

This Week's Citation Classic

CC/NUMBER 46
NOVEMBER 14, 1983

Barker S B & Summerson W H. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* 138:535-54, 1941.
[New York Hospital, Depts. Medicine and Biochemistry, Cornell Univ. Medical College, New York, NY]

This procedure achieved the sensitive and specific quantitation of lactate in biological fluids, eliminating the previous need for oxidation, distillation, and titration. Heating in concentrated sulfuric acid produced acetaldehyde which was directly determined by the purple color formed with p-hydroxydiphenyl in the presence of cupric copper. [The SCJ[®] indicates that this paper has been explicitly cited in over 2,500 publications since 1961. Of these, 70 were in 1980, 70 in 1981, and 55 in 1982.]

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August 23, 1983

"At the present time, it is difficult to appreciate the importance attached to lactic acid in carbohydrate metabolism during the 1930s and 1940s. Measurement of this stabilized form of pyruvate was an essential aspect of many studies, at both *in vivo* and *in vitro* levels—exercise and Warburg tissue slice experiments being good examples of each. Determination of changes in glycogen, glucose, and lactate levels was essential in those days. Of the three, lactate was by far the most laborious, since the Friedemann, Cotonio, and Shaffer¹ (F-C-S) procedure was the standard and involved an incredibly tricky distillation apparatus in which the acetaldehyde produced by permanganate oxidation of lactate was received in an excess of bisulfite. The final eye-straining titration required addition of 0.002N iodine solution to the faintest possible blue-grey endpoint with starch indicator.

"Many laboratory groups which viewed the complicated array of glassware with mixed devotion and loathing tried one or another of the colorimetric procedures proposed for lactate, but found them unreliable. Summerson's primary interest was the development of colorimetric methods for his photoelectric instrument and I was anx-

ious to have a less temperamental procedure than the F-C-S. The effectiveness of the collaboration was probably enhanced by the fact that we worked in separate laboratories. As individually independent investigators, we routinely subjected each other's results to careful scrutiny and reconciled any discrepancies. The relatively long-lived success of the published Barker-Summerson procedure can undoubtedly be explained by the combination of our rigorous control of each step plus the elimination of a complicated distillation and painstaking titration. Simplicity was achieved by carrying out both stoichiometric acetaldehyde production and its reaction with p-hydroxydiphenyl in the same solution contained in a single appropriate test tube.

"Although this was a technologically primitive era, as viewed nowadays, nonetheless several advances were crucial to the success of the method. Quantitative dispensing of highly purified H₂SO₄ required the use of an all-glass system, including a grease-free, accurately ground standard taper stopcock. Thorough but contamination-free mixing of copper-calcium hydroxide reagents with biological solutions being analyzed was greatly facilitated by the opportune invention of 'Parafilm,' sometimes mistranslated as paraffin. Above all, making a long series of color readings in very concentrated sulfuric acid was practical only by the advent of photoelectric colorimeters which accepted closely standardized test tubes. Early variable results, as well as one laboratory's claim that lead was necessary, caused us to test various metallic ions, with the important discovery of a considerable enhancement of color development by added copper or by a mixture of ferrous and ferric iron.

"This procedure, for its day, was remarkably sensitive, one of the first to be accurate in the microgram range. In fact, its very sensitivity was the source of most of the contamination problems as other laboratories adopted it. Lactate is present in sweat and saliva at easily detectable levels. Rigorous manipulative standards had to be adopted, e.g., avoiding handling of pipette tips or sneezing over open test tubes. After some two decades of wide acceptance, it was eventually superseded by spectrophotometric analysis utilizing lactic dehydrogenase.

"For a report of recent work in the field, see reference 2."

1. Friedemann T E, Cotonio M & Shaffer P A. The determination of lactic acid. *J. Biol. Chem.* 73:335-58, 1927.
2. Byers F M. Automated enzymic determination of L(+) and D(-)lactic acid. *Meth. Enzymology* 89:29-34, 1982.