

The technique developed is by no means perfect. The coloured precipitate produced can only be a rough guide to the sites of the enzyme activity. Therefore efforts are continued to refine the technique.

1. Ellis, R. A. and Montagna, W. *J. Histochem. and Cytochem.* **6** (1958) 201.
2. Friede, R. L. *J. Histochem. and Cytochem.* **7** (1959) 34.
3. Goldberg, B., Wade, O. R. and Jones Jr., H. W. *J. Natl. Cancer Inst.* **13** (2), (1952) 543.
4. Shimizu, N. and Okada, M. *J. Histochem. and Cytochem.* **5** (1957) 459.
5. Takeuchi, T. *J. Histochem. and Cytochem.* **6** (1958) 208.
6. Takeuchi, T. and Kuriaki, H. *J. Histochem. and Cytochem.* **3** (1955) 153.
7. Yasuda, K., Furusawa, H. and Ogata, N. *Okajimas Folia Anat. Japon.* **31** (1958) 161.

## The Phosphotransferase Activity of Alkaline Phosphatases. Studies with Hydroxyamino Acids and Amines as Acceptors

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In an extensive study Morton<sup>1</sup> recently reported that alkaline phosphatase from calf intestinal mucosa catalysed the synthesis of phosphate esters by direct transfer of the phosphate group from phosphomonoesters to suitable hydroxy compounds. He also used serine, threonine, and ethanolamine as acceptors with some presumptive evidence of a positive result. However, he had no authentic marker compounds for comparison with the suspected phosphorylated components. Clear evidence for the formation of such compounds has now been obtained.

Radioactive glycerophosphate was used as donor. It was prepared according to Bublitz and Kennedy<sup>2</sup> using AT<sup>32</sup>P and glycerokinase. A simplified method for assay of enzyme activity was used based on the observation of Ernster *et al.*<sup>3</sup> Some radioactive inorganic phosphate formed in the reaction was separated on a Dowex-1 column.

In a series of experiments 10 mg of glycerophosphate, containing 0.46 mC <sup>32</sup>P, and 10 mg

of a preparation of alkaline bone phosphatase<sup>4</sup> containing 420 Portmann<sup>5</sup> units/mg N were incubated at pH 9.8 in a bicarbonate buffer for 10 min with 1 mmole of serine, threonine, glucosamine, or ethanolamine, respectively, in a total volume of 2 ml. After heating glycerophosphate was separated from the formed phosphorylated product by gradient elution on a Dowex-1 formate column<sup>6</sup>. The total radioactivity of the fractions measured in a L.K.B. Robot Scaler gave the following values in counts per minute:

	With enzyme	Without enzyme
Phosphorylserine	375 000	26 000
Phosphorylthreonine	26 000	460
Phosphorylglucosamine	67 000	41 000
Phosphorylethanolamine	912 000	150 000

Some interesting features are observed, the large proportion of phosphorylserine to phosphorylthreonine also found in hydrolysates of casein and animal phosphoproteins<sup>6</sup>. Of similar interest is the dominance of phosphorylethanolamine over phosphorylglucosamine also found in hydrolysates of the protein fraction from *E. coli*<sup>7</sup>. The significance of these observations with regard to possible ways for the formation of phosphoproteins is, however, unclear. The yield obtained in the enzymatic transphosphorylation was rather small, usually less than 1 μmole. The specific activity calculated as counts per minute per μg of phosphorus had about the same value for all compounds. In controls without enzyme smaller amounts of phosphorylated amino acids and amines were formed. Of interest for the present problem is the recent isolation of a free phosphorylated amino acid from extracts of *L. casei*<sup>8</sup> together with some phosphopeptides.

1. Morton, R. K. *Biochem. J.* **70** (1958) 139.
2. Bublitz, C. and Kennedy, E. P. *J. Biol. Chem.* **211** (1954) 951.
3. Ernster, L., Zetterström, R. and Lundberg, O. *Acta Chem. Scand.* **4** (1950) 942.
4. Ågren, G., Zetterquist, Ö. and Ojamäe, M. *Acta Chem. Scand.* **13** (1959) 1047.
5. Portmann, P. *Z. Physiol. Chem. Hoppe-Seyler.* **309** (1957) 87.
6. Ågren, G., de Verdier, C.-H. and Glomset, J. *Acta Chem. Scand.* **8** (1954) 1097.
7. de Verdier, C.-H. and Ågren, G. *Acta Chem. Scand.* **12** (1958) 361.
8. Ågren, G. *Acta Soc. Med. Upsaliensis* **64** (1959) 379.