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Metabolic Effects of Adrenalectomy in Eviscerated Rats (40476)

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The role of kidneys and adrenal glands is blood glucose and amino nitrogen homeostasis in eviscerated animals has been extensively reported (1–4). It is well established that the liver is the major site for blood glucose regulation. Studies to quantify the role of the liver using eviscerated preparations in absence of the kidneys and/or the adrenal glands have not been reported in the literature.

We have previously (5) studied the effect of nephrectomy or hepatectomy on several metabolic parameters in eviscerated rats. This paper presents data on the effect of adrenalectomy on the specific role of the liver and the kidneys in carbohydrate, protein and lipid metabolism.

Methods. Eight different surgical procedures (5, 6) corresponding to the possible combinations of eviscerated rats with or without liver, with or without kidneys and with or without the adrenals were utilized in this study.

Eviscerated rats (Evs)	Adrenalectomized (Adx)	Nonadrenalectomized (N.Adx)
With functional liver (L) and kidneys (K)	Adx + L + K	N.Adx + L + K
With kidneys and nonfunctioning liver	Adx − L + K	N.Adx − L + K
With functioning liver (L) and nephrectomized	Adx + L − K	N.Adx + L − K
Nephrectomized and non-functioning liver	Adx − L − K	N.Adx − L − K

The surgical procedures have been described previously (5, 6) and at least 10 rats were utilized in each surgical group. Adrenalectomy was carried out 4 days before evisceration. The animals were exsanguinated via abdominal aorta puncture and sacrificed serially in groups of 10 rats at 3, 6, 24, 48, or 72 hr postoperatively after being anesthetized with sodium amytal Lilly (5 mg/100 g b.w.). Aliquots of blood were taken for chemical measurements of blood glucose (BG), plasma free fatty acids (FFA), blood ketone bodies (KB), plasma amino nitrogen (AN) and blood urea nitrogen (BUN) according to methods described previously (5).

Since the survival of the Evs rats is markedly reduced, we used this survival time as a standard to sacrifice the Evs + L + K at intervals up to 72 hr. The Evs + L − K and Evs − L + K were sacrificed at intervals up to 6 hr. The Evs − L − K had the lower ± survival time and were sacrificed at 3 hr after surgery. In the group of adrenalectomized-eviscerated rats + L + K, the rats did not survive more than 24–30 hr, so the animals were sacrificed up to 24 hr.

In the groups of Evs N.Adx control rats, 10 animals were submitted to a sham operation (non eviscerated) while in the groups of Evs Adx rats, another 10 rats were submitted to adrenalectomy and sham evisceration.

Results. Blood glucose (Fig. 1). As expected the highest levels of BG was observed in N.Adx Evs rats + L + K, followed in order by + L − K, − L + K and by − L − K. In the Adx Evs all the values were significantly lower ($p < .001$) than the N.Adx Evis rats, with one exception. The values of the Adx − L − K were not significantly different from the N.Adx − L − K at 3 hr after surgery. Another interesting observation was that the Adx + L − K were not statistically different from the Adx + L + K at the 6 hr BG determination.

Free fatty acids (Fig. 2). The lipolytic process taking place in Evs animals (5) is more intense in the N.Adx − L − K rats at the 3-hr period than in the other groups of N.Adx Evs rats ($p < .001$). In the Evs Adx rats there is no statistically significant difference at 3 hr between the − L − K and the − L + K groups. Six hours after surgery the N.Adx − L + K group has the highest level of FFA

