Renal Gluconeogenesis in Eviscerated Diabetic Rats

(epinephrine/cortisol/lactic acid)

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ABSTRACT Experiments were carried out in eviscerated rats having intact kidneys to examine the effects on body glucose of some conditions known to stimulate overall gluconeogenesis. Preliminary experiments confirmed the enhancing effect of renal ligation on falling blood glucose concentration after evisceration. Adrenodemedullation of alloxan-diabetic rats did not affect blood glucose concentration, but comparison with completely adrenalectomized rats again supported previous conclusions that the adrenocortical hormones, in the presence of the kidneys, maintained the blood glucose at higher concentrations after evisceration. Calculated changes in total body glucose concentration over a 4-hr period after evisceration of several groups of alloxan-diabetic rats with intact kidneys showed that epinephrine, like cortisol, consistently reduced losses of total body glucose compared to the controls. An intravenous infusion of lactic acid so diminished the fall in total body glucose in diabetic rats that changes actually became positive, which indicated a net addition of glucose to the body fluids. Thus, the increased rate of disappearance of blood glucose that follows renal ligation in eviscerated animals results in large part from the interruption of renal gluconeogenesis, and the kidneys as well as the liver must be taken into account when considering the total glucogenic capacity of the body.

The rate at which blood glucose decreases after hepatectomy is often used as a measure of glucose utilization in the remaining tissues of the body, the muscle being the most important. The method requires the assumption that appreciable amounts of glucose no longer enter the circulation once liver function has been interrupted by ligation or excision. Because of the conflicting evidence regarding the effects of exogenous adrenocortical hormones on blood glucose in such animals, earlier work was carried out (1) in which alloxan-diabetic rats were functionally hepatectomized by an evisceration procedure (2), including ligation of the liver and kidneys and removal of the gastrointestinal tract. The results clearly showed that the well-known hyperglycemic effects of cortisol, such as those found in the adrenalectomized-diabetic whole rat (3), are in large part dependent upon the presence of the intact liver.

Nevertheless, some additional observations in similar rats whose kidneys were left unligated at evisceration suggested that the ensuing changes in total body glucose might also be influenced by renal factors. Since the kidney is the only organ other than the liver known to carry on gluconeogenesis

at all, and since the subjects' production of glucose was presumably increased by both insulin deficiency and cortisol excess, one possibility to be considered was that much of the excess body glucose found in renal-intact compared to renal-ligated eviscerated animals was the result of new glucose added to the body by the kidneys. It has been well established in various preparations, including eviscerated normal rats (4), that the kidneys can produce some glucose. Nevertheless, the extent of the renal contribution to either normal or abnormal carbohydrate metabolism as a whole is still unknown. In recent years Krebs (5) has been able to show remarkable increases in glucose production in renal cortex slices *in vitro* when the tissues were taken from rats that had been subjected to some conditions known to stimulate gluconeogenesis, notably low carbohydrate diet or exercise. Some of these results have now been confirmed in the isolated perfused rat kidney as well (6). On theoretical grounds it has been estimated that the two kidneys together may supply as much new glucose to the body as the liver itself (5). We have therefore observed changes in body glucose after evisceration of diabetic rats with intact kidneys under conditions that usually enhance the formation of new glucose in the intact animal.

METHODS

Male Sprague-Dawley rats were housed in individual cages in rooms having a temperature of approximately 25°C and an automatically operated 12-hr light-dark cycle. They received a diet of moderately high carbohydrate content and water *ad lib*.

Diabetic, adrenalectomized-diabetic, and adrenodemedullated-diabetic rats weighing 200-300 g were prepared for evisceration as follows.

Diabetes

Rats (200 ± 50 g) were anesthetized with ether after an overnight fast and severe diabetes was produced by an intravenous injection of 6 mg/100 g body weight of alloxan. Starting on the day after alloxan was injected, daily subcutaneous injections of 1-2 U of a long-acting insulin preparation were required to maintain the animals in good condition. Fast-acting (crystalline zinc) insulin (2-5 U) was substituted for long-acting insulin on the day preceding evisceration, adrenalectomy, or adrenodemedullation.

