Utilization of glucose, alanine, lactate, and glycerol as lipogenic substrates by periuterine adipose tissue in situ in fed and starved rats

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Abstract A technique previously used to study placental transfer in pregnant rats, consisting of labeled tracer infusion through the left uterine artery, was employed to determine the utilization of lipogenic substrates by periuterine adipose tissue in the fed and 48-hr starved female virgin rat. After 20 min infusion with either D[U-^{14}C]glucose, L-[U-^{14}C]alanine, [U-^{14}C]glycerol or L-[U-^{14}C]lactate, the radioactivity appearing in periuterine adipose tissue ^{14}C-labeled lipids from the left side was always higher than that appearing in tissue from the right side. Negligible radioactivity was detected in the tissue from either side when the infusion was done with non-metabolizable derivatives such as L-[1-^{14}C]glucose or [1-^{14}C]α-aminoisobutyric acid. Simultaneous infusion of L-[U-^{14}C]alanine and an alanine transaminase inhibitor (aminooxyacetic acid) into the left uterine artery completely blocked the conversion of the alanine into periuterine adipose tissue ^{14}C-labeled lipids. The utilization of the infused substrate for fatty acids and glyceride-glycerol synthesis by the tissue was quantified by taking into account the infused radioactivity, the difference in the amount of ^{14}C-labeled lipids appearing in periuterine adipose tissue on the left and the right sides, the arterial plasma concentration of the studied metabolite, and the uterine horn blood flow. In fed animals, the highest fatty acid synthesis was found with lactate, followed by glucose, alanine, and glycerol. This process was intensely decreased with all the substrates in 48-hr starved rats. In fed animals the synthesis of glyceride-glycerol was highest with glucose followed by lactate, alanine, and glycerol. In the starved rats it did not change with lactate and decreased with both alanine and glycerol. Besides validation of the technique for the study of periuterine adipose tissue metabolism in situ, results show the efficient utilization of lactate as lipogenic and of glucose as glycerogenic substrates. On the basis of the known capacity of adipose tissue to synthesize alanine from other amino acids, we propose that in the fed state this tissue may use different amino acids for lipogenesis. The negligible use of glycerol by periuterine adipose tissue in situ contrasts to its known utilization in vitro indicating that, whereas glycerokinase activity in the tissue may have a role in the re-utilization of glycerol released into the tissue, it does not contribute to the use of circulating glycerol.—**Palacín, M., M. A. Lasunción, and E. Herrera.** Utilization of glucose, alanine, lactate, and glycerol as lipogenic substrates by periuterine adipose tissue in situ in fed and starved rats. *J. Lipid Res.* 1988. **29:** 26–32.

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Rat white adipose tissue metabolism has been extensively studied in in vitro preparations by using either tissue homogenates (1), fat pad pieces (2–5), or isolated adipocytes (6–9). Studies in vivo are required to determine whether findings from in vitro experiments may be extrapolated to the intact animal and how endogenous events such as changes in the availability of substrates, nervous activity, blood flow, etc. affect the animal's adipose tissue metabolism. By using 2-deoxy-[1-^{3}H]glucose, which is not metabolizable but is taken up by adipose tissue by the same carrier as glucose, Ferré et al. (10) were able to quantify glucose utilization in vivo in rat adipose tissue. However, this method does not allow the determination of the metabolic fate of the physiological substrate in the tissue. Many attempts have been made to study in vivo adipose tissue metabolism by giving a radioactive substrate intravenously to the intact animal and determining the appearance of labeled components in the tissue thereafter. This procedure is unsatisfactory because, before reaching adipose tissue, most of the tracer is rapidly taken up, and even transformed, by other organs (mainly the liver). To overcome these problems we have developed a technique to study rat periuterine adipose tissue metabolism in situ based on the method reported by us for determining metabolite placental transfer in the pregnant rat (11). The technique consists in the infusion of ^{14}C-labeled substrates through the left uterine artery so that the periuterine adipose tissue on the left side receives the infused substrate directly whereas the right side receives it after it has been circulated throughout the entire rat. Comparison of the radioactivity present in periuterine adipose tissue lipids from either side and adequate correction by specific activity and

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artery blood flow allows estimation of the utilization of the infused substrate by the tissue. In addition to a description of this technique and its validation in this report, we have used the technique to compare the use of glucose, glycerol, alanine, and lactate in lipid synthesis by periuterine adipose tissue in fed and 48-hr starved rats.

