Zinc 65 Secretion by the Exocrine Pancreas of Chicks Fed Soybean Meal[1] (37218)

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Zinc 65 is a useful tool to investigate the cellular regulation of Zn-containing enzymes. Zinc is a constituent of many enzymes or enzyme systems, among which are the carboxypeptidases, carbonic anhydrases, alkaline phosphatase and glutamic dehydrogenase (1). The form of administered Zn influences absorption and secretion of the element in the body (2). It is reported that $^{65}ZnCl_2$ is slowly excreted by the intestines, whereas ^{65}Zn-EDTA is rapidly excreted.

Injected ^{65}Zn is usually taken up by both the acinar and the islet cells of the pancreas. However, the turnover is higher in the acinar cells (3). A soybean meal diet fed to baby pigs with cannulated pancreatic ducts elicited a greater pancreatic secretion than when skim milk protein was fed. Feeding soybean meal diet resulted in secreting five times as much amylase, protease and lipase and twice the volume of pancreatic juice secreted by similar pigs fed a milk diet. This phenomenon of increased secretion was attributed to conceivable traces of the soybean trypsin inhibitor activity, which remained intact in the soybean meal diet used (4, 5). Pekas, Thompson and Hays (5) did report that a correlation existed between the ^{65}Zn activity in pancreatic juice and the total protein secreted.

The present study was undertaken to investigate the cellular regulation of Zn enzyme synthesis in the pancreas and to determine whether or not inhibitors or other components in soybean meal have an effect on the rate of synthesis and release of pancreatic enzymes, particularly the carboxypeptidases, using the incorporation and depletion of ^{65}Zn as an index.

Experimental Procedure. Chicks were fed diets containing unheated or autoclaved soybean meal for 4 wk, at which time they were fasted for 8 hr. Composition of the experimental diet was essentially that used by Salman (6). After this fasting period, ^{65}Zn in HCl[3] was administered intramuscularly at the level of 7 μCi/100 g body weight, the chicks were allowed 24 hr of feeding time from their respective previous diets.

A period on feed allowed for gradual build-up of ^{65}Zn activity in the pancreas and other organs, which was shown to be increasing greatly within 32 hr. After this postinjection period the chicks were fasted and 3 chicks from each diet were sacrificed after 6, 12, 16 or 22 hr of fast, corresponding to 30, 36, 42 and 48 hr of isotope administration. Other groups of three chicks were fed at 16 hr, either the same diet as before the fast or the alternate diet, for periods of 2 and 6 hr. Pancreata, livers, intestines and total excreta from individual chicks collected in polyethylene bags were removed, weighed and subsequently measured for gamma activity. The total excreta was collected in polyethylene bags attached to each individual pen. A well-type scintillation counter equipped with a thallium activated 2-in. NaI crystal was used to measure activity. The data were expressed as percentage of dose injected.

Results and Discussion. The data herein indicate that the pattern of ^{65}Zn secretion is related, if not parallel, to reported data on protein secretion from the pancreas (8). This is in support of the evidence shown by Pekas, Thompson and Hays (5) that a correlation existed between ^{65}Zn activity and

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[3] The ^{65}Zn in HCl was purchased from New England Nuclear Corp., Boston, MA.

total protein secreted from the pancreas of pigs. The percentage of injected ^{65}Zn per gram of wet tissue was higher in pancreata of chicks fed unheated soybean meal for 4 wk than in those fed autoclaved soybean meal. The pancreas tissue activity from replicate chicks showed very small deviations on a unit weight basis. Accumulation of the isotope was consistently higher in pancreata of fasted chicks which previously had been fed unheated soybean meal (Fig. 1). Feeding unheated soybean meal after 16 hr of fasting resulted in continuous depletion of the pancreas of its zymogen content as reflected by the rapid fall in the percentage of ^{65}Zn activity per gram of tissue. This type of response was observed when autoclaved soybean meal was fed to chicks after the fasting period. Initially, the ^{65}Zn activity per gram of pancreas did drop slightly after feeding, but it increased after 2 hr, presumably due to increased synthesis and/or decreased release of the ^{65}Zn proenzymes (Fig. 1). The results discussed are similar and complementary to published data (8) on pancreatic enzyme where tryptic and chymotryptic activities were much higher when unheated soybean meal was fed. The rate of depletion of the pancreas in response to feeding was higher when the unheated soybean

