not grown. Similarly, sterile EL alone does not support growth and the medium of Elliott(2) at full and at half-concentration is toxic.

DISCUSSION

Most procedures for obtaining sterile, free-living ciliates embody one modification or another of the principles established by Parpart(8). Parpart axenized paramecia by means of a series of rapid washes of single animals, interrupted by one lasting several hours for egestion of spores and their subsequent removal by dilution during further washes. The importance of swimming as a means of dislodging contaminants caught in cilia and cell surface irregularities was emphasized by Hetherington(4).

Others exploited trophic responses to increase the efficiency of the washing and dilution process (3,1,10) or, more simply, centrifugation(5).

Efficiency of the washing and dilution procedures has been enhanced by the use of antibiotics. Seaman (9) obtained sterile *Colpidium campylum* with relatively few centrifugations using penicillin. Elliott(2) sterilized *Tetrahymena* with penicillin + streptomycin after a minimum of pipette transfers.

In this study, penicillin + streptomycin were successful only when applied to detached and swimming animals. Detached animals were necessary because of the difficulty of controlling contaminants which stuck to their bodies and stalks, and the glass to which they adhered. The association of bacteria and some species of *Vorticella* was noted and figured by Noland and Finley(7). Perhaps yeasts and bacteria were washed away from detached vorticellas as they swam to and fro to be diluted beyond consequence in the series of baths. Presumably, bacteria were also controlled by long term exposure to antibiotics.

I am indebted to R. W. Harkaway and L. Mattman, both of the Biology Department, Wayne State University for, respectively, the disc test and the *Pseudomonas* and *Aerobacter* cultures, and to Dr. A. M. Elliott, Zoology Dept., University of Michigan, for his suggestion to use penicillin + streptomycin.

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J. PROTOZOOL. 6(2), 171-177 (1959).

Vegetative Mutants and Clonal Senility in *Tetrahymena**

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SYNOPSIS. Eight strains of *Tetrahymena pyriformis* were examined in multiple daily isolation series over ~ 800 generations. Dead and defective lineages appeared in all clones. In 7, the defective sub-lines appeared at a constant characteristic rate; the 8th showed an increasing rate of production of two defects. A breeding analysis of certain persistent morphological and growth rate variants suggests that the variations derive from macronuclear alterations. The relationship between these cellular changes and "senility" in metazoa and other protozoa is discussed.

SENILITY and mortality in protozoa have occupied many investigators for more than half a century (see 12), but no generalizations applicable to all protozoa, or indeed all ciliates, have developed. Studies such as those of Calkins(2) on *Uroleptis mobilis*, Jennings(6,7) on *Paramecium bursaria*, and, more recently, Sonneborn(12) on *P. aurelia*, appear to establish for these forms a life cycle terminated by senility and death if sexual reorganization does not occur. On the other hand, some closely-related forms have been maintained under laboratory conditions for dozens of years and for thousands of cell generations without sexual reorganization and apparently without age-correlated degeneration. *Tetrahymena pyriformis* is

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TABLE I. Strains studied and code designations.

Kind of strain	Strain designation	Source	Code
Amicronucleate	E	Elliott (3)	A-1
	UM-2845	Gruchy (5)	A-2
Variety 1 (inbred)	N-56-10-2(III)	"Family A" (8)	B-1
	N-55-8-15(V)		B-2
	N-55-9-44(II)		B-3
	N-55-12-2(I)		B-4
Variety 1 (cross-bred)	N-56-17-19(III)	"Family A" × Alp-4, Michigan	C-1
	N-56-21-23(IV)	"Family B" × UM-226, Vermont	C-2

perhaps the prime example of such an organism, but systematic investigations of these problems in *Tetrahymena* have not been undertaken and, indeed, studies comparable in scope to those cited could not have been undertaken before the discovery of sexual strains(4).

While maintaining isolation cultures of *Tetrahymena* for other purposes, I observed degenerative changes which resembled those described as symptoms of senility in other organisms. Further investigation showed significant differences between the changes in *Tetrahymena* and those in e.g., *Paramecium*.

