and Endocrine Variations. J. Nutr., 84:58.

21. Stutts, Ensel C., W. E. Briles and H. O. Kunkel. 1957. Plasma Alkaline Phosphatase Activity in Mature Inbred Chickens. Poul. Sci., 36:269.

22. Wiese, A. C., G. H. Benham, C. A. Elvehjem and E. B. Hart. 1941. Further Bone Phosphatase Studies in Chick Perosis. Poul. Sci., 20:255.

23. Hurwitz, S. and Paul Griminger. 1961. The Response of Plasma Alkaline Phosphatase, Parathyroids and Blood and Bone Minerals to Calcium Intake in the Fowl. J. Nutr., 73:177.

24. Luecke, Richard W., Mary E. Olman and Betty V. Baltzer. 1968. Zinc Deficiency in the Rat: Effect on Serum and Intestinal Alkaline Phosphatase Activities. J. Nutr., 94:344.

25. Forbes, R. M. 1961. Excretory Patterns and Bone Deposition of Zinc, Calcium and Magnesium in the Rat as Influenced by Zinc Deficiency, EDTA and Lactose. J. Nutr., 74:194.

26. Lease, J. G., B. D. Barnett, E. J. Lease and D. E. Turk. 1960. The Biological Unavailability to the Chick of Zinc in a Sesame Meal Ration. J. Nutr., 72:66.

27. Lease, J. G. and W. P. Williams Jr. 1967. Availability of Zinc and Comparison of In Vitro and In Vivo Zinc Uptake of Certain Oil Seed Meals. Poul. Sci., 46:233.

28. Likuski, H. J. A. and R. M. Forbes. 1964. Effect of Phytic Acid on the Availability of Zinc in Amino Acid and Casein Diets Fed to Chicks. J. Nutr., 84:145.

29. Lease, J. G. and W. P. Williams Jr. 1967. The Effect of Added Magnesium on the Availability of Zinc with Some High-Protein Feedstuffs. Poul. Sci., 46:242.

30. Nielsen, F. H., M. L. Sunde and W. G. Hoekstra. 1966. Effect of Dietary Amino Acid Source on the Zinc-Deficiency Syndrome in the Chick. J. Nutr., 89:24.

31. Coleman, B. W., E. M. Reimann, R. H. Grummer and W. G. Hoekstra. 1969. Antagonistic Effect of Dietary Arginine on Zinc Deficiency in Chicks. Fed. Proc., 28:761.

32. Davis, Susan S. 1970. Effect of Certain Amino Acid, Aspirin and Zinc Supplements on the Zinc-Deficiency Syndrome of Chicks Given Four Protein Sources. Master's Thesis. Montana State University, Bozeman, Montana.

33. Lease, J. G. 1971. Effect of Dietary Histidine on Tibia Alkaline Phosphatase and Leg Deformities of Chicks Given a Low-Zn Sesame Meal Diet. Fed. Proc., 30:643.

34. Lease, J. G. 1968. Effect of a Soluable Fraction of Oil Seed Meals on Uptake of ^{65}Zn from Ca·Mg.^{65}Zn·Phytate Complexes by the Chick. J. Nutr., 96:126.

35. Nutritional Requirements of Poultry. 1966. National Academy of Sciences, National Research Council, p. 3.

36. Lease, J. G. 1967. Availability to the Chick of Zinc-Phytate Complexes Isolated from Oil Seed Meals by an In Vitro Digestion Method. J. Nutr., 93:523.

37. Moog, Florence. 1961. The Functional Differentiation of the Small Intestine. VIII. Regional Differences in the Alkaline Phosphatases of the Small Intestine of the Mouse from Birth to One Year. Develop. Biol., 3:153.

APPENDIX

Leg Scores

Chick tibiae were scored with the following designations:

0 - normal leg development;

1 - slight deformity in one leg;

2 - slight deformity in both legs;

3 - severe deformity in one leg; and

4 - severe deformity in both legs.

The degree of leg deformity was determined by the shortness of the tibia and the enlargement of the hock joint. A difference of 1 in leg scores was considered significant.

