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The Sources of Sodium in Rabbit Pancreatic Juice (39870)

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Introduction. In recent years, numerous studies of pancreatic exocrine secretion have utilized *in vitro* preparations of the rabbit pancreas (1-6). In these studies the pancreas was excised from the animal, mounted in a chamber, and immersed in a buffer solution with the main pancreatic duct cannulated. The final pancreatic secretion was then collected from the cannulated duct. The results of such experiments have been used to prepare new models for the mechanism of electrolyte and water secretion by the pancreatic tissue.

Clearly, several important differences exist betwen such an *in vitro* preparation and the *in vivo* pancreas. First, the nerves known to innervate the pancreas are severed in this preparation, which destroys any contribution they may make to the control of secretory rate or composition of the final pancreatic juice. Second, the *in vitro* preparation is deprived of the normal circulation of blood to the tissue and thereby must obtain metabolites, hormones, and pancreatic juice constituents from the bath in which it is held. Such substances then must diffuse through the mesenteric tissue into the cells where they are used rather than being supplied by the blood. This may be extremely important to the action of secretin on pancreatic secretion since the response of the *in vitro* isolated pancreas to secretin added to the bathing solution has been shown to be much less than that seen for the *in vivo* pancreas (3, 6). Although the unstimualted secretory rate of the *in vitro* rabbit pancreas generally equals or exceeds that of most *in vivo* preparations (2, 3), the reduced effectiveness of secretin, as well as our observation that circulatory and secretory ductal vessels appear to be intimately associated within the pancreatic tissue, leads us to question the applicability of the *in vitro* preparation as a model of the *in vivo* pancreatic exocrine secretion. In view of the high secretory rate of the *in vitro* pancreas, the question also arises as to whether the rabbit pancreas which, as a sheet-like structure, obtains some or all of its exocrine secretory components from the intraperitoneal fluid bathing the abdominal viscera. To investigate these possibilities, we have conducted studies to determine the relative contributions of extravascular and intravascular sources of a pancreatic juice constituent to the final pancreatic secretion.

Materials and methods. Male New Zealand white rabbit weighing approximately 2.5 kg were anesthesized by an intraperitoneal injection of 0.65 ml/kg body weight of Dial-urethane (Ciba Pharmaceutical Co., Summit, N. J.). The animals were immobilized in a supine position on a heated animal board, and the left femoral artery was cannulated with PE-60 tubing. This cannula was used for monitoring of arterial blood pressure and for collection of arterial blood samples for determination of plasma pH, pCO_2, ^{24}Na, and ^{22}Na. The pH and PCO_2 of blood were determined using a Radiometer pH meter 27 with micro pH electrode and blood gas attachments (Radiometer, Copenhagen, Denmark).

The abdomen was opened via a midline incision, both ureters were ligated, and the first duodenal loop containing the pancreas was brought through the incision. The pancreas of the rabbit is contained within the mesoperitoneum of the first duodenal loop. Using an approach similar to that of Schulz *et al.* (7), this loop is mobilized by cutting the peritoneal attachment of the distal limb of the loop to the rectum as far back as the root of the mesentery. The pancreas is located in the second of three layers of connective tissue and is a diffuse organ with small lobules scattered throughout this duodenal mesentery. The section of large bowel

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which transverses this mesenteric loop was then carefully dissected away, and the tissue was mounted on a dish-shaped glass holder heated to ~38° by circulating water. A small amount of warm (~38°) buffer was then put on the exposed mesentery until all other preparations had been made. The main pancreatic duct was then cannulated with a polyethylene catheter (PE 10) adjacent to its site of entry into the duodenum. Collections of the final pancreatic juice were subsequently made into tared polyethylene vials.

After a period of about 15 min during which the animal was allowed to stabilize, an approximately 1-ml bolus of ^{22}Na-labeled Krebs–Henseleit buffer was injected through the femoral artery, and the isotope was allowed to distribute throughout the animal for about 15 min. At this time the nonlabeled buffer was removed from the pancreatic mesentery and replaced with ^{24}Na-labeled buffer so that the pancreatic mesentery was in contact with the ^{24}Na-labeled buffer.

