(Biological Laboratory, University College, New York University.)

Isolation of *Glaucoma ficaria* KAHL in bacteria-free cultures, and growth in relation to p_H of the medium.

By

David F. Johnson.

(With 7 figures in the text.)

Introduction.

The investigations dealing with the pure culture of ciliates fall into two general groups. In the first, bacteria-free pure cultures have been established, the ciliates being grown in peptone solutions or similar media, dependent upon saprozoic nutrition. In the second group of investigations, the protozoa have first been freed from bacteria and then transferred to media containing particulate food in the form of known species of dead or living yeasts, bacteria or other microorganisms.

The earlier work on bacteria-free cultures of ciliates includes the classical research of LWOFF (1924), who succeeded in isolating *Glaucoma piriformis* in a peptone medium. PETERS (1920) had previously reported the establishment of bacteria-free cultures of "*Paramecium*" (later designated as *Colpidium colpoda*) in a complex salt solution containing glucose, three amino acids and ammonium lactate, but bacteria were later recovered (GATENBY and COWDRY, 1928, pp. 412—413) from these cultures. PETERS (1929) did finally succeed in growing a bacteria-free strain of "*Colpidium colpoda*" (from PETERS' description, apparently *Colpidium campylum*) in his original medium to which killed yeast or 'glaxo' had been added. OEHLER (1924) reported the isolation of bacteria-free strains of *Colpoda steinii* and

Colpoda cucullus in spinach broth, the former species having been isolated in 1919 and the latter in 1922. *Colpoda steinii* was grown also in clear egg albumin and peptone solution, bouillon and other clear media, free from solid particles, as well as in several media containing heat-killed bacteria and yeast, finely chopped meat, powdered egg albumin and cell fragments. MILKOVITCH (1929) succeeded in isolating another strain of *Glaucoma piriformis* in a peptone medium containing blood serum. BUTTERFIELD (1929) established bacteria-free cultures of *Colpidium* (probably *Colpidium campylum* in peptone solution, and HETHERINGTON (1933) obtained bacteria-free cultures of *Colpidium campylum*, *Glaucoma scintillans* and *Loxocephalus granulosus* in yeast-extract and peptone-glucose solutions. ELLIOTT (1933) isolated *Colpidium striatum* and a year later LOEFER (1934) obtained bacteria-free cultures of *Paramecium bursaria*, both investigators using a Difco tryptone medium.

OEHLER was the first worker to obtain pure cultures of ciliates with dead microorganisms as a source of food. In 1919 he reported growth of *Colpoda steinii* on dead bacteria and yeasts. E. and M. CHATTON (1923, 1929) grew *Glaucoma scintillans* and *Glaucoma piriformis* on dead bacteria. In this group also belongs the excellent work of GLASER and CORIA. In 1930 they reported methods for freeing protozoa from associated bacteria, and were successful in cultivating *Trichoda pura* on artificial media. They were unable to propogate *Paramecium caudatum* in the absence of living microorganisms, but did obtain cultures on yeast and one species of bacteria. In later papers (GLASER, 1932; GLASER and CORIA, 1933) the establishment of pure cultures of *Paramecium caudatum* and *Paramecium multimicronucleata* has been reported. These investigators used a medium containing liver extract, sterile kidney tissue and heat-killed yeast; the yeast could be replaced by *Staphylococcus aureus* or *Staphylococcus albus*. The *Paramecium* cultures of GLASER and CORIA are undoubtedly free from living bacteria (BROWN, 1934), but the fact that their medium contains dead bacteria or yeasts makes necessary a technical distinction between their work and that of LWOFF, ELLIOTT, LOEFER and others mentioned above. On the other hand, GLASER and CORIA (1935) established cultures of *Trichoda pura* and *Chilodon cucullulus* in bouillon, a medium similar to that used by other investigators.

At the present time nine species of ciliates have been grown successfully in bacteria-free cultures, and three additional species have been maintained in cultures containing dead microorganisms as a source of food. It is obvious, therefore, that the scepticism of

LWOFF (1929, 1932) was not justified. In 1929 LWOFF stated that *Glaucoma piriformis* is the only ciliate capable of continued existence in synthetic particle-free media, despite OEHLER's (1924) results with *Colpoda steinii*. In 1932, also, LWOFF criticized the results obtained by GLASER and CORIA on the basis that their tests for bacterial sterility were inadequate. In addition to their own convincing tests for sterility, GLASER and CORIA have submitted their cultures to other workers (BROWN, 1934) for rigid tests, so that their results cannot be questioned.

