GROWTH STUDIES ON CILIATES

VII. Comparative Growth Characteristics of Four Species of Sterile Ciliates

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During the past year experiments have been conducted on four species of holotrichous ciliates in pure culture in order to establish their nutritional requirements and some of their characteristics of growth. It is now possible to report the results of these experiments and to attempt an analysis of some of the factors of growth, both favorable and unfavorable.

In the ever widening field of protozoan physiology the quest is going on for more species which can be used for precise experiments. These species should be able to grow and reproduce in the absence of other microorganisms if complete control is to be obtained. Up to the present time it seems likely that the only genus of ciliate which has remained in successful pure culture is *Tetrahymena* (Furgason, 1940). Various names have been applied to pure-culture ciliates by different authors but, as Furgason has succeeded in showing, they were probably dealing with strains of *Tetrahymena geleii.* In a previous paper of this series (Kidder, Lilly and Claff, 1940) a description was given of a saprozoic ciliate which was referred to the genus *Glaucoma.* This organism (*G. vorax*) was described before access was had to Furgason's excellent work. I am now of the opinion that our ciliate belongs to the genus *Tetrahymena* and therefore it will be referred to in the future as *Tetrahymena vorax.*

Paramecium bursaria was cultured bacteria-free by Loefer (1936) but these cultures were subsequently lost. Because of the inclusions of *Chlorella* in this species the status of "pure culture" is questionable.

The four species to be dealt with in the following report are *Tetrahymena geleii* (strain W), *T. vorax, Glaucoma scintillans* and *Colpidium campylum.* All of these organisms were sterilized and established in pure culture in this laboratory and remain available to other investigators who may be interested in them for experimental purposes.

Material and Methods

Isolation and Sterilization

Tetrahymena geleii (strain W) was isolated from Mill Pond in Woods Hole, Massachusetts in July, 1939. It was sterilized in the migration-dilution apparatus described by Claff (1940) and established in pure culture.

Tetrahymena vorax is the strain previously described from this laboratory (Kidder, Lilly and Claff, 1940).

Glaucoma scintillans (strain A) was isolated from Mill Pond in July, 1939. It was sterilized in the migration-dilution apparatus of Claff and established in pure culture. Strain B was isolated from a freshwater stream near Providence, Rhode Island in May, 1940. It

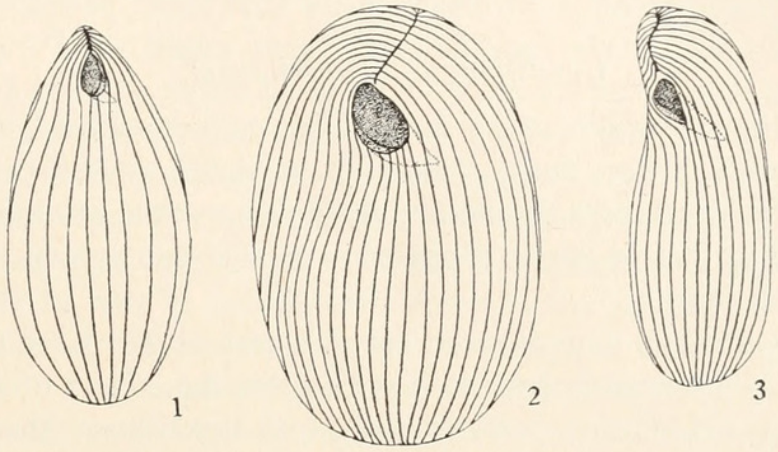

Fig. 1. *Tetrahymena geleii* (strain W). × 800. Total number of ciliary meridians = 17 — 19.

Fig. 2. *Glaucoma scintillans*. × 800. Total number of ciliary meridians = 35 — 40.

Fig. 3. *Colpidium campylum*. × 800. Total number of ciliary meridians = 27 — 30.

was sterilized and established in the same manner as strain A. Strain B will be discussed only in reference to adaptation to sterile conditions as it was not used in any of the other comparative studies.

Colpidium campylum was isolated from a freshwater stream near Providence, Rhode Island in September, 1939. It was sterilized by migration across a fluid-filled Petri dish (details of this method given elsewhere, Kidder, 1940) and established in pure culture.

Description of Species

Because of the confusion which has resulted from lack of adequate description of experimental material, three figures are presented which

show the diagnostic characteristics of those strains which were used and which have not been figured previously. These figures were prepared from opal blue treated material and indicate the distribution of the ciliary lines and the position of the mouth. Figure 1 is of *Tetrahymena geleii* (strain W) and corresponds almost exactly to the figures given by Furgason (1940) for this species. Figure 2 represents *Glaucoma scintillans* and Fig. 3 *Colpidium campylum*. No figure is given of *Tetrahymena vorax* as descriptions have been previously presented (Kidder, Lilly and Claff, 1940).

Studies of *Glaucoma scintillans* and *Colpidium campylum* have been made using the silver technique of Klein (1926) and the relief method of Bresslau (1922). Comparisons with slides prepared from different strains which were used in a previous study (Kidder and Diller, 1934) show that the present organisms are the same species as those reported at that time.

Conditions of Experiments

All qualitative studies were made from cultures grown in the specially designed Pyrex flasks described in detail elsewhere (Kidder, 1941). Organisms were counted by the direct method after appropriate dilutions. Qualitative observations were carried out on material grown in Pyrex test tubes.

Incubation of all experimental cultures was at 27° C. ± 0.2°.

Except in the experiments designed to test the effects of the age of the inoculum, all cultures were started from logarithmic phase ciliates. For uniformity the following ages of inocula were always used: *Tetrahymena geleii* (strain W)—18 hours; *T. vorax*—24 hours; *Glaucoma scintillans* and *Colpidium campylum*—48 hours.

