TABLE III

EFFECT OF P-L-SERINE ON HYDROLYSIS OF ^{32}P-D-SERINE

Substrates added	*Radioactivity released as P_i (counts/min)*
1. 2 μmoles ^{32}P-D-serine	8565
2. 2 μmoles ^{32}P-D-serine + 2 μmoles 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 P_i was determined by a method based on that of ERNSTER, ZETTERSTROM AND LINDBERG[3]. The specific activity of the ^{32}P-D-serine was 104,000 counts/min μmole.

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

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O-Phosphoserine phosphatase*

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

$$\text{O-phosphoserine} \longrightarrow \text{serine} + \text{orthophosphate} \quad (1)$$

$$\text{O-phospho-L-serine} + {}^{14}C_3\text{-L-serine} \longrightarrow {}^{14}C_3\text{-O-phospho-L-serine} + \text{L-serine} \quad (2)$$

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 μmoles/10 min/mg protein at 38°. The final concentration of the components in the test system were 0.01 *M* $MgCl_2$; 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[1]. 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[2] 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[3] 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[4] showed that the L-serine inhibition is uncompetitive.

The exchange of $^{14}C_3$-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[2].

*** The following abbreviations are used: PS, O-phosphoserine; L-PS, O-phospho-L-serine; D-PS, O-phospho-D-serine; $^{14}C_3$-L-serine, uniformly labelled L-serine; S.A., specific activity (counts/min/μmole).

incorporation of $^{14}C_3$-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 $^{14}C_3$-L-PS in the presence of a pool of L-serine. The specific activity of the $^{14}C_3$-L-PS dropped, and the observed dilution of the $^{14}C_3$-L-PS corresponded to the incorporation of $^{14}C_3$-L-serine observed on Table I-A.

TABLE I

THE EXCHANGE OF SERINE WITH PHOSPHOSERINE

Additions	*Initial S.A. of L-PS* $\cdot 10^{-5}$	*Final S.A. of L-PS* $\cdot 10^{-5}$	*μMole of L-serine exchanged*
A. L-PS, 3.0 μmoles $^{14}C_3$-L-serine, 3.2 μmoles (S.A. = $2.38 \cdot 10^5$ counts/min/μmole)		0.580	0.99
B. $^{14}C_3$-L-PS, 3.3 μmoles L-serine, 3.0 μmoles	2.21	1.72	0.98

Each tube contained 10 μmoles $MgCl_2$; 50 μmoles 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 × *g*. The tubes were incubated at 32° 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 × 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[5]. The radioactivity was determined in a gas-flow counter on stainless-steel planchets. The μmoles of L-serine exchanged were calculated by the method of DUFFIELD AND CALVIN[6].

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}P-labeled orthophosphate into PS under the conditions of a typical exchange experiment.

A proposed mechanism consistent with our data is as follows:

$$\text{Enzyme} + \text{PS} \rightleftharpoons \text{Enzyme} - \text{PS} \rightleftharpoons \text{Enzyme} - \text{P} + \text{Serine}$$

$$\downarrow$$

$$\text{Enzyme} + \text{Orthophosphate}$$

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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