Plasma Immunoreactive Insulin (IRI) and Immunoreactive Glucagon (IRG) After Evisceration with and Without a Functional Liver

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SUMMARY

Fed male Wistar rats were eviscerated by two procedures. The first group of eviscerated rats were left with a nonfunctional liver in situ while the second group were eviscerated by a newer technic, developed in this laboratory, that preserves liver function.[7] The animals were maintained on a regimen of saline and antibiotic treatment, and abdominal aortic blood was drawn at intervals up to seventy-two hours postoperatively from animals with a functional liver and up to six hours postoperatively in those with nonfunctional liver status. Blood concentrations of glucose, immunoreactive insulin, and immunoreactive glucagon were measured. Our results indicate that even with a functional liver, totally pancreatectomized eviscerated rats maintained normal amounts of plasma IRI and IRG for more than twenty-four hours. IRI and IRG were measurable even at forty-eight hours postoperatively. At the same time, these animals developed abnormally high blood glucose levels, which were sustained despite the presence of "normal" IRI in plasma. It had been suggested that the presence of measurable IRI and IRG in the classically prepared eviscerated animal was due to a deficit liver destruction of these substances. To the contrary, our data suggest that even with a functional liver, the eviscerated preparation maintains a circulating level of insulin and glucagon-like materials forty-eight hours after the known sources of such substances are removed. The physiologic meaning of immunoassay results following pancreatectomy are thus difficult to interpret. DIABETES *24*:637-40, July, 1975.

Bioassay procedures for the determination of serum insulin (or insulin-like activity) have produced ambiguous data in which there is evidence for the continued presence of such substances after total pancreatectomy in dogs,[1-3,12-14] cats,[2] and human beings.[11] The two-stage total pancreatectomy in dogs carried out by Kosaka et al.[4] resulted in normal levels of blood glucose (BG) after one and one-half to four hours. In addition, oral or intravenous glucose tolerance tests (IVGTT) before and immediately after acute pancreatectomy were not significantly different. Serum insulin was not measured in these experiments.

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Rasio et al.[5] also found a normal IVGTT in dogs exposed to a glucose load forty minutes after pancreatectomy. In these animals, the level of serum IRI was found to be significantly lower than normal. Repetition of the IVGTT three hours and forty minutes later showed impairment of glucose tolerance with very low serum IRI levels. Chieri et al.[6] found that partially or completely pancreatectomized dogs infused with saline did not show significant changes in BG or IRI levels during the first ninety minutes postoperatively. When the pancreatectomized dogs were given an IVGTT, these investigators found a normal glucose tolerance with a rise in IRI levels for up to fifty minutes postsurgery. To account for their data, Chieri et al. suggested that there might be some residual pancreatic tissue or some extrapancreatic GI tissues that can bind or store insulin.

In order to clarify these conflicting data, we used eviscerated animals with no GI or pancreatic tissues. These animals were prepared with either a functional or nonfunctional liver in situ. Eviscerated animals with functional hepatectomy had already been shown to have measurable levels of IRI and IRG.[10] No data were available for the eviscerated animal with a functional liver as measured by the ability to produce bile, glucose, urea, and ketone bodies.[15] We prepared such animals and measured serum levels of glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) and compared these data with those obtained from eviscerated rats with no liver function.

METHODS

Fed male Wistar rats (280-380 gm. body weight) were anesthetized with sodium amytal (4 mg./100 gm. body weight) and eviscerated according to the technic described by Russell.[7] This surgical technic results in the excision of the gastrointestinal tract, pancreas, and spleen. The liver remains in situ but is not functional because it completely lacks a blood

supply. Other groups of fed male rats were eviscerated in a similar fashion but retained a functional liver.

The surgical procedure used was the following: An incision was made in the midline of the abdominal wall, starting at the midpoint between the sternum and the pubis and extending upward to the lower edge of the sternum. Pressing gently against both sides of the abdomen then causes protrusion of the gastrointestinal viscera, including the spleen. These organs are moved to the right side of the animal and covered with a sponge moistened with warm 0.9 per cent saline. The descending colon is carefully separated from the small intestine and a ligature is placed around the colon at the level of the rectum. A second ligature is placed 3-5 mm. higher, and the rectum is severed between both ligatures.

The next step is designed to separate the stomach and the abdominal esophagus from their ligaments and other membranous tissues connecting the upper GI tract with the liver. Two ligatures are placed around the esophagus, and this organ is then sectioned between the ties. Care must be taken such that the ligatures include the esophageal blood vessels. The next step is to gently move all the exposed viscera to the left side of the animal in order to expose the celiac trunk, the superior mesenteric artery, and the portal vein.

