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Effects of glucagon, glucose, adrenaline and insulin infusion on blood glucose level in the common African toad (*bufo regularis*)

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Summary

Male toads, weighing 70-123 g, were divided into 13 groups with 8 toad in each group. Animals in each group were fasted overnight before the experiments. Toads in groups 1 to 4 were infused for 30 minutes with adrenaline, 5 ugkg^{-1} min^{-1}, glucose, 5.5 mg kg^{-1} min^{-1}; glucagon 2 ugkg^{-1} min^{-1}; and insulin 2000uU kg^{-1} min^{-1}, respectively. Blood samples for blood glucose measurement were taken before, during and after each infusion. The experiment was repeated in groups 5 and 6 using 3.5 mg kg^{-1} min^{-1} and 7.5 mg kg^{-1} min^{-1} of glucose respectively. Toads in groups 7 to 9 were pretreated with prazosin, 0.2 mg/kg and those in groups 10 to 12 were pretreated with propranolol, 0.5 mg/kg. After pretreatment, glucose, glucagon and insulin infusions were repeated in the alpha-blocked and beta-blocked toads, respectively. Group 13 was infused with 0.7% saline and served as the control. The results showed that infusions of adrenaline, glucose and glucagon resulted in significant hyperglycaemia while insulin caused hypoglycaemia. The hyperglycaemic response to glucose was dose-dependent. The experiments using blockers showed that the glycaemic effects of glucagon and insulin are mediated via beta adrenoceptors, that for glucose is via alpha adrenoceptors and from an earlier study, the glycaemic response of adrenaline is through both alpha and beta adrenoceptors.

Keywords: *Glucagon, glucose, adrenaline, insulin, common African toad.*

Résumé

Les crapauds male, peasant 70-123g ont été divisé en 13 groupes ayant 8 crapauds par groupe. Les animaux dans chaque groupe ont été maintenuent à jeun pendant une nuit ayant les experiences. Les crapauds dans les groupes 1 à 4 ont été infusé pendant 30 minutes avec l'adrenaline, 5 ugkg^{-1} min^{-1}, les glucose 5.5 mg kg^{-1} min^{-1}, glucagon 2 ugkg^{-1} min^{-1}, et l'insuline 2000U kg^{-1} min^{-1} de manière retrospective. Les specimens sanguin pour la mesure du taux de glucose ont été prise avant, durant et après chaque infusion. Les experiences ont été repétés dans des groupes de 5 et 6 crapuds en utilisant 3.5 mg kg^{-1} min^{-1} et 7.5 mg kg^{-1} min^{-1} de glucose respectivement. Les crapauds dans les groupes 7 à 9 ont été pré-traité avec la prozasin (0, 2mg/kg) et ceux des groupes 10 à 12 avec la propanolol (0,5mg/kg). Après le pre-traitement, les infusions du glucose, glucagon et insuline ont été repéte dans les crapauds alpha-blocqu et beta-bloqu respectivement. Les animaux du groupe 13 ont été infusè avec 0.7% de saline normale et ont servit de controle. Le resultats montrent que les infusions d'adrenaline, glucose et glucagon ont resulté à une hyperglycemic significative, alorsque l'insuline a causé une hypoglycemie. La response hyperglycemique au glucose a été dependante de la dose. Les experiences utilisant les bocqueurs montrent que l'effet glycemique du glucagon et de l'insuline sont medié par les adrenorecepteurs beta, et ceux du glucose via les adrenorecepeurs alpha. Les resultants d'autres études preliminaires ont aussi montré que la response glycemique à l'adrenlaine se fait à travers les adrenoreceptors alpha et beta.

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Introduction

Several workers have studied blood glucose levels in amphibians and have recorded values lower than those in mammals. Reports on blood glucose levels in frogs show wide species variation. Thus, fasting blood glucose levels were found to be 38 mg/dl in *rana temporaria* [1], 10 mg/dl in *rana catesbiana* [2], 50-70 mg/dl in *rana pipiens* [3], 40-50 mg/dl in *rana clamitans* [4], 94 mg/dl in *bufo regularis* [5] and sometimes totally absent (zero) in *bufo bufo* and *rana catesbiana* [7]. Although metabolism in amphibians does not depend upon closely regulated blood glucose levels [8], some regulation of amphibian blood glucose does occur and most of the hormones involved in the regulation in mammalian carbohydrate metabolism have been shown to be active in amphibians [9].

