equiv., 84.1. Found: C, 57.45; H, 4.80; neut. equiv., 83.5, 83.9.

Triethyl 1,3,5-Benzenetriacetate (II).—In the best of several runs, 57.1 g. (0.227 mole) of I, m.p. 197–204°, was refluxed with 500 ml. of ethanol, 250 ml. of benzene and 5 ml. of sulfuric acid. Working up in the usual way yielded 68.7 g. (90%) of II as a light yellow oil, b.p. 165–193° at 0.05 mm. Redistillation of a portion for analysis gave II as a water-white oil, b.p. 166–167° at 0.1 mm.

Anal.[4] Calcd. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.22; H, 7.20.

A solution of 2.0 g. of II in 10 ml. of acetic acid and 20 ml. of concd. hydrochloric acid was slowly distilled to a volume of 5 ml. After addition of 10 ml. of acetic acid and cooling an almost quantitative yield of I was obtained. The melting point and neutral equivalent were identical to that above mentioned.

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Phosphorylation of Adenylic Acid by the Phosphate Anhydride of Leucine and Chromatographic Analysis of the Resulting Products

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Received May 17, 1954

The phosphate anhydrides of amino acids prepared recently in this Laboratory[1] are labile, reactive compounds. It was of interest to determine whether they would phosphorylate lower-energy phosphates, *e.g.*, adenylic acid, to adenosine diphosphate or triphosphate.

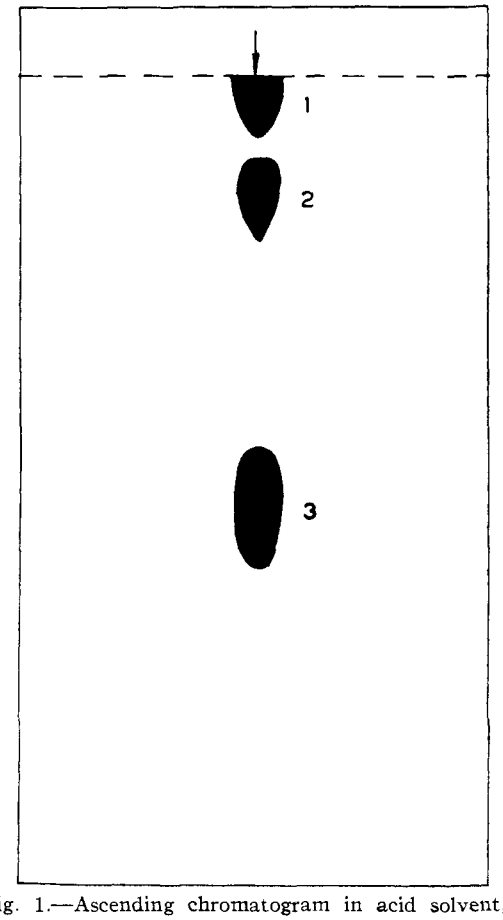

Fig. 1.—Ascending chromatogram in acid solvent, developed with phosphorus reagents; reaction mixture, leucine phosphoanhydride + adenylic acid: Spot 1, ATP + ADP + peptides; spot 2, AMP + inorganic pyrophosphate: spot 3, inorganic orthophosphate.

(1) A. Katchalsky and M. Paecht, This Journal, **76**, 6042 (1954).

