FULL PAPER *Internal Medicine*

Use of ^{13}C-Acetate Breath Test for Assessment of Gastric Emptying in Horses

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ABSTRACT. This study aimed to establish and standardize a breath test that uses ^{13}C-acetate in a liquid diet for evaluation of gastric emptying in horses. Seven adult healthy thoroughbreds were used in this study. They were given ^{13}C-acetate (125 mg, 250 mg, or 500 mg) in a test meal (2000 m*l* liquid diet) via an intranasal catheter. ^{13}C concentrations in the exhaled CO_2 were measured in samples taken before and after test meal administration using an infrared absorption spectroscope. In the 500 mg ^{13}C-acetate group, $\Delta^{13}CO_2$ showed a steep gradient immediately after meal administration compared to the 125 mg and 250 mg groups. Therefore, t_{max} in the 500 mg group was easier to determine than in the 125 mg and 250 mg groups. In the 500 mg group, GEC, half-empty time (t1/2), calculated t_{max} (t_{lag}), and t_{max} were 1.95 ± 0.28 (mean ± SD), 229.2 ± 57.0 (min), 139.2 ± 22.2 (min), and 124.0 ± 28.4, respectively. Differences in CV observed in the 500 mg group were lower than those in the 125 mg and 250 mg groups. This study demonstrates that the ^{13}C-acetate breath test is useful for evaluating gastric emptying in horses since it is non-invasive and does not require set up of special facilities or equipment. Optimum evaluation of gastric emptying in horses can be achieved with 500 mg of ^{13}C-acetate given in a liquid diet.
KEY WORDS: ^{13}C-acetate breath test, equine, gastric emptying, gastrointestinal dysfunctions.

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Horses frequently develop upper gastrointestinal (GI) dysfunctions, such as anorexia and gastric ulcer, due to harsh training and stress [5, 6]. These conditions accompany delayed gastric emptying, indicating injury of the gastric mucosa by voluminous residual gastric juice, which then develops into a severe gastric ulcer [1, 3]. For this reason, understanding the mechanism of gastric emptying is an important step in the prevention and treatment of upper GI dysfunctions in horses. Historically, radioscintigraphy using radioactive markers has been the gold standard for evaluation of gastric emptying ability [8]. However, this method is not suited for general use, since it requires special equipment. Consequently, the establishment of a non-invasive, simple, and objective gastric emptying test for horses has been awaited. Recently, a breath test that uses stable isotope ^{13}C-labeled compounds has been clinically applied to detect gastric emptying disorders in humans [9], and the use of this test in horses has long been anticipated. In horses specifically, ^{13}C-octanoic acid delivered in solid food has been evaluated as breath test labeled compound. However, absorption of ^{13}C-octanoate in the small intestine has been show to be unstable [2, 11, 13]. In humans and dogs, on the other hand, gastric emptying ability is assessed mainly by ^{13}C-acetate delivered in a liquid diet. Against this background, this study aimed to establish and standardize a breath test that uses ^{13}C-acetate in a liquid diet for evaluation of gastric emptying in horses.

MATERIALS AND METHODS

Animals: Seven healthy adult horses (thoroughbred, mean age: 9.3 ± 5.9 years, mean body weight: 527.1 ± 37.5 kg) were used in this study. The horses were raised in a horse barn and fed twice daily (hay: 7 kg/day, wheat bran: 1 kg/day, oats: 3 kg/day). This study was approved by the animal welfare and ethics committee at the Obihiro University of Agriculture and Veterinary Medicine.

Breath collection technique: Exhaled breath samples were collected using a silicone tube with a sheet that seals a nostril (Fig. 1). The tube was inserted into the right nostril, and the left nostril was closed manually as the animal breathed (Fig. 2). Breath released from the tube was collected into breath collection bags specifically made for this purpose (200 m*l* and 1,000 m*l*, Otsuka Pharmaceutical, Japan).

Administration of the test meal: The test meal used in this study consisted of a 2,000 m*l* liquid diet containing 18% protein, 20% fat, and 62% sugar (RACAL, 1 m*l*/kcal, Otsuka Pharmaceutical, Japan). The liquid diet had ^{13}C-acetate (99% 1–13 acetic acid, Wako Pure Chemical Industries, Japan) added to it, and an intranasal catheter was used to enable oral administration.