Previous studies have reported the effect of adrenaline [4,10,11,12,13] glucagon, [7,11,13] glucose [4,7], and insulin [14] on blood glucose levels in various species of frogs. However, there has been no study of the effects of glucagon, glucose or insulin on the blood glucose level in the common African toad, *Bufo regularis*. Also, there has been no study, as far as we know, in which the effects of glucagon, adrenaline, insulin and glucose were compared simultaneously in the same species of frog or toad. The present study was therefore carried out to investigate the effects of infusions of glucagon, glucose, insulin and adrenaline on blood glucose level in the common African toad, *Bufo regularis*. Since adrenoceptors have been found to play a role in the glycaemic effects of these four substances in mammals, [15] the role of adrenergic receptors in the observed responses in the toad will also be investigated.

Materials and methods

Experiments were carried out on adult male toads weighing 70-123 g. Toads were collected randomly as ealier described [16]. Each animal was fasted 24-48 hours and then anaesthetized with sodium pentobarbitone, 3.0 mg 100^{-1}g given intraperioteneally (ip). A fine cannula was introduced into a brachial artery and advanced into the truncus arterious and secured with a ligature. The anterior abdominal vein was also cannulated. The arterial and venous cannulae were for blood sampling and drug infusion, respectively. Fluidity of blood was maintained by i.v. injection of heparin.

Core body temperature was recorded in all toads by means of a Type RM 4 thermocouple probe and TE-3

electrical thermometer (Electorlaboratoriet, DK). The thermocouple was inserted about 2.0 cm deep into the cloaca of each toad. Body temperature was thereafter maintained within ± 1.0 ^{0}C of the initial value with the aid of intermittent heating by means of a table lamp fitted with a 100 watt bulb. Infusion of glucose and hormones was by means of an infusion/withdrawal pump-type Truth-All (Japan) at a rate of 0.26 ml hr^{-1}. In 40 of the toads, blood pressure (BP), heart rate (HR) and haematocrit (Hct) were measured. Blood pressure measurement was via a BP transducer connected to the arterial cannula, while HR was from ECG recorded via needle electrodes connected to the limbs of the toad.

The animals were divided into 13 groups of eight toads per group. In each animal, basal blood sample (0.05 ml per sample) was drawn directly into a micropipette from the cannula placed in the truncus arteriosus. Each sample was immediately transferred into 2.95 ml of protein precipitant and mixed thoroughly. Blood glucose was later estimated using a modified glucose oxidase method [17]. After basal blood sample collection, toads (untreated) in groups 1,2,3 and 4 were given a 30 min. i.v. infusion of glucose, 5.5 mg kg^{-1} min^{-1}, adrenaline 5 ug kg^{-1} min^{-1}, glucagon (sigma, USA) 2 ug kg^{-1} min^{-1} and insulin (Novo Lab., DK) 2000 uU kg^{-1} min^{-1}. Each infusion was in a total volume of 0.13 ml given in 30 min. Blood samples for glucose estimation (0.05 ml per sample) were taken at 5 min, 10 min, 15 min, 20 min, 25 min and 30 min, (during infusion) as well as 45 min, 60 min and 90 min post-infusion. After each sample, 0.7% saline (0.05 ml) was injected into the animal to maintain blood volume.

Toads in groups 5 and 6 were infused with 3.5 mg kg^{-1} min^{-1} and 7.5 mg kg^{-1} min^{-1} of glucose respectively, to assess the effect of glucose load on the glycaemic response.

Toads in groups 7 and 8 were pretreated with bolus i.v. injection of prazosin, 0.2 mg kg^{-1}. After allowing 30 min for the drug to take effect, the animals were given an i.v. infusion of glucose, glucagon and insulin, respectively, as in the untreated groups. Toads in groups 10, 11 and 12 were also pretreated with bolus injection of propranolol, 0.5 mg kg^{-1} i.v. Thirty minutes was also allowed for the drug to take effect and thereafter the animals were given i.v. infusion of glucose, glucagon and insulin, respectively, as in untreated groups. Toads in group 13 were infused i.v. with 0.7% NaCl and served as controls. In groups 5 to 13, sample collections and glucose estimation were repeated as in groups 1 to 4. Effects of blockers on the response to adrenaline was not included in the present study since this was reported recently from our laboratory [6].