MATERIALS AND METHODS

Virgin female Wistar rats maintained in a light (12 hr on-off cycles) and temperature ($22 \pm 2°C$) controlled room were studied either fed or after a 48-hr starvation when the rats weighed 170–180 g. The rats were anesthetized with sodium pentobarbital (33 mg/kg, dissolved in 0.9% NaCl, i.v.) and subjected to the surgical procedure already described for the pregnant rat (11). In short, after laparotomy, the hypogastric trunk, the superior gluteal, the superior external pudendal, and the deep circumflex arteries of the left side were clamped. A PE-10 cannula (Intramedic) was introduced countercurrent into the left external iliac artery to the level of the left uterine artery. After surgery the abdomen was closed and a solution of 0.9% NaCl was immediately infused at the rate of 12.5 μl/min by means of a peristaltic pump. After 20 min of infusion the medium was changed to 0.9% NaCl supplemented with 10 μCi/250 μl of one of the following tracers: D-[U-^{14}C]glucose (sp act 257 mCi/mmol), L-[1-^{14}C]glucose (sp act 58 mCi/mmol), L-[U-^{14}C]alanine (sp act 10 mCi/mmol), [1-^{14}C]-α-aminoisobutyric acid (sp act 58 mCi/mmol), [U-^{14}C]glycerol (sp act 16 mCi/mmol) or L-[U-^{14}C]lactate (sp act 171 mCi/mmol) from The Radiochemical Centre, Amersham, UK. This infusion with the tracer was kept at a rate of 12.5 μl/min for 20 min. Where indicated, the infusion medium was supplemented with aminooxyacetic acid (80 μg/μl). In some experiments another cannula (PE-50, Intramedic) was placed into the right jugular vein and 10 μCi of one of the ^{14}C-labeled substrates dissolved in 250 μl of 0.9% NaCl was infused at the rate of 12.5 μl/min for 20 min, while the left uterine artery was infused with a plain saline solution at the same rate. After the infusion, blood was collected from the abdominal aorta with heparinized syringes and periuterine adipose tissue from the left and right horns was dissected separately, thoroughly rinsed with 0.9% NaCl, and frozen in liquid N_2. Weighed aliquots of the tissue from each side were used for lipid extraction in chloroform–methanol 2:1 (by vol) (12) and fractionation as described elsewhere (13). Radioactivity measurements were done in a xylene/Triton X-100/PPO/POPOP-based scintillation cocktail, with a counting efficiency that was always above 93%. Plasma was separated immediately after blood collection and aliquots were deproteinized with 10% $HClO_4$ and neutralized with saturated $KHCO_3$. Protein-free supernatants were used for glucose (14), alanine (15), and lactate (16) determination. Glycerol concentration was determined in other plasma aliquots (17) after deproteinization with cold acetone (18). When labeled D-glucose, L-alanine, or L-lactate was used as tracer, labeled glucose, alanine, and lactate were isolated from 100 μl of deproteinized plasma supernatants by passing them over microcolumns (0.5 cm i.d.) filled to 2.5 cm with cationic resin (AG 50 W-x8, 200–400 mesh in hydrogen form, Bio-Rad Laboratories) and 2.5–5 cm with anionic resins (AG 1-x8, 200–400 mesh in chloride form, Bio-Rad Laboratories). Columns were rinsed sequentially with 5 ml of deionized water, 5 ml of 0.5 M formate, and 5 ml of 2 N NH_4OH for the successive elution of [^{14}C]glucose, [^{14}C]lactate, and [^{14}C]alanine. Recovery of labeled standards added to rat plasma before deproteinization was $97 \pm 2\%$ for D-[U-^{14}C]glucose in the water eluate, $88 \pm 3\%$ for L-[U-^{14}C]lactate in the formate eluate, and $91 \pm 2\%$ for L-[U-^{14}C]alanine in the NH_4OH eluate, whereas contamination of the other tracers in each of the eluates was always less than 0.4%. When [U-^{14}C]glycerol was the tracer administered, labeled glycerol, glucose, and lactate were isolated from the acetone-deproteinized plasma by ascending paper chromatography using the upper phase of butanol–water–methanol–formic acid 320:320:80:1 (by vol) as described elsewhere (19). Recovery for ^{14}C-standards added to plasma was 86 ± 0.4% for [U-^{14}C]glycerol, 92 ± 0.7% for D-[U-^{14}C]glucose, and 90 ± 0.5% for L-[U-^{14}C]lactate.