Blood samples were then taken every 15 min for immediate analysis of radioactivity. The final pancreatic juice was also collected for six consecutive 15-min periods into tared polyethylene vials, weighed, and analyzed for radioactivity. The buffer on the pancreatic mesentery was occasionally stirred at ~3-min intervals to ensure that the solution on the surface of the mesentery was uniform. Samples of this buffer were also taken for analysis of radioactivity.

Since ^{24}Na has a half-life of only 15 hr, all samples of blood, pancreatic juice, and ^{24}Na buffer were counted immediately upon completion of the experiment to determine the total ^{22}Na + ^{24}Na activity. The samples were then stored and recounted 10 days later after the ^{24}Na had decayed, leaving only much-longer half-life ^{22}Na. The ^{24}Na content of each sample was then determined by subtracting the ^{22}Na activity from the ^{22}Na + ^{24}Na activity determined in the first analysis.

Results. The concentration of ^{22}Na in both blood and pancreatic juice samples for two experiments is presented in part A of Table I. The appearance of ^{22}Na in pancreatic juice is quite constant throughout the 90-min experiment, indicating that the system is in a reasonable isotopic steady state. Although the amount of ^{22}Na added to the circulating blood of the two animals is very different (22.6×10^3 versus 81.7×10^3 cpm/ml), respectively, in the two experiments, the ratio of ^{22}Na in pancreatic juice to that of whole blood is a constant 2.4 for both experiments. This supports the notion that the

TABLE I. ^{22}Na AND ^{24}Na ACTIVITY IN BLOOD AND PANCREATIC JUICE.

	Experiment No. 1		Experiment No. 2	
Sample time (min)	Pancreatic juice	Whole blood	Pancreatic juice	Whole blood
		(A) ^{22}Na (10^3 cpm/ml)		
15	25.2	8.7	83.9	33.7
30	22.1	9.0	79.5	37.4
45	21.6	9.4	78.0	29.7
60	21.6	10.6	80.2	32.0
75	22.3	10.5	85.2	33.9
90	23.0	8.9	83.5	36.3
	$\bar{X} \pm SD = 22.6 \pm 1.4$	$\bar{X} \pm SD = 9.5 \pm 0.8$	$\bar{X} \pm SD = 81.7 \pm 1.2$	$\bar{X} \pm SD = 33.8 \pm 1.1$
Juice-to-blood ratio	22.6/9.5 = 2.4		81.7/33.8 = 2.4	
		(B) ^{24}Na (10^3 cpm/ml)		
15	201.0	3.9	10.5	0.6
30	162.8	4.9	13.3	1.2
45	205.6	6.7	15.2	1.1
60	201.8	8.1	14.4	1.5
75	199.6	7.1	15.0	1.4
90	163.3	7.0	14.5	1.6
	$\bar{X} \pm SD = 189.0 \pm 20.2$	$\bar{X} \pm SD = 6.3 \pm 1.6$	$\bar{X} \pm SD = 13.8 \pm 1.8$	$\bar{X} \pm SD = 1.2 \pm 0.4$

two animal preparations are behaving identically.

Part B of Table I presents the ^{24}Na activity in both pancreatic juice and blood. Again the two experiments differ significantly in the total amount of ^{24}Na appearing in the pancreatic juice or blood from the ^{24}Na-labeled buffer pooled on the pancreatic mesentery. This is primarily due to the fact that the concentration of ^{24}Na in the pooled buffer is 8.9×10^5 cpm/ml in Experiment 1 and 9.2×10^4 cpm/ml in Experiment 2.

Not only was ^{24}Na found to be absorbed from the pooled buffer into the circulatory system (Table I, part B), but a small amount of ^{22}Na was found in the pooled buffer. The concentration of ^{22}Na in the pooled buffer at the midpoint of Experiment 1, calculated by a linear extrapolation, was 6×10^3 cpm/ml of buffer, while that in the buffer of Experiment 2 at the same point in the experiment was 31.8×10^3 cpm/ml of buffer. This midpoint concentraiton can be used to estimate the contribution of ^{22}Na absorbed into the pooled buffer to the final pancreatic juice.

Discussion. In order to elucidate the magnitude of the contribution made by the two potential sources of Na in pancreatic juice, we must make some adjustments to the experimental data. These manipulations are presented in Table II and detailed below.

