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Rise in Serum Immunoreactive Glucagon After Intrajejunal Glucose in Pancreatectomized Dogs.* (32576)

KEITH D. BUCHANAN,† JAMES E. VANCE,‡ TERUAKI AOKI, AND ROBERT H. WILLIAMS
Departments of Medicine and Surgery, University of Washington, Seattle

Bioassayable glucagon has been reported to be present in the gastrointestinal tract and pancreas of man and other animals(1,2) but a different immunological behavior of intestinal and pancreatic glucagon has been shown (3,4). A rise of serum immunoreactive glucagon (IRG) has been demonstrated after alimentary glucose(5,6). Ohneda *et al*(6) reported that the rise in IRG after alimentary glucose was of enteric origin, but Samols (3) considered it was derived from the pancreas. Because it is crucial to know the relative contribution of the intestinal and pancreatic IRG to serum IRG level under various conditions, we have studied serum IRG in pancreatomized dogs under basal conditions and following alimentary glucose.

Methods and materials. Immunoreactive insulin (IRI) was assayed by the double antibody technique of Morgan and Lazarow (7). IRG was assayed by a sensitive double antibody technique based on the same principles as the insulin assay. Trasylol (Bayer Ltd.) was added to the incubation media in the glucagon assay (1000 Kallikrein inhibitor units per 300 μl serum) to prevent the degradation of labelled and unlabelled glucagon by serum(8). Glucagon antibody was raised in guinea pigs and used in a final concentration of 1:1875 in the assay, and crystalline beef-pork glucagon (Eli Lilly and Co.) was used as the standard reference in the assay. Details of the glucagon assay method are in preparation for publication. IRG was extracted from tissues by the method of Kenny (9). Plasma glucose was measured in the Technicon Autoanalyzer. Mongrel dogs, male Wistar rats and a squirrel monkey were used in the studies.

TABLE I. Immunoreactive Glucagon in Tissues.

	mμg/g wet organ			
	Human	Dog	Monkey	Rat
Pancreas	3700 (1)	2200 (3)	2500 (1)	275 (6)
Stomach	—	0 (2)	—	
Duodenum	—	0 (2)	—	}
Jejunum	290 (1)	43 (3)	—	} 44 (3)
Ileum	—	—	—	}
Colon	—	99 (2)	—	}
Rectum	120 (1)	—	—	} 58 (3)
Adrenal	—	0 (1)	—	—
Kidney	—	0 (2)	—	0 (1)
Liver	—	0 (1)	—	0 (1)
Spleen	—	—	—	0 (1)
Heart	—	—	—	0 (1)
Diaphragm	—	—	—	0 (1)
Thymus	—	—	—	0 (1)

No. of organs assayed is shown in parentheses.

Results. Table I shows that high levels of IRG were detected in the pancreas of 4 species of animals, and that the intestine contained smaller amounts, detectable from the jejunum to the rectum.

Fig. 1 demonstrates the inhibition slopes(10) of extracts of human intestine and pancreas. The human pancreas and rectal inhibition slopes and those of the monkey and dog pancreas (not shown), were all parallel to the beef-pork standard, suggesting their immunological identity. However, the human jejunal and dog jejunal slopes were signifi-

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‡ Fellow, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