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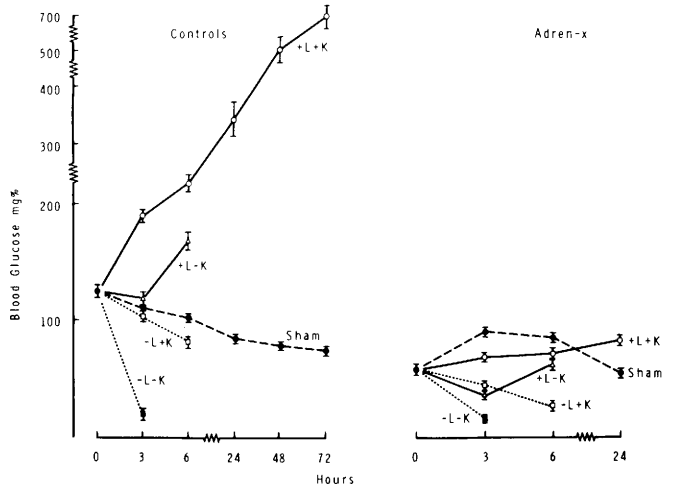

FIG. 1. Blood glucose levels (mg%) in rats eviscerated in each of the four conditions comparing fed with fed 96 hr adrenalectomized animals. Each value represents the average of 10 measurements with its corresponding S.E.

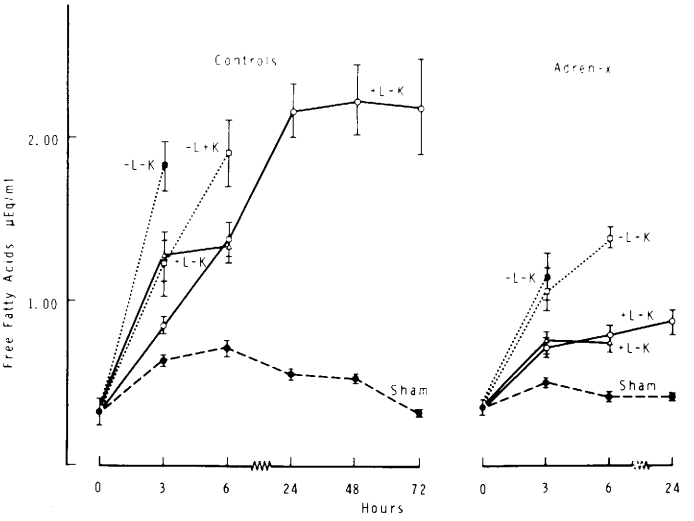

FIG. 2. Plasma free fatty acid levels (µEq/ml) in rats eviscerated in each of the four conditions comparing fed with fed 96 hr adrenalectomized animals. Each value represents the average of 10 measurements with its corresponding S.E.

which is signifciantly higher than the other two groups of N.Adx + L + K and N.Adx + L − K ($p < .001$). At 24 hr the N.Adx + L + K reach a plateau and the concentration of FFA remains non significantly different for the 48 and 72 hr determinations. In the Adx Evs rats all the levels of FFA were in general significantly lower ($p < .001$) than the N.Adx Evs animals. In the Adx Evs rats it was interesting to observe that in the absence of the liver, with or without the kidney, there was no significant difference at 3 hr after evisceration. Furthermore, in Adx Evs + L with or without K, the FFA levels were not significantly different.

Ketone bodies (Fig. 3). In the N.Adx rats the production of KB was significantly higher in the Evis + L − K than in the Evs + L + K ($p < .001$ at 3 hr and $p < .025$ at 6 hr). In the Evs − L + K there was a significant fall ($p < .05$) of the KB levels at 6 hr when compared with the initial 0-hr value. No sig-

nificant change was observed at 3 hr in the Evs − L − K rats.

In general the production of KB in the Adx Evs rats were significantly lower than in the Evs N.Adx ($p < .001$). There was no statistical difference between Adx Evs + L + K and + L − K at either 3 or 6 hr, nor was there a difference between Adx Evs − L + K and − L − K at 3 hr.

