Reprinted from "The Microscope," July-August, 1953. (4 *New Zealand Avenue, Barbican, London, E.C.* 1)

THE ELIMINATION OF XYLENE FROM HISTOLOGICAL TECHNIQUE

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(Translated from *Mikroskopie*, Wien, VII, 1952, Heft 5 & 6, p. 208, by D. S. SPENCE, M.B.)

Translator's Note

Owing to the various advantages of isopropyl alcohol in microscopical work, its use has recently been advocated; and, judging from its properties, it would seem that it has been much and surprisingly neglected. Its chemical formula is $CH_3 \cdot CH(OH) \cdot CH_3$, and it is sold about 99 per centum by weight, by Laporte Chemicals Ltd. Some with water is on the market. The specific gravity of the commercial alcohol is about ·786, and its price recently was 3s. 9d. a pint. It mixes, according to the writer's experiments, with water in all proportions, ethyl alcohol, chloroform, paraldehyde, glacial acetic acid, Acetic Acid B.P., glycerol, Phenol Liquefactum (liquefied carbolic acid), ether, oil of turpentine, acetone, xylene, melted hard paraffin, heated liquid paraffin, and toluene. It dissolves Canada balsam, Silicone D.C. 804, and castor oil, but does not dissolve shellac completely. It boils about 82° C. The danger of poisoning by inhalation or by absorption through the skin is very small, and comparatively large doses have to be swallowed to cause harm.

The following is the translation of the article by Joseph Hauser "Ausschaltung des Xylols in der histologischen Technik", and seeming to show that isopropyl alcohol is the answer to the wishes of many private microscopical workers. The writer's friend, Mr. A. G. Palmer, a laboratory technician, cut some sections according to the method described, and said he was very favourably impressed by the results.

THE employment of xylene in histological technique is fairly widespread. As well as its use in paraffin infiltration, it is used for the removal of hard paraffin, as intermediary before enclosure in Canada balsam, and as solvent for the latter. As well as good properties, xylene has also bad ones which are detrimental to its use in histological technique, namely the causation of considerable shrinking of tissue, and its poisonousness after prolonged inhalation (in the Innsbruck University Hospital severe liver diseases were confirmed after working with xylene).

In my opinion it is now quite possible to eliminate xylene from microscopical technique, as seems practicable in the way described as follows:

(1) Infiltration with hard paraffin, using isopropyl alcohol

R. Romeis says, with regard to the use of xylene in paraffin infiltration, in "Mikroskopische Technik" (München, 1948), page 94: "Toluene and xylene make the preparations hard, and are, on account of their higher boiling points (111°–140° C.), more difficult than the considerably more rapidly evaporating benzene, to remove from the sections and the paraffin. They are for that reason better replaced in the technique of infiltration by benzene." A. Dietrich, in his article on "Isopropyl Alcohol for histological uses", *Zentralblatt der Pathologie*, Vol. XLVII, 1929, page 117, (Isopropylalkohol für histologische Zwecke), recommends isopropyl alcohol for the hardening of pieces of tissue, and also for dehydration,

nd as an intermediate liquid before xylene or chloroform. E. K. Doxtader also has reported on its use in paraffin infiltration, in " Isopropyl alcohol in the paraffin infiltration technic ", in *Stain Technology*, Vol. XXIII, 1928, page 1, and in *Mikroskopie*, Vol. V, 1950, page 302; the process here is really shorter than for infiltration through benzene and methyl benzoate. The use of isopropyl alcohol in paraffin infiltration is equally recommended by G. Hartwich and R. Piechocki in " Über die Verwendung des n-propylalkohols in der mikroskopischen und makroskopischen Technik ", *Zoologische Anzeiger*, Vol. CXLVII, 1951, page 210. I have now tested Doxtader's procedure, and can thoroughly confirm its usefulness. It has the advantages over infiltration using benzene, that it is really shorter, and produces much less shrinking in the objects. Hartwich and Piechocki, however, doubt that the danger of shrinking is largely avoided or generally eliminated by using isopropyl alcohol. Against this, Doxtader asserts that isopropyl alcohol really produces less shrinking than ethyl alcohol, as can also be confirmed by me. J. W. Boellaard, author of an article, " Über Umbauvorgänge in der rechten Herzkammerwand während der Neugeborenen- und Säuglingsperiode " (On reconstructive processes in the wall of the right chamber of the heart in the newborn and suckling), soon to be published in the *Zeitschrift für Kreislaufforschung*, in a letter from the Biological Institute of the Philosophical Academy at Pullach near München, gave me the following information: " Moreover, I can only confirm the assertions of Doxtader that the tissue shrinks less than with the use of ethyl alcohol. The extremely delicate intercellular connective tissue of the newborn heart is as good as not shrunken at all ". This method is specially adapted for very small objects, in connection with which, moreover, there exists the danger of loss or injury through the numerous manipulations which are independently necessary. In the histology of Turbellaria many beautiful results have been achieved through its help, and this summer it has proved itself very useful in embryological technique also in the Biological Institute of Saria in Barcelona. B. Romeis also informed me by letter that he had infiltrated numerous objects by this method with good success. This method has the further advantage that isopropyl alcohol mixes, in a warm state, in all proportions with hard paraffin. This makes it possible to take the object directly from the alcohol series into hard paraffin, and an intermediary is unnecessary.

