

used. The pieces should be thoroughly shellacked. Any parts of the geological pattern may be painted on a fresh cast with the air-brush. This is done by protecting the surrounding parts of the cast with the pieces of the stencil. After a single color has been applied to several casts, and has become dry, other colors may be blown on in succession until the whole geological pattern has been applied to the casts. Lakes and major streams may be blown on with the air-brush through a sheet lead stencil which has been drawn down to fit the topography. Complicated stream patterns and printing require hand work.

EDWARD J. FOYLES

UNIVERSITY OF ROCHESTER

A METHOD FOR CONCENTRATING AND FIXING FREE-LIVING PROTOZOA ON COVER GLASSES

IN making slides of free-living protozoa the greatest difficulty usually encountered is the sticking of large numbers of organisms on the slide or cover glass, as the case may be. This difficulty is greatly overcome by the following method for fixing on the cover glass. By folding paper with a porousness comparable to that of mimeographing paper, make a box about 20 mm deep and of a size into which the cover glass closely fits. Smear the cover glass thinly with egg albumen and place it face up into the box. Stand the box on blotting paper and fill it with

fixative to a depth of 4 mm, or more if the cultures are poorly populated. Into the fixative pipette an equal, or less, amount of water containing the protozoa. The fluid seeps out and is absorbed by the blotting paper. Because the seepage is uniform and relatively slow, the organisms are left securely fastened and evenly distributed on the cover glass. When only a thin film of the fluid still envelops the organisms, remove the cover glass and drop it face down into a dish of the fixative. After fixation, follow the usual procedure for the fixing solution and stain selected. Reverse the above process, when fixing rhizopods, by putting them into the box, allowing them to become attached and then adding the fixative.

The various fixing solutions and stains give good results with the above method. Navaschin's solution is particularly good for sticking the organisms on the cover glass. Since the crystal violet-iodine staining method leaves the cytoplasm almost colorless, yet stains the nucleus well, it is excellent for studying nuclear divisions in total mounts of protozoa. With this stain and the above method for fixing, the nuclear behavior in ex-conjugants of *Paramecium caudatum* is easily followed; the chromosomes are especially sharply defined.

J. T. BALDWIN, JR.

THE BLANDY EXPERIMENTAL FARM AND
THE MILLER SCHOOL OF BIOLOGY
UNIVERSITY OF VIRGINIA

SPECIAL ARTICLES

THE INCIDENCE OF THE DISEASE-PRODUCING AMOEBA (*ENDAMOEBA HISTOLYTICA*) IN 1060 COLLEGE FRESHMEN AND ITS SIGNIFICANCE

DURING November, 1933, the newspapers of the country carried items concerning an "outbreak" of amoebic disease in Chicago, with reports of 100 or more cases and a number of deaths. These reports gave the impression that the disease-producing amoeba (*Endamoeba histolytica*) is extremely rare and mostly confined to the tropics.

While it is true that amoebic disease is more prevalent in the tropics and that the incidence of the parasite tends to become lower in the more temperate regions, nevertheless, it has been amply demonstrated that this amoeba is world-wide in its distribution and is not uncommon in the more temperate climates. In fact, the first case of amoebic dysentery to be reported in the literature, by Lösch in 1875, was found in a northern locality, St. Petersburg (now Leningrad), Russia. Since then the disease and the parasite have been extensively studied in many parts of the world.

Craig¹ has called attention to the probability that between 5 per cent. and 10 per cent. of the population of the United States harbor *Endamoeba histolytica*, while, in our more southern states, Faust,² Meleney^{3, 4} and others have found a still higher incidence of infection. In order to determine the incidence of *E. histolytica* and other intestinal Protozoa in college students, the present authors added an examination of a single ordinary stool to the regular medical ex-

¹ C. F. Craig, "The Amoebiasis Problem," *Jour. Am. Med. Assoc.*, xcvi: 1615-1620, 1932; "The Pathology of Amoebiasis in Carriers," *Am. Jour. Trop. Med.*, xii: 285-299, 1932.

² E. C. Faust, "A Study of the Intestinal Protozoa of a Representative Sampling of the Population of Wise County, S. W. Virginia," *Am. Jour. Hyg.*, xi: 371-384, 1930; "The Incidence and Significance of Infestation with *Endamoeba histolytica* in New Orleans and the American Tropics," *Am. Jour. Trop. Med.*, xi: 231-237, 1931.

