

Whatever is the explanation, it seems that this is the first observed case of an association (other than coagulation of electrically charged colloidal particles) that requires an activation energy.

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#### GLUCOSE, A CONSTITUENT OF ALKALINE PHOSPHATASE<sup>1</sup>

Sir:

Alkaline phosphatase of swine kidney, found in the ribonucleoprotein particles of the microsomes<sup>2</sup> and released as an active fragment by proteolysis,<sup>2,3</sup> has been isolated in an apparently homogeneous condition by application of the procedures previously described<sup>3</sup> plus ion-exchange chromatography with Ecteola cellulose.<sup>4</sup> In a typical purification 2,000,000 units activity<sup>2</sup> with a specific activity of over 100,000 units per mg. total N (micro-Kjeldahl) was placed on a column of Ecteola 5 × 100 cm. and, after thorough washing with water, was eluted with a gradient of barium acetate at pH 9 varying from 0.01 to 0.05 M in 10 liters of solution. The active material was eluted symmetrically near 0.03 M and was concentrated by the barium procedure<sup>3</sup> to yield about 1,500,000 units material with a specific activity of 295,000 to 310,000 on the basis of total N. Rechromatography on Ecteola-cellulose, on Deae-cellulose<sup>4</sup> or on Dowex-2<sup>3</sup>, paper electrophoresis and paper chromatography (ethanol-1 M ammonium acetate, 70-30) revealed no dissociation of absorbancy at 280 mμ from activity. The material was free of peptidase and diesterase activity when tested undiluted, amino acids were without effect on the activity and at no time was it possible to demonstrate dialyzable cofactors other than magnesium ion. The absorbancy in the ultraviolet was characteristic of protein with a maximum at 278 and a minimum at 250 mμ. However, in the course of treatment with dilute acid (0.1 to 1.0 M at 100°) the absorbancy was found to increase remarkably and, at the end of 2 hr., the absorbancy at 278 was nearly tripled. There was a parallel release of reducing material,<sup>5</sup> of ninhydrin reactive material,<sup>6</sup> and of material reacting with phosphomolybdate.<sup>7</sup> Paper chromatography (propanol-water, 80-20) separated a phosphomolybdate and ninhydrin reactive material from a ninhydrin negative but aniline hydrogen phthalate positive<sup>8</sup> (brown color) material with the same  $R_f$  as glucose. The untreated material in the cysteine methods of Dische<sup>9</sup> gave

(1) These studies were supported by grants from the U. S. Public Health Service. Detailed studies of the purification, the effects of amino acids and of divalent metal ions will be described by C. Lea.

(2) F. Binkley, J. Davenport and F. Eastall, *Biochem. Biophys. Research Com.*, **1**, 206 (1959).

(3) F. Binkley, V. Alexander, F. E. Bell and C. Lea, *J. Biol. Chem.*, **228**, 559 (1957).

(4) E. A. Peterson and H. A. Sober, *THIS JOURNAL*, **78**, 751 (1956).

(5) N. Nelson, *J. Biol. Chem.*, **153**, 375 (1944).

(6) S. Moore and W. H. Stein, *ibid.*, **176**, 367 (1948).

(7) O. Folin and V. Ciocaltu, *ibid.*, **73**, 627 (1927).

(8) S. M. Partridge, *Nature*, **164**, 443 (1949).

(9) Z. Dische, in D. Glick, "Methods of Biochemical Analysis," Vol. 2, Interscience Publishers, Inc., New York, N. Y., 1955, p. 313 ff.

in the general reaction a product identical with the aldohexoses and in the secondary reaction of hexoses gave a product identical with glucose. A solution of alkaline phosphatase containing 30 μg. N per ml. after 2 hr. at 100° with 1 N HCl was found to contain 38 μg. glucose as determined by the reducing sugar method as determined by the cysteine reaction on the untreated material. The hydrolysate was found to contain approximately 25 μg. glucose as determined with glucose oxidase.<sup>10</sup>

The ninhydrin-phosphomolybdate positive material was found to migrate as a cation at pH 8 and to be unstable in more alkaline solutions. Other reactions of the compound were an immediate reaction with  $K_3FeCN_6$  and  $FeCl_3$  (blue) and an immediate reaction with  $K_3FeCN_6$  alone (blue). Both these reactions were destroyed by previous treatment with traces of cupric ion with exposure to air. In a study of model compounds, ptenolic compounds appear to have been eliminated but similar reactions have been observed with tri- and tetra-substituted pyrimidines; from the absorbancy in the ultraviolet, from studies of the model pyrimidines and from analogy with the proposed structure for vicine,<sup>11</sup> it is suspected that the material may be a diamino-5-hydroxypyrimidine attached, in the active material, to the glucose by a glycosidic linkage at the 5-hydroxy position.

(10) "Glucostat," Worthington Biochemical Corp., Freehold, N. J.  
(11) A. Bendich and G. C. Clements, *Biochim. Biophys. Acta*, **12**, 462 (1953).

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#### THE PHOSPHINOUS ACID $(CF_3)_2POH$ AND THE DIPHOSPHOXANE $(CF_3)_2POP(CF_3)_2$ <sup>1</sup>

Sir:

We have recently isolated the new-type compounds  $(CF_3)_2POH$  and  $(CF_3)_2POP(CF_3)_2$  as stable liquids contrasting with the apparently complete instability of the corresponding hydrocarbon derivatives.<sup>2</sup> Evidently the highly electronegative  $CF_3$  groups lower the power of phosphorus lone-pair electrons to bond either  $H^+$  or  $(CF_3)_2P^+$  coming from O. Thus these new  $(CF_3)_2P$  compounds do not undergo the rearrangements  $R_2POH \rightarrow R_2POH$  and  $R_2POP(R)_2 \rightarrow R_2P-PR_2$  which probably represent the first stages of decomposition when R is a hydrocarbon group.

**Synthesis and Characterization of the Diposphoxane.**—The reaction  $2(CF_3)_2PI + Ag_2CO_3 \rightarrow CO_2 + 2AgI + (CF_3)_2POP(CF_3)_2$  (room temperature, repeated shaking with fresh silver carbonate) gave yields above 79%. The unused  $(CF_3)_2PI$  (1%) was converted by AgCl to the easily

(1) This research was supported by the United States Air Force under Contract AF 33(616)-5435 (Subcontract No. 1) monitored by the Materials Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

(2) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 144. We also have found that reactions expected to form  $(CH_3)_2POH$  give nearly quantitative yields of  $(CH_3)_2PH$  and  $(CH_3)_2POOH$ ; and attempts to make  $(CH_3)_2POP(CH_3)_2$  also give products suggesting disproportionation.