Evidence for Natural Chelates Which Aid in the Utilization of Zinc by Chicks

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Results have been presented indicating that certain natural feedstuffs, such as casein, liver extract, and distillers dried solubles, contain a factor or factors capable of improving both absorption and metabolic utilization of zinc. Since zinc utilization also is improved by supplementing the diet with the chelating agent ethylenediaminetetraacetic acid, it appears that the active principles in the natural feedstuffs may be natural chelates which normally function to improve transport and utilization of required mineral nutrients.

During the past three decades, we have witnessed the discovery, isolation, and identification of many vitamins and required nutrient factors previously recognized only as "unidentified chick growth factors." The chick was used as the chief experimental animal, and vitamin G was found to be riboflavin; the filtrate factor turned out to be pantothenic acid; factor R, vitamin B_{10}, and vitamin B_c were found to be pteroylglutamic or folic acid. The quest for such factors using chicks still continues in many nutrition laboratories. As each new factor is identified, it is added to the purified basal diet in adequate amounts, and then the search goes on for further nutrients.

Purified casein was used as the source of protein in the basal diets employed in most of the early work on unidentified chick growth factors. Thus, it was the casein diet that was used when zinc was recognized as a required nutrient. From very brief unpublished studies in the authors' laboratory, it appeared that the amount of added zinc necessary in the purified diet containing casein as the source of protein was approximately 4 to 5 mg. per kg. of diet. This amount of zinc, therefore, was routinely added to the mineral mixtures used in the authors' studies and those of most other research workers investigating unidentified growth factors required by the chick.

In recent years, other protein sources were employed in an effort to refine the purified basal diet to make it a more critical assay diet for the study of unidentified growth factors. Isolated, washed soybean protein apparently was very good for this purpose. With such a diet in 1955, Morrison *et al.* (*4*) at Cornell University reported evidence of the existence of an unidentified mineral nutrient required by the chick. The basal diet was believed to contain adequate amounts of all nutrients required by the chick. However, the addition of a mixture of special feedstuffs, such as milk by-products, fish solubles, and corn distillers dried solubles, or simply the ash of corn distillers dried solubles, improved the growth of chicks receiving this diet. A summary of 18 experiments conducted on this problem by Morrison and associates (*3*) is presented in Table I.

In 1957, O'Dell and Savage (*6*), reporting on the effects of zinc and corn distillers dried solubles as supplements to a purified soybean protein diet similar to that used by the Cornell workers, showed that zinc additions to this diet improved growth in a manner similar to that obtained by the Cornell workers from the ash of unidentified growth factor sources. In 1958, O'Dell *et al.* (*5*) showed that the zinc requirement of chicks receiving the purified diet containing soybean protein was approximately 35 mg. per kg. of diet. Since the basal diet contained about 15 mg. per kg., it appeared that approximately 20 mg. of added zinc was required per kg. of diet. Subsequent studies (*7*) on the zinc requirement, using soybean protein diets, sometimes indicated an even higher requirement.

Thus, Dam *et al.* (*1*) found that addition of zinc in high amounts produced the same growth and prevention of enlarged hocks and improvement of the poor feathering as had been obtained with the various special feedstuff sources of "unidentified chick growth factor" (UGF). This finding was difficult to reconcile, however, with the fact that the special sources of UGF, such as liver extract and distillers dried solubles, contained far too little zinc to account for their beneficial effects.

These supplements must therefore possess some property which allows chicks to attain near normal growth and prevention of enlarged hocks on lower amounts of zinc than are needed when pure zinc salts are added to the basal diet. Further work indicated that the major reason for the high zinc requirement under these conditions was that some property of the soybean protein supplement severely reduced the availability of zinc for chicks.

Recent studies by Kratzer *et al.* (*2*), confirmed by work here, indicated that the amount of zinc added by Morrison and coworkers (*3, 4*) to the purified diet used in the Cornell studies on the unidentified mineral factor is almost sufficient if the zinc present in the isolated protein of the basal diet is available to the chicks. Kratzer and associates found that the availability of the zinc in the soybean protein was greatly enhanced by addition to the diet of an effective chelating agent. The further work in the authors' laboratory indicates that a variety of natural materials may act in a manner similar to that of synthetic chelating agents, thereby causing a marked improvement in the availability to the chick of the zinc in the basal diet. The results of a series of studies with White Plymouth Rock × Vantress male chicks, comparing the effects of a liver extract and ethylenediaminetetraacetic acid (EDTA), an excellent chelating agent, are shown in Tables II, III, and IV. The results presented in these tables show that 15 mg. of added zinc per kg. of diet was insufficient for maximum growth and prevention of enlarged hocks as compared to that obtained upon addition of 60 mg. of zinc per kg. of diet. On the other hand, the addition of 1.5% liver extract produced normal growth in the presence of 15 mg. of added zinc per kg. of diet, and when

