Inhibition of Inorganic Phosphate Transport by Amino Acids in Rat Kidney-Cortical Slices *in vitro*.* (31766)

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The mechanisms controlling renal excretion of inorganic phosphate (P_i) are complex. Although the role of parathyroid hormone is well known(1) the effects of vitamin D are poorly understood since the response *in vivo* to vit D administration at moderate doses may be to enhance tubular reabsorption whereas at high doses a phosphaturic response is observed(2). Under certain experimental conditions *in vivo*, the tubular reabsorption of phosphate may be decreased following administration of p-amino hippurate(3), bicarbonate(4), acetoacetate(5), and glucose(6,7). Infusions of amino acids have a similar effect (8,9). The selective inhibition of tubular reabsorption of P_i by the L-enantiomorph of a neutral amino acid and not by the corresponding D-enantiomorph and the lack of an effect by basic amino acids suggested stereospecificity of this inhibitory effect(9). Furthermore, indole decreases the uptake of P_i by Ehrlich ascites cells(10).

The present studies were undertaken in an attempt further to define the role of amino acids in regulating the uptake of P_i by rat kidney cortical slices.

Materials and methods. (^{32}P) P_i (carrier free) and (^{3}H-methoxy) inulin were obtained from New England Nuclear, L-(U-^{14}C) alanine and D-(1-^{14}C) alanine were obtained from Nuclear-Chicago and were chromatographically pure by ascending paper chromatography (butanol-acetic acid-H_2O, 4:1:2, V/V). Non-radioactive amino acids were purchased from Nutritional Biochemicals. Glycylglycylglycine was purchased from Mann Research Laboratories. The 2,4-dinitrophenol was obtained from Eastman Chemical.

Male Sprague-Dawley rats weighing 200-300 g were used in all experiments. Techniques used in the preparation and incubation of kidney cortex slices and determination of total tissue water and extracellular space have been described(11,12). Two tissue slices weighing a total of 60-80 mg, were incubated aerobically (O_2:CO_2, 95:5) in modified Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C, 60 min. The Ca^{2+} and P_i concentrations in all media were 1.25 and 1.17 mM/l respectively. All tissue slices were preincubated in the control media at 23°C for 10 minutes, lightly blotted and carefully transferred to the incubation media containing (^{32}P) P_i (.2-.6 μc/incubation) and the non-radioactive amino acid as described for each experiment. A minimum of 5 incubations was performed for each determination. At the conclusion of each incubation, the flasks were placed quickly in an ice-water bath and the free intracellular P_i pool was extracted with boiling distilled water. Aliquots (200 μl) of this aqueous extract and the remaining medium were each transferred to aluminum planchets and counted in a gas-flow detector (Nuclear-Chicago) with an efficiency of 15% for the system used. A sufficient number of counts were recorded for each sample to insure a maximum of 2% counting error.

Results are expressed as the distribution ratio which is the ratio of cpm/ml intracellular H_2O:cpm/ml extracellular H_2O.

Results. The ability to inhibit accumulation of (^{32}P) P_i in kidney cortical slices by incubation at room temperature (Fig. 1) and by varied concentrations of dinitrophenol (Table I) support the utilization of the present techniques to demonstrate active transport of P_i *in vitro*.

The addition of varying concentrations to the incubation mixture of the L-isomers of leucine, lysine, arginine, phenylalanine, tryptophan or of glycine markedly inhibited the net transport of (^{32}P) P_i (Fig. 2 and 3).