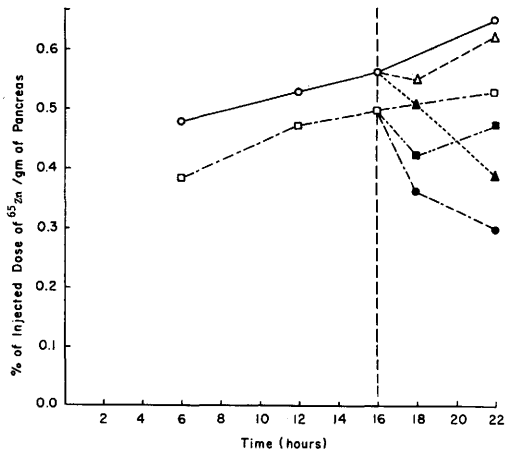

FIG. 1. Concentrations of ^{65}Zn (% of dose/g) in the pancreas of 4-wk-old chicks. (○—) unheated, fasted; (△- -) unheated, fed, autoclaved; (▲- - -) unheated, fed, unheated; (□— -) autoclaved, fasted; (■— - -) autoclaved, fed, autoclaved; (●— — - -) autoclaved, fed, unheated.

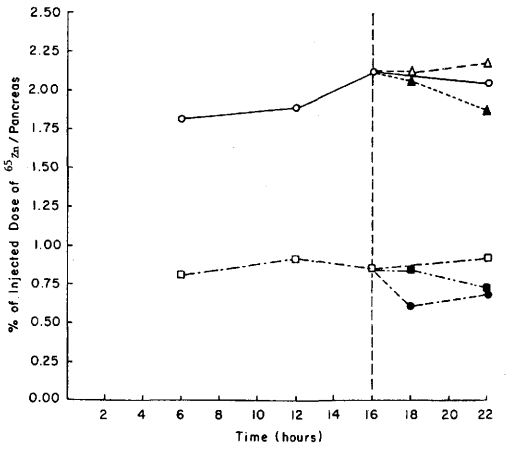

FIG. 2. Concentration of ^{65}Zn (% of dose) in the pancreas of 4-wk-old chicks fed soybean meal with respect to time. (○—) Unheated, fasted; (△- -) unheated, fed, autoclaved; (▲- - -) unheated, fed, unheated; (□— -) autoclaved, fasted; (■— - -) autoclaved, fed, autoclaved; (●— — - -) autoclaved, fed, unheated.

meal was fed without bearing to the adaptation period regime. The continuous depletion effect of the unheated soybean meal as a result of feeding, coupled with the failure to resynthesize after a 2-hr feeding time, which was indicated in enzyme work (8) and ^{65}Zn activity in this study, suggest that the synthesis of trypsin, X-trypsin, carboxypeptidase A and B are under the same coordinate control.

The total activity of ^{65}Zn per pancreas was almost twofold higher in the pancreata of chicks fed unheated soybean meal (Fig. 2). This is closely correlated with tryptic activity for pancreata in chicks fed unheated soybean meal. (8).

The unusually higher levels of ^{65}Zn activity per pancreas in chicks fed unheated soybean confirms an earlier report from this laboratory that the enlargement was a result of hyperplasma (9). In that report, histological examination did not indicate any variance in the acinar cells in pancreata of chicks fed autoclaved or unheated soybean meal. Furthermore, that work indicated that no differences were present in the moisture, DNA, RNA and protein levels of such pancreata on a unit weight basis.