Sterility tests on solid and in liquid media, incubated at room temperature and at 37° C., were carried out according to the methods outlined in previous studies of this series (Kidder and Stuart, 1939; Kidder, Lilly and Claff, 1940; Dewey and Kidder, 1940; Kidder, 1941) and, unless otherwise stated, all cultures were bacteria-free.

Method of Evaluation of Data

Attention should be called to an important point regarding the presentation and evaluation of data. The method often employed (Hall and Elliott, 1935; Hall, 1939; Hall and Schoenborn, 1939; etc.) of comparing the final concentration of cells (X) to the initial concentration (Xo) and expressing the result as the ratio X/Xo may lead to erroneous conclusions. The time selected for the final concentration count is arbitrary and may represent a point on the growth curve beyond

the cessation of logarithmic growth. No information is obtained regarding the activity of the cultures during the earlier phases of growth. The same criticisms apply to the method developed by Elliott (1939) where total protoplasmic volumes are compared, unless estimations are made in the early stages of the growth of the cultures. Therefore it seems not only desirable but necessary to follow the growth of cultures by taking numerous samples at regular intervals. The culture flasks used in these experiments were designed for such a procedure (Kidder, 1941).

Experimental Results

Physical Condition of Medium

Tetrahymena geleii (strain W) and *T. vorax* are both able to utilize dissolved proteins. This fact was immediately apparent upon the initial sterilization. They began rapid reproduction when placed in any of the standard peptone media or in Difco yeast extract. The addition of particles to such media did not increase the growth rate or the yield. These ciliates correspond to the other strains of *Tetrahymena,* therefore, in their ability to grow and reproduce in dissolved materials. Evidence is still lacking regarding Lwoff's (1932) contention that saprozoic ciliates are able to take in polypeptides through the pellicle. We still do not know whether extracellular enzymes are released which might hydrolyse proteins. If it can be shown that no such proteolytic enzymes are released into the medium, then it seems fairly certain that nutritive materials, even in the dissolved state, enter food vacuoles by way of the cytostome. This conclusion would be justified when it is noted that at least five strains of *Tetrahymena* (tested by V. C. Dewey in this laboratory) have been found to exhibit perfectly normal growth characteristics in dissolved casein. It seems highly improbable that whole protein molecules could be absorbed through the pellicle.

Both *Glaucoma scintillans* and *Colpidium campylum* are dependent upon particles of nutritive materials. This fact was noted by E. and M. Chatton (1923) for *G. scintillans* when they were able to obtain growth on dead *B. coli* but not on dissolved proteins. Hetherington (1933) reports the establishment of *G. scintillans* in yeast autolysate but the ciliates failed to reproduce beyond a few divisions and the cultures were presumably discarded.

When *Glaucoma scintillans* (strain A) was first sterilized single ciliates were placed in 2 per cent proteose peptone broth. After many days only a few divisions had occurred and it was apparent that the medium was inadequate. Difco yeast extract (1 per cent) and liquid yeast autolysate (10 per cent) were no better. Those ciliates placed in par-

ticulate Yeast-Harris (Kidder, Lilly and Claff, 1940; Kidder, 1940), however, reproduced quite rapidly while those placed in a mixture of Yeast-Harris and proteose peptone yielded thriving cultures (Fig. 4). Yeast-Harris or the mixture with proteose peptone which had been rendered particle-free by filtration gave no growth. It was later found that strain B and *Colpidium campylum* likewise require particles in the me-

