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# The Origin and Turnover of D-Serine in Brain

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**The origin of D-serine was investigated using microdialysis probes to administer radiolabeled glucose, glycine, and L-serine directly into rat brain. In these experiments the labeling of D-serine was found to be determined only by the radioactivity present in the L-serine pool, regardless of the precursor employed, indicating that L-serine is the direct precursor of the D-isomer. Its rate of synthesis was 4.6 ± 1.2 %/h; 9.2 nmol/g/h). This rate of synthesis is in agreement with that found in the mouse after a loading dose of intraperitoneally injected L-[$^3$H]-serine (4.1 %/h). These rates are also consistent with the degradation rates of D-serine in rat and mouse brain, determined in pulse labeling experiments (4.1 and 3.8 %/h, respectively). Synthesis within the brain from L-serine therefore is adequate to account for the turnover of the brain D-serine pool: contributions from other sources, including the diet, must be minimal. Independence from dietary sources was also demonstrated by the failure of labeled D-serine administered in the drinking water to label the brain pool unless very high doses were given. These results suggest that D-serine in the brain is formed directly by the racemization of L-serine.** 

D-serine is present in mammalian brain at a concentration of 0.2 to 0.3 $\mu$mol/g (1). Its distribution within the brain correlates with the distribution of NMDA receptors (2). More recent studies have shown that D-serine is localized to particular cell types and to definite brain regions (3, 4). These results strongly suggest that D-serine plays a physiological role as a co-agonist (5) at the NMDA excitatory amino acid receptor, where it acts at the strychnine-insensitive glycine modulatory site (6). D-serine, injected directly into the brain, has been shown to have behavioral effects in animal models including the inhibition of the abnormal activity induced by phencyclidine (7, 8). The ability of D-serine to reverse the behavioral effects of phencyclidine has led to the suggestion that that D-serine might be useful in the treatment of schizophrenia (7). This may be the case even though the concentration of D-serine is not depressed in schizophrenic brain (9)

In the present report we are concerned with the origin and turnover of D-serine in rat and mouse brain. The rate of degradation was determined after labeling the D-serine of brain with intraperitoneally injected D-[$^3$H]-serine. Synthesis rates were obtained both from an intraperitoneally injected load of L-[$^3$H]-serine and from the rate of labeling of D-serine following the introduction of various radioactive precursors directly into brain using microdialysis probes. From the data obtained in these experiments we conclude that the turnover rate of D-serine in rodent brain is about 4 %/h and that the immediate precursor of D-serine is the L-isomer.

## MATERIALS AND METHODS

*Materials.* Radioactive precursors were obtained from American Radiolabeled Chemicals, New England Nuclear, and Amersham. Amino acids and D-amino acid oxidase (porcine, Type II) (EC 1.4.3.3) were purchased from Sigma. N-tert.-butyloxycarbonyl-L-cysteine (t-BOC) came from Calbiochem, solvents from Fisher and J. T. Baker and scintillation fluid from National Diagnostics. Biogel P6DG was purchased from BioRad. Molecular weight cutoff filters were from Amicon.

*Animals.* The Sprague-Dawley rats and C57B mice that were used were bred at our animal facility.

*Serine isolation and amino acid analysis.* A BioRad HPLC system equipped with a Pickering ion exchange column was used for the isolation of serine and for routine amino acid analyses. A portion of the effluent was analyzed by post column orthophthalaldehyde (OPA) derivatization (10), fluorometric detection with an Aminco fluorometer, and quantitation with a Shimadzu CR6A integrator. The remainder of the effluent was collected and reserved for D- and L-serine analysis and radioactivity measurements.

*Serine resolution.* Serine was resolved into D- and L- isomers using an HPLC system composed of a Waters pump, an Alltech C18 reversed phase column (Adsorbosphere HS, 5 $\mu$m) and a Kratos fluorescence monitor. In a variant of the method of Hashimoto (11) the

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Abbreviations used: CSF, cerebrospinal fluid; i.p., intraperitoneal; MW, molecular weight; OPA, o-phthalaldehyde; PCA, perchloric acid; TCA, trichloroacetic acid; t-BOC, (N-tertiary-butyloxycarbonyl)-L-cysteine.

