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The Zinc Content of Bile and Pancreatic Juice in Zinc-Deficient Swine (41021)[1]

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Abstract. The content of zinc, magnesium, calcium, bilirubin, and bile acids was determined in the hepatic bile of zinc-deficient and control swine. Zinc deficiency was produced by dietary zinc restriction while pair fed zinc supplemented animals served as controls for observations of hepatobiliary functions. Pigs weighing 5 kg were placed in two groups and fed the respective diets for 6 weeks; hair and skin abnormalities as well as decreased weight gain were present in the zinc-deficient group by this time. Hepatic bile and pancreatic juice were obtained from each animal after careful isolation and cannulation of the pancreatic and bile ducts. Animals were studied for a 3-hr period while under constant secretin stimulation and chloralose anesthesia. During this period, there was a progressive decrease in the biliary concentrations of bilirubin, bile acids, and magnesium in the bile of both groups, while zinc and calcium levels were not altered. The zinc concentrations in pancreatic juice were reduced in the zinc-deficient animals. The zinc content of the serum, liver, kidney, and pancreas was also decreased in this group. The calcium and magnesium content of the serum and organs was similar in the two groups with the exception of decreased magnesium in the kidney of the zinc-deficient pigs. Low content of zinc in the liver and serum of the deficient animals was associated with a zinc concentration in the bile comparable to the control group; however, the relative content of zinc in pancreatic secretion and bile was found to be altered in zinc deficiency. In control animals 60% of the zinc is in the pancreatic secretion and 40% in the bile. These percentages are reversed in zinc-deficient animals in which 60% of the zinc is derived from the bile.

Many studies using zinc radioisotopes have been conducted seeking to explain the absorption of zinc and the amount and source of the endogenous excretion of this element. Early studies with intravenously injected ^{65}Zn indicated that pancreatic acinar cells showed a rapid intake of zinc which then appeared in the pancreatic secretions. Only "small" quantities were eliminated in the bile and duodenal juice (1, 2) according to the reported appearance of radioactivity in these two secretions. Pancreatic juice contained the highest level of ^{65}Zn and was present for a longer period of time (3). In a study with a T tube in the common duct, the biliary and urinary radioactivity was measured for 24 hr and 0.05% of ^{65}Zn was excreted in the bile as compared to 0.15% in the urine (4). In a prolonged study of patients, again using ^{65}Zn the sum of bile, duodenal and pancreatic secretion failed to account for total endogenous stool content of ^{65}Zn (5).

In another evaluation of organs and body fluids following ^{65}Zn injection in dogs and humans, the higher initial biliary excretion was seen in the first hour. The lower level of radioactivity in Hours 3 to 6 were interpreted as representing hepatic excretion of ^{65}Zn (6). Rat intestine has been shown readily to incorporate ^{65}Zn into the cellular mucosa following an intravenous injection of ^{65}Zn (7).

The purpose of this investigation was to determine the actual zinc content of bile in swine, to note changes in biliary secretion

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in zinc-deficient pigs, and to determine the relation of biliary zinc to pancreatic zinc and other constituents of bile following secretin stimulation.

Material and Methods. Twelve mixed-breed weanling pigs (Yorkshire–Hampshire and Poland–China) weighing between 4.0 and 5.8 kg (mean 4.9) were randomly divided into two groups. Animals were individually housed in stainless-steel-fenced, tiled-floor pens. After a 5-day adaptation period, six animals were given 300 g/day of a zinc-deficient diet which they consumed in total. It contained 3.15 μg zinc/g and was a modification of the Forbes and Yohe diet (8) as obtained from Teklad Test Diets, Madison, Wisconsin, see Table I. The trace element content of the diet was measured in our laboratory and found to be as described. The remaining six animals were given a similar quantity of the same diet by paired feeding plus a zinc additive which increased the zinc content to 128.15 μg/g. The amount of diet given in relation to weight was as suggested by Tumbleson (9). Total consumption of diet given during the first 30 days of the study was followed by a steady decline in the amount eaten. The average amount of diet consumed per day at time of sacrifice was 120 g. Zinc-free water was supplied *ad libitum* to all animals.

TABLE I. COMPOSITION OF ZINC-DEFICIENT DIET[a]

Ingredient	Amount (g/kg diet)
Casein	50.0
Egg white solids	143.0
Corn oil	120.0
Non-nutritive fiber (cellulose)	70.0
Corn starch	100.0
Sucrose	469.8
Vitamin mix[b]	15.0
Biotin	0.004
Salt mixture[c]	32.2

[a] The mean total consumption by each animal was approximately 11.1 kg.