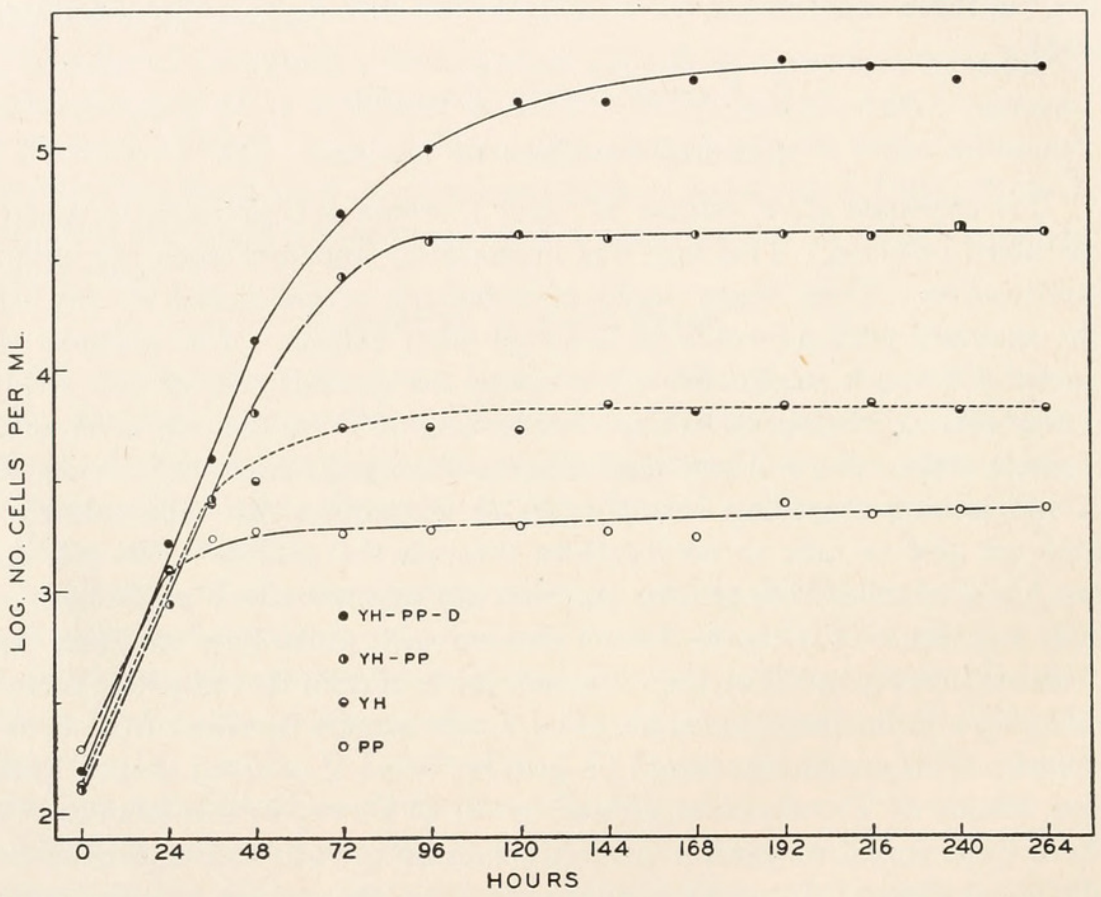

FIG. 4. *Glaucoma scintillans.* Growth curves constructed from the average data of 5 separate experiments. YH = 1 per cent Yeast-Harris; PP = 2 per cent proteose peptone; YH — PP = 1 per cent Yeast-Harris plus 2 per cent proteose peptone; YH — PP — D = 1 per cent Yeast-Harris plus 2 per cent proteose peptone plus 0.5 per cent dextrose.

dium and it was in this way that pure cultures of these strains were established.

Glaucoma and *Colpidium* appear to possess feeding mechanisms which are stimulated to ingestion only by solid particles. The slight amount of growth obtained in proteose peptone broth can be accounted for by the few particles which are invariably present after sterilization. When these particles are used up reproduction ceases.

After considerable experimentation the following basic medium was

adopted as the best for general use in dealing with *Glaucoma* and *Colpidium*—

Brewers Yeast-Harris	10 grams
Pyrex distilled water	1 liter

This is brought to a boil and filtered, first through cotton and then through Schleicher and Schüll No. 595 filter paper. This does not remove the finer particles of the broken yeast cells and the resulting solution is slightly turbid. To this liter of 1 per cent Yeast-Harris is added 20 grams of Difco proteose peptone and the whole sterilized in the autoclave at 15 pounds pressure for 20 minutes. This forms the base for the other experimental materials or may be used without additions. The concentrations given appear to be near optimum for these two species of ciliates as both higher and lower concentrations were inferior for growth.

Chemical Condition of Medium

No experiments on the inorganic requirements are to be described here as all of the media used contain sufficient inorganic constituents for all species (Lwoff, 1932).

In all media used the peptones, proteoses and proteins offered an adequate source of nitrogen. For the two species of *Tetrahymena* it was possible to test the relative effectiveness of various types of protein products on growth rate and maximum concentration. Various yeast products were tested as a substitute for and in combination with the standard proteose peptone. One per cent Difco yeast extract, one per cent filtered Yeast-Harris and 10 per cent liquid yeast autolysate were used. The rate of reproduction (as calculated by the formula

$$g = \frac{t \log 2}{\log b \log a},$$

where $g =$ the generation time and $t =$ the time in hours during which the population has been increasing, $a =$ the number of cells per unit volume at the beginning and $b =$ the number of cells at the end of time, t) in the case of *T. geleii,* was slightly lower in all three types of yeast media than in proteose peptone (Table I). The generation time for *T. vorax* was more than doubled (as compared with 2 per cent proteose peptone) in both yeast extract and yeast autolysate and was somewhat greater in Yeast-Harris (Table I). Some product of yeast autolysis (present in both the extract and the autolysate) seems to inhibit the reproduction of this species.

In the above experiments the various yeast factors were presented along with the yeast proteins. There remained the possibility that some

TABLE I

Tetrahymena. Comparison of growth in protein media. Average of four experiments.

Medium	Generation time in hours	
	T. geleii (strain W)	*T. vorax*
1 per cent yeast extract	3.51	7.07
10 per cent liquid yeast autolysate	3.34	7.90
1 per cent Yeast-Harris (particulate)	3.65	4.61
2 per cent proteose peptone	2.78	3.54

of these factors might stimulate growth if more adequate protein products were present. Consequently a "yeast vitamin concentrate—Harris" which is practically free of native protein was added to a basic medium of proteose peptone. Various concentrations were tested on *Tetrahymena geleii* (strain W) and the results are given in Table II.

TABLE II

Tetrahymena geleii (strain W). Comparison of growth after the addition of various concentrations of Yeast Vitamin Concentrate (Harris) to a basic medium of 2 per cent proteose peptone plus 0.5 per cent dextrose. Average of two experiments.

Percentage Yeast Vitamin Conc.	Generation time	Population per ml. at end of log. phase	Maximum yield
	hours		*cells/ml.*
0	3.37	48000	310000
0.025	3.14	52000	330000
0.05	2.95	67000	380000
0.1	2.70	70000	400000
0.2	2.74	54000	365000

The reproductive rate increased with the concentration up to 0.1 per cent but was slightly lowered at 0.2 per cent. The addition of yeast concentrate consistently lowered the reproductive rate of *T. vorax*.

The addition of yeast vitamin concentrate to the particulate medium used for *Glaucoma* and *Colpidium* had no significant effect up to a concentration of 0.2 per cent although higher concentrations caused inhibition of growth. These observations are of little significance, however, as the basic medium must contain rather high concentrations of the yeast factors.

The addition of a separate source of carbon to the basic proteose peptone medium for *Tetrahymena* and the particulate medium for *Glaucoma* and *Colpidium* increased the length of the logarithmic phase and the maximum yield of all species except *T. vorax*. As was mentioned in the initial report on *T. vorax* (Kidder, Lilly and Claff, 1940) dextrose

TABLE III

Tetrahymena vorax. Comparison of growth after the addition of various concentrations of dextrose to a basic medium of 2 per cent proteose peptone. Average of four experiments.

