Pancreozymin-Cholecystokinin Injected by Portal and Systemic Routes.* (28249)

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Secretin has been observed to produce significantly less pancreatic secretion when injected intraportally than when given by the systemic route(1,2). This difference was thought to be due to partial inactivation of secretin on passage through the liver, probably by an enzyme(3). It seemed of interest to ascertain whether a similar effect of different routes of injection would be obtained with pancreozymin and cholecystokinin.

Methods. The best available preparation of these hormones is Cecekin-Vitrum, made according to the method of Jorpes and Mutt (4). It consists of a mixture of potent cholecystokinin and pancreozymin and is standardized according to its cholecystokinin activity; its pancreozymin activity is constant.

Fasted, male or female, mongrel dogs were anesthetized with nembutal (25 mg/kg, i.v.). The abdomen was opened and pylorus and cystic duct were ligated; a small caliber plastic tube filled with saline was secured in the dome of the gall bladder and connected to Statham capsule strain gauge which recorded on a dynograph. Blood pressure from a carotid artery was recorded in like manner. After tying off the accessory pancreatic duct(s), a plastic tube was introduced into the major duct. Fine polyethylene tubing was introduced into the portal vein through an adjoining mesenteric vein and into a jugular vein. A control period of 1 hour, with minimal basal secretion, elapsed before hormones were injected. Two injections of Cecekin were given to each dog: the first intraportally and after a 3-hour interval, the second intrasystemically; the order of injections alternated in subsequent experiments between portal-systemic or systemic-portal. Animals were divided into 2 groups which differed in method of injection. Group 1 (12 dogs): Cecekin (20 Ivy dog units) was given within 1-2 seconds and washed down with saline; pancreatic secretion was collected on ice for 15 minutes. Maximum gall bladder pressure was recorded in this group. Group 2 (10 dogs): Cecekin (75 Ivy dog units) was given by constant infusion pump (0.194 ml/min) for 1 hour; pancreatic juice was collected during the hour of infusion and for the following 15 minutes. Volume of secretion was noted and amylase and trypsin concentration determined. *Amylase.* Pancreatic secretion was diluted in 0.04 M phosphate buffer pH 7.2 and assayed by Somogyi's method(5). *Trypsin.* Pancreatic secretion, diluted 1:10 in buffer pH 6, was incubated at 37°C with an equal volume of enterokinase solution for 45 minutes to activate trypsinogen (Viodenum powder† was used as source of enterokinase; solution was made by shaking 0.5 g powder in 50 ml distilled water and filtering). An aliquot of the incubated juice was diluted in 0.04 M phosphate buffer pH 7.2 and Anson's method(6) used for assay. A trypsin unit is defined as that amount of enzyme which will liberate 1 mg of tyrosine in a 10 minute incubation period at 37°C.

We should mention here for the interest of others occupied with similar technics, that reflux of bile into the pancreatic duct was observed in one dog, although an accessory duct had been ligated. In this animal 2 accessory ducts existed, running in different directions. The unligated duct ran into the common bile duct and after the latter had been ligated below, the backflow of bile into the main pancreatic duct ceased. This is an extremely rare condition which we have seen only once; it reminds us of atavism: all pancreatic ducts in the rat terminate in the common bile duct.

Results and discussion. (Table I). Al-

* Supported by National Science Foundation grant. The department is supported in part by Michael Reese Research Foundation.

† Kindly furnished by Viobin Corp., Monticello, Ill.

though volume of secretion in Group 1 was not changed by the route of injection, a significantly smaller volume was noted in Group 2 when Cecekin was given slowly by the portal in contrast to the systemic route. As Ivy (7) has shown that Cecekin contains 0.5-1.0 unit of secretin per unit of cholecystokinin, this hormone, and not pancreozymin, probably, is responsible for the increase in volume seen over a minimal basal secretion, and also for the differences in volumes produced by different routes of injection, the latter observation confirming previous work(1,2).

No differences in amylase or trypsin concentration of pancreatic secretion were noted, regardless of route or mode of injection of pancreozymin. However, total enzyme output cannot be compared here, for it is dependent particularly on volume which is affected by secretin; we know that less pancreatic secretion is obtained after portal rather than after systemic injection of secretin(1,2). A study of the total output of amylase and trypsin under these experimental conditions will have to await a pancreozymin preparation free of secretin.

It is of interest that the 2 methods of injection should produce different results only with regard to volume of secretion. Possibly, prolonged stimulation of the pancreas by constant infusion and the relatively larger dose of Cecekin containing secretin accentuated the volume differences. These factors did not seem to play a role with regard to enzyme concentration.

Contraction of the gall bladder by cholecystokinin (Group 1) remained the same whichever route of injection was employed. Thus, under these experimental conditions, the physiological effect of cholecystokinin, unlike that of secretin(1,2), seems to be unchanged by intraportal injection.

