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Concentration of chromatographic effluent for gel electrophoresis

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Abstract

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This is a technique we have developed for use in the purification of dehydrogenases from Neurospora but it may hove other applications. In the purification of enzyme extracts the fractions of effluent collected from the chromatographic column ore much too dilute for direct application to supporting material for gel electrophoresis. A simple method of concentrating the effluent was needed to allow detection of bands of protein or isoenzymes after electrophoresis. This method is bared on the technique first described by Horowitz and Fling (1962 NN#2: 19) who placed dilute solutions of tyrosinase in dialysis bags to dialyze against solid sucrose. We reversed the positions of the sucrose and the sample. Sucrose granules are placed in a dialysis bag of large diameter. The top surface of the bag is flattened so that a row of filter paper pieces can be placed in close contact with the surface. The bog is chilled and cold fractions of effluent are pipetted onto the surface until a layer of liquid is formed on top of each piece. Water and electrolytes ore absorbed into the sucrose below and in on hour or so a second application, if necessary, can be made or the filter paper piecer can now be inserted into starch or acrylamide gel for electrophoresis. This method allows a large number of samples to be concentrated simultaneously and requires very little expenditure of equipment or material. The pipetted amounts of effluent also give a control of the amount of protein or enzymes to be subjected to electrophorerir. - - - Pediatric Laboratory, University of Michigan Medical Center, Ann Arbor, Michigan.