Blood flow in the uterine horn was studied in other animals kept under the same environmental and experimental conditions as above. The method previously described by us (20) was followed using pentobarbital-anesthetized rats with polyethylene catheters inserted into the right femoral and the right carotid arteries, which were injected with ^{99m}Tc-labeled albumin microspheres (Sovin Biomedica SpA, Saluggia, Italy). Blood flow to the left uterine horn was calculated as described by Rosso and Kava (21).

Expression of the results

An aliquot of the solution to be administered was always counted to determine the total radioactivity (TR) given to each animal. All radioactivity values in plasma and tissues were corrected by the factor of $1x10^6$/TR to obtain the cpm value for each parameter. In this way, TR was always considered to be 1×10^6 cpm. Results were expressed as mean ± SEM and statistical comparison between the groups was done with the Student's *t* test. The periuterine adipose tissue's utilization of each of the infused metabolites for lipid synthesis was calculated by the following formula. Metabolite converted to lipids (nmol/g fresh tissue wt per min) = (Lipids*L – Lipids*R) × (Met) × Φ/Met*, where Lipids* denotes the labeled lipids (cpm/g) in left (L) and right (R) periuterine adipose

tissue, Met is the arterial plasma concentration of the infused metabolite, Met* is the radioactivity of the infused metabolite (always corresponding to 1×10^6) and Φ is the blood flow to the left uterine horn. The latter value was 0.158 ± 0.012 ml/min in fed animals ($n = 10$) and 0.127 ± 0.023 ml/min in 48-hr starved animals ($n = 8$), with no statistical difference between the two groups ($P > 0.05$). A sample calculation, using the mean values shown in Tables 1 and 2 for fed animals, for the estimation of glucose utilization for total lipid formation by periuterine adipose tissue is: $(5368 - 845) \times 5.80 \times 0.158/1 \times 10^6 = 4.14$ nmol/g of fresh tissue per min = 24.8 natom C/g of fresh tissue per min, where 5368 corresponds to cpm in ^{14}C-labeled lipids in adipose tissue from the left side, 845 is cpm in ^{14}C-labeled lipids in adipose tissue from the right side, 5.80 is the arterial plasma glucose concentration (expressed as mmol/l), 0.158 is the blood flow to the left uterine horn (expressed as ml/min), and Met* is 1×10^6 cpm.

RESULTS AND DISCUSSION

As shown in **Table 1**, after the infusion with D-[U-^{14}C]glucose, L-[U-^{14}C]alanine, [U-^{14}C]glycerol, or L-[U-^{14}C]lactate through the left uterine artery in fed or 48-hr starved rats, radioactivity present in lipids of periuterine adipose tissue from the left side was significantly higher than that from the right side. When either L-[1-^{14}C]glucose or [1-^{14}C]α-aminoisobutyric acid was the infused tracer, practically no radioactivity was found in lipids from the tissues of either side (Table 1). The L-glucose analog is known not to be recognized by the D-glucose carrier (22) whereas α-aminoisobutyric is a non-metabolizable amino acid which is known to be mainly transported by the A system (23). These findings clearly show that periuterine adipose tissue is able to take up and use either D-glucose, L-alanine, glycerol, or L-lactate for lipid synthesis, and observed values are the result of neither contamination nor unspecific tracer utilization. The specificity of the use of L-alanine was further tested by infusing aminooxyacetic acid simultaneously with L-[U-^{14}C]alanine through the left uterine artery. This compound is a known transaminase inhibitor (24). As is also shown in Table 1, aminooxyacetic acid completely blocked the utilization of L-[U-^{14}C]alanine in tissue lipid synthesis; in its presence no radioactivity was detected in periuterine adipose tissue lipids from either the left or the right side. This finding allows us to emphasize the validity of this method for detecting the specific metabolic utilization by the tissue of any substrate for lipid synthesis. Observed differences between the radioactivity present in the periuterine adipose tissue from the left and the right uterine horn when the tracer was infused through the left uterine artery are not a consequence of blood circulation disturbances on either side having been produced by the surgical procedure. When either D-[U-^{14}C]glucose, L-[U-^{14}C]-alanine, or [U-^{14}C]glycerol were infused through the jugular vein simultaneously to saline infusion via the left uterine artery, no difference was found in the radioactivity in periuterine adipose tissue ^{14}C-labeled lipids from either the left or the right sides (data not shown). These findings and the higher amount of ^{14}C-labeled lipids present in the tissue on the left side versus the tissue on the right side after the infusion of a metabolizable tracer through the left uterine artery indicate the direct use of the infused substrate by the tissue from the left side. Values in the tissue from the right side correspond to the tracer having reached the tissue after being diluted (and partially transformed) in the whole animal. We therefore consider that the difference in the amount of ^{14}C-labeled lipids in the periuterine tissues from either side corresponds to the net amount of the infused tracer having been directly taken up and metabolized by the tissue. As the in-

