Splanchnic glucagon kinetics in exercising alloxan-diabetic dogs

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Coker, Robert H., D. Brooks Lacy, Mahesh G. Krishna, and David H. Wasserman. Splanchnic glucagon kinetics in exercising alloxan-diabetic dogs. J. Appl. Physiol. 86(5): 1626–1631, 1999.—The purpose of this study was to define the relationship between arterial immunoreactive glucagon (IRG) and IRG that perfuses the liver via the portal vein during exercise in the diabetic state. Dogs underwent surgery >16 days before the experiment, at which time flow probes were implanted in the portal vein and the hepatic artery, and Silastic catheters were inserted in the carotid artery, portal vein, and hepatic vein for sampling. Dogs were made diabetic with alloxan injected intravenously ~3 wk before study (AD) or were studied in the nondiabetic state (ND). Each study consisted of a 30-min basal period and a 150-min moderate-exercise period on a treadmill. The findings from these studies indicate that the exercise-induced increment in portal vein IRG can be substantially greater in AD compared with ND, even when arterial and hepatic vein increments are not different. The larger IRG gradient from the portal vein to the systemic circulation in AD dogs is a function of a twofold greater increase in nonhepatic splanchnic IRG release and a fivefold greater hepatic fractional IRG extraction during exercise. In conclusion, during exercise, arterial IRG concentrations greatly underestimate the IRG levels to which the liver is exposed in ND, and this underestimation is considerably greater in dogs with poorly controlled diabetes.

METHODS

Animal care and surgical procedures. Experiments were performed in five AD (25.0 ± 1.0 kg) and six ND (24.0 ± 1.0 kg) dogs that had been fed a standard diet (Pedigree beef dinner, Waltham, Vernon, CA; and Wayne Lab Blox, Allied Mills, Chicago, IL): 51% carbohydrate, 31% protein, 11% fat, and 7% fiber on the basis of dry weight. The catecholamine data for four of the AD dogs included in this study have been published previously (1). The dogs were housed in a facility that met guidelines of the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Animal Care and Use Subcommittee. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg atropine and 15 mg/kg pentothal sodium presurgery, and 1.0% isoflurane inhalation during surgery). Silastic catheters (0.04-in. ID) were inserted in the portal vein and common hepatic vein. In addition, an incision in the neck region allowed the isolation of the carotid artery, into which a Silastic catheter (0.04-in. ID) was inserted and advanced to the aortic arch. After insertion, catheters were filled with saline that contained heparin (200 U/ml), and the free ends of the catheters were knotted. Ultrasonic transit-time flow probes (Transonic Systems, Ithaca, NY) were fitted and secured to the portal vein (1.0 ml/min resolution; relative accuracy ±2%) and hepatic artery (0.2 ml/min resolution; relative accuracy ±2%) for measurements of blood flow. Approximately 4 days after surgery, dogs that were to become diabetic were given a 65 mg/kg intravenous dose of alloxan (BDH Chemicals, Poole, UK) dissolved in a citrate buffer (pH 4.0). After glycospuria was detected, dogs were treated with regular and isophane (neutral protamine Hagedorn) pork insulin to keep glycospuria <1%. The last injection of intermediate-acting insulin was given 48 h before study, and the last injection of short-acting insulin was given 18 h before study. Dogs maintained on porcine insulin do not develop insulin antibodies (8).

At least 7 days after surgery, dogs were accustomed to running on a motorized treadmill. Dogs were not exercised 48 h before the experiment. Animals were used only if they had 1) a leukocyte count <18,000/mm³, 2) a hematocrit >36%, 3) a good appetite (consumption of daily food ration), and 4) normal stools.

The liver releases glucose into the circulation at an increased rate during exercise in the poorly controlled diabetic state despite the existing hyperglycemia (16, 17). The mechanisms that sustain endogenous glucose production rate of appearance (Rglyc) in the presence of hyperglycemia are not fully understood. However, there is evidence that immunoreactive glucagon (IRG) plays an important role (19). The role of IRG is difficult to assess, because arterial IRG levels do not provide an accurate assessment of IRG concentrations in hepatic sinusoidal blood, inasmuch as ~80% of hepatic delivery is via the portal vein (18). The portal vein is also the first vessel that coalesces with the pancreatic vein. For this reason, small alterations in the arterial concentrations of IRG may be associated with much larger changes in the portal vein concentration of IRG (7, 18). The portal vein is the conduit between the pancreas and the liver and, although this is an efficient anatomic arrangement, it makes the IRG concentration at the liver extremely difficult to assess in conscious humans, because this vein is largely inaccessible. Therefore, the use of an animal model that permits portal vein catheterization is necessary to measure the concentration of IRG at the liver (18). The purpose of this study was to assess the relationship of arterial to portal vein IRG concentration and to determine the mechanisms (hepatic fractional IRG extraction and nonhepatic splanchnic IRG release [NSGR]) that distinguish alloxan-diabetic dogs (AD) from nondiabetic dogs (ND) during rest and exercise.