The group of diabetic rats that was prepared for evisceration received their crystalline-zinc insulin 18-24 hr before operation. The rats that failed to develop polydipsia, ketonuria, and weight loss after substitution of crystalline-

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zinc insulin were eliminated, while those with severe diabetes and various degrees of acidosis were selected for evisceration.

Adrenalectomy and diabetes

A second group of rats was adrenalectomized after the development of diabetes and was then maintained with daily subcutaneous injections of 0.5 mg of deoxycorticosterone in oil until evisceration 7–14 days later. They received no further insulin.

Adrenodemedullation and diabetes

A third group of diabetic rats underwent adrenodemedullation instead of total adrenalectomy. That is, one adrenal was removed completely; the other was enucleated and the capsule was returned to the retroperitoneal space. During the first week after adrenodemedullation, saline was substituted for water as a drinking fluid. The reinstitution of treatment with long-acting insulin became necessary after 1–2 weeks, however, because of the reappearance of glycosuria signalling functional regeneration of the cortical cells from the capsule. Evisceration was carried out 1 month after adrenodemedullation, when the diabetic hyperglycemia can be expected to be well maintained by such adrenal cortex autotransplants (3). Adrenodemedullated diabetic animals were then prepared and selected for evisceration in the same manner as the other diabetic rats.

Evisceration

On the day before evisceration, neomycin (1 g/l) was added to the rats' drinking water, but they continued to receive food until anesthetized. Rats were then eviscerated under pentobarbital anesthesia by a one-stage procedure (2). In this procedure, the blood supply to the viscera is ligated and all the organs of the digestive tract are removed except the liver which is left *in situ*, although it is completely isolated from the circulation. Similarly, the kidneys were ligated by placing the ligature on both renal pedicles and leaving the kidneys *in situ*. When solutions were to be infused intravenously, a polyethylene cannula containing saline and 200 U of an aqueous solution of heparin (Liquemin sodium, Organon) was placed in the femoral vein before evisceration.

After operation the animals were placed in a box where their temperature (measured rectally) was maintained at 37°C. Animals showing signs of hemorrhage or respiratory distress were discarded, for shock alters glucose utilization and lactic acid production peripherally (7), and anoxia and lowered blood pressure probably also have adverse effects on renal gluconeogenesis (8).

After evisceration all rats received 2 ml of 0.9% saline or other fluid per hour either subcutaneously or intravenously by means of an infusion pump. The effects of epinephrine were studied in several series of experiments in which 0.02 mg of epinephrine protected by glutathione was added to the hourly subcutaneous injections of saline. Glutathione in saline alone was injected into the control animals. Cortisol was administered at the time of evisceration as an injection of 5 mg of microcrystals of the free alcohol suspended in 1 of the 2 ml of saline injected subcutaneously. L-Lactic acid solution was given intravenously as a 2.7% solution $\frac{1}{4}$ neutralized by the addition of NaOH. The rate of lactic acid infusion was adjusted according to the amounts of endogenous lactic acid found in the blood after epinephrine treatment of eviscerated rats, as reported by Winternitz *et al.* (9).

FIG. 1. Effect of leaving the kidneys intact on the fall in blood glucose concentration after evisceration of adrenodemedullated diabetic rats. A, adrenodemedullated, kidneys ligated ($n = 10$); B, adrenodemedullated, kidneys intact ($n = 14$); C, adrenalectomized, kidneys intact ($n = 16$). Means $\pm$ SE.

Glucose in the blood and urine was analyzed as before (1) from samples taken from the tail and bladder at hourly intervals.

RESULTS

Effect of the kidneys

The fall in blood glucose concentration after evisceration of adrenodemedullated-diabetic rats is shown in Fig. 1. The findings were essentially the same as those previously described in diabetic rats having intact adrenals. The initial blood glucose was between 450–500 mg/100 ml and the subsequent pattern of response of the blood glucose to evisceration was determined by whether or not the kidneys were ligated along with the liver at evisceration. The rapid fall in blood glucose that occurred after combined hepatic and renal ligation is shown in curve A. When the experiment was continued longer, some of the animals actually died in hypoglycemic convulsions. On the other hand, if the kidneys remained intact after evisceration (curve B), the blood glucose concentration fell very little during the next 4 hr, which indicates that the net change in total body glucose remained small, especially when it is recalled that some glucose was lost in the urine. Provided there was no difference in peripheral utilization of glucose in the latter, the best explanation for these findings would be that new glucose was added to the circulation by the kidneys.