Study design: The test meal containing ^{13}C-acetate in three doses (125 mg, 250 mg, and 500 mg) was administered after a 12-hr fast. Breath samples were collected before the test meal was given and 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, and 240 min after meal administration. For breath collection before test meal administration, a large breath collection bag (1,000 m*l*, Otsuka Pharmaceutical, Japan) was used. Breath samples after test meal administration were collected into 2 bags (200 m*l*). The concentration greater of $^{13}CO_2$ in each breath sample was determined, and samples with a $^{13}CO_2$ concentration more than 2% were used as study samples. The breath test was repeated at intervals of more than 1 week.

Measurement of ^{13}C concentration: The concentration of ^{13}C in each sample taken before and after test meal administration was determined using an infrared absorption spectro-

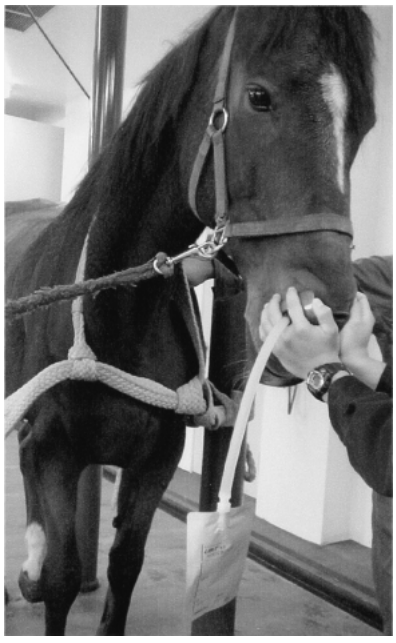

Fig. 1. Breath collection technique. After inserting the breath collection tube into the right nostril, the left nostril was closed manually as the animal breathed. The exhaled breath from the tube was collected into a breath collection bag specifically made for this purpose (200 m*l*, Otsuka Pharmaceutical, Japan).

scope (UBiT-IR300, Otsuka Electronics, Japan). Changes in $^{13}CO_2$ concentration in the exhaled breath expressed as $\Delta^{13}CO_2$ (‰: $\delta^{13}Ct - \delta^{13}Co$) were then calculated. For data analysis, the average of 2 measurements at each time point was used. Samples with a $^{13}CO_2$ concentration of less than 0.5% were discarded.

Data analysis: The excretion rate of ^{13}C (% dose/hr) was determined using a program developed by Chihiro Nosaka (Kyowa Hakko Kogyo, Japan) that incorporates Ghoos' theory [2], and the production rate of $^{13}CO_2$ was estimated to be constant at body surface area/min of $0.1561/m^2$ [7].

Body surface area was calculated using the following formula: Body surface area (m^2) = 10.5 × body weight (g) $^{2/3}$/ 10,000 [7].

The excretion rate of ^{13}C (% dose/hr) was obtained using the χ^2 probability density function model as follows: % dose/hr = at^be^{-ct} (t = time; a, b, c = regression constants). The gastric empting coefficient [GEC = ln(a)] and t_{lag} (t_{lag} = b/c) were also calculated. The time corresponding to the maximum $^{13}CO_2$ concentration in the exhaled breath was taken as t_{max}, and cumulative ^{13}C recovery rates (C % D, % dose) were determined from the cumulative time of the % dose/hr value using the following formula: C % D=m(1-e-^{kt}) $^{\beta}$(k, m, β= regression constants). Half-empty time ($t_{1/2}$) was calculated using the following formula: $t1/2 = (-1/\kappa) \times \ln(1-2^{-1/\beta})$.

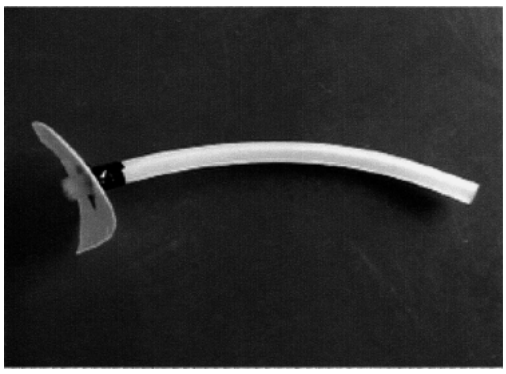

Fig. 2. Exhaled breath collection tube. Exhaled breath samples were collected using this silicon tube with a sheet that completely seals the nostril.