Mixed cultures of single species of ciliates and other living micro-organisms (bacteria, yeast, algae) have been established by numerous investigators. Investigations of this type constitute a distinct phase of the pure culture of ciliates, differing from that discussed above, and will be considered in detail by the writer in a later paper (JOHNSON, MSS). For further details concerning the pure culture of ciliates, the reader is referred to the reviews of LWOFF (1932) and SANDON (1932).

In the present investigation the writer has established bacteria-free cultures of *Glaucoma ficaria* (KAHL), and has attempted to determine satisfactory culture methods as well as the relation of p_H of the medium to growth of the ciliate. Comparisons have been made with *Glaucoma piriformis*, also available in bacteria-free culture, the strain having been obtained from Dr. A. M. ELLIOTT who in turn had received it from Dr. A. HETHERINGTON. The writer wishes to express his appreciation to Professor R. P. HALL for his suggestions and criticisms during the course of the investigation.

Material and methods.

The strain of *Glaucoma ficaria* (KAHL, 1926) used in the present investigation is a pure line derived from a single ciliate isolated from a hay infusion, the material having been obtained originally from a pond in the Bronx Zoological Park. The ciliates were washed free of bacteria in the manner described by PARPART (1928). This method has had wide usage and is a relatively simple procedure, but it is rather unsatisfactory due to the fact that so few ciliates can be washed at one time.

The difficulties encountered by the writer in obtaining growth of *Glaucoma ficaria* in the absence of bacteria more or less parallel those of ELLIOTT (1933) with *Colpidium striatum*. In the first attempts the ciliates lived for a few days, gradually becoming smaller and

finally dying with indications of starvation. Successful results were finally obtained after growing the ciliates in a tryptone medium with a mixed population of bacteria for a period of three months, during which transfers were made every two or three days. In each transfer a few bacteria were carried over with the inoculum of ciliates, and as a result in the earlier transfers the cultures became slightly cloudy during the first twelve hours of incubation, with clearing occurring after 24 hours. Inoculation of agar plates from such clear cultures showed that the bacteria remained numerous during the first few weeks. Later on, however, similar plate tests showed that the number of bacteria in the transfer cultures was decreasing steadily. At regular intervals during the three-month period, ciliates were washed free of bacteria and placed in sterile tryptone medium. Ultimately, bacteria-free cultures were established in this way.

The bacteria-free strain of *Glaucoma ficaria* has now been maintained in the laboratory for more than 18 months in Difco tryptone medium. Bacteriological tests have been made at intervals during the course of the investigation, and these have always failed to show bacterial contamination of the stock.

ELLIOTT (1933) applied the term "acclimazation period" to such growth of the ciliates for a period of time in a nutritive medium containing bacteria. He pointed out that this method has certain advantages in that the ciliate becomes more and more dependent upon food materials in solution as the subculturing continues and the bacterial population declines. This is undoubtedly true but, in the opinion of the writer, it is not the only contributing factor in the isolation of ciliates. One disadvantage of PARPART'S method of washing ciliates is that relatively few bacteria-free specimens may be obtained at one time. In such a method, the failure to obtain successful bacteria-free cultures may be due largely to the possibility that a few washed ciliates cannot withstand the initial shock of the new environment, since the conditions, even though favorable, may be far from optimal for growth of the species. In the present investigation it happened that the particular medium used was favorable for growth of the ciliates, as determined by later results. It seems probable, however, that, given a fairly satisfactory medium and an initial inoculum of several hundred organisms instead of the few obtained by PARPART'S method, enough ciliates would have survived the initial shock to insure the production of successful cultures earlier in the investigation. This is indicated by GLASER and CORIA'S (1933) success in growing *Paramecium caudatum* bacteria-free. These workers

used an initial inoculum of approximately 870 specimens obtained by the use of OGATA tubes, or 'migration pipettes'. Perhaps the labor entailed in OGATA's procedure is more than offset by the results obtained, as compared with the technique of PARPART. There is obviously a real need for a more efficient method of mass sterilization of ciliates in order to accelerate the development of this interesting field of research.

For experimental purposes the ciliates were grown in Pyrex culture tubes. Experimental cultures were incubated at 28° C. in a water bath, while stock cultures were maintained at room temperature. The p_H of the medium was adjusted by means of a LA MOTTE roulette comparator, readings being subject to an error of 0.1. In determining amounts of growth under given sets of conditions, the organisms were counted by means of the SEDGWICK-RAFTER counting cell and the WHIPPLE micrometer. The technical procedures followed were essentially the same as those described by ELLIOTT (1933), as indicated below.

Development of suitable culture media.

Series I.