[b] Vitamin mix, Teklad test diets, Madison, Wisconsin.

[c] Salt mixture/kg of diet: 2.75 g KCl, 24.177 g $CaHPO_4$, 1.868 g $CaCO_3$, 1.79 g $MgSO_4$, 1.0 g NaCl, 0.016 g $CuSO_4$, 0.004 g KIO_3, 0.5 g ferric citrate (16.7% Fe), 0.065 $MnSO_4 \cdot H_2O$, 0.0003 g Na_2SeO_3, 0.008 g $CoCl_2 \cdot 6H_2O$.

The diet was continued until the deficient animals exhibited extreme weakness plus the physical indices of zinc deficiency (10) (usually about 40 days). The animals, an experimental and its pair fed control, were then fasted overnight, anesthetized with chloralose (70 mg/kg iv), the abdominal cavity was opened, the cystic duct was ligated at the cystic duct-bile duct juncture, a clamp was placed at the duodenopyloric junction, and both the bile and pancreatic ducts were cannulated. None of the animals were found to possess a secondary pancreatic duct.

Hepatic bile was collected in 10-min individual collections for a 3-hr period, during which time the animals were given intravenous secretin at the rate of 0.5 units/min in 1 ml of isotonic saline. Secretin was obtained from GIH Research Unit, Chemistry Department, Karolinska Institutet, Stockholm, Sweden. Pancreatic secretion collections were made at identically timed periods. At the completion of the 3-hr secretin study, the animals were sacrificed and tissue and blood samples were taken.

The bile and pancreatic as well as tissue specimens were stored in trace element-free containers at −20° until analyzed.

Zinc, magnesium, and calcium were determined in bile by atomic absorption spectrophotometry (11, 12) after comparing dilute bile with appropriate dilution of nitric acid digests of bile for accuracy and reproducibility. The mean value of the two sets of determinations for zinc were: (a) dilution only, 6.27 μmole/liter, (b) digested, 6.12 μmole/liter. The largest variation between like aliquots was 0.46 μmole/liter.

Bile acids were enzymatically determined by the Admirand and Small (13) modification of the method of Talalay.

Total bilirubin was obtained by means of the direct diazo reaction as developed by Nosslin (14).

Trace elements in serum, digested tissue, and pancreatic juice were also analyzed by atomic absorption spectrophotometry (11, 12). A comparison was made between control and deficient group means for all the parameters studied using the unpaired *t* test.

TABLE II. EFFECTS OF DIETARY REGIMEN ON SWINE

Groups	Initial weight	Kill weight (weight in kg)	Average weight gain	Average days on diet
Controls (6)[a]	4.9 ± 0.6[b]	9.2 ± 1.0	4.4 ± 1.3	41
Zinc deficient (6)	4.8 ± 0.5	6.9 ± 1.3	2.0 ± 1.4	40
P values[c]		<0.010	<0.025	

[a] Number of pigs studied.
[b] Mean ± SD.
[c] Significant *P* values as obtained by *t* test.

Results. In Table II, a comparison is made of the initial and terminal weights, and the weight gains during the 40 to 41 days of the experiment. The mean initial weight was comparable in the two groups thus the difference in weight gain appeared to be indicative of greater growth on the same caloric intake when adequate zinc was available.

The zinc, magnesium and calcium content of the liver, pancreas, kidney, and serum are shown in Table III. Zinc in serum and in all the organs studied was significantly decreased in the zinc-deficient animals while the calcium and magnesium, except in the kidney, were unaltered.

In the zinc-deficient animals there was a 50% decrease in the volume of bile when compared to the controls; the volume of pancreatic juice, however, was essentially unchanged, Table IV. The concentration of zinc in the pancreatic juice was one-fourth

TABLE III. TRACE ELEMENT CONCENTRATION OF TISSUE AND SERUM

Tissue	Zinc	Magnesium	Calcium
	(μg/g wet weight)		
Liver			
control (6)[a]	234.0 ± 59.3[b]	201.6 ± 13.8	40.0 ± 3.8
Zinc deficient (6)	31.9 ± 6.6	177.8 ± 32.4	47.0 ± 8.8
P values[c]	<0.001		
Pancreas			
control (6)	119.2 ± 65.8	217.7 ± 11.0	91.1 ± 18.1
Zinc deficient (6)	23.9 ± 4.5	196.1 ± 22.4	102.7 ± 19.5
P values	<0.01		
Kidney			
control (6)	36.0 ± 7.2	169.3 ± 5.8	62.9 ± 5.3
Zinc deficient (6)	19.6 ± 1.3	146.0 ± 9.7	56.7 ± 4.9
P values	<0.001	<0.005	
	(μg/ml)		
Serum			
control (6)	1.32 ± .40	23.8 ± 2.8	97.3 ± 10.0
Zinc deficient (6)	0.47 ± .06	21.5 ± 2.9	90.8 ± 9.0
P values	<0.001		

[a] Number of pigs studied.
[b] Mean ± SD.
[c] Significant *P* values as obtained by *t* test.