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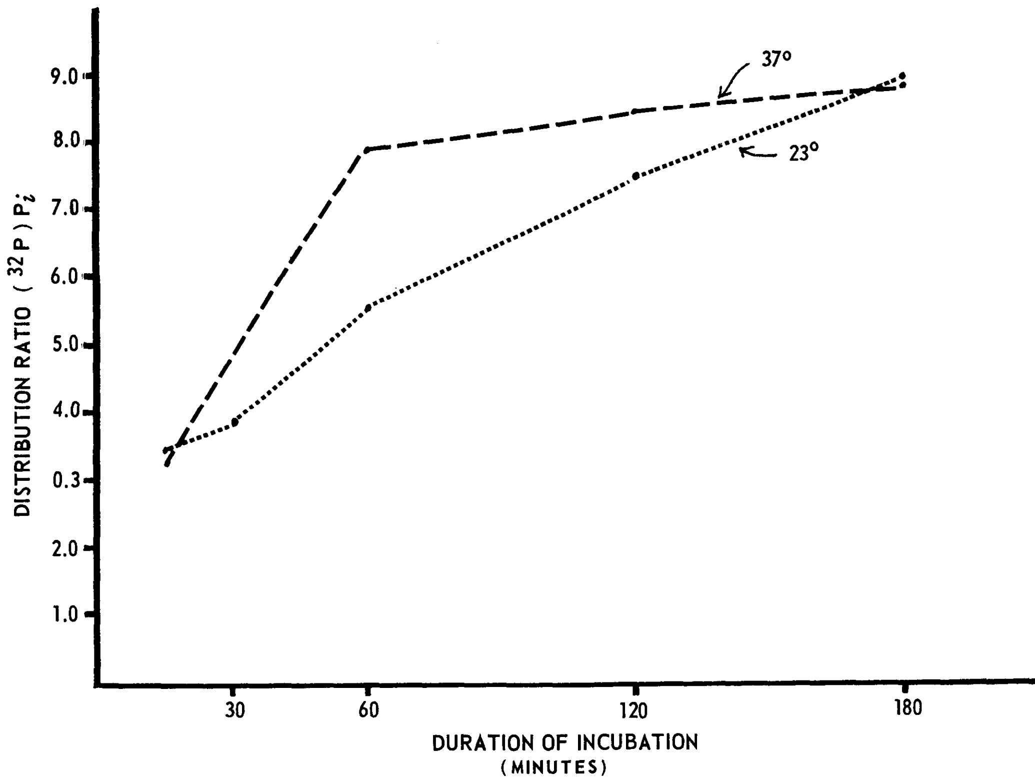


FIG. 1. Effect of temperature on net accumulation of (^{32}P) P_i in rat kidney cortex slices. Each point represents mean distribution ratio of 5 incubations.

TABLE I. Inhibition of (^{32}P) P_i Accumulation in Rat Kidney Cortex Slices by 2, 4-Dinitrophenol.

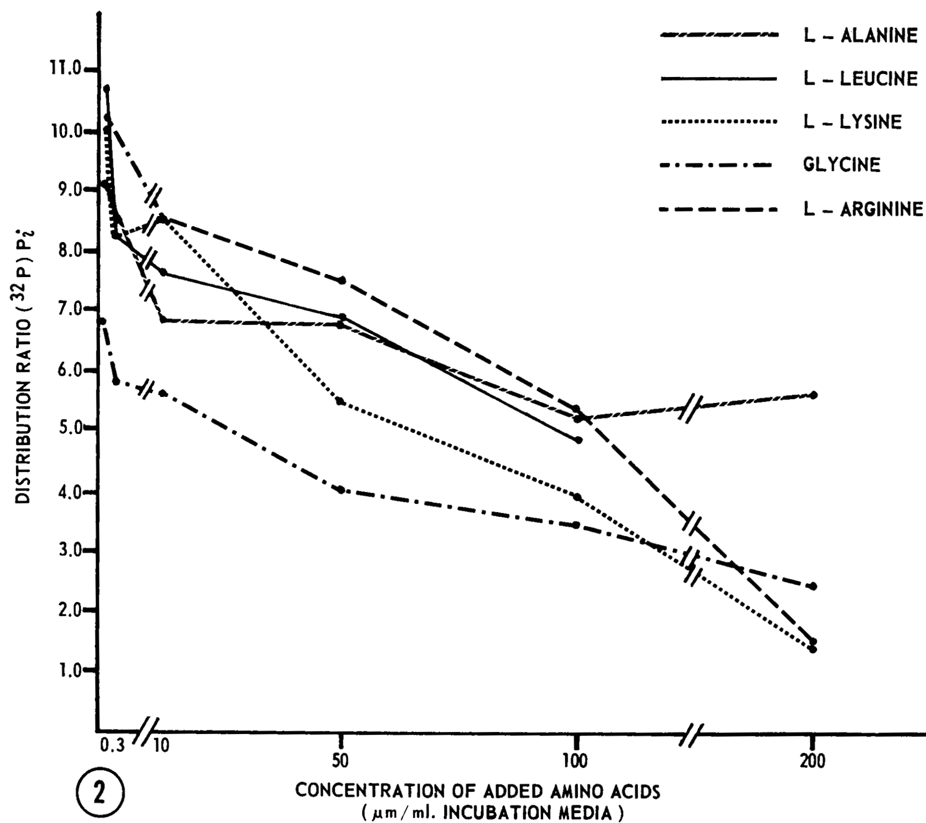
	Distribution ratio†	Significance
Control*	8.58 ± .72	
2, 4-Dinitrophenol*		
5 × 10^{-5} M	4.34 ± .75	<.001
1 × 10^{-4}	4.28 ± .41	
2 × 10^{-4}	1.74 ± .37	