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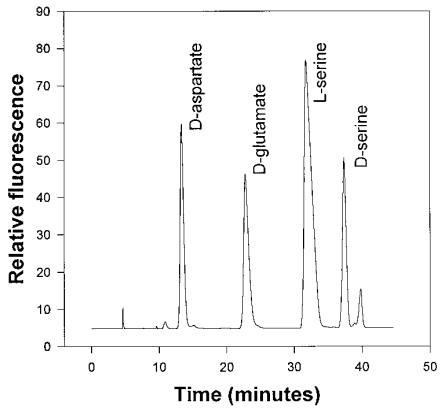


**FIG. 1.** The separation of D- and L-serine as t-BOC-cysteine OPA derivatives. Serine, previously isolated from a mouse brain by ion exchange chromatography, was resolved on a reversed phase column as described. Note the absence of glutamine between the L- and D-serine peaks.

sample is derivatized with OPA and t-BOC-L-cysteine and the resulting diestereoisomers separated on the reversed phase column using a 25 mM $NaH_2PO_4 \cdot H_2O$:25 mM/Na acetate buffer, pH 5.7, containing 14 % acetonitrile. With this solvent system D-serine was sufficiently separated from L-serine (see Fig. 1) that a radioactive peak of a few hundredths percent of that in L-serine could be quantitated. After passage through the fluorescence detector the effluent was collected for scintillation counting in a Packard Tri Carb 2000. We found that with t-BOC-L-cysteine the glycine derivative is unstable and radioactive material bleeds off the column throughout the run. It was necessary therefore, when radioactive glycine was the label, to first isolate the serine using the ion exchange column.

*Isotope administration.* For experiments involving microdialysis a CMA/Microdialysis infusion pump and free moving live animal setup were used. The microdialysis probes were CMA 10 s (3 mm dialysis surface) with the appropriate CMA guide cannula. The procedure used for implantation of the probes into the striatum was as described previously (10). For labeling experiments, the radioactive amino acid was dissolved in an artificial CSF (150 mM Na, 3.0 mM K, 1.4 mM Ca, 0.8 mM Mg, 1.0 mM $PO_4$ and 155 mM Cl) and infused at a rate of either 1 or 3 $\mu$l per minute. As isotope was infused through the microdialysis probe the effluent was collected and frozen. At the end of the perfusion the probe was removed and the animal decapitated. The forebrain was homogenized in ice cold 3 % (wt/vol) perchloric acid (PCA), frozen and reserved for amino acid analysis.

For measurement of the synthesis rate of D-serine from L-serine, a loading dose (15 $\mu$mol/g) of labeled serine was injected i.p. into C57B mice. The injected solution was 500 mM L-serine containing 80 $\mu$Ci/ml of L-[3-$^3$H]-serine. At the appropriate time the brain was removed and processed as above.

For the administration of D-serine in drinking water, solutions of 0.24 and 10 mM were given as the sole source of water. The respective specific activities were 4.2 $\mu$Ci/100 ml and 74 $\mu$Ci/100 ml. The solution was changed each morning and the concentration of D-serine in the remaining fluid was determined by amino acid analysis.

*The isolation of serine from brain protein.* For the determination of the specific activity of protein bound amino acids, the pellet from the PCA extract was homogenized in 3 % PCA, centrifuged, homogenized in 5 % (wt/vol) tricloracetic acid (TCA), centrifuged, and homogenized in 5 % TCA. This homogenate was then heated in a boiling water bath for 20 min, cooled, and centrifuged. The pellet was once again extracted with 5 % TCA. Thereafter the pellet was homogenized in methanol, then $CHCl_3$-methanol 2:1 (vol/vol), again in $CHCl_3$-methanol, and finally in ether. Two to three mg of dry white protein from the air dried pellet was hydrolyzed in 200 $\mu$l of constant boiling 6 N HCl by heating the capped tubes in boiling water for 4 h. The hydrolysates were dried in a vacuum centrifuge, dissolved in water, centrifuged and a portion of the sample reserved for analysis.

