BRIEF COMMUNICATION

The Use of Aluminium Lake of Nuclear Fast Red in Plant Material Succesively with Alcian Blue

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Abstract. The successive staining alcian blue/aluminium lake of nuclear fast red was proved a useful tool for studies on plant root tip. A simple and reliable procedure is given resulting in blue cell walls, almost colourless cytoplasm and red nuclei. Attempts were made to apply spectrophotometry and paper chromatography to overcome the confusions in manufacturers' labelling of the dye and to check the lake formation.

Nuclear staining with aluminium lake of nuclear fast red** is currently used in pathologist's praxis. Since the procedure is simple and reliable it seemed promising to try it in our studies of differentiating plant root tissues especially in combination with cell wall staining with alcian blue.

Concerning status questions the review by Harms 1965 is the most useful. The dye named Kernechtrot was introduced into microtechnique by Domagk 1932, 1933 in connection with Becher's studies on the staining abilities of lakes of synthetic dyes. Somewhat later the procedure of Domagk was used by Herzberg 1934a, b for staining nuclei in the material processed to reveal the presence of viruses. Questions on the identity of the dyes used by Domagk and Herzberg seem to be hardly answerable. This makes problematic the labelling of some samples as "nuclear fast red Herzberg". Moreover, concerning this point, the supports of the views of Gurr 1965 are lacking. The prescription by Domagk has been included in many manuals of microtechnique, sometimes with such differences like the addition of acetic acid, the presence of conserving substances etc. The chemical formula of nuclear fast red was unknown until recently, when it was included in Colour Index under the No. 60760 as 1,3-dioxy-4-amino-antraquinone-2-sulphonic acid or 1-amino-2,4-dioxy-3-sulphonic acid (Harms 1965). It was this substance which McGee Russell (1955, 1958) used for calcium determination. As a result of the studies of Müller 1963, Müller and Chytíl 1966 and Sams and Davies 1967 the following 2 points must be taken in to account: 1. the chemical identity of the sample, since several different sub-

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** In German, the dye is named Kernechtrot. The Czech name jádrová or jaderná červen stálá is recommended for use, the term pravá jaderná červen being regarded as improper (cf. Němec 1962). Some other names were also used, e.g. the name calcium red was suggested by McGee-Russell 1958.
stances may be sold under the same name, 2. the application of the plain solution of the dye and the use of its lake, the latter acting as a basic nuclear stain whereas the plain dye is an acidic one, the lake formation being a school example of "Umladung" (Harms 1965).

Two samples of the dye were used in the present work, Kernechtrot Grübler a gift from doc. MUDr. R. Kodousek, CSc., and Kernechtrot Serva obtained through the kindness of prof. MUDr. Z. Lojda, DrSc. Both samples were found satisfactory for routine aluminium lake* staining of nuclei in human pathological material in donators’ laboratories. 0.1% solutions were prepared either in H2O or in 5% aluminium sulfate. Using a water bath, all solutions were brought to about 80 °C, kept at this temperature for about 10 min and, then, filtered after getting cool. They were used for several weeks. Whereas no differences were visible comparing the plain solution and the lake of Grübler, the plain solution of the Serva in H2O was clear and dark red in incident day light, whereas the lake of this dye was less transparent, opalescent and of definitely brownish shade.

For spectrophotometric measurements both the plain and the lake solutions were diluted with H2O, Grübler 1 : 20, Serva 1 : 40. The readings were taken with Specord (Zeiss, Jena) in 10 mm glass cuvettes against H2O. The results are given on Fig. 1. The shape of the curves of the plain solutions of the 2 dye samples is not identical. Moreover, the curves are not in agreement with those given by Müller 1963. No differences were revealed between the plain solution and the lake of Grübler, but the relevant curves of Serva are quite unlike which could be regarded as a proof of the lake formation in the latter case.

Our attempts in paper chromatography cannot be regarded as decisive for lake formation. About 0.025 ml of the solutions used currently were placed as 1 cm strips on Whatman 1. Some simple alcoholic media were used with the ascending procedure. The results with 25% ethanol, 50% ethanol, 3% acetic acid in 50% ethanol and 3% pyridine in 50% ethanol are given on Fig. 2. It seems, that the differences between the 2 dye samples are greater here than those between the given dye and its lake. The question remains of the effect of the free aluminium and sulphate ions on the movement of the lake spots.

Root tips of broad bean (Vicia faba L. cv. Chlumecký) were used throughout the present work (Adámková and Beneš 1966), both sections of paraffin — embedded material (Ca-formol fixation overnight at laboratory temperature, overnight wash in water, dehydration and clearing in ethanol — tertiary butanol series) and frozen sections (1 h fixation in Ca-formol at ice-bath temperature, washing, keeping and sectioning the objects in 5% ethanol, see Beneš 1973). The sections were deparaffinized, stained as given below, dehydrated and cleared in ethanol-xylene series and mounted in Canada balsam. The Gama medium cannot be used, since the dye diffuses within and out of the sections. For checking the colour intensity a 5 grade scale was used (0 — unstained, 5 — very intensively stained). If not otherwise stated, the same results were obtained with both frozen and paraffin sections.

When staining the sections in lake solutions, practically the same results were obtained after 5 and 30 min dying period with both samples of the dye. The colour intensity of cell walls was 2 to 3, cytoplasm about 1, nuclei 4 or 5. There was a difference in colour: Serva results in purple shade, Grübler in red. However, with Serva, the nuclei in root cap are reddish in comparison with the nuclei of other parts of the same section. Substantial differences were observed in a comparison of the plain dye and the lake, with both dye

* The term lake will be used here for dye solutions prepared as given here with aluminium sulphate regardless of the validity of the direct proof of lake formation.