Percentage dextrose	Generation time	Population per ml. at end of log. phase	Maximum yield
	hours		*cells/ml.*
0	3.55	10000	70000
0.5	4.60	7000	42000
1.0	5.01	5500	32000
2.0	9.56	890	6000

decreases the division rate in direct proportion to its concentration (Table III) and the maximum yield is lowered in the same manner.

Experiments were conducted to test the ability of the four species of ciliates to ferment some of the more common carbohydrates. One polysaccharide (Difco soluble starch), four disaccharides (Difco saccharose, Difco maltose, Difco lactose and Difco cellobiose), three monosaccharides (Difco dextrose, Difco levulose and Difco galactose) and two pentose sugars (Special Chemicals arabinose and Difco xylose) were used. To the two types of basic media 0.5 per cent of the above carbohydrates and 0.02 per cent brom thymol blue were added. These media were dispensed in Pyrex tubes and sterilization was accomplished in the autoclave at 15 pounds pressure for 12 minutes. After cooling each type of media was inoculated with the four species of ciliates and the results were noted by the color change of the indicator after 96 hours in the case of *Tetrahymena* and 240 hours in the case of *Glaucoma* and *Colpidium.*

TABLE IV

Fermentation of carbohydrates. All carbohydrates added to basic protein media in 0.5 per cent concentrations. Medium contained 0.02 per cent brom thymol blue. Six experiments.

Carbohydrate	Organism			
	Tetrahymena geleii (strain W)	*Tetrahymena vorax*	*Glaucoma scintillans*	*Colpidium campylum*
starch	+	+	+	−
sucrose	−	−	−	+
maltose	+	+	+	+
lactose	−	−	−	−
cellobiose	+	+	+	−
dextrose	+	+	+	+
levulose	+	+	+	+
galactose	−	−	−	−
arabinose	−	−	−	−
xylose	−	−	−	−

The results of these fermentation experiments are given in Table IV. *Colpidium* alone failed to utilize starch and cellobiose. On the other hand, *Colpidium* was able to utilize sucrose while the other three species were not. None of the ciliates fermented galactose although Colas-Belcour and Lwoff (1925) report fermentation of this monosaccharide by their strain of *Tetrahymena* (*Glaucoma piriformis*). Galac-

