

### A MODIFICATION TO THE "INCUBATION MIXTURE FILM METHOD" FOR THE HISTOCHEMICAL LOCALIZATION OF LACTIC DEHYDROGENASE\*

Recently Fahimi and Amarasingham (*Fed. Proc.* **22**: 195, 1963; *J. Cell Biol.* **22**: 29, 1964) have shown that approximately 89% of the lactic dehydrogenase contained in a muscle section diffuses into the fluid medium after 10 minutes of incubation as recommended by standard procedures. To prevent such diffusion they used a gelatin film containing all of the incubation mixture ingredients. By this means they were able to improve the intensity of the reaction and the localization of lactic dehydrogenase in tissue sections of rabbit skeletal muscle.

Although their method seems quite simple, it was observed in our laboratory that it is not devoid of some significant inconveniences: (1) The final gelatin concentration of 2.5% described in the original method seems inadequate in that the quick evaporation occurring during and shortly after application of the gelatin results in considerable retraction of the film. (2) The contact of the tissue sections, particularly those of a large size, with the gelatin film when prepared on a rigid glass surface is sometimes irregular. Such uneven contact favors the formation of air bubbles and in turn areas of negative staining. (3) The detachment of the sections from the gelatin film after incubation requires considerable traction and/or side sliding of the cover glass. This results in wrinkles and damaged areas in the tissue sections.

Trying to avoid the inconveniences just described, we have introduced to the original incubation mixture film method some modifications that seem to extend considerably its versatility and use: (1) To retard the retraction and evaporation of the film, a higher concentration of gelatin was used. One part of the incubation mixture (prepared as described originally) was mixed with one part of a 6.5% buffer-gelatin solution (Tris buffer 0.2 M, pH 7.4) resulting in a final gelatin incubation mixture concentration of 3.25%. With this gelatin concentration the retraction is negligible one hour after the gel film is formed. (2) To improve the contact with the tissue sections the gelatin substrate film was prepared on a flexible base. Any commercial polyvinyl sheet of a thickness ranging from 0.09 to 0.13 mm can be used. In our experiments a chlorpolyvinyl sheet 0.09 mm thick served the purpose. A rectangle of the appropriate size is cut from the plastic sheet and

stretched over a rigid frame or piece of glass. The cover of a staining jar measuring 10.5 × 8.5 cm provided a suitable frame. The edges are secured over the opposite surface with gummed tape. Care should be taken to stretch the plastic sheet to produce a flat, even surface. By means of a pipette 1.6 cc of the gelatin incubation mixture is spread over the surface of the stretched plastic. The film is allowed to gel in the dark for at least 15 min, after which it is ready for use. Strips of the desired size are cut off from the plastic gelatin film. Contact is readily achieved simply by applying the film gently and without pressure over the tissue

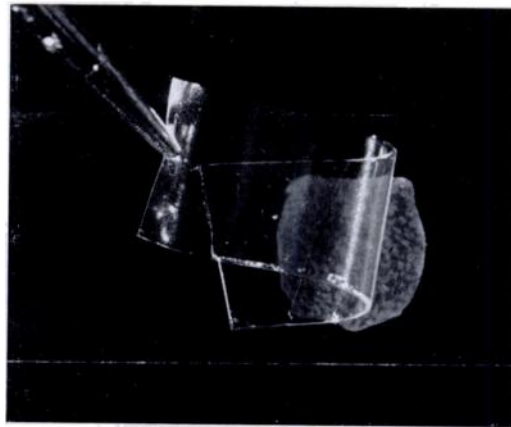


FIG. 1. Method of applying the "Flexible Incubation Mixture Film" over a mounted tissue section.

section (Fig. 1). With this method the contact is significantly improved, thus preventing uneven staining and formation of bubbles. (3) The combination of the plastic base with a higher concentration of gelatin assures easy detachment of the film. Hence there is no need for traction or sliding of the mounted section. After incubation the film can be removed directly either by stripping it off with a forceps or melting it away under warm water (45°C). The slides are washed thoroughly with running warm water and fixed in neutral buffered formalin. The wrinkles and damaged areas observed with this procedure are negligible.

After studying a number of tissues a good localization and a strong reaction for lactic dehydrogenase activity without diffusion was observed (results to be reported elsewhere). The advantages of the plastic gelatin film were particularly obvious

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when sections of whole rat organs such as heart, liver, and kidney, or whole mouse embryo sections were incubated. Furthermore, the time required to prepare the plastic gelatin film is considerably shorter when compared to individual slide spreading. The amount of material needed was also found to be less as compared to the original method. About 20 sections can be incubated with each 1.6 cc gelatin film.

This plastic gelatin film method has also been

used for the localization of succinic dehydrogenase,  $\alpha$ -glycerophosphate dehydrogenase and NADH-diaphorase.

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