It can be shown, however, that leaving the kidneys intact does not invariably support the blood glucose concentration after evisceration. Fig. 1, curve C, confirms previously reported changes in blood glucose concentration after evisceration of diabetic rats from which the entire adrenal—cortex as

TABLE 1. *Effect of epinephrine on the change in blood glucose concentration of adrenalectomized-diabetic or adrenomedullated-diabetic rats after evisceration without ligation of the kidneys*

			Blood glucose (mg/100 ml)					
			No. rats	Initial	Decrease after evisceration (hr)			
Group		1			2	3	4	
<i>Adrenalectomized-diabetic</i>								
1	Control	16	493 ± 15	81 ± 10	173 ± 10	260 ± 16	360 ± 17	
2	Epinephrine	13	541 ± 29	121 ± 19	156 ± 15	222 ± 19	283 ± 19	
3	Epinephrine and cortisol	19	483 ± 14	67 ± 8	107 ± 7	137 ± 12	210 ± 11	
<i>Adrenodemedullated-diabetic</i>								
4	Control	14	474 ± 13	29 ± 11	81 ± 7	107 ± 9	100 ± 10	
5	Epinephrine	19	503 ± 13	44 ± 12	47 ± 6	52 ± 10	47 ± 10	

Values are means ± SE. Epinephrine: 0.02 mg/hr subcutaneously. Cortisol: 5 mg of microcrystalline free alcohol in 1 ml of saline subcutaneously at evisceration.

well as medulla—was removed. As reported before (1), even the presence of intact kidneys did not prevent the rapid decline in blood glucose in totally adrenalectomized-diabetic eviscerated rats. The difference between curve *B* and *C*, then, is due to the regenerated adrenal cortex. Since the rising concentration of amino acids in the plasma of eviscerated diabetic rats is known to be dependent on the adrenocortical hormones (10), the failure of the kidneys to form glucose after adrenalectomy might be explained by the failure of sufficient substrate to accumulate in the blood.

Epinephrine

Since exercise is one of the stimulants to gluconeogenesis that has been shown to stimulate the formation of glucose from renal cortex slices *in vitro*, changes in the blood glucose concentration were studied after epinephrine treatment of adrenalectomized or adrenomedullated-diabetic eviscerated rats. Table 1 shows that the fall in blood glucose concentration was less whenever epinephrine was given. In agreement with earlier work, treatment with cortisol further decreased the fall in blood glucose concentration after evisceration (1). The effect of the adrenocortical hormones is also apparent, of course, when adrenalectomized-diabetic controls (Group 1) are compared with those having an endogenous supply of hormones from regenerated adrenal cortices (Group 4).

Change in total body glucose after evisceration

A more quantitative estimate of the changes occurring in the glucose balance of diabetic rats after evisceration without renal ligation should, however, include the glucose that is excreted in the urine. Changes in total body glucose concentration can be calculated by dividing the change in blood glucose concentration over the experimental period by 4 to express it in mg/100 g body weight, and subtracting the total amount of glucose found in the urine during the same period, also expressed as mg/100 g body weight (1). Changes in the small amounts of glycogen stored by the renal cortex are considered negligible. The effect of epinephrine on total body glucose concentration so calculated is shown in Table 2. There was a reduction in loss of total glucose after evisceration when epinephrine was given to completely adrenalectomized-diabetic rats (Groups 1 and 2), and this reduction was accentuated by cortisol treatment at operation by about 26 mg/100 g. Changes in this range might be expected from ob-

serving the time course of the effect of cortisol alone in similar preparations. It has been found to be expressed by the formula: $y = -3.98 + 2.34x \pm 0.21$ in mg/100 g per hr (1); this predicts, after a 4-hr period, at least 22 mg/100 g more glucose attributable to the cortisol given.