It is evident from the ^{24}Na blood levels that a significant amount of ^{24}Na moves in the circulatory system, as well as into the pancreatic juice. Once the ^{24}Na enters the bloodstream, it can be secreted into the pancreatic juice in a manner identical to that of

TABLE II. THE CONTRIBUTIONS OF VASCULAR AND EXTRAVASCULAR SOURCES TO PANCREATIC SODIUM SECRETION.

	^{24}Na, Marker of extravascular sodium source ($\times 10^3$ cpm/ml)	
	Experiment No. 1	Experiment No. 2
(*A*) Total pancreatic juice activity	189.0	13.8
(*B*) Blood isotope levels	6.3	1.2
(*C*) Pancreatic juice activity secreted by route buffer → blood → p. juice (2.4 {Table I} × *B*)	15.1	2.9
(*D*) Pancreatic juice activity due to direct buffer → p. juice route (*A* − *C*)	173.9	10.9
(*E*) Activity of buffer source	890.0	92.0
(*F*) Specific activity of buffer source (*E* ÷ 140 μEq/ml)	6.4×10^3 cpm/μEq of Na	657 cpm/μEq of Na
(*G*) Contribution of extravascular buffer source to p. juice sodium: (*D* ÷ *F*)	27 μEq/ml	16 μEq/ml
	^{22}Na, Marker of vascular sodium source ($\times 10^3$ cpm/m)	
	Experiment No. 1	Experiment No. 2
(*H*) Total activity in pancreatic juice	22.6	81.7
(*I*) Average activity in buffer	6.0	31.8
(*J*) Contribution of blood → buffer → p. juice route ({*A* ÷ *E*} × *I*)	1.3	4.7
(*K*) Pancreatic juice activity due to direct blood → p. juice route (*H* − *J*)	2.13	77.0
(*L*) Activity of whole blood source (Table I)	9.5	33.8
(*M*) Activity of plasma source (2 × *L*)	19.0	67.6
(*N*) Specific activity of plasma source (*M* ÷ 139 μEq of Na/ml of plasma)	136 cpm/μEq of Na	485 cpm/μEq of Na
(*O*) Contribution of blood plasma source to pancreatic juice sodium (*K* ÷ *N*)	157 μEq/ml	159 μEq/ml
(*P*) Fraction of sodium contributed by extravascular pooled buffer: *G* ÷ (*G* + *O*)	0.15	0.09

^{22}Na. Since we wish to use ^{24}Na to trace the movement of Na into the pancreatic juice from extravascular source alone (the intraperitoneal fluid), we must correct for its absorption into the blood supply and subsequent secretion. To make this correction, we can take advantage of the observation that the ^{22}Na concentration in pancreatic juice is 2.4 times greater than its whole blood concentration (Table I). Quite obviously, ^{24}Na should behave identically to ^{22}Na, and we would expect its activity in the pancreatic juice to be 2.4 times its whole blood concentration. Thus, the direct contribution of the intraperitoneal fluid (bathing buffer) to the pancreatic juice will be the observed ^{24}Na levels of the jucie (Table I, part B) less that amount secreted through the blood, or 2.4 times the observed blood levels. The ^{24}Na derived directly from the buffer bath then in Experiment 1 is: 189×10^3 ($2.4 \times 6.3 \times 10^3$) or 173.9×10^3 cpm/ml of juice. Experiment 2, the value is 13.8×10^3 (2.4×1.2) or 10.9×10^3 cpm/ml (Table II, line D).

Since the pooled buffer was measured to have 140 μEq/ml of Na and had a ^{24}Na activity in Experiment 1 of 8.9×10^5 cpm/ml, the specific activity of the buffer was 8.9×10^5 cpm/ml $\div$ 140 μEq/ml or 6.4×10^3 cpm/μEq of Na. As seen on line G of Table II, the contribution of the pooled buffer to the final pancreatic juice is then 1.74×10^5 cpm/ml $\div$ 6.4×10^3 cpm/μEq of Na or 27 μEq of Na/ml of final juice. The specific activity of Experiment 2 is 9.2×10^4 cpm/ml $\div$ 140 μEq/ml or 657 cpm/μEq of Na, and the contribution of the pooled buffer to the final juice is 10.9×10^3 cpm/ml $\div$ 657 cpm/μEq of Na or 16.3 μEq of Na/ml of final juice.

