

## RESEARCH NOTE

### MORPHOLOGY OF THE CYST OF *BLASTOCYSTIS HOMINIS*

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*Blastocystis hominis* is a common human parasite which is probably transmitted through a cystic stage via the oral-fecal route (Boreham and Stenzel, 1993). A number of publications have appeared on the morphology of the cyst at the ultrastructure level (Stenzel and Boreham, 1991; Zaman *et al*, 1993; Stenzel *et al*, 1993) but so far its light microscopic appearance has not been adequately described. This is clearly needed, as laboratory diagnosis is based on light microscopy. There are two possible reasons for the lack of this information; the first is the relatively small size of the cyst (3-5  $\mu\text{m}$  in the electron microscope) and the difficulty in separating it from the non-cystic stages of the parasite. A concentration technique has recently been described which obviates this problem by removing all the non-cystic stages and selectively concentrating the cysts (Zaman and Khan, 1994). Briefly the technique is as follows:

1. Approximately 5 g of feces are thoroughly mixed in 15-20 ml of distilled water and sieved through gauze to remove coarse particles.
2. Sediment is re-suspended in 15-20 ml of distilled water and centrifuged at 300g for 20 minutes. This is repeated 3 times.
3. The centrifuged deposit is mixed in 1 ml of distilled water and layered on 10 ml of Ficoll-Paque (Pharmacia) column. This is centrifuged at 2,000g for 20 minutes.
4. *Blastocystis* cysts band about 0.5 cm below the top. Most, but not all, of the bacteria and other fecal debris becomes pelleted at the bottom of the tube.
5. The concentrated material in the band is carefully removed with a pasteur pipet and re-centrifuged in 20 ml of distilled water to remove Ficoll-Paque. The suspension of cysts is now available for establishing cultures and studying its morphology.

The essence of the technique is the use of the

distilled water which breaks up all the non-cystic stages (vascuolar, granular and amoeboid forms). The fragility of the non-cystic stages to distilled water has been known for a long time and has been used to remove *Blastocystis* from *Entamoeba histolytica* cultures (Smedley, 1956).

The main morphological features of the cyst are as follows:

1. Shape - using phase-contrast the cyst appears as a sharply demarcated polymorphic, but mostly oval or circular dense body, surrounded by a loose outer membranous layer (Fig 1, 2). Without phase-contrast the membranous layer is not readily seen. The space between the membranous layer and the cyst often shows fine particulate matter and occasionally larger structures such as bacteria (Fig 2). Cysts may be seen without the layer.
2. Size - mean diameter of 20 cyst with the membranous layer is 12.65  $\mu\text{m}$  (SD = 1.14). Without the

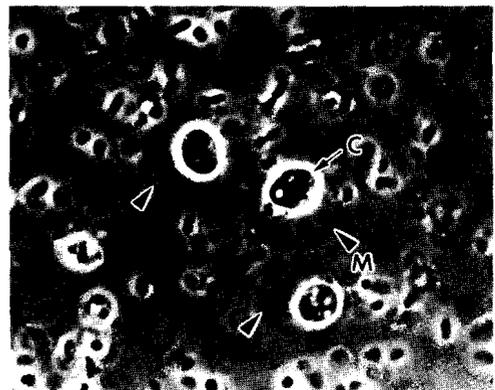


Fig 1-3 cysts are visible. Arrow heads mark the membranous layer (M) and arrow marks the cyst (C). Phase-contrast  $\times 1,000$ .

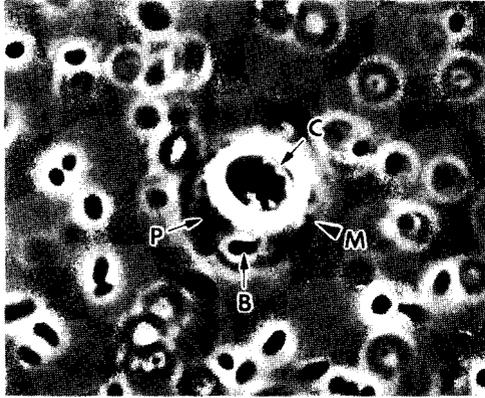


Fig 2-A cyst is visible in the center.  
M = membranous layer, B = bacteria,  
P = particulate matter, C = cyst.  
Phase-contrast X 1,200.

membranous layer (cyst only) it is  $6.65 \mu\text{m}$  (SD = 2.32).

3. Cyst contents - a number of large and small circular bodies are visible inside the cyst, which are probably mitochondria and nuclei. Their numbers could not be assessed accurately.

The membranous layer seen in phase-contrast, corresponds to the "fibrillar layer", described around the cyst at the ultrastructure level (Stenzel and Boreham, 1991) and is the easiest diagnostic feature which differentiates it from cysts of other small intestinal protozoa, such as *Cryptosporidium* sp. In addition, unlike the *Cryptosporidium* sp the *Blastocystis* cysts do not take up acid-fast stain.

The size of the cysts in the light microscope appears to be slightly larger than that observed by electron microscope. This is probably due to the flattening of the specimen caused by the weight of the cover glass. The proof that these were cysts of *Blastocystis* and no other parasite was obtained by culturing them *in vitro*. In the case of every positive cyst containing specimen (so far over 42) luxuriant growth of *Blastocystis* was obtained in Jones' medium (1946) in 2-3 days after inoculation. Further studies on the morphology of the cysts are being conducted using various staining techniques to identify its internal structures.

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