MATERIALS AND METHODS

The 8 strains of *Tetrahymena* studied (Table I) consisted of 2 amicronucleate strains, 4 inbred strains of variety 1 and 2 cross-bred strains of variety 1. They were grown in *Aerobacter*-Cerophyl media(9, 10).

The study began with the isolation of 30 single cells from each of the 8 strains into individual depression slides. The next day, after a population had developed, a single organism was removed from each culture and placed in another depression. On each subsequent day single cells were isolated from the day-old cultures started the previous day until 100 serial cultures had been produced from each original isolation. On the 2nd day after a cell was isolated, the culture it produced was examined and its characteristics were recorded. Under these conditions a normal (*N*) culture undergoes about 8 fissions/day, but the culture medium in a depression slide can support only 13-14 cell divisions. Hence, a culture growing at the normal rate exhausts the medium in 48 hr. and has begun to starve; dividing cells are absent. Cultures which grow at a slightly slower rate (*S* cultures), *i.e.*, at ~ 6 fissions/day, produce a thriving population in 48 hours, removing the bacterial pellet from the bottom of the depression, but still contain many dividing cells. Cultures growing still more slowly, i.e., 4 fissions/day, reach in 48 hr. the stage reached by normal cultures in 24 hr.; the bacterial pellet is visible in the bottom of the depression; the cultures are designated as "pellet" (*P*) cultures. Cultures growing still more slowly are "degenerate" (*D*) cultures. Occasionally an isolated cell failed to divide at all, ending the lineage (*X*). Terminated lines were replaced by isolations from "normal" sister lines. This classification is crude but does describe the alterations which occurred in the cell lineages.

Some starved cultures yield small rounded immobile cells which resemble cells described previously as genetically determined anomalies in highly inbred lines (8) and, like them, are amicronucleate. Cultures showing some cells of this type are designated semi-amicronucleate (*A*) and terminate quickly, since the frequency of amicronucleate cells usually rises sharply and such cells are not capable of continued growth. Morphological variants (*M*) are also encountered occasionally. Some of these will be described.

The original plan included the study of conjugation after various periods of growth, and crosses were made between the initial cultures able to conjugate and between derived cultures after 10, 20, and 30 transfers. These crosses were scored for frequencies of non-viable conjugants and for frequencies of non-conjugant pairs. Since no marked changes in the frequencies of anomalies were observed in these crosses, the procedure was discontinued after 30 transfers, but a few crosses were made with normal cultures retained after the experiment was completed. These later crosses differed from the earlier ones but were limited in number.

RESULTS

1. Gross Death Rates. Some lines in all series were terminated by death. The data on rates of termination (Table II) support two conclusions: (*a*) Strains differ in gross death rates. Excluding strain B-1, which appears special, the probability that a single cell would fail to produce a culture varied from 0.0057 in strain C-2 to 0.0249 in strain A-2. The death rates are not clearly correlated with the type of clone. One amicronucleate strain had a high death rate, the other a very low one; one of the cross-bred strains showed a low death rate but the other a death rate within the

TABLE II. Probabilities for terminated lines (cells giving no growth/total isolations) at different times after initiation of transfer series.

Strain	Transfer interval 1–20	21–40	41–60	61–80	81–100	Mean	Total isolations
A-1	.010	.015	.008	.003	.007	.0078	2550
A-2	.027	.025	.025	.028	.019	.0249	2974
B-2	.021	.012	.015	.003	.018	.0139	2963
B-3	.028	.041	.013	.019	.012	.0227	2953
B-4	.012	.032	.018	.015	.020	.0194	2989
C-1	.003	.022	.013	.015	.022	.0150	2987
C-2	.000	.009	.008	.005	.007	.0057	2967

TABLE III. Line terminations classified according to conditions in preceding cultures. NX, normal; AX, semi-amicronucleate; MX, defective morphologically; PX, defective in growth rate.

