l'électrophorèse en 2-ème dimension. Le seul inconvénient de ce procédé est la difficulté de mettre en évidence les aminoacides non substitués, par la réaction à la ninhydrine, à cause de la présence de substances donnant une réaction positive à la ninhydrine dans les amines volatiles utilisées et de l'existence d'une large bande d'éthanolamine formée au cours du développement chromatographique en 1-ère dimension*. Sur les diagrammes de la Fig. 1 ont été indiquées les positions des artéfacts (S_1, S_2, S_3) issus de la décomposition de la S-mono-DNP-cystéine**. Les deux produits dérivés principaux S_2 et S_3 sont indistingables, par ce procédé d'analyse, respectivement de la N,N'-di-DNP-cystine (pour S_2) et de la N,S-di-DNP-cystéine (pour S_3).

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* Cette mise en évidence est facile sur les chromato-électrogrammes réalisés avec le système: *n*-butanol–diéthylaminoéthanol–eau (80:5:15) puis diéthylaminoéthanol 0.025 *M*.

** Produit préparé à pH 5.2 selon réf. 5 et donnant une seule tache, très distincte de celle de la N,N'-di-DNP-cystine et de la N,S-di-DNP-cystéine, par chromatographie dans le système *n*-butanol–acide acétique–eau (70:7:23) puis phénol saturé d'eau; notons que la S-mono-DNP-cystéine est une substance peu stable.

An effect of the sample pH on the separation of phosphates by ion-exchange chromatography*

Ion-exchange separation of the acid soluble organic phosphates of protein-free trichloroacetic acid extracts of milk[1] showed in preliminary studies that inorganic orthophosphate was eluted as two peaks if the pH of the extract was 4. If the pH of the extract was adjusted with ammonia to 6.4, the first peak decreased in area while the second increased.

These observations indicated the elution of inorganic phosphate from the ion-exchange resin was dependent on the pH of the sample put on the column, and that orthophosphate could, under certain circumstances, behave as two distinct compounds. Since the effect to our knowledge has not been the subject of previous investigation, this note appeared to be worthwhile.

Potassium dihydrogen phosphate (41 mg) containing 10 mg of phosphorus was dissolved in 10 ml of water. The solution (pH 4.7) was charged to a 25 × 1 cm Dowex

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1 — X8 column in the chloride form, followed by 100 ml of water. Concave gradient elution (using a two-reservoir system with the second reservoir in the shape of a cone) with 0–1 *M* NH_4Cl was carried out. The effluent from the column was collected in 10 ml fractions and was analyzed for the presence of inorganic phosphate according to BARTLETT[2].

A solution (110 ml, pH 8.0) of disodium hydrogen phosphate containing 10 mg of phosphorus was chromatographed the same way. The two elution diagrams (Fig. 1 and Fig. 2) illustrate the effect of the pH of the sample solution. At pH 4.7 two peaks

