

TABLE III  
EFFECT OF P-L-SERINE ON HYDROLYSIS OF  $^{32}\text{P}$ -D-SERINE

Substrates added	Radioactivity released as $\text{P}_i$ (counts/min)
1. 2 $\mu\text{moles } ^{32}\text{P}$ -D-serine	8565
2. 2 $\mu\text{moles } ^{32}\text{P}$ -D-serine + 2 $\mu\text{moles P-L-serine}$	275

Conditions of incubation were the same as in Table I, except that the time of incubation was 20 min. The radioactivity released as  $\text{P}_i$  was determined by a method based on that of ERNSTER, ZETTERSTROM AND LINDBERG<sup>3</sup>. The specific activity of the  $^{32}\text{P}$ -D-serine was 104,000 counts/min  $\mu\text{mole}$ .

A previous study of the enzymic hydrolysis of phosphoserine has been carried out by ICHIHARA AND GREENBERG<sup>2</sup> who did not observe the reactions described by NEUHAUS AND BYRNE<sup>1</sup> and by us.

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<sup>1</sup> F. NEUHAUS AND W. L. BYRNE, *Biochim. Biophys. Acta*, 28 (1958) 223.

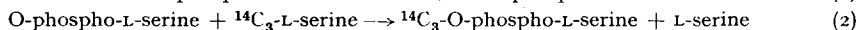
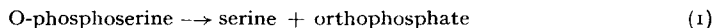
<sup>2</sup> A. ICHIHARA AND D. M. GREENBERG, *J. Biol. Chem.*, 224 (1957) 331.

<sup>3</sup> L. ERNSTER, R. ZETTERSTROM AND O. LINDBERG, *Anal. Chem.*, 4 (1950) 942.

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### O-Phosphoserine phosphatase\*

The following reactions have been found to be catalyzed by enzyme preparations from chicken and rat liver\*\*.



The phosphatase activity, reaction 1, was purified 20 fold starting with aqueous extracts of the acetone powder of chicken liver. The purified enzyme preparation dephosphorylates L-PS\*\*\* at the rate of 1.81  $\mu\text{moles}/10 \text{ min}/\text{mg}$  protein at 38°. The final concentration of the components in the test system were 0.01  $M$   $\text{MgCl}_2$ ; 0.05  $M$  succinate buffer, pH 5.90; 0.01  $M$  substrate and enzyme. After deproteinization, the orthophosphate formed was determined by the method of DRYER, TAMMES AND ROUTH<sup>1</sup>. With respect to reaction 1, the purified preparation is highly specific for PS. At low substrate concentrations it is specific for L-PS ( $K_m = 5.8 \cdot 10^{-5} M$ ) while at high substrate concentrations it will dephosphorylate D-PS ( $K_m = 4.2 \cdot 10^{-3} M$ ) which confirms the observation of BORKENHAGEN AND KENNEDY<sup>2</sup> that rat-liver preparations dephosphorylate D-PS as well as L-PS. Identical maximum velocities were observed for both isomers. The only other substrate dephosphorylated at any appreciable rate is *p*-nitrophenyl phosphate which is due to a contaminating, uncharacterized phosphatase. These results are in contrast to the conclusion of ICHIHARA AND GREENBERG<sup>3</sup> that the major pathway for the cleavage of PS was carried out by a non-specific phosphatase present in rat-liver extracts.

L-Serine was found to be a very effective inhibitor ( $K_i = 5.9 \cdot 10^{-4} M$ ) of the phosphatase activity. DL-Homoserine (0.05  $M$ ) and DL-threonine (0.05  $M$ ) were without effect. LINEWEAVER-BURK plots<sup>4</sup> showed that the L-serine inhibition is uncompetitive.

The exchange of  $^{14}\text{C}_3$ -L-serine with L-PS, reaction 2, occurs at a significant rate in chicken- and rat-liver homogenates. This exchange is illustrated for a chicken-liver homogenate by the rapid

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\*\* Similar and independent results are being published simultaneously by BORKENHAGEN AND KENNEDY<sup>2</sup>.

\*\*\* The following abbreviations are used: PS, O-phosphoserine; L-PS, O-phospho-L-serine; D-PS, O-phospho-D-serine;  $^{14}\text{C}_3$ -L-serine, uniformly labelled L-serine; S.A., specific activity (counts/min/ $\mu\text{mole}$ ).

incorporation of  $^{14}\text{C}_3$ -L-serine into an unlabelled L-PS pool as shown in Table I-A. The reverse experiment, Table I-B, was carried out by incubating a pool of  $^{14}\text{C}_3$ -L-PS in the presence of a pool of L-serine. The specific activity of the  $^{14}\text{C}_3$ -L-PS dropped, and the observed dilution of the  $^{14}\text{C}_3$ -L-PS corresponded to the incorporation of  $^{14}\text{C}_3$ -L-serine observed on Table I-A.