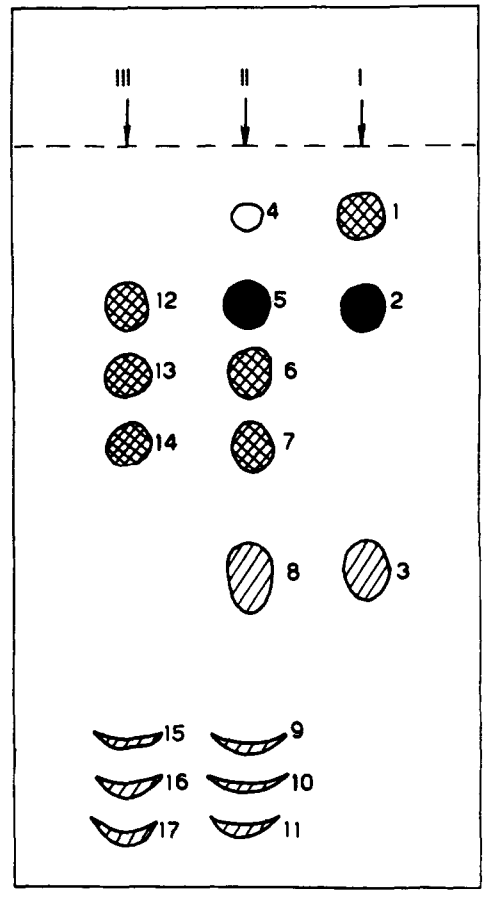

Fig. 2.—Descending chromatogram in alkaline solvent, after the orthophosphate has been cut off, developed with phosphorus reagents. Column I: control mixture, ATP + ADP + AMP + inorg. pyrophosphate + inorg. orthophosphate. Spot 1, ATP; spot 2, ADP + inorg. pyrophosphate; spot 3, AMP. Column II: reaction mixture, phosphate anhydride of leucine + adenylic acid in water solution. Spot 4, ATP; spot 5, ADP + inorg. pyrophosphate; spots 6, 7, 9, 10, 11, peptides; spot 8, AMP. Column III: control solution, phosphate anhydride of leucine in water solution. Spot 12, inorg. pyrophosphate; spots 13, 14, 15, 16, 17, peptides.

The phosphorylation reaction in aqueous solutions was analyzed chromatographically. Since, during this reaction, numerous other reactions take place which lead to the formation of polypeptides and inorganic phosphates, the reaction mixture is a rather complicated system. It can however be shown that adenosine diphosphate is obtained, and that even traces of adenosine triphosphate are detectable. This indicates that the phosphoanhydrides of amino acids should be classified as reactive phosphates.

Experimental

Phosphorylation.—In 1 ml. of an aqueous solution of $m/50$ adenylic acid, 1 equivalent of leucine phosphate anhydride was dissolved and allowed to stand at room temperature for about an hour. Then the mixture was analyzed chro-

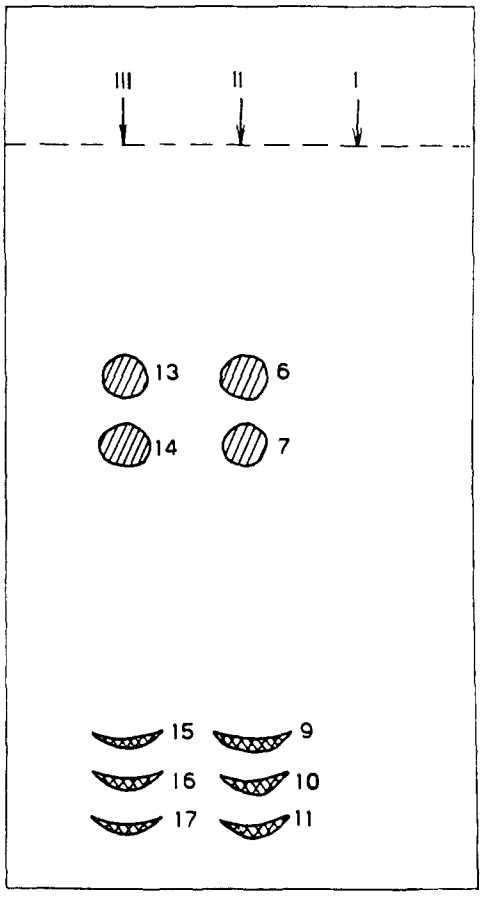

Fig. 3.—Descending chromatogram in alkaline solvent, after the orthophosphate has been cut off, developed with ninhydrin: columns I, II and III and spots as in Fig. 2.

matographically using the Eggleston and Hems method.[2]

Chromatography.—First an ascending chromatogram was made, using 90 cc. of isopropyl alcohol + 60 cc. of 90% formic acid as solvent. Figure 1 is a typical run for a reaction mixture composed of leucine phosphate and ADP. The rapidly wandering orthophosphate (Spot 3 Fig. 1) is cut off from the rest of the chromatogram. Subsequently the paper was turned upside down and the chromatogram spread out in the descending direction with an alkaline solvent—240 cc. of propanol + 120 cc. of ammonia + 40 cc. of 0.002 *M* ethylenediamine tetraacetate.

