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This Week's Citation Classic ™

Mauzerall D & Granick S. The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.* **219**:435-46, 1956. [Rockefeller Institute for Medical Research, New York, NY]

Delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) in biological fluids are separated by the use of ion exchange resins. A pyrrole is quantitatively formed by condensing ALA with acetyl acetone. Both the pyrrole and the PBC are determined colorimetrically with a modified Ehrlich's reagent. The factors influencing the optimal conditions for these reactions are discussed [The *SCI*® indicates that this paper has been cited in over 960 publications since 1956.]

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"In 1955, Sam Granick realized the importance of a simple assay for the crucial metabolite δ-aminolevulinic acid (ALA). For a fresh graduate in physical organic chemistry, having just joined Granick at the Rockefeller Institute, this was a breeze. However, the adage on the time required to solve an 'easy' problem held true: multiply the guess by two and go to the next higher time scale. There existed an easy color reaction for the next intermediate in the biosynthetic pathway, porphobilinogen (PBG): the Ehrlich reaction. Why not convert ALA to a pyrrole and use the same reaction? One less reagent on the rack. But (and still a but for the few of us interested in the photosynthetic origin of life) ALA→PBG has a low yield *'in vitro.'* Therefore, back to the Knorr pyrrole synthesis and use of *β*-dicarbonyl compounds. This is where the escalation to the higher time scale occurred. Suitable study showed that the conditions could be specified for the quantitative yield of pyrrole using ALA. Writing this reminds me that I never published that work, something about which Granick always chided me.

"Since we had a (nearly) quantitative reaction for ALA, the Ehrlich color reaction definitely needed improvement. It faded characteristically faster than the technician could insert the tube into the Beckman DU (1955). Rates were competing with equilibria, and it was a textbook case of kinetic analysis which was carried out on an analog computer built by C.C. Yang. It was great fun–I could simulate autocatalytic reactions just by the leakage in the capacitors. Unfortunately, all this was chopped out of the *Journal of Biological Chemistry* paper by a referee. Some things do not change that much.

"The molar extinction for the Ehrlich reaction was doubled, and the stability of the color much increased. This was accomplished by increasing the acidity of the solvent: perchloric acid in acetic acid *versus* hydrochloric acid in water. In a fit of worry just before publishing, I strongly heated some of the 'Special Ehrlich Reagent' (taking suitable precautions). It decomposed quite vigorously but did not detonate. Relieved, we sent the manuscript off with a warning that the reagent be disposed of periodically.

"The 'normals' in the test assay included Granick and myself as is usual. Only later[1] was it found that these 'normals' were actually high (possibly because of the presence of lead in the urban environment). Granick tried for many years to interest the drug companies in a 'packaged' column for the analysis. At that time there was no interest in what has now become an active enterprise. So it goes.

"I believe this article is quoted because it is a useful analytical method for an important metabolite. Its presence reflects the genetic defects in hereditary hepatic porphyrias or tyrosinemia and the effects of environmental hazards such as lead. Recent reviews summarize the use of this assay.[2,4] The choice of the particular problem was Granick's. It was a result of his constant probing and questioning. He let me indulge in theorizing to try to answer his questions. It was a good way to start."

1. **Sassa S, Gramick S & Kappas A.** Effect of lead and genetic factors on heme biosynthesis in the human red cell. *Ann. NY Acad Sci.* **244**:419-40, 1975.
2. **Lien L F & Beattie D S.** Comparisons and modifications of the colorimetric assay for delta-aminolevulinic acid synthase. *Enzyme* **28**:120-32, 1982.
3. **Bishop D F, McBride L & Desmick R J.** Fluorometric coupled-enzyme assay for delta-aminolevulinate synthase. *Enzyme* **28**:94-107, 1982.
4. **Sassa S.** Delta-aminolevulinic acid dehydratase assay. *Enzyme* **28**:133-43, 1982.