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Introduction

Certain protozoa have often been the cellular animals of choice for research and teaching in the past 100 years. In 1926, Asa Schaeffer (20) placed "the amoeba" at the pinnacle for animals that serve as primary research tools, along with the dog and the frog. *Paramecium* (one or more of its species) and *Euglena* (one or more of its species) are other protozoa (the latter, also an alga) well-known and oft-employed in both teaching and research.

In the past 40 years, however, another protozoan has gained prominence in research, equalling — even superseding — the three mentioned above.

Its generic name: *Tetrahymena.*

Tetrahymena

One or more of its species, particularly various strains of *Tetrahymena pyriformis*, serve more often, now, as research tools than those of any other, ciliate, protozoan genus. Its use, also, in teaching laboratories, particularly where durable cells are needed for observation and experiment, increases steadily, and it is now available from most commercial suppliers of biological materials, as well as (in clonal strains) from the American Type Culture Collection in Rockville, Maryland.

Tetrahymena pyriformis was seen and described by Leeuwenhoek (17) and first named *Leucophrys pyriformis* by Ehrenberg (7). It was used in cellular growth studies before 1900 by the Algerian librarian, Maupas (18), whose hobby was protozoology. It did not acquire its present generic name, however, until the year 1940, when W.H. Furgason (10) clearly distinguished and separated it from other small genera of ciliates with which it had been confused *(Glaucoma, Colpidium, Leucophrys*, and others).

Since then — its identity cleared in 1970 by International Rules (13) — it has rapidly gained its present favor in biological research and teaching.

Its "virtues" for laboratory use are many: 1. It is easily found, being ubiquitous in freshwaters the world over. 2. It grows easily at room temperature and requires no special incubators. 3. It can feed omnivorously — on bacteria, yeasts, cellular debris, on other smaller protozoa, as a scavenger, a saprozoan, a parasite, or even (one species) as a cannibal! 4. It can be raised on a simple, nutrient soup, completely free of other organisms (i.e., axenically). 5. Its food requirements are completely known (12). 6. It is hardy and withstands much stress in laboratory experiments. 7. It is a good example of a well-adapted, ciliated protozoan for classroom study. 8. It is a sturdy animal cell, useful in a wide variety of researches, either as an animal or as a cell.

As an animal, it is a ciliated protozoan. As a cell it is a complex, but still relatively unspecialized eucaryotic cell. Its present classification is as follows, after Corliss (14).

Kingdom — Protista
Subkingdom — Protozoa
Phylum — Ciliophora
Subphylum — Oligohymenophora
Class — Hymenostomatea
Order — Hymenostomatida
Suborder — Tetrahymenina
Family — Tetrahymenidae
Genus — *Tetrahymena*
Species — *pyriformis* (or other species)

In shape, the majority of its species are more-or-less pyriform, i.e., meaning *pear*-shaped, *not* flame-shaped, as some assume; see Corliss (6). It is broadly rounded at the base, narrower and rounded at the tip (the anterior end, in swimming), and is covered with rows of cilia from anterior to posterior.

The mouth is (technically) ventral, near the anterior end in an oral ("buccal") depression that contains, along its left (the *protozoan's* left) wall, three small, dense clumps (membranelles) of cilia. On the right edge of the oral depression there is a double, partly-fused row of cilia which, because of its coordinated movements, is called an *undulating membrane* (Fig. 1). The organism gets its generic name, *Tetrahymena*, from these four, oral membranes that comprise its feeding apparatus.

Culture

Growing *Tetrahymena* for study is a "cinch"! All one needs is some filtered pond water, or *Chalkley's solution (2), an easily compounded

**Chalkley's solution: sodium chloride (NaCl) 0.1 gram; potassium chloride (KCl) 0.004 gram; calcium chloride 0.006 gram; water (H_2O; glass-distilled or de-ionized), 1,000 milliliters (ml).*

equivalent, in a clean glass container. The *Tetrahymena* and the food can be added. Mote (19) found that bacteria which were grown in an "alfalfa tea" are more-than-adequately nutritious for *Tetrahymena* and other ciliates. You may start a clone by selecting a single ciliate by micropipette, transferring it to the growth medium (water and food) in the container. The container should be loosely covered to prevent entry of "foreigners." A one-pint to one-quart glass jar with a screw-cap lid, loosely, but partly screwed on, is a good container.