In seeking to trace the contribution of vascular Na using ^{22}Na, we must realize that the total ^{22}Na found in the final pancreatic juice is both from the blood Na secreted directly into the pancreatic juice and from the blood Na absorbed into the pooled buffer and subsequently secreted into the pancreatic juice. Since 21 and 15% of the ^{24}Na in the pooled buffer source is found in the final pancreatic juice of Experiments 1 and 2, respectively, we can assume that the same fraction of the ^{22}Na which diffuses into the pooled buffer will then also be secreted into the pancreatic juice. Hence, the contribution of the route of Na secretion, blood $\rightarrow$ buffer $\rightarrow$ pancreatic juice, for Experiment 1 is 21% of 6×10^3 or 1260 cpm/ml (line J, Table II). Likewise, the contribution of this pathway to Experiment 2 ^{22}Na pancreatic juice values is 15% of 31.8×10^3 or 4.7×10^3 cpm/ml of buffer. Note that the contribution of this pathway is small, only 6% of the ^{22}Na found in the final pancreatic juice in both experiments. The total ^{22}Na contributed to the pancreatic juice directly from the blood is then 21.3×10^3 cpm/ml in Experiment 1 and 77.0×10^3 cpm/ml in Experiment 2 (line K, Table II).

If we assume that one-half of the blood volume is formed elements and protein (our own measurements of hematocrit in such experiments are 0.52 to 0.56), the ^{22}Na concentration in the blood plasma is $2 \times 9.5 \times 10^3$, or 19×10^3 cpm/ml (line M, Table II). Using flame photometry, we have determined the sodium concentration of rabbit plasma to be 139.3 $\pm$ 6.1 ($\pm$SD) mEq/liter; thus, the specific activity of the plasma source of Na will be the ^{22}Na activity per milliliter of plasma divided by the plasma Na concentration. Hence, the specific activity of the plasma ^{22}Na in Experiment 1 (line N, Table II) is $19 \pm 1.6 \times 10^3$ cpm/ml 139.3 μEq/ml, or 136 cpm/μEq. Since there are 21.3×10^3 cpm/ml of ^{22}Na coming directly from the blood plasma Na pool, the contribution of this pool to the final juice is seen in line O of Table II to be about 157 μEq of Na/ml. On the other hand, the ^{22}Na concentration of plasma in Experiment 2 is about 67.6×10^3 cpm/ml and the plasma Na concentration is 139.3 μEq/ml; the ^{22}Na specific activity of Experiment 2 is 485 cpm/μEq of Na. Consequently, the contribution of the plasma sodium pool is 77.0×10^3 cpm/ml $\div$ 485 cpm/μEq of Na = 159 μEq of Na/ml, which is essentially identical to that of Experiment 1 (line 0, Table II). Note that both experiments show that this plasma Na may actually be concentrated in pancreatic juice secretion over plasma. This is at least qualitatively in agreement with our flame photometer concentrations of 153.3 $\pm$ 5.8 and 139.3 $\pm$ 6.1 μEq Na/ml of Na for pancreatic juice and blood plasma, respectively.

Although one must be careful in the inter-

pretation of calculations like those above, the results clearly show that the *in vivo* pancreas relies almost exclusively on its blood supply for secreted sodium. If one assumes that the pooled buffer ^{24}Na and the plasma ^{22}Na represent separate and independent sources for pancreatic secreted Na, our calculations show that the extravascular pooled buffer can account for only 9 to 15% of the total pancreatic juice sodium (line P, Table II).

With vascular Na being preferred so greatly in the *in vivo* pancreas preparation, one must question whether the use of the buffer-bathed *in vitro* pancreas preparation is physiologically meaningful when investigating the nature and source of electrolyte secretion by the pancreas.

Summary. Using an *in vivo* rabbit pancreas preparation, we have shown that approximately 85–90% of the sodium found in rabbit pancreatic juice comes directly from the circulating blood of the animal as opposed to fluids bathing the peritoneum. This conclusion is reached using data from radiotracer experiments in which the blood and synthetic peritoneal fluid Na is labeled with ^{22}Na and ^{24}Na, respectively. The appearance of the various isotopes in the pancreatic juice has been used to determine the relative contributions of the two Na sources. The preference of the *in vivo* pancreas for vascular Na suggests that studies of pancreatic secretory mechanisms done *in vitro* may not be physiolgoically meaningful.

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