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This Week's Citation Classic

Lipmann F & Tuttle L C. A specific micromethod for the determination of acyl phosphates. *J. Biol. Chem.* **159**:21-8, 1945.
[Biochemical Research Laboratory, Massachusetts General Hospital, Boston, MA]

The discovery of acetyl phosphate as a metabolic intermediary made a simple, sensitive method for its determination desirable. Acetyl phosphate is an acid anhydride, and a described qualitative method for determination of acetyl chloride with hydroxylamine converts it to the hydroxamic acid which gives a bright purple color on addition of ferric chloride. There was no difficulty converting this into a quantitative method applicable also to the determination of other reactive acyl derivatives under a variety of conditions. [The *SCI*® indicates that this paper has been cited over 850 times since 1961.]

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"I was somewhat surprised to learn that during the period 1961-1975 this colorimetric method still seems to have been extensively used, because radioactive compounds came into use during those years. Now that we are able to assay very small amounts so easily by the use of radioactivity, this often seems preferable to colorimetry. However, we subsequently found this method very useful for detecting activated carboxylic acid derivatives of various kinds, and it has become valuable for detecting them as metabolic intermediaries.

"To give some examples, we found that the determination of acyl phosphates required a concentration of hydroxylamine of only 0.02 M. However, in pork liver extracts, it appeared that due to the presehce of an esterase, the salts of fatty acids in general could be converted to hydroxamate, but concentrations of 2-3 M hydroxylamine were needed. This indicated that by reacting with the esterase, an activated acyl intermediary formed which would react with the high concentration of hydroxylamine to form hydroxamate. Furthermore, it was known that at strongly alkaline reaction, any ester would give hydroxamates. However, unusually reactive esters yielded hydroxamates at the much lower pH of 5.5.

"An hydroxamate formation at low pH became of considerable interest in the case of amino acid activation. This was found to be biphasic: the primary reaction with ATP leads to an aminoacyl adenylate, followed by aminoacyl transfer on the same enzyme to the 3'-terminal of a low molecular ribonucleic acid called the transfer RNA (tRNA). The aminoacyl adenylate and the 3'- (or 2'-) amino acid ester with tRNA are in reversible equilibrium. Therefore, the ester link between the carboxyl group of the amino acid and the tRNA had to be of the energy-rich type, comparable to the phosphoanhydride in aminoacyl adenylate. Accordingly, we found this aminoacyl-tRNA reactive with hydroxylamine at a pH of 5.5 where ordinary aminoacyl esters are completely nonreactive.

"It is my impression that the popularity of the hydroxamate method is due to the versatility of the hydroxamate reactivity for various acyl derivatives, using different pH or different concentrations of hydroxylamine as indicators of the type of reaction product being studied."