THE ELIMINATION OF ADMINISTERED ZINC IN PANCREATIC JUICE, DUODENAL JUICE, AND BILE OF THE DOG AS MEASURED BY ITS RADIOACTIVE ISOTOPE (Zn^{65})*

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Several experimental findings suggest that the pancreas is concerned with the metabolism of zinc. In 1927 Drinker *et al.* (1) reported that the long continued ingestion of large amounts of zinc leads to fibrotic changes in the pancreas but in no other tissues of the cat. This striking effect upon the pancreas has been confirmed by Scott and Fisher (2). The fibrous proliferation in this gland was restricted to the acinar portion, the islet tissue remaining unaffected. Numerous papers dealing with zinc in crystalline insulin have appeared (3–9), but their significance with respect to the metabolism of zinc in the animal body is as yet not clear. Scott and Fisher found that the zinc content of the pancreas is reduced in the diabetic patient (10). This observation was not confirmed by Eisenbrand and Sienz (11).

In the present investigation further evidence is provided for the view that the acinar portion of the pancreas is concerned with the metabolism of zinc. It is shown with the aid of the radioisotope (Zn^{65}) that administered zinc is eliminated by pancreatic juice. The elimination of radiozinc in duodenal juice and bile was also studied.

EXPERIMENTAL

Preparation of Dog with Pancreatic Fistula (Dog IV).—The pancreatic fistula was prepared by a modification of the Elman and McCaughan procedure (12). The lesser or proximal pancreatic duct was doubly ligated and divided between ligatures. The greater or distal pancreatic duct was cannulated with a glass cannula and the pancreatic juice drained to the outside through a system of rubber tubing that had a flange at the site of emergence from the abdominal wall in order to prevent extrusion of the tubing. The rubber tubing was wrapped in omentum. All connections between the pancreas and duodenum were then cut, except for certain easily identifiable major blood vessels and the space filled with omentum. This procedure served to destroy any aberrant duct communications and to prevent their recanalization.

Preparation of Dogs with Pancreatic and Biliary Fistulae (Dogs I and III).—The pancreatic fistulae were prepared by the method described above. The biliary

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fistulae were prepared by a modification of the Rous-McMaster principle (13). The common bile duct was doubly ligated and divided between the ligatures. The gall bladder was excised and the cystic duct cannulated. The cannula system was identical with the one used for the pancreatic fistula. The rubber tubes from both pancreatic and biliary fistulae were brought out of the abdominal cavity through a single stab wound in the right or left upper rectus region.

Preparation of Dog with Biliary and Duodenal Fistulae (Dog VI).—The common bile duct was cannulated by the same method used for cannulating the cystic duct. The common duct was then ligated distal to the cannula and divided between the ligature and cannula. The gall bladder was not removed. The duodenal fistula was made as follows: The duodenum was separated from the stomach at the pylorus and the two cut ends inverted. The distal end of the duodenum was divided just proximal to the ligament of Treitz and the distal end of the bowel closed. The continuity of the gastrointestinal tract was reestablished by means of an anterior gastrojejunostomy. A Dragstedt type of intestinal cannula (14) was then inserted into the lower part of the isolated loop of duodenum through a stab incision on the antimesenteric border of the bowel after which the lower end of the duodenal loop was closed. In order to exclude pancreatic juice from the loop of duodenum the two main pancreatic ducts were doubly ligated, divided between the ligatures, and all connections between the pancreas and the duodenum separated except for a few major vessels. Omentum was then inserted between the bowel and pancreas so as to serve as a barrier to the possible establishment of drainage of pancreatic juice into the loop of duodenum. Thus the duodenal fistula was free of all but duodenal secretions. The cannula was surrounded with omentum at the point where it emerged from the loop of bowel. It was brought to the outside through a right rectus stab wound.

Preparation of Dog with Biliary, Duodenal, and Pancreatic Fistulae (Dog VIII).—The biliary fistula was produced by cannulating the common bile duct and leaving the gall bladder *in situ*. The pancreatic fistula was prepared as described above. The duodenal fistula was also prepared as described above except that the main pancreatic duct was cannulated.

All dogs were anesthetized with nembutal. It was administered intravenously in sufficient amounts (25 to 30 mg. per kilo) to produce the desired anesthesia.