*The purification of labeled L-serine.* In experiments where labeled L-serine was used as a precursor, it was necessary to reduce the contamination with labeled D-serine to essentially zero (less than 0.02 % D) by incubating with D-amino acid oxidase (DAO). To remove the ammonium sulfate in the DAO preparation, a column containing 6 ml of Biogel P6DG was washed with 50 mM Tris, pH 8.5, containing 0.01 mM FAD. DAO (80 $\mu$l, 5 mg/ml) was mixed with catalase (20 $\mu$l, 1 mg/ml) and 400 $\mu$l of the same buffer and applied to the column. The first 2.3 ml of effluent was discarded. The next 1.0 ml contained the enzymes. The labeled serine was dried and dissolved in the enzyme solution to which was added 0.1 ml of 10 mM D-serine. After incubation for 1.5 h at 37°C another 0.1 ml of 10 mM D-serine was added and the incubation continued for 2 hours. The DAO and catalase were removed from the L-serine with a Centricon 10 filter (10,000 MW cutoff). In initial trials some of the isotopes failed to react with DAO which was apparently due to contamination in the label's solvent. For this reason isotopes were routinely isolated by passage on to a desalting column (Dowex 50×8, 0.3 × 3 cm), eluted with ammonia and dried.

## RESULTS AND DISCUSSION

### *The Turnover of the D-Serine Pool of the Brain*

The accumulating evidence that D-serine is physiologically active at NMDA receptors suggested that, as is the case for other receptor agonists, D-serine is synthesized within the brain. The rate of synthesis can be found from the rate of decay of the D-serine pool after its labeling with radioactive D-serine, since, at equilibrium, synthesis and decay rates must be the same. We therefore determined the turnover time of D-serine in rat and mouse brain after an intraperitoneal injection of D-[$^3$H]-serine (Fig. 2). The decay followed

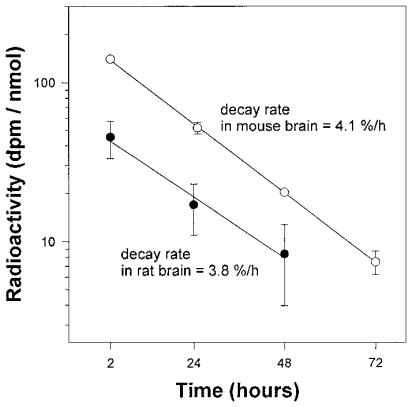


**FIG. 2.** The D-serine decay rate in mouse and rat brain after pulse labeling. Mice were injected i.p. with 2 ml/100 g body weight of D-[$^3$H]-serine (17 $\mu$Ci/ml, 21 Ci/mmole) in saline. Rats received an injection of 0.25 ml/100 g (60 $\mu$Ci/ml, 3 Ci/mmole). Four animals were used for each point. Serine was isolated from brain extracts by ion exchange chromatography and the specific activity of D-serine determined. L-serine contained almost no radioactivity. The plots shown are first order linear regressions. The standard deviation at each point is indicated.

first order kinetics with a half life of 16.9 h for mouse and 18.2 h for the rat. The replacement rates were 4.1 and 3.8 %/h, respectively. Since the D-serine pool is about 200 nmol/g in both species, the synthesis rate is 8 nmol/g/h.

*Precursor Studies Using Microdialysis*

For studies of the conversion of possible radiolabeled precursors to D-serine, we selected perfusion through microdialysis probes as a useful method for isotope administration. This procedure restricts metabolism to that occurring within the brain, reduces isotope dilution by large whole-body pools, and permits the maintenance of relatively stable levels of precursor radioactivity over a long period of time. In addition to indicating product precursor relationships, microdialysis experiments can also provide information about the rate of interconversion of metabolites. If steady state conditions between the probe and the surrounding brain are reached quickly, synthesis rates can be calculated directly from the ratios of product to precursor specific activities divided by the perfusion time. However, if the gradient of precursor specific activity continues to increase during the perfusion, the average specific activity of the precursor will be lower than its final value, and the calculated rate would be too low. Since amino acids are incorporated into proteins continuously over the period of perfusion, the specific activity of an amino acid in the protein hydrolysate serves as a direct measure of its integrated specific activity during the perfusion. The synthesis rate of brain protein in adult rats is known to be 0.65 %/h (12). Therefore, the synthesis rate of D-serine from L-serine as a precursor can be calculated as:

$$\frac{\text{specific activity of free D-serine (dpm/nmol)}}{\text{specific activity of L-serine in protein (dpm/nmol)}} \times 0.65\ \%/\text{h} = \text{Synthesis rate (\%/h)}.$$