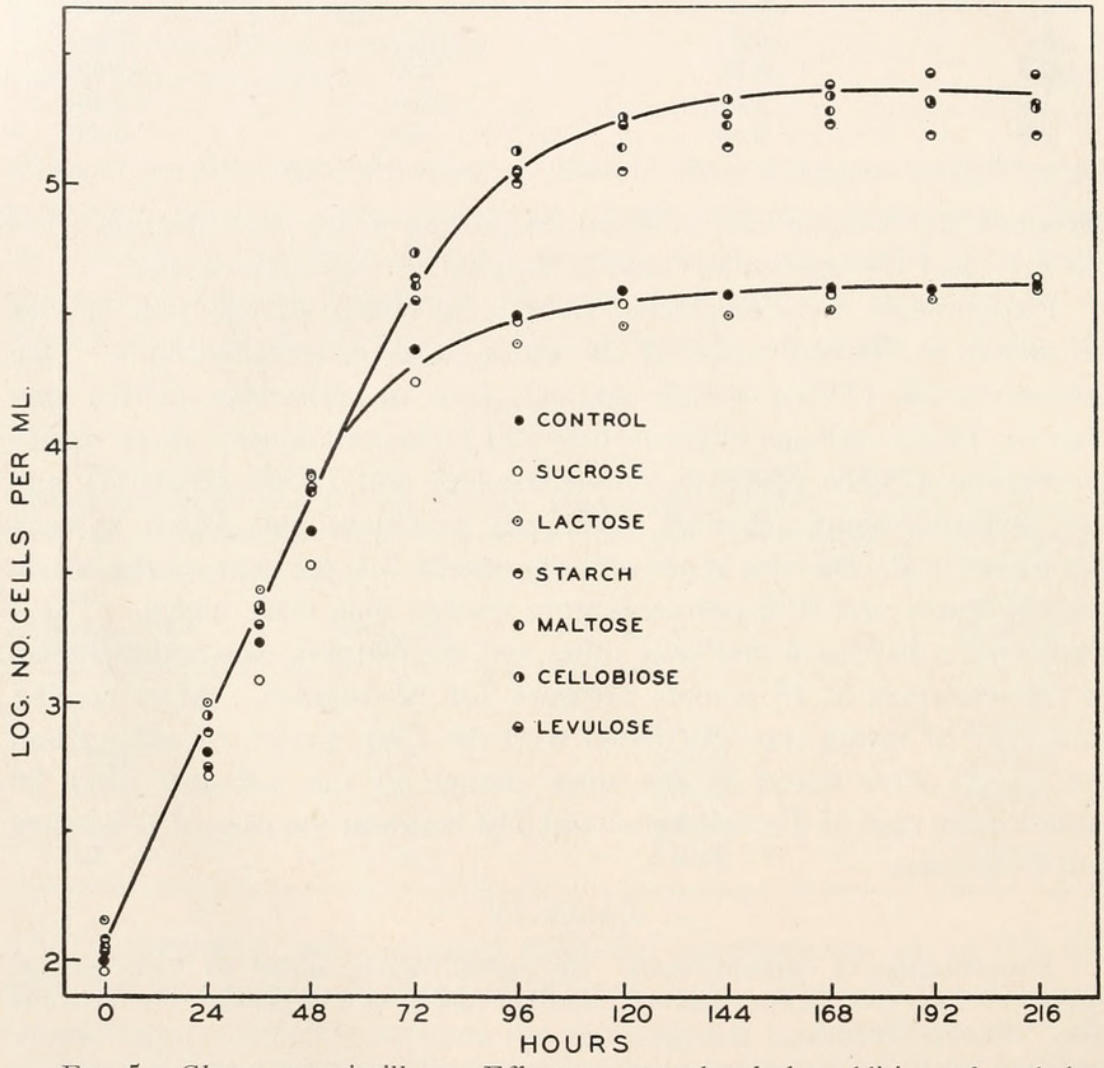

FIG. 5. *Glaucoma scintillans.* Effect on growth of the addition of carbohydrates to basic medium (1 per cent Yeast-Harris plus 2 per cent proteose peptone). All carbohydrates added in 0.5 per cent concentrations. Average of 3 experiments.

tose, arabinose and xylose so inhibited the growth of all the species that these carbohydrates were not used in the quantitative growth studies. All of the other carbohydrates were re-investigated in growth flasks and the cultures followed by frequent counts. The indicator was omitted but otherwise the media were as above.

The division rate, length of logarithmic phase and maximum yield were slightly increased by all of the carbohydrates fermented by *Tetra-*

hymena geleii (strain W). These increases were small but constant. No significant differences could be detected between any of the media containing fermentable carbohydrates. The acidity rose from an initial pH 6.8 to a final pH 4.8 in those flasks containing starch, maltose, cellobiose, dextrose or levulose while it fell in all others, including the control flasks, to pH 7.2.

Tetrahymena vorax, although it was able to ferment starch, maltose, cellobiose, dextrose and levulose (initial pH 6.8–final pH 5.4) was dis-

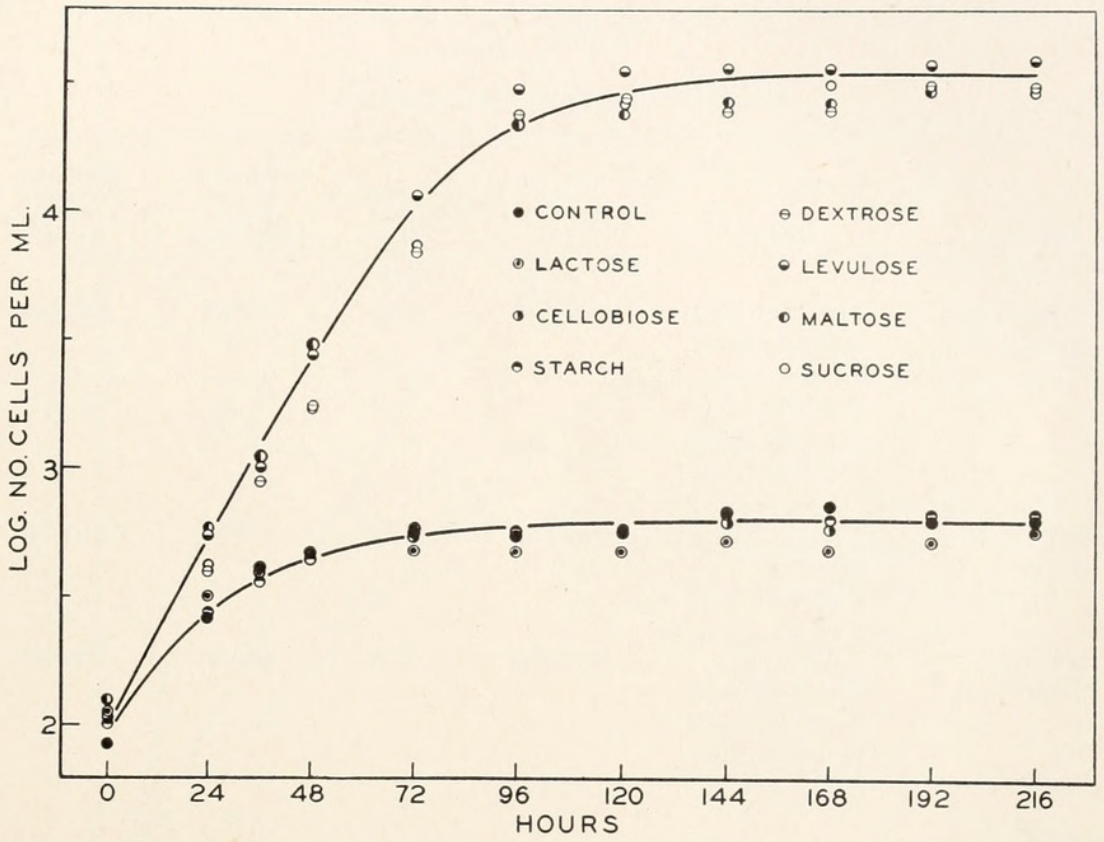

FIG. 6. *Colpidium campylum.* Effect on growth of the addition of carbohydrates to basic medium (1 per cent Yeast-Harris plus 2 per cent proteose peptone). All carbohydrates added in 0.5 per cent concentrations. Average of 5 experiments.

tinctly inhibited in its growth. Reproductive rate, length of logarithmic phase and maximum yield were decreased wherever fermentation occurred (see Table V for dextrose) and were unaffected (as compared with controls) in media containing carbohydrates which were not attacked (sucrose, lactose).

The most striking results of the addition of carbohydrates were found in the cases of *Glaucoma* and *Colpidium.* These results are given in Figs. 5 and 6.

The growth of *Colpidium* without carbohydrate was very slow and the maximum yield was low (not greater than 800 per ml.). The addi-

tion of 0.5 per cent dextrose, levulose, sucrose or maltose increased the rate of growth during the logarithmic phase and the maximum yield was increased to over 40,000 per ml. in some cases. For practical purposes, therefore, an additional source of carbon is a necessity for this ciliate.

