*Nature*, **168, 786- 787, (1951)**
Separation of enzymes by paper chromatography
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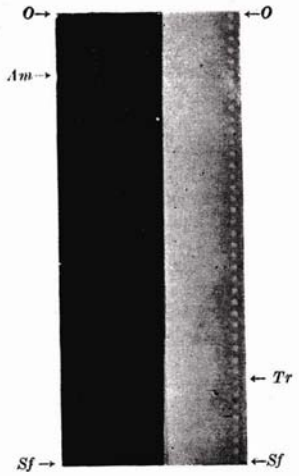
## Separation of Enzymes by Paper Chromatography

In a recent publication[1] from this laboratory, a technique for the study of the chromatographic behaviour of enzymes on paper was described. The ease with which enzymes can be readily identified on the paper chromatogram by the agar-plate method enabled us to study the chromatographic behaviour of some of the important enzymes. In the course of preliminary experiments with amylases, phosphorylases and phosphatases from various sources, we found that some enzymes did not move at all while others moved considerable distances on the paper. This observation prompted us to attempt the separation of enzymes by paper chromatography.

Apart from the work of Mitchell, Gordon and Haskins[2], who report that some separation of adenosine deaminase from amylase has been achieved by their 'chromatopile' method, no really complete separation of enzymes by paper chromatography has been reported. In a recent note by Wallenfels and Pechman[3], which appeared after we sent our communication[1] on the technique for the detection of enzymes on paper, the separation of the enzymes of a mould extract by a micro-electrophoretic technique was described. However, by adopting the usual paper chromatographic technique and using aqueous acetone or sodium chloride or a suitable combination of these two as developing solvents, we have been successful in achieving a few good separations of the individual enzymes from mixtures prepared artificially or obtained from natural sources such as mould or tissue extracts.




Chromatogram showing the separation of amylase from trypsin : *O*, origin ; *Sf*, solvent front ; *Am*, amylase ; *Tr*, trypsin

As a typical example of the separation that can be so readily obtained by the paper chromatographic method, we reproduce a photograph of the separation of amylase from the proteinase, trypsin, present in commercial trypsin powder (Pfanstiehl Chemical Company). From the photograph, it can be seen that the amylase shows little or no tendency to move, whereas trypsin has moved quite a distance from the starting line, and a complete separation of the two enzymes is thus obtained. As it is necessary to adhere to our procedure to be able to reproduce successfully the above separation, we are giving below the essential features of our method.

A 2 per cent aqueous solution of commercial trypsin powder was prepared and centrifuged to remove the suspended impurities. By means of a micro-pipette (11 μl.) two spots of the enzyme solution were placed, at an interval of 4 cm., on the starting line of the filter paper strip (Whatman No. 1, 40 × 8 cm.). The chromatogram was run at low temperature (0–5° C.), the developing solvent being 50 per cent aqueous acetone containing 1·5 per cent sodium chloride. After the solvent front had advanced a distance of 25 cm. from the starting line, the filter paper strip was removed from the chromatographic chamber and cut into two strips (*A* and *B*) longitudinally, to locate the positions of the two enzymes. The position of the amylase on strip *A* was detected by the method previously described[1]. The elegant and *simple method of Wallenfels* and Pechman[3] for the detection of proteinases was used for locating the trypsin on the chromatogram. Accordingly, strip *B* was placed on the wetted gelatin side of a 35-mm. ciné-film strip about 12 in. long. The combination was placed in an incubator at 30° C. and water was sprayed, at intervals, on to the paper to keep it moist. After about two hours, which was sufficient for the trypsin to liquefy the gelatin, the paper was removed from the film strip. The position of the enzyme, trypsin, on the paper is indicated by liquefaction of the gelatin, resulting in the formation of a clear zone.

It is interesting to note that neither aqueous acetone nor water alone is able to bring about any appreciable movement of trypsin. However, the addition of sodium chloride in concentrations as little as 0·5 per cent to the aqueous acetone or water results in the trypsin moving a considerable distance from the starting line. Further, we have observed that the use of saline solutions enhances the definition of the spots by arresting tailing.

Details of the other separations of enzymes so far achieved by us will be published elsewhere.

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July 7.

[1] Giri, K. V., and Prasad, A. L. N., *Nature*, **167**, 859 (1951).
[2] Mitchell, H. K., Gordon, M., and Haskins, F. A., *J. Biol. Chem.*, **180**, 1071 (1949).
[3] Wallenfels, K. W., and Pechman, E. V., *Ang. Chem.*, **63**, 44 (1951).