A small modification of this procedure, resembling the dioxan method, is shortly described as follows:—

The fixed and washed material is dehydrated by isopropyl alcohol. To this end it is taken out of water directly into 60 per cent isopropyl alcohol, as Doxtader recommends also; therein it remains for a longer or shorter time according to its size. For penetration one must generally allow 15 to 30 minutes for each millimetre of thickness. If the object is surrounded by an epithelium

the longer time must be chosen; if not, the shorter may be used. Isopropyl alcohol penetrates only slowly into tissues, and so produces only quite negligible shrinking.

From the 60 per cent alcohol the material is taken into 90 per cent, left in it for the same time, and then into absolute, which is changed once; in each of these it remains ten minutes for each millimetre of thickness. Finally the material is taken to another change of isopropyl alcohol, warmed up to a temperature of 45° to 55° C., corresponding to the melting point of the hard paraffin, and left in it for ten minutes to an hour.

Thence the material now goes either directly into melted hard paraffin, or, for very delicate objects, into a mixture of equal parts of isopropyl alcohol and hard paraffin at 50° C., and thence into pure hard paraffin. After about half an hour the isopropyl alcohol has nearly completely evaporated, so that the material can now be taken into pure hard paraffin, wherein it remains, according to its size, from half an hour to a day. Before pouring out, it is recommended to change the material for ten to thirty minutes into the hard paraffin to be poured out.

Miss Haas, of the Innsbruck Zoological Institute, infiltrated the eggs of *Macrostomum* in an even shorter way with good results, using isopropyl alcohol. She brought the eggs, previously fixed, and stained with eosin, into a large volume of absolute isopropyl alcohol, and thence, after dehydrating for half to a whole hour, by means of a fine pipette directly into melted hard paraffin, which was cooled after one or two hours.

(2) For the removal of hard paraffin

The sections go into absolute isopropyl alcohol, warmed to 50° C., for four or five minutes, then into the same at room temperature, and lastly through 90 per cent isopropyl alcohol and distilled water into the staining liquid.

(3) For the mounting of stained sections in Canada balsam

The interposition of xylene, after dehydration by an ascending series of alcohols, before mounting, is unnecessary. G. Hartwich and R. Piechocki, in the paper already quoted, indeed mention that it is possible to proceed in this manner, using Caedax or Euparal, without discussing this possibility in connection with mounting in Canada balsam also; they mention only that normal propyl alcohol may be used as intermediary between 96 per cent ethyl alcohol and xylene. According to my experiments, however, isopropyl alcohol also can be used with success as intermediary for mounting in Canada balsam. For this purpose, the section comes, after staining or differentiating is finished, into twice-changed isopropyl alcohol for three minutes or so; then all superfluous fluid is cleared carefully from the underside and edge of the slip, Canada balsam dropped on the section soaked in isopropyl alcohol, and the cover laid on. (For thick mounts, readers will, it is hoped, use the Exposure

Method.—D.S.S.). The solution of Canada balsam in xylene or in isopropyl alcohol must be fairly thin. With regard to clearing, there is no difference between sections mounted in this way and those mounted in Canada balsam in the usual way through xylene.

Thanks are due to the Editor and Publishers of *Mikroskopie*, Wien, for consenting to the publication of the foregoing translation.—Editor, *The Microscope*.

THE CAMPFIELD PRESS, ST. ALBANS