RESULTS

Since a special silicon tube equipped with a plastic sheet that seals the nostril and prevents breath leakage was used for breath collection, it was possible to obtain stable measurements with little effort.

As shown in Fig. 3, $\Delta^{13}CO_2$ in horses given 125 mg or 250 mg ^{13}C-acetate gradually increased after test meal administration, reaching a maximum of 3.5‰ (105 min) or 7.5‰ (120 min), respectively, and then remained almost unchanged until the end of the experimental period (240 min after test meal administration). However, in horses that received 500 mg, ^{13}C-acetate $\Delta 1^{13}CO_2$ rapidly increased after test meal administration, reaching a peak (16‰) at 105 min after treatment, and then slowly decreased to about 13‰ by the end of the experimental period. The t_{max} in horses given 500 mg ^{13}C-acetate was, therefore, easier to distinguish than that in horses treated with 125 mg or 250 mg ^{13}C-acetate. Moreover, $\Delta^{13}CO_2$ in horses given 500 mg of ^{13}C-acetate exhibited bimodal peaks, one occurring 105 min after test meal administration and the other appearing thereafter (175 min after test meal administration).

Changes in ^{13}C expiration rates (% dose/hr) were similar to those in $\Delta^{13}CO_2$ in all groups (Fig. 4). On the other hand, cumulative ^{13}C recovery rates (C % D, % dose) gradually increased after test meal administration reaching maximum values, i.e. 4.8 ± 0.6 (125 mg), 9.7 ± 2.1 (250 mg), and 17.8 ± 2.9 (500 mg), at 240 min after treatment (Fig. 5).

In horses given 500 mg of ^{13}C-acetate, GEC, half-empty time ($t_{1/2}$), calculated t_{max} (t_{lag}), and t_{max} were 117.2 ± 16.8 min 229.2 ± 57.0 min, 139.2 ± 22.2 min (min), and 124.0 ± 28.4 min, respectively (mean ± SD, Table 1). Differences in CV (Coefficient of variation) observed in the horses given 500 mg ^{13}C-acetate were lower than those in horses treated with 125 mg or 250 mg ^{13}C-acetate .

DISCUSSUION

The breath test, which generally loads stable isotopes, including ^{13}C-labeled compounds, is used to detect slight

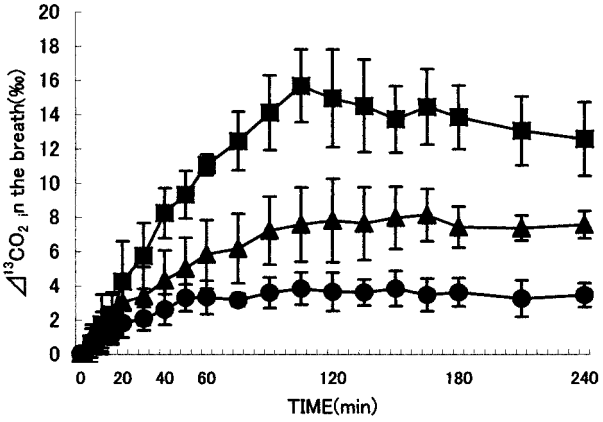

Fig. 3. Breath $^{13}CO_2$ content curves. ●: 125 mg ^{13}C-acetate group, ▲: 250 mg ^{13}C-acetate group, and ■: 500 mg ^{13}C-acetate group. Data are expressed as the mean ± SD (n=7). For the 500 mg ^{13}C-acetate group, t_{max} was easily recognized as compared to the 125 mg and 250 mg ^{13}C-acetate groups.

