ions respectively, it is to be expected that there will be resonance of the positive charge in both these ions. While this would favour $H_3O^+$, it seems reasonable to suppose that this resonance will also confer some stability to the $H_2O^+$ ion. Furthermore, the dipole interaction energies of a proton with an $H_2O$ molecule on the one hand and with an OH radical on the other, should be of the same order of magnitude.

In view of these considerations, it is probable that whenever OH radicals are formed in aqueous solution, they may be present to a certain extent, in the form of (hydrated) $H_2O^+$ ions according to the equilibrium (2).

Hydroxyl radicals are known to play an important part in the reactions of hydrogen peroxide[2] and also in the action of ionising radiations on water[3].

Recent studies have shown that a number of reactions of OH radicals in aqueous solution exhibit a pH-dependence, which can, in some cases, be accounted for by assuming processes involving the $H_2O^+$ ion.

For instance, in the hydroxylation of certain monosubstituted benzene derivatives by OH radicals, it was found that the ratio of the hydroxylated *ortho-*, *para-*, and *meta*-isomers shows a distinct pH-dependence[4].

The presence of this ion may also have a bearing on the interaction of two OH radicals in aqueous systems. In the gaseous state the experimental evidence is strongly in support of the view that hydrogen peroxide is *not* formed by the recombination of two OH radicals (2 OH = $H_2O_2$)[5]. However, this may be different in aqueous solutions, where it appears that, under suitable conditions, hydrogen peroxide can be formed directly by some interaction of OH radicals. It is suggested that, in acid solutions, hydrogen peroxide formation may take place according to:

$$H_2O^+ + OH \longrightarrow H_3O_2^+ \qquad (3a)$$

$$H_3O_2^+ \rightleftharpoons H_2O_2 + H^+ \qquad (3b)$$

In general, therefore, in all the reactions of OH radicals in aqueous systems, processes involving the participation of the $H_2O^+$ radical ion may have to be taken into account.

A detailed study of the pH-dependence of such reactions is now in progress and may give more information regarding the basicity of the OH radial in solution.

J. Weiss

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*Zusammenfassung*

Theoretische Überlegungen führen zur Annahme, dass das in der Gasphase wohlbekannte Molekülion $H_2O^+$ auch in wässeriger Lösung unter geeigneten Bedingungen eine gewisse Stabilität besitzt. Es ist zu erwarten, dass dieses Ion, welches als protoniertes OH-Radikal aufgefasst werden kann, bei Reaktionen in saurer, wässeriger Lösung eine gewisse Rolle spielt.

[2] *Cf.* J. Weiss, Advanc. Catalys. *4*, 343 (1952).

[3] J. Weiss, Nature *153*, 748 (1944); Brit. J. Radiol. Suppl. *1*, 56 (1947).

[4] H. Loebl, G. Stein, and J. Weiss, J. chem. Soc. *1950*, 2704; *1951*, 405. – G. R. A. Johnson, G. Stein, and J. Weiss, J. chem. Soc. *1951*, 3275. – G. Stein and J. Weiss, J. chem. Soc. *1951*, 3265.

[5] K. F. Bonhoeffer and T. G. Pearson, Z. physikal. Chem. [B] *14*, 1 (1931).

## PRO EXPERIMENTIS

### A New Fixative for Smoked Kymographic Tracings

By the difficulties encountered in using the traditional fixatives of alcoholic solutions of natural shellacs, we were induced to look for a new synthetic plastic substance for the fixation of smoked kymographic tracings.

The ideal properties for such a surface coating agent are: to be inexpensive, readily prepared, quick in drying and rendering the record permanently soft and flexible without any damaging effect to the smoked surface.

Such a fixative suitable for everyday use can be prepared from commercially available polybutyl methacrylate solutions. The available lacquer[1] contains 50% of the polymer in solution, it is transparent with a pale straw colouring.