Summary. When pancreozymin-cholecystokinin (Cecekin) was administered to dogs by constant infusion, volume of pancreatic secretion was significantly less after portal than after systemic injection; when given in a single dose, volume remained the same regardless of route of injection. As Cecekin contains secretin, the effect on volume is due,

TABLE I. Comparison of Volume and Enzyme Content of Pancreatic Secretion and of Maximum Gall Bladder Pressure with Portal *vs* Systemic Route Of Injection of Pancreozymin-Cholecystokinin.

		Portal			Systemic		
	No. of dogs	Mean ± S.D.*	Range		Mean ± S.D.*	Range	
Group 1							
Vol, ml	12	2.42 ± 1.33	.16– 4.50		2.80 ± 1.24	.8– 5.0	
Amylase units per ml	12	44,533 ±20,786	25,500 –94,000		42,600 ±15,964	19,600 –79,500	
Trypsin units per ml	11	210 ± 37	153 – 289		196 ± 31	151 – 238	
Max gall bladder pressure, cm of saline	12	11.6 ± 7.8	0 – 27		11.2 ± 6.6	.5– 33.0	
Group 2							
Vol, ml	10	11.9 ± 7.4	2.5 – 27.0		15.7 ± 9.3	3.4– 30.5	
Amylase units per ml	10	23,640 ± 8,961	8,400 –42,600		19,165 ± 7,549	6,500 –33,400	
Trypsin units per ml	10	140 ± 36	82 – 208		130 ± 39	80 – 213	

* Standard deviation.

Group 1: Volume: $t = 1.449$, $P > 0.1$. Amylase: $t = 0.352$, $P > 0.7$. Trypsin: $t = 1.045$, $P > 0.3$. Gall bladder pressure: $t = 0.311$, $P > 0.7$.
Group 2: Volume: $t = 3.088$, $P < 0.02$. Amylase: $t = 1.791$, $P > 0.1$. Trypsin: $t = 1.221$, $P > 0.2$.

probably, to this hormone and not to pancreozymin. Neither the route (portal *versus* systemic) nor the mode of injection (single dose *versus* constant infusion) changed the amylase or trypsin concentration of pancreatic secretion. With the experimental procedures employed, the physiological effect of cholecystokinin seems to be unmodified by intraportal injection.

1. Necheles, H., Ogawa, T., Chiles, Th., Levinson, M., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 110.

2. Skillman, J. J., Silen, Wm., Harper, H. A., *Am. J. Physiol.*, 1962, v202, 347.

3. Bridgwater, Anna B., Kuroyanagi, Y., Chiles, Th., Necheles, H., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 852.

4. Jorpes, J. E., Mutt, V., *Ann. Int. Med.*, 1961, v55, 395.

5. Somogyi, M., *J. Biol. Chem.*, 1938, v125, 399; *ibid.*, 1945, v160, 61, 69.

6. Anson, M. L., *J. Gen. Physiol.*, 1938, v22, 79.

7. Ivy, A. C., Janecak, H. M., *Acta Physiol. Scand.*, 1959, v45, 220.

Received February 11, 1963. P.S.E.B.M., 1963, v112.

Marrow Distribution in Rat Femurs Determined by Cell Enumeration and Fe^{59} Labeling.* (28250)

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Anatomical, hematokinetic and radioisotopic procedures have been used to determine the distribution of bone marrow in man and laboratory animals(1-14). Although the rat femur is frequently the source of marrow for hematological studies, no definitive information is available on the relative and absolute distribution of marrow within the whole bone (diaphysis and epiphyses) of the animal. Bone marrow estimations, made either as cellular numbers per mg or cells per mm^3 of marrow are usually based on marrow recovered solely from femoral diaphyses. This is because technical difficulties encountered in recovering marrow from the bone spicules in the epiphyses make these areas inaccessible to routine quantitation and because of the assumption that epiphyseal marrow content is negligibly small.

* This investigation was supported in part by NIH grant. Part of this work was performed at Brookhaven National Laboratory under the auspices of U. S. Atomic Energy Commission.

† Part of this work was carried out during the tenure of a Postdoctoral Fellowship from Nat. Inst. Health.

‡ Postdoctoral Fellow of U. S. Public Health Service.

The purpose of the present investigation was to determine the marrow nucleated cellular content, weight and distribution within the entire rat femur using both direct cell enumeration as well as radioiron labeling procedures.

Methods. A. Diaphyseal marrow quantitation. Male rats (260-280 g) of a modified Long-Evans strain were used in all phases of this study. Cellular quantitation of femoral diaphyseal bone marrow was accomplished by a modification of the method of Fruhman and Gordon(5). The femur was split lengthwise and the exposed marrow aspirated by mouth into a weighed hematocrit tube (1.3-1.5 mm $\times$ 75 mm, Clay-Adams "Red Tip") with a rubber tubing assembly which was attached to one end of the tube. The amount of marrow recovered from the diaphysis was estimated by subtracting the initial empty tube weight from the final tube weight after it contained the marrow.§ The marrow was then

§ It would now seem erroneous to estimate total marrow mass, as reported earlier, by first weighing the entire intact femur and the then dried bony casing cleaned of marrow (the difference in weight representing total marrow mass). This is due to the consider-