contact with the plaque surface, especially when the fluid is moving downwards. Aspiration of agar is to be avoided. The resulting suspension is thoroughly mixed with an equal volume of 2% sodium phosphotungstate solution of pH 7.0 in a small polyethylene container. A drop of the mixture is then placed on a collodion-and-carbon coated copper grid and withdrawn immediately with the same pipette or with filter paper. As soon as the preparation has dried, it is examined in an electron microscope with double condenser illumination.

Besides some bacteria, several bacteriophage particles are nearly always found on scanning the first preparation made from the mixture, which is kept at hand until the morphological type of the bacteriophage is documented. Even small plaques usually yield a sufficient number of particles. In this way it is, for instance, possible to differentiate between a mixture of morphologically different bacteriophages causing different plaques on one hand and a morphologically uniform population of bacteriophages causing different plaque types in one and the same Petri dish on the other hand.

Zusammenfassung. Beschreibung einer Präparationstechnik, durch die von einzelnen mit einem kurzen Glas- oder Metallzylinder von der Umgebung abgegrenzten Plaques mit zwei Tropfen Suspensionsmedium genügend Bakteriophagen für eine Untersuchung im Negativkontrastverfahren gewonnen werden.

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Department of Hygiene and Medical Microbiology, University of Berne (Switzerland), February 18, 1966.

Rodent Preimplantation Stages: Mounting 'in toto' and Alterations Due to Anisotonic Solutions

While demonstrating enzyme reactions of the whole egg with the employment of the mounting 'in toto' technique[1] after successful reaction, we observed in more than 2000 examined eggs of the white rat and the golden hamster (*Mesocricetus auratus* Waterhouse), that even slightly anisotonic solutions cause severe alterations in shape and size of the eggs. Concomitant with these alterations are intracellular artefacts, which make it difficult to draw correct conclusions (see Figures 1–3). We had not considered sufficiently this fact in a preliminary note[2].

By performing the reaction on free eggs, as is required for certain enzyme reactions[3], those alterations are avoidable only if exclusively isotonic solutions are used prior to mounting. Deviations of isotonicity frequently occur during mounting with pasting the eggs on a slide, when the small amount of solution surrounding the egg becomes hypertonic through evaporation. Finally, the eggs shrink in spite of good preservation after incubation. Therefore attention must be directed also to a suitable mounting technique.

Mounting procedure. Applying the following procedure, we have succeeded in preparing whole eggs which, after the enzyme reaction with isotonic solutions, differ neither in size nor in shape from recently gained tubal eggs (see Figure 4).

The eggs ready for mounting are transferred in Tyrode's solution or in fixing agent. Preferably, one egg at a time is aspirated by a finely drawn glass pipette and brought on a clean slide coated immediately before mounting with

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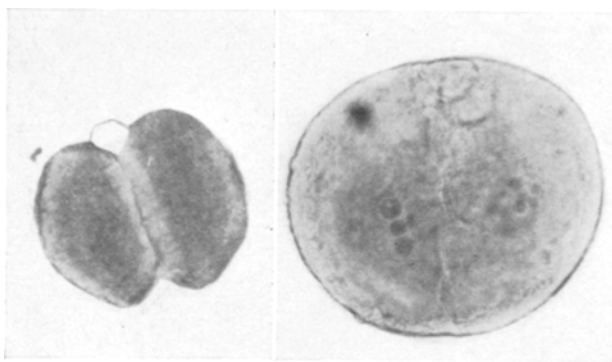
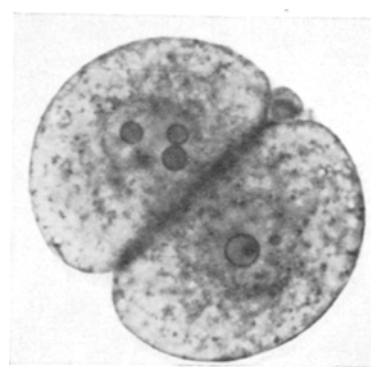
[2] P. Elmiger and K. S. Ludwig, Experientia *21*, 635 (1965).
[3] P. Elmiger, Experientia *21*, 670 (1965).



a b

Fig. 1a. 2-cell stage, R 505_3, calculated age 57 h, glucose-6-phosphate dehydrogenase, × 500. Hypertonic incubation solution. Shape rather well preserved. Size excessively diminished. Nucleoli hardly recognizable. Due to artefacts the cleavage plane and the peripheral zones of both blastomeres seem to be free of the reaction product.

