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Citation Classics

Warren, Leonard. The thiobarbituric acid assay of sialic acids. *Journal of Biological Chemistry* 234:1971-5, 1959.

A colorimetric assay was developed for the measurement of sialic acids in which the products of periodate oxidation are coupled with thiobarbituric acid to form a red chromophore. Since only free sialic acids are measured the reaction could be used for the detection and measurement of neuraminidase (sialidase) which hydrolytically releases sialic acids from their bound form. [The *SCI*® indicates that this paper was cited 2,656 times in the period 1961-1975.]

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"In the mid-fifties the biosynthesis of small molecules was regarded as a praiseworthy subject for study. I had the good fortune to work on the biosynthesis of the purine ring in an exciting laboratory (under J.M. Buchanan, in the Department of Biology, Division of Biochemistry, at M.I.T.) and after obtaining my doctorate it was only natural that I apply my knowledge and skills to determine how other compounds of biological interest were made. Not wanting to remain in the purine field, forever one of Buchanan's students, I decided to look for new sets of compounds to investigate. The structure for sialic acid was formulated at that time by A. Gottschalk and after some reading I decided to look into its mode of biosynthesis. On paper there were a few obvious pathways for its synthesis and these I investigated after arrival at the N.I.H. in Bethesda. Little progress was made, mainly because the existing assays (Direct Erlich, resorcinol and others) were not specific or sensitive enough. In retrospect I'must have observed some of the desired *in vitro* synthesis but the backgrounds were so high and variable that I had little faith in the results.

"At that time, A. Weissbach and J. Hurwitz at the N.I.H. had just completed a study on 2-keto, 3-deoxygluconic acid (KDG) metabolism in bacteria and had used the thiobarbituric acid reaction to assay the compound. [1,2] There were striking analogies between the structures of KDG and sialic acid (N-acetylneuraminic acid) except for one difference that theoretically ruled out the application of the thiobarbituric acid test to the measurement of sialic acid; the amino group on sialic acids was always blocked with an acetyl or glycolyl group. This would prevent the formation of a fragment (β-formylpyruvic acid) from the first four carbons of sialic acid upon oxidation with periodate. Never letting mere theory interfere with my experiments, I used the Weissbach-Hurwitz procedure, after attempting to remove the amino blocking group. I obtained small and variable amounts of color. By systematically varying every parameter that could be altered I nudged the molar extinction co-efficient to about 57,000 in a reproducible way, tested biological materials, and devised methods for corrections. The entire exercise took about 2 months and, in fact, the resulting publication reflects a lack of extensive experience with the method. Exploiting this assay, it was not long before the precise pathway of biosynthesis of sialic acid was determined. The assay was found to be convenient in the measurement of neuraminidase activity because it measured only free sialic acid. Its widespread use over the years attests not only to its usefulness, reliability, and sensitivity but to the fact that it was devised at a time when membranes, cell surfaces, and glycoproteins were becoming very popular with biochemists, virologists, immunologists, and those studying the process of malignancy.

"It pleases me to be identified with this assay. At the same time, the published work has taken on a separate life of its own so that when I consult the paper it is as if someone else had written it. I suppose someone else did, a young aspiring biochemist."

1. **Weissbach A & Hurwitz J.** The formation of 2-keto-3-deoxyheptonic acid in extracts of E. Coli B.I. Identification. *Journal of Biological Chemistry* 234: 705-9, 1959.
2. **Hurwitz J & Weissbach A.** The formation of 2-keto-3-deoxyheptonic acid in extracts of E. Coli B. II. Enzymic studies. *Journal of Biological Chemistry* 234:709-12, 1959.