Isozymic Heterogeneity in Tetrahymena Strains

Abstract. *Examination of electrophoretic mobility patterns indicates that the strain designations of 44 classical strains of* Tetrahymena *are confused. It is suggested that new designations be made on the basis of phenotypic similarity.*

The ciliate *Tetrahymena pyriformis* is widely used for experimental purposes in biochemistry, physiology, and genetics. Well over half of the published studies (more than 2500 in 50 years) employ a limited number of "classical," usually amicronucleate strains, representing a small number of isolates from nature. These strains are difficult to distinguish on morphological grounds (*1*), although patterns of covariation of cortical structures (*2*) offer some help. Serological affinities (*3*) have not been explored sufficiently to provide a basis of discrimination. The most commonly used "classical" strain is GL, which was derived from a single cell isolated into axenic media by A. Lwoff in 1922. Strain GL has achieved international distribution because it was the first *Tetrahymena* isolated into pure culture. Many other *Tetrahymena* strains have also been isolated into pure culture (*1*) and have been given various designations.

Recently, a broad survey of many enzymes in *Tetrahymena* (*4*) showed that electrophoretic resolution of isozyme mobilities provided a powerful procedure for distinguishing between *Tetrahymena* strains. The present study of the classical strains was undertaken in an attempt to identify their possible evolutionary derivations from wild mating populations. However, early observations showed profound differences among strains of identical designations and presumably of common origin. This observation provoked a more systematic exploration of variation within and between strains (*5*). The study of these strains revealed quite unexpected results and should be of concern to such a large number of laboratories that a preliminary presentation of the results seemed indicated. Both Frankel (*6*) and McCoy (unpublished) come to similar conclusions on the basis of cortical analysis.

The strains were grown, harvested, homogenized, subjected to electrophoresis, and stained as previously described (*4*). Each strain was run several times and all strains were run with a reference strain of breeding syngen 1, C2-368-3x, on the gels. The enzymes studied were tyrosine aminotransferase, NAD-malate dehydrogenase (both supernatant and mitochondrial forms) (*7*), NADP-isocitrate dehydrogenase (two forms), NAD-glutamate dehydrogenase, FDP-aldolase, tetrazolium oxidase, propionyl esterases, and acid phosphatases. Some of the activities, particularly the esterases and the phosphatases, were represented on the gels by several discrete bands, but for the present analysis these were treated as a unit. The strains to be compared were either absolutely similar with respect to the electrophoretic mobilities for a given isozyme pattern or they were considered to be different. When extracts of some strains were subjected to electrophoresis, some enzymes were never detected for reasons not completely understood. For present purposes these negative results were ignored instead of treated as comparative data. An average of eight comparisons was employed; 95 percent of the comparisons were based on seven or more points.

The results with the strains designated as GL are summarized in a half-matrix (Table 1) which records the percentage of identical enzyme phenotypes for each pair of strains. Strain GL-1 is indistinguishable from GL-2, -3, -6, and -8, but only one third (three of nine) of its enzymes possess the same mobilities as the enzymes of GL-5. Strain GL-1 shares *no* common enzyme mobilities with GL-4, -9, or -10. Because of recent studies of variations within and between syngens (*4*), we have set 67 percent as the lower limit of variation for inclusion within a phenotypic set. Therefore, GL-7 is included in the same set as GL-1, -2, -3, -6, and -8 (phenotype set or "phenoset" *A*), and GL-4, -5, -9, and -10 belong to other classes. Strains GL-4 and -9 are indistinguishable and are designated as set *B*; GL-5 and -10 are indistinguishable and belong to set *C*. Strains which have been historically designated as GL, therefore, consist of three distinctive classes with molecular divergences among them of a very large order.

When comparisons are made among other classical strains, again striking differences are found among samples labeled identically. Seven E strains (supposedly amicronucleate) were divided into four classes; one of these classes (containing four strains) possessed sufficient similarity to include it with phenotype set *A* of GL (Table 2). One E strain belonged to set *B*; two others (both having micronuclei) are the first strains in two additional sets, *D* and *E*. Three of four W strains examined belonged to set *A* of GL; the fourth belonged to set *C*. Three of four strains labeled HS or HSM belonged to set *D*, with the E strain. All four of these strains have micronuclei; the fourth HS strain (also micronucleate) was placed in a sixth pheno-

Table 1. Similarity comparisons for GL strains. Values represent the percentage of similarity for the enzyme patterns observed.

	GL-1	GL-2	GL-3	GL-6	GL-8	GL-7	GL-4	GL-9	GL-5	GL-10
GL-1										
GL-2	100									
GL-3	100	100								
GL-6	100	100	100							
GL-8	100	100	100	88						
GL-7	88	85	83	88	88					
GL-4	0	0	20	0	14	0				
GL-9	0	0	0	0	0	0	100			
GL-5	33	40	20	0	0	0	12	14		
GL-10	0	0	0	0	0	0	14	0	100	

Table 2. Similarity comparisons of strains within phenotype set *A* and with strains of other sets. Strains E-7, W-2, GL-4, and GL-10 are members of other sets. Values represent the percentage of similarity for the enzyme patterns observed.

