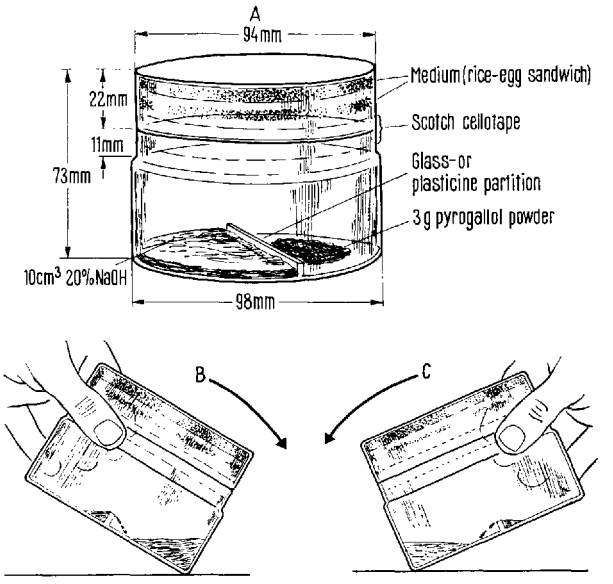
PRO EXPERIMENTIS

A Novel Rice-Egg Sandwich for Mass Propagation of *Entamoeba histolytica* on Agar Plates

For the in vitro propagation of *E. histolytica*, many methods have been widely used. The successful mass propagation of *E. histolytica* on agar plates, however, is only a recent achievement (YOUSSEF[1]). An important modification, which renders the plate culture of *E. histolytica* suitable for drug assay, amenable to direct, frequent, and even continuous observation and other important studies, is reported below.

The rice-egg sandwich. This can easily be prepared: Sterile agar plates containing 0.7% table salt – after solidifying – are covered with a warm egg-blood or serum mixture prepared as follows: With sterile precautions, egg white mixed with 5–10% defibrinated blood or serum is warmed to 45°C. A minimal amount of sterile agar-thioglycolate-table salt solution cooled to about 50°C is added in calculated amounts so that the final concentration of agar in the new mixture is around 0.2%, sodium thioglycolate around 0.1%, and table salt about 0.7%. Sterile Difco rice powder (1–2 g/plate) is then distributed carefully and as homogeneously as possible all over the plate.

Inoculation of Entamoeba histolytica. E. histolytica from acute dysenteric stools – one or two loopfuls of mucus – is inoculated in the centre of the plate. A sterile 22 mm coverglass is gently applied to the amoeba inoculum and the sandwich is then completed by pouring on the coverglass enough sterile agar agar (1.5%)-sodium thioglycolate (0.1%)-table salt (0.7%) solution, cooled to about 50°C, so that an agar layer that completely seals the rice-egg layer is formed. The rice-egg layer, which is temporarily isolated from air, however, must be permanently isolated. The culture is incubated at 37°C in an oxygen-CO_2-free atmosphere (with the aid of pyrogallol-sodium hydroxide solution) in a Mcleod's plate, or preferably in the all-glass plate used by the author (see Figures A, B, C – the thickness of the rice-egg layer and the agar seal is exaggerated, particularly the former). The culture plate may be examined directly under the microscope but it must be replaced in an oxygen-CO_2-free atmosphere after each examination. *E. histolytica* was observed growing in swarms, with the individual amoebae actively creeping in the sandwich, devouring and competing with each other, and gathering on the rice granules like a swarm of ants on a piece of sugar but with greater activity and vigour. A rounded macro-colony of *E. histolytica* was seen in the sandwich, which rapidly increased in diameter. This colony originated from the inoculum and spread centrifugally towards the periphery of the plate in the form of spreading lysis of the opaque rice layer (note analogy with the amoeba previously isolated, YOUSSEF[2]).



Résumé. Modification importante de la méthode de culture en masse d'*Entamoeba histolytica* sur plaques d'agar (YOUSSEF[1]) permettant la détection rapide de l'action anti-amibienne directe des composés et quelques autres observations intéressantes.

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Bacteriological Laboratory, Luxor (Egypt), February 9, 1965.

[1] K. A. YOUSSEF, Exper. *21*, 170 (1965).
[2] K. A. YOUSSEF, Exper. *20*, 463 (1964).
[3] Note added in proof: The method as described is suitable only for short periods of cultivation. Work is going on to overcome these difficulties.

A Staining Pool for Mammalian Eggs

In order to determine the enzyme content histochemically on mammalian egg cells and early cleavage stages, we developed a staining pool which renders it possible to change solutions without a dislocation of the egg, so that repeated transfers of the egg from one solution to the other are avoided. At the same time the pool permits the staining of unfixed eggs, as needed for succinate dehydrogenase and DPNH-diaphorase, for example.

Description of the staining pool. The pool consists of a 'Perspex'-plexiglass plate which is thicker but of the same size as an underlying slide, both held together by two brackets. The middle of the 'Perspex' plate contains in its lower half a cylindrical hole 5 mm in diameter continuous with a funnel in the upper half of the plate. On the underside of the plate there are several grooves which, together with the underlying slide, make up small channels for the depletion of the pool; at the same time closing the cylindrical hole from the bottom (Figures 1 and 2). In order to get a steady outflow from the pool, a system of channels with measured relative diameters has been constructed. The fluid leaves the pool through four symmetrically arranged channels with a sectional area of 2 mm · 20 μ, dimensions which prohibit the escape of the egg cells and the blastulae of rodents. The four radial channels flow into

a large ring-channel; the latter is drained by two smaller channels with a sectional area of 3 mm · 150 μ which are in opposition to one another (Figure 1). On each side, the fluid then flows into a larger round channel drilled vertically and horizontally in the plate (Figure 2).

