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Metabolic Studies in Eviscerated Rats with Functional Livers (38708)

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(Introduced by L. S. Lilienfield)[1, 2]

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The classic one stage, surgical technique of rat evisceration was established by Russell in 1942 (1). A two-stage evisceration procedure was also described by Reinecke (2, 3) and by Ingle (4). In Russell's technique, the animal was not hepatectomized. The liver remained *in situ*. These eviscerated rats, however, were considered to be functionally hepatectomized and have provided excellent test animals in which to study various hormonal effects on the overall metabolism of caloric substrates as well as the role of the kidneys in the metabolism of these substrates in peripheral tissues.

Since inulin, glucagon and the gastrointestinal hormones play an important role in liver metabolism (5), we devised an evisceration procedure in which the operated animals retained a functional liver. With this in mind, a gastroenteropancreatosplenectomy was performed in rats using a technique similar to that described by Russell but with the added advantage of preserving liver function. We used this preparation to measure certain parameters of substrate metabolism and the data obtained were compared with those reported by investigators using eviscerated rats with nonfunctioning livers.

Methods. Fed male rats weighing 250–350 g were anesthetized with sodium amytal (4 mg/100 gbw). 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% 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 procedure 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 coeliac 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 using surgical silk (Deknatel 4-0). Muscle and skin are included in the same suture. The animals were then injected subcutaneously every 8 hr with warm saline solution (0.9% NaCl at 1 ml/100 gbw) and antibiotics

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(0.5 mg/rat, Principen/*N*, Squibb and Sons, Inc.) and the rats were maintained at a room temperature of 22–24° (6–8). A control group of rats was subjected to Russell's evisceration procedure (1) and treated in the same way.

Groups of ten previously fed eviscerated male rats with functional livers were sacrificed at 3, 6, 24, 48 and 72 hr after operation, and blood samples were collected from the abdominal aorta. An equal number of control animals which were functionally hepatectomized were sacrificed at the same time intervals and samples of aortic blood collected. Finally, a group of ten fed, intact male rats were sacrificed and the blood samples collected were used as a zero hour reference.

Chemical determination of glucose (9), amino nitrogen (10), free fatty acids (11) and ketone bodies (13) were carried out in all blood samples.

Results. Table I shows the concentration of blood glucose (BG) in eviscerated rats with non-functional livers (ERNFL). Six hr after evisceration, the BG dropped 33.1% from preevisceration levels. The eviscerated rats with functional livers (ERFL), however, had an actual increase of 87.9% in the BG during this same 6 hr time interval postevisceration. This elevation of BG was maintained for up to 72 hr following surgery. At this time, the percent increase of BG over control levels was 466%.

Table I also shows that 6 hr after evisceration in the ERNFL, the level of ketone bodies in blood decreased by 50% from initial concentrations. During the same time interval, the ERFL had an *increase* of 475% of blood ketone bodies. Seventy-two hr after the operation, these ERFL showed a 991.7% increase in ketones as compared to the initial concentrations taken as 100%.

The urea levels in the ERNFL did not change significantly during the 6 hr postevisceration period, but the ERFL had a 6 hr increase in urea concentration of 118.7%. This rise continued for 48 hr and reached a plateau at 72 hr (Table I).

Table II shows that the plasma free fatty acids (FFA) were significantly increased 6 hr postevisceration in animals with either a functional or nonfunctional liver (307.5% in the ERNFL and 342.5% in the ERFL). At 72 hr, the level of FFA in the ERFL

TABLE I. BLOOD GLUCOSE, KETONES AND UREA CONCENTRATION IN FED MALE EVISCERATED RATS WITH (ERFL) OR WITHOUT (ERNFL) A FUNCTIONAL LIVER. EACH VALUE REPRESENTS THE MEAN OF 10 ANIMALS PLUS THE STANDARD ERROR.

Parameter	Condition		Before surgery	Hours after surgery 3	6	24	48	72
Blood glucose		Avr.	124	104	83			
mg/100 ml	ERNFL	SE ±	3.1	2.0	3.2	—	—	—
		%	100	84	67			
		Avr.		189	233	340	505	702
	ERFL	SE ±	—	7.5	12.6	33.6	52.3	45.1
		%		152	188	274	407	566
Ketone bodies		Avr.	1.2	1.0	0.6			
mg/100 ml	ERNFL	SE ±	0.2	0.2	0.2	—	—	—
		%	100	83	50			
		Avr.		2.1	6.9	8.0	14.0	13.1
	ERFL	SE ±	—	0.2	0.7	0.8	0.8	2.6
		%		175	575	667	1167	1092
Urea mg/100 ml		Avr.	16	20	15			
	ERNFL	SE ±	0.7	1.3	1.4	—	—	—
		%	100	125	94			
		Avr.		26	35	54	76	70
	ERFL	SE ±	—	1.1	3.2	3.3	5.6	5.8
		%		162	219	337	475	437

TABLE II. FREE FATTY ACIDS, AMINO NITROGEN AND SURVIVAL DATA FROM ERFL AND ERNFL. EACH VALUE REPRESENTS THE MEAN OF 10 ANIMALS PLUS THE STANDARD ERROR.