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Experimental procedures. All studies were conducted in dogs after an 18-h fast. The catheter ends and Doppler leads were accessed through small skin incisions made under local anesthesia (2% lidocaine; Astra Pharmaceutica Products, Worcester, MA) immediately before experiment. The contents of the exposed catheters were then aspirated, and the catheters were flushed with saline, connected to Silastic tubing, and secured to the back of the dog with quick-drying glue. The protocol consisted of a basal period (−30 to 0 min) and a moderate exercise period (0–150 min). Exercise was performed on a motorized treadmill at a speed of 4 miles/h at a 12% grade.

Blood-sample collection and analysis. Blood samples were drawn from the carotid artery, hepatic vein, and portal vein at t = −30 and 0 min during the basal period and at t = 50, 100, and 150 min during the exercise period to assess splanchnic insulin, glucagon, and other hormonal factors. Immunoassays for insulin, glucagon, and IRG were performed using specific antibodies against each hormone. The interassay coefficient of variation (CV) was 10%. Plasma glucose concentrations were determined by using the glucose oxidase method, and with the use of a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Glucagon and insulin antisera were obtained from Dr. R. L. Gingerich (Washington University School of Medicine, St. Louis, MO), and the standard glucagon and 125I-glucagon were obtained from Linco Research (St. Louis, MO).

Calculated metatarsus. The equations described below were used to calculate the portal vein-to-arterial IRG gradient, net NSGR, hepatic hormone delivery, estimated sinusoidal IRG levels, net hepatic fractional IRG extraction, and net hepatic IRG uptake, such that the arterial, portal vein, and hepatic vein IRG concentrations ([IRG]a, [IRG]pv, and [IRG]hv, respectively) are as found in the following calculations. HAF and PVF are the hepatic artery and portal vein blood flows.

The portal vein-to-arterial IRG gradient was calculated by using the equation
\[
[IRG]_a - [IRG]_p
\]

(1) NSGR was calculated by using the equation
\[
([IRG]_a - [IRG]_p) \times PVF
\]

(2) Hepatic IRG delivery was calculated by using the equation
\[
([IRG]_a \times HAF) + ([IRG]_p \times PVF)
\]

(3) Estimated sinusoidal IRG levels were calculated by using the equation
\[
[IRG]_a \times HAF/[HAF + PVF] + [IRG]_p \times PVF/[HAF - PVF]
\]

(4) Net hepatic fractional IRG extraction was calculated by using the equation
\[
([IRG]_a \times HAF/[HAF + PVF] + [IRG]_p \\
\times PVF/[HAF + PVF]) - ([IRG]_a/[IRG]_p) + ([IRG]_a \times PVF/[HAF + PVF])
\]

(5) Net hepatic IRG uptake was calculated by using the equation
\[
E_3 \times E_5
\]

(6) IRG uptake was determined by using the two-compartment approach described by Mari (10) during a constant-rate infusion (0.30 μCi/min) of radioactive glucose ([3-3H]glucose; NEN, Boston, MA).