Care of Animals.—The dogs were fed twice daily. Each meal consisted of 200 to 300 gm. of cooked meat supplemented with vitamin concentrates. Dogs with pancreatic fistulae received in addition 100 gm. of raw pancreas twice daily, while those with bile fistulae were fed 5 gm. of bilron (Eli Lilly) with each meal. To compensate for the loss of fluids, each animal received intravenously 500 to 1000 cc. of Ringer's solution daily.

For the first few days after the operation, the pancreatic juice, duodenal juice, and bile were drawn off once daily, measured, and discarded. Bile and pancreatic juice were withdrawn aseptically. When it became apparent that the secretions were being produced in amounts adequate for the tests radiozinc was administered.

The preparation of zinc chloride containing Zn^{65} has been described elsewhere (15). It was injected intravenously into a hind leg vein of the dog. Blood samples were removed from the jugular vein or a vein of the foreleg. The blood was heparinized, centrifuged, and the plasma separated. 2 cc. samples of plasma were transferred to rectangular (3 × 6 cm.) dishes made from aluminum foil that had been lined with a piece of lens paper. The plasma was then dried over a hot plate. The lens paper served to spread the liquid uniformly over the entire surface and to prevent splattering during the evaporation. The weight of the lens paper was such that its absorption of the radiations of the Zn^{65} was negligible. The sides of the dish were then flattened, the entire aluminum foil with its contents wrapped in cellophane, and sealed with scotch tape in a way that only one thickness of cellophane came between the dried plasma and the aluminum wall of the Geiger counter. Its radioactivity was determined after the manner described in a previous paper (15).

The samples of bile and pancreatic juice obtained from dogs I and III were treated in a manner similar to that of plasma described above. In dogs IV, VI, and VIII, in which larger amounts of these secretions were dealt with, the bile and pancreatic juice were first treated with an excess of HCl in order to neutralize their alkalinity, and the mixture evaporated to dryness on a steam bath. The residues were then ashed in a muffle furnace thermostatically controlled at 450°. The determination of the radioactivity of the ash has been described elsewhere (15).

Duodenal juice was obtained from dogs VI and VIII. The juice secreted under these conditions was not clear and contained cellular material. The samples of juice were therefore centrifuged. Radiozinc was determined in the supernatant fluid and in sediment separately. They were first treated with HCl and then ashed as described above.

RESULTS

Pancreatic Juice.—The excretion of Zn^{65} was measured in five dogs. The results for dog III are recorded in Fig. 1, and those for dogs I and IV in Table I. The first samples were obtained as early as 30 minutes after the intravenous injection of the labeled zinc. By this time radiozinc had already appeared in the secretions of the pancreas. The total amount of Zn^{65} eliminated by this route is significant. In dog III over 10 per cent of the injected Zn^{65} was eliminated by way of the pancreatic juice in 14 days (336 hours). In 7 days dog I excreted 6.5 per cent of the radiozinc in the pancreatic juice. A total of 4.6 per cent of the Zn^{65} appeared in the external secretion of the pancreas of dog IV in 7 days.

The concentration of Zn^{65} in the *plasma* of dog III is shown in Fig. 1. Following its intravenous introduction into the blood stream, radiozinc disappeared rapidly from the plasma. At the 2 hour interval plasma of dog III contained 0.00064 per cent of the injected Zn^{65} per cc., whereas at 1.5 to 2 hours pancreatic juice contained 0.0036 per cent per cc. At 48 hours the Zn^{65} content of plasma was not measurable; yet significant amounts were being excreted into the pancreatic secretions at this time. Radiozinc continued to be excreted for as long as 14 days in dog III despite the fact that measurable amounts of Zn^{65} were no longer detectable in its plasma (Fig. 1). Similar results were obtained in dog I (Table I).

Bile.—The first studies of radiozinc in bile were made in dogs I and III.

