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LABORATORY INVESTIGATION

GFR increases before renal mass or ODC activity increase in rats fed high protein diets

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GFR increases before renal mass or ODC activity increase in rats fed high protein diets. Consumption of a high protein diet causes renal hypertrophy and increased glomerular filtration rate (GFR). To determine the relationship between increases in GFR, renal ornithine decarboxylase activity (ODC), arginase activity, and renal growth, dietary protein intake was increased from 8.5% to 40% in 50 male Sprague-Dawley rats (HP). Forty-one rats remained on 8.5% protein as time controls (LP). Eight to 17 animals were killed daily for measurement of kidney weight (kidney wt), ODC and arginase activities, total kidney protein and DNA content. GFR increased within the first 24 hours after the increase in dietary protein and reached a maximum within 48 hrs. ODC increased from 9.7 ± 0.8 U/g to a peak of 170 ± 35 U/g at 48 hours, decreasing to a stable value of 28.6 ± 8.0 U/g at 72 hours and 25.4 ± 5.1 U/g at 168 hours, a value significantly greater than that at time zero. Arginase activity did not change. Kidney wt as percent body weight (body wt) increased after the initial increase in both GFR and in ODC activity. The peak in ODC activity corresponded with the maximum increase in GFR and preceded the increase in renal mass. After GFR stabilized, ODC activity decreased to a plateau and renal growth relative to body wt ceased. The increase in kidney weight was accompanied by a parallel increase in total kidney protein. Kidney protein/kidney DNA ratio increased significantly by 96 hours, indicating that renal hypertrophy had occurred. The sequence of these events suggests that increasing GFR may trigger the rise in ODC activity.

Increased dietary protein intake triggers renal growth [1] and initiates glomerular hemodynamic changes that result in an increase in glomerular filtration rate (GFR) [2–4]. Renal growth and increased GFR are also linked in several models of renal disease. Renal hypertrophy occurs following unilateral nephrectomy, and in this case, single nephron glomerular filtration rate increases prior to the onset of renal growth [5]. The effects of unilateral nephrectomy and increased dietary protein intake on both GFR and renal mass are additive [6]. Increased GFR and renal hypertrophy are likewise characteristic of early diabetes mellitus [7, 8].

Ornithine decarboxylase (ODC, EC4.1.1.17) is the rate controlling enzyme for the biosynthesis of growth regulatory polyamines [9, 11], and an increase in ODC activity should coincide with or precede tissue growth. Renal ODC activity is known to increase after fasting rats are fed a single casein meal [12, 13]. Although it is known that both GFR and renal ODC activity are increased acutely by a single protein meal, the temporal relationship between the change in GFR and the change in ODC activity after increased dietary protein ingestion is not defined. In other experimental models where renal hypertrophy occurs, renal growth is proportional to the increase in single nephron GFR (SNGFR) [14, 15]. Increased tubular reabsorption of solute that occurs as a consequence of the increased GFR [16] has been proposed to be the stimulus for renal growth. If this postulate can be extended to the renal hypertrophy that follows increased protein consumption, then renal ODC activity should increase following or in parallel with the rise in GFR caused by supplemented dietary protein intake, and should preceed the increase in renal mass. Sequential measurements of changes in both GFR and renal ODC activity might therefore reveal a relationship between the renal hemodynamic alterations occurring after an increase in protein intake and the initiation of renal growth.

Arginase (EC 3.5.3.1) is present in large concentration in the kidney [17], second only to liver in activity, and might also play a growth regulatory function, since the product of its reaction, ornithine, is the substrate for ODC. Arginase activity increases in both liver and kidney when rats are fed following a 24 hour fast [18], and is increased in the kidney of the chick fed purified crystalline amino acid diets [19]. Thus changes in renal arginase activity might occur also at the time of rapid renal growth and might increase in response to dietary protein content. Hepatic arginase activity is quite sensitive to dietary protein intake, increasing when protein intake is high [20], and decreasing when protein intake is low [20–22]. The relationship between renal arginase activity and dietary protein intake in mammals is unknown, as are the temporal relationships between increasing GFR, changes in the activity of renal ODC and the renal growth that occur subsequent to an increase in dietary protein. We therefore measured sequential changes in GFR, renal ODC and arginase activities, and renal mass in normal male rats after an increase in dietary protein intake.