In the outer part of the two larger round channels two short PVC tubes are inserted. Their free ends are attached by a Y-shaped coupling piece, made of polyethylene, to a rubber tube 1 m in length. In this way there is a closed system from the pool to the free end of the rubber tube. Once filled, the staining pool is placed on a table and the free end of the rubber tube is inserted into a large bottle on the floor. As in a continuous suction drainage with a siphon, the fluid travels through the system of channels and tubes, depleting the pool. Fluid-flow can be easily regulated by a bracket on the rubber tube.

Handling of the staining pool. Eggs gained by flushing the tubae uterinae with Tyrode's solution are transferred with a finely drawn pipette to the pool which also contains Tyrode's solution. The latter is then replaced by other solutions, according to the desired histochemical reaction. To avoid air bubbles rising from the fluid into the channel system, the solutions must be kept at room temperature, and to avoid the inflow of air the pool should never be completely depleted. The solutions must be added slowly, e.g. with a finely drawn pipette, so that the eggs retain their position on the bottom of the pool. At the end of the reaction the pool is depleted until the meniscus almost reaches the bottom of the pool. If necessary, the stained eggs are pushed with a needle into the centre of the pool. Thereafter the pool content is frozen with CO_2; the brackets which hold the 'Perspex' plate and the slide together are removed, plate and slide being separated by sliding one against the other. The frozen pool-content is pushed through the hole in the plate onto a slide which is covered with plastic tape. The latter has a hole in its middle in order to prevent the dispersion of the pool content upon thawing. If the freezing of the eggs is observed to be disadvantageous, the eggs are transferred directly from the pool to a slide. Finally, the fluid around the eggs is removed with a finely drawn pipette and the eggs are embedded in glycerine-gelatine[1].

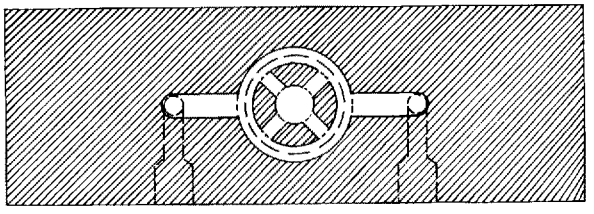

Fig. 1. Underside of the 'Perspex' plate (natural size).

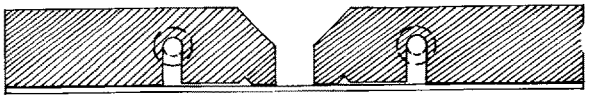

Fig. 2. Longitudinal section through the staining pool (natural size).

Zusammenfassung. Beschreibung und Handhabung eines Färbetrichters mit basaler Abflussvorrichtung zur Durchführung histochemischer Reaktionen an Eizellen und frühen Furchungsstadien von Säugern. Der Färbetrichter ermöglicht ein mehrmaliges Wechseln der Lösungen ohne die Eier anzurühren, wodurch ein mehrmaliges Pipettieren der Eier vermieden werden kann.

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Anatomisches Institut der Universität Basel (Switzerland), August 14, 1965

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

Homogenisator für kontinuierliches Homogenisieren zur präparativen Gewinnung von Zellorganellen

Im Zusammenhang mit der Isolierung der Cerebrosidsulfatase[1], eines Enzyms, das nur in sehr geringer Konzentration in Säugerorganen enthalten ist, war es erforderlich, Homogenate von kg-Mengen Gewebe herzustellen, um eine lysosomenhaltige Partikelfraktion durch differentielles Zentrifugieren zu gewinnen. Ein Starmixgerät konnte infolge der Labilität der Lysosomen nur zur Vorzerkleinerung benützt werden. Daher wurde der gebräuchliche Potter-Elvehjem-Homogenisator[2] so modifiziert, dass eine kontinuierliche Passage von 10 l Gewebesuspension aus 2–4 kg Gewebe möglich wurde.

Dieser kontinuierliche Durchfluss wird durch eine Schlauchquetschpumpe erreicht, die das in einer 0,25 m Saccharoselösung vorzerkleinerte und suspendierte Material ansaugt, durch eine untere Öffnung in das feststehende Homogenisiergefäss treibt und an dem rotierenden Teflonpistill (1200 U/min) vorbeipresst bis das Homogenat durch eine obere Öffnung abfliesst (Figur). Durch dieses Prinzip wird ein rascher Durchfluss (120 bis 150 ml/min) mit regulierbarer Geschwindigkeit gewährleistet, so dass eine lokale Überhitzung vermieden wird. Bei den bisher beschriebenen Homogenisatoren[3] erfolgte die kontinuierliche Passage tropfend durch das Eigengewicht des Homogenates. Das sich während der Passage um etwa 3° erwärmende Gesamthomogenat wird in gekühlten Gefässen aufgefangen und insgesamt 3–4mal durch den kontinuierlichen Homogenisator geschickt. Eine Verstopfung des Spaltes zwischen Pistill und Homogenisiergefäss tritt nicht ein, wenn die schlachtfrischen Organe von zähen Anteilen befreit und im Starmix 30 sec bei 6000 U/min vorzerkleinert worden sind (400 g Gewebe und 400 ml Saccharoselösung).

[1] E. Mehl und H. Jatzkewitz, Hoppe-Seyler's Z. *339*, 260 (1964).
[2] V. R. Potter und C. A. Elvehjem, J. biol. Chem. *114*, 495 (1936).
[3] K. Lang und G. Siebert, Biochem. Z. *322*, 360 (1952). – E. S. Harris und J. W. Mehl, Science *120*, 663 (1954).