alteration in membrane permeability (Jacob, Bischel, and Herschler, 1964). Reversible alteration in membrane protein structure and/or the removal of fatty acids from the cell membrane may occur. These findings imply facilitation of movement of some substances by changes in membrane porosity.

As with most staining techniques, it is important that solutions be changed after frequent use. It is also helpful to the interpreter to have a control specimen with known *P. carinii* stained simultaneously with the test specimen. We found that necropsy lung sections from *P. carinii* pneumonitis can be maintained in refrigeration several months for this purpose.

The procedure has been carried out with excellent results by technicians after brief orientation.

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A simple method for studying nuclear division in free-living soil amoebae

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The study of mitotic figures is an essential investigation for the classification of free-living amoebae. Singh (1950) described a method requiring the growth of strains on a thin agar film attached to a glass slide. After fixation, cells which had penetrated the agar layer were found attached to the surface of the slide. Alternatively, trophozoites may be removed in a small volume of distilled water deposited on culture plates and transferred to slides for fixation and staining. In our experience, the latter technique fails to produce sufficient cells for easy study and those that are visible appear shrunken and hyperchromatic. The method of Singh, as the author states, requires considerable practice and patience.

Materials and Methods

CULTURE OF AMOEBAE

A variety of free-living amoebae, including pathogenic isolates of *Naegleria fowleri* (Carter, 1970), were grown on plates of 1.25% agar (Difco Laboratories) in distilled water. After solidification, the agar surface was spread with a culture of *Escherichia coli* ATCC 11229 to within approximately 3 cm of the periphery. After drying, the plates were inoculated in the centre using a micropipette charged with a distilled water suspension of amoebae. Cultures were incubated at 37°C for 24 to 48 hours and then examined under a stereoscopic zoom microscope (Olympus, Tokyo).

PREPARATION OF AGAR BLOCKS

Blocks are cut from selected areas of heavy growth under stereomicroscopic control using a sterile blade (Swann Morton no. 11) mounted on a suitable handle. Blocks are lifted out on the point of the scalpel blade and placed, surface upwards, on a glass slide. At this stage, the presence of abundant amoebae can again be checked by microscopy.

PREPARATION OF SLIDES

Clean glass slides are pressed gently onto the surface

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Fig. 1 *Naegleria fowleri*. Agar block impression. Haematoxylin and eosin $\times 400$.

Fig. 2 *Naegleria fowleri*. Early prophase. Haematoxylin and eosin $\times 1000$.

Fig. 3 *Naegleria fowleri*. Metaphase. Haematoxylin and eosin $\times 1000$.

of the block, quickly reversed and flooded with fixative (Nissenbaum, 1953). When, as occasionally happens, the block adheres to the upper slide, it is gently detached with the scalpel blade. The successful transfer of cells can be readily confirmed by in-

specting the surface of the fixed slide macroscopically, using transmitted light. Preparations are taken to water through iodine-alcohol and 70% alcohol, and then stained with haematoxylin and eosin.

Results

Typical slides show large numbers of trophozoites, especially with those species forming a growing edge. Shrinkage is minimal and intracellular detail is preserved (Fig. 1). With an oil-immersion objective, numerous mitotic figures can be identified and all phases of mitosis are readily seen (Figs. 2 and 3).

The method is rapid, simple, and allows many areas of growth to be examined, representing not only those with mitotic figures but also those containing cysts. The size of the block removed leaves most of the culture intact and allows further incubation and observation.

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