Methods

Protocol 1

Male Sprague-Dawley rats (120 g) were obtained from Bantin Kingman Farms (Fremont, California, USA) and kept in temperature regulated, standard 12-hour light/dark cycled rooms. All animals were fed Purina purified protein diet 5769 (Ralston

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Table 1. Change in glomerular filtration rate and in renal ornithine decarboxylase and arginase activities in rats after an increase in dietary protein intake

Time *hrs*	0	24	48	72	96	168
Dietary protein increased from 8.5% to 40% (HP)						
Glomerular	1.17	1.76[a,e]	2.03[a,d]	2.05[a,d]	1.86[a,e]	ND
filtration	±0.11	±0.13	±0.19	±0.15	±0.15	
ml/min	*N* = 14	*N* = 14	*N* = 13	*N* = 14	*N* = 12	
Ornithine	9.87	28.1[c]	170.4[c]	28.6	ND	25.4[c,f]
decarboxylase	±1.8	±4.42	±35.0	±8.0		±5.1
activity	*N* = 4	*N* = 7	*N* = 7	*N* = 4		*N* = 3
U/g kidney						
Arginase	ND	18.1	15.6	ND	ND	18.1
activity		±2.2	±1.76			±1.33
U/g kidney		*N* = 7	*N* = 7			*N* = 3
Protein	143	149	156	ND	156	
mg/g kidney	±9.2	±3.9	±8.4		±2.9	
N	4	5	5	5	5	
Protein	68	82	80	ND	109[a,d]	
mg/mg DNA	±2	±7	±4		±5	
Kidney weight	0.83	0.87[f]	0.92[c,d]	1.0[c]	0.98[c,d]	0.92[f]
% body weight	±0.03	±0.03	±0.01	±0.04	±0.04	±0.05
Body weight	172	180	177	182	207	196
g	±6	±5	±5	±6	±7	±15
Kidney weight	1.42	1.56	1.62[b,f]	1.81[b]	2.02[a,d]	1.79[a,d]
g	±0.04	±0.04	±0.01	±0.1	±0.07	±0.04
Dietary protein intake maintained at 8.5% (LP)						
Glomerular	1.13	1.08	1.05	1.05	1.09	ND
filtration	±0.07	±0.09	±0.07	±0.06	±0.06	
ml/min	*N* = 14	*N* = 11	*N* = 11	*N* = 14	*N* = 14	
Ornithine	9.87	4.07	15.56	ND	ND	5.09
decarboxylase	1.8±	±3.27	±2.54			±1.58
activity	*N* = 4	*N* = 3	*N* = 3			*N* = 3
U/g kidney						
Arginase	ND	21.03	13.96	ND	ND	5.09
activity		±3.26	±2.04			±1.58
U/g kidney		*N* = 3	*N* = 3			*N* = 3
Protein	143	160	148	ND	150	
mg/g kidney	±9.2	±13.3	±2.7		±4.7	
Protein	68	76	74	ND	75	
mg/mg DNA	±2	±4	±3		±4	
Kidney weight	0.83	0.77	0.79	ND	0.68[c]	0.70[c]
% body weight	±0.03	±0.02	±0.02		±0.03	±0.03
Body weight	172	172	181	ND	201	184
g	±6	±11	±11		±3	±13
Kidney weight	1.42	1.33	1.41	ND	1.37	1.28
g	0.04	±0.11	±0.07		±0.06	±0.06

Dietary protein intake was increased from 8.5% to 40% in HP, but remained unchanged in LP. GFR (the renal clearance of [^{51}Cr]EDTA) was continuously monitored. Several animals were killed at times indicated for measurement of renal ornithine decarboxylase and arginase activity and kidney weight. Results are means ± SE, *N* represents the number of measurements at each time point. ND indicates not measured.