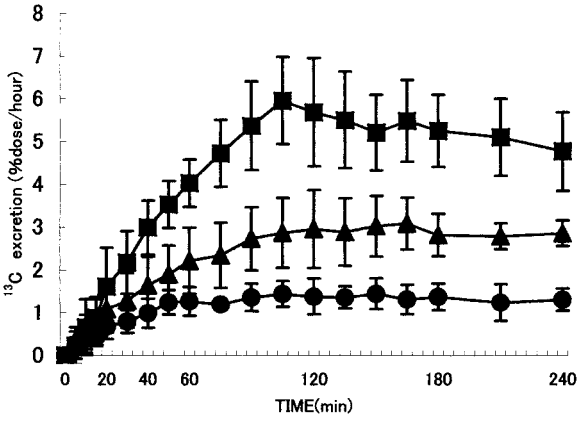

Fig. 4. ^{13}C expiration rate. ● shows the ^{13}C expiration rate fitting curve. The time when breath $^{13}CO_2$ content reached the actual maximum measured value is indicated by t_{max}. This time conformed with the time of maximum ^{13}C expiration. The time when the ^{13}C expiration rate fitting curve reached the actual maximum measured value is indicated by t_{lag}. Although the ^{13}C expiration rate showed bimodal peaks, the maximum value on the fitting curve used in the calculation was the middle point.

changes in the concentration of labeled substances, such as the concentration of $^{13}CO_2$ exhaled at different times or after a certain period of time [2]. The ^{13}C-labeled compound used in this study (^{13}C-acetate) enabled us to perform an open study, since it can be handled anywhere with no restrictions on its disposal. Evaluation of gastric emptying generally uses a breath test with ^{13}C-octanoate [4, 13] in solid food or ^{13}C-acetate [10, 11] in a liquid diet as the labeled compounds. In this test, the gastric emptying rate is determined from changes in the exhaled $^{13}CO_2$ concentration at different times. When gastric emptying increases, the amount of the ^{13}C-labeled compound absorbed in the

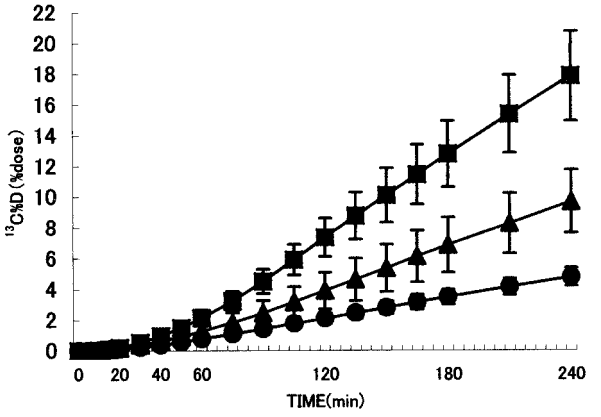

Fig. 5. ^{13}C expiration rates. ●: 125 mg ^{13}C-acetate group, ▲: 250 mg ^{13}C-acetate group, and ■: 500 mg ^{13}C-acetate group. Data are expressed as the mean ± SD (n=7).

intestine per unit of time and the $^{13}CO_2$ concentration in the exhaled breath increase. Based on this principle, infrared absorption spectroscopy is used to calculate $\Delta^{13}CO_2$ (‰), which shows the $^{13}CO_2$ content in exhaled CO_2. When gastric emptying is facilitated, the initial gradient of the $\Delta^{13}CO_2$ curve (‰) and the peak value become higher [4]. On the other hand, when gastric emptying is delayed, the amount of ^{13}C-labeled compound absorbed in the intestine per unit of time decreases resulting in a decrease in both the initial gradient of the $\Delta^{13}CO_2$ curve and the peak value. Therefore, gastric emptying in horses can be evaluated using changes in the $^{13}CO_2$ content in the $\Delta^{13}CO_2$ curve.

Generally, ^{13}C-octanoate is mixed with egg yolk when solid food is prepared for the breath test since this compound has high affinity for egg yolk [13]. However, solid food does not allow prompt absorption of ingested ^{13}C-octanoate after exiting the stomach, and can delay the increase in $^{13}CO_2$ concentration in exhaled breath [2, 4]. Additionally, ^{13}C-octanoate is likely to be affected by absorption and metabolism of ingredients in the test meal, such as fat. For these reasons, the standard test meal used in humans consists of a liquid diet that can easily be standardized and that is less likely to be affected by absorption, metabolism, and excretion [10]. Therefore, for evaluation of gastric emptying in horses, we selected a liquid diet as the test meal for this study, and decided to use water-soluble ^{13}C-acetate as the ^{13}C-labeled compound. Moreover, since a fair number of calories are required to evaluate gastric emptying in horses, we used a liquid diet equivalent to 2 M calories in this study (RACOL, 2,000 m*l*).