TABLE 1. Appearance of ^{14}C-labeled lipids in periuterine adipose tissue from fed and 48-hr starved rats after 20 min infusion of various labeled substrates through the left uterine artery

	CPM in ^{14}C-Labeled Lipids/g Fresh Tissue					
	Fed Rats			48-Hr Starved Rats		
Infused Tracer	Tissue from Left Side	Tissue from Right Side	P^a	Tissue from Left Side	Tissue from Right Side	P
D-[U-^{14}C]Glucose	5368 ± 784 (4)[b]	845 ± 303 (4)	< 0.05	12844 ± 3642 (4)	522 ± 80 (4)	< 0.05
L-[U-^{14}C]Alanine	38907 ± 8556 (5)	1777 ± 709 (5)	< 0.01	962 ± 56 (4)	182 ± 47 (4)	< 0.01
[U-^{14}C]Glycerol	5392 ± 1930 (4)	518 ± 176 (4)	< 0.05	1216 ± 366 (4)	174 ± 29 (4)	< 0.05
L-[U-^{14}C]Lactate	12747 ± 3096 (4)	303 ± 115 (4)	< 0.05	2296 ± 329 (4)	305 ± 81 (4)	< 0.05
L-[1-^{14}C]Glucose	7 ± 4 (3)	6 ± 5 (3)	NS			
[1-^{14}C]Aminoisobutyric acid[c]	4 (2)	1 (2)				
L-[U-^{14}C]Alanine + aminooxyacetic acid	9 ± 4 (4)	1.3 ± 0.3 (4)	NS			

All values were corrected by considering 1×10^6 cpm as the total radioactivity of each tracer administered to each rat.
[a] P, statistical comparisons between tissues from the left and the right sides for each parameter in the same animal.
[b] Means ± SEM; number of rats per group in parentheses.
[c] Values with [1-^{14}C]-α-aminoisobutyric acid correspond to the mean of two separate experiments.

fused labeled metabolite is given in tracer amounts and is immediately mixed with the endogenous pool in the uterine artery, quantification of the actual amount of the substrate being used by the tissue must take into account its specific activity in artery blood plasma. **Table 2** summarizes the arterial plasma concentrations and specific activities after 20 min infusion of the tracers to fed and to 48-hr starved rats. Plasma concentrations of glucose, alanine, and lactate were significantly reduced in the starved versus the fed rat and their specific activities after the respective infusions with D-[U-^{14}C]glucose, L-[U-^{14}C]alanine, or L-[U-^{14}C]lactate were significantly higher in the starved animals. Glycerol concentration and [^{14}C]glycerol specific activity in arterial plasma were not found to differ between fed and 48-hr starved rats after the infusion of [U-^{14}C]glycerol (Table 2). Comparative specific activities reached among the different tracers indicate that [^{14}C]glycerol was the "least diluted" tracer followed by [^{14}C]alanine, -glucose, and -lactate, and this order was the same in starved or in fed animals in spite of the observed differences in the specific activity values between these two groups (Table 2).