[a] $P < 0.001$ compared to time 0
[b] $P < 0.005$ compared to time 0
[c] $P < 0.025$ compared to time 0
[d] $P < 0.001$ compared to low protein control animals
[e] $P < 0.005$ compared to low protein control animals
[f] $P < 0.05$ compared to low protein control animals

Purina, Richmond, Indiana, USA) containing 8.5% protein as casein ad libitum for a period of 7 to 14 days. Rats were then placed in metabolic cages for basal collections of urine for a period of 48 hours, during which time they remained on the low protein diet. The food was mixed with water to form a slurry. Following this 48 hour period, rats were either switched to Purina diet 5779 containing 40% protein as casein (HP), or remained on the 8.5% protein diet as time controls (LP). The two diets were isocaloric and contained identical quantities of Na, K, P, and fat. The dietary alteration was accomplished by an isocaloric replacement of carbohydrate—a 50/50 mixture of dextrose and dextrin, with casein. Fifty animals were switched to the high protein diet (HP). Forty-one animals remained on the 8.5% diet as low protein time controls (LP) and eight animals were killed at the end of the baseline collection as time zero controls. Glomerular filtration rate was measured in 14 animals in each dietary group studied in this protocol. Data from this protocol appear in Table 1 and Figure 1. Eight to 17 animals were killed with an intraperitoneal injection of sodium pentobarbital at time 0, at 24, 48, 72, and at 96 hours, and three animals from each group were killed at one week (168 hours) for measurement of renal arginase and ODC activities and for

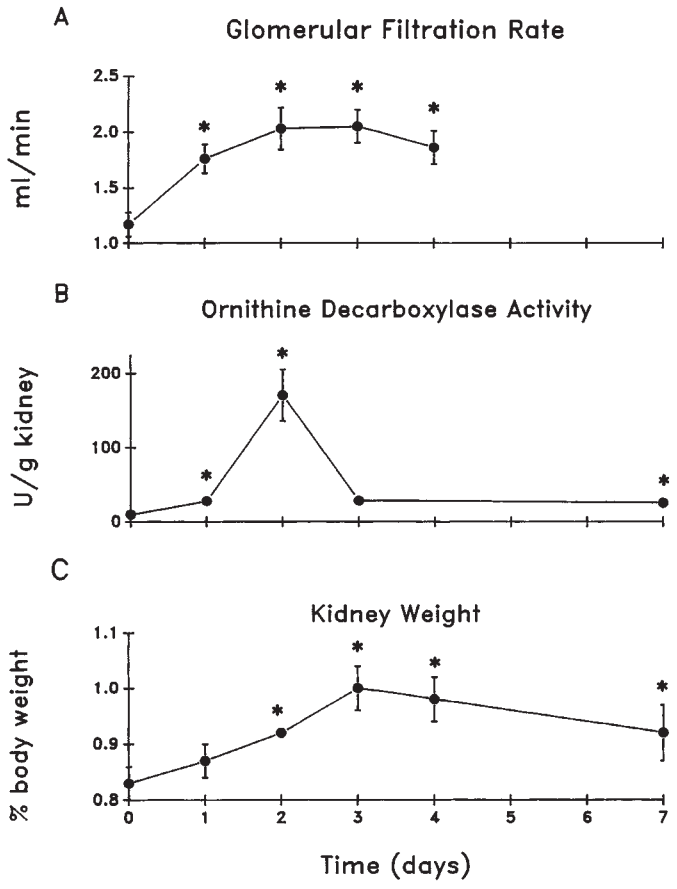

Fig. 1. *Sequential changes in GFR (renal clearance of [^{51}Cr]EDTA), renal ornithine decarboxylase activity, and fractional renal weight that occur in rats after dietary protein intake is increased from 8.5% to 40%.* The bracketed points depict mean values ± SEM. * connotes a value that is significantly greater ($P < 0.05$) than that at time zero.

measurement of renal protein and DNA content. All animals were killed between 9 and 11 a.m. The postprandial state was confirmed in each rat by demonstration of food in the stomach at autopsy. Kidneys were removed, decapsulated, weighed and homogenized separately (right and left). One kidney (left or right chosen randomly) was assayed for ODC activity [23] and the other was assayed for arginase activity [10]. Kidneys from four to five rats in each dietary group were analyzed for protein and DNA content at times 0, 24, 48, and 96 hours.

Protocol 2

In addition, GFR was measured for 12 consecutive days in an additional 10 rats, 5 of which were maintained on the low protein diet and 5 of which were switched to the high protein diet for the last 10 days during which GFR was measured. These latter studies were performed in order to assure that changes in GFR occurring within the first four days following dietary protein augmentation were not transient.

