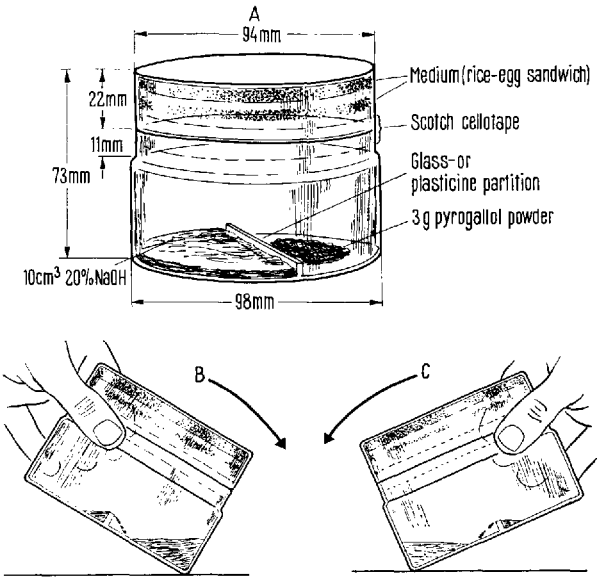
PRO EXPERIMENTIS

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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[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