The breath collection technique used for humans consists of holding the breath for few seconds, expiring, and collecting the breath expired toward the end of exhalation. Since such a technique is not suitable for horses, the exhaled breath of the horse at rest was collected into a 200 m*l* breath collection bag using a tube specifically designed for this purpose. In this study, since the $^{13}CO_2$ contents in expired CO_2 and $\Delta^{13}CO_2$ (‰) were calculated, fluctuations in mea-

Table 1. GEC, $t_{1/2}$, t_{lag}, and t_{max} recorded after administration of 125 mg, 250 mg, and 500 mg of ^{13}C-acetate

500 mg	GEC	$t_{1/2}$ (min)	t_{lag} (min)	t_{xam} (min)
Horse No. 1	91.2	231.6	141.6	105.0
Horse No. 2	131.4	189.0	132.6	105.0
Horse No. 3	118.8	255.0	139.2	105.0
Horse No. 4	126.0	205.8	132.0	120.0
Horse No. 5	138.0	172.8	114.0	105.0
Horse No. 6	114.6	207.0	129.0	165.0
Horse No. 7	100.2	343.2	184.8	165.0
Mean±SD	117.2±16.8	229.2±57.0	139.0±22.1	124.0±28.4
CV	0.14	0.25	0.16	0.23

250 mg	GEC	$t_{1/2}$ (min)	t_{lag} (min)	t_{max} (min)
Horse No. 1	12.0	354.0	220.2	240.0
Horse No. 2	72.6	264.6	147.0	150.0
Horse No. 3	97.8	214.8	121.8	120.0
Horse No. 4	43.85	384.6	231.0	165.0
Horse No. 5	49.8	633.0	245.4	135.0
Horse No. 6	73.8	238.2	133.2	135.0
Horse No. 7	97.2	193.2	123.6	120.0
Mean±SD	63.9±30.9	326.1±152.7	174.6±55.0	157.5±43.2
CV	0.48	0.47	0.31	0.28

125 mg	GEC	$t_{1/2}$ (min)	t_{lag} (min)	t_{max} (min)
Horse No. 1	25.2	277.2	141.0	105.0
Horse No. 2	21.6	343.2	174.0	180.0
Horse No. 3	49.2	186.0	120.6	105.0
Horse No. 4	2.4	379.2	207.6	240.0
Horse No. 5	33.0	264.6	65.4	40.0
Horse No. 6	46.2	172.2	110.4	135.0
Horse No. 7	58.2	202.2	98.4	60.0
Mean±SD	33.7±19.1	260.7±79.6	131.1±47.8	124.0±69.0
CV	0.57	0.31	0.36	0.56

surements caused by breathing forms were minimal. However, due to the precision of measurment equipment, the CO_2 concentration in the exhaled breath was required to be greater than 2%. Thus, the exhaled breath was collected twice at rest, and this led to stable measurements.

The exhaled breath was collected at a total of 19 time points, i.e. at 5-min intervals from 0–20 min, at 10-min intervals from 20–60 min, at 15-min intervals from 60–180 min, and at 30-min intervals from 180–240 min. The recommended number of time points for breath collection in humans is 12, taken between 0–90 min [11]; however, since the duration of gastric emptying in horses is longer than in humans, we extended the measuring time to 240 min. Although a limited number of breath collections is preferred when evaluating normal clinical gastric emptying, a fair number of breath samples are required to evaluate delayed gastric emptying.

In this study, t_{max} was defined as the time when the $^{13}CO_2$ content in the exhaled breath reaches a maximum, which reflects the actual measurements of the $^{13}CO_2$ contents in breaths ($\Delta^{13}CO_2$). When t_{max} is determined using IR absorption spectroscopy, it is known to correlate with $t_{1/2}$ (50% gastric emptying time), which can also be determined by IR absorption spectroscopy [2, 8]. Thus, t_{max} in the breath test can be used as an indicator to estimate and evaluate gastric emptying.

