

Cain A J. The use of Nile Blue in the examination of lipoids. *Quart. J. Microsc. Sci.* 88:383-92, 1947.
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This paper provided a review and critique of previous work on the dye Nile Blue as a histochemical reagent for lipoids in tissue sections and a recipe for applying it to distinguish between "neutral" and "acidic" lipoids. [The *SCJ*[®] indicates that this paper has been cited in over 205 publications.]

Validation of a Histochemical Test

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When I returned to Oxford from war service to work for a D. Phil., I didn't know what I wanted to do, except that I didn't want to work in ecology or genetics. This was because I was scared stiff of the two zoology staff members who taught those subjects. (In fact, after my histochemical period, I've worked in just those fields for the rest of my life.) My tutor, Peter Medawar, suggested that I should go round the staff to see what they were doing, beginning next door. Next door was John R. Baker, who told me, "I have just invented a histochemical test." I went no further. I was fond of test-tube chemistry, and anyway, how do you invent a histochemical test? My first assignment was to find out whether Baker's acid haematein test for phospholipines was valid—and if I'd found it wasn't, he certainly would have accepted the disproof. He had no regard whatever for status and retracted his own errors in print immediately after he had convinced himself of them.

This brought up the whole question of how to determine the validity of histochemical tests. The authority was Lison, and he dismissed Nile Blue as having no more significance than any other combination of a lipid colorant and a basic dye; what he hadn't said

was, what was that significance. So I did a number of experiments with what few more or less purified lipoids were available to try to fill the gap. It turned out that the original introducer of the dye was on the right track, and one could distinguish between "neutral" (in some sense) lipoids colouring red and "acidic" lipoids (fatty acids, lipines, etc.) staining blue.

In those days, almost any distinction between types of lipid was of interest—for example, it might enable one to follow variations in lipid metabolism within cells or tissues. So I published it, giving a careful critique of previous work (which had led to a different interpretation because the methods of presenting the substances investigated to the dye were unsatisfactory). I also gave a recipe for applying the dye to tissue sections, and I can only conclude that people have gone on quoting the paper simply because there is a recipe.

John told me I'd been mentioned in print and gave me the reference. With trembling hands I turned over the pages of the volume and found at the very bottom of a page, "The beautiful work of Cain on Nile Blue has shown that...." Self-preening set in. I turned the page and found that the author had got it wrong. (He was in fact an excellent worker, who had read too quickly—I refuse to embarrass him by giving the reference.) I've never bothered since to look up any references to me.

Surely there are far better techniques for separating kinds of lipoids now; the paper should have been superseded years ago. I've never regretted my work on lipid histochemistry; it showed me that papers can be decorated with recondite structural formulae and based on apparently scientific procedures and still be worthless. This paper had no impact on my further career. What it all really shows is the heterogeneity of the class of *Citation Classics*. To call this paper a classic (however qualified) because people have gone on citing it (I hope while improving or rejecting it, but I fear not) is downright misleading.

[Editor's note: Professor Cain's Nile Blue paper is indeed still cited: see, for example, references 1 and 2.]

1. Wagner U A & Maier W A. Nile blue as a stain for exoerythrocytic forms of *Plasmodium yoelii*. *Trans. Roy. Soc. Trop. Med. Hyg.* 83:70, 1989.
2. Labandeira-Garcia J L, Guerra-Seijas M J & Suarez-Nuñez J M. Quantitative histochemical study of changes with age in intracellular lipids of xiphisternal cartilage in rat and rabbit. *Anat. Anzeiger* 164:255-64, 1987.