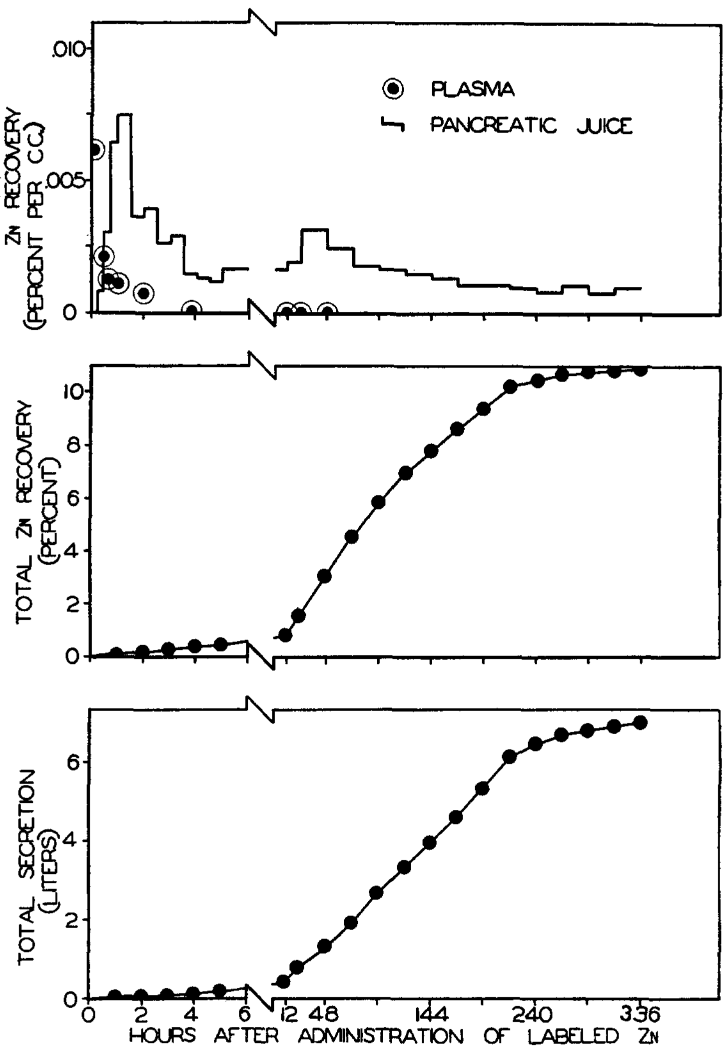

FIG. 1. The excretion of Zn^{65} in pancreatic juice of dog III. Fistulae of the cystic bile duct and of the pancreatic duct were prepared. The excretion of Zn^{65} in the bile during the interval studied was negligible. This dog weighed 17.0 kilos. It received intravenously 16.3 μg. of labeled zinc as the chloride.

2 cc. samples of the bile excreted during the first 5 hours after the injection of the labeled zinc were analyzed. Measurable amounts of radiozinc were not found in these small samples; hence in dogs VI (Table II) and VIII (Table III) larger samples of bile were taken for analysis. By the end of 8 days, dog VI excreted

TABLE I

The Excretion of Intravenously Injected Zn^{65} into the Pancreatic Juice of Dogs I and IV

1	2		3	4	5	6
Dog	Interval after injection of radiozinc		Total flow*	Recovery of Zn^{65} in pancreatic juice as per cent of the injected Zn^{65}		Recovery of Zn^{65} per cc. plasma as per cent of the injected Zn^{65}
				Per cc. for interval‡	Total*	
	days	*hrs.*	*cc.*			
I§	0	0.5	6	0.0024	0.01	0.0023
	0	0.75	9	0.0056	0.02	
	0	1	12	0.0065	0.04	0.0015
	0	1.5	17	0.0052	0.07	
	0	2	20	0.0065	0.09	
	0	2.5	24	0.0105	0.13	0.0012
	0	3	31	0.0065	0.17	
	0	3.5	37	0.0057	0.21	
	0	4	41	0.0056	0.23	
	0	4.5	44	0.0091	0.26	0.0008
	0	5	48	0.0083	0.29	
	0	11	130	0.0028	0.51	0.0006
	0	23	490	0.0021	1.25	0.0004
	1	10	715	0.0031	1.98	
	2	0	850	0.0039	2.51	Not detectable
	3	0	1250	0.0031	3.71	
	4	0	1530	0.0024	4.34	
	5	0	1940	0.0025	5.34	
	6	0	2210	0.0025	6.01	
	7	0	2530	0.0017	6.55	
IV‖	0	11	400	0.00152	0.61	
	0	21	810	0.00137	1.17	
	1	13	1220	0.00096	1.57	
	2	0	1670	0.00107	2.05	
	3	0	2420	0.00085	2.70	
	4	0	3170	0.00070	3.22	
	5	0	3620	0.00094	3.65	
	6	0	4220	0.00090	4.18	
	7	0	4720	0.00080	4.59	

* After injection of Zn^{65}.

