CHROMATOGRAPHIC SEPARATION OF PHOSPHATE COMPOUNDS*

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The widely used method of Hurlbert et al. (1) for the separation of nucleotides has two features that can be disadvantageous in certain cases. 1. The removal of ammonium formate cannot be easily accomplished. 2. Acid labile compounds, such as creatine phosphate might suffer breakdown during the separation procedure.

It was found that using Dowex-1 - bicarbonate columns and potassium bicarbonate as eluent a satisfactory separation of a group of phosphate compounds could be achieved. This system has the advantage of operating at a nearly neutral pH and most of the $KHCO_3$ can be rapidly removed as potassium perchlorate and CO_2. Under the conditions described below the residual salt concentration after the removal of potassium perchlorate is 0.3%. Thus, e.g., 90 percent of the salt can be removed from a 3 percent $KHCO_3$ solution.

The Dowex-1-Cl resin (X - 10) was washed in 8 percent $NaHCO_3$ solution until chloride free: this was followed by extensive washing with H_2O. The phosphate compounds were added in a volume of 5 ml to an 18 x 1 cm column connected to a gradient elution system and a fraction collector, as described by Hurlbert et al. (1). The mixing vessel contained 500 ml H_2O, and the elution was carried out at room temperature at a rate of 10 drops/min, collecting fractions of 100 drops. The fractions were neutralized with perchloric acid; the potassium perchlorate was removed by centrifugation after standing at -15° for 1 hr. The

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eluent added to the mixing vessel was initially 3 percent $KHCO_3$, and 4 percent and 7 percent solutions were substituted after the collection of 120 and 220 fractions, respectively.

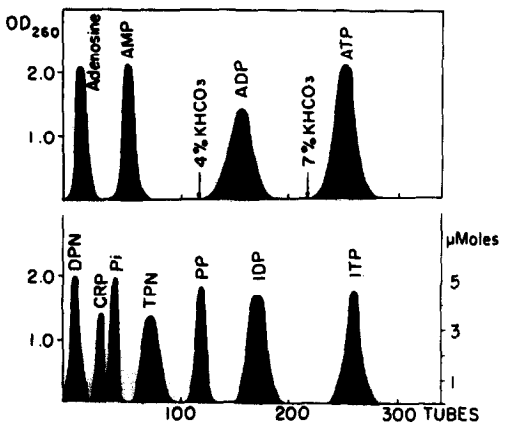

Fig. 1. Separation of phosphate compounds on a Dowex-1-bicarbonate column.

The upper and lower portions of the figure represent two different runs. 20 µmoles of each of the phosphate compounds were added to the column and the elution was carried out as described in the text.
L.h.s. ordinate: optical density of the nucleotide fractions at 260 mµ.
R.h.s. ordinate: the concentration of creatine phosphate, inorganic ortho- and pyrophosphates in µmoles/fraction.
Abscissa: number of fractions collected.

The method as described is most satisfactory for the separation of adenosine mono-, di-, and triphosphates and for the separation of creatine phosphate from inorganic phosphate (Fig. 1). The recovery of the creatine phosphate is between 85 - 90 percent. When 10 µmoles of inorganic orthophosphate labelled with 25 µCuries of P^{32} and 5 µmoles of creatine phosphate were run simultaneously no radioactivity could be detected in the creatine phosphate containing fractions. Although no complete separation could be achieved between glucose-6 phosphate and creatine phosphate, glucose-1-6 diphosphate and TPN, phosphoenolpyruvate and pyrophosphate, each of these compounds can be determined in the pre-

sence of the other by properly selected methods.

REFERENCE

1. Hurlbert, R.B., Schmitz, H., Brumm, A.F., and Van R. Potter. J. Biol. Chem. 209, 23, 1954.