The decrease in total body glucose of adrenomedullated-diabetic rats 4 hr after evisceration is only 18.5 mg/100 g, which most probably reflects the influence of endogenous adrenocortical hormones. Nevertheless, the addition of epinephrine again results in a significant reduction in glucose losses in the body after evisceration. In fact, the change was now positive (+1.5), which indicates that more glucose may actually have been added to the body fluids than was utilized.

Changes in total glucose in the body after epinephrine treatment of adrenalectomized or adrenomedullated-diabetic eviscerated rats minus the control changes should indicate the effect of the hormone itself. Taken together, therefore, the findings reported in Table 2 indicate that the effect of epinephrine in all cases was to decrease the loss of body glucose by approximately 20 mg/100 g over the 4-hr period after evisceration.

Lactic acid infusion

One explanation for the effect of epinephrine might be that the kidneys, like the liver, dispose of lactic acid entering the blood during anaerobic glycolysis in the peripheral tissue by converting it to glucose. Renal gluconeogenesis was therefore tested by the infusion of a partially neutralized 2.7% solution of lactic acid into severely diabetic eviscerated rats. The results (Table 3) show that infusion of lactic acid, like epinephrine, decreases the fall in blood glucose concentration as compared to simultaneously infused diabetic control rats receiving saline. The blood glucose concentration actually was elevated in many animals, and large amounts of glucose appeared in the urine. The calculated changes in total body glucose are shown in Table 2: it will be seen that lactic acid infusion results in a net gain in glucose in the body. The difference from the control value, moreover, representing the effect of the lactic acid, is approximately 32 mg/100 g over a 4-hr period of extra glucose added to the body fluids.

DISCUSSION

The present experiments were undertaken to determine what contribution renal gluconeogenesis might make to the changes

TABLE 2. *Calculated changes in body glucose concentration in diabetic rats without renal ligation*

	No. rats	Change in body glucose (mg/100 g)*	Difference from control
<i>Adrenalectomized (Table 1)</i>			
Control	16	-82.9 ± 4.6	—
Epinephrine	13	-65.5 ± 5.1	17.4
Epinephrine and cortisol	19	-39.3 ± 3.1	43.6
<i>Adrenomedullated (Table 1)</i>			
Control	14	-18.5 ± 2.8	—
Epinephrine	19	$+1.5 \pm 2.5$	20.0
<i>Eviscerated only (Table 3)</i>			
Saline-infused	10	-5.0 ± 4.0	—
Lactic acid-infused†	14	$+26.7 \pm 5.1$	31.7

Values are means $\pm$ SE.

* Adjusted to include loss of glucose in the urine.

† 75.5 mg/100 mg per 4 hr.

in blood glucose that follow evisceration. The secretion of some glucose by the kidneys has been noted in a variety of liverless animals in the past, for example, eviscerated rats (4, 11, 12), rabbits (13), cats (14), and hepatectomized dogs (15, 16). Reineke, moreover, attributed the prolonged survival of eviscerated normal rats having intact kidneys to the prevention of fatal hypoglycemia (4). Although such experiments did not appear to indicate any very significant role in the intact body for this extrahepatic source of glucose, our finding that, in eviscerated rats having kidneys, excess amounts of glucose are consistently associated with diabetes, cortisol treatment (1), or epinephrine treatment and with lactic acid infusion seem best explained in terms of higher rates of renal gluconeogenesis. This conclusion is borne out by evidence (5) of similar effects on glucose production from various precursors *in vitro* added to renal cortex slices taken from rats previously subjected to some of the other conditions known to predispose the whole animal to higher rates of gluconeogenesis, notably fasting, a low carbohydrate diet, and exercise.