Fig. 1b. 2-cell stage, R 7_3, calculated age 57 h, DPNH-diaphorase, × 500. Hypotonic incubation solution. Marked deformation of both blastomeres. Size hardly altered because of subsequent shrinkage in the relatively hypertonic Tyrode's solution. The peripheral zones of both blastomeres seem to contain no reaction product.



c

Fig. 1c. 2-cell stage, R 50_5, calculated age 57 h, DPNH-diaphorase, × 500. Solutions isotonic. Shape and size not altered. Correct distribution of the reaction product.

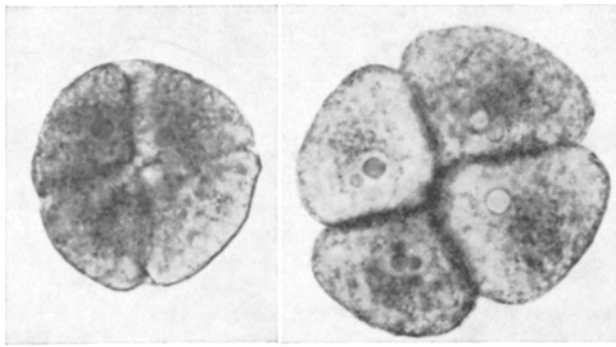


Fig. 2a. 4-cell stage, R 29_1, calculated age 62 h, DPNH-diaphorase, × 500. Hypertonic incubation solution. All blastomeres flattened through shrinkage. Size markedly reduced. Correct localization of the reaction product impossible.

Fig. 2b. 4-cell stage, R 65_9, calculated age 64 h, DPNH-diaphorase, × 500. See Figure 1c.

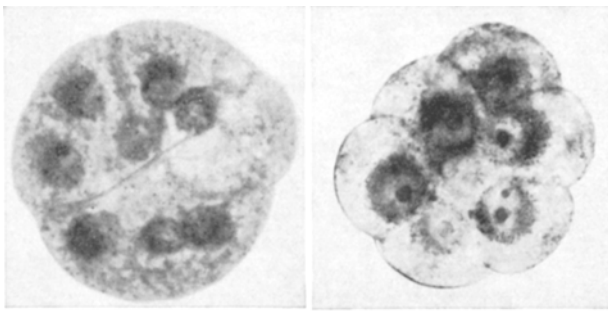


Fig. 3a. 8-cell stage, R 45_1, calculated age 81 h, DPNH-diaphorase, × 500. Hypotonic incubation solution. For mounting Tyrode's solution diluted with distilled water 4:1. All blastomeres markedly enlarged and deformed by swelling. Distribution of the reaction product altered.

Fig. 3b. 8-cell stage, R 91_6, calculated age 80 h, DPNH-diaphorase, × 500. See Figure 1c.

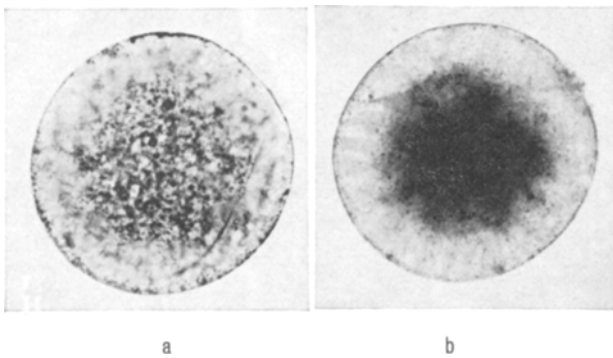


Fig. 4a. 1-cell stage (fertilized), R 94_2, calculated age 29 h, directly mounted in Tyrode's solution after flushing of the tube, × 500. Fig. 4b. 1-cell stage (fertilized), R 95_1, calculated age 30 h, DPNH-diaphorase, × 500. Both eggs are similar in size and shape. In both eggs shadows of the polar bodies at 2 o'clock.

