A Culture Method of growing *Entamoeba histolytica* and Other Anaerobic Amoebae for the Study of their Nuclear Division

THE mode of nuclear division in *Entamoeba histolytica* and other anaerobic amoebae is not clearly understood. There are two main reasons for this: first, difficulty is encountered in obtaining the various normal stages of nuclear division; second, unsuitable cytological techniques are used to distinguish between chromatic and non-chromatic substances. Dobell[1] developed a culture method of obtaining large numbers of *E. histolytica* trophozoites in an actively growing condition. He grew amoebae by the Boeck–Drbohlav technique in a tube into which a coverglass was placed. The amoebae crept over the coverglass, where they continued their development, fed on the accompanying bacteria and rice starch and multiplied. Because they adhered to the glass, they could be readily obtained from the culture by removing the coverglass, and then fixed and stained. It should be emphasized that by this method large numbers of bacteria and rice starch particles also remain attached to the coverglass. This makes any study of nuclear structure and nuclear division difficult.

Singh[2,3] developed a method for the culture of small free-living amoebae; this enabled him to obtain without any difficulty all stages of nuclear division in nine species of amoebae. The amoebae were grown on thin films of non-nutrient agar on slides. They were then fixed according to normal cytological methods and the slides brought to water. At this stage the film of agar was removed by giving the slide a gentle shake in water; this left the majority of the amoebae attached to the slide.

The agar film culture method of Singh[2] has been modified to provide large numbers of trophozoites of *E. histolytica*, *E. invadens* and *E. moshkovskii* in a normal and actively growing condition. Thin films of non-nutrient agar (0·8–1·0 per cent agar depending on the quality of agar; 0·85 per cent sodium chloride) were made on coverslips. One or two small drops of hot melted agar were placed on 22 × 22 mm number '0' coverslips and quickly covered by round coverslips (16–18 mm diameter). When the agar had solidified, the top coverslip was gently pushed from one side and removed, leaving the film of agar on the lower coverslip. The films were kept in moist Petri dishes until required.

Twenty-four to forty-eight hour old amoebae growing in modified Boeck and Drbohlav medium (M/40 phosphate buffer in 0·85 per cent sodium chloride was used to dilute inactivated horse serum 1/7; *p*H 7·2) with mixed bacterial flora and rice starch were pooled and inoculated in large numbers in hollow ground slides (ground glass slides with a circular cavity 16 mm in diameter and 4 mm deep). The cavities were filled with diluted horse serum and a small quantity of rice starch (Difco) was added. They were then sealed with coverslips containing agar films, keeping the film downwards. Care was taken to avoid air bubbles. Any extra fluid around the coverslip was removed by filter paper. The cavity slides were then inverted and incubated at 37° C. There was profuse growth of amoebae on the agar films. After various intervals of time, ranging from 16 h to 24 h, the slides were inverted and the coverslips with the films gently removed with a pair of forceps. The amoebae were quickly fixed in Carnoy's solution (absolute alcohol six parts, chloroform 3 parts and glacial acetic acid one part) for 30–45 min. They were then placed in 90 per cent alcohol for 24 h and gradually brought to water. At this stage the films were gently detached around the periphery with a fine needle and removed by gently shaking the coverslips in water. The majority of the amoebae remained attached to the coverslips, and were stained with iron alum haematoxylin. It seems that amoebae sink through the agar, as observed by Singh[2] in the case of free-living amoebae, and remain adhered to the glass after the film has been removed.

It is hoped that by this method it will be possible to find normal, young and actively dividing amoebae. As the amoebae are confined to a small area, it should be easy to locate dividing individuals under an oil-immersion lens. The amoebae can be stained by suitable techniques so as to show up the chromatanic and non-chromatanic substances at various stages in the nuclear division.

Shaffer[4] has recently shown that the trophozoites of *E. histolytica* in Shaffer–Frye or CLG medium[5] possess two or more nuclei. From 2 to 25 per cent of the amoebae were binucleate between 8 h and 16 h and continued for 120 h of incubation. No binucleate amoebae were present at 4 h or 8 h of incubation. A few trophozoites which contained from three to five nuclei were found without any apparent relation to the growth cycle of the culture. Based on these observations, Shaffer[4] has suggested that nuclear division and cell division may be separate phenomena that do not necessarily proceed in parallel.

We have found that on a single coverslip preparation made from 16–24 h old cultures of *E. histolytica* and *E. invadens* there were plenty of trophozoites with two or three nuclei. Amoebae with four or five nuclei were less frequent. Amoebae containing six, seven and eight nuclei have also been observed; multinucleate amoebae also occurred with *E. moshkovskii*. The nuclei and cell cytoplasm in multinucleate *E. histolytica*, *E. invadens* and *E. moshkovskii* appeared to be quite normal. Our observations support Shaffer's[4] suggestion that nuclear and cell division are probably two separate phenomena.

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J. P. DUBEY*
S. R. DAS

Central Drug Research Institute,
Lucknow, India.

* Present address: Department of Bacteriology, University of Sheffield.

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[5] "CLG, cysteine-lactalbumin hydrolysate-glucose medium," see McDade, J. J., and Shaffer, J. G., *Amer. J. Trop. Med. Hyg.*, **8**, 540 (1959).

Effect of Spinal Lesions on the Colour Change of the Minnow (*Phoxinus phoxinus* L.)

THE dorsal and lateral surfaces of the minnow (*Phoxinus phoxinus* L.) change colour on pale and dark backgrounds. This colour change is effected by changes in the condition of the melanophores which are found on these surfaces of the minnow. Large macromelanophores and small micromelanophores can be seen under the binocular microscope, and the differential distribution of these two types gives rise to a prominent pattern which is to be observed particularly during the paling and darkening of the fish. Black-adapted minnows taken from a black background and placed on a white one pale rapidly during the first few minutes under nervous control, then continue to pale at a slower rate under hormonal control[1]—maximum pallor is reached after some hours. The macromelanophore pattern pales more slowly than the micromelanophores and it is still visible after the fish has been paling for one minute but has more or less disappeared after 3 min. On a black background the fish darkens rapidly during the first few minutes and maximum darkness is attained after some hours.

A melanophore aggregating centre exists in the medulla oblongata of the minnow from which autonomic pigmentomotor fibres pass caudally to their spinal outflow levels

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