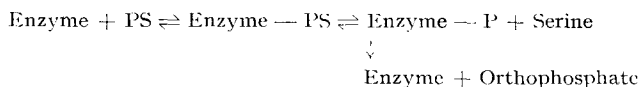
TABLE I  
THE EXCHANGE OF SERINE WITH PHOSPHOSERINE

Additions	Initial S.A. of L-PS, $10^{-3}$	Final S.A. of L-PS, $10^{-3}$	$\mu\text{Mole of L-serine}$ exchanged
A. L-PS, 3.0 $\mu\text{moles}$ $^{14}\text{C}_3$ -L-serine, 3.2 $\mu\text{moles}$ (S.A. = $2.38 \cdot 10^5$ counts/min/ $\mu\text{mole}$ )		0.580	0.99
B. $^{14}\text{C}_3$ -L-PS, 3.3 $\mu\text{moles}$ L-serine, 3.0 $\mu\text{moles}$	2.21	1.72	0.98

Each tube contained 10  $\mu\text{moles}$   $\text{MgCl}_2$ ; 50  $\mu\text{moles}$  Tris(hydroxymethyl)aminomethane buffer, pH 7.4; 0.1 ml of chicken-liver homogenate; and additions as indicated in a final volume of 1.0 ml. The homogenate was prepared by homogenizing for 1 min 10 g liver in 50 ml 0.25  $M$  sucrose containing 0.001  $M$  ethylenediaminetetraacetate, pH 7.4, and was used after centrifuging for 5 min at  $600 \times g$ . The tubes were incubated at  $32^\circ$  for 30 min and then deproteinized. After the protein-free filtrate had been adjusted to pH 8.0, it was added to a Dowex-1 (200-400 mesh) column ( $1 \times 20$  cm) in the chloride form. The column was washed with 3 column volumes of 0.012  $M$  HCl and the PS was eluted with 2.5 column volumes of 0.015  $M$  HCl. PS was determined by the method of TROLL AND CANNAN<sup>5</sup>. The radioactivity was determined in a gas-flow counter on stainless-steel planchets. The  $\mu\text{moles}$  of L-serine exchanged were calculated by the method of DUFFIELD AND CALVIN<sup>6</sup>.

When activities toward reactions 1 and 2 were determined at each step of the purification procedure, it was found that the activities fractionated in a parallel manner. The exchange activity, reaction 2, was measured in a system in which the phosphatase was inhibited 80% by L-serine. The phosphatase activity of reaction 1 was measured in a test system in which initial rates were measured so that L-serine accumulation had no effect. Reactions 1 and 2 show an absolute requirement for a divalent cation. The ratio of the phosphatase activity at pH 5.90 to the exchange activity at pH 7.12 is 1.0. The pH optimum for reaction 1 is 5.9-6.6 while for reaction 2 it is pH 6.9-7.3. Simple reversal of reaction 1 as an explanation for reaction 2 was ruled out by showing that the enzyme did not catalyze a detectable incorporation of  $^{32}\text{P}$ -labeled orthophosphate into PS under the conditions of a typical exchange experiment.

A proposed mechanism consistent with our data is as follows:



Since no acceptors other than L-serine have been found for reaction 2, it is doubtful that the enzyme functions as a transferase. The effective inhibition by L-serine suggests that PS phosphatase could control serine biosynthesis from carbohydrate precursors.

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<sup>1</sup> R. L. DRYER, A. R. TAMMES AND J. I. ROUTH, *J. Biol. Chem.*, 225 (1957) 177.

<sup>2</sup> L. F. BORKENHAGEN AND E. P. KENNEDY, *Biochim. Biophys. Acta*, 28 (1958) 222.

<sup>3</sup> A. ICHIHARA AND D. M. GREENBERG, *J. Biol. Chem.*, 224 (1957) 331.

<sup>4</sup> H. LINEWEAVER AND P. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.

<sup>5</sup> W. TROLL AND R. K. CANNAN, *J. Biol. Chem.*, 200 (1955) 803.

<sup>6</sup> R. B. DUFFIELD AND M. CALVIN, *J. Am. Chem. Soc.*, 68 (1946) 557.

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