Table I. Summary of Results of 18 Experiments on the Unknown Mineral Requirement of Growing Chicks

Treatment	Average Weight at 4 Weeks, Grams	Response over Basal Diet, %
Basal diet	318	..
+ 6% distillers dried solubles	380	19
+ ash of 6% distillers dried solubles	357	12
+ 5 UGF[a]	418	31
+ ash of 5 UGF	390	23

[a] 3% Fish solubles, 3% dried whey, 3% dried grass juice, 3% penicillin mycelium meal, and 6% distillers dried solubles.

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3% liver extract was added to the diet in the presence of only 10 mg. of added zinc, maximum growth was obtained. Analysis of variance showed highly significant improvements in growth from addition of liver extract to the low levels of zinc, and no significant improvement from adding liver extract to chicks receiving 60 mg. of zinc per kg. of diet (Table II). Chemical analyses for the zinc content of the liver extract indicated that 3% of liver extract supplied only approximately 1.5 mg. of zinc per kg. of diet.

The results presented in Table II could be interpreted to indicate that the liver extract contained a second factor which supplemented the zinc and coincidentally produced growth and improvement of leg weakness equivalent to that which was achieved by a

Table II. Effect of Zinc and Liver Extract on Growth of Chicks

		0% Liver Extract			1.5% Liver Extract			3.0% Liver Extract		
Group	Zinc Added,[a] Mg./Kg.	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %
I	5	281[b]	1.99	73	356	1.86	73	410	1.74	70
	10	380	1.85	69	466	1.64	57	514	1.64	26
	15	457	1.67	46	524	1.62	30	556	1.63	8
II	60	524	1.60	5	545	1.58	5	560	1.56	3

Analysis of Variance

Source of variation	Degrees of freedom	Mean square	F value[c]
Treatments	11	16,196	30
Group I (zinc deficient)	8	15,944	29
Zinc	2	41,016	76
Liver extract	2	22,366	42
Zinc × liver extract	4	197	N.S.
Group II (zinc adequate)	2	654	N.S.
Group I *vs.* Group II	1	49,289	92
Error	12	535	

[a] Added to soy protein basal diet C of Zeigler *et al.* (7). [b] Results are average of two lots of 15 male chicks per lot. [c] Denotes significance at 0.01 level.

Table III. Effect of EDTA on the Zinc Requirement of Chicks

		No EDTA			EDTA, 150 Mg./Kg.			EDTA, 300 Mg./Kg.		
Group	Zinc Added,[a] Mg./Kg.	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %
I	0	159[b]	2.40	66	296	1.83	66	417	1.74	60
	5	240	1.93	76	513	1.60	49	563	1.57	9
II	60	592	1.56	5	583	1.55	7	587	1.53	2

Analysis of Variance

Source of variation	Degrees of freedom	Mean square	F value[c]
Treatments	8	56,481	153
Group I (zinc deficient)	5	50,600	137
Zinc	1	65,860	179
EDTA	2	88,945	241
Group II (zinc adequate)	2	40	N.S.
Group I *vs.* Group II	1	198,767	540
Error	9	367	

[a,b] Same as in Table II. [c] Denotes significance at 0.01 level.

Table IV. Effect of Liver Extract and EDTA on the Zinc Requirement of the Chick

(All values not followed by the same letter are significantly different from each other at the 0.05 level)

	None			EDTA, 300 Mg./Kg.			3.0% Liver Extract			10.5% Liver Extract		
Zinc Added,[a] Mg./Kg.	4-Week weight, grams	Feed/gain	Incidence of enlarged, hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %
0	...	...	..	...	...	...	...	...	..	357ef	1.88	68
5	206[b]	2.14	65	459d	1.52	4	316f	1.83	70	494bcd	1.60	20
15	377c	1.72	57	454d	1.71	3	478bcd	1.63	9	...	...	..
60	466cd	1.59	9	511abc	1.61	3	517ab	1.63	4	545a	1.66	3

Analysis of Variance

Source of variation	Degrees of freedom	Mean square	F value[c]
Treatments	11	19,633	48
Error	12	402	

[a,b] Same as in Table II. [c] Denotes significance at 0.01 level.