Amino nitrogen (Fig. 4). At the 3 hr observation, the highest level of blood AN was observed in the N.Adx Evs − L − K ($p < .001$). At th 6 hr determinations the highest level was in the N.Adx Evs − L + K followed by + L − K and by + L + K. The differences between each group and the sham operated were statistically significant ($p < .001$). In the Adx Evs group 3 or 6 hr the highest level of AN was in the − L + K group. This was statistically higher ($p < .001$) than the other three types of evisceration, or the sham Adx Evs group. There was no statistical differ-

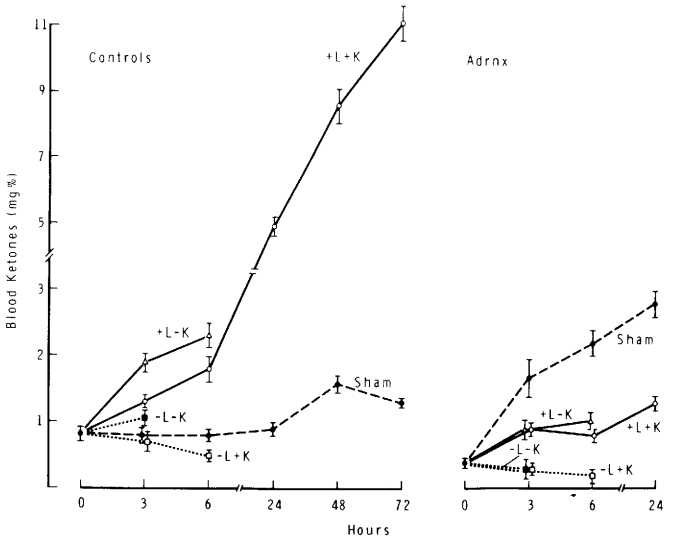

FIG. 3. Blood ketones (mg%) in rats eviscerated in each of the four conditions comparing fed with fed 96 hr adrenalectomized animals. Each value represent the average of 10 measurements with its corresponding S.E.

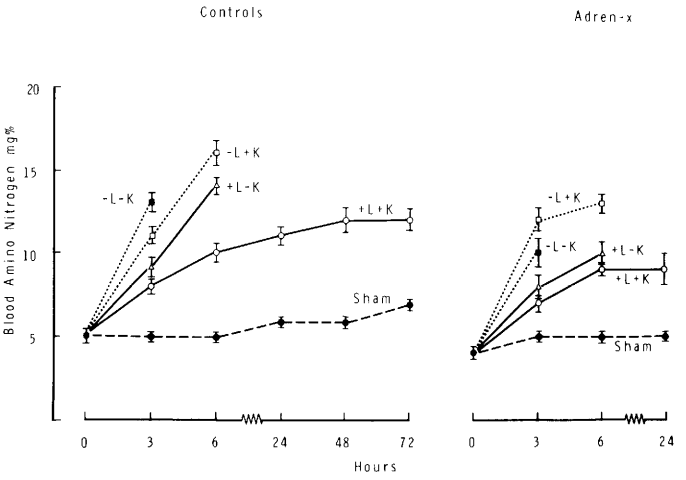

FIG. 4. Blood amino nitrogen levels (mg%) in rats eviscerated in each of the four conditions comparing fed with fed 96 hr adrenalectomized animals. Each value represents the average of 10 measurements with its corresponding S.E.

ences between Adx Evs + L + K and + L − K; they were significantly higher than the sham Adx Evs ($p < .001$).

Urea nitrogen (Fig. 5). There are no significant differences in BUN between the N.Adx and the Adx groups, except at 24 hr; the value for the N.Adx Evs + L + K is significantly higher ($p < .01$) than in the Adx group. At 3 hr the BUN values of Evs − L + K in both groups are significantly lower than the respective sham operated rats as well as to the other three types of eviscerations ($p < .001$).