Parameter	Condition		Before surgery	Hours after surgery 3	6	24	48	72
Free fatty acids		Avr.	0.40	0.89	1.23			
μEq/ml	ERNFL	SE ±	0.03	0.06	0.08	—	—	—
		%	100	222	307			
		Avr.		0.85	1.37	2.16	2.21	2.18
	ERFL	SE ±	—	0.05	0.09	0.16	0.26	0.31
		%		212	392	540	552	545
Amino nitrogen		Avr.	2.0	8.0	14.8			
mg/100 ml	ERNFL	SE ±	0.2	0.1	0.5	—	—	—
		%	100	400	740			
		Avr.		8.5	9.8	10.7	11.7	11.6
	ERFL	SE ±	—	0.7	0.6	0.6	0.9	0.7
		%		425	490	535	585	580
Mortality	ERNFL	Dead/survival	—	0/10	0/10	—	—	—
	ERFL	Dead/survival	—	0/10	0/10	1/10	4/10	6/10

was 445% higher from initial value. The plasma amino nitrogen (PAN) (Table II) during this period increased by 649% from the initial concentration in the ERNFL and by 390% in the ERFL). Rats with functional livers survived for longer periods of time, showed a levelling of the PAN by 24 hr (Table II).

Eviscerated rats without liver function could only be salvaged for about 6 hr (Table II). All such animals were dead by 9 hr. With a functional liver, however, eviscerated rats could be kept alive for as long as 72 hr. Of these animals, 90% were alive after 24 hr, 60% at 48 hr and 40% at 72 hr. Without intravenous nutritional supplement all the ERFL were dead at 96 hr.

Discussion. The eviscerated rat has been used since 1942 (1) to study acutely peripheral tissue metabolism and the effects of drugs and hormones on this metabolism. The absence of a functional liver, however, has limited the usefulness of such preparations in longer term experiments. In addition, the presence of a functional liver in the absence of insulin, glucagon and the intestinal hormones makes it possible to dissect out the direct action, if any, of each of these substances on either the peripheral tissues or the liver itself. The isolated liver perfusion has not been entirely satisfactory due to the absence of peripheral tissue-liver interactions and the lack of any regulation by either nervous system or hormonal factors.

In our newer technique, the liver is maintained with its arterial, venous and lymphatic circulation but without its portal blood inflow. In this preparation, we have eliminated the direct influence of the endocrine pancreas and the endocrine intestine. All other hormones, however, are secreted as usual into the systemic venous circulation and can act on the liver. In addition, since insulin, glucagon, the intestinal hormones and probably certain splenic hormones are secreted directly into the liver, the immediate effect of the evisceration surgery with the liver function preserved is to precipitate a rapid form of diabetes mellitus. The blood levels of glucose, ketones, urea, FFA and amino nitrogen rise rapidly in the postoperative period. For the most part, with the exception of FFA and PAN, this is opposite to the effect observed in eviscerated rats with nonfunctional livers. Eviscerated rats with functional livers can be maintained in a viable condition for up to 72 hr by the administration of only physiological saline which compensates for the rapid dehydration which would otherwise ensue in these animals.

It is of interest that even in the absence of

the known sources of glucagon and intestinal hormones, the gluconeogenic activity of the liver is very high if insulin is absent. This is consistent with Cahill's recent observation regarding the totally pancreatectomized human (14). He concludes that when exogenous insulin is made available "a glucagon-free existence may occur without major abnormality." It is apparent that insulin is the critical component in the regulation of carbohydrate metabolism in the liver. One of the problems, however, in interpreting data obtained using the usual immunoassay methods for circulating levels of either insulin or glucagon, is demonstrated by our findings that even after evisceration, the serum levels of IRG were normal or above normal (15). The nature of these circulating substances is unknown.

Comparison of the data obtained from the classical eviscerated preparation and the eviscerate animal with a functional liver, reveals that the fall in BG previously reported in the ERNFL is not as rapid as expected. Only when the ERNFL were fasted prior to the evisceration does a profound hypoglycemia rapidly occur. The ERFL showed a continuous rise in the blood glucose particularly in previously fed animals. This indicates an active process of liver gluconeogenesis and glycogenolysis.

Lipid metabolism was significantly altered in the absence of the liver, with a steady decline in ketone body formation despite a simultaneous rise in plasma FFA. With the liver functioning, an elevation in ketone bodies was observed up to 48 hr postsurgery, with a plateau achieved by 72 hr. This was paralleled by a similar rise in plasma FFA. As expected, blood urea levels fell in the absence of liver function while a rise in blood urea was observed for more than 48 hr if liver function was relatively intact. Pan followed a similar pattern in the two types of animals.

Thus, it is apparent that in the absence of the endocrine pancreas, peripheral tissue catabolism is accelerated whether or not the liver is functional. The fate of these products of catabolism, however, as reflected in plasma concentrations of carbohydrates, lipids and nitrogenous compounds obviously depends on the presence of the liver. The eviscerated rat with a functioning liver represents a tool for characterizing peripheral, hormonal and hepatic contributions to the overall metabolic changes observed in the intact animal following feeding or fasting.

Summary. A new technique is described for evisceration in the rat in which liver function is preserved. These animals lack all known sources of glucagon and insulin and are capable of active gluconeogenesis, urea formation and ketone body production by the liver. Measurements of blood levels of the metabolites of the caloric substrates showed that, unlike the classical eviscerate preparation, these animals maintain high blood glucose, urea and ketone body levels for up to 72 hr as contrasted with the profound decrease in these constituents in the absence of the liver. Survival time is also significantly extended from about 6 hr in rats lacking liver function to 72 hr or more when the liver is viable. This new surgical preparation is a valuable tool for studying the role of the liver in absence of the known gastroentero-pancreatic hormones. It would also be utilized as a model of "acute" diabetes.

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