The mean blood glucose level in all the groups studied was calculated and paired t-test was applied to assess the statistical significance of differences [18]. *P* values of 0.05 or less was taken as statistically significant.

Results

The results are shown in Tables 1, 2 and 3 and in Figures 1 to 4. Table 1 shows the body weight, temperature and fasting blood glucose in 104 toads as well as the haematocrit, blood pressure and heart rate in 40 toads.

Table 1: Some basal physiological values recorded in the toad, *Bufo regularis*

	Range	Mean ± S.E.
Body weight (gm)*	70 - 123	87.4 ± 19.3
Temperature (^{0} C)*	24.1 – 27.9	26.5 ± 1.1
Packed cell volume (%)**	40.0 – 48.5	46.5 ± 4.2
Blood Prressure (mmHg)		
(i) Systolic	53 - 60	56.4 ± 2.6
(ii) Diastolic	24 – 38	32.7 ± 6.0
Heart rate (per min)**	60 – 78	68.0 ± 5.6
Fasting blood gulocse (mg/dl)*	61.2 – 118.5	87.4 ± 11.3

(*) indicates n = 104
(**) indicates n = 40

Table 2: Blood glucose levels (mg/dl; Mean ± SE) in toads infused with adrenaline, glucagon, insulin, glucose and 0.7% saline

	TIME (MIN)							
Infusion	0"	5"	10"	15"	30"	45"	60"	90"
Adrenaline	96.6	104.5	112.2**	125.9**	135.0**	129.7**	132.3**	126.0**
5 ug kg^{-1} min^{-1}	± 4.0	± 7.3	± 6.8	± 2.0	± 5.6	± 4.1	± 8.1	± 7.5
Glucagon	79.1	91.9*	98.0**	125.7**	110.9**	93.4*	87.4	84.4
2 ug kg^{-1} min^{-1}	± 6.9	± 5.4	± 5.2	± 5.4	± 7.4	± 4.4	± 5.2	± 2.5
Insulin	88.0	85.4	74.6	72.1*	65.8**	64.4**	60.2**	54.8**
200 uU kg^{-1} min^{-1}	± 7.0	± 4.5	± 7.2	± 4.6	± 6.7	± 6.1	± 5.3	± 6.6
Glucose	65.2	91.4	101.3*	106.0**	119.7**	137.1**	133.7**	131.4**
5.5 mg/kg/min	± 3.1	± 5.0	± 4.9	± 6.8	± 3.7	± 5.1	± 4.8	± 8.2
Saline	87.2	84.4	85.1	91.5	80.6	81.8	80.6	82.5
0.7% NaCl	± 3.9	± 5.9	± 5.9	± 4.6	± 9.0	± 5.5	± 6.2	± 4.6

Note: Asterisk (s) indicate level of significance; *P* values; * = 0.05; ** 0.025; n= 8 for each Infusion.

Table 3. Effect of graded doses of glucose infusions and saline on blood glucose levels (mg/dl; Mean ± SE) in toads

	TIME (MIN)						
Substance Infused	0"	5"	10"	15"	30"	45"	60"
Glucose 7.5 mg kg^{-1} min^{-1}	89.3 ± 7.7	101.7** ± 7.6	143.0** ± 7.6	105.1** ± 8.6	185.7** ± 9.1	195.4** ± 9.9	194.6 ± 11.2
Glucose 7.5 mg kg^{-1} min^{-1}	85.2 ± 3.1	91.4 ± 5.0	106.0** ± 6.8	119.7** ± 3.7	137.1** ± 5.1	133.7** ± 4.8	131.4** ± 8.2
Glucose 3.5 mg kg^{-1} min^{-1}	80.1 ± 1.4	81.0 ± 2.9	90.1** ± 3.7	104.3** ± 5.8	97.1** ± 2.7	96.7** ± 4.9	95.3** ± 3.0
Saline 0.7% NaCl	85.2 ± 3.9	84.4 ± 5.9	86.5 ± 4.6	80.6 ± 5.0	81.8 ± 5.5	82.3 ± 6.2	82.5 ± 4.6

Note. Asterisk(s) indicate level of significance; P values; * = 0.05; ** 0.025; n = 8 for each Infusion.