Since a Difco tryptone medium was used in the isolation of *Glaucoma ficaria*, it seemed desirable to determine the concentration of this peptone most suitable for growth of the ciliates. Accordingly. tryptone was added to 0.2 % K_2HPO_4 solution in concentrations ranging from 0.1 to 3.0 %. The p_H of each type of medium was adjusted to 5.6, since in a preliminary experiment the optimal p_H was found to lie betwen 5.0 and 6.0. Each medium was tubed in 9.5 cc amounts and then autoclaved and inoculated with *Glaucoma ficaria*. The initial concentration was found to be 200 organisms per cubic centimeter. Cultures were incubated for 72 hours, and then fixed for determination of the final concentration. The results are expressed graphically in figure 1 as x/x^0 (ratio between final and initial concentrations of organisms).

Most abundant growth occurred in the 1.5 % tryptone medium, while reasonably good growth was supported by concentration ranging from 0.5 to 2.0 %. Moderate growth occurred in tryptone concentrations of 0.1—0.5 %, but concentrations as high as 3.0 % seemed to be lethal. Series I was repeated, with similar results; hence, a 1.5 % tryptone medium has been used subsequently in the maintenance of stock cultures of *Glaucoma ficaria*.

Series II.

A similar procedure was followed in determining the optimal concentration of yeast-extract for growth of *Glaucoma ficaria*, since this material has been used extensively (e. g., HETHERINGTON, 1933; ELLIOTT, 1935; LOEFER, 1934, MSS; PHELPS, 1935). In this series the following was used as a basic medium:

Tryptone .	5.0 gm
K_2HPO_4 . . .	2.0 gm
Distilled water	1.0 liter

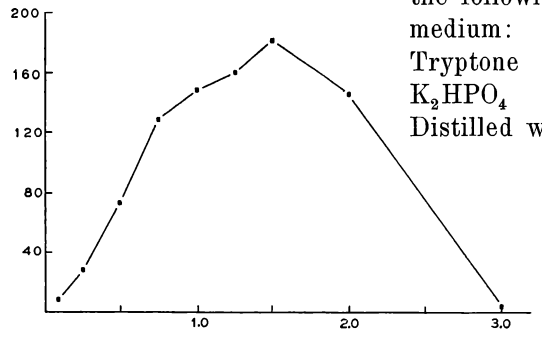

Fig. 1 *Glaucoma ficaria;* x/x_0 (ratio offi nal to initial concentration of organisms) plotted against concentration (%) of tryptone.

This tryptone medium was prepared in bulk and Difco yeast-extract was added to different portions of it in concentrations ranging from 0.03 to 8.0 %. Tryptone medium alone was used as a control. The same procedure was followed as in series I. The initial count was 190 organisms per cubic centimeter, and the initial p_H was 5.8. Final p_H and final concentrations were determined after incubation for 72 hours. In yeast concentrations of 0.03 to 0.5 %, the p_H had risen to 6.2; in the higher concentrations to 6.1. The results are expressed x/x_0 in figure 2. Maximum growth occurred in a concentration of 0.5 % yeast-extract, and acceleration of growth was noted in concentrations of 0.03 to 2.0 %. Concentrations of 3.0 % and higher were decidedly inhibitory.

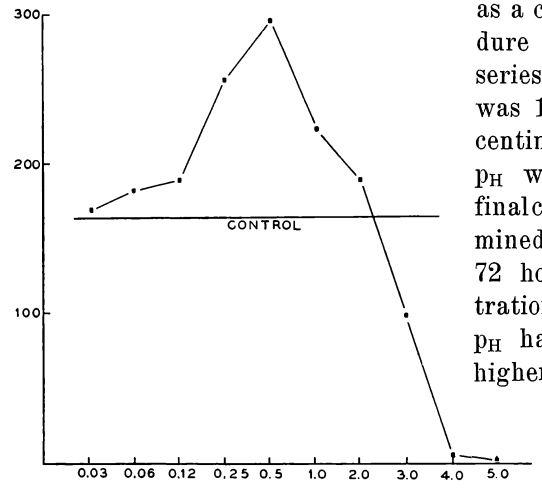

Fig. 2. *Glaucoma ficaria;* x/x_0 (ratio of final to initial concentration of organisms) plotted against concentration (%) of yeast-extract.

Series III.

In order to determine the relative values of other protein derivatives for use in culture media, growth of *Glaucoma ficaria* and *Glaucoma piriformis* was compared in the following Difco products (1.0 % concentrations in 0.2 % K_2HPO_4 solution): tryptone, proteose-peptone, bacto-peptone, bacto-protone, neopeptone, bacto-veal, bacto-liver, gelatin and casein. The initial concentration of *Glaucoma ficaria* was 100 per cc; of *Glaucoma piriformis*, 50 per cc. Cultures were incubated for 72 hours. Initial and final p_H determinations are listed in Table 1.

