Eine leichte Methode zum Aufkleben von Kunststoffschnitten

Das Gemisch von Methylmethacrylat-Polyäthylenglykol hat sich als günstiges Einbettungsmittel für lichtmikroskopische Untersuchungen erwiesen: Man kann organische Substanzen, auch chitinhaltige Objekte, sehr dünn schneiden und erhält wenig Artefakte in Form von Verzerrungen[1–4]. Allerdings bereitet das Aufkleben der Schnitte auf den Objektträger einige Schwierigkeiten; beim Auflösen des Kunststoffes in Aceton schwimmen die Objekte – besonders die kleinen – leicht weg. Durch folgendes einfaches Verfahren lässt sich diese Schwierigkeit nahezu vollständig vermeiden. Die von CATHEY[3] angegebene Lösung wird auf den nach APÁTHY vorbehandelten Objektträger getropft, die gestreckten Schnitte werden aufgelegt und mit einer Lage von Filterpapier bedeckt. Ein zweiter Objektträger wird dann auf das Filterpapier gelegt[2] und die beiden Objektträger werden mit gewöhnlichen Wäscheklammern von allen Seiten fest aufeinander gepresst; bei meinen Versuchen wurden hierbei in der Regel acht Klammern verwendet. Bei Schnitten vom Kopf des Mehlkäfers *Tenebrio* (etwa 2 mm × 1,5 mm) und vom Kopf des Goldauges *Chrysopa* (etwa 1,2 mm × 0,5 mm) kann man schon nach 3 h Verweildauer im Thermostaten (38–40 °C) mit dem Färben beginnen.

Bei diesem Verfahren erübrigt sich ein Andrücken der einzelnen Schnitte mit dem Finger auf das Filterpapier, welches bisher notwending war und eine gewisse Geschicklichkeit erforderte. Auch eine Nachbehandlung mit einer dünnen Zelloidin-Schicht ist überflüssig.

Summary. An easy, safe and time-saving method to avoid the coming off of sections of plastic embedding media from the slide is described.

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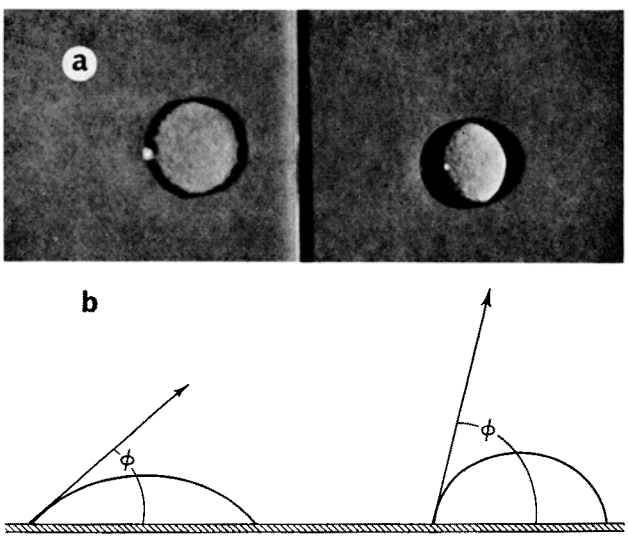
Hydrophobic Surfaces for Serological Reactions

Several workers have pointed out the need for hydrophobic, or water repellent, surfaces for certain types of serological reactions[1–5]. A coating of the test surface with Formvar has been used by several workers[1,4,5]. Initially we employed Formvar-coated surfaces in our laboratory; however, such surfaces were often unsatisfactory. In some microprecipitin tests under mineral oil the Formvar film was either ruptured when the reactants were mixed or became detached from the glass surface. When used in agar plates for gel diffusion tests, the Formvar was sometimes ruptured or, more often, it became clouded if the agar was too hot when poured into the plate as has been pointed out by BALL[1].

Although we have used other water repellent agents, including the ones recommended by CROWLE[2,3], none provided the long lasting, clear, hydrophobic surfaces comparable to those produced by use of Silicone GE Dri-Film SC 87[6,7]. Since plastic petri dishes, which are ordinarily hydrophobic, sometimes became cloudy and were readily scratched, glass petri dishes coated with Silicone GE Dri-Film appeared to be superior. If one takes reasonable care in washing glass petri dishes coated with this product, they may be re-used many times before a recoating is necessary. This is an advantage over certain other water repellents which must be reapplied to the glass surface prior to re-use.

The following simple procedure has been utilized with Silicone GE Dri-Film as the water repellent agent. One or two drops are spread evenly with a cotton swab or cheese cloth pad over the surface to be made hydrophobic. Excess repellent is then removed by wiping with lint-free cloth or lense paper. Careful removal of excess repellent will insure the production of an optically clear, hydrophobic surface.