* 5 incubations at each concentration.
† Mean ± standard deviation.

When this phenomenon was observed at varying intervals of time utilizing 10 μmoles/ml of L-alanine, significant inhibition was noted at 60, 120, and 180 minutes ($p<.025$, $<.025$, and $<.05$, respectively) (Fig. 4). However, when the concentration of L-alanine was increased to 100 μmoles/ml, inhibition was more pronounced when compared with the controls at all intervals (15, 30, 60, 120 min, $p<.025$, $<.005$, $<.001$, $<.001$, respectively). Although the data are insufficient to quantitate the relative potency of inhibition by each of the amino acids tested, L-tryptophan seemed to be the most effective inhibitor at all concentrations tested.

In a further attempt to define the specificity of the inhibition by amino acids of P_i transport, the tripeptide, glycylglycylglycine was added to the incubation media (Fig. 3). No significant decrease in net (^{32}P) P_i transport was observed until the concentration of the tripeptide was increased to 100 μmoles/ml ($p<.001$). The inhibitory effect at this concentration may have resulted from hydrolysis of the tripeptide to the monomer unit with secondary inhibition resulting from the accumulation of the latter.

Although previous studies from this laboratory(4) demonstrated that the inhibition of tubular reabsorption of P_i in the intact kidney by amino acid infusion was stereospecific, the present studies with rat kidney cortex slices do not differentiate between the effects of D- and L-amino acids (Fig. 5). Thus, although there was a greater inhibitory effect



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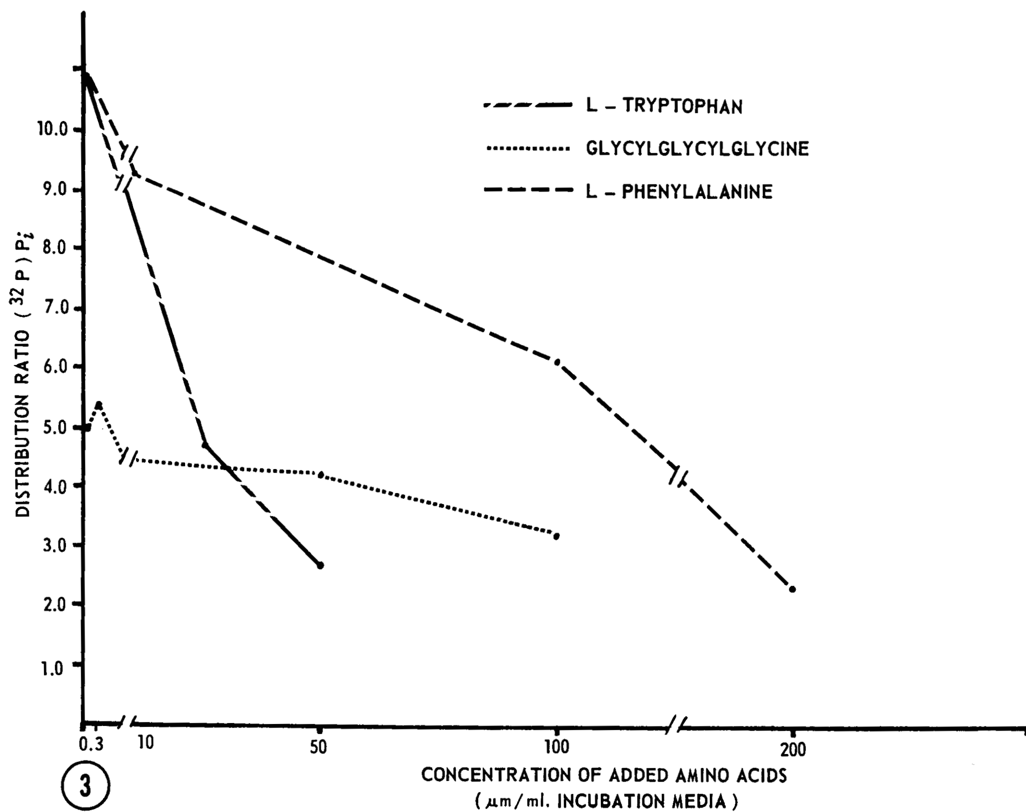