Discussion. In a previous paper (5) we described the changes observed in several blood metabolites when the liver function is preserved in an eviscerated animal. In absence of all known abdominal sources of glucagon, insulin or gastrointestinal hormones, the eviscerated rat is capable of active gluconeogenesis, urea formation and ketone body production by the liver for up to 72 hr; whereas in the absence of the liver these metabolites decrease rapidly. The role of the kidneys and of the adrenals in these changes has been evaluated in the present study.

It has been shown that adrenalectomy in rats produces a decrease in urea nitrogen levels and increased urea excretion, decreased blood sugar levels and diminished liver glycogen stores (7). In fact there is a significantly greater production of glucose in the Evs controls than in the Adx Evs rats. Our data supports the concept that in the absence of adrenal glands, gluconeogenesis proceeds at a slower than normal rate (8). The elevation of blood sugar in the Evs control animals depends on the presence of a functioning liver. In the Adx Evs rats the elevation of blood sugar is significant 24 hr after eviscertation and in the presence of the kidneys and a functioning liver. In fact, when the animals are nephrectomized, the functioning liver in the Adx rats maintained the glycemia level at that of the zero hr. In the N.Adx Evs rats with kidneys and non functioning liver, the level of the blood sugar 6 hr after surgery is maintained by the kidneys at 65.7% of its initial value, while in the Adx Evs − L + K the levels of blood sugar was 46.9% of the zero hr level, indicating that kidney gluconeogenesis was severely impaired in the absence of the adrenal gland. Early studies demonstrated that Adx Evs rats require more glucose to maintain blood sugar than N.Adx Evs rats (9, 10) and that the eviscerated rat is particularly sensitive to the presence or absence of adrenal cortex (9). Although it is evident that in the Adx animals the gluconeogenic process is affected in both organs, the liver (8) and the kidneys (11), it is interesting to note that in absence of both in the Evs rats the decline of the BS that leads to death is

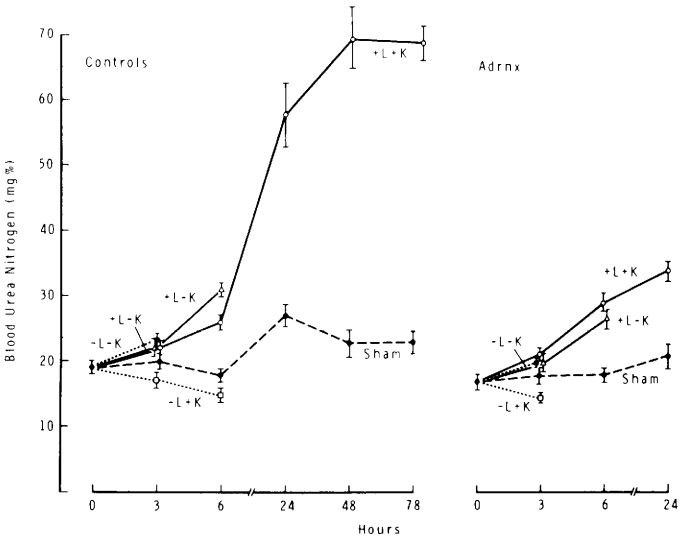

FIG. 5. Blood urea nitrogen levels (mg%) in rats eviscerated in each of the four conditions comparing fed with fed 96 hr adrenalectomized animals. Each value represents the average of 10 measurements with its S.E.

greater in N.Adx (−85.7% from initial value) than in the Adx ones (−36.4%) at 3 hr after surgery (Fig. 1). However the final glycemia levels were not significantly different in the groups of Evs rats which suggests a more rapid utilization of glucose by N.Adx Evs than in the Adx ones. Although the utilization of sugar in the Adx − L − K seems to be reduced (12, 13) the lower initial levels of blood sugar due to the decreased peripheral protein catabolism and the lack of liver and kidney glycogen contribution, results after 3 hours in the same level of hypoglycemia in both groups.