Growth is recorded as x/x_0 in figure 3. With each species, good results were obtained with tryptone, proteose-peptone and Bacto-liver, while growth was poor in Bacto-peptone, casein and gelatin. A comparison of the two species shows that maximum growth of *Glaucoma ficaria* was supported by Bacto-liver, with tryptone second and pro-

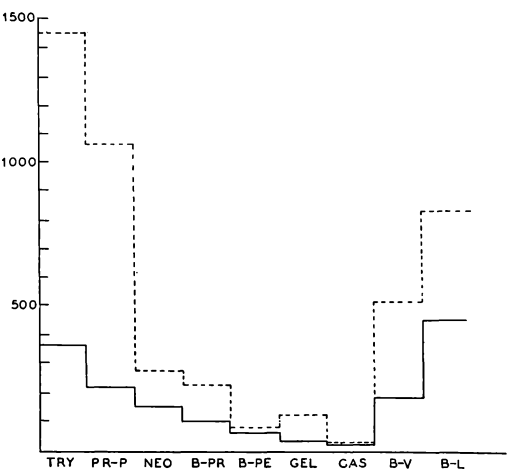

Fig. 3. *Glaucoma ficaria* (continuous line) and *Glaucoma piriformis* (broken line); x/x_0 (ratio of final to initial concentration of organisms) in different types of media; TRY, Tryptone; PR-P, Proteose-peptone; NEO, Neopeptone; B-PR, Bacto-protone; B-PE, Bacto-peptone; GEL, Gelatin; CAS, Casein; B-V, Bacto-veal; B-L, Bacto-liver.

Table 1.
Initial and final p_H of the different media used in series III.

Substance Tested	*Glaucoma ficaria*		*Glaucoma piriformis*	
	Initial p_H	Final p_H	Initial p_H	Final p_H
Tryptone	5.7	6.1	5.7	5.8
Proteose-peptone	5.8	6.1	5.8	5.9
Bacto-peptone	5.9	6.0	5.9	5.9
Bacto-protone	5.7	5.8	5.7	5.8
Neopeptone	5.7	5.8	5.6	5.7
Bacto-liver	5.6	5.8	5.5	5.7
Bacto-veal	5.7	5.9	5.7	5.8
Gelatin	5.5	5.7	5.5	5.6
Casein	5.6	5.6	5.6	5.6

teose-peptone third; while for *Glaucoma piriformis*, Bacto-liver ranked third, with tryptone and proteose-peptone first and second, respectively. Additional differences were noticed in Bacto-peptone and gelatin, Bacto-peptone being the better for *Glaucoma ficaria* and gelatin for *Glaucoma piriformis*. The relative division rates of the two species are also strikingly different, as indicated in figure 3. Similar differences in growth rates have been noted by ELLIOTT (1935) for *Colpidium striatum* and *Colpidium campylum*.

Although Bacto-liver supports better growth of *Glaucoma ficaria* than does a tryptone medium, the former is less satisfactory for experimental purposes on account of the coaglum formed when the medium is heated. Tryptone, on the other hand, gives a clear, particle-free solution which is much more suitable for the purposes of the present investigation.

Growth in relation to p_H of the medium.

Series IV.

In the first attempt to determine the relation between p_H and growth of *Glaucoma ficaria* the same medium (tryptone, 0.5 %; K_2HPO_4, 0.2 %) was employed as had been used for isolation of the ciliate in bacteria-free cultures. The p_H of the different sets of culture tubes ranged from p_H 3.6 to 8.6, and the initial concentration of organisms was 370 per cc. After incubation for 72 hours, the final p_H was determined in each case, and the tubes were fixed for counting. Growth occurred from p_H 4.0 to 8.6, and reached a maximum at 5.6, with a secondary high point at 7.2 as compared with 6.8 and 7.5. In most of the tubes of this series there was a change in p_H toward neutrality, the changes being most marked in the lower acid range. In view of these changes growth was plotted (Fig. 4) in relation to both initial and final p_H, the resulting curves being similar in appearance. Similar changes in p_H were noted by ELLIOTT (1933) in his cultures of *Colpidium striatum* in low concentrations of tryptone.

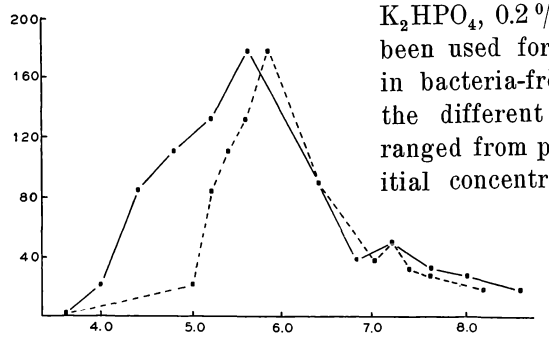

Fig. 4. *Glaucoma ficaria*, growth in relation to p_H; x/x_0 (ratio of final to initial concentration of organisms) plotted against initial p_H (——) and final p_H (------).