Application of the formula indicated in Methods to the values shown in both Tables 1 and 2 allows us to quantify the actual use by the tissue of each of the studied metabolites for total lipid synthesis. As tissue lipids were fractionated into both fatty acids and glyceride-glycerol, radioactivity present in each of these fractions was applied to the formula in order to quantify the actual amount of the infused substrate converted into each of these moieties. **Table 3** shows these values for both fed and 48-hr starved rats. As can be seen, lactate is the substrate most efficiently used for lipid synthesis in fed animals, followed by glucose, alanine, and glycerol. Proportional contribution of fatty acids and glyceride-glycerol synthesis also differs among the different substrates. Both lactate and alanine are converted primarily into fatty acids while a lesser proportion is converted into glyceride-glycerol. Glucose is converted equally into each of the two fractions and glycerol is mainly converted into glyceride-glycerol (Table 3). Starvation provoked a marked decrease in fatty acid synthesis from glucose, alanine, glycerol, and lactate. The starved rats' glyceride-glycerol synthesis from glycerol, and alanine was also decreased, whereas it was not significantly affected from either lactate or glucose (Table 3).

Although it is not possible to directly compare present findings with published results from in vitro adipose tissue preparations—which, besides being far from the in vivo condition, offered the adipocytes unphysiological amounts of the substrate under study in most cases— some extrapolations may be made. The observed use of lactate for fatty acid synthesis by periuterine adipose tissue at a higher proportion than glucose agrees with reported findings from both rat hepatocytes (25) and adipocytes (8, 26) which show the superiority of lactate over glucose as a lipogenic substrate for these tissues. This conclusion does not agree with other reports showing that in vitro adipose tissue converts lactate to fatty acids at a very low rate (27). The reason for the discrepancies is not clear, but, besides providing carbon atoms for the generation of both *sn*-glycero-3-phosphate and fatty acids via its conversion into pyruvate, lactate can alter the cytoplasmic redox state of the adipocyte (28) to favor both glycerogenesis and fatty acid synthesis. Besides substantiating the efficient use of lactate as a lipogenic substrate in periuterine adipose tissue, the present findings show that whereas glycerogenesis is unaffected, fatty acid synthesis is intensely impaired in the starved rat, as expected. In the starved condition fatty acid esterification in rat adipose tissue is known to be either ummodified or enhanced (29) and we propose that *sn*-glycero-3-phosphate synthesized from lactate may play an important role in keeping that pathway active. The results of alanine as substrate for fat-

TABLE 2. Arterial plasma concentrations in fed and 48-hr starved rats of glucose, alanine, lactate, and glycerol and their specific activities after 20 min infusion of the respective labeled substrates through the left uterine artery

Concentration	Fed Rats	48-Hr Starved Rats	P^a
Glucose (mM)	5.80 ± 0.17 (4)[b]	3.50 ± 0.60 (4)	<0.01
Alanine (μM)	399 ± 42 (5)	213 ± 31 (5)	<0.01
Lactate (mM)	4.00 ± 0.48 (4)	2.24 ± 0.26 (4)	<0.05
Glycerol (μM)	196 ± 39 (4)	145 ± 17 (4)	NS
Specific activity[c]	*cpm/μmol*		
[^{14}C]Glucose	2008 ± 362 (4)	3929 ± 138 (4)	<0.01
[^{14}C]Alanine	3207 ± 320 (5)	9209 ± 1591 (5)	<0.001
[^{14}C]Lactate	219 ± 17 (4)	608 ± 56 (4)	<0.01
[^{14}C]Glycerol	11240 ± 1437 (4)	12931 ± 1089 (4)	NS

[a] *P*, statistical comparison between fed and 48-hr starved rats.
[b] Mean ± SEM; number of animals in group in parentheses.
[c] Specific activity values were corrected by considering 1 × 10^6 cpm as the total radioactivity of the respective tracer administered to each animal.