The levels of FFA were greater in the sham control than in the adrenalectomized animals in accordance with an early observation of Samuels and Conant (14). Although adrenalectomy does not abolish the increase in FFA mobilization during fasting, it decreased the magnitude of this response (15). In adipose tissue cortisol and its analogs increase net release of FFA and inhibit glucose uptake (16, 17). Moreover, in insulin deficient animals, lipolytic hormones promote FFA release while glucocorticoid limits reesterification of FFA by inhibiting glucose metabolism (18). Thus there are two reasons for the lower levels of FFA in all types of Adx Evs rats although the zero hr control values (in fed condition) are essentially the same.

The plasma levels of ketone bodies in nontreated diabetes are elevated in presence of the adrenal glands. This condition is improved by adrenalectomy (17, 18). In all groups of Adx Evs rats the levels are lower than for the N.Adx Evs ones. At 6 hours, the levels are higher in the Evis + L − K, because in absence of the kidney there is both lack of excretion of ketone bodies and lack of utilization of acetoacetate, preferentially used by the kidney over glucose or lactate (19).

Plasma amino nitrogen levels were depressed in all groups of Adx Evs animals agreeing with the findings of Bondy *et al.* (20). Although glucocorticoids increase hepatic uptake of amino acids they have no effect per se on liver protein catabolism (21, 22). This is why in our preparations levels are higher when liver was non functional in Adx and N.Adx animals; lowest when the liver was functioning. Adrenalectomy does not appear to affect the kidney's role in protein metablism, although the kidney is known to preferentially extract certain amino acids (23); thus in the nephrectomized rats the level of amino nitrogen is higher than the nonnephrectomized ones.

Blood urea nitrogen levels are not significantly affected by the absence of the adrenal glands in either the control or eviscerated animals, for the first 6 hr. After that period the blood urea levels are signficantly elevated in the N.Adx Evs + L + K. This finding fits with the previous observation that these animals have the lowest level of blood amino nitrogen, indicating the large capability of the liver to catabolize protein and amino acids, and thus to synthetize urea (21). This increase also correlates with the active process of gluconeogenesis observed in this N.Adx Evs + L + K, which mimics an acute diabetic state (5).

In conclusion, our data show that the liver is much more effective relative to the kidney in maintianing blood sugar levels in Evs Adx and N.Adx animals. In the N.Adx Evs rats the kidneys could sustain up to 80% of normal blood glucose in absence of a functional liver. This role is effective only in the presence of the adrenal glands. The levels of all other metabolites studies were lower in the Adx animals. Catabolic activity in the Evs rats produced by the lack of insulin and leading to a short survival was counteracted by the presence of a functioning liver, allowing a survival of 72–96 hr without other treatment except saline injection described in methods. This protective effect of the liver was shortened in the absence of the adrenal glands. In the absence of the kidney the protective effect of the liver was shortened to 24–36 hr. The role of the kidney in the Evs − L + K rats was also diminished in the absence of the adrenal glands.

Summary. Sprague Dawley rats were adrenalectomized (Adx) and then eviscerated (Evs) 4 days later. Various surgical procedures were used during the evisceration to preserve or eliminate liver and/or kidney function. Non-Adx Evs rats were used as controls for each group. Abdominal aortic blood was used to measure: blood glucose (BG), urea nitrogen (BUN) and ketone bodies (KB) as well as plasma free fatty acids (FFA) and amino nitrogen (AN), 3–72 hr

after evisceration. All eviscerated rats lack insulin and with the liver *in situ* can maintain a hyperglycemia if the adrenals are present. Adx Evs rats do not develop this diabetic pattern. Furthermore, non Adx rats without a functional liver can sustain 80% of normal BG if the kidneys are *in situ* but Adx Evs rats require the liver as well. In the abence of the kidneys, protein metabolites are accumulated in the blood in both Adx and non Adx Evs animals.

FFA elevations occur in the Adx-Evs animal but are markedly lower than in the non-Adx preparation. The absence of insulin partially overrides the effect of Adx on this parameter.

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