RESULTS

Arterial, portal vein, hepatic vein, and estimated hepatic sinusoidal IRG concentrations, and the portal vein-to-arterial IRG gradient. Arterial IRG was similar in AD (63 ± 10 pg/ml) and ND (48 ± 5 pg/ml) during the basal period. Arterial IRG was significantly higher in AD compared with ND at t = 150 min (83 ± 12 vs. 59 ± 3 pg/ml, respectively) during exercise. However, the exercise-induced increment in arterial IRG was not different between AD and ND at t = 150 min (20 ± 11 vs. 11 ± 5 pg/ml, respectively). Basal portal vein IRG was higher in AD compared with ND (86 ± 16 vs. 58 ± 6 pg/ml, respectively; P < 0.05). Portal vein IRG was ~2.5-fold greater in AD compared with ND at t = 150 min (233 ± 48 vs. 95 ± 7 pg/ml, respectively; P < 0.05) during exercise. In addition, the exercise increment in portal vein IRG was significantly greater in AD compared with ND at t = 150 min (147 ± 54 vs. 37 ± 13 pg/ml, respectively). Although hepatic vein IRG was not significantly different between AD and ND during the basal period (74 ± 16 vs. 52 ± 6 pg/ml, respectively), hepatic vein IRG was higher in AD compared with ND in response to exercise (117 ± 15 vs. 83 ± 6 pg/ml, respectively; P < 0.05). The exercise-induced increment in hepatic vein IRG levels was not different between AD and ND at t = 150 min (43 ± 15 vs. 31 ± 10 pg/ml, respectively). Estimated hepatic sinusoidal IRG was higher in AD compared with ND during the basal period (81 ± 18 vs. 54 ± 6 pg/ml, respectively; P < 0.05). During exercise, hepatic sinusoidal IRG was also markedly higher in AD compared with ND at t = 150 min (199 ± 42 vs. 86 ± 7 pg/ml, respectively; P < 0.05; Table 1). The portal vein-to-arterial IRG gradient was higher in AD compared with ND during the basal period (23 ± 11 vs. 7 ± 2 pg/ml, respectively; P < 0.05). This difference was increased by exercise, as the portal vein-to-arterial IRG gradient was about four-fold higher at t = 150 min in AD compared with ND (151 ± 37 vs. 37 ± 6 pg/ml, respectively; P < 0.05; Fig. 2).

Net hepatic IRG uptake, net hepatic fractional IRG extraction, and NSGR. Net hepatic IRG uptake was similar in AD and ND during the basal period (0.2 ± 0.1 vs. 0.1 ± 0.1 ng·kg⁻¹·min⁻¹) but rose in AD compared with ND at 150 min of exercise (1.7 ± 0.6 vs. 0.3 ± 0.1 ng·kg⁻¹·min⁻¹, respectively; P < 0.05; Fig. 3). Net hepatic fractional IRG extraction increased (P < 0.05) from 0.08 ± 0.02 during the basal period to 0.35 ± 0.07 at 150 min of exercise in AD. In contrast, hepatic fractional IRG extraction was not significantly affected...
by exercise in ND (Fig. 3). NSGR was greater in AD compared with ND during the basal period (0.5 ± 0.3 vs. 0.1 ± 0.0 ng·kg⁻¹·min⁻¹; P < 0.05). NSGR was also greater in AD compared with ND at t = 150 min during exercise (1.4 ± 0.4 vs. 0.5 ± 0.1 ng·kg⁻¹·min⁻¹, respectively; P < 0.05; Fig. 2).

Arterial, portal vein, hepatic vein, and estimated hepatic sinusoidal IRI concentrations. Basal arterial IRI was lower in AD compared with ND (5 ± 1 vs. 10 ± 1 µU/ml, respectively; P < 0.05). Arterial IRI was similar in AD and ND (4 ± 1 vs. 5 ± 1 µU/ml) at 150 min of exercise. Basal portal vein IRI was lower in AD compared with ND (9 ± 1 vs. 22 ± 3 µU/ml, respectively; P < 0.05). Portal vein IRI was similar in AD and ND at t = 150 min during exercise (15 ± 3 vs. 17 ± 3 µU/ml, respectively). During the basal period, hepatic vein IRI was lower in AD compared with ND (6 ± 1 vs. 14 ± 2 µU/ml, respectively; P < 0.05). Hepatic vein IRI was lower at t = 150 min during exercise in AD compared with ND (4 ± 1 vs. 7 ± 1 µU/ml, respectively; P < 0.05; Fig. 4). Estimated basal hepatic sinusoidal insulin was much lower in AD compared with ND (9 ± 2 vs. 18 ± 2 µU/ml, respectively; P < 0.05). At 150 min of exercise, estimated hepatic sinusoidal insulin rose by 4 µU/ml in AD and fell by 4 µU/ml in ND (Table 1). As a result, estimated hepatic sinusoidal IRI was not different during exercise in the two groups.

Blood-flow measurements. Hepatic artery and portal vein blood flows were similar during the basal and exercise periods in AD and ND (Table 2).

Arterial glucagon concentrations and kinetics. Arterial glucagon concentrations were approximately threefold higher in AD compared with ND during the basal period (P < 0.05). Ra was higher in AD compared with ND during the basal period (P < 0.05). Ra was lower at 150 min of exercise in AD than in ND (P < 0.05) (Table 3).