Strain	Numbers of lines terminated				Total isolations
	NX	AX	MX	PX	
A-1	14	—	5	3	2550
A-2	13	—	1	60	2974
B-2	19	10	1	11	2963
B-3	27	8	3	29	2953
B-4	18	8	1	30	2989
C-1	9	13	2	21	2987
C-2	6	2	1	8	2967

range of the inbred series. (*b*) Except for B-1, the rates of termination showed no systematic trend during 100 transfers (up to 800 fissions). Changes in death rates may have occurred during the previous history of the clones (100-200 fissions since conjugation in the B and C clones; an unknown but certainly much longer period in the A clones) and later changes cannot be excluded. On the other hand, extensive experience with younger clones and limited experience with older clones (up to 2500 fissions) suggest similar rates of termination in these periods.

2. *Differential Death Rates.* Terminations of lineages reflect precursor conditions. In some cases a cell isolated from an apparently normal culture will fail to reproduce; these are *NX* deaths. The immediate prior history of a clone may include one or more serial cultures with depressed growth rates; these are lumped as *PX* deaths. In other cases the preceding cultures show the semi-amicronucleate condition (*AX* deaths) or morphological variants (*MX* deaths), perhaps in addition to showing depressed growth. The instances of death in 7 of the series classified according to previous history are recorded in Table III. The amicronucleate strains do not, of course, manifest death related to the loss of micronuclei for they, unlike the micronucleate strains, can reproduce without micronuclei. Aside from this, however, differences in the frequencies of death associated with particular precursor conditions are apparent.

Of significance in the interpretation of these results is the relationship betwen precursor conditions and the probability of death. Clones producing amicronucleate cells are destined for early termination; in no instance have cultures been observed to produce appreciable numbers of such cells and to recover and yield persistent normal cultures in later transfer generations. Although following detection a semi-amicronucleate line may persist from 1-10 transfers, the mean persistence is 3-4 transfers and the probability of termination at a particular transfer is ~ 30%. These values are biased to the extent that cultures producing small numbers of amicronucleates are classified as normal. Some *NX* deaths are probably *AX* deaths.

Unlike the semi-amicronucleate lines, the lineages with depressed growth show considerable variety. Some deteriorate progressively from 8 fissions/day to zero fissions, over a period of 3-30 transfers. Others stabilize at a depressed level or improve and return to normal after a variable number of depressed transfer cultures. Grouped together the probability of termination in lines growing no faster than 4 fissions/day is ~ 10% in all series.

The morphological variants are infrequent and diversified. Some anomalies become progressively more severe, some stabilize, and some disappear. Some stable mutants have been maintained for 50 or more transfers.

In all cases it is hard to tell precisely when the primary damage occurs and when it is repaired if a line recovers. One unique morphological mutant appeared in several different lines at different times; all these lines had been derived recently (5-10 transfers previously) from the same source through replacement of terminated lineages. The only reasonable explanation for this observation is that the primary alteration had occurred at some time prior to its overt manifestation. This difficulty in determining when cellular damage or repair occurs makes quantitation difficult.

Strain B-1 differed from the other 7 strains examined. It began resembling the other clones in its properties; during the first 10 transfers the gross death rate was only ~ 0.01. During this time, however, several lines acquired depressed growth rates, and between the 10th and 20th transfers progressively larger fractions slowed down. Unlike similar lines in other series, these slow lines were uniform in growth rate and stable. In addition to these changes, again after about 10 transfers, the rate of production of semi-amicronucleate lines began to rise sharply. These defects appeared only in the normal lines, not in the slow lines, and as usual led to early termination. At the 20th transfer only one normal line remained; the rest were either semi-amicronucleate or "pellet" cultures. In the 21st depression all isolations were made from the normal line in hopes of maintaining the series, but within 3 transfers all lines became semi-amicronucleate and were lost. This clone appeared therefore to differ significantly from the others and behaved like certain clones in *P. aurelia*. The sublines underwent a fixed sequence of changes, either toward slow growth or toward the semi-amicronucleate condition; normal lines could not be maintained.

3. *Behavior of Vegetative Mutants at Conjugation.* Since many of the variations in growth rate or morphology persisted for long periods of growth, they could be considered as "hereditary" variations in a

broad sense. To elucidate the physical bases of the intra-clonal variations, several persistent variants were chosen from the B and C series for cross-breeding analyses. No crosses could be made, of course, with the amicronucleate strains. A brief description of the vegetative mutants used in this study follows.