Measurement of GFR

Glomerular filtration rate was continuously monitored from 48 hours prior to and for up to 10 days after the change in diet. An osmotically driven microinfusion pump (Alza Corp., Palo Alto, California, USA) was loaded with 350 μCi to 1 mCi of [^{51}Cr]Ethylenediaminetetraacetic acid (EDTA; NEN, Boston, Massachusetts, USA). The chromium was obtained as the chloride and was reacted with excess sodium EDTA at pH 6.0 to form the chromium chelate. The pumps—0.2 cc volume—were inserted subcutaneously in the back of the neck immediately prior to the first baseline urine collection while animals were all eating the 8.5% protein diet. Blood (250 μl) was obtained daily, between 9 and 11 a.m., from a tail vein without anesthesia. GFR was calculated daily by dividing total 24-hour urine counts by serum counts, factored for time of collection. Samples were counted in a gamma counter (Searle 1185, Searle Analytics Inc. Des Plaines, Illinois, USA). Aside from the use of [^{51}Cr]EDTA, the clearance technique employed here is identical to that described by Jobin and Bonjour [24].

Enzyme assays

Arginase was assayed as previously described [17]. Briefly, the kidneys were weighed and then homogenized in 9 volumes (vol/wt) of 0.25 M sucrose - 0.01 M Tris-HCl (Sigma Chemical Co., St. Louis, Missouri, USA), pH 7.5, using a teflon motor-driven homogenizer of the Potter-Elvehjim type. Ten up and down strokes were used. Manganous chloride, 2 M, was added to a final concentration of 0.01 M, and the homogenate was vortexed and allowed to stand overnight at 4°C to insure complete activation. Enzyme activity increases to a plateau within 12 hours and ceases to change thereafter. To assay arginase in the homogenate, 100 μl of enzyme solution containing 1 μmol of $MnCl_2$ and 1 μmol of Tris-HCl, pH 9.5, was added to 0.9 ml of a solution containing 100 μmol of arginine (Sigma) at pH 9.5. The final volume was 1 ml, and the final concentration of each reagent was 0.001 M $MnCl_2$, 0.001 M Tris-HCl, 0.1 M arginine. Samples were incubated at 37°C for 10 minutes. The pH remained constant at 9.5 during this period. The reaction was terminated by addition of 1 ml of 10% trichloroacetic acid (TCA) and the sample centrifuged to remove precipitated protein. A volume of 2.5 ml of Erlich's reagent (0.4 M p-dimethylamino-benzaldehyde in 3.6 N H_2SO_4) was added to 1 ml of the supernatant. Absorbance at 450 nm was determined after 20 minutes. Correction was made for the contribution of arginine to the color value. All assays were carried out in triplicate. A unit of enzyme activity is defined as that amount of arginase necessary to produce 1 μm of urea per minute at pH 9.5 at 37°C at a concentration of arginine of 100 mM.

Ornithine decarboxylase activity was determined by the method of Seely and Pegg [23]. The kidneys were removed, decapsulated and finely chopped with scissors. All procedures were performed on ice. The chopped tissue was suspended in a buffer containing 67 mM Na phosphate pH 7.2, 1 mM EDTA, 1 mM dithiothreitol (DTT), and homogenized with a Potter-Elvehjim teflon homogenizer. The homogenate was centrifuged at 100,000 g for 20 minutes at 4°C in a Beckman L5-65 ultracentrifuge. The supernatant was assayed for ODC activity. Assays were carried out in 25 ml Ehlermeyer flasks or in disposable glass tubes. The reaction vessel was sealed with a rubber stopper through which was suspended a polypropylene well (Kontes Glass Co., Vineland, New Jersey, USA) containing 0.5 ml of a solution of methanol and phenylethylamine in a ratio of 1:2. The reaction mixture contained a total volume of