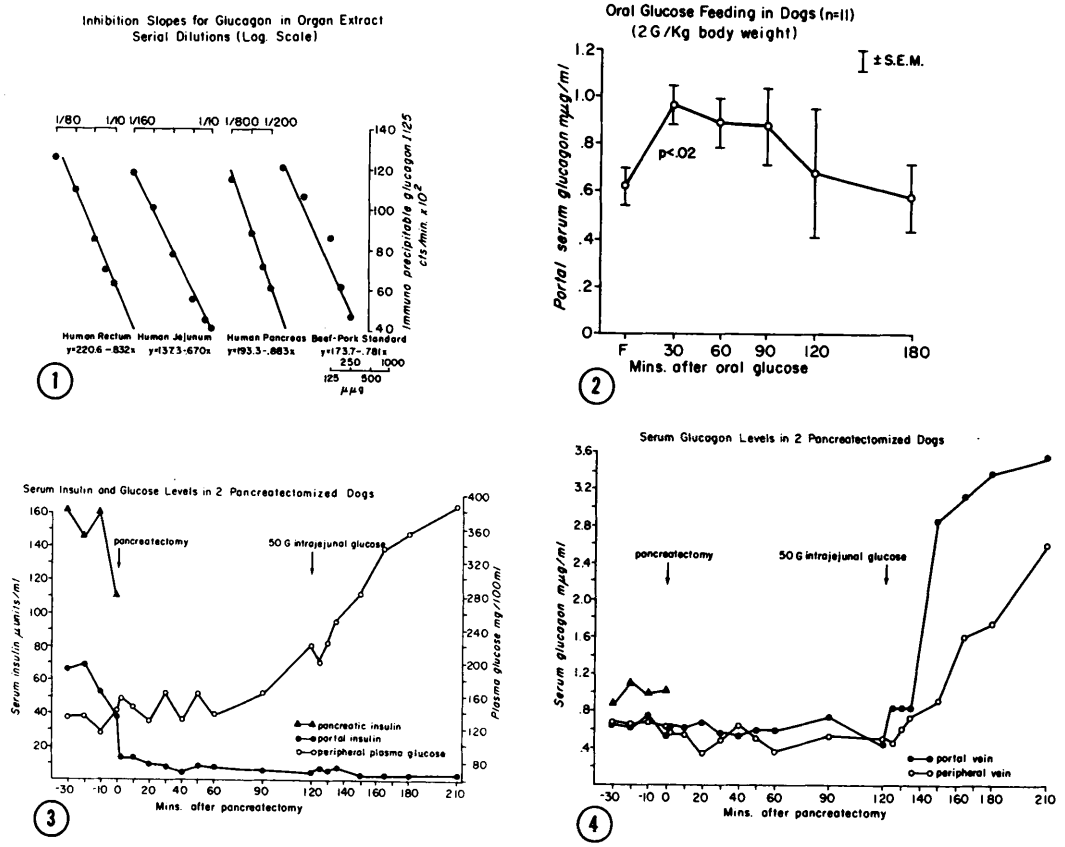

FIG. 1-4. Note: Fig. 2. There is a significant rise ($p<.02$) in serum glucagon after oral glucose between fasting and 30 minutes.

cantly shallower than human pancreas and dog pancreas, respectively ($p<.01$ in both instances), suggesting an immunological difference between pancreatic and jejunal glucagons.

Following oral glucose, 2 g/kg body weight, portal vein IRG rose significantly in conscious dogs (Fig. 2). After pancreatectomy in two anaesthetized dogs (Fig. 3) serum immunoreactive insulin fell to near zero, and did not rise after 50 g intrajejunal glucose, but serum glucagon fell only slightly in a peripheral vein, and not at all in the portal vein (Fig. 4). Following intrajejunal glucose, there was a dramatic rise in IRG in both dogs (Fig. 4), which was greater than that seen in the intact conscious animals. Prior to pancreatectomy the highest levels of serum insulin and glucagon were recorded in the pancreatic vein. There was a distinct portal-peripheral vein insulin gradient, but no portal-peripheral vein glucagon gradient. Following intrajejunal glucose, the level of glucagon in the portal vein, which in the dog drains the gastrointestinal tract apart from the anal canal, was higher than in the superior mesenteric vein which drains only the jejunum and ileum. There was no difference in the glucagon levels between the peripheral vein and the superior mesenteric vein.

Discussion. We have found that the half life of pancreatic IRG injected intraportally into 2 dogs to be 3 and 4 minutes, respectively, (unpublished observations), so that following pancreatectomy, pancreatic IRG would be expected to virtually disappear from the circulation within 1 hour. There is little doubt, therefore, from our experiments on the pancreatectomized animal, that gut IRG makes a substantial contribution to the immunoassayable serum glucagon both in the basal state and following alimentary glucose. This

confirms Ohneda *et al's*(6) findings that the glucagon rise after oral glucose is enteric in origin in dogs, but is contradictory to Samols *et al's*(3) findings in man. We have found IRG to be present in the intestine from the jejunum to the rectum. Because of the much higher level of IRG after glucose in the portal vein than in the superior mesenteric, it appears that the colon and/or rectum may contribute to the rise in IRG seen after oral glucose. Therefore it is clear that in the absence of an efficient method of distinguishing between intestinal and pancreatic IRG in serum, in order to study glucagon secretion one must resort to study of animals with multiple catheters *in situ,* pancreatectomized or eviscerated animals, or *in vitro* pancreatic or gut systems, before the source of IRG can be determined and the results correctly evaluated.

It is interesting to speculate as to what is the physiological role of intestinal IRG. A different immunological behavior(3,4) and different molecular weights for gut and pancreatic IRG(4) have already been recognized. Valverde *et al*(12) have shown that, unlike pancreatic glucagon, intestinal IRG does not appear to possess the ability to stimulate glycogenolysis, activate the adenyl cyclase system or stimulate the release of pancreatic insulin. The rise of circulating gut IRG after oral glucose would certainly be consistent with an insulin stimulating role. It was interesting to note that in our experiments in the pancreatectomized dog, the rise in gut glucagon was more dramatic than in the intact animal, suggesting that insulin deficiency may augment its release.

Summary. Immunoreactive glucagon was detected in the pancreas and the small and large intestines of 4 different animals. Immunological differences existed between pancreatic and jejunal glucagon in the human and dog, but pancreatic and rectal glucagon appeared similar. A rise in serum immunoreactive glucagon was demonstrated in unanaesthetized dogs after oral glucose. Basal serum glucagon levels in dogs fell only slightly after pancreatectomy. A marked rise in serum immunoreactive glucagon occurred after intrajejunal glucose in pancreatectomized dogs, suggesting its enteric source. An insulinogenic role for intestinal glucagon is considered.

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