A simple technique for the preservation of vital dyes in fixed and sectioned embryos

By J. PERTUSA

From the Instituto Biológico de Sarriá, Barcelona

In embryological work using vital dyes it is highly desirable to be able to study the distribution of the dyes in fixed material, whether examined in toto or after embedding in paraffin and serial sectioning. However, both fixation and dehydration present problems for the preservation of colour in vitally stained cells. Some fixatives preserve some dyes but, so far as I am aware, none will preserve all the vital dyes in common use. On the other hand, ethyl alcohol destroys or dissolves all vital dyes and its use in dehydration is thus undesirable. Among the fixatives that have been proposed are those of Golowin (1902), Mitamura (1923), Parat & Painlevé (1925), and Tchéou Tai Chuin (1930) for neutral red; that of Izquierdo (1955) for toluidine blue; that of Gérard (1925) for Trypan blue; that of Turchini (1919) for methylene blue; that of Lehmann (1929) for Nile blue.

I have found that the use of isopropanol saturated with mercuric chloride both fixes and dehydrates dyed material without loss of colour. It is not, however, a good fixative from the cytological point of view.

Since isopropanol mixes with paraffin, material fixed in it can be passed directly to paraffin without an intermediate agent. Thus the two methods suggested are as follows:

(a) For sections. Fix in isopropanol saturated with mercuric chloride and change the fluid several times to ensure complete dehydration, pass to paraffin, routine cutting and mounting of sections, dewaxing and clearing using xylene and Canada balsam (Mayer’s albumen does not affect the dye). When embryos or very delicate eggs are to be fixed, they must be hardened by placing them in distilled water saturated with mercuric chloride before they are transferred to isopropanol. If cytological detail is required, the material can be fixed in some other fixative and then dehydrated in isopropanol and mercuric chloride.

(b) For whole mounts. Material fixed and dehydrated as above is passed to an essential oil and then to xylene before mounting in Canada balsam.

The stains that I have used have been obtained from Messrs Gurr of London.

1 Author’s address: Instituto Biológico de Sarriá, Calle Dr Amigant 31, Barcelona 17, Spain.
The stains tested were: Neutral red, Neutral red iodide (vital), Vital new red, Brilliant vital red, Trypan red (vital), Toluidine blue, Trypan blue, Nile blue, Methylene blue. The tests I made with these stains were only to see if the fixative described was able to fix them. No account was taken of the fact that some are more or less toxic to the embryo.

**SUMMARY**

The use of a saturated solution of mercuric chloride in isopropanol for the fixation and dehydration of embryonic material stained *intra vitam* preserves the colour of the dye while permitting permanent whole mounts or histological sections to be prepared.

**RÉSUMÉ**

Une technique simple pour préserver les teints vitaux dans les embryons fixés et sectionnés

L'emploi d'une solution saturée de chlorure mercurique dans l'isopropanol pour fixer et déshydrater les matériaux embryonnaires teints *intra vitam* préserve la couleur de la teinture et, en même temps, rend possible la préparation des spécimens entiers et des sections histologiques.

**REFERENCES**


(Manuscript received 13 September 1965)