Table V. Evidence of a Chelating Effect in Corn Distillers Dried Solubles (DDS)

		No DDS			Ash = 6% DDS			6% DDS		
Group	Zinc Added,[a] Mg./Kg.	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %	4-Week weight, grams	Feed/gain	Incidence of enlarged hocks, %
I	0	...	...	..	243	2.05	80	276	1.87	64
	5	248[b]	2.03	67	386	1.72	72	402	1.70	67
	10	324	1.78	67	427	1.69	55	463	1.61	50
	15	417	1.64	49	...	...	..	...	...	..
II	60	561	1.57	7	553	1.49	3	569	1.54	6

Analysis of Variance

Source of variation	Degrees of freedom	Mean square	F value[c]
Treatments	11	27,491	215
Group I (zinc deficient)	8	13,690	107
DDS *vs.* ash + none	1	6,188	48
Ash *vs.* none	1	1,452	11
Added zinc	2	49,588	387
DDS *vs.* ash + none × zinc	2	95	N.S.
Ash *vs.* none × zinc	2	1,256	10
Group II (zinc adequate)	2	136	N.S.
Group I *vs.* Group II	1	96,306	753
Error	12	127	

[a,b] Same as in Table II. [c] Denotes significance at 0.01 level.

very high level of zinc. The results also could be interpreted to indicate the presence in liver extract of a possible zinc-containing vitamin which is required at a much lower zinc content level to produce maximum growth in a manner analogous to the effect of vitamin B_{12} as compared to cobalt in some animals. A third possibility is that the liver extract contained an agent which was effective in chelating iron or other minerals in the soybean protein, thereby rendering that zinc more available for use by the chick.

To check these hypotheses, the experiment was conducted which is presented in Table III. The results of this experiment show that the addition of EDTA at a level of 300 mg. per kg. to the diet containing only 5 mg. of added zinc per kg. of diet promoted growth which was approximately equivalent to that obtained with the addition of 60 mg. of zinc per kg. of diet in the absence of the chelating agent. The results presented in Table IV show a direct comparison of the effect of EDTA and that of liver extract upon the zinc requirement. In the presence of 5 mg. of added zinc per kg. of diet, the addition of either 300 mg. of EDTA or 10.5% liver extract resulted in markedly improved growth. In the presence of 15 mg. of added zinc per kg., 3% liver extract caused growth approximately equivalent to that obtained with 60 mg. of zinc added alone.

Addition of 10.5% liver extract, which supplied approximately 5 mg. of zinc per kg. of diet, produced growth which was superior to that obtained with 15 p.p.m. of zinc alone (Table IV). When fed together with 60 p.p.m. of zinc, it produced growth which was significantly superior to that obtained with 60 p.p.m. of zinc alone. These results are similar to many others obtained in the authors' laboratory and also to those reported by O'Dell and Savage (*6*), indicating the existence of at least one additional unknown growth factor.

Direct evidence indicating a chelating effect in corn distillers dried solubles is presented in Table V. These results show that 6% of corn distillers dried solubles, which contributed approximately 5 mg. of zinc per kg. of diet, actually produced a growth response which was equivalent to that obtained by the addition of twice that amount of added inorganic zinc.

On the other hand, supplementation of the diet with the ash of 6% corn distillers dried solubles increased chick growth only in proportion to the inorganic zinc content of the ash.

Work with radioactive Zn^{65} indicated that while the effect of EDTA and other chelating substances may be due to an improvement in the availability of the zinc contained in the basal soybean protein diet, evidence also has been obtained indicating a more efficient metabolic utilization of zinc in the presence of the chelating agent(s).

Chicks which had been starved for 24 hours were given about 15 grams of the specific diet under test and then an oral dose of Zn^{65}. Following dosing, no further feed was given. After 24 hours, the chicks were sacrificed, and the Zn^{65} contents of the bones (tibias), muscles, and livers were determined.

These studies showed that the Zn^{65} uptake by the bones, muscles, and livers was increased almost twofold when the casein diet was fed, as compared to feeding the soybean protein diet. Also, in studies with colostomized hens, both EDTA and liver extract decreased fecal excretion and increased urinary excretion of Zn^{65}.

On the other hand, when the soybean protein diet supplemented with 3% liver extract was fed, a signfficant reduction in retention of Zn^{65} by the tibia was noted as compared to the soybean protein diet alone. EDTA also reduced tibia deposition of Zn^{65}, but the reduction was not significant. Nevertheless, these data indicate that the effects of liver extract and EDTA may not be due simply to improving the absorption of zinc, but perhaps to some improvement in zinc metabolism.

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