Series V.

After the establishment of 1.5 % as the optimal concentration of tryptone for growth of *Glaucoma ficaria*, it seemed desirable to determine the relation of p_H to growth of the ciliates in this more favorable medium. It was also expected that, with the better buffering system, p_H changes might be avoided to some extent. For purposes of comparison, *Glaucoma ficaria* and *Glaucoma piriformis* were used. The initial p_H ranged from 4.0 to 9.5. The initial concentration, in the case of *Glaucoma ficaria*, was 340 per cc, and for *Glaucoma piriformis*, 355 per cc. All cultures were incubated for 72 hours, and then final p_H and final concentrations were determined. The changes in p_H were slight (0.1 to 0.3) as compared with those observed in series IV. The growth range (initial p_H) extended from p_H 4.0 to 9.5 for *Glaucoma ficaria*, and from 4.0 to 8.9 for *Glaucoma piriformis* (Fig. 5). Growth of *Glaucoma ficaria* reached its maximum in the medium with an initial p_H of 5.4. For *Glaucoma piriformis*, on the other hand, a bimaximal curve was noted, with peaks at 5.1 and 6.7. In series IV a suggestion of a bimaximal growth curve was noted for *Glaucoma ficaria*, but there was no evidence for this in the more favorable 1.5 % tryptone medium.

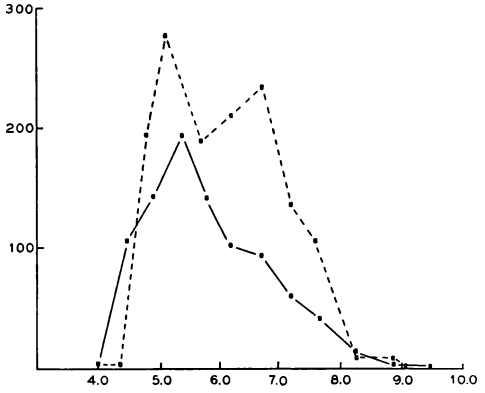

Fig. 5. Growth of *Glaucoma ficaria* and *Glaucoma piriformis* in relation to p_H of the medium; x/x_0 (ratio of final to initial concentration of organisms) plotted against initial p_H; continuous line indicates growth of *Glaucoma ficaria* and broken line that of *Glaucoma piriformis*.

The experiment was repeated for both species, and the same general results were obtained.

Series VI and VII.

At this point in the investigation it seemed desirable to determine whether or not the type of peptone used in the medium might alter the p_H range and optimum. In series VI a Bacto proteose-peptone medium (proteose-peptone, 1.0 %; K_2HPO_4, 0.2 %) was used

for both *Glaucoma ficaria* and *Glaucoma piriformis* in the p_H range 4.1—8.8. The initial count was 375 per cc for *Glaucoma ficaria*, and 405 per cc for *Glaucoma piriformis*. All cultures were incubated for 72 hours. Only slight p_H changes (0.0—0.2) were noted, and growth of each species occurred throughout therange (Fig. 6). An interesting feature of this medium was the appearance of a bimaximal growth curve for *Glaucoma ficaria*, with peaks at 5.1 and 6.7. For *Glaucoma piriformis* the growth curve was also bimaximal, the peaks (p_H 4.8 and 7.4) being more widely separated.

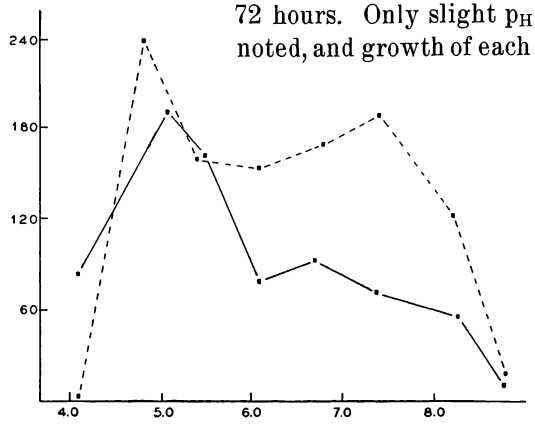

Fig. 6. Growth of *Glaucoma ficaria* (———) and *Glaucoma piriformis* (- - - - - -) in relation to p_H; x/x_0 plotted against initial p_H of Bacto proteose-peptone medium.