Strains B-1-22, B-3-15, B-4-6 and C-1-28 were sublines which had grown at slow rates (~ 4 fissions/day) for from 5 to 50 transfers. At starvation the last 3 clones were also characterized by the formation of unusually small cells.

The morphological variants were a more interesting series. Strain B-2-26 produced monsters, ranging from simple tandem formations of two cells which had failed to separate completely at division to large masses of protoplasm containing dozens of macronuclei and in some cases hundreds of micronuclei. The imperfectly organized cortex in these latter formations projected in many spurs, giving an "ameboid" or star-shaped appearance. Occasionally single cells budded off the parental mass, but when isolated these singles again gave rise to cultures containing many monsters. The essential defect in this strain appeared to be a failure of cytoplasmic division to keep pace with protoplasmic increase and nuclear division, but a failure of micronuclear and macronuclear synchrony is also indicated by the disproportionate numbers of the two kinds of nuclei in the monsters. As the nutrient was restricted in the growth of the cultures, cytoplasmic division appeared to continue after the cessation of growth; the average size of the monsters decreased and the frequency of single cells increased. A fully starved culture was hardly distinguishable from a normal culture. When crosses are made, pairing usually involves only the singles or the simple monsters containing two or three macronuclei.

Strain B-3-25 also produced monsters, but unlike the highly multiple structures in B-2-26, these were simple giants. Each culture contained a small fraction of large cells containing only one macronucleus, clearly visible even in living cells.

Fruitless attempts were also made to cross a third morphological mutant of a still different type. Strain C-2-18 grew at maximal rate and appeared normal during periods of rapid growth, but when the nutrient was exhausted the cells rounded up, became vesiculated and lysed. Since starvation was required for conjugation, no breeding analysis was possible.

In the summary of crosses (Table IV), the data for each strain are presented without regard for the mate. Since some of the crosses were made between mutants, some of the data appear in the table twice. A large fraction of the pairs died without producing viable progeny, but the probabilities of death at conjugation vary among the mutants. Among the pairs which survived conjugation most were classified as "non-conjugant" since they failed to become sexually immature after separating. Non-conjugation does not necessarily imply that no nuclear reorganization occurs, but only that the old macronucleus is retained (10). In clones derived from non-conjugant pairs, the mutant trait persisted in nearly all cases. A cross of mutant cell with a normal cell yielded one mutant clone and one normal clone from the separated mates; a cross of two different mutants yielded two distinctive mutant clones. Rarely, a mutant trait expressed by a parent was not observed in the progeny, but these cases may reflect only the variability also encountered in vegetative reproduction. In contrast, the progeny of all pairs classified as true conjugants were normal for the traits followed.

TABLE IV. The behavior of vegetative mutants at conjugation.

Strain	Pairs studied	Pairs died	Non-conjugant pairs: Trait persists	Non-conjugant pairs: Trait lost	True conjugant pairs: Trait persists	True conjugant pairs: Trait lost
B-1-22	87	38	47	1	0	1
B-2-26	175	66	104	1	0	4
B-3-15	59	25	33	0	0	1
B-3-25	118	54	63	1	0	0
B-4- 6	113	63	39	0	0	11
C-1-28	24	3	14	0	0	7

4. Breeding Studies of Normal Lineages. Initially crosses of normal clones throughout the "aging" interval were planned. This procedure was followed with cultures prior to the initiation of isolation series and after 10, 20, and 30 transfers. Since the frequency of death and non-conjugation was very low, and seemed not to be increasing, the procedure was stopped. A few normal clones were retained after 100 transfers, however, and these were subsequently crossed among themselves. Surprisingly, all the progeny of these crosses were either inviable or non-conjugant. Clearly, some alteration had occurred between the time the cultures were last crossed at 30 transfers and the time of the final crosses, and these alterations were not reflected in defects during vegetative reproduction. Unfortunately, the number of clones retained after 100 transfers was too small to estimate the frequency of such cryptic defects and no data are available indicating the time at which the alterations occurred. Since the mutant clones crossed were derived late in the experiment period, their behavior at conjugation may not be directly related to their obvious defects.

