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This Week's Citation Classic

Bandurski R S & Axelrod B. The chromatographic identification of some biologically important phosphate esters, *J. Biol. Chem.* **193**:405-10, 1951. [Kerckhoff Labs. Biol., Calif. Inst. Technol., Pasadena, and Enzyme Res. Div., Bur. Agr. and Indust. Chem., Agr. Res. Admin., US Dept. Agr., Albany, CA]

The objective of this research was to provide a means for separating and identifying phosphate esters involved in glycolysis in higher plants. At the time we didn't know whether plants had a glycolytic system or whether they did oxidative phosphorylation and made ATP. These ideas were only then being developed in animal and microbial systems. Our procedure was based on two-dimensional paper chromatography of plant extracts with successive development in an acidic and in a basic solvent. The solvents finally selected gave the best overall resolution of the intermediates involved in plant glycolysis. [The *SCI*® indicates that this paper has been cited over 475 times since 1961.]

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"The frequent citation of this paper reflects not a classic quality in the paper but simply the needs of biochemistry for methods. When the work was done, paper chromatography was only then being developed as a sensitive and rapid means of detecting chemical compounds in complex biological mixtures. Although paper chromatography was already quite useful for separating sugars, amino acids, and organic acids, it could not readily be applied to phosphorylated compounds since the phosphorus so dominated the chemical properties of the compounds that they were never sufficiently separated. The solvents Axelrod and I described in the paper gave reasonably adequate resolution of the ten or so major phosphorylated compounds occurring in biological extracts.

"A further difficulty with chromatography of phosphorylated compounds on paper was that methods of visualizing the compounds caused almost total destruction of the paper. Here is where chance entered in. Axelrod and I had sprayed a paper chromatogram with the then used Hanes-Isher-wood reagent. This reagent contained strong acids and had the paper been heated to hydrolyze phosphorus-containing compounds, the paper would have disintegrated. There was insufficient phosphorus for detection so we examined the sprayed sheet in a dark room under ultraviolet illumination. To our astonishment, a blue spot gradually became visible. Apparently, the ozone generated by the UV lamp, in the presence of acid and molybdate from the spray, easily degraded even resistant esters to inorganic phosphate which then reacted to form the blue colored phosphomolybdate product. Thus, accidental use of an ultraviolet lamp led to a way for degrading organophosphates to inorganic phosphorus.

"Now, 30 years later, we realize how primitive our methods were—but we did have the correct idea and that was to try to spread out all the compounds being made and then in a gross way to try to decide what major metabolic products were being made and what major perturbations the experimental treatments were causing.

"Also, the work illustrates the principle of serendipity. One starts out with a reasonable project and a humble and simple question and all else follows automatically. The methods are developed as one needs them and then from the methods come answer after answer, each one leading to a new level of understanding. Accident plays a role, of course, as it did in our case, but accident only provides the opportunity; it is the determination to answer a question that provides the progress.

"The reference cited illustrates the dwarfing complexity of the work done since our primitive publication."[1]

1. **Stahl E,** ed. *Thin-layer chromatography: a laboratory handbook.* New York: Springer-Verlag, 1977. 1041 p.