*Glucose, Glycine, and L-Serine as D-Serine Precursors*

Incomplete stereospecificity along known pathways of L-serine formation or the racemization of L-serine itself, seemed the most likely mechanism of D-serine formation. Therefore radioactive glucose, glycine and L-serine were selected as potential D-serine precursors in the microdialysis experiments. Since these metabolites are interconvertible, experiments were designed to compare their relative rates of conversion to D-serine. The perfusion of L-serine and glycine labeled in specific positions was also used to distinguish between alternative pathways to D-serine.

The first compound tested was D-[U-$^{14}$C]-glucose (Table 1, experiment 1). Both D- and L-serine were labeled confirming that D-serine is formed within the brain. If D-serine is formed directly from L-serine, the minimum rate of synthesis would be 2.8 %/h, not far below the rate of 3.8 %/h found in the turnover experiment.

The possible conversion of glycine to D-serine was then studied by perfusing [2-$^{3}H_2$]-glycine, [1-$^{14}$C]-glycine, and [2-$^{14}$C]-glycine in one single and two double label experiments (Table I, exp. 2-4). In all cases glycine labeled both the L-serine and D-serine pools. From the specific activities found at the end of the perfusions, the relative rates of D-serine formation from glycine and L-serine can be calculated. The rate of D-serine formation from L-serine was closer to the expected value than was that from glycine, suggesting the direct conversion of L- to D-serine. However, other pathways that convert glycine to D-serine are not ruled out by these results.

When [2-$^{3}H_2$]-gly and L-[1-$^{14}$C]-glycine were administered in a double label experiment (Table I, exp. 3), the ratio of $^{3}$H in L-serine to $^{3}$H in glycine was found to be the same as the ratio of $^{14}$C in L-serine to $^{14}$C-glycine at the end of the infusion. Thus, L-serine made from [2-$^{3}H_2$]-glycine has the same specific activity (two moles of tritium per mole) as the glycine from which it was derived. There was however a difference in the ratios found in D-serine, indicating that about 26% of the tritium in [2-$^{3}H_2$]-glycine (or L-serine) was lost in the conversion to D-serine. This finding was confirmed in the double label experiment with [2-$^{14}$C]-glycine and [2-$^{3}H_2$] glycine (Table I, exp. 4). Here the ratio of $^{3}$H/$^{14}$C in D-serine was again 73 % of that in L-serine. However, the ratio of $^{14}$C transferred to L-serine to that of the $^{3}$H transferred was almost 2. Taken together, the two double label experiments show that when two moles of glycine are converted to one mole of L-serine and one mole of $CO_2$ by the glycine oxidase system (13) both of the C2 carbons of the two glycines, but only two of the four $^{3}$H atoms originally present on C2 of the two glycines are retained. Note that the synthesis rates shown in Table I are corrected for the different efficiencies of these labels.

The synthesis rate of D-serine was then examined using preparations of L-serine from which all traces of D-serine had been removed by treatment with D-amino acid oxidase. With L-[1-$^{14}$C]-serine (Table I, exp. 5), the rate of synthesis of D-serine was comparable to that found when the label in the L-serine pool was derived from glucose or glycine. In this experiment, the glycine pool was also labeled but if D-serine were derived from glycine, the rate of synthesis would have to be many times the rate that was found when glycine itself was administered. That glycine does not make a significant contribution to D-serine synthesis was confirmed by the results of the L-[3-$^{3}$H]-serine perfusion (Table 1, exp. 6). In this case D-serine also was labeled at a rate similar to that from the other labeled L-serine pools,