Series VII, in which a Bacto-peptone medium (Bacto-peptone, 1.0%); H_2HPO_4, 0.2% was used, was started simultaneously with series VI, with the same initial count and p_H range. At the end of 72 hours final p_H and final concentrations were determined. The p_H changes observed were slight, just as in series VI. In this medium both species showed unimaximal growth (Fig. 7), with an optimum at 6.0 for *Glaucoma ficaria* and one at p_H 5.3 for *Glaucoma piriformis*.

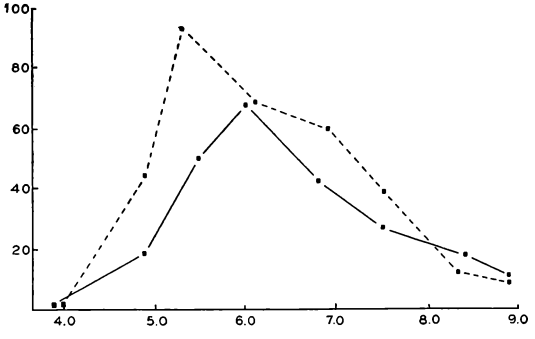

Fig. 7. Growth of *Glaucoma ficaria* (———) and *Glaucoma piriformis* in relation to initial p_H; Bacto-peptone medium; x/x_0 plotted against initial p_H.

Discussion.

The present investigation shows that there are certain similarities and certain differences between *Glaucoma ficaria* and *Glaucoma piriformis* in the relations of growth to hydrogen ion concentration. The

p_H range is practically the same for the two species: p_H 4.0—9.5 for *Glaucoma ficaria*, and p_H 4.0—8.9 for *Glaucoma piriformis*. In general, the optimum is somewhat lower for *Glaucoma piriformis* (p_H 4.8—5.3) than for *Glaucoma ficaria* (p_H 5.1—6.0). In 1.5% tryptone medium the growth curve of *Glaucoma piriformis* was distinctly bimaximal, and that of *Glaucoma ficaria* unimaximal. In the other two media the growth curves for the two species differed in form in each case.

ELLIOTT (1933) noted a bimaximal type of growth curve in observations on the relation of p_H to growth of *Colpidium striatum* in a tryptone medium. The same author (ELLIOTT, 1935) reported that bot *Colpidium striatum* and *Colpidium campylum* showed bimaximal growth-p_H curves in Bacto-peptone, but not in Bacto proteose-peptone, Bacto-protone or Bacto-veal. Both *Glaucoma ficaria* and *Glaucoma piriformis*, on the other hand, showed bimaximal growth-p_H curves in proteosepeptone and unimaximal curves in Bacto-peptone. An additional difference between the two genera is noted in the fact that maximal growth of *Colpidium* always occurred on the alkaline side of the range, while growth of *Glaucoma* reached a maximum on the more acid side of the range. In the case of *Paramecium bursaria* LOEFER (MSS), using a tryptone medium, has found a p_H range of 5.3—8.0, with an optimum at 6.7—6.8. With this species, there was no indication of a bimaximal growth-p_H curve. The investigations just mentioned are the only ones in which growth in relation to p_H of the medium has been investigated in bacteria-free cultures.

The investigations in which bacterized cultures have been used are much more numerous, as indicated in table 2.

It is interesting to note that in bacterized cultures two species showed the bimaximal type of growth in relation to p_H. MOREA (1927) found that *Colpoda cucullus* had optima at p_H 6.5 and 7.5, and DARBY (1929) obtained maximal growth of *Stylonychia pustulata* at p_H 6.7 and 8.0.

There is considerable variation in the extent of the p_H range for various species of ciliates. The smaller ciliates have a relatively wide p_H range, e. g., *Colpidium* sp. (MILLS, 1931), p_H 4.5—8.0 and *Colpoda cucullus* (MOREA, 1927), p_H 5.5—9.5. On the other hand some of the larger ciliates have a rather restricted p_H range, e. g., *Spirostomum ambiguum* (SAUNDERS, 1924, p_H 6.8—7.5; *Spirostomum* sp. (MOREA, 1927), p_H 6.5—8.0; *Holophrya* sp. (PRUTHI, 1926), p_H 6.5—7.4; *Plagiopyla* sp. PRUTHI, 1926), p_H 6.9—7.5; and *Amphileptus* sp. (PRUTHI, (1926), p_H 6.8—7.5). Other forms are intermediate between these two extreme groups in their reaction to the hydrogen ion concentration.

Table 2.
p_H range and p_H optima for various species of ciliates.

