Iowa Science Teachers Journal

Volume 8 | Number 2 Article 4

1970

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Introduction

We so frequently use *Amoeba proteus* for observation and experimentation in our classes in Cell Structure and Function, and Physiological Protozoology at the University of Kansas, that it has been necessary to avoid buying the many needed cultures from any of the major biological suppliers. Consequently, we have had to devise a reasonably reliable method of our own to culture that amoeba.

Materials and Methods

Prescott and James (1955) raised *Amoeba proteus* with the ciliated protozoan, *Tetrahymena pyriformis* as its food. *Tetrahymena* is easily raised bacteria-free on a proteose-peptone and salts medium (Elliott and Hayes, 1953), or on a 1 per cent (weight/volume) yeast-extract medium (Kozloff, 1956) (see Table 1). Griffin (1960) determined the requirement of *Tetrahymena* per *Amoeba* per day. Nardone (1957, 1958) used a modification of the Prescott-James method, plus antibiotics, attempting to get a bacteria-free strain of *Amoeba*. Noble (1960) raised protozoa in the cold.

Table 1
Peptone Medium

proteose-peptone	2 g
K_2HPO_4	0.25 g
$MgSO_4$	0.25 g
KCl	0.25 g
Na Acetate	2 g
Ferric Cl	trace
H_2O	1 liter

Our method, now in use for nearly two years, combines, partly, the methods of those researchers for raising *Amoeba proteus*. We have eliminated the need for antibiotics by keeping the cultures refrigerated except when feeding them. While the method eliminates only some of the tedium of frequent transfers, it enables us to: (1) grow *Amoeba proteus* in the balanced saline solution also used in experimentations; (2) keep them virtually free of bacterial and mold contaminants; (3) reduce their rate of multiplication (and thereby the need for frequent feeding and transfers); and (4) have a large number of clean, healthy amoebas available at almost any time.

Starting with amoebas from a commercial source, we stir the recently received culture with a glass rod, or shake it gently while tightly capped, to make the amoebas release their adhesions to the bottom and sides of the shipping jar. Then we empty the amoebas and fluid into a sterilized

glass Petri dish. After an hour or so, when the amoebas have attached to the glass bottom of the Petri dish, the fluid is decanted off and replaced by several ml of Chalkley's solution (see Table 2). This is swished gently

Table 2
Chalkley's Solution

NaCl	0.1 g
KCl	0.004 g
$CaCl_2$	0.006 g
H_2O	1 liter

around to wash the amoebas without dislodging them, is decanted and replaced with more Chalkley's solution.

The Petri dish is then placed in the refrigerator and left overnight. Sometime near noon the next day (amoebas are most active at mid-day) the Petri dish is removed from the refrigerator and allowed to warm to room temperature.

While the Petri dish (and the amoebas) are warming to room temperature, we centrifuge (at 1,000 rpm for 15 sec) tube-cultures of the ciliated protozoan *Tetrahymena pyriformis,* an adequate food for *Amoeba proteus.* We then decant their nutrient medium, replace it with Chalkley's solution, swish the tube, or tip it back and forth several times, to swirl and wash the *Tetrahymena,* repeat the centrifugation and decantation, rewash, recentrifuge and finally decant all but about 1 ml of fluid which contains the *Tetrahymena* at the bottom of the tube. This sounds involved, but takes less than 5 minutes.

These *Tetrahymena* are then pipetted into the Petri dish containing the amoeba culture; and the amoebas are allowed to feed on them for half an hour to one hour. By then the amoebas have ceased feeding and have attached to the glass bottom of the dish.

We then carefully decant off the fluid and the remaining swimming *Tetrahymena,* add Chalkley's solution, cover the dish, and replace it in the refrigerator.