The bile duct is then cannulated with a polyethylene tube (no. PE 10) inserted toward the liver in order to allow continuity of bile secretion. The other end of the cannula is inserted in the rectum to provide an effluent pathway for the bile. A double ligature is then placed around the superior mesenteric artery, and then the blood vessel is severed between the ligatures. In this way the stomach, small and large intestines, pancreas, mesentery, and spleen can be excised. When the portal vein is ligated, care must be taken to exclude the hepatic artery. This arterial flow is vital for maintenance of liver function. Section of the hepatic artery produces a functional hepatectomy.

The abdominal incision was closed with surgical silk (Deknatel 4-0). Muscle and skin are included in the same suture. The animals were then injected subcutaneously every eight hours with warm saline solution (0.9 per cent NaCl at 1 ml./100 gm. body weight) and antibiotics (0.5 mg. per rat, Principen/N, Squibb) and the rats were maintained at a room temperature of 22-24°C.

Evidence for continued liver function during the seventy-two-hour postevisceration period is provided by the fact that the blood glucose increased from 124 to 702 mg. per 100 ml., the ketone body levels increased from 1.2 to 13.1 mg. per cent, and the urea levels increased from 16 to 70 mg. per cent.[15] Since the liver is critical for the maintenance of these blood constituents, the continuous rise observed in blood glucose, urea, and ketone bodies, as well as the uninterrupted bile secretion, can be explained only by the continued functioning of the liver.

This new surgical procedure retains the arterial blood flow to the liver and allows for bile excretion through a polyethylene cannula into the stump of the rectum. The kidney and adrenals are left intact in both types of eviscerated rats with functional liver (ERFL) or nonfunctional liver (ERNFL).

Groups of ten rats were anesthetized (sodium amytal 4 mg./100 gm.) and blood was collected from the abdominal aorta before the operation and ½,1,2,3,4,6,24,48 and 72 hours after. ERNFL were studied for six hours postoperatively and blood samples from groups of ten rats each were collected under anesthesia at ½,1,2,3,4,5, and 6 hours after surgery.

Blood glucose was measured by the Glucostat method (Worthington Biochemical, Freehold, N.J.). Levels of plasma insulin and glucagon were measured by radioimmunoassay methods. Blood was obtained from the abdominal aorta in syringes rinsed with 10 per cent solution of ethylenediaminotetraacetate (EDTA) into tubes containing 500 units kallikrein inactivator (0.1 ml. of Trasylol). The plasma was separated immediately and stored at −20°C. for no more than two weeks. Glucagon immunoassay (IRG) was measured by the Unger et al. method.[8] Insulin was measured by the Morgan and Lazarow method.[9]

RESULTS

Table 1 shows that the level of blood glucose (BG) in the ERNFL decreased by 25 mg. per 100 ml. (−20.3 per cent) six hours after evisceration. On the other hand, the ERFL showed an increase in BG of 204 mg. per 100 ml. (+166 per cent) during the same period. This rise continued and the BG reached a level which was 683 mg. per 100 ml. (+555.3 per cent) above normal by seventy-two hours.

The levels of IRI in the ERNFL did not change significantly during the first three hours after surgery. The fourth-hour sample showed a significant IRI decrease of 13 μU. per milliliter ($p<0.0001$). This lower level of IRI did not change significantly during the fifth and sixth hours. In the ERFL the IRI did not change significantly from control values up to twenty-four hours postevisceration. At forty-eight to seventy-two hours, the IRI level decreased significantly ($p<0.001$).

TABLE I

Blood levels of glucose, IRI, and IRG in eviscerated rats with and without a functional liver
Each number is the average of ten animals with its standard error