As the viscosity of this solution is high, a 1 to 7 dilution of the concentrate is made with dry acetone.

The results obtained with this fixative correspond fully with requirements outlined above.

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*Department of Pharmacology, University of Ghent, Belgium, March 28, 1956.*

*Résumé*

Une dilution de polybutyl méthacrylate dans de l'acétone anhydre (rapport 1 à 7) est proposée comme fixateur d'enregistrements sur papier enfumé. Ce nouveau fixateur est incolore, sèche vite, rend les enregistrements flexibles et est peu coûteux.

[1] Available as Vinalak No. 5909 from Vinyl Products Ltd., Carshalton, Surrey, England.

## PRO EXPERIMENTIS

### Efficacy of some Histochemical Techniques for Acid Mucopolysaccharides

In an earlier investigation[1] we studied, by chemical methods[2], the acid mucopolysaccharide content of the rats skin (hyaluronic and chondroitinsulphuric acids) and its variations under diverse experimental conditions. The histochemical examination by metachromasia with thionine was simultaneously made with a portion of the same material, fixed in a 4% formalin solution in 90° alcohol. In accordance with Lison's recommendations[3] for avoiding false reactions, use was made of an aqueous 0·5% solution of that stain in an acid medium (pH 3·2) and of Apathy's syrup for mounting. Some sections were previously incubated for 5 h at 37°C with 1:25,000

[1] E. Del Conte, J. Della Sala, and M. Stux, Acta endocrinol. *20*, 343 (1955).

[2] R. H. Pearce and E. M. Watson, Canad. J. Res. [E] *27*, 43 (1949).

[3] L. Lison, *Histochimie animale; méthodes et problèmes* (Gauthier-Villars, Paris 1936).

testicular hyaluronidase, while other sections, which served as controls, were incubated with saline solution and with filtered saliva. This procedure being followed, the identity of the results of the chemical and those of the histochemical methods was complete; a deeper metachromatic staining was found in the derms of skins which, by chemical means, proved to be richer in acid mucopolysaccharides and *vice versa*. On the other hand, the staining was not altered by ptyaline and it disappeared completely after treatment with hyaluronidase, which made it possible to ascribe it to the hyaluronic and chondroitinsulphuric acids, both being hydrolyzed by this enzyme[4].

Further study of successive sections of the same material by other histochemical methods widely used for polysaccharides, such as those of McMANUS[5] with periodic acid-SCHIFF and HALE's[6], improved by RINEHART and ABUL-HAJ[7], with colloidal iron, have enabled us to demonstrate, in the light of such chemical data as we possess, their relative efficiency for the demonstration of acid mucopolysaccharides. A mild staining was obtained with both methods in the derms of the various skins, while no significant change was appreciated corresponding to the varying concentration of the studied substances. No change in the degree of staining which could be attributed to the hyaluronic or chondroitinsulphuric acids was likewise noticed after treatment with ptyaline and hyaluronidase under the abovementioned conditions; by contrast, the response to colloidal iron appeared to be more intense in some cases.

These results confirm that, despite its well-known affinity to various polysaccharides, McMANUS' method does not stain the acid mucopolysaccharides[8] and that HALE's method, which was specifically suggested for these substances, is not more valuable for this purpose[9]. The metachromatic reaction, however, notwithstanding some objections[10], is quite simple and, if duly carried out with adequate precautions and supported by incubation with hyaluronidase, retains an undeniable practical value in the demonstration of acid mucopolysaccharides[11].

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*Résumé*

L'efficacité relative de certaines méthodes histochimiques utilisées habituellement pour la mise en évidence des mucopolysaccharides acides a été étudiée sur des peaux de rats soumises à des conditions expérimentales diverses. Les différentes concentrations des substances en étude étaient connues, ayant été quantitativement déterminées par la méthode chimique de PEARCE et WATSON. La réaction métachromatique, réalisée avec les précautions nécessaires et avec le concours de la digestion par l'hyaluronidase, nous a fourni une image fidèle de la concentration de mucopolysaccharides acides. Ce n'est pas le cas lorsqu'on emploie l'acide périodique-SCHIFF ou le fer colloïdal qui ne semblent pas être très utiles pour la mise en évidence des substances étudiées.