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FIG. 2. and 3. Effect of varied concentrations of L-amino acids on net accumulation of (^{32}P) P_i. Each point represents mean distribution ratio of at least 5 incubations.

with L-alanine than with D-alanine at a concentration of 50 μmoles/ml ($p < .025$), the same concentration of D-leucine was more inhibitory than L-leucine ($p < .005$). Furthermore, lower concentrations of the L- and D-isomers of alanine and leucine were equally inhibitory (Table II).

In order to relate the inhibition by L- and D-amino acids of P_i accumulation in rat kidney slices to the net transport of the amino acids, tracer quantities of L- and D-(^{14}C) alanine were incubated with rat kidney slices in a system similar to that described above, and the distribution ratios of the amino acids and their metabolites were compared. After 45 minutes of incubation, the distribution ratios of radioactivity from L- and D-(^{14}C) alanine were 2.73 $\pm$ .41 and .61 $\pm$ .10, respectively. Hence, there was no net transport of D-alanine. Ascending paper chromatography of the intracellular water extracts of the L-(^{14}C) alanine incubation revealed 3 distinct radioactive peaks, one of which had an Rf similar to that of alanine. However,

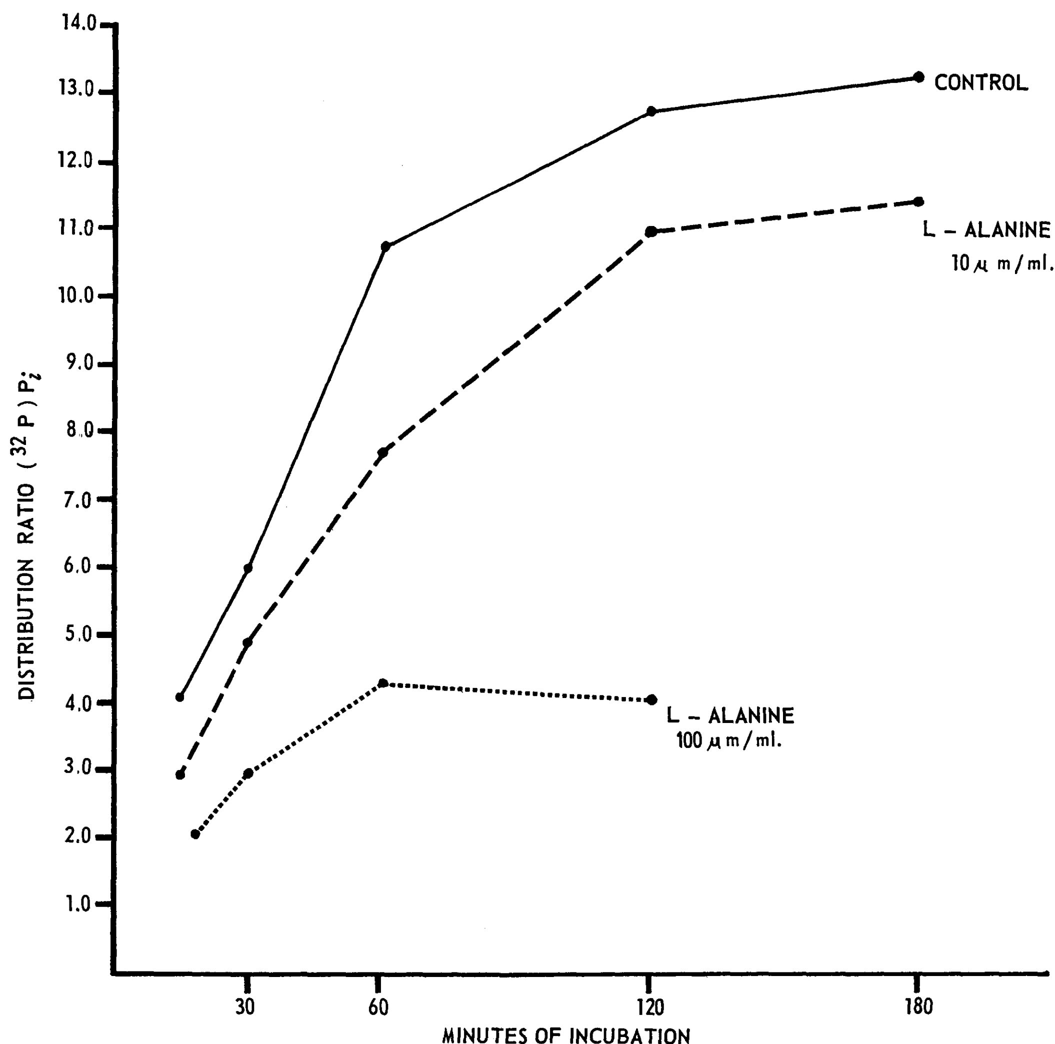

FIG. 4. Inhibition of net (^{32}P) P_i accumulation by L-alanine (10 μm/ml, 100 μm/ml) over time in 2 experiments. Each point represents mean distribution ratio of 5 incubations.

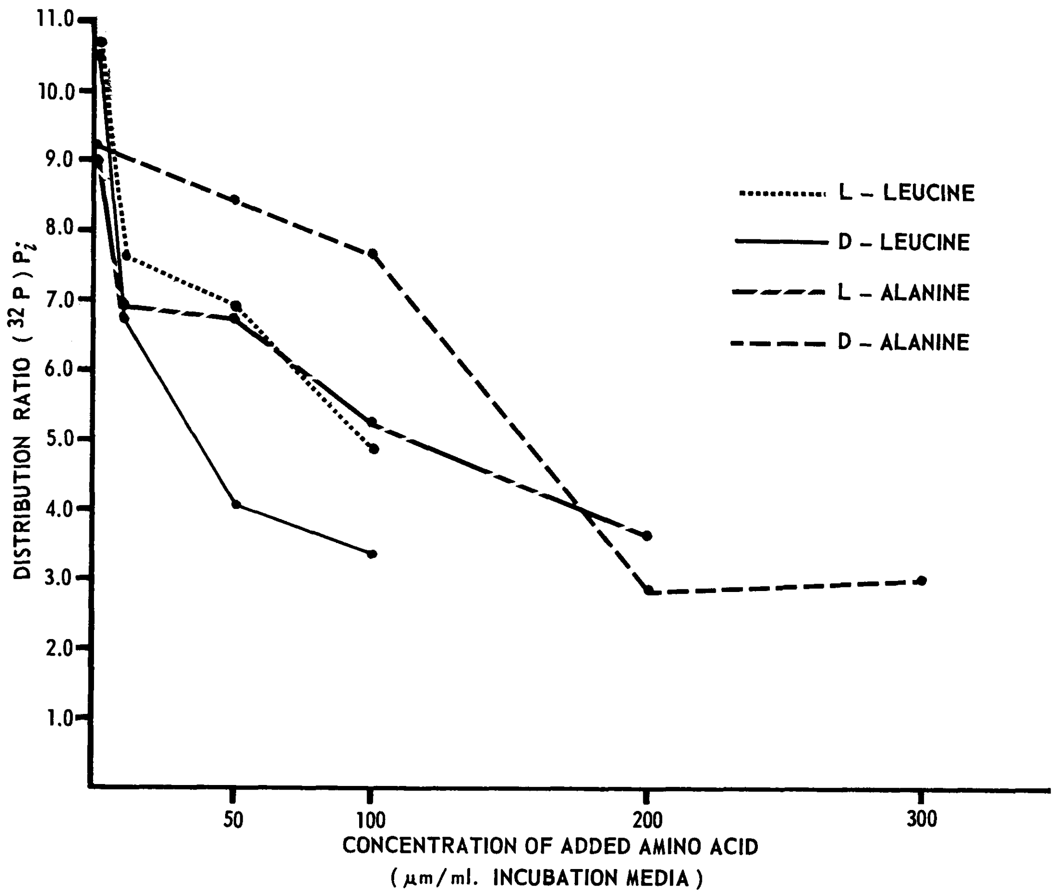

FIG. 5. Relative effects of L- *vs.* D-alanine and leucine on net (^{32}P) P_i accumulation. Each point represents mean distribution ratio of 7 incubations.