Species and author	p_H range	Optimum
Colpidium sp. (PRUTHI, 1926)	6.0—8.5	—
**Colpidium* sp. (MILLS, 1931)	4.5—8.0	6.0
Colpoda cucullus (MOREA, 1927)	5.5—9.5	6.5 and 7.5
Gastrostyla sp. (PRUTHI, 1926)	6.0—8.5	—
Spirostomum ambiguum (SAUNDERS, 1924)	6.8—7.5	7.4
Spirostomum sp. (MOREA, 1927)	6.5—8.0	7.5
Holophrya sp. (PRUTHI, 19 6)	6.5—7.4	—
Plagiopyla sp. (PRUTHI, 1926)	6.9—7.5	—
Amphileptus sp. (PRUTHI, 1926)	6.8—7.5	7.1—7.3
Stylonychia pustulata (DARBY, 1929)	6 0—8.0	6.7 and 8.0
Paramecium sp. (SAUNDERS, 1924)	—	7.8—8.0
Paramecium sp. (PRUTHI, 19 6)	7.0—8.5	7.8—8.0
Paramecium aurelia (MOREA, 1927)	6.0—9.5	approx. 7.0
P. caudatum (DARBY, 1929)	5.3—8.2	7.0
P. aurelia (DARBY, 1929)	5.7—7.8	6.7
P. multimicronucleata (JONES, 1930)	4.8—8.3	approx. 7.0
P. aurelia (PHELPS, 1934)	5.9—8.2	"no differences"

* Calculated from rate of food vacuole formation.

PRUTHI (1926) reported that *Paramecium* sp. never appeared in his infusions until the hydrogen ion concentration had reached p_H 7.0 or above. He also reported that p_H 6.0 was lethal to *Paramecium*. However, the results of later investigations do not support his findings, e. g., *Paramecium aurelia*, p_H range, 6.0—9.5 (MOREA, 1927), 5.7—7.8 (DARBY, 1929) and 5.9—8.2 (PHELPS, 1934); *Paramecium caudatum*, p_H range, 5.3—8.2 (DARBY, 1929); *Paramecium multimicronucleata*, p_H range, 4.8—8.3 (JONES, 1930).

In infusions containing a mixed population of bacteria MOREA (1927) found p_H 7.0 was optimal for growth of *Paramecium aurelia*, and DARBY (1929) for the same form reported maximal growth at p_H 6.7. On the other hand, PHELPS (1934) found no differences in the division rate of *Paramecium aurelia* from p_H 5.9—8.2 when the ciliates were cultured in a pure line of *Erythrobacillus prodigiosus*. He attributed these results to the constant supply of food, the bacteria, available for the ciliates in his experiments. However, the work of ELLIOTT (1933) on *Colpidium striatum*, (LOEFER (MSS) on *Paramecium bursaria* and that of the writer on *Glaucoma ficaria* and *Glaucoma piriformis* were carried out in synthetic media which provided a constant food supply, and the division rate of all these species was markedly affected by changes in hydrogen ion concentration. Moreover, the writer (MSS) has recently found that when grown in suspensions of *Bact.* (*Erythrobacillus*) *prodigiosus* the division

rate of *Glaucoma ficaria* was approximately the same from p_H 4.5—8.6. But in suspensions of *Bacillus flourescens*, *Bacillus mucosum capsulatum*, and *Proteus vulgaris* the ciliate showed a bimaximal growth-p_H curve, with peaks at p_H 5.0—5.2 and p_H 7.6. Therefore, it seems probable that the results obtained by PHELPS (1934) with *Paramecium aurelia* were due not to a constant food supply but rather to the unique effects of this particular bacterium on the growth of the ciliates. A more detailed account of these findings will appear later (JOHNSON, MMS).

Maximal growth of *Glaucoma ficaria* occurred in a 1.5% tryptone medium and in a 0.5% yeast-extract medium. ELLIOTT (1935) obtained good growth of *Colpidium striatum* in concentrations of tryptone ranging from 1.0 to 3.0%. In the case of *Paramecium bursaria*, however, LOEFER MSS) has noted a greater sensitivity to concentrations of tryptone: a concentration of 0.5% was optimal, while higher concentrations were distinctly less favorable. This writer also noted a more striking sensitivity of *Paramecium bursaria* to yeast-extract; the optimal concentration was 0.03%, while growth was descreased in 0.125%, inhibited in 0.25%, and the organisms were killed in a 0.5% solution. In both tryptone and yeast-extracct media, therefore, *Glaucoma ficaria* is decidedly less sensitive than *Paramecium bursaria* in its growth requirements, Perhaps other ciliates, like *Paramecium bursaria*, are sensitive to the concentration of the medium. With such species this factor obviously becomes important in attempts to obtain bacteria-free cultures. LOEFER (MSS) has considered this problem in more detail.

Summary.