Bone Preparation for Analysis

The right tibia was removed from six chicks randomly selected from each cage, most of the flesh was removed. Three bones were reserved for zinc analysis and three bones were reserved for AP determination. All bones were stored at -30°C until time of analysis.

Intestine Preparation

Starting at the pyloric valve, the length of the intestine sample was arbitrarily set as 2.5 cm. past the attachment of the pancreas to the intestine. This comprised most of the duodenum. This sample was taken from the same three chicks whose bones were set aside for AP analysis. The intestinal contents were gently squeezed out and the intestine rinsed twice with deionized water. The excess water was

gently squeezed off, the intestine labeled and put on crushed dry ice. The frozen intestine was stored in the freezer until all intestines from one cage were done; then all placed in a plastic bag and stored at -30°C until time of assay.

Tibia Zinc Analysis

1. The extraneous flesh was removed after dipping the bones in boiling deionized water for one minute, all excess tissue, including the epiphyseal plate cartilage was removed.
2. The three bones from one cage were wrapped together in paper toweling, crushed and securely tied; they were extracted in a Soxhlet extractor for 20 hours with 95% alcohol, 20 hours with ethyl ether, dried at a constant temperature of 50°C, cooled in desiccators and weighed.
3. The extracted bones were ashed at 550°C for 24 hours, cooled in desiccators and weighed.
4. The ash, dissolved in 0.1 N HCL, was analyzed for zinc by an atomic absorption method.[1] A Perkin-Elmer 290-B Spectrophotmeter was used; the wave length was 2138 Å; acetylene was the fuel, the oxidant was air. Metallic zinc was dissolved in 0.1 N HCL for

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[1]Courtesy of Mr. Vincent Haby, Plant and Soil Science, Montana State University, Bozeman.

the standard. The concentration was varied from 0 to 5 ppm over 0 to 100% transmission.

Alkaline Phosphatase Method

The amount of p-nitrophenol liberated from the p-nitrophenyl phosphate by AP was measured at 410 mu[2]. The methods were based on those used by Starcher and Kratzer [9] and Luecke, et al. [24].

Solutions[3]

1. 1 N HCL: 8.85 ml of concentrated acid in 100 ml water.
2. 1 M $MgCl_2$: 9.4 gm $MgCl_2$ in 100 ml water.
3. 1 M Buffer Substrate at pH 10: 4.45 gm 2-amino-2-methyl-1-propanol[4] in 50 ml flask, add 22.5 ml 1 N HCL, put solution in 50 ml volumetric flask and dilute to 50 ml with water. Add this 50 ml solution to 210 mg p-nitrophenyl phosphate diluted to 50 ml with water. Add 0.2 ml 1 M $MgCl_2$ to final solution.
4. Chloroform water: 2 ml chloroform in 100 ml water.
5. 0.25 N NaOH: 5 gm NaOH in 500 ml water.
6. Working Standard: (Stable one day, discard excess) Accurately pipette into a 100 ml volumetric flask 0.5 ml p-nitrophenol standard solution[4]. Add 0.25 N NaOH to make 100 ml, mix thoroughly.

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[2] A Spectronic 20 was used, Bausch and Lomb.

[3] Deionized water was used throughout.

[4] Sigma Chemical Company, St. Louis, Missouri. Stored at 8°C.

Method

Prepare buffer substrate. Pipette 1.5 ml into each (15 x 1.8) test tube and store at -30°C.

Six samples of either bone or intestine were analyzed at one time along with a buffer substrate-chloroform water blank.

Preparation of Calibration Curve

1. Prepare the working standard.
2. Pipette the solutions indicated in columns (2) and (3) of the table below into seven labeled test tubes.

(1)	(2)	(3)	(4)	(5)	(6)
Tube No.	ml Working Standard	ml 0.25 N NaOH	Percent Transmittance	Optical Density	Equivalents p-nitrophenol
1	.50	5.9			.50
2	1.0	5.4			1.0
3	1.5	4.9			1.5
4	2.0	4.4			2.0
5	3.0	3.4			3.0
6	4.0	2.4			4.0
7	5.0	1.4			5.0

3. Read and record the percent transmittance of each tube at 410 mu using 6.4 ml of 0.25 N NaOH as a blank. This gives a range of approximately 14-80% transmittance. The optical density is found on the chart that comes with the spectrophotometer. A curve is prepared for each day an experiment is run. The optical density of the standards is plotted as the ordinate and the equivalents of p-nitrophenol on the abscissa.