Feeding is done thereafter every 2 to 3 days. Experiments indicate that for adequate growth feeding is needed at least once within each four-day period, and that longer intervals between feedings may be detrimental, although we have successfully let a culture go 7 to 8 days occasionally without feeding. In other instances, upon feeding after such long fasts, the amoebas died after gorging themselves—perhaps of indigestion?

If we require larger numbers of the amoebas, we grow them in the same way, except that we keep them at room temperature (20-22° C), and feed them once each day. Such cultures enter an almost perfectly circadian cycle of growth, doubling in numbers each day.

Results

We have been able to keep sturdy clean cultures of *Amoeba proteus* going throughout the year by this method, and can start new ones easily when desired.

The method is not absolutely foolproof, however. We occasionally lose cultures, probably because of lack of the few trace factors required from a minimal number of bacteria and yeasts in the diet, (see Prescott, 1958), but we rarely lose all cultures at the same time. Besides the apparent demise from gorging after exces-

sive starvation already mentioned, we find that the peptone medium in which the *Tetrahymena* grow is toxic to the amoebas. Consequently, careful repeated washing of the *Tetrahymena* is required before they are fed to the amoebas. In one instance we may have lost a culture because the *Tetrahymena,* although adequately washed, were so full of peptone-containing gastrioles ("food vacuoles") that the peptone from them poisoned the amoebas as they digested the *Tetrahymena.*

Therefore, we now keep the *Tetrahymena* in the saline wash-fluid for about an hour before the final rewashing and feeding to the amoebas to allow for digestion of the peptone in the *Tetrahymena's* gastrioles.

Discussion

Although *Amoeba proteus* and *Tetrahymena* tolerate a wide variety of ionic fluids (Nardone, 1958) we use Chalkley's solution because it is the most simply concocted, and consists of readily available chemical salts. As a relatively simple solution it is also amenable to many predictable ionic adjustments by a variety of other to-be-added chemicals, so that its experimental utility is excellent. We also find it best to use glass-distilled water (we doubly distill it) as the solvent for the Chalkley's solution.

There are also more elaborate media for raising *Tetrahymena;* but either the proteose-peptone medium or the yeast extract medium adequately nourish the *Tetrahymena.* The amoebas derive their critical vitamins and trace minerals adequately by digesting the *Tetrahymena,* and from the few bacteria or yeasts which may be present.

LITERATURE CITED

Chalkley, H. W. 1930. Stock cultures of amoebae. *Science,* 71: 442.

Elliott, A. M. and Hayes, R. E. 1955. *Tetrahymena* from Mexico, Panama and Colombia, with special reference to sexuality. *J. Protozool.,* 2: 75-80.

Kozloff, E. N. 1956. Experimental infection of the gray garden slug, *Deroceras reticulatum* (Müller) by the holotrichous ciliate, *Tetrahymena pyriformis* (Ehrenberg). *J. Protozool.,* 3: 17-19.

Griffin, J. L. 1960. An improved mass culture technique for the large free-living amebae. *Exptl. Cell Res.,* 21: 170-78.

Nardone, R. M. 1957. Growth of *Pelomyxa carolinensis* on a single protozoan species. *J. Protozool.,* 4 (suppl): 19.

Nardone, R. M. and Ashton, A. K. 1958. The behavior of *Pelomyxa carolinensis* and *Amoeba proteus* under controlled conditions. *J. Protozool.,* 5 (suppl): 22.

Noble, G. A. 1960. Cold culture. *J. Parasitol.,* 42: 581-84.

Prescott, D. M. 1956. Mass and clone culturing of *Amoeba proteus* and *Chaos chaos. Compt. rend. trav. Lab. Carlsberg, Sér. chim.,* 30: 1-12.

Prescott, D. M. 1959. Microtechniques in amoebae studies. *Ann. N.Y. Acad. Sci.,* 78: 655-61.

Prescott, D. M. and James, T. W. 1955. Culturing *Amoeba proteus* on *Tetrahymena. Exptl. Cell Res.,* 8: 256-58.