³ H. E. Meleney, "Community Surveys for *Endamoeba histolytica* and Other Intestinal Protozoa in Tennessee," *Jour. Parasitol.*, xvi: 146-153, 1930; H. E. Meleney, E. L. Bishop and W. S. Leathers, "Investigations of *Endamoeba histolytica* and Other Intestinal Protozoa in Tennessee." "III. A State-wide Survey of the Intestinal Protozoa of Man," *Am. Jour. Hyg.*, xvi: 523-539, 1932.

aminations of incoming students at a professional school in Philadelphia. In the fall of 1931, 401 freshmen were examined, and the results have been published.⁵ Since then, 299 and 360 were added in 1932 and 1933, respectively, making a grand total of 1,060, of whom 351 were women and 709 were men.

From the examination of a single stool per person, it was found that 2.3 per cent. of the women and 4.9 per cent. of the men, or 4.1 per cent. of the total, harbored *E. histolytica*. It has long been held that a single examination of a group of individuals reveals only from 30 per cent. to 40 per cent. of the actual incidence of this amoeba. It is, therefore, safe to estimate that our incidence of 4.1 per cent. based upon one examination probably indicates a true incidence of as much as 10 per cent.

Over 90 per cent. of these 1,060 students came from homes in Pennsylvania or New Jersey, and few had traveled extensively either in the United States or abroad. Hence it is probable that these students became infected before coming to college, so that the incidence found indicates the presence of the parasite in these districts.

It should, of course, be emphasized that most of the students harboring *E. histolytica* were "carriers," as are most of those who harbor this amoeba in temperate climates. The "carrier" shows no symptoms of disease, but discharges cysts which are the infective stages, capable of infecting other persons, some of whom might succumb to the disease. In rural districts and other places where sewage disposal facilities are inadequate, it is known that flies are effective agents in the dissemination of such cysts. In the cities, food-handlers in public eating places who are carriers, and who may be careless or uncleanly in their personal habits, are the most probable sources of infection. Just why some persons when infected by this amoeba become ill, while others do not, is not exactly known. It seems probable, however, that some individuals have a greater natural resistance to this parasite than others and also that some strains of the parasite may be more virulent than others.

Our data support Craig's belief that between 5 per cent. and 10 per cent. of our population harbor the disease-producing amoeba and, if this is true, it is not surprising that a number of people contracted the disease in Chicago this past summer. It may also be seriously questioned whether or not the incidence of amoebic disease was so different from that at other times and in other large centers of population. Since amoebic dysentery is considered to be so rare by many medical men, the true nature of such cases is likely to be overlooked. Amoebic disease is admittedly diffi-

cult to differentiate from other disturbances of the digestive tract and normally requires the finding of the amoebae themselves in the feces or in the tissues obtained at autopsy. With four other amoebae resident in the human bowel, proper diagnosis requires special training in the technique of examination and in the differentiation of these different types of parasites. It may be significant that recognition of the disease in Chicago apparently came at the end of the summer, when deaths occurred and autopsies revealed the nature of the disease in its true light.

In consideration of the above statements, it may be suggested that: (1) The general public, as well as medical men, should be made aware of the wide-spread distribution of *Endamoeba histolytica* and should appreciate the methods by which this potentially pathogenic parasite is disseminated. (2) Public health officials might well arrange for adequate examination of the food-handlers in public eating places to detect carriers among them, positive cases being treated to eliminate the parasite. (3) Many diarrheas, dysenteries and other disturbances of the digestive tract are due to causes other than the presence of amoebae; hence a diagnosis of amoebic disease is scarcely justified unless *Endamoeba histolytica* be found in the stools or detected in material obtained with the proctoscope or in pathological tissues. (4) In view of the fact that four other kinds of amoebae occur in the large intestine of man, two of them with greater frequency than *E. histolytica*, it should be especially emphasized that special training is required before one can hope to recognize the different types and distinguish *E. histolytica* from the others.

D. H. WENRICH

R. M. STABLER

J. H. ARNETT

UNIVERSITY OF PENNSYLVANIA

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⁵ J. H. Arnett, D. H. Wenrich and R. M. Stabler, "A Survey of 401 College Freshmen for Intestinal Protozoa," *Am. Jour. Trop. Med.*, xiii: 311-315, 1933.