an invisible thin film of paraffin oil. In such a way a markedly vaulted droplet of about 2–3 mm in diameter develops, in which the egg is enclosed. The egg should be placed centrally in the droplet, if not, it may be displaced towards the light refracting border after the coverslip is set in place. As soon as possible the small droplet of Tyrode's solution is then covered with mounting medium (MM) 'Harleco' (in Xylene), whereby one must be certain that it is completely enclosed by the MM. The MM must be trickled down from a short distance, because without that the coherent Tyrode's droplet would be fractionated into many small droplets (Figure 5). Thereafter the preparation is carefully covered with a coverslip, whose size must be selected in accordance with the amount of MM. Too large a coverslip leads to flattening of the eggs through pressure, while too small a coverslip makes the observation with high resolution impossible. If the size of the Tyrode's droplet, as well as the amount and the viscosity of the MM are chosen correctly, the Tyrode's solution extends from the slide to the coverslip. In this way an optical bordering plane between the Tyrode's solution and the MM is avoided and the egg lies freely on the bottom of an 'aquarium' bordered laterally by MM (Figure 6).

Almost half a year ago we mounted eggs, as described, on which we had performed a reaction for DPNH-diaphorase, using MTT[4] after short prefixation with glutaraldehyde[5]. Until now these eggs have not shown any alterations after storage in the refrigerator at 4 °C. However, mounting in a mild fixing agent, e.g. glutaraldehyde, may prove to give better long-term results.

Discussion. For certain enzyme reactions the techniques of DALCQ[1], LUDWIG[6] and DALCQ and PASTEELS[7], whereby the eggs are pasted on a slide prior to the reaction, are not suitable. DALCQ[8,9] and ISHIDA and CHANG[10] refer to mounting 'in toto' after the reaction. In some of DALCQ's photographs, deformations of the eggs are recognizable

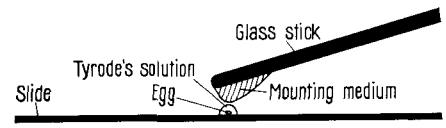


Fig. 5. Application of mounting medium (MM) to cover the droplet of Tyrode's solution containing the egg. Observe the position of the glass stick, as well as its proximity to the slide.

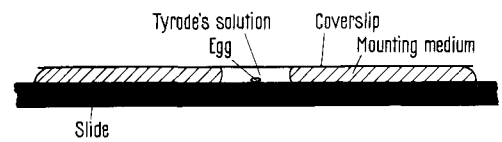


Fig. 6. Mounted egg at the bottom of an 'aquarium', the lateral wall of which consists of mounting medium.

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(see Figures B and C[8] and Figures 1, 3, 5, 8–10, 14, 15a, 16–20)[9]. ISHIDA and CHANG transfer the eggs to a slide between four vaseline spots, anchoring them between slide and coverslip. Thereafter the edges of the coverslip are sealed with nail-polish. This method is rather complicated, and in their photographs as well, the eggs show some deformation (see Figures 1 and 2)[9].

Compared with the techniques of pasting the eggs before or after the reaction, those described by ISHIDA and CHANG[10] and by us show several advantages for delicate histochemical reactions: isotonicity is preserved during the mounting, uneven adhesion to the slide is excluded and protein-precipitants or other pasting agents, as needed for the pasting technique, are avoided. Furthermore, the techniques are applicable without modification for all preparations and with any mode of fixation, as long as the reaction can be done on free eggs and the reaction product is not water-soluble.