In a comparative study of the hydrophobic qualities of Formvar-coated glass surfaces with glass surfaces coated with Silicone GE Dri-Film SC 87 the physical appearance of water droplets on both surfaces were measured. Chemically clean glass microscope slides were coated with Formvar by dipping them into a 1% solution of Formvar in chloroform and allowing them to dry. A



(a) Vertical view of 25 λ of water placed on a Formvar-coated glass slide on the left and the same volume of water on a silicone-coated glass slide on the right (Silicone-GE-Dri-Film SC-87). (b) Tracings from a photograph of the horizontal view of the same 2 drops in (a). The contact angle (ϕ) for each drop is indicated. (The photograph of the horizontal view was enlarged before tracing to facilitate drawing the contact angles.)

second lot of slides was rubbed with a cotton swab saturated with the Silicone GE Dri-Film. Excess Silicone was removed by rubbing with a dry cotton swab. An equal volume of distilled water (25 λ) was deposited on each surface from a micropipette. The appearance of the water drops can be seen in a vertical view in Figure (a). It is obvious that the drop on the right, which is on the Silicone surface, occupies less area than the one on the Formvar-coated surface on the left. When equal volumes of saline solution and serum were also deposited on the 2 surfaces, the results were the same as illustrated with the water drops.

According to JIRGENSONS and STRAUMANIS[8], the wetting of a surface is a function of the cosine of the contact angle (ϕ) between the surface and a tangent to the liquid drop surface at the edge of the drop. Wetting is perfect if $\phi = 0$ ($\cos \phi = 1$). Conversely the water repellent or hydrophobic quality of the surface increases as ϕ increases ($\cos \phi$ decreases). Figure (b) illustrates the fact that the contact angle of the drop on the Silicone GE Dri-Film surface is greater than the contact angle of the drop on the Formvar-coated surface on the left. This means that the Silicone-coated surface actually has a greater degree of hydrophobicity than does the Formvar-coated surface. This increased hydrophobicity is another quality of the Silicone GE Dri-Film coated surface which makes it more useful than repellent surfaces produced by other agents.

We have successfully used Silicone GE Dri-Film coated surfaces for microprecipitin tests under oil and agar gel double diffusion tests; presumably such surfaces would be helpful in other serological tests if a hydrophobic surface is desired.

Résumé. Le silicone GE Dri-Film SC 87 produit une surface hautement imperméable aux réactions sérologiques. On démontre expérimentalement qu'il est plus hydrophobique que le formvar.

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Bulk Staining of Ovules and Ovaries to Note the Percentage of Well-Organized Embryo Sacs in Sterile and Semi-Sterile Plants Utilized for Breeding Purpose

When sterile and semi-sterile plants (where, at times, either of the sexes alone is functional) are utilized for breeding purposes, it is necessary for a breeder to know the percentage of viable pollen grains and well-organized embryo sacs, so that appropriate techniques can be applied during controlled matings. While fertility tests for pollen are quite simple – iodine or tetrazolium – there are no such quick methods by which female gametes could be tested, except smears; but there again the 'full picture' of the ovule is lost and older ovules present difficulties while squashing. A quick paraffin method described here is found to be very useful.

Material and method. Flower buds of normal and hybrid *Oryza sativa* at different stages of their development were fixed in formalin-acetic-alcohol and processed further as follows: (1) Fixed material; if possible dissect out ovaries and ovules. (2) Carry dehydration through alcohol grades as routine. (3) Alcohol-xylene mixture, 1:1. Dissolve the stain-fast green and leave till deeply stained. (4) Pure xylene. (5) Infiltration, embedding and cutting as routine. (6) Affix paraffin ribbons (with stained sections) on slide and use xylene to remove the paraffin completely. (7) Mount in canada balsam.

This method is found to be very useful (Figures 1–4) especially with plants such as haploids, triploids, autotetraploids and semi-sterile/sterile hybrids and mutants, in which one needs to know whether the embryo sac is developed and if so whether its organization is normal, on the basis of which breeding experiments can be planned. However, the ultimate test of viability lies in actually pollinating with the viable pollen and looking for a seed-set, on similar lines as the pollen viability is ultimately decided on its capacity to germinate and fertilize.

Merits. (1) Large number of ovaries and ovules can be sectioned and observed. (2) The laborious and tedious procedure of staining is avoided, saving time and alcohol. (3) A reliable percentage of normal embryo sacs is obtained, because a large number of buds can be observed and it helps a breeder to plan his experiments accordingly. (4) Percentage of non-functional embryo sacs and the exact stage(s) when they degenerate can also be noted[1].

Résumé. La maculation massive des ovaires et ovules d'*Oryza sativa* L. a été faite avec du vert rapide et de alcool:xylol (1:1) par étapes. Après section il fut possible de noter le pourcentage des sacs embryonnaires viables et non-viables plus particulièrement dans des plantes

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