When chicks were fed after 16 hr of fast-

TABLE I. Total ^{65}Zn Activity (% of Dose per Tissue) in Fasted Chicks That Were Previously Fed Unheated or Autoclaved Soybean Meal.[a]

Soybean meal	Fast (hr)	Intestines	Liver	Gallbladder	Excreta
			% of dose per tissue		
Autoclaved	6	2.2	4.7	0.10	3.1
Unheated	6	2.6	3.4	0.10	3.6
Autoclaved	12	2.4	4.7	0.11	3.3
Unheated	12	2.7	3.9	0.09	4.9
Autoclaved	16	2.3	4.5	0.11	3.6
Unheated	16	2.4	3.4	0.11	5.9
Autoclaved	22	2.5	6.2	0.10	4.7
Unheated	22	2.3	5.2	0.10	6.8

[a] Each value is a mean of three chicks.

ing, the total percentage of ^{65}Zn activity per pancreas dropped continuously when unheated soybean meal diet was the source of dietary protein. This contrasts with almost no variation when autoclaved soybean meal was fed. However, feeding the alternate diets after the fasting period resulted in a higher depletion when unheated soybean meal was fed. This is compared with slight increase when autoclaved soybean meal was fed (Fig. 2). The percentage of ^{65}Zn/tissue was almost always higher in the livers of chicks fed autoclaved soybean meal. This is in contrast to higher activity in the excreta and intestines of chicks fed unheated soybean meal. Zinc 65 activity in the gallbladder was not different in chicks fasted on 6, 12, 16 and 22 hr (Table I). The data shown on livers, intestines, gallbladders and excreta showed small deviations and in a few cases variations up to 25%, yet on an average organ basis the differences were abundantly clear. The excreta ^{65}Zn activity increased as a function of time in the fasted chicks, irrelative of the nature of the diet previously fed. However, this activity was constantly higher for the chicks previously fed unheated soybean meal (Table I).

Chicks fed after a 16-hr fasting period had a lower ^{65}Zn activity in their gallbladder, indicating a discharge of the gallbladder as a result of feeding. However, the ^{65}Zn activity released from this latter source into the gastrointestinal tract is not a major contributor to the high activity in the intestines. After a 2-hr period on feed, the intestines in chicks fed previously unheated soybean meal had a higher ^{65}Zn activity,

TABLE II. Total ^{65}Zn Activity (% of Dose per Tissue) in Chicks Fed Previous or Alternate Diet After 16 hr of Fasting.[a]

Soybean meal[b]	On feed (hr)	Intestine	Liver	Gallbladder	Excreta
			% of dose per tissue		
Autoclaved → autoclaved	2	2.3	5.3	0.13	3.9
Autoclaved → unheated	2	2.6	3.2	0.09	4.5
Unheated → unheated	2	2.7	3.3	0.12	6.1
Unheated → autoclaved	2	2.8	2.6	0.12	4.9
Autoclaved → autoclaved	6	2.1	4.9	0.05	5.2
Autoclaved → unheated	6	2.0	3.0	0.08	6.2
Unheated → unheated	6	2.2	3.7	0.07	6.6
Unheated → autoclaved	6	2.3	4.4	0.08	5.5

[a] Each value is a mean of three chicks.
[b] The arrow (→) indicates a change in diet.

but this was not the case when feeding time was extended to 6 hr, because of the excretion of the chyme from the tract and the failure of the pancreas to synthesize ^{65}Zn containing enzymes as fast as it discharged them into the duodenum (Table II).

The results presented above indicate that the pancreata from chicks fed unheated soybean meal had higher activity of ^{65}Zn than chicks fed autoclaved soybean meal. Excreta of these chicks also had a higher activity than chicks fed autoclaved soybean meal. Variations in the total activities may be a reflection of the weights of organs that are variable among chicks. It is concluded from these results that the ^{65}Zn activity in the pancreata of chicks decreases rapidly as a result of feeding unheated soybean meal after the fasting period, regardless of previous dietary conditions. The ^{65}Zn activity data complement and support previous reports (8, 9) that unheated soybean meal induces a hyperplastic type change in the organ and that the synthesis of the Zn-containing enzymes is higher. The data support the hypothesis (8) that unheated soybean meal results in increased release rate and a failure to increase synthesis to compensate for continuous depletion.

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