‡ The value given in this column is for the interval between the corresponding time shown in column 2 and the time directly above it.

§ This dog weighed 18.0 kilos. It received intravenously 16.3 μg. of labeled zinc as the chloride.

‖ This dog weighed 13.0 kilos. It received intravenously 1.63 μg. of labeled zinc as the chloride.

into its bile 0.4 per cent of the injected Zn^{65}. Dog VIII failed to eliminate more than 0.1 per cent in 15 days. In no case were the amounts of radiozinc eliminated by this route comparable to the amounts excreted in pancreatic juice. Thus in 7 days, dogs I, III, and IV excreted into the pancreatic juice 6.5, 8.6, and 4.6 per cent of the administered radiozinc.

Duodenal Juice.—Considerable amounts of the injected Zn^{65} appeared in the intestinal juice obtained from the duodenum (Tables II and III). At the end of 11 days, the radiozinc contained in the total material eliminated by the

TABLE II

*The Excretion of Radiozinc in the Bile and Duodenal Juice of Dog VI**

Interval after Zn^{65} injection		Bile			Duodenal juice			
		Total flow	Recovery of Zn^{65} as per cent of the injected Zn^{65}		Total flow	Recovery of Zn^{65} as per cent of the injected Zn^{65}		
						Supernatant		Sediment‡
			Per cc.	Total§		Per cc.	Total§	Total§
days	*hrs.*	*cc.*			*cc.*			
0	6	6	0.0038	0.03	63	0.00071	0.05	0.06
0	22	16	0.0029	0.05	300	0.00060	0.19	0.20
2	0	31	0.0013	0.07	580	0.00058	0.35	0.35
3	0	86	0.0018	0.17	700	0.0027	0.67	0.44
4	0	163	0.00055	0.21	780	0.0056	1.14	0.51
5	0	288	0.00044	0.27	930	0.0015	1.35	0.60
6	0	413	0.00036	0.31	1240	0.0061	3.20	1.12
7	0	603	0.00018	0.35	1550	0.0014	3.63	1.42
8	0	726	0.00058	0.42	2010	0.0012	4.18	2.17
10	0	—	—	—	2250	0.0013	4.50	2.41
11	0	—	—	—	2720	0.00048	4.72	2.85

* This dog weighed 15.8 kilos. It received intravenously 3.26 $\mu g.$ of labeled zinc as the chloride.

‡ Obtained by centrifugation. The volume of sediment represented less than 5 per cent of the total volume of duodenal juice.

§ After injection of Zn^{65}.

duodenal loops of dogs VI and VIII was respectively 7.6 and 7.9 per cent of the administered Zn^{65}. In dog VI the supernatant fluid after the 3rd day contained somewhat more of the Zn^{65} than the sediment obtained by centrifugation, whereas in dog VIII the latter fraction contained the larger proportion of the radiozinc. This is not surprising since there is no reason to believe that the composition of the sediment was the same in both animals.

In view of the large amounts of radiozinc found in the sediment (probably cellular debris) conclusions regarding the significance of duodenal secretions in the elimination of zinc should be withheld at present. It has been shown previously that after its intravenous injection relatively large amounts of radiozinc are recovered in both muscular and mucosal layers of the small intestine (16).