	E-1	E-4	W-1	W-3	S-3	S-5	E-7	W-2
GL-2	80	100	100	100	100	100	12	33
GL-3	100	88	100	100	100	100	14	20
GL-7	71	78	83	71	83	80	0	33
GL-8	85	89	85	100	83	100	14	28
GL-4	0	0	0	0	0	0	100	11
GL-10	0	0	0	0	0	0	14	71

typic set, *F*. Six samples of strains designated S were examined and all were very similar among themselves and to the strains in GL, phenotype set *A*. Table 2 shows several E, W, and S strains compared to GL strains of phenotype sets *A*, *B*, and *C*. Most of the strains are included in set *A*, but several strains belong to set *B* or *C*. Note that comparisons between strains of different sets show very little similarity.

Several explanations for the described situation can be entertained. Perhaps the strains of *Tetrahymena* under laboratory culture undergo very rapid molecular change, so that in a relatively short period of time (two to five decades) two sublines of a strain may become demonstrably different in most of their proteins examined. A second possibility is that controlling loci remain on or off in quasi-stable ways; each strain might be expressing only a fraction of its potential enzyme activity at any one time. Alternatively, the strains so different in their molecular properties did not have a recent common origin, but have inadvertently acquired labels that misrepresent their origins. Unprecedented molecular plasticity is rendered unlikely by the observation that two GL strains, supposedly separated for over 25 years, are virtually indistinguishable. Strain GL-8 was obtained from the laboratory of E. Zeuthen, who obtained his strain from G. W. Kidder in 1947; Kidder's strain is represented by GL-7 (obtained by our laboratory in 1972). These two strains differ by a single variation in one of the seven esterase bands. Moreover, the detailed identity of some of GL, E, W, and S strains would require an unusual convergent evolution and is scarcely explained by random molecular drift. Our provisional interpretation is that the six phenotypic sets represent six collections of wild cells. The variation within sets represents either latent variations in the original isolates (as in a heterozygous state or a macronuclear mosaic), the variations due to control processes, or else mutations which have been established during laboratory culture. The decision as to which phenotypic set corresponds to which original isolate is more difficult to adjudicate.

Regardless of the means whereby they have arrived at their present status, the classical strains of *Tetrahymena* do not constitute a molecularly closely similar set. If the variability of supposedly identical strains (which are used for so many different kinds of biological investigations) is as great as indicated by their electrophoretic mobility patterns, then this variation must be taken into account when comparing the data and conclusions derived from different laboratories. We are depositing two strains of each phenoset in the American Type Culture Collection, Rockville, Maryland, and the Culture Centre of Algae and Protozoa, Cambridge, England, to facilitate the availability of these strains.

D. BORDEN*
G. S. WHITT
D. L. NANNEY†
Zoology Department, University of Illinois, Urbana 61801

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7. Abbreviations: NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; and FDP, fructose diphosphate.
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* Present address: Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201.
† Address requests for reprints to D.L.N.

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Cyclic Adenosine Monophosphate, Metabolites, and Phosphorylase in Neural Tissue: A Comparison of Methods of Fixation

Abstract. *Fixation of rat brain tissue by freeze-blowing, microwave irradiation, immersion of whole rats in liquid nitrogen, and decapitation into liquid nitrogen indicates that postmortem changes in metabolites and enzyme forms are minimal in freeze-blown brains. Cyclic adenosine monophosphate levels are lowest in microwave-irradiated brains, which has been interpreted by some investigators to indicate rapid fixation and minimal anoxia. However, the changes in phosphocreatine, adenosine triphosphate, lactate, and phosphorylase clearly demonstrate that fixation by freeze-blowing or immersion in liquid nitrogen more closely approximate the state in vivo.*

Preparation of tissue for the study of metabolite levels, transformation of enzyme forms, and cofactors involved in metabolic regulation demands that metabolic processes be stopped as quickly as possible. The enormously high metabolic rate of the brain coupled with its limited energy reserves means that substantial changes can take place if the fixation is not sufficiently rapid.

Recently, two new techniques have been developed, one of freeze-blowing the brain and another of fixation by microwave irradiation. The freeze-blowing of the brain fixes the tissue in less than 1 second and has been shown to preserve the energy reserves of the brain better than immersion in liquid nitrogen or microwave irradiation (*1*, *2*). However, this technique precludes the use of brain tissue for regional studies. In another study, microwave irradiation was used to fix the brain for the measurement of cyclic adenosine monophosphate (cyclic AMP) levels (*3*). The results indicated that cyclic AMP concentrations were in good agreement with measurements made by quick-freezing and offered the possibility of regional studies. However, total inactivation of the enzymes involved in cyclic AMP synthesis and degradation required at least 20 seconds of exposure (*4*), suggesting that changes in some metabolites or forms of enzymes or both might occur. After irradiation of mouse heads 1 second after decapitation, Nelson and Mantz (*5*) found that there was a differential effect on the enzymes of glycolysis resulting in low levels of lactate but breakdown of glycogen and adenosine triphosphate (ATP). In the present report, substances which are sensitive indicators of the degree of anoxia or ischemia or both in the brain have been measured in rat brains which have been fixed by immersion in liquid nitrogen, freeze-blowing, or microwave irradiation. The levels of phosphocreatine, lactate, ATP, cyclic AMP, and the conversion of phosphorylase *b* to *a* have been studied.

Male Wistar rats (Carworth Farms) weighing 200 to 250 g were starved overnight prior to use. Fixation of brain tissue was accomplished in the following ways: (i) the whole animal was immersed in liquid nitrogen for 5