TABLE IV. VOLUME AND ZINC CONCENTRATION OF SECRETIONS

Groups	Pancreatic secretion		Bile secretion	
	Total volume (ml/3 hr)	Zinc (μmole/liter)	Total volume (ml/3 hr)	Zinc (μmole/liter)
Control (5)[a]	59.9 ± 34.4[b]	7.98 ± 6.39 (6)	61.8 ± 22.1	5.30 ± 1.64
Zinc deficient (5)	53.5 ± 19.0	2.01 ± 0.93 (6)	31.4 ± 8.5	5.00 ± 1.60
P values[c]			<0.025	

[a] Number of pigs studied.
[b] Mean ± SD.
[c] Significant *P* values as obtained by *t* test.

as much in deficient animals as in controls. The range was 3.9 to 19.7 μmole/liter in the controls and 1.0 to 3.3 in the zinc-deficient animals. The biliary zinc concentration was identical in the two groups, but the total zinc excretion in the bile was decreased in the zinc-deficient animals because of the lower bile output (Table IV).

In Fig. 1, the concentrations of zinc, calcium, magnesium, bilirubin, and bile salts are shown for various times during the 3-hr study period for both zinc-deficient and control animals. There appears to be no significant difference between them. During the last hour of the experiment the concentrations for all substances studied were slightly lower.

Discussion. The responses to secretin stimulation of the pancreas in control and zinc-deficient pigs were similar to those previously described (15) using smaller doses of secretin in larger pigs.

In this study the relatively high content of zinc in the bile was not expected. The control group produced 0.16 μmole Zn/hr in pancreatic juice and 0.11 μmole Zn/hr in bile. Zinc-deficient pigs produced 0.03 μmole Zn/hr in the pancreatic juice and 0.05 μmole Zn/hr in the bile.

The observed differences in pancreatic juice were due to variations in zinc concentration, while in the bile the zinc concentration was stable and the variation arose from changes in volume.

The zinc found in bile appeared to be minimal in ^{65}Zn studies (5, 6). It has no recognized function in bile at this time. ^{65}Zn is taken up in large amounts by the liver when injected intravenously. The biliary zinc excretion that we have noted was quite independent of the serum zinc level.

There is insufficient evidence to support

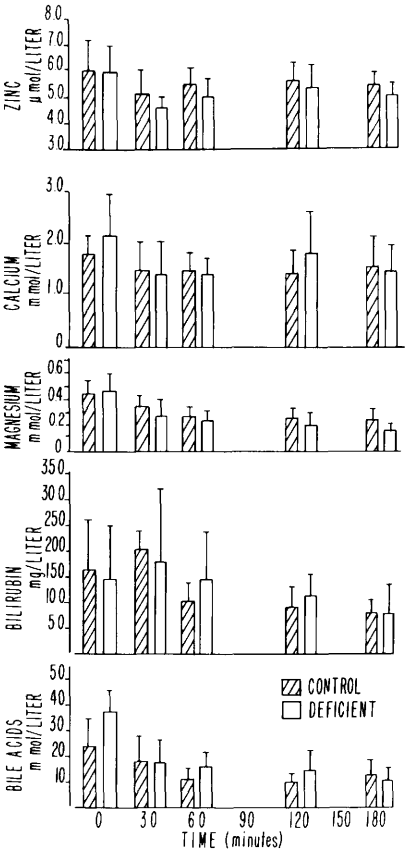

FIG. 1. The concentrations of zinc, calcium, magnesium, bilirubin, and bile salts in bile from zinc-deficient and control swine. Mean ± SD.

the concept of an enterohepatic zinc circulation. However, the continued biliary zinc excretion with decreased hepatic and serum zinc content does support the concept that the zinc which appeared in the bile was complex in derivation.

The analysis of the zinc in the bile and pancreatic juice of the zinc-deficient animals indicated that the zinc output was decreased to one-third that in the control animals and that the distribution of zinc in the two fluids was altered. In controls 60% of the zinc was in the pancreatic secretion and 40% in the bile. These percentages were reversed in zinc-deficient animals in which 60% of the zinc was derived from the bile.

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