DISCUSSION

1. Production of Defective Cells in Clones of Tetrahymena. All 8 clones of *Tetrahymena* in this study

gave rise to defective lines. The mode of production differed in one clone and its characteristics will be discussed separately. In the other 7 clones, defective lines were infrequent and the rate of appearance remained constant over the period of observation. Production of defective lines seems to be a random process having different probabilities in different clones. Different kinds of "accidents" occur within a single clone, but different clones show distinctive rates of production and distributions of kinds of anomalies. Many alterations undoubtedly have intrinsic origins in imperfect cellular machinery, but others may result from accidents imposed from the outside, i.e., laboratory accidents such as cellular damage by micropipettes or improperly cleaned glassware. A distinction between the two kinds of causes is not easy and may not be meaningful since intrinsic factors probably govern the responses of the cells to environmental circumstances, at least within certain limits.

Although some alterations are sudden (like those associated with semi-amicronucleates), most are gradual. A morphological variant may appear initially in very low frequency ($< 1\%$) in a culture; isolations from this source yield many sub-lines similar to the original and a few which depart from the norm in either direction. Selection of the extremes may lead to the production of sub-clones which are almost entirely normal and of sub-clones in which nearly all cells are visibly abnormal. Similar observations are available for some of the growth-rate variants. Because the transitions are gradual, they are difficult to quantify, but they resemble the changes occurring in lines unstable for mating type(1,9) which have been quantified. In this latter case an analysis of the variation led to an explanation of stabilization based on the assortment of differentiated sub-nuclei in the macronucleus.

Although the vegetative mutants have not been explored to the same extent as the clones of unstable mating type, their qualitative features may be explained in the same way. Moreover, the disappearance of the traits at conjugation (when the macronucleus also disappears) is most readily explained by a macronuclear basis for the mutant traits. Quite possibly some of the vegetative mutants, unlike the mating type variants, are due to classically conceived genic or chromosomal mutations, and their apparent instability is due to segregation in the compound macronucleus (see 11).

This interpretation cannot be easily applied to the one aberrant clone. Here the alterations occurred not at a constant rate, but at an increasingly rapid rate to one of two defective cell types. The transitions from the normal to the abnormal states were, moreover, very rapid and the condition once established (in the slow growth variant) was remarkably stable. No explanation based on random cellular accidents and nuclear segregation seems reasonable. These are determined unidirectional alterations from normal to abnormal and appear to involve a single unitary determination. Yet, the information available from breeding analyses (clone B-1 in Table IV) suggests a macronuclear basis for these alterations also. This behavior is reminiscent of the pattern of senescence Sonneborn(12) observed in *P. aurelia* and that of the genetically determined semi-amicronucleates in *Tetrahymena*(8). The factors controlling such alterations are not understood.

2. Cellular Senility, Clonal Senility and Experimental Design. Difficulties arise in relating senility in higher forms to "senility" in unicellular forms. A protozoan may be considered a complete organism analogous to an entire metazoan; but in another sense the individual protozoan is comparable to one of the cells in a higher organism and the protozoan clone is the closest analogy to a multicellular organism. Neither analogy is exact; the latter is defective in that it ignores the cellular differences and cellular interactions in higher forms. Nevertheless, for consistency, the following discussion will be based on the analogy between the protozoan clone and the multicellular organism.

Senility in a higher form is an "organismic" concept, definable as a state of lowered vitality occuring regularly in the life span prior to death. Senility may be reflected in alterations in many tissues and functions. The relationship between changes in the organism as a whole and changes at the cellular level is not clear, but the common assumption that aging is primarily a supracellular phenomenon is by no means established. Senility in a protozoan clone, to be comparable, must however be associated with reduced vitality of the clone as a whole and must lead to the extinction of the clone. The death of some cells, like the death of some cells in a metazoan, is not equivalent to senescence of the organism.