0.25 ml, consisting of 5 μl of 20 mM L-ornithine, 10 μl of L-[1-^{14}C] ornithine (Amersham, Arlington Heights, Illinois, USA), 55 mCi/mmol (0.1 μCi of ^{14}C ornithine was used per assay flask), 5 μl of 2 mM pyridoxol 5'-phosphate, 12.5 μl of 25 mM DTT (both prepared fresh), and 2.5 μl of 0.05 M Tris pH 7.5 [24]. The reaction was started by the addition of 0.215 ml of supernatant and was incubated in a shaking temperature controlled bath for 30 minutes at 37°C. The reaction was stopped by injection of 0.83 ml of 3.6 N H_2SO_4 through the stopper. The acidified reaction mixture was allowed to stand overnight at room temperature in order to trap all liberated $^{14}CO_2$. The polypropylene well was placed in a 10 ml vial and mixed with PCS scintillation fluid (Amersham) and counted in a Searle Model 6880 β counter (Searle Co., Chicago, Illinois, USA). A unit of activity was defined as the amount of enzyme releasing 1 nmol of $^{14}CO_2$ per 30 minutes at 37°C [18]. All assays were performed in triplicate and corrected for blanks incubated without enzyme.

Kidney protein was determined by the method of Lowry et al [25] and kidney DNA was determined colormetrically using diphenylamine [26].

Statistics

Comparison between groups was by Student's unpaired *t*-test. Comparison within groups was by paired *t*-tests with the Bonferoni adjustment when multiple comparisons were made [27]. Results are presented as the mean ± the standard error of the mean.

Results

In the animals in protocol 1 changed to a high protein diet, GFR increased significantly at 24 hours from 1.17 ± 0.11 ml/min to 1.76 ± 0.13 ml/min ($P < 0.001$), and at 48 hours, to 2.03 ± 0.19 ml/min ($P < 0.001$). GFR at 48 hours was significantly greater than at 24 hours ($P < 0.005$). GFR did not change thereafter (Fig. 1, Table 1). GFR did not change in the rats that remained on the low protein diet (time controls). GFR was significantly greater in the HP animals compared to LP at 24 hours, and remained significantly greater throughout the 96 hour period of measurement (Table 1). Similarly, in animals studied in protocol 2, GFR increased significantly at 24 hours, from 1.04 ± 0.07 ml/min to 1.30 ± 0.07 ml/min ($P < 0.03$), was increased further at 48 hours to 1.69 ± 0.17 ml/min ($P < 0.02$ vs. day 0), was 1.82 ± 0.17 ml/min at 72 hours ($P < 0.005$ vs. day 0), was 1.68 ± 0.08 ml/min at 96 hours ($P < 0.005$ vs. day 0), and at ten days was 1.63 ± 0.12 ml/min ($P < 0.005$ vs. day 0). There was essentially no variation in GFR between 96 hours and ten days. GFR was unchanged in animals maintained on the low protein diet and after 24 hours was significantly less than GFR in the animals switched to the high protein diet.

In animals studied in protocol 1, renal ODC activity increased significantly at 24 hours in the HP animals (Fig. 1, Table 1) and increased further, to a sharp peak at 48 hours. At 72 hours, ODC activity returned to the level of activity measured at 24 hours, but only 17% of the peak value, and remained at that increased level of activity at 7 days. Ornithine decarboxylase activity did not change in the LP time control animals (Table 1).

Kidney weight in the HP group, relative to body weight, began to increase only after 48 hours and reached a plateau at 72 hours, one day after the maximal increase in GFR and after the peak in ODC activity (Table 1).

Arginase activity did not vary either with time or with changes in dietary protein intake (Table 1).

Total protein per kidney increased significantly, from 210 ± 15 mg/kidney to 310 ± 8 mg/kidney by 96 hours after initiation of dietary protein augmentation. The amount of protein per gram of kidney weight, however, was not affected by diet. In contrast, total kidney DNA did not increase following dietary protein augmentation, so that by 96 hours, the protein/DNA ratio was significantly increased in the animals fed the high protein diet (108.8 ± 4.5 vs. 67.8 ± 1.7 at time 0, $P < 0.0002$ and vs. 75.0 ± 3.5 in animals maintained on the low protein diet, $P < 0.001$). There was no change in kidney weight, kidney protein or kidney DNA in the LP animals.

