

This Week's Citation Classic.

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Aminoff D. Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochemical J.* 81:384-92, 1961. [Public Health Research Institute of the City of New York, Inc., New York, NY]

Two colorimetric procedures, the thiobarbituric acid (TBA) and alkali-Ehrlich, have been developed for the determination of free sialic acid in the presence of the bound sugar. Complementing each other, and those for the determination of total sialic acid in sialoglycoconjugates, it is now possible to follow the chemical and enzymatic release of sialic acid from many biologically important compounds. [The SC® indicates that this paper has been cited over 1,050 times since 1961.]

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"G.K. Hirst, whom I joined in 1957 at the Public Health Research Institute of the City of New York, Inc., requested that I develop an assay for neuraminidase. This enzyme is found in many bacterial extracts and also in the myxo group of viruses.

"My approach was determined by my previous experience. I had carefully re-examined the Morgan-Elson assay (MEA) for N-acetylhexosamines while working on my PhD thesis with W.T.J. Morgan in England. A. Gottschalk was visiting our labs at about that time. He had come from Australia, where he was working on the identification of the product released by the action of influenza virus on ovomucin. He found anomalies in the behavior of the product in the Morgan-Elson assay, and came to us for advice

"The cause of the anomalies was not resolved until a few years later, when the material released by the virus was

identified as sialic acid. The similarity of the colors given by sialic acid and N-acetylhexosamines in MEA convinced me that this assay could also be adapted to determine sialic acid. The remaining requirement, to determine *free* in the presence of the *bound* sialic acid, was ultimately achieved. The alkali-Ehrlich assay, however, is not very sensitive, and is of value only in certain circumstances.

"In search of a more sensitive technique to determine *free* sialic acid in the presence of the *bound* compound, my thoughts kept reverting back to my previous experience with the use of periodate in Conway units to determine the acetaldehyde released from *free* fucose. However, the oxidation products of sialic acid give no volatile material other than formaldehyde. In this, it does not differ from the other common hexoses found in glycoconjugates. The method would thus lack specificity.

"However, one day, while visiting with E. Racker at the same Institute, I observed Dan Levine, one of his graduate students, set up a colorimetric assay for deoxyribose. The method involved oxidation with periodate and the interaction of the oxidation product, malonaldehyde, with TBA to give a pink color. The implications hit me like a thunderbolt, for is not sialic acid also a deoxy sugar?! Rushing back to my own lab, I quickly established that free sialic acid readily reacted, while sialoglycoproteins did not. Far from ideal at the outset, it was laboriously refined and readied for use.

"The reasons for the article having been cited so much are attributable to: a) Sialoglycoconjugates play an important role in many biological systems, b) There is a great need for the determination of *free* in the presence of *bound* sialic acid, c) The TBA assay is extremely sensitive, specific, and reproducible. The only other assay that meets these requirements is Warren's TBA assay,¹ which was developed independently."

1. Warren L. The thiobarbituric acid assay of sialic acids. *J Biol. Chem.* 234:1971-5, 1959.