The situation is somewhat different with *Glaucoma*. The division rate during the first 48 hours is not appreciably changed when a fermentable carbohydrate is added. Without a separate source of carbon, however, the end of the logarithmic phase is reached rather suddenly and

TABLE V

Summary of growth characteristics.

Organism	Medium	Optimum pH	Generation time	Population at end of log. phase	Maximum concentration per ml.
			hours		
Tetrahymena geleii (W)	2 per cent proteose peptone, 0.5 per cent dextrose, 0.1 per cent Yeast Vitamin Conc.	5.6–8.0	2.69	58000	395000
Tetrahymena vorax	2 per cent proteose peptone	6.2–7.6	3.52	12000	110000
Glaucoma scintillans	1 per cent Yeast-Harris, 2 per cent proteose peptone, 0.5 dextrose	5.6–6.8	7.37	40000	270000
Colpidium campylum	"	5.4	11.56	7200	41000

the curve flattens, the concentration (about 42,000 per ml.) remaining relatively constant thereafter for many days. The addition of sucrose or lactose has no significant effect upon the cultures, but the addition of dextrose, levulose, maltose, cellobiose or starch causes an increase in the length of the logarithmic phase, a long phase of negative growth acceleration and a final yield in excess of 200,000 per ml.

Optimum pH

A number of experiments were conducted to determine the optimum pH limits for the four species of ciliates. For these experiments three

types of media were used: 2 per cent proteose peptone for *Tetrahymena vorax;* 2 per cent proteose peptone plus 0.5 per cent dextrose for *T. geleii* (strain W); 1 per cent Yeast-Harris, 2 per cent proteose peptone, 0.5 per cent dextrose for *Glaucoma* and *Colpidium.* The pH was adjusted through a wide range of values (from pH 4.8 to pH 8.6) with HCl and NaOH.

The results of these experiments are contained in summary form in Table V.

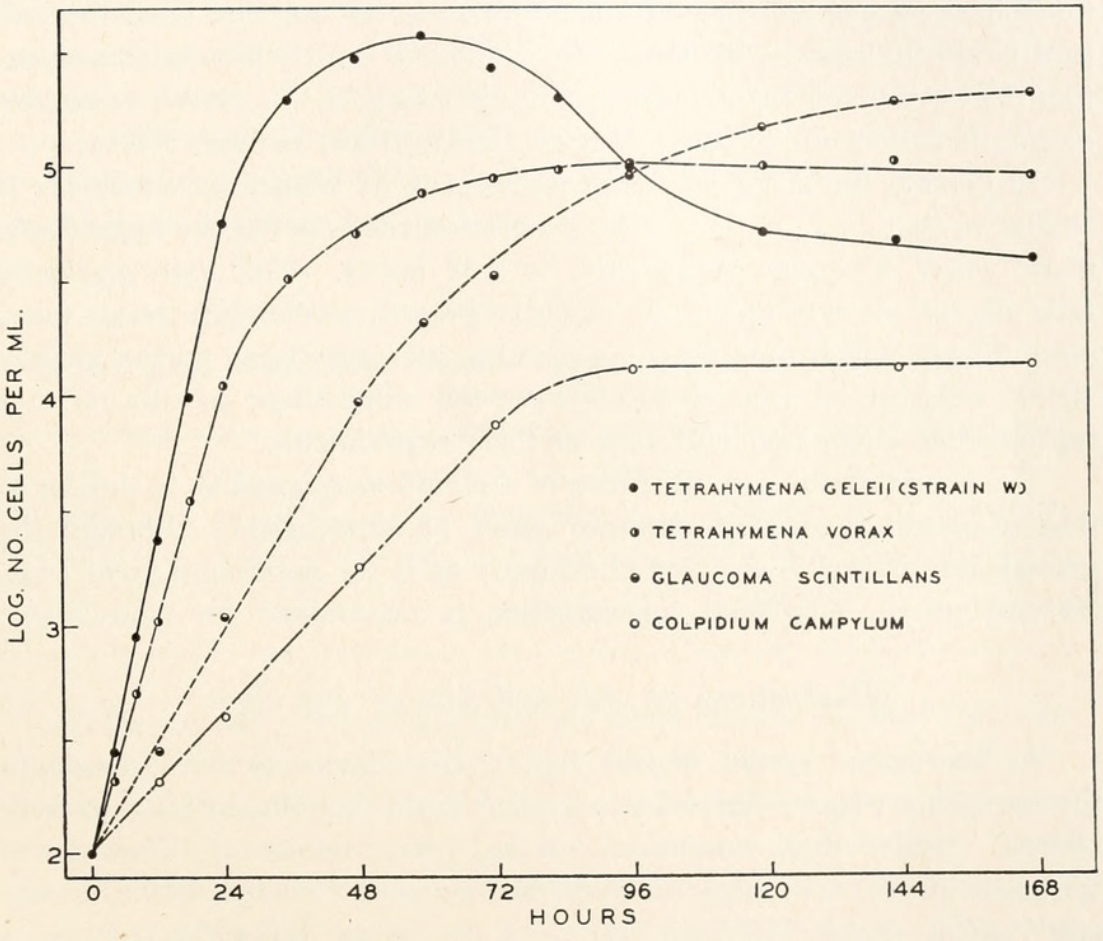

FIG. 7. Graphic comparison of the growth characteristics of four species of ciliates. Media as given in Table IX.

General Comparison of Growth Characteristics

Several interesting facts are brought out when the four species of ciliates are compared during the various phases of their growth. Figure 7 is a graph prepared from various experiments, each species growing under optimum conditions. A summary of data is given in Table V.