	Hours		0	½	1	2	3	4	5	6	24	48	72
Glucose mg./100 ml.	Evisc. no func. liver	Avr.	123	114	119	119	120	108	103	98	—	—	—
		S.E.±	2.7	2.2	7.7	6.6	5.6	2.8	2.5	3.5	—	—	—
	Evisc. with func. liver in situ	Avr.	123	140	169	170	177	179	—	327	387	649	806
		S.E.±	2.7	5.6	13.9	9.6	9.9	5.5	—	29.3	12.9	20.0	25.2
Significance between 2 groups			—	<0.001	<0.001	<0.001	<0.001	<0.0005	—	<0.0005	—	—	—
Insulin μ/ml.	Evisc. no liver	Avr.	40	48	47	38	35	27	29	24	—	—	—
		S.E.±	6.5	5.9	5.6	5.9	3.0	1.7	2.6	2.2	—	—	—
	Evisc. with func. liver in situ	Avr.	40	29	31	30	33	45	—	34	39	14	6
		S.E.±	6.5	3.2	4.7	5.6	7.2	5.3	—	8.7	8.3	3.1	2.2
Significance between 2 groups			—	0.01	N.S.	N.S.	N.S.	0.005	—	N.S.	—	—	—
Glucagon pg/ml.	Evisc. no liver	Avr.	214	184	263	339	320	281	470	490	—	—	—
		S.E.±	28.9	26.8	34.5	17.3	32.3	15.7	23.2	30.7	—	—	—
	Evisc. with func. liver	Avr.	214	259	270	269	260	313	—	456	613	322	155
		S.E.±	28.9	13.7	24.1	18.7	22.1	45.4	—	29.2	16.8	18.2	12.8
Significance between 2 groups			—	N.S.	N.S.	0.01	N.S.	N.S.	—	N.S.			

In the ERNFL the level of IRG increased significantly at the second, third, fifth, and sixth hour ($p<0.001$) samples. In the ERFL the level of IRG increased significantly ($p<0.001$) only six hours after surgery as compared with control IRG values. At twenty-four and forty-eight hours, the elevation in IRG was still highly significant ($p<0.001$). Seventy-two hours after the operation, the concentration of IRG returned to the initial level.

Comparison of the two groups of rats reveals that after a thirty-minute postoperative interval, the concentration of glucose is significantly different between the ERNFL and the ERFL ($p<0.001$). This difference increases with time during the next six hours.

In addition, a significant difference in the IRI in the ERNFL and the ERFL groups was observed at the thirty-minute interval after the surgery. At this time, the levels in the ERFL were lower than in the ERNFL, but by the fourth hour the ERNFL had a significantly lower IRI level than the ERFL. At the sixth hour, the IRI levels were not significantly different in the two groups.

Apart from the significant difference in IRG levels observed at the end of the second hour, no other differences in this parameter were found at any time as between the two groups of animals.

DISCUSSION

The continued presence of measurable serum immunoreactive glucagon levels (IRG) in pancreatectomized animals that has been observed by several investigators[3,12] suggested either that the animals had residual pancreatic tissue or that there might be another source of serum IRG from the rest of gastroenterosplenic complex.

Previous evisceration technics used to study these phenomena were complicated by the rapid deterioration of the animals when liver function was absent. We designed a surgical technic, therefore, which preserved major liver function in the otherwise eviscerated animal. This procedure enabled us to remove all suggested sources of serum IRI and IRG while maintaining a viable animal for many hours. It had also

been proposed that the maintenance of serum IRI and IRG levels in the classic eviscerated preparation was the result of a lack of destruction of these hormones due to the absence of the liver. We found, however, that even with a functional liver, in situ, eviscerated rats also maintained normal amounts of serum IRI for more than twenty-four hours. These animals were viable for up to ninety-six hours postoperatively when only saline and antibiotic treatment were instituted. IRI was still measurable at forty-eight hours. IRG also stayed high during this time. It is impossible to account for the persistence of high IRI and IRG levels in such animals solely on the basis of a lack of liver metabolism of the hormones.

Of further interest is that, with the liver functioning, eviscerated animals show a form of acute diabetes despite the fact that serum levels of IRI are in the normal range. Only after forty-eight hours is a marked drop in IRI and IRG observed. This discrepancy between the metabolic state and the level of circulating IRI and IRG may be an artifact of the method used for the immunoassay complicated by the metabolic ketosis present in these rats. Another possible artifact may lie in the interaction of the immunoassay with fragments of insulin or glucagon catabolites. Under the novel conditions of our experiments it is possible that nonspecific polypeptides were produced that skewed the results obtained. This can be verified only by further separation of polypeptides in each sample. It should be noted, however, that we used the standard technics for the immunoassay determinations of active hormones of the pancreas. Data obtained in this way have been the basis of published reports by many investigators. This raises questions about the meaning of immunoassay results following evisceration. In any event, the rapid development of a severely diabetic state concurrent with the persistence of high serum levels of IRI and IRG makes it necessary to reevaluate what is actually being measured by the immunoassay under circumstances in which all known sources of these substances have been removed but a functioning liver remains in situ.

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