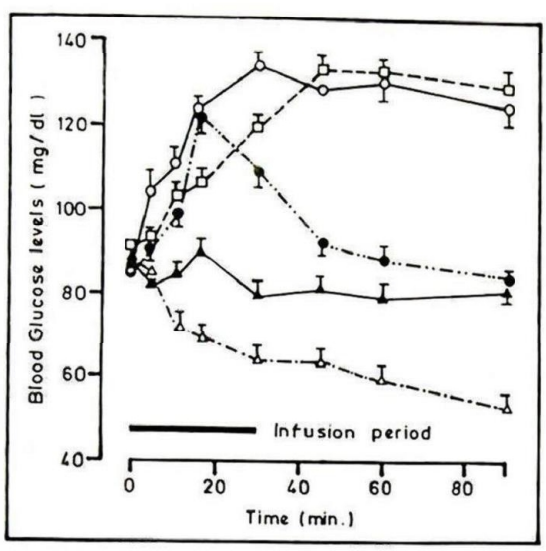

Fig. 1 Effects of infusions of adrenaline (O——O), glucose (□——□), glucagon (●— —●), insulin (Δ— —Δ) and 0.7% saline (▲——▲) on blood glucose levels in *bufo regularis*.

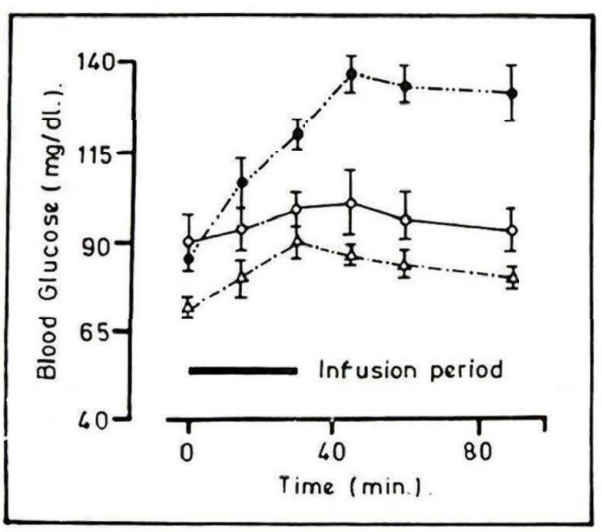

Fig. 2 The effects of glucose infusion (●---●), the pretreatment with pruzosin (O——O) and propranolol (Δ---Δ) before glucose levels in *bufo regularis*

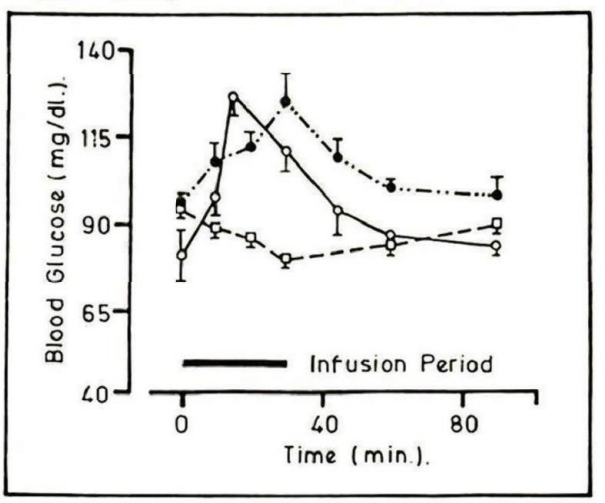

Fig 3: The effects of glucagon infusion (O——O) and pretreatment with pruzosin (●— —●) and propranolol (□— —□) before glucagon infusion on blood glucose levels in *bufo regularis*

Effects of glucose, glucagon, adrenaline and insulin

The mean (± SE) fasting glucose level in the toad, *bufo regularis*, was 87.4 ± 11.3 mg dl^{-1}. Infusion of glucose (5.5 mg kg^{-1} min^{-1}), glucagon or adrenaline caused a significant increase in blood glucose level (Table 2 and Fig. 1). In the glucagon experiments, the blood glucose level had increased to a significant level at 5 min of infusion, reached a peak at 15 min and between 15 and 30 minutes while the infusion was still on, blood glucose level had started to decrease. At 30 min post-infusion, blood glucose had returned to basal level. Adrenaline and glucose-induced hyperglycaemia reached their peaks at 10 min and 45 min, respectively, and blood glucose remained elevated and did not show a decrease throughout the post-infusion period.