Tibia Method

1. Remove one bag of tibiae from the freezer. With a cheesecloth remove all flesh but retain the epiphyseal plate cartilage.
2. Weigh cleaned tibiae on analytical balance.
3. Wrap bone in slick white paper and crush. The crushed bone is homogenized[5] with 10 ml of water for 8 minutes in an ice bath, poured into a 15 ml centrifuge tube and kept in an ice bath.
4. The six samples are centrifuged at 2500 rpm for 10 minutes. The supernatant is decanted into labeled test tubes.
5. Dilute 0.1 ml of the supernatant to 5 ml with water. Use Cyclo-Mixer to insure mixing. Add 0.2 ml[6] of diluted supernatant and 0.3 ml of chloroform water to the buffer substrate tubes.
6. Incubate for exactly 30 minutes in a 38°C water bath. Add 4.5 ml of 0.25 N NaOH to stop the reaction.
7. Pour part of each tube contents into a spectrophotometer tube. Use the chloroform-water blank to standardize the machine. Read per cent transmittance at 410 mu. Plot the sample optimal density on the calibration curve prepared for that day.

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[5] Virtis "45" homogenizer. The Virtis Company, Inc., Gardiner, New York.

[6] Concentration may be varied if per cent transmission is above 80% or below 20%.

Calculations

Multiply the ml value obtained from the plotting of the optical density by 6.95 to find the micrograms of p-nitrophenol in the 0.2 ml sample.

Multiply the microgram value by 2,500 to get the micrograms in the whole bone. Divide the results obtained from the above step by 1,000 to get the mg of p-nitrophenol liberated from the total bone.

Divide the mg value by the weight of the bone to get the mg p-nitrophenol liberated per gram of bone.

Intestine Method

1. Remove one cage bag of intestines from freezer. Weigh each intestine on an analytical balance. Starting at the pyloric valve end remove approximately one gram for the sample. Weigh section removed on analytical balance.
2. Cut sample into small pieces and put into a straight-sided Virtis homogenizer flask into which 20 ml of water has been placed. Keep flasks on crushed ice until all intestines from one cage are weighed and put into flasks.
3. Homogenize each intestine for 2.5 minutes, keeping the flasks on ice. Pour homogenized intestine into labeled clean centrifuge tubes and centrifuge at 2,500 rpm for 15 minutes. Pour off supernatants into labeled test tubes and store at -30°C until analysis is done.

4. Remove intestine tubes for one cage from freezer. Thaw tubes in a beaker of cool water. After thawing, cork tubes and invert to mix contents. Pipette 0.1 ml of supernatant into labeled tube and dilute to 10 ml with water. Use Cyclo-Mixer to insure mixing.
5. Place tubes containing buffer substrate and 0.3 ml of chloroform water in 38°C water bath, add 0.1 ml of diluted substrate[7] and incubate for exactly 15 minutes.
6. At the end of the incubation time remove tubes from water bath and immediately add 4.5 ml of 0.25 N NaOH to each to stop the reaction.
7. Pour part of contents of each tube into spectrophotometer tube. Use chloroform-water blank to standardize machine. Read per cent transmittance at 410 mu. Convert sample values into optical density values and plot on calibration curve prepared for that day.

Calculations

Multiply ml values obtained from the plotting of the optical density by 6.95 to find the micrograms of p-nitrophenol liberated in the 0.1 ml sample.

Multiply the microgram value by 20 to get the milligrams of p-nitrophenol liberated for the segment used. Divide this value by the

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[7] Concentration may be varied if per cent transmittance is below 20% or above 80%.

weight of the segment used to obtain the mg p-nitrophenol liberated per gram of intestine.

Multiply the per gram value by the weight of the total segment taken to get the mg p-nitrophenol liberated for the total intestine.

Finch, Betty
Tibia and intestine alkaline phosphatase activity and zinc status of the chick ...