An evaluation of the changes in a protozoan clone requires careful attention to the conditions of growth and to the experimental design employed. Consider a study in which a clone is divided into many subclones, each maintained through serial transfer of single cells. Although cellular "error control" is highly efficient, it is not perfect and defective cells will be produced occasionally through cellular accidents. Hence, sub-lines will be terminated. If the experiment is continued more and more sub-lines will be lost and the average growth rate (including the dead and defective lines) will fall. Eventually all sub-lines will become extinct. The result will occur regardless of the rate of cellular accidents so long as the accident

rate is finite and the experiment is continued long enough.

This experimental design may be modified in several ways which alter the effects of the accidents—not on the cells themselves, but on the characteristics of the clone. If instead of allowing lines to terminate, extinct lines are replaced by isolations from normal sister lines, the properties of the clone will not change in the same way. If accidents occur at a low rate, the fraction of defective lines at any time will be small and replacement will not be difficult; the clone may persist indefinitely. If the rate is high, but constant, large numbers of sub-clones may be required for indefinite persistence.

Also of importance is the nature of the accidents. If accidents merely reduce the vitality of the cell, but do not prevent its indefinite multiplication, many lines may acquire abnormalities and the replacement of dead lines by normal cells becomes difficult. Hence, an experimental design which involves replacement of lines which become defective may yield different results from one which involves replacement only of terminated lines. Finally, an experimental design which maintains a clone in a single large mass culture (as *Tetrahymena* is usually maintained) approximates the design employed when sub-clones are maintained and defective lines replaced. Natural selection in the mass culture will eliminate both the dead and the defective cells.

These considerations suggest that clonal decline and extinction are certain, unless morbid cells are replaced by some method. Since clonal extinction is also certain, regardless of experimental design, if the rate of production of defective cells increases with the age of the culture, the fact of clonal extinction must be interpreted with caution.

Studies on the protozoa suggest two distinct mechanisms of production of defective cells. Most of the observations on *Tetrahymena* are consistent with the idea of random cellular accidents and with the observation of vitality in mass cultures over periods of many years. Other observations, however, suggest an additional mechanism operating in some clones. The increased abnormalities observed at conjugation in "old" lines of *Tetrahymena* may be explained by random cellular accidents involving the micronuclei (which are protected from natural selection during vegetative life), but the determined decline leading to death in clone B-1 and in the genetic semi-amicronucleates is more difficult to explain with an accident hypothesis. Similarly, Sonneborn's observations on *P. aurelia* establish a progressive and predetermined decline in all sub-lines which is incompatible with random alterations.

A final question concerns the relationship, if any, between these two kinds of cellular alteration and the changes ascribed to senility in higher forms. If a multicellular organism is to be analogized with a protozoan clone, certain restrictions must be placed on the analogy. Clearly the metazoan is not comparable to a single mass culture of a protozoan in which the cells are similar and can replace each other without complication. The metazoan is more nearly comparable to a protozoan clone broken up into many subclones which are restricted in their abilities to replace each other when defects appear. The situation is even more complex since certain lineages constantly undergo division (epidermis, hematopoietic tissue, etc.) and others divide practically not at all after maturity (nervous and muscular tissues). The effect of cellular accidents would depend in part on the nature of the tissue and in part on the nature of the alterations. Accidents occurring at a constant rate—dependent on the genetic constitution, the tissue function and the cellular environment—could reduce the efficiency of a tissue. The reduced efficiency might be detected only after it fell below a certain threshold (giving the appearance of an accelerated rate of decline) or it might be detected in some tissue distantly removed from the primary defect (obscuring the source of the damage), but no major conceptual difficulty arises in applying the mechanism of random cellular accidents to higher forms.

The determined cellular decline of the sort found in *Paramecium* cannot be applied to higher forms without important modifications. It involves a limit on the number of cell divisions possible and an accelerated rate of production of defective cells. Yet tissue culture studies on many metazoans (like mass culture studies of *Tetrahymena*) show that individual tissues can be perpetuated indefinitely. At least the tissues capable of *in vitro* culture do not manifest this determined decline if sufficiently large numbers are maintained to permit overgrowth to replace defective cells.

