

15  $\mu$ moles  $\text{MgSO}_4$ , and the following as potassium salts at pH 7.6: ribose-5-phosphate, 6  $\mu$ moles; phosphate, 60  $\mu$ moles; adenosine triphosphate, 3  $\mu$ moles; 3-D-phosphoglycerate, 15  $\mu$ moles.

Sup. F was the high-speed (25,000 g) supernatant fraction of 25% liver-0.25  $M$  sucrose homogenates. The chicken liver fractions were obtained as follows: Sup. F was made 0.01  $M$  with potassium phosphate buffer pH 7.4, and 0.30, 0.45, 0.65 and 1.30 volumes (referred to original volume) of acetone ( $-20^\circ$ ) were added stepwise (maintained at  $-5^\circ$ ) and the precipitates collected and taken in water to give F I, II, III, and IV respectively. RNA was recovered from the trichloroacetic acid (TCA) precipitates of the reaction mixture, after repeated washing with 0.3  $M$  TCA, by the method of PAIN AND BUTLER<sup>6</sup>. RNA was measured by the orcinol reaction or by its ultraviolet light absorption. RNA samples were plated in thin layers (self-absorption correction, 10% or less) on copper planchets and counted to a probable error of 4%. Figures in the table are counts/min/mg RNA.

TABLE II  
RELATIVE INCORPORATION OF PRESUMED PYRIMIDINE PRECURSORS

Precursor	counts/min/mg RNA	Precursor	counts/min/mg <sup>a</sup> RNA
Orotate**	46	UMP**	516
C- $\beta$ -alanine*	9.6	Uracil***	1.5
Hydouracil*	12.6	Uridine****	3.5
C $\beta$ ARP**	125	C $\beta$ AR****	3.9
HUMP**	148	Hydouridine****	2.7

The components and conditions were as described in the legend of Table I. Fraction III and IV were combined and used in the experiments of the Table. \*, \*\*, \*\*\*, \*\*\*\*, S.A. 1, 0.9, 0.5 and 0.3  $\mu\text{C}/\mu\text{mole}$ , respectively.

\* Uncorrected figures. The Geiger tube used gives about 3% of the actual S.A.

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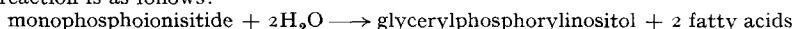
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## The enzymic breakdown of monophosphoinositide by phospholipase B preparations

Recent work has shown that phospholipase B preparations prepared from *Penicillium notatum* will attack lecithin providing certain activating lipids obtainable from liver are added to the system in small quantities<sup>1</sup>. These lipids have been isolated and identified as monophosphoinositide (phosphatidyl inositol) and a polyglycerol phospholipid<sup>2</sup>. While studying the mechanism of this

activation it has been found that monophosphoinositide by itself can be rapidly attacked by the *P. notatum* enzyme. Acid soluble-P and water-soluble combined inositol were found to be liberated in the incubation medium in approximately equimolar proportions. From the same digest a water-soluble phosphate ester was isolated by chromatography: this contained glycerol and inositol in equimolar proportions. On paper chromatography in a number of solvents it ran to a position identical with that of glycerylphosphorylinositol prepared by the mild alkaline hydrolysis of monophosphoinositide and distinctly different from that of inositol monophosphate. Fatty acid titration showed the liberation of two fatty acids for every water-soluble P atom formed. This indicates that the net reaction is as follows:



Purified phospholipase B, prepared from ox pancreas by the method of SHAPIRO<sup>3</sup> and which did not attack lecithin, rapidly liberated water-soluble combined inositol from monophosphoinositide which suggests that a similar reaction occurs with this enzyme.

It cannot yet be concluded that the active centre of phospholipase B is responsible for the removal of both fatty acids from the phospholipid. However, the pH optimum for the reaction (*P. notatum* enzyme, pH 3.3, pancreas enzyme, pH 6.1) are identical with the pH optima for their respective attacks upon lysolecithin. Moreover, lysolecithin inhibits both enzymes. Up to the present only monoacylated phospholipids (lysolecithin and lysophosphatidyl ethanolamine) have been considered by definition as substrates for phospholipase B.

The attack of both enzymes on monophosphoinositide can be completely inhibited by sufficient quantities of lecithin. With the *P. notatum* enzyme glycerylphosphorylcholine is liberated, but with the pancreas enzyme no such activity occurs. The enzymes also differ in their behaviour towards the addition of calcium: the *P. notatum* enzyme is not affected, or slightly inhibited, whereas the pancreas enzyme is markedly stimulated.

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