When gluconeogenesis is stimulated by insulin deficiency and glucocorticoid excess in intact animals, hyperglycemia and glycosuria accompanied by negative nitrogen balance are prominent features of the syndromes that develop. Although hepatic gluconeogenesis is known to be increased, the role of the kidney in determining the extent of these disturbances is not yet clear. Nevertheless, it was found earlier (1) and confirmed here that both the adrenal cortex and the kidney are important for the maintenance of the blood glucose of diabetic rats after evisceration. Plasma concentrations of amino acids, which are among the substrates converted to glucose by both isolated perfused rat kidneys (6, 19) and renal cortex slices *in vitro* (5), rise rapidly after cortisol treatment of eviscerated adrenalectomized-diabetic rats (10). Possible states of increased activity of the enzyme systems carrying on gluconeogenesis in renal cortical tissue, moreover, have been indicated by the finding of both insulin-sensitive increases in renal gluconeogenesis in slices taken from diabetic rats (17) and stimulating effects of the glucocorticoids on glucose production (18) *in vitro*. For these reasons, it does not seem unlikely that some of the extra amino acids in the plasma of

TABLE 3. *Effect of intravenous lactic acid infusion on change in blood glucose concentration of diabetic rats after evisceration without ligation of the kidneys*

	No. rats	Blood glucose (mg/100 ml)				
		Initial	Decrease after evisceration (hr)			
			1	2	3	4
Saline	10	445 ± 13	54 ± 11	64 ± 21	86 ± 15	83 ± 6
Lactic acid*	14	391 ± 22	36 ± 17	14 ± 25	53 ± 29	53 ± 31

Values are means $\pm$ SE.

* 75.5 mg/100 g per 4 hr.

the adrenalectomized-diabetic eviscerated rat treated with cortisol may be converted to glucose and added to the circulation by the unligated kidneys.

Furthermore, the results presented here suggest that the Cori cycle continues to operate after evisceration when the kidneys are left unligated. Thus, one consequence of epinephrine treatment appeared to be an increase in the total body glucose in all groups tested. There are several possible sources for the extra glucose, but any effects epinephrine may have on the peripheral tissue utilization of glucose should be minimal in severely diabetic animals because those that have been described in eviscerated normal rats are apparently insulin-dependent (20). On the contrary, the finding (in an additional series of experiments) of excess glucose in the body after the infusion of lactic acid itself supports the view that the effects of epinephrine on body glucose were probably secondary to the mobilization of lactic acid from the peripheral tissues. Lactic acid can serve as a precursor of glucose in isolated perfused rat kidneys (6, 19) and during the perfusion of the kidneys in hepatectomized cats (14). Nor are these observations surprising in view of the behavior of renal cortex slices taken from (fasted) rats exposed to a medium containing lactate or pyruvate as a substrate (5).

Calculations which took urinary glucose into account indicated earlier that the kidneys of eviscerated rats when stimulated responded with increased amounts of glucose production in the same general range, on a weight basis, as the *in vitro* studies would predict. Application of similar methods of calculation to the values in Table 1 shows that the extra glucose after epinephrine treatment compared to control values amounts to approximately 5 mg/100 g per hr in either adrenomedullated or completely adrenalectomized eviscerated diabetic rats (Table 2). It is interesting to note that, in contrast to the liver, these results in the kidney do not reveal synergism, nor a permissive relationship between the adrenal cortical and medullary hormones (21, 22) on gluconeogenesis, but indicate instead that the maximum rates of glucose production previously ascribed to the kidneys were apparently increased further by epinephrine. Thus it was not surprising to find (Table 2) substantial additions of glucose to the body fluids during the infusion of lactic acid, amounting to a total of +27 mg/100 g in 4 hr. Table 2 shows that although, according to the present interpretation, renal gluconeogenesis was apparently already proceeding at an accelerated rate in the kidneys of severely diabetic rats used, 8 mg/100 g per hr more glucose was found after 4 hr of lactic acid infusion than in the controls. It would be difficult to explain a positive balance of

glucose of this magnitude in terms of the function of any extrahepatic tissue other than the kidney cortex.

There is reason to believe, then, that the kidney, particularly in diabetes or other states which enhance gluconeogenesis, can play an important role in the overall production of glucose in the body, and the rate of production of new glucose by the renal cortex may be determined, as has previously been suggested on the basis of findings in more isolated preparations, by the condition of the animal and the availability of various substrates.

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