TABLE III

*The Excretion of Radiozinc in the Bile and Duodenal Juice of Dog VIII**

Interval after Zn^{65} injection		Bile			Duodenal juice			
		Total flow‡	Recovery of Zn^{65} as per cent of the injected Zn^{65}		Total flow‡	Recovery of Zn^{65} as per cent of the injected Zn^{65}		
						Supernatant		Sediment§
			Per cc.	Total‡		Per cc.	Total‡	Total‡
days	*hrs.*	*cc.*			*cc.*			
0	12	95	0.000025	0.002	110	0.00038	0.04	0.04
1	0	—	—	—	165	0.00042	0.07	0.10
1	12	—	—	—	425	0.00041	0.17	0.29
2	0	340	0.000050	0.011	700	0.00015	0.21	0.50
2	12	405	0.000055	0.015	925	0.00041	0.31	0.65
3	0	475	0.000051	0.019	1120	0.00041	0.38	0.75
3	12	565	0.000082	0.026	1620	0.00067	0.72	1.07
4	12	735	0.000028	0.031	2620	0.00026	0.98	1.62
5	12	890	0.000045	0.038	3020	0.00043	1.15	2.08
6	12	1200	0.000029	0.047	3770	0.00017	1.28	2.60
7	12	1550	0.000019	0.053	4620	0.00031	1.55	3.10
8	12	1780	0.000016	0.057	5270	0.00068	1.99	3.68
9	18	1980	0.000020	0.061	6240	0.00037	2.35	4.18
10	18	2130	0.000016	0.063	6890	0.00034	2.57	4.63
11	18	2400	0.000023	0.070	7500	0.00037	2.81	5.05
12	18	2710	0.000014	0.074	8110	0.00050	3.12	5.37
13	18	3040	0.000014	0.079	—	—	—	—
14	18	3340	0.000012	0.083	9310	0.00035	3.58	5.95

* Fistulae of the common bile duct, duodenal, and pancreatic duct were prepared in this dog. Pancreatic juice, however, was not obtained. This dog weighed 24.9 kilos. It received intravenously 37.5 μg. of labeled zinc as the chloride.

‡ After injection of Zn^{65}.

§ Obtained by centrifugation. The volume of sediment represented less than 5 per cent of the total volume of duodenal juice.

DISCUSSION

The tissues in which the highest concentrations of radiozinc appeared after its intravenous injections were liver and pancreas (16). Maximum deposition in the tissues of the dog was observed 8 hours after the injection; at this time the liver of the dog contained 0.34 per cent of the injected Zn^{65} per gm. and the pancreas 0.28 per cent per gm. The maximum amount of radiozinc found in the *whole* pancreas at a single interval, namely 8 hours, was 3.1 per cent; in 7 days the amount was reduced to 0.7 per cent. A better index of the rôle of the pancreas in zinc metabolism, however, is afforded by the present investigation, in which the elimination of the administered labeled zinc was measured in the external secretion of this organ over a period of several days. A large proportion of the intravenously introduced radiozinc is excreted into the pancreatic juice. Thus over a period of 7 days dogs I, III, and IV excreted 5 to 9 per cent of the injected Zn^{65} by way of the pancreatic juice. In dog III, a total of approximately 11 per cent of the radiozinc was eliminated by the gland in a period of 14 days. It is of interest to note here that in contrast to the pancreas and its secretion (*i.e.* pancreatic juice) very little radiozinc was eliminated by way of the bile despite the fact that large concentrations of Zn^{65} were found in the liver at all intervals between 3 hours and 7 days after the injection of the radiozinc (16). In view of the fact that a tracer dose of radiozinc was injected into the dogs, *i.e.* an amount of zinc that did not increase significantly the previously existing concentrations in the animal, these findings may be interpreted to mean that pancreatic juice is a normal excretory pathway for zinc. The high concentration of Zn^{65} in the pancreatic juice (the secretion of the acinar cell), as compared with the exceedingly low concentrations of this isotope in plasma, shows that the acinar cell concentrates zinc. It is of interest to consider the possibility that this element may be concerned with an enzyme, protein in nature, elaborated by the acinar cells.

SUMMARY

The excretion of intravenously injected *labeled* zinc into pancreatic juice, duodenal juice, and bile of the dog was investigated. The use of the radioactive isotope permitted the injection of minute amounts of zinc, amounts that were negligible when compared with the total amount of zinc already present in the animal.

1. A large proportion of the injected Zn^{65} was eliminated by way of the external secretion of the pancreas. As much as 11 per cent was excreted in the pancreatic juice in 14 days.

2. Very little of the administered radiozinc appeared in the bile. The maximum excretion observed for this pathway was 0.4 per cent in 8 days.

3. Radiozinc was also found in large amounts in the juice obtained from an isolated loop of the duodenum.

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