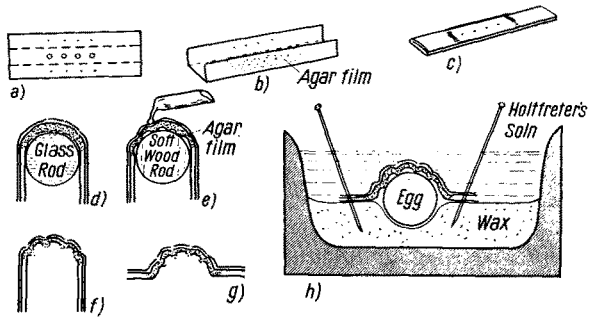
PRO EXPERIMENTIS

A Modified Vital Staining Technique for Amphibian Eggs

During a stay at the Hubrecht Laboratory, International Embryological Institute, Utrecht (Holland), the need arose for a technique facilitating the application of regular rows of vital stain marks of different size and spacing on amphibian eggs. The techniques used up till now are best suited for the application of single marks. The number of marks that can be held in position simultaneously is limited in these techniques. HASSA (Cf. NIEUWKOOP c. s. 1955)[1] used a thin collodion film with holes, which was fixed over the egg. By keeping a stained agar block on top of the film, the dye was allowed to pass through the holes in the film, resulting in a row of marks. The technique described here makes use of a strip of stained agar film which is made to protrude through holes in a strip of tin foil.



Various stages in the preparation of tin foil strip to hold stained agar film for vital staining of amphibian eggs.

A very thin agar film is made by pouring melted 3% agar on a clean, polished stainless steel plate. When dry, the film comes off automatically. It is then immersed for 24 h in a 1% solution of Nileblue sulphate, and dried again on the steel plate.

A strip of tin foil of about 15 mm long and 3–4 mm broad is cut out and divided longitudinally into three equal zones by lines (Figure *a*). Under a dissecting microscope provided with an ocular micrometer, a row of minute holes of proper spacing is made in the middle zone. The edges of the holes are smoothed with a glass rod with a ballend. In the lateral zones, at a level with the holes, small punctures are made, which will later serve as markers. The strip is now folded as shown in Figure *b*, sterilized in 70% alcohol (about 10 min), rinsed in sterile water, and placed with some water in the groove on a clean, sterile glass slide. A strip of stained agar film of the required size is cut out and placed in the groove. The sides of the tin foil strip are carefully folded one over the other and slightly pressed under another clean glass slide (Fig. *c*).

The tin foil strip with the agar strip inside is now curved smoothly around a glass rod of the same diameter as the egg (Fig. *d*). It is then transferred to a rod of soft wood of equal diameter, and, with a fine glass ball, depressions are made in the tin foil over the positions of the holes, indicated by the punctures. Hereby the agar is made to protrude through the holes, and at the same time the parts between the holes become concave (Fig. *e* and *f*). Finally the strip is immersed in sterile water, so as to make the agar swell and protrude further.

The staining of the naked egg is illustrated by Figure *h*. The strip should be treated with gentle pressure only, and the surface of the tin foil should not make contact with the egg surface, since the latter tends to stick to the tin foil. The marks usually attain the required intensity in maximally 30 to 45 min.

With the necessary modifications, this technique may also be applied to the avian, and probably to the reptilian egg.

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Zusammenfassung

Es wird eine Methode beschrieben, die es ermöglicht, mit Hilfe eines gefärbten Agarfilms, der durch Löcher in Stanniolpapier gepresst wird, am Amphibien-Ei regelmässige Reihen von Vitalfarbmarken zu setzen.

[1] P. D. NIEUWKOOP *et al.*, Origin and establishment of organization patterns in embryonic fields during early development in amphibians and birds, in particular in the nervous system and its substrate, I, Proc. Kon. Nederl. Akad. Wetensch. Amsterdam [C] *58*, 2, 219 (1955).

PRO EXPERIMENTIS

Zur Wahl der organischen Lösungsmittel bei der Gewinnung von Chloroplasten in nichtwässerigem Milieu

Die präparative Isolierung morphologischer Zellen- und Gewebebestandteile kann in wässerigem und in nichtwässerigem Milieu (Behrens-Methode) vorgenommen werden. Beide Methoden haben Vor- und Nachteile. Während beim Arbeiten in wässerigem Milieu Fermente, Proteine und andere wasserlösliche Substanzen verloren gehen, wird in nichtwässerigem Milieu den morphologischen Zellbestandteilen ein Teil der Lipide entzogen.

Im Zusammenhang mit den kürzlich beschriebenen Arbeitsgängen zur Gewinnung von Chloroplasten in nichtwässerigem Milieu[1–4] schien es uns von Bedeutung, den Lipidverlust zu ermitteln, welchen gefriergetrocknetes Blattpulver bei Suspension in verschiedenen organischen Solventien erleidet, um die organischen Lösungsmittel mit dem geringsten Lösungsvermögen für Lipide bei der Gewinnung von Chloroplasten heranzuziehen.

[1] M. BEHRENS und R. THALACKER, Naturwissenschaften *44*, 621 (1957).
[2] U. HEBER, Ber. dtsch. bot. Ges. *70*, 371 (1957).
[3] C. R. STOCKING, Plant Physiol. *34*, 56 (1959).
[4] R. THALACKER und M. BEHRENS, Z. Naturf. *14b*, 443 (1959).