TABLE 3. Estimated utilization of glucose, alanine, glycerol, and lactate for the synthesis of fatty acids and glyceride-glycerol by periuterine adipose tissue in fed and 48-hr starved rats

	natom C per g Fresh Tissue Wt per Min			
	Fed Rats		48-Hr Starved Rats	
Substrate	Fatty Acids	Glyceride-Glycerol	Fatty Acids	Glyceride-Glycerol
Glucose	12.1 ± 1.5	12.6 ± 1.1	0.60 ± 0.18[a]	26.2 ± 6.84
Alanine	5.79 ± 1.23	0.72 ± 0.12	0.009 ± 0.003[b]	0.051 ± 0.027[b]
Glycerol	0.078 ± 0.003	0.165 ± 0.042	0.002 ± 0.001[a]	0.051 ± 0.018[c]
Lactate	23.3 ± 7.11	1.56 ± 0.18	0.45 ± 0.15[c]	0.99 ± 0.18

Mean ± SEM of four or five rats per group. The estimation takes account of the ^{14}C-labeled lipid moiety present in the tissue from both uterine sides after the infusion of the labeled substrate through the left uterine artery, the uterine blood flow, the endogenous concentration of the substrate, and the administered radioactivity, as described in Materials and Methods.

Statistical comparison of starved versus fed animals: $^{a}P < 0.001$; $^{b}P < 0.01$; $^{c}P < 0.05$.

ty acid synthesis in periuterine adipose tissue in vivo agree with previous findings with isolated adipocytes in vitro (6, 30) and the known presence of alanine aminotransferase in this tissue (31). The observed block in L-[U-^{14}C]alanine utilization for lipid synthesis by aminooxyacetic acid, which is an inhibitor of alanine aminotransferase (24), further supports the active dependence on that enzyme in the use of alanine as lipogenic substrate by this tissue. In in vitro preparations it has been reported that the presence of glucose was required in the incubation medium to facilitate the metabolism of alanine and other amino acids in adipose tissue (6, 32) but this condition is fulfilled in the in vivo situation where the tissue is bathed with the glucose present in the irrigating blood. It has been shown that adipose tissue can release alanine (33, 34) derived both from the net protein breakdown and the degradation of branched-chain amino acids (which together with the present findings showing the use of alanine as lipogenic substrate indicate that in the fed state adipose tissue can efficiently use other amino acids) after converting them into pyruvate, for the synthesis of fatty acids. In the starved condition alanine production by adipose tissue is either unaltered (34) or enhanced (33), but the block in the use of alanine for lipid synthesis observed here would suggest that the net alanine release by this tissue must be enhanced. Since adipose tissue may constitute a significant percentage of body weight, it is proposed that in the fasted, instead of in the fed state, fat constitutes an important source of circulating alanine, actively contributing to the glucose-alanine cycle (35, 36).

The observed conversion of glucose in equal parts into both fatty acids and glyceride-glycerol by periuterine adipose tissue in the fed animals and mostly into glyceride-glycerol in the starved animals agrees with previous findings in in vitro preparations (5, 8, 9, 29). Fatty acid formation in the periuterine adipose tissue of fed animals from glucose was similar to that from alanine and much lower than that from lactate. This indicates that in spite of the well-known effect of glucose enhancing lipogenesis (6, 9, 37, 38), this metabolite does not constitute a preferential substrate in this pathway. It is, however, a major contributor to glyceride-glycerol synthesis. This process is enhanced slightly in the starved animal, a finding that agrees with the known active re-esterification of endogenous released free fatty acids which is even greater in the starved than in the fed state (28, 39). Under conditions of enhanced lipolysis, this mechanism of reesterification avoids the free fatty acid accumulation in the tissue and overcomes its potential inhibitory action on adipocyte metabolism (40).

Present findings on the use of glycerol as a lipogenic substrate in periuterine adipose tissue agree with the recognized presence of glycerokinase in this tissue (41, 42) and with reports of in vitro preparations showing the capacity of this tissue to use glycerol for both fatty acid and glyceride-glycerol synthesis (3, 6, 13, 43, 44). According to present results, this process is, however, quantitatively very limited in vivo in comparison to the use of the other substrates studied. This conclusion agrees with previous findings (45, 46) and it is proposed that the presence of glycerokinase activity in adipose tissue may be relevant to the reutilization of glycerol released into the tissue but not to the utilization of circulating glycerol.

In summary, the technique herein described allows direct study of the metabolism of rat periuterine adipose tissue in situ taking into account the endogenous substrates available at physiological concentrations and the blood flow to the tissue. In this way it is possible to determine how either endogenous (nerve stimulus, hormonal changes) or exogenous factors (nutrition or environment modifications) affect tissue metabolism in vivo.

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