chromatograms of the extract from the D-(^{14}C) alanine incubation had only a major peak (>90%) corresponding with alanine along with another more indistinct peak. Chromatography of the media from both incubations revealed only alanine. Further identification of the metabolites from the L-(^{14}C) alanine incubation by ion exchange

TABLE II. Effect of Small Concentrations of Added L- and D-Amino Acids on (^{32}P) P_i Accumulation.

	Distribution ratio‡ (L-amino acid)	Significance	Distribution ratio (D-amino acid)	Significance
Control	8.38 ± 1.69		8.54 ± 1.26	
Alanine,* .038 µM/ml	7.35 ± 1.40	>.05	8.02 ± 1.03	>.05
.075	7.53 ± 1.47	>.05	8.14 ± 1.19	>.05
.15	6.31 ± .96	<.025	6.83 ± .92	<.025
.30	6.21 ± 1.08	<.025	6.30 ± .78	<.025
1.0	4.20 ± .35	<.005	4.78 ± .50	<.005
5.0	4.58 ± .40	<.005	4.35 ± .43	<.005
Control	6.14 ± .67			
Leucine,† .3 µM/ml	5.79 ± .79	>.05	5.82 ± .94	>.05
3.0	4.79 ± .85	<.025	4.78 ± .89	<.025
7.0	4.65 ± .81	<.025	5.06 ± .62	<.025

* 5 incubations at each concentration. † 6 incubations at each concentration. ‡ Mean ± standard deviation.

chromatography(13)§ indicated the presence of alanine and aspartate or glutamate.

Discussion. In contrast with the stereospecific inhibition of tubular reabsorption of P_i by the infusion of neutral amino acids observed in previous studies(4), the present investigation revealed significant inhibition of the net transport of (^{32}P) P_i by both L- and D-enantiomorphs of neutral amino acids as well as by aromatic and polar amino acids.

Other studies utilizing rat kidney cortex slices(1) have demonstrated an inhibitory effect of D-galactose, D-fructose and D-glucose on the accumulation of α-amino isobutyric acid, glycine, cycloleucine and valine. No effect on uptake of histidine, lysine or phenylalanine was observed. The same authors(7) later confirmed these observations with infusions of glucose, galactose, and fructose into human subjects who had significant increases in the excretion ratios of P_i and alpha-amino nitrogen. These studies suggested that the inhibition was due to a toxic effect of the hexoses or their metabolites in the renal tubular transport system.

The present report supports the suggestion by Fox *et al*(7) as to the role of toxic interference by simple metabolizable substances with kidney tubular reabsorptive mechanisms and not by a competition at a common transport site. Thus, despite interference with accumulation of (^{32}P) P_i by both L- and D-amino acids, isotopic studies with the corresponding (^{14}C) amino acids could demonstrate no net transport of the D-isomer and a correspondingly diminished metabolic degradation. The preference of many metabolic systems for the L-rather than the D-isomer of amino acids also has been demonstrated in studies of the transport systems of the Ehrlich ascites tumor cells(14) and in the brain(15). The relationship of renal toxins to abnormalities in renal reabsorptive mechanisms of amino acids, sugars, and phosphate has been well documented(16).

§ Performed in the Laboratory of Dr. Richard Von Korff, Dept. of Biochemistry.

Summary. The net accumulation of (^{32}P) inorganic phosphate in rat kidney cortex slices *in vitro* is inhibited by addition to the incubation mixture of L- or D-amino acids. In contrast with L-(^{14}C) alanine, there was no net transport of D-(^{14}C) alanine. Possible mechanisms of the inhibitory effect are discussed.

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