Insulin caused a significant decrease in blood glucose level. Insulin-induced hypoglycaemia became significant at 15 min of infusion (Table 2) and hypoglycaemia was progressive throughout the post-infusion observation period.

Effect of graded doses of glucose

The three doses of glucose infused produced significant increases in blood glucose in a dose-dependent manner (Table 3). The low, medium and high doses produced peak increase of blood glucose levels of 30%, 61% and 120% respectively, and these increases occurred 30 min, 45 min and 60 min into the experiment, respectively (Table 3). Blood glucose remained significantly elevated and did not

decrease throughout the 60 min post-infusion observation period at the three dose levels.

Effects of Propranolol and prazosin

These are shown in Figures 2, 3 and 4. The hyperglycaemic response to glucose infusion was abolished by prazosin. After treatment with prazosin, glucose infusion produced a mere 12% insignificant increase in blood glucose level. Propranolol produced a marked reduction, but did not abolish the hyperglycaemic response to glucose infusion. Thus, glucose infusion after propranolol treatment caused a peak 25% increase in blood glucose level as against a 61% increase in the untreated group.

Propranolol totally abolished the hyperglycaemic response to glucagon infusion (Figure 3). Prazosin delayed the attainment of the peak hyperglycaemic response to glucagon by 10 min and the peak response was reduced from 62% increase over basal value in the untreated group to 30% in the prazosin treated group.

Propranolol totally abolished the hypoglycaemic response of insulin while prazosin delayed the onset of hypoglycaemia (Figure 4).

In the untreated group, hypoglycaemia started at 10min of infusion and increased progressively (-16% to -39%) to the end of the 60 min post-infusion observation period. In the prazosin-treated group, the hypoglycaemic effect did not start until 60 min into the experiment and at 90 min, blood glucose had already fallen to -24% of basal value.

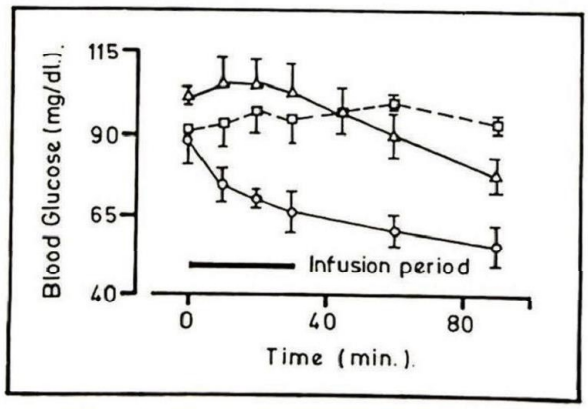

Fig. 4 The effects of insulin infusion (O——O) and pretreatment with pruzosin ((Δ——Δ) and propranolol (□—□) before insulin infusion on blood glucose levels in *bufo regularis*

Effect of 0.7% NaCl

Infusion of 0.7% NaCl had no effect on blood glucose level (Figure 1), blood pressure and heart rate.

Discussion

It is well known that numerous factors influence blood sugar values in vertebrates. The factors include analytical technique, genetic strain, sex, age, nutritional status, environmental conditions, anaesthesia and method of handling the animals [19, 20]. In addition, a fall in blood volume due to repeated sampling can cause a marked increase in blood glucose [7]. Measurements of blood pressure, heart rate and body temperature in this study were used as indices of assessing the stability of the animals throughout the duration of the experiment. Only male toads were used in our studies. The stable records of BP, HR, temperature and glucose levels in the control group showed that our results were not influenced by these variables. The fasting blood glucose in *bufo regularis* in the present study is significantly higher than the values published for frogs and *bufo bufo* [1-5], but similar to he value recently published for *bufo regularis* [6]. The lowest fasting glucose of 61.2 mg dl^{-1} in this study suggests that the exceedingly low blood sugar levels reported for amphibians [21] does not apply to *bufo regularis*. The reason for this is not clear and any attempt to explain this at present will be speculative. It is also of interest to note that while frogs with total absence of glucose survived and live a normal life [6,7], some toads in the present study died following infusion of insulin. Whether death was due to insulin shock as described by Smith [14] or due to severe hypoglycaemia is difficult to say. Our animals were anaesthetized and any tendency to convulsive siezures usually arising from insulin shock would have been obscured by the anaesthesia. Further experiments in conscious toads will be required to clarify this point.