In the human ^{13}C-acetate breath test, 100 mg of ^{13}C-acetate is used [2, 4]. However, since our study subjects were adult thoroughbred horses weighing 500 kg, we administered 125 mg, 250 mg, and 500 mg of ^{13}C-acetate and compared the results. In horses treated with 125 mg and 250 mg of ^{13}C-acetate, t_{max} in the $^{13}CO_2$ content curve was unclear, and its standard deviation (SD) and t_{lag} were large. In contrast, a distinctive breath $^{13}CO_2$ content curve with clear t_{max} values was noted in the horses treated with 500 mg of ^{13}C-acetate. When the cost of ^{13}C-acetate was taken into account, using 500 mg of ^{13}C-acetate was considered best for conducting the ^{13}C-acetate breath test in horses.

In the horses treated with 500 mg ^{13}C-acetate, two peak values for t_{max} were found. Generally, bimodal peaks arise from changes in GI movement patterns that are accompanied by gastric emptying [12]. In other words, the first peak appears as gastric contents are excreted since gastric contents and the gastric excretion rate decrease. When the gastric contents decrease, communicable and strong constrictions develop from the pyloric portion of the stomach and the duodenal bulb towards the anus. The excretion rate of gastric contents temporarily increases, giving rise to the second peak. When two peaks are observed in the $^{13}CO_2$ content curve, it is generally recommended to consider only the larger peak, or in some cases the first peak, for determination of t_{max} [2]. However, in this study, both peaks were considered appropriate for determination of t_{max}.

Other than the tmax obtained from the breath $^{13}CO_2$ content curve, $t_{1/2}$ (half-emptying time), t_{lag} (lag phase), and GEC are also commonly used as indicators of gastric emptying [12]. The half-emptying time $t_{1/2}$, is the time required to excrete half of the total ^{13}C. The lag phase, t_{lag}, is the time required for test meal to be stored, mixed, and reduced in the stomach for excretion, and is equivalent to the time when ^{13}C excretion reaches a maximum. GEC corresponds to the gradient of the first half of the gastric emptying rate curve. The smaller the value of GEC, the more gastric emptying is delayed. In this study, $t_{1/2}$, t_{lag}, and GEC were calculated using an analysis program that incorporates Ghoos' theory [2]. The ^{13}C expiration rate was expressed as % dose/hr, and was calculated using the folding formula: % dose/hr = Δ^{13}C (‰) × 0.0112371 × 10 × body surface area × 300/(dosage × concentration/molecular weight). Body surface area in this formula was calculated based on Orr's theory [7]: body surface area (m^2) = 10.5 × body weight $(g)^{2/3}$/10,000.

Although the actually measured t_{max} and the calculated t_{lag} were expressed separately, the changes in breath $^{13}CO_2$ content and the changes in ^{13}C expiration rate calculated by the analysis program (^{13}C excretion curve, % dose/hr) were the same. This finding suggests that gastric emptying can be evaluated by actually measuring the peak of the breath $^{13}CO_2$ content curve. Nonetheless, for the calculation of t_{lag}, the mid point of bimodal peaks on the breath $^{13}CO_2$ content curve is treated as the peak. As a result, t_{lag} becomes higher than t_{max}, which can be problematic. Consequently, the use of measured t_{max} is recommended with respect to manipulation of pharmacological data.

The breath $^{13}CO_2$ content curve is divided into two sections; 0 min to t_{max} and t_{max} to the latter half. Each section reflects phases that include gastric emptying of ^{13}C-labeled compounds, digestion and absorption in the small intestine, liver metabolism, and breath expiration. In other words, it is suggested that the first half of the curve reflects gastric emptying and small intestinal absorption against liver metabolism and breath expiration, and the latter half reflects liver metabolism and breath expiration against gastric emptying and small intestinal absorption [12]. Thus, evaluation of liver function in horses can be made by assessing changes in the $^{13}CO_2$ content that appears in the latter half of the curve.

In summary, this study demonstrates that the ^{13}C-acetate breath test is useful for evaluating gastric emptying in horses since it is non-invasive and does not require set up of special facilities or equipment. Optimum evaluation of gastric emptying in horses can be achieved with 500 mg of ^{13}C-acetate given in a liquid diet Finally, use of the breath test in conjunction with the $t_{1/2}$, t_{lag}, and GEC indicators for diagnosis, treatment, and prevention of upper GI dysfunctions in horses appears very promising.

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