biosynthesis of the coenzyme, a shortage might be postulated of the sulphydryl groups, notably cysteine, that are needed for the formation of pantotheine.

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Separation of Mono-*n*-butyl Phosphate and Di-*n*-butyl Phosphate by means of Ion Exclusion

Many different methods have been applied by several authors to the separation of mono- and di-*n*-butyl phosphate. Kumler and Eiler[1], who were interested in obtaining pure samples for the determination of their dissociation constants, used fractional precipitation of barium salts. Extraction by organic solvents (*n*-amylic alcohol or dibutylic ether) was applied with a good yield by Stewart and Crandall[2]; di-*n*-butyl phosphate is preferentially extracted into the organic phase. With the aim of obtaining mono- and di-*n*-butyl phosphate labelled with phosphorus-32, after the isotopic transfer of phosphorus-32 from $H_3{}^{32}PO_4$ to the esters, the exchange resin 'Dowex 2 *X*–8', 100–200 mesh, chloride form, was used by Higgins and Baldwin[3] to separate the three components from each other. Thus the mixture at *p*H 9 was fixed at the top of the resin bed; *ortho*-phosphoric acid, mono- and di-*n*-butyl phosphate were consecutively obtained by eluting with 0·05 *M* hydrochloric acid and with 0·5 *M* potassium chloride.

Separations of the same type were also carried out in our laboratory with satisfactory results, by using 'Amberlite *IRA* 400', carbonate form, and 0·1 *M* sodium carbonate and 0·1 *M* sodium chloride as selective eluants.

To investigate another way of separation, some ion exclusion experiments were carried out by us. This method needs no regeneration of the resin, which acts with a different mechanism from that of common ionic exchange. Many factors are reported to determine the limitations of ion exclusion[4]; among them are: rate of flow of solution through the column; volume of feed and concentration ratio of the two components; operating temperature; size of resin particles and percentage of cross-linkage; degree of dissociation of compounds to be separated. In principle, separation through ion exclusion is effective when compounds involved possess different degrees of dissociation, because one component acts as an 'ionic substance' in respect to the other 'non-ionic'.

Even though mono- and di-*n*-butyl phosphate have nearly the same dissociation constants[1] (for mono-*n*-butyl phosphate $K' = 1{\cdot}29 \times 10^{-2}$, $K'' = 1{\cdot}40 \times 10^{-7}$ and for di-*n*-butyl phosphate $K = 1{\cdot}90 \times 10^{-2}$) a fairly good separation was obtained by operating in the conditions reported below. A bed of 50 c.c. of 'Dowex 50 *X*–8', 50–100 mesh, hydrogen form, having a cross-section of 1·1 cm.², was fed with 9 ml. of a solution containing 0·071 m.moles/ml. of mono-*n*-butyl phosphate and 0·037 m.moles/ml. of di-*n*-butyl phosphate at a flow-rate of 6·25 ml./hr. cm.². While eluting at room temperature (18° C.), with distilled water at the same rate of flow, fractions of 2·2 ml. were automatically collected. Results are shown in Fig. 1, where mono-*n*-butyl phosphate is shown appearing after 18 ml. of effluent and di-*n*-butyl phosphate after 38 ml., as was expected on a theoretical base. Furthermore, it should be pointed out that mono-*n*-butyl phosphate, in spite of its slightly lower dissociation constant, is eluted first. The yield of separation is 95·5 per cent in pure mono- and 89 per cent in pure di-*n*-butyl phosphate, since in one fraction alone the two components were both present. Rate of flow can be raised to 9 ml./hr. cm.² and feed concentration varied at about ±10 per cent without influencing the separation yield, which is lowered only when the amount of resin is reduced by 20 per cent.

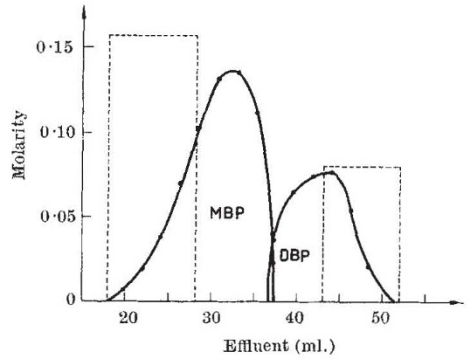

Fig. 1. Elution curves for mono- and di-*n*-butyl phosphate. Dashed lines indicate the theoretical shape of the separation

To prepare significant amounts of pure compounds, seven semi-continuous cycles were carried out, on 650 c.c. of resin, 5·2 cm.² cross-section. Each cycle employed 100 ml. of feed solution containing 0·061 m.moles/ml. of mono-*n*-butyl phosphate and 0·028 m.moles/ml. of di-*n*-butyl phosphate, at a rate of flow of 9 ml./hr. cm.².

All the experiments described here were performed on solutions of mono- and di-*n*-butyl phosphate obtained synthetically from phosphorus pentoxide and butanol, and brought to suitable concentrations by dilution with water.

Analyses of fractions were carried out by potentiometric titration with 0·1 or 0·01 *N* sodium hydroxide; in some cases a purity control by paper chromatography was effected[5].

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