The sustained hyperglycaemia following glucose infusion in the present study is similar to the effects of glucose injection in *rana catesbiana* [7] and *rana clamitans* [4]. It contrast however with the findings in mammals where blood glucose levels returned to basal levels in less than 1hr, post-glucose injection; [7,22] amphibians have a lower glucose clearance rate than mammals and do not use sugar as their main energy source [8]. The latter probably explains the prolonged hyperglycaemia following glucose injection or infusion in amphibians. Since prazosin abolished glucose-induced hyperglycaemia, it is reasonable to conclude that this effect is mediated via alpha adrenoceptors.

The hyperglycaemic effect of adrenaline in this study is similar to the results of previous studies in toads [5,6] and frogs [4,10-13]. The mechanisms by which adrenaline increases blood glucose in amphibians is well documented [11]. Endogenous secretion of adrenaline and nor-adrenaline occurs in frogs and toads [23-26]. With respect to the receptors involved in the effects of adrenaline, it should be noted that great species differences exist in the receptors mediating the metabolic responses to catecholamines [27]. Even with a single response in a single species, there are conflicting reports regarding the adrenergic receptors involved [28]. Although the present study did not include investigation of receptors involved in adrenaline-induced hyperglycaemia, recent studies in our laboratory have shown that both alpha and beta adrenoceptors are involved in adrenaline-induced hyperglycaemia in *bufo regularis* [6].

The increase in blood glucose caused by glucagon in this study is consistent with its reported action in amphibians [7,11,13] and mammals [29-31]. In the present study, significant hyperglycaemia was attained within 5 minutes of commencing the infusion of 2 ug kg^{-1} of glucagon and within 60 min, blood glucose had returned to the resting level, whereas, in the bullfrog [7], hyperglycaemia was not present until 20 min after intra-arterial injection of 30-200 ug kg^{-1} of glucagon and the raised blood glucose was sustained throughout the 90 min of the experiment. In *rana pipiens* [11], hyperglycaemia was not present until 1hr after intra-peritoneal injection of 1 mg kg^{-1} of glucagon and the raised blood glucose lasted well over 5 hrs. These differences are unlikely to be due to the differences in dosage or routes of administration. If anything, the higher doses used in these other species should

act faster, but this was not so. Also, there is no pharmacological basis for such a wide differences in onset of action for the same drug administered (glucagon) by the intravenous, intra-arterial or intra-peritoneal route. Species difference may well account for this. Glucagon raised blood sugar in the frog and toad by causing the breakdown of liver glycogen [11,32-34] through stimulation of adenylcyclase enzyme [35] and subsequent increase in cyclic AMP [36]. Both adenylcyclase and CAMP have been identified in the toad's liver [37]. The total annulment of glucagon-induced hyperglycaemia by propranolol suggests that a beta-adrenergic mechanism is responsible for this effect.

The hypoglycaemia produced by insulin in this study agrees with its well-known pharmacological action. Hypoglycaemia became significant in 15 min in the present study unlike in earlier studies in which it required well over 1hr to become evident [7,11]. The present study also shows that beta adrenoceptors mediate the hypoglycaemic action of insulin in the toad.

We have shown in this study that the common African toad, *bufo regularis* responds to adrenaline, insulin, glucagon and glucose administration in a manner similar to that of different species of frogs and mammals. Since frogs and toads produce insulin [4], adrenaline [23-26] and glucagon [11], we conclude that the endogenous release of these hormones under the appropriate stimuli most probably play important roles in glucose homeostasis in these amphibians. We also conclude that in the toad, the glycaemic effects of glucagon and insulin are mediated via beta adrenoceptors, that for glucose is -via alpha adrenoceptors and from an earlier study, the glycaemic response to adrenaline is through both alpha and beta receptors.

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