Discussion

Polyamine levels are elevated in tissues during periods of rapid growth [9–11], such as during embryogenesis or during compensatory hypertrophy. The rate limiting enzyme for the generation of these amines is ODC [9–11], which generates putrescine, a precursor of spermidine and spermine, from ornithine. The latter is an amino acid generated by the action of arginase on arginine. The content of arginase in kidney is second only to that in the liver [17, 18] and is not homologous to the hepatic isozyme [17, 28, 29], an enzyme that plays a pivotal role in the urea cycle. Since renal arginase activity, as well as ODC activity have been reported to increase in the rat after ending a total fast [12, 18], it was reasonable to postulate that the renal isozyme of arginase might have been responsible for providing substrate for ODC in response to increased dietary protein intake and would therefore be expected to increase in activity following an isocaloric increase in dietary protein intake. Since the activity of renal arginase did not change, we feel that such a hypothesis is unlikely. The absence of an increase in renal arginase activity suggests that renal ornithine generation is not a limiting factor in the regulation of renal polyamine metabolism.

Renal ODC activity is increased after animals are exposed to a variety of agents or perturbations that promote renal growth, such as administration of androgens [30], induction of experimental diabetes mellitus [31], following unilateral nephrectomy [32, 33], or the refeeding of amino acids to fasted animals [12, 13]. We observed that renal ODC activity is significantly increased by 24 hours after dietary protein augmentation, and continues to increase to a peak value at 48 hours. ODC activity subsequently declines, but remains elevated compared to animals maintained on a low protein diet. The increase in renal ODC activity is paralleled by a corresponding increase in GFR both at 24 and at 48 hours. GFR remains elevated after 48 hours, but once GFR ceases to increase, renal ODC activity declines. The temporal relation between the change in ODC activity and the change in GFR suggests that increasing GFR, or some consequence of increasing GFR, such as augmented renal reabsorption of solute [16, 34, 35], may be one trigger for the increase in ODC activity.

The temporal relationships that we observed differ somewhat from what occurs following either a single protein containing meal, administration of amino acids after fasting, or following partial renal ablation. Renal ODC activity increases only tran-

siently, and within only four hours following administration of either a single meal to previously fasted animals or following parenteral administration of amino acids [12, 13]. However, GFR also increases within only a few hours after these stimuli, and the increase in GFR, like the augmentation in renal ODC activity, is not sustained [36, 37]. Similarly, following unilateral nephrectomy, renal ODC activity is maximal at six to nine hours after nephrectomy and decreases again by 24 hours [32, 33], a pattern similar to that observed following a single protein meal. In the uninephrectomy model, the increase in ODC activity may precede the increase in whole kidney GFR that has occurred by 24 to 48 hours post-nephrectomy [38]. However, the early events following uninephrectomy are not well characterized, and the precise temporal relationship between the change in GFR and the increase in renal ODC activity following nephrectomy is not known. In rats undergoing partial renal infarction of a solitary kidney, GFR expressed as a function of the weight of viable kidney increases significantly within only 160 minutes of infarction [39]. Thus, it is possible that early small increases in GFR or single nephron GFR may trigger the early rise in ODC activity following reduction in renal mass.

One possible explanation for the differences in the temporal sequence of renal ODC activity that we observed in animals switched to a high protein diet is that the experimental technique we used was somewhat different than that previously described for either protein refeeding [12, 13] or unilateral nephrectomy [32, 33]. The stimulus we employed was a continuous one rather than a single meal. Additionally, dietary protein intake was increased in animals already consuming a diet that contained sufficient amino acids and calories to allow growth.

Despite the differences in the experimental design and the differences in the temporal relationship between the change in renal ODC activity and the inciting stimulus (uninephrectomy vs. dietary protein augmentation), we observed renal hypertrophy four days after augmenting dietary protein intake and renal hypertrophy occurred three days following nephrectomy. Thus the renal growth occurring in response to each of these perturbations exhibited a similar time course. Since ODC is the rate limiting step for the synthesis of growth regulatory polyamines, it would seem logical to propose that an increase in the activity of ODC in response to a stimulus resulting ultimately in renal hypertrophy might be causally related to the hypertrophy. However, when the increase in ODC activity after uninephrectomy was prevented by treatment with difluoromethylornithine, compensatory hypertrophy was not prevented [33]. Thus compensatory renal hypertrophy is not dependent on an increase in ODC activity, at least in the case of unilateral nephrectomy. However the increases in renal ODC activity may be linked to the augmentation of GFR observed both in the renoprival model and in animals fed a high protein diet.

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