Table 1. Hepatic sinusoidal IRI and IRG concentrations during rest and 150 min of moderate-intensity exercise in nondiabetic and alloxan-diabetic dogs

<table>
<thead>
<tr>
<th>Condition of Dogs</th>
<th>n</th>
<th>Basal</th>
<th>Moderate Exercise</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 min</td>
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<tr>
<td>Hepatic sinusoidal IRI, µU/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>18 ± 2</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>9 ± 2†</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Hepatic sinusoidal IRG, pg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>54 ± 6</td>
<td>75 ± 5†</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>81 ± 18*</td>
<td>137 ± 30†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of dogs. IRI, immunoreactive insulin; IRG, immunoreactive glucagon. *Difference between 2 groups, P < 0.05; † difference from basal period, P < 0.05.
DISCUSSION

The results of this study demonstrate that the exercise-induced increment in portal vein IRG is greater in AD compared with ND, even at a time when the increment in arterial IRG is not significantly different. The greater portal vein increment during exercise in AD was due to an approximately twofold greater increase in NSGR in AD. Previous studies have reported elevated concentrations of IRG in the portal vein compared with the peripheral circulation at rest (5) and that this difference is exaggerated during exercise (18). The present study provides evidence that the portal vein-to-arterial IRG gradient increases with exercise in poorly controlled diabetes, and this increase is substantially greater than that in the normal healthy state. The reason that the arterial increment in IRG was similar in both groups was that hepatic fractional IRG extraction was increased in AD compared with ND. This served to dampen increases in the IRG concentration exiting the liver (i.e., hepatic vein IRG concentration).

The mechanism behind the exercise-induced increase in IRG release may relate to increased sympathetic drive to the pancreas. Studies from our laboratory have shown that sympathetic drive to nonhepatic splanchnic tissue is increased to a greater extent with exercise in AD (2). The increase in sympathetic drive is evidenced by elevations in portal vein norepinephrine and a threefold increase in nonhepatic splanchnic norepinephrine spillover in AD dogs compared with normal dogs during exercise (2). Prior studies have supported the possible influence of adrenergic stimulation on pancreatic IRG secretion in normal animals (15). First, it has been shown that α- and/or β-adrenergic stimulation increases IRG release from pancreatic α-cells (13). Second, sectioning the splanchnic nerve

Table 2. Blood-flow measurements during rest and 150 min of moderate-intensity exercise in nondiabetic and alloxan-diabetic dogs

<table>
<thead>
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<th>Condition of Dogs</th>
<th>n</th>
<th>Basal</th>
<th>Moderate Exercise</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>50 min</td>
<td>100 min</td>
</tr>
<tr>
<td>Portal vein blood flow, ml·kg⁻¹·min⁻¹</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>25±2</td>
<td>18±1†</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>26±3</td>
<td>19±1†</td>
</tr>
<tr>
<td>Hepatic artery blood flow, ml·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>6±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>7±1</td>
<td>7±1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of dogs. †Difference from basal period, P < 0.05.
has been shown to result in reductions in the exercise-induced IRG response in the dog (3). Last, adrenergic blockade can attenuate the increment in arterial IRG during exercise in rats (9).

Although basal arterial IRG levels in the 18-h-fasted dog range from ~20 to 60 mg/ml under our assay conditions, the concentration of the 3,500-molecular-weight glucagon level is less (~10–50 mg/ml). This difference is due to the use of antibodies that crossreact with proteins other than glucagon. The crossreactivity is generally accepted, since physiological changes in IRG are thought to primarily reflect alterations in the level of 3,500-molecular-weight glucagon (18). It is important to note that the hepatic fractional extraction of 3,500-molecular-weight glucagon may be underestimated from measurements of IRG because of the presence of larger peptides that crossreact with the 3,500-molecular-weight glucagon (7). The liver extracts the 3,500-molecular-weight component more effectively than the other glucagon-like immunoreactive proteins which comprise the total IRG (6). The presence of IRG fractions other than the 3,500-molecular-weight component in the peripheral circulation may explain why net hepatic fractional IRG extraction in ND is slightly lower in our study than in those that have measured the 3,500-molecular-weight-glucagon fraction specifically (7). Our data are similar to those from Ishida et al. (4), who showed that the hepatic fractional extraction of total IRG ranges from 10 to 20% in the dog.

