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The detection of pyruvate and phosphoenolpyruvate on chromatograms

A comprehensive technique has been described by the author[1] for the separation and identification on paper chromatograms of the phosphorylated compounds of the glycolytic pathway and related reactions. Unfortunately, in two of the solvents most useful for the separation of phosphates, namely the third solvent (GW3) described by GERLACH, WEBER AND DÖRING[2] and the isobutyric acid solvent of KREBS AND HEMS[3], phosphoenolpyruvate and inorganic phosphate have nearly the same R_F value. Since many of the mixtures which may be studied by paper or thin layer chromatography contain considerable amounts of inorganic phosphate the phosphoenolpyruvate spot is obscured by the larger overlying spot of orthophosphate when chromatograms are treated with an acid molybdate reagent and subjected to ultraviolet irradiation. For easy identification of phosphoenolpyruvate a reagent is required that does not depend on reaction with the phosphate group.

Such a reagent has now been developed based upon the rapid catalytic breakdown of phosphoenolpyruvate to pyruvate and inorganic phosphate in the presence of mercuric ion[4] and the detection of pyruvic acid as the highly fluorescent 2-hydroxy-3-methylquinoxaline formed by condensation with *o*-phenylenediamine in acid[5]. The latter reaction also serves as an easy and sensitive means of detecting pyruvic acid itself.

Paper chromatograms are dipped through a solution of 0.1 *M* mercuric nitrate in *N* nitric acid diluted with 2 volumes of ethanol. When the papers are dry they are dipped through a mixture of 5 ml 2 % *o*-phenylenediamine hydrochloride, 5 ml 2 *N* sulphuric acid, and 20 ml of ethanol. Pyruvate and phosphoenolpyruvate appear as pale yellow spots with a green fluorescence under both short and long wave ultraviolet light. The spots appear at room temperature but may be intensified a little by heating for 2 min at 90°; longer heating increases the colour of the background and reduces the fluorescence. After treatment with mercury, 2 μg/cm^2 of phosphoenolpyruvate and 1 μg/cm^2 of pyruvate may be detected. If the mercury treatment is omitted phosphoenolpyruvate does not show up but as little as 0.5 μg/cm^2 of pyruvate may be detected. The previous treatment with mercuric nitrate reduces the fluorescence given by pyruvate by about half. The comparison of fluorescent spots on duplicate chromatograms, one of which has been treated with mercuric ions, and the other that has had this treatment omitted appears to provide a very specific way of detecting phosphoenolpyruvate.

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Department of Biochemistry, McGill University, Montreal, Que. (Canada) T. WOOD

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