The growth rate of *Tetrahymena geleii* (strain W) is higher than any strain of ciliate in pure culture so far reported ($g = 2.69$ hrs.).

Unlike strain P (Phelps, 1935; 1936) and strain H (Kidder, 1941) of this species the negative acceleration period of strain W is quite long and the stationary phase is short. The concentration declines rapidly after the culture is approximately 60 hours old (initial inoculum of 100 cells per ml.) but the death rate decreases later (at about 120 hours) so that a concentration of 30,000–40,000 cells per ml. is maintained for many days.

Tetrahymena vorax grows at a regular rate ($g = 3.52$ hrs.) only during the first 24 hours. Thereafter a long negative growth acceleration phase ensues and the stationary phase is not reached until the culture is approximately 96 hours old. There is no decline in concentration, however, for many days so, in this respect, *T. vorax* resembles strains P and H of *T. geleii* (Phelps, 1935, 1936; Kidder, 1941).

In general the shape of the growth curve of *Glaucoma scintillans* is similar to that of *T. vorax*. The generation time during the logarithmic phase (first 48 hours of growth) is 7.37 hours. This rate gradually falls off and an extremely long negative growth acceleration phase takes place during which time the concentration increases from approximately 40,000 cells per ml. to over 200,000 per ml. The shape of this curve is reproducible under the conditions of these experiments.

The shape of the growth curve of *Colpidium campylum* is similar to that of strain H of *Tetrahymena geleii* (Kidder, 1941) although the growth rate is very low ($g = 11.56$ hrs.) as is the maximum yield (41,000 per ml.). This final concentration is maintained for many days.

Observations on Age and Size of Inoculum

As was stated earlier in this report, the ciliates used as inocula in the foregoing experiments were all taken from their logarithmic growth phases. Under these conditions no lag phase occurred. This corresponds to previous findings on controlled cultures (Phelps, 1935; Dewey and Kidder, 1940; Kidder, 1941). A lag phase invariably occurs if the ciliates which form the inoculum are taken from cultures which have passed the logarithmic growth phase. This statement holds for all four species used in the present study and has been reported for other strains and species (strains P and H of *Tetrahymena geleii,* Phelps, 1935; Kidder, 1941; *Perispira ovum,* Dewey and Kidder, 1940). The length of the lag phase increases, up to a certain point, in direct relation to the age of the parent culture.

The course of the growth is not dependent upon the size of the inoculum of *Tetrahymena geleii, T. vorax* or *Glaucoma scintillans.* Experiments on this point were conducted using large growth flasks (1

liter capacity) containing 500 ml. of media. Single ciliates were inoculated into these flasks. In order to insure the inoculation of active, single organisms they were first isolated into small containers made from the lower portion of shell vials. These containers had been previously placed in Petri dishes and sterilized. After the single ciliates had been isolated and checked under the dissecting binocular for number and activity, the whole container was lifted with sterile forceps and dropped into the culture flask. The initial inoculum is, in this case, 0.002 cells per ml. After growth has proceeded for sufficient time so that samples include enough cells for determination of numbers, the generation time is calculated and compared to the control flask which has received the usual inoculum of 100 cells per ml. No significant difference between the generation times in high and low inoculum cultures of the three species was obtained (Table VI). In no case did these single ciliates fail to establish perfectly normal cultures.

TABLE VI

Effect of size of inoculum. Figures represent generation time in hours. Volume of medium = 500 ml.

No. of cells per ml. inoculated	Organism			
	Tetrahymena geleii (strain W)	*Tetrahymena vorax*	*Glaucoma scintillans*	*Colpidium campylum*
100	2.69	3.52	7.37	11.66
0.002	2.71	3.48	7.41	18.25

Colpidium campylum did not give the same results (Table VI). In a number of the flasks inoculated with single ciliates no growth occurred. In those cultures which became established the generation time was significantly increased (18.25 hours as compared with 11.66 hours in the controls) and, what is more striking, the maximum yield was always very low (8,000 per ml. as compared with 40,000 per ml. in the controls). This species does not follow the same course as the other three and would seem to correspond to the reports of Robertson (1921–1927) on non-sterile organisms and of Mast and Pace (1938) on *Chilomonas*. Considering the general characteristics of *Colpidium,* however, I believe that there may be an explanation of the apparent " allelocatalytic " effect. Some substance or condition of the medium may be slightly detrimental to this ciliate. When large numbers of organisms are introduced no single cell receives a lethal amount of the toxic material. When a single ciliate is introduced into a large amount of medium it accumulates enough of the toxic substance to cause its death in some

cases or to injure it in others. When the injury is sub-lethal it nevertheless permanently affects the cells. The lowering of the maximum yield in single-cell-inoculated cultures which become established seems to favor this hypothesis.

Discussion

No complete analysis of the protein requirements of any ciliate is available at the present time due to a number of factors. Lwoff (1932) and Lwoff and Lwoff (1937) have obtained some data on their strain of *Tetrahymena geleii* (*Glaucoma piriformis*) but until all of the supplementary factors in relation to nutrition are more perfectly known this knowledge must remain incomplete. Neither *Glaucoma scintillans* nor *Colpidium campylum* appears to offer satisfactory experimental material for studies along this line. They both require particles. The particles obtained from powdered yeast cells are of nearly unknown chemical constitution. About all we can say concerning these particles is that they are very complex. To these particles appear to be adsorbed the molecules of proteoses and peptones necessary for the optimum growth. An attempt was made to substitute animal charcoal (Norit) for the powdered yeast. *Colpidium* failed to ingest these particles and while *Glaucoma* did ingest them at first (black food vacuoles), they later refused to do so and very little growth resulted. Various other inert materials which were tried proved no more successful. There may well be other types of particles (such as precipitated proteins, etc.) which could be substituted but no appreciable advantage would be gained. Casein, a well-known protein, was used successfully to supply the particles but the growth was never as good as in the Yeast-Harris medium, even though a filtrate of the Yeast-Harris was added.