[4] M. B. MATHEWS, S. ROSEMAN, and A. DORFMAN, J. biol. Chem. *188*, 327 (1951).

[5] J. F. A. McMANUS, Nature *158*, 202 (1946).

[6] C. W. HALE, Nature *157*, 802 (1946).

[7] J. F. RINEHART and S. K. ABUL HAJ, Arch. Path. *52*, 189 (1951).

[8] D. V. DAVIES, Stain Techn. *27*, 65 (1952). – A. G. E. PEARSE, *Histochemistry; theoretical and applied* (J. & A. Churchill Ltd., London 1953). – G. GOMORI, *Microscopic histochemistry; principles and practice* (The University of Chicago Press, Chicago 1952).

[9] D. V. DAVIES, Stain Techn. *27*, 65 (1952). – G. GOMORI, *Microscopic histochemistry; principles and practice* (The University of Chicago Press, Chicago 1952).

[10] B. SYLVÉN and H. MALMGREN, Labor. Invest. *1*, 413 (1952).

[11] A. G. E. PEARSE, *Histochemistry; theoretical and applied* (J. & A. Churchill Ltd., London 1953). – R. E. MANCINI (personal communication).

## PRO LABORATORIO

### Ultramikrotom mit mechanischem Vorschub

Durch die grundlegenden Arbeiten der Schulen von PORTER und SJÖSTRAND ist gezeigt worden, dass hochauflösende Elektronenmikroskopie von Dünnschnitten nur möglich ist, wenn die Schnittdicke unter 300 Å liegt. Das beste erreichbare Auflösungsvermögen beträgt ungefähr $^1/_{10}$ der Dicke des Objektes[1]. Auf verschiedener Grundlage wurden mehrere brauchbare Mikrotome entwickelt. Diese unterscheiden sich im wesentlichen nach der Art des Vorschubs und der Bewegung, die der Objekthebel beschreibt. Allen erfolgreichen Instrumenten ist gemeinsam, dass der Block nie hinter dem Messer zurückgeführt wird. Bei diesem Zurückführen wird der soeben abgehobene Schnitt auf den Block zurückgezogen; dadurch wird dieser benetzt, was ein weiteres Schneiden verunmöglicht. Thermischen Vorschub benutzen die Mikrotome von SJÖSTRAND[2], HODGE, HUXLEY und SPIRO[3], v. BORRIES[4] und GAUTIER[5]. In allen diesen Mikrotomen wird der Block in einer zum Messer parallelen Ebene rotiert. Im Mikrotom von FERNANDEZ-MORAN[6] hingegen wird er in einer zum Messer senkrechten Ebene bewegt. Im einzigen Mikrotom mit mechanischem Vorschub (PORTER und BLUM[7]) wird ein langer Objekthebel mit Hilfe einer Führung seitlich am Messer vorbei zurückgeführt. Ein minimaler mechanischer Vorschub von 200 Å kann eingestellt werden.

In dem hier zu beschreibenden Mikrotom (vgl. Abb. 1) verwenden wir ebenfalls einen mechanischen Vorschub, jedoch eine rotierende Bewegung des Blocks in einer zum Messer senkrechten Ebene[8]. Das Mikrotom ist für Handantrieb vorgesehen.

Der Vorschubmechanismus wurde unverändert übernommen aus dem früher mit DANON[9] entwickelten und von Trüb, Täuber & Cie fabrizierten Mikrotom: er ist zusammengesetzt aus der Bewegung einer Mikrometer-

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[2] F. S. SJÖSTRAND, Exper. *9*, 114 (1953).

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