the supernatant will normally bring the colour into the range where its optical density can be measured on a colorimeter or spectrophotometer. A different final dilution ratio may be required for some other materials, such as denatured proteins exceptionally rich or poor in lysine. A blank is included with every group of analyses.

The method as described is at present being utilized in a programme of development of specific electron stains for protein[6], as a guide to the extent and specificity of reactions, and has been applied to rat liver, ram sperm and *Amoeba proteus*, with ethanol, osmium tetroxide and formalin as fixatives. While results of these investigations lie outside the scope of this communication, attention may be directed here to the need for caution in interpreting ninhydrin data, in certain instances. In particular, there is some indication that, after osmium fixation, many more NH_2 groups may be available for reaction than are revealed by ninhydrin. This apparent 'blocking' has not yet been explained, but the observation underlines the need to apply a multiplicity of analytical techniques when attempting to interpret cytochemical reactions.

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The Periodic Acid – Schiff Reaction

The periodic acid–Schiff (PAS) reaction—the term was introduced by McManus[1,2]—is one of the most widely used histochemical methods. In a recent review of "Periodate Oxidation Techniques" McManus[2] states that the first publication, describing the demonstration of mucin and some other structures by the Schiff reagent after treatment with periodic acid, appeared in *Nature* in 1946[3]. In 1948 Hotchkiss[4] published his description of the PAS reaction, which had been applied already in 1947 by Gersh[5] and by Catchpole[6]. Also in 1947 Marchese[7] used the PAS reaction for the demonstration of glycogen, but independently Lillie[8] used acidified periodate for the same purpose. Since other histochemical texts[9–11] give essentially the same version of the development of our knowledge of the PAS reaction, we should like to direct attention to the work of A. L. Shabadash, whose publications on the PAS reaction apparently are not widely known outside the U.S.S.R.

At a meeting of the Institute for Biology and Biochemistry of the Academy of the Medical Sciences of the U.S.S.R., held on February 14, 1946, Shabadash presented a paper on "A Rational Method for the Histochemical Determination of Glycogen, and its Theoretical Basis". This paper was published in 1947 (ref. 12). After surveying critically the methods in use for the fixation and demonstration of glycogen Shabadash discusses Bauer's method[13], and goes on to say that he experimented with several other oxidants, among them potassium permanganate, but that he was unable to produce the reactive aldehyde groups necessary for the interaction with leukofuchsin. " . . . After a whole row of experiments and investigations I settled on periodic acid which was proposed by Malaprade (1927) and used by Jackson and Hudson (1937) for the study of the structure of starches. . . . I investigated the action of dilute (0·01 mol.) solutions of periodic acid on glycogen *in vitro* and in control preparations (sections of liver) with subsequent treatment with leukofuchsin. The results proved the correctness of the theoretical assumptions. The brightness and intensity of the colour reaction was superior to that of any of the methods published in the literature or known to me; it differed from them not only in the degree of sensitivity, but also in its chemical basis . . . " Shabadash realized that this method was applicable not only to the identification of glycogen, but also of polysaccharides in general. He used both periodic acid and sodium or potassium periodate in water in dilutions which, depending on the amount of glycogen present in the tissues, varied from 0·01 mol. (0·23 gm. per cent) periodic acid to 0·002 mol. periodate. The time of exposure varied from 15 to 25 min. No reducing rinse, only distilled water, was used as a wash. After 20–25 min in leukofuchsin the sections were rinsed in a sulphite wash.

Several points are of interest in this publication.

(1) Shabadash discusses the nature of the PAS reaction, its specificity, the necessity of enzymatic digestion for control purposes, and the possibility of substances other than complex carbohydrates responding to this treatment, at length, so that his paper is unquestionably the most detailed account of the PAS reaction published at that time.

(2) Although Shabadash extensively quotes the work of earlier writers who have investigated the histochemical demonstration of glycogen or other carbohydrates, he does not mention the first publication by McManus[3] of whose work he, presumably, did not know.

(3) Shabadash's paper appears to be the result of a prolonged, systematic search for a specific histochemical method for the detection of glycogen.

(4) In the paper summarized here Shabadash states that the colour obtained is stable and permits the preservation of histological sections under ordinary conditions for years. He then adds: "I have preparations which have preserved their original brightness for seven years". He makes a similar claim in another publication, an extensive monograph on the histochemistry of the normal central nervous system, which appeared in 1949 (ref. 14). In a footnote on p. 104 he states "My preparations have retained their brightness over a period of five to seven years". According to these claims, then, Shabadash had used the PAS reaction as a histochemical tool some time between 1939 to 1944. It should be pointed out that the work of Hotchkiss, started in 1945, remained unpublished for three years, so that Shabadash cannot have known of it.

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