Figures 2 and 3 represent two descending chromatograms spread out by the alkaline reagent. The chromatographic runs were usually carried out with two controls for comparison of rates, designated on Figs. 2, 3 and 4 as: (I), a solution containing a mixture of ATP (adenosine triphosphate), ADP (adenosine diphosphate), AMP (adenylic acid) and inorganic ortho- and pyrophosphates; and (III), a solution of free leucine phosphate. Figure 2 was developed by ninhydrin for free amino acid and polypeptides obtained by the self polymerization of leucine phosphate. It will be observed from Fig. 3 that polypeptides of various degrees of polymerization are formed both in the control and in the reaction mixture (III), Spots 13, 14, 15, 16, 17 and (II) 6, 7, 9, 10, 11, and inorganic pyrophosphates appears as a result of self-phosphorylation (Fig. 2, (III) spot 12 and part of (II) spot 5). However, Fig. 2 discloses that in addition to the phosphate salts of the peptides, adenosine diphosphate (Fig. 2, (II) spot 5) and traces of adenosine triphosphate have been obtained.

As the spot for adenosine diphosphate coincided with that for inorganic pyrophosphoric acid, the two substances were separated by the upper phase of the following mixture of solvents: 20 g. of *p*-toluenesulfonic acid + 60 ml. of *t*-amyl alcohol + 300 ml. of water.[3] This solvent, though not suitable for the separation of ATP from ADP, discriminates clearly between the inorganic pyrophosphate and ADP. The chromatograph represented by Fig. 4 demonstrates that phosphorylation has taken place, and that adenosine diphosphate, (II) spot 5, has been formed by the interaction of leucine phosphate with adenylic acid.

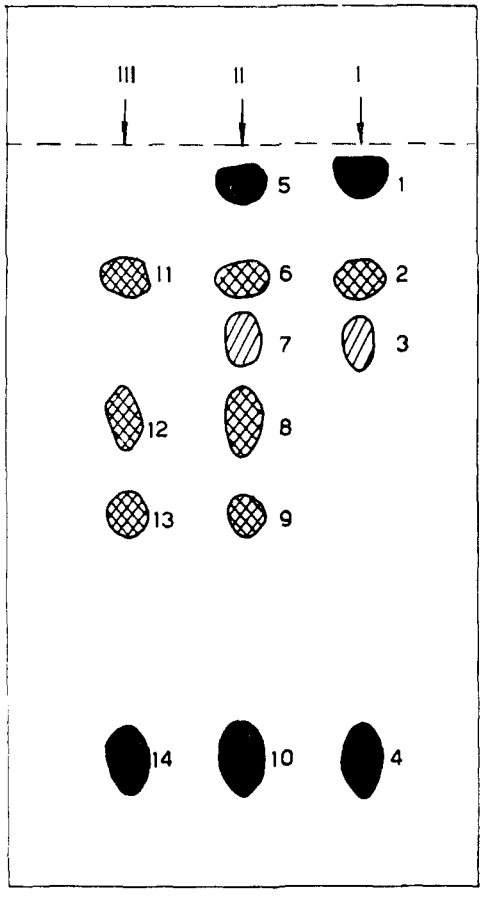

Fig. 4.—Descending chromatogram in *p*-toluenesulfonic acid solvent: columns I, II, III as in Fig. 2. Spot 1, ATP + ADP; spots 2, 6, 11, inorganic pyrophosphate; spots 3, 7, AMP; spots 4, 10, 14, inorg. orthophosphate; spot 5, ADP; spots 8, 9, 12, 13, peptides.

Spot 5, containing ADP formed by the reaction described, was washed out from another undeveloped chromatogram and its phosphorus content determined after 7 minutes of hydrolysis. The analytical results confirmed the fact that ADP was formed.

Acknowledgment.—It is a pleasure to thank Dr. Avidor for numerous helpful suggestions with regard to the chromatographic analysis.

(2) L. V. Eggleston and R. Hems, *Biochem. J.*, **52**, 156 (1952).

(3) W. Bartley, communicated by Dr. Avidor.

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Conditions for Rapid Hydrolysis of Some Proteins by Dowex 50 Catalysis[1]

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RECEIVED MAY 28, 1954

Application of ion-exchange resins as catalysts for the essentially complete hydrolysis of proteins

(1) This work was supported by the Herman Frasch Foundation.