In comparison with the technique of ISHIDA and CHANG, it appears to us that ours offers some advantage in that it is more simple. In addition, the eggs lie completely free and they can be dislocated for purposes of observation by sliding the coverslip many hours after mounting. One disadvantage must be mentioned: experience is required for a presentable demonstration of all the eggs found in one animal[11].

Zusammenfassung. Anisotonische Lösungen verursachen bei der Durchführung histochemischer Reaktionen an freien Eiern von Nagern Artefakte. Es wird eine einfache Montagetechnik nach Durchführung der Reaktion beschrieben, bei der intrazelluläre Veränderungen vermieden und weder Form noch Grösse der Eier durch Anisotonie verändert werden. Die Technik erlaubt jede Art von Fixation und jede Reaktion, solange das Reaktionsprodukt wasserunlöslich ist.

P. ELMIGER

Anatomisches Institut der Universität Basel (Switzerland), May 9, 1966.

[11] This investigation was supported by a research grant from the Ford Foundation.

Purification of Ferritin-Labelled Immunoglobulins

Ferritin has thus far proved to be the most commonly used label for identifying immunoglobulins in the electron microscope. This probably owes much to the careful work of its first proponents[1], and to the ease and certainty with which labelled molecules can be detected. Purification of the crude mixture obtained after the coupling reaction is, however, rather tedious. RIFKIND et al.[2] recommend three sequential centrifugations at 100,000 *g* for 4 h to remove the uncoupled γ-globulin, free ferritin being allowed to remain. BOREK and SILVERSTEIN[3] suggested the use of continuous flow paper electrophoresis to isolate the conjugate, but this has not been much used (see, however, BAXANDALL et al.[4]). The present note describes the use of a zone electrophoretic separation which forms a convenient preparative method for obtaining the conjugate free of impurity.

Ferritin (twice crystallized; Nutritional Biochemicals Corp.) was coupled to γ-globulin by means of toluene 2,4-diisocyanate (K. and K. Laboratories). The method used was derived by combination of those described by SINGER and SCHICK[1] and RIFKIND et al.[2]. After the crude reaction mixture has been dialysed into 0.05 *M* phosphate buffer pH 7.5, it contains, in addition to the conjugate, unreacted γ-globulin, free ferritin, and ferritin which has reacted with diisocyanate alone. These can be removed by zone electrophoresis using polyvinyl powders[5,6], because γ-globulin is slow-travelling and remains near the origin; ferritin, on the other hand, travels faster than the conjugate, while ferritin-diisocyanate is the fastest travelling of the three major contaminants.

The procedure used is that described by FAHEY and MCLAUGHLIN[6]; Pevikon C 870 being obtained from the Shandon Scientific Co. Ltd., 65 Pound Lane, London N.W. 10, and Breon Vinyl 425, said to be the equivalent

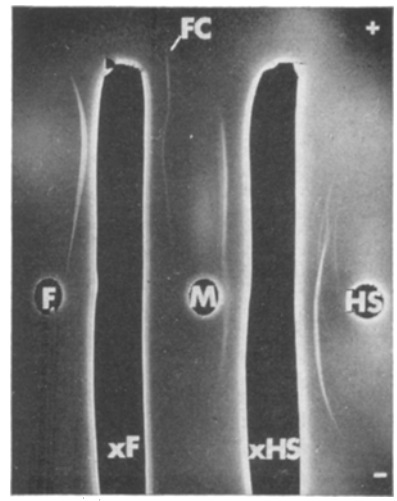

Fig. 1. Immunoelectrophoresis on agar gel in veronal buffer pH 8.2 ionic strength 0.05. Plate photographed after drying, fixing, and staining with Ponceau red. F, purified horse ferritin; FC, presumed ferritin-diisocyanate complex; xF, goat anti-ferritin antiserum; M, crude reaction mixture; xHS, rabbit anti-human serum antiserum; HS, human serum.

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