**TABLE 1**

Perfusion of Potential Substrates through Microdialysis Probes in the Brain and D-Serine Synthases Rates

| | | | | | | Synthesis rate of D-serine (%h) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Specific activity in the brain after perfusion (dpm/mol) | | | Approximate rate | | Corrected rate from protein hydrolysate |
| Exp. | Precursor | Time (h) | glycine | L-serine | D-serine | glycine→ D-serine | L-serine→ D serine | L-serine→ D-serine |
| 1 | D-[U-$^{14}$C]-glucose | 5.0 | | 19.5 | 2.71 | | 2.78 | |
| 2 | [2-$^{3}$H]-glycine | 5.0 | 491 | 153 | 11.4 | 0.62[a] | 2.01[a] | 4.3[a] |
| 3 | [2-$^{3}$H]-glycine + | 4.6 | 1748 | 427 | 19.6 | 0.34[a] | 1.34[a] | 3.6[a] |
| | [1-$^{14}$C]-glycine | | 2049 | 481 | 31.3 | 0.33 | 1.40 | 3.7 |
| 4 | [2-$^{3}$H]-glycine + | 3.3 | 3038 | 710 | 33.8 | 0.46[a] | 1.96[a] | |
| | [2-$^{14}$C]-glycine | | 3673 | 1476 | 94.7 | 0.40[b] | 1.97 | |
| 5 | L-[1-$^{14}$C]-serine | 5.0 | 1229 | 2954 | 218 | 3.5 | 1.48 | 4.0 |
| 6 | L-[3-$^{3}$H]-serine | 5.7 | 7.82 | 1182 | 141 | NC[c] | 2.34 | |
| 7 | L-[3-$^{3}$H]-serine + | 5.2 | 23.8 | 995 | 75.2 | NC[c] | 1.44 | 6.5 |
| | L-[1-$^{14}$C]-serine | | 299 | 681 | 53.1 | 3.4 | 1.49 | 5.6 |

*Note.* Each row includes the data from one perfusion. Specific activities were determined as described.

[a,b] Indicate that the rate has been multiplied by an isotope factor to account for differences in efficiency of conversion to L- or D-serine relative to [1-$^{14}$C]-glycine. [a]The factor for [2-$^{3}$H]-glycine is 1.35 and [b]the factor for [2-$^{14}$C]-glycine, is 0.5. For example the rate for the first [2-$^{3}$H]-glycine labeling experiment shown was (11.4 dpm/nmol/153 dpm/nmol)/5 hours = 0.0149/h = 1.49 %h. This rate is then multiplied by the isotope factor, 1.35, to give 2.01 %h. See text for details.

[c] The rates cannot be calculated. The specific activity of the glycine is less than the specific activity of the D-serine so, no matter what the conversion rate, the D-serine cannot be coming exclusively from glycine.

even though little or no radioactivity could have come from glycine.

To confirm the relative contributions of C1 and C3 of L-serine to the D-serine pool, we carried out a double label experiment in which L-[1-$^{14}$C]-serine and L-[3-$^{3}$H]-serine were perfused together (Table I, exp. 7, and Fig. 3). The rates of synthesis were found to be identical. Since the conversion of L-serine to glycine would result in the loss of the C3 carbon, and its $^{3}$H, glycine cannot be an intermediate in D-serine formation.

### *The Rate of Synthesis of D-Serine in Rat and Mouse Brain*

The rate of synthesis of D-serine in rat brain perfusion experiments was initially calculated from the ratio of the D-serine to L-serine specific activities divided by the perfusion time and corrected for labeling efficiencies. As discussed above an additional correction can be applied to these rates. We assume that the average specific activity of free L-serine is equal to that of the L-serine incorporated into brain protein during the perfusion period, rather than that of the final free L-serine specific activity in the brain. The application of this correction to six of these rates, where we determined protein bound L-serine specific activities, is shown in Table I. The average rate of synthesis was 4.8 ±1.6 %/h.

As a further confirmation of these conclusions, the synthesis rate of D-serine was determined in mice after loading with labeled L-serine. A large dose of precursor was injected to force exogenous labeled amino acid into the brain in an attempt to establish a constant precursor specific activity over the period of incorporation. While this approach works well with essential amino