Exercise resulted in a threefold increase in net hepatic fractional IRG extraction in AD. In contrast, fractional extraction was only negligibly increased in ND. It has been shown that the 3,500-mol-wt IRG is more readily increased by exercise than are the larger IRG fractions that comprise the remainder of the immunoreactivity (5). Because the 3,500-molecular-weight IRG is extracted to a greater extent by the liver compared with the larger IRG fractions (6), hepatic fractional IRG extraction may increase simply because the composition of the IRG delivered to the liver was changed and not because the control of IRG uptake directly at the liver was changed. One may hypothesize, therefore, that the increase in hepatic fractional IRG extraction during exercise in AD is related to a primary and selective increase in the secretion and hepatic delivery of the 3,500-molecular-weight fraction during exercise. One may further speculate that the hepatic fractional IRG extraction was not greatly influenced by exercise in ND, because the increase in IRG, specifically the 3,500-molecular-weight IRG, was less. It is unlikely that an increase in hepatic IRG delivery, without a change in the composition of IRG, affects fractional extraction. When the hepatic delivery of IRG was increased experimentally, without altering IRG composition, the hepatic fractional extraction of IRG did not increase (7). One cannot rule out that there is a specific effect of exercise on the extraction of glucagon by the liver in diabetic dogs. However, we have shown that hepatic fractional extraction of IRG can increase with exercise, even in ND dogs, when hepatic IRG delivery, and presumably the percentage of IRG that was 3,500 molecular weight, was increased to a greater extent (18).

Arterial IRI in ND falls gradually during exercise. On the other hand, AD dogs had no further reduction in the already low arterial IRI levels in response to exercise. Although arterial IRI did not change in AD dogs, there was an exercise-induced increase in portal vein IRI levels. The reason for the paradoxical increase in portal vein insulin is unclear. α-Adrenergic stimulation suppresses insulin secretion, and β-adrenergic stimulation increases insulin secretion (13). One could hypothesize that α-adrenergic mechanisms are impaired in AD, isolating β-adrenergic effects. The fact that arterial levels are constant at a time when portal vein IRI levels are increased also suggests that net fractional IRI extraction is increased during exercise in dogs with poorly controlled diabetes.

In the present study, the increased IRG concentration at the liver in AD actually resulted in a lower rise in Ra compared with ND. The prevailing hyperglycemia (12) and the elevation in portal vein IRI (14) in AD most likely restrained the stimulatory effect of the larger increment in portal vein IRG on Ra. The exercise-induced responses of norepinephrine (2, 19) and cortisol (19), like portal vein IRG, are also exaggerated during exercise and may work to stimulate hepatic glucose production in exercising AD dogs.

In summary, this study demonstrates that the exercise-induced increase in portal vein IRG is greater in AD than in ND dogs, even though the increments in arterial and hepatic vein IRG are similar in the two groups. This increase in portal vein relative to arterial and hepatic vein IRG in AD dogs is due to concomitant increases in NSGR and net hepatic fractional IRG extraction. An increase in sympathetic drive to the pancreatic α-cells could be the cause of the exaggerated IRG secretion and increased portal vein IRG concentration in AD during exercise (13). The elevated exercise-induced increments in portal vein IRG in the poorly controlled diabetic state are likely to contribute to the stimulation of glucose production, despite the existing hyperglycemia. Finally, these studies emphasize the need to be cautious when the hepatic effects of IRG are extrapolated from arterial IRG measurements.

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Table 3. Arterial glucose concentration and endogenous glucose production during rest and 150 min of moderate-intensity exercise in nondiabetic and alloxan-diabetic dogs

<table>
<thead>
<tr>
<th>Condition of Dogs</th>
<th>n</th>
<th>Basal</th>
<th>50 min</th>
<th>100 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial glucose concentration, mg/dl</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>108 ± 4</td>
<td>112 ± 4</td>
<td>116 ± 4</td>
<td>119 ± 4†</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>349 ± 25*</td>
<td>360 ± 33*</td>
<td>360 ± 33*</td>
<td>382 ± 33*</td>
</tr>
<tr>
<td>Endogenous glucose production, mg·kg⁻¹·min⁻¹</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>6</td>
<td>3.3 ± 0.3</td>
<td>8.1 ± 1.8†</td>
<td>7.8 ± 1.6†</td>
<td>8.9 ± 1.7†</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>5</td>
<td>3.7 ± 0.6</td>
<td>6.4 ± 0.9†</td>
<td>6.0 ± 0.9†</td>
<td>5.6 ± 0.7††</td>
</tr>
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</table>

Values are means ± SE; n, no. of dogs. *Difference between 2 groups, P < 0.05; †difference from basal period, P < 0.05.
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