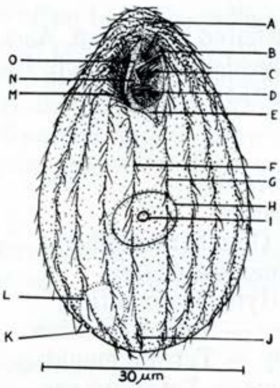

Fig. 1. *Tetrahymena pyriformis:* A — Origin of ciliary rows; B, C, D — Membranelles of the buccal cavity; E — cytopharynx; F — 1st ciliary meridian; G — last ciliary meridian; H — nucleus; I — nucleolus; J — cytopyge; K — pore of water-expelling vesicle; L — water-expelling vesicle; M — undulating membrane; N — buccal cavity; O — oral rim.

In several days to a week, *Tetrahymena* should become numerous, even abundant. Mote (loc. cit) gives a *regimen* for feeding which is useful.

For longer-term cultures, bacterial growth can be promoted by adding powdered milk (a *very* small amount, i.e., a small pinch) about once a week (9).

Axenic cultures are nearly as easy, requiring a little more care and equipment to prevent contamination. They are raised in flasks (Ehrlenmeyer-type or equivalent) usually screw-capped, or plugged with sterile cotton. The "soup" (usually 1 gram of protease-peptone in 99 ml of distilled, or deionized, water) must be sterilized by "autoclaving" (a small, household, pressure-cooker is adequate) before the axenic *Tetrahymena* are added. Routine, bacteriological methods of transfer are required to maintain the sterile conditions during transfer by sterile pipette (1), but those methods can be mastered with a little care and practice.

In the classroom, *Tetrahymena* (one or another strain or species) can be used for observation or experiment in a variety of classes: 1) In General Biology, Zoology or Invertebrate Zoology as an example of a common, not-too-complex ciliate; 2) in Entomology as a faculative pathogen in the insect hemolyph; 3) in Genetics to demonstrate physiological inheritance of enzymes, serotypes, or mating types, or the morphological inheritance of numbers of ciliary rows; 4) in Developmental Biology to demonstrate cell division, conjugation, and morphogenesis of the oral structures; 5) in Cellular Biology to demonstrate cellular growth-cycles, sites of cellular enzymes, phagocytosis, pinocytosis, water-intake and excretion, cyclosis and other cellular phenomena; 6) in Population Biology to demonstrate growth of populations on different nutrients and under varied external conditions; 7) in Biochemistry to determine and demonstrate cellular requirements, enzymes, RNA or DNA content, biochemical cycles, etc.; 8) in Pharmacology to test the toxicity of drugs; 9) in Sanitary Engineering as a bioassay for the presence of and toxicity of pollutants; 10) in Limnology or Ecology as an example of a highly adaptive organism; and many other uses.

Research-wise, its utility is even greater. The taxonomy and classification of the genus *Tetrahymena* and the (at least) 14 species in that group have been thoroughly discussed (4) by Dr. John O. Corliss now of the University of Maryland in many papers since 1947 (5). He has also repeatedly surveyed the rapidly-growing literature on *Tetrahymena*, showing that the number of papers published on research with it has jumped phenomenally from about 250 papers between 1676-1943 to nearly 1,400 in 1963 (3)! That figure, by Dr. Corliss' estimates (5) and my own has more than trebled between 1963-1976, being now approximately 4,000 papers, numerous reviews and at least two books!

Most of these papers are by researchers in biochemistry, molecular biology, and cellular physiology (about 60%); about 15% deal with its genetics and cytogenetics; about 20% are concerned with its cell structure and systematics; about 3% with its morphogenesis; and the remaining 2% with its ecology and its role as a parasite. These figures, as Corliss says (3, 5) are — and can only be — approximate, because of the continuing and burgeoning of research on and newly published papers concerning *Tetrahymena*.

Recently, the importance of *Tetrahymena* as a "test-object" (15) in biological research and teaching has resulted in a major book, *The*

Biology of Tetrahymena (8), edited by Dr. A.M. Elliott, Professor Emeritus of Biology, University of Michigan. That book, published in 1973 by Dowden, Hutchinson and Ross, of Stroudsburg, Pennsylvania, contains 13 chapters, authored (or co-authored) by 19 specialists in research with *Tetrahymena*. The General Bibliography, containing over 1,700 referenced papers and books, succinctly indicates the impact of *Tetrahymena* on teaching and research in biology! Dr. Corliss' (5) most recent survey of the literature on *Tetrahymena* is an excellent guide to the many uses to which that protozoan has been put as an animal and/or cell of choice in successful researches. Nearly 1,500 people have done published research on *Tetrahymena*, with more daily becoming involved, a record not likely to be exceeded by researchers on another ciliated protozoan!

Tetrahymena has become, as Dr. G.W. Kidder of Woods Hole, Massachusetts (who pioneered with it in biochemical researches) calls it, "a star performer" in teaching and research (11, 16)!

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