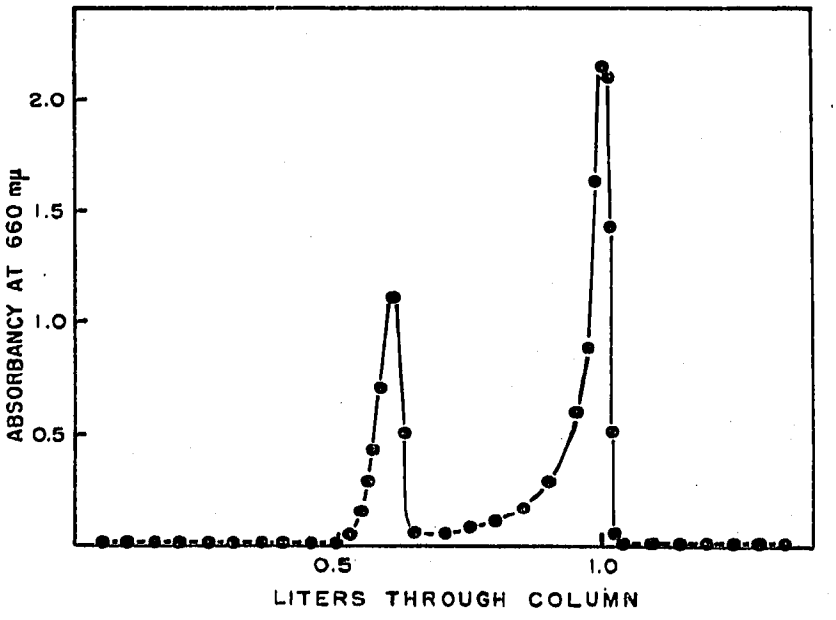

Fig. 1. Elution of inorganic phosphate. pH of sample = 4.7.

were obtained, while only one appeared at pH 8.0. The last peak in the first diagram occupied a position identical to the single peak in the second diagram.

The results showed that the ionic form in which inorganic phosphate was initially absorbed by the column was to some degree determined by the pH of the sample solution.

At pH 8.0 the phosphate is predominately present as monohydrogen phosphate ions, while pH 4.7 favors the dihydrogen phosphate ion. The relative amounts of divalent *vs.* monovalent ions absorbed by the resin will also be influenced by the rate constant of the exchange reactions:

$$2\ RCl + HPO_4^- \leftrightharpoons R_2HPO_4 + 2\ Cl^- \quad (1)$$

$$RCl + H_2PO_4^- \leftrightharpoons RH_2PO_4 + Cl^- \quad (2)$$

where R designates the cationic groups of the resin. The results indicate that at pH 8.0 the conditions are such that virtually all of the orthophosphate is absorbed as divalent ions.

It is reasonable to believe that this effect of pH of the sample solution on the valency of ions absorbed on the resin is not limited to orthophosphate but also may

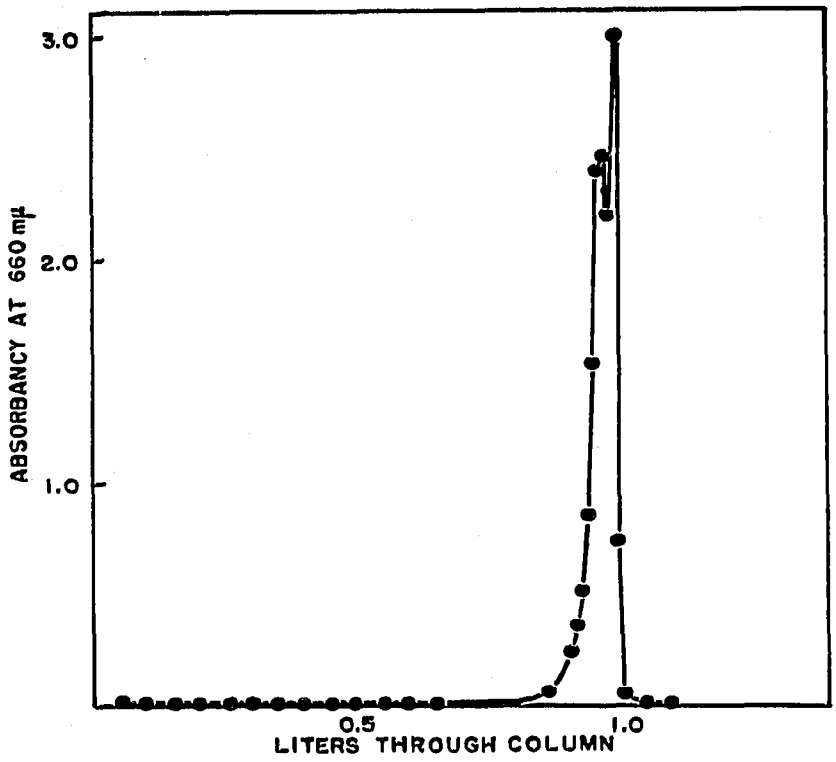

Fig. 2. Elution of inorganic phosphate. pH of sample = 8.0.

occur with organic phosphates, nucleotides, etc. Thus the possibility exists that artifactual peaks may occur in ion-exchange chromatography of polyvalent ions unless the pH of the sample is properly adjusted.

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