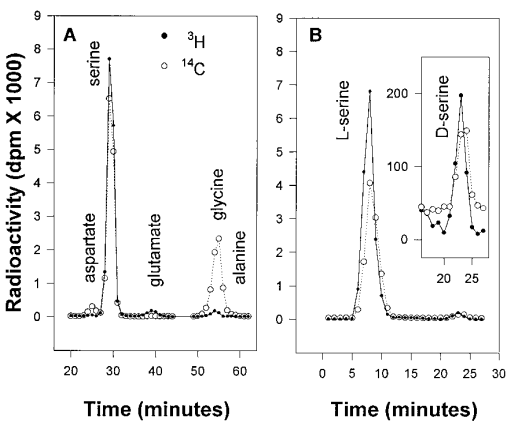


**FIG. 3.** Differential labeling of D-serine in a double labeling perfusion experiment with L-[1-$^{14}$C]-serine and L-[$^{3}H_3$]-L-serine. (A) The distribution of label in brain free amino acids resolved by ion exchange chromatography after perfusion. Note that while L-[1-$^{14}$C]-serine labels glycine, almost no tritium from L-[3-$^{3}$H]-serine was incorporated. The positions of the amino acids determined from the fluorescence is indicated. (B) The resolution of D- and L-serine as t-BOC derivatives. The inset shows the radioactivity in the D-serine peak on an expanded scale. Note that D-serine, like L-serine and unlike glycine, contains substantial amounts of $^{3}$H.

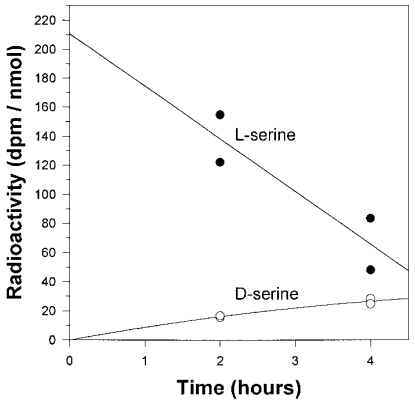


**FIG. 4.** D-serine synthesis after an L-[$^3H_3$]-serine load in mouse brain. The specific activity of D- and L-serine are measured two and four hours after injection. Serine was isolated from brain extracts by ion exchange chromatography and the specific activity of D- and L-serine determined.

acids such as valine (14) and phenylalanine (15), L-serine is so rapidly metabolized that the specific activity in the brain is not constant over the period of incorporation (see Fig. 4). Nevertheless by measuring the specific activities of L-serine at two points we can calculate the average specific activity of L-serine over the interval. The rate of synthesis of D-serine from L-serine in an L-serine load was found to be 4.6 %/h. Rates based on L-serine as the precursor are again reasonably close to those found from the decay curve of D-serine in mouse brain (Fig. 2) and for those calculated for rat brain from both the perfusion and decay experiments.

### *Exogenous Contribution of D-Serine*

It seemed possible that some component of the D-serine in the brain might be supplied by the diet. We therefore fed labeled D-serine in the drinking water of mice to determine to what extent D-serine might enter the brain by this route. Concentrations of 0.24 and 10 mM D-serine were used. By replacing the solution in the water bottle each day, the concentration was maintained at over 90 % of the initial value during the experiment. At the lowest level, no radioactive D-serine could be detected in the brain after five days. The mice drink over 5 ml of water per day so the amount of D-serine consumed at 0.24 mM is about 1.2 $\mu$mol per day versus a total brain content of 0.06 $\mu$mol (0.2 $\mu$mol/g $\times$ 0.3 g brain) i.e. 20 times the brain content. Even when the concentration in the drinking water was raised 40 fold to 10 mM, the specific activity in the brain reached only about 25 % of that in the water after three days though the specific activity in the plasma was close to equilibration. With a half-life of 16.9 h, the D-serine in the brain should have been 94 % replaced in 3 days. From these data it is clear that D-serine in the diet does not play a role in establishing or maintaining the D-serine level in the brain.

### *Conclusions*

It has been suggested that the glycine cleavage system might be involved in the synthesis of D-serine (4) and evidence has been presented (16) which might support this possibility. However the data that we have presented indicates L-serine, rather than glycine, is the direct precursor of D-serine. It has also been suggested that D-serine might be derived from L-phosphoserine (17). This seems unlikely since L-phosphoserine originates from glucose (18) rather than L-serine.

We conclude that D-serine in the brain is manufactured exclusively in the brain and that the precursor is L-serine. This conversion may be catalyzed by a specific racemase since we have found that partially purified brain extracts, in the absence of pyridoxal phosphate or other added cofactors, can convert L-serine to D-serine.

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