

## Citation Classics

**Barka T & Anderson P J.** Histochemical methods for acid phosphatase using hexazonium pararosanalin as coupler. *J. Histochem. Cytochem.* **10**:741-53, 1962.

**The authors analyze problems inherent in histochemical demonstration of acid phosphatase activity and describe methods which provide reliable, accurate localizations. [The SC<sup>®</sup> indicates that this paper was cited 778 times in the period 1962-1976.]**

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"Our paper is cited frequently, presumably because it describes a useful azo-dye technique for the histochemical localization of acid phosphatase activity. We felt the paper offered more. It reflected our long experience with the classical Gomori method; pointed out its pitfalls and the causes of artifacts, e.g., nuclear staining, obvious in many published reports; described a workable modification of Gomori's technique; and even alluded to some interesting substrate-specificity of phosphatases in mesangial cells of the kidney. Whatever the merits of the paper, the method described apparently fulfilled a need and became accepted for the simple reason: given the proper chemicals it always works.

"The method, and finally the publication, evolved almost spontaneously. The proper ingredients were, however, present: our long-standing interest in histochemical methods, and particularly phosphatases, going back to the late forties; a stimulating environment in close contact with Leonard Ornstein and B.J. Davis, who were in the exciting phase of

developing the poly-acrylamide gel electrophoresis and discovered that hexazotized p-rosoanilin is an excellent coupler in azo-dye methods; and the great surge of interest in acid phosphatases due to the formulation of the lysosome concept by DeDube and to the insight of Novikoff in identifying the lysosomes in hepatocytes by using electron microscopic and histochemical methods.

"At that time we were also engaged in studying lysosomes using Gomori's technique and were not spared occasional frustrations. We knew a great deal about the workings of Gomori's method and adapted an acceptable variation but, in the search for a more reliable method for acid phosphatases, we tried Davis and Ornstein's new diazonium compound with various naphthol substrates. The extraordinary capability of hexazonium pararosanalin as a capture reagent became apparent when I first attempted acid phosphatase localization using alpha-naphthyl phosphate as the substrate and briefly reported this method in 1960.<sup>1</sup>

"Minor technical problems (diffusion, etc.) prompted us to search for more substantive substrates and the substituted naphthol-AS compounds of Burstone appeared promising. Dr. Anderson synthesized a number of these substrates and reported improved localization of acid phosphatase in the nervous system using naphthol-AS-BI phosphate.<sup>2</sup> After further study, we settled on naphthyl-AS-TR phosphate as the most suitable substrate for this hexazonium coupler in the acid phosphatase reaction.

"Of course, we are gratified by the sustained interest in our paper, but do not fail to appreciate the originality of the contributions of Menten, Young and Green, Burstone and Ornstein and Davis. On a personal level, my memory goes back to many conversations with Paul Anderson, perhaps over a glass of Hungarian apricot brandy, when he reminisced about one of his most remarkable teachers, George Gomori, at the School of Medicine of the University of Chicago, who started all that, after all."

### REFERENCES

1. **Barka T.** A simple azo-dye method for histochemical demonstration of acid phosphatase. *Nature* **187**:248-9, 1960.
2. **Anderson P J & Song S K.** Acid phosphatase in the nervous system. *J. Neuropathol. Exp. Neurol.* **21**:263-74, 1962.