It appears strange that *Tetrahymena vorax* is inhibited by some factor in yeast while the reproduction of *T. geleii* (strain W) is accelerated. This situation is also true of the fermentable carbohydrates. No answer to the question of these specific differences is available at present. It will be interesting to compare the supplementary requirements of these two species with the yeast factor question in mind.

Living organisms as food have been found to be a necessity for a number of species of ciliates (Phelps, 1934; Kidder and Stuart, 1939; etc.). This is not the case with *Glaucoma* and *Colpidium,* however. The most apparent difference between growth on a favorable bacterium and in pure culture is rate of reproduction. The living bacteria accelerate growth. This is not true in the case of *Tetrahymena,* where no species of food organism tested was as favorable for growth as the dissolved protein materials.

The observations on the carbohydrates are interesting in showing specific differences in enzyme production. While all four species of ciliates used in this investigation produce an amylase and a maltase, none of them produce lactase. *Colpidium campylum* stands alone in producing invertase and failing to produce cellobiase. All species ferment dextrose and levulose and fail to ferment galactose, arabinose and xylose.

With the exception of galactose and the pentose sugars, the carbohydrates which were not fermented did not influence the growth of any of the ciliates, although Elliottt (1935) reports some cases where acceleration of growth resulted without acid fermentation. These cases, however, must be questioned as he calculated acceleration by yield after a given time (usually 72 hours). The reason for questioning the validity of this method has been given in a previous section of this report.

Galactose, arabinose and xylose were found to be inhibitory to all four species of ciliates. Elliott (1935) reports inhibition of *Tetrahymena geleii* (strains H and E) by galactose, while Colas-Belcour and Lwoff (1925) record the fermentation of galactose by their strain of *T. geleii* but give no data regarding growth.

In the experiments designed to test the effect of the initial pH of the medium upon the growth of the ciliates investigated there was no indication that two optima exist as was reported by Elliott (1933) for his strain of *Tetrahymena geleii.* In fact, there were no significant differences in generation time, length of logarithmic phase or maximum yield over a wide pH range in the case of *T. geleii* (strain W), *T. vorax* or *Glaucoma scintillans. Colpidium campylum* reproduces faster, for a greater length of time and to greater final concentrations when the pH of the medium is low (pH 5.4).

No data are available from these experiments as to the factors which limit the period of maximum reproductive rate or cause the death of the organisms during the later stages of the cultures. It should be pointed out that the growth characteristics given are valid only under the conditions outlined and might well be changed somewhat by varying these conditions. The accumulation of volatile products of metabolism, such as CO_2, or the reduction of O_2 tension could be largely overcome by aeration. Phelps (1936) found that aeration increased the length of the logarithmic phase of *Tetrahymena geleii* (strain P) but did not alter the generation time in the early stages of growth.

A point of some interest which should be brought out is what Elliott (1933) and Johnson (1935) called "acclimatization." These authors report the necessity for gradually reducing the number of bacteria in the process of sterilizing their ciliates (*Tetrahymena*). These observations

were not confirmed on the ciliates used in this study. In every case establishment after complete sterilization followed immediately upon the presentation of an adequate medium. Another type of acclimatization was noted, however, in the case of *Glaucoma scintillans*. The growth rate (strain A) increased steadily during the first three months of sterile culture. The first calculations were based upon cursory data so this point was checked with strain B. One week after its initial isolation (May 27, 1940) growth flasks were inoculated and the generation time during the logarithmic phase was determined and found to be 12.21 hours. Cultures started June 10 grew more rapidly (generation time 10.64 hours) while those started on July 12 and September 20 were increasingly rapid (9.81 hours and 8.98 hours, respectively). Strain B, therefore, repeated what had been noted for strain A and although this strain does not reproduce as rapidly as strain A, even after four months, the same tendency of gradual adaptation to the sterile medium is shown.

Summary

1. The growth characteristics of four species of holotrichous ciliates (*Tetrahymena geleii, T. vorax, Glaucoma scintillans* and *Colpidium campylum*), grown in pure culture, are given.

2. The two species of *Tetrahymena* are able to utilize dissolved nutritive materials while *Glaucoma* and *Colpidium* are dependent upon particulate materials in the media.

3. The growth of *T. geleii* is slightly accelerated by some factor in yeast and by the presence of fermentable carbohydrates (dextrose, levulose, maltose, cellobiose and starch) while inhibition of the growth of *T. vorax* results when these materials are present.

4. The maximum yield of *Glaucoma* and *Colpidium* is greatly increased by fermentable carbohydrates.

5. *Colpidium* fails to ferment cellobiose but, unlike the other three species, does ferment sucrose.

6. Galactose, arabinose and xylose, while not fermented by any of the four species of ciliates, inhibit the growth of all.

7. The optimum range of pH values for *T. geleii* (strain W) is wide (pH 5.6 — pH 8.0); *T. vorax* is slightly more limited (pH 6.2 — pH 7.6); *Glaucoma* is limited to the acid range (pH 5.6 — pH 6.8), while *Colpidium* grows best at pH 5.4.

8. In the cases of *Tetrahymena geleii, T. vorax,* and *Glaucoma scintillans* when single ciliates from the logarithmic growth phase are inoculated into 500 ml. of media (initial inoculum = 0.002 cells per ml.) there is no lag phase and the generation time is not reduced (as compared with controls).

9. Single *Colpidium campylum* inoculated into 500 ml. of media often die. When a culture is established the generation time is longer and the maximum yield is smaller than when many cells are inoculated. It is suggested that these results are correlated with slight toxicity of the medium.

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