Finally, neither of these analogies is necessarily pertinent. Senility in higher forms may not be the result of cellular aberrations primarily, but of defects at higher levels of organization. If, however, cellular accidents and intra-clonal population dynamics are important in senescence (as they certainly are in carcinogenesis), the difficulties in describing the "primary" metabolic deficiency in the aging process are more readily understood.

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J. PROTOZOOL. **6**(2), 177-182 (1959).

Studies on Trichomonads. III. Inhibitors, Acid Production, and Substrate Utilization by 4 Strains of *Tritrichomonas foetus*

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SYNOPSIS. Inhibitors, acid production, and substrate utilization by 4 strains of *Tritrichmonas foetus* (BP-3, BP-4, A-1, and A-2) were studied manometrically. All used glucose, galactose, mannose, fructose, sucrose, maltose, trehalose, glycogen, starch, lactate, and pyruvate. Strain A-1, with the highest aerobic and anaerobic endogenous rates, used these substrates less than did the others. Strain BP-3 did not use lactose; strains BP-4 and A-2 did not use raffinose aerobically and only slightly anaerobically; strain A-1 used both nearly as well as maltose and sucrose. All were strongly inhibited by iodoacetate and, if tested in the presence of glucose, aerobically or anaerobically, by fluoride, arsenite, hydroxylamine, and 8-hydroxyquinoline. Aerobically, 2,4-dinitrophenol produced stimulation which was greater in the presence of glucose; anaerobically, it produced inhibition which was, in some cases, comparable to the effects produced by the other inhibitors. Fluoride, arsenite, azide, and hydroxylamine, although producing insignificant inhibitory effects on endogenous O_2 consumption, reduced and, in some cases, abolished motility of all strains. All 4 strains produced acid under anaerobic and aerobic conditions; strain A-1 produced more than the others. Lactic acid accounted for 30-51% of the acid produced in all strains.

Strain A-1 more closely resembled the nasal trichomonad of swine (strain PN-610) than did strain BP-1. (Doran(3)). The writer believes that the swine nasal strain is a highly adapted strain of *T. foetus*.

RYLEY(8), Suzuoki & Suzuoki(9), and Doran(3) studied similar aspects of the carbohydrate metabolism of *Tritrichomonas foetus* by manometry and other biochemical techniques. These workers differ as to the utilization of various carbohydrates, effect of enzyme inhibitors, utilization of lactate and pyruvate, and production of lactic acid as one of the main end products of metabolism (Table I).

Do the differences in Table I reflect strain differences or are they merely due to variations in experimental procedure or treatment of organisms prior to experimentation? (*a*) The organisms were maintained axenically in different types of media. Read & Rothman(7) found that the metabolism of *T. vaginalis*, strain 2, was quite different when the organisms were grown on two different kinds of media. (*b*) Composition and total molarity of the suspending media during metabolic studies were different. Ryley(8) and Doran(3) used suspending solutions having molarities of 0.130 M and 0.106 M, respectively; Suzuoki & Suzuoki(9) used a 0.230 M solution of which 0.138 M was NaCl. Doran(3) compared the metabolic rates of strain BP-1 in saline solutions of different strengths and found that organisms suspended in solutions containing 0.134 M NaCl had metabolic rates ½ those of organisms suspended in solutions with optimal NaCl. (*c*) Different pH's were used. Ryley(8) and Doran(3) used 6.2 and 6.4, respectively. The Japanese workers(9) found the optimum pH range to be 7.0-7.6 and, although they did not state the pH of their experiments, one assumes that they used a pH in the optimal range. Doran(3) reported that O_2 consumption varied with pH, and Ryley(8) found that the effects of azide and 2,4-dinitrophenol on O_2 uptake were different at different pH's. There are additional differences in procedure. The molarities of the substances tested were often different. Suzuoki & Suzuoki(9) found 70% inhibition with 0.1 M 2,4-dinitrophenol—quite a high concentration; the increase in total molarity from 0.203 M to 0.303 M might easily account for most of the inhibition. Suzuoki & Suzuoki(9) and Doran(3) found significant inhibition with fluoride; Doran also observed inhibition with arsenite and 8-hydroxyquinoline. These workers tested