The method used in isolating *Glaucoma ficaria* in bacteria-free culture is outlined, and suitable culture media are described. Growth of *Glaucoma ficaria* and *Glaucoma piriformis* is compared in different media and in relation to p_H of the media. Growth of *Glaucoma ficaria* occurs within the p_H range 4.9—9.5 and that of *Glaucoma piriformis* within the p_H range 4.0—8.9. In general, the p_H optimum is somewhat lower for *Glaucoma piriformis* (4.8—5.3) than for *Glaucoma ficaria* (5.1—6.0). The type of growth-p_H curve for either species depends upon the type of medium used. Growth characteristics of *Glaucoma* and *Colpidium* are compared. An explanation is suggested for PHELPS' (1934) findings that the division rate of *Paramecium aurelia* is unaffected by changes in the hydrogen ion concentration.

Literature cited.

Brown, J. H. (1934): Pure cultures of Paramecium. Science Vol. 80 p. 409—410.

Butterfield, C. T. (1929): A note on the relation between food concentration in liquid media and growth. Pub. Health Reports Vol. 44 p. 2865—2872.

Chatton, E. & M. (1923): L'influence des facteurs bacteriens sur la nutrition, la multiplication et la sexualite des Infusoires. C. R. Acad. Sci. T. 176 p. 1262—1264.

— (1929): Les conditions de la conjugaison du Glaucoma scintillans en culture lethobacteriennes. Action directe et specifique de certains agents zygogenes. Ibid. T. 188 p. 1315—1317.

Darby, H. H. (1929): The effect of hydrogen-ion concentration on the sequence of protozoan forms. Arch. f. Protistenk. Bd. 65 p. 1—37.

Elliott, A. M. (1933): Isolation of Colpidium striatum Stokes in bacteria-free cultures and the relation of growth to pH of the medium. Biol. Bull. Vol. 65 p. 45—56.

— (1935): Effects of certain organic acids and protein derivatives on the growth of Colpidium. Arch. f. Protistenk. Bd. 84 p. 472—494.

Gatenby, J. B. and E. V. Cowdry (1928): Bolles Lee's Microtomist's Vade-mecum. Philadelphia, Blakiston.

Glaser, R. W. (1932): Culture of certain Protozoa, bacteria-free. Journ. Parasit. Vol. 19 p. 23. Abstract.

Glaser, R. W. and N. A. Coria (1930): Methods for the pure culture of certain protozoa. Journ. Exp. Med. Vol. 51 p. 787—806.

— (1933): The culture of Paramecium caudatum free from living microorganisms. Journ. Parasit. Vol. 20 p. 33—37.

— (1935): The culture and reactions of purified protozoa. Am. Journ. Hygiene Vol. 21 p. 111—120.

Hetherington, A. (1933): The culture of some holotrichous ciliates. Arch. f. Protistenk. Bd. 80 p. 255—280.

Johnson, D. F. (1936): Growth of Glaucoma ficaria Kahl in cultures with single species of other microorganisms. MSS.

Jones, E. P. (1930): Paramecium infusions histories. I. Hydrogen-ion changes in hay and hay-flour infusions. Biol. Bull. Vol. 59 p. 274—285.

Kahl, A. (1926): Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. Arch. f. Protistenk. Bd. 55 p. 197—438.

Loefer, J. B. (1934): Bacteria-free cultures of Paramecium. Science Vol. 80 p. 206—207.

— (1936): Bacteria-free cultures of Paramecium bursaria and concentration of the medium as a factor in growth. MSS.

Lwoff, A. (1924): Le pouvoir de synthese d'un protiste heterotrophe: Glaucoma piriformis. C. R. Soc. Biol. T. 91 p. 344—345.

— (1929): Mileux de culture et d'etretien pour Glaucoma piriformis (Cilie). Ibid. T. 100 p 635—636.

— (1932): Reserches biochemiques sur la nutrition des Protozoaites. Le pouvoir synthese. Monographies de l'Institute Pasteur 158 pp. Paris.

Mills, S. M. (1931): The effect of the hydrogen-ion concentration on Protozoa, as demonstrated by the rate of food vacuole formation in Colpidium. Brit. Journ. Exp. Biol. Vol. 8 p. 17—29.

Milkovitch, G. (1929): Action du serum humain sur un infusoire, Glaucoma piriformis. C. R. Soc. Biol. T. 100 p. 417—420.

Morea, L. (1927): Influence de la concentration en ions H sur la culture de quelques infusoires. C. R. Soc. Biol. T. 97 p. 49—50.

Oehler, R. (1919): Flagellaten- und Ciliatenzucht auf reinem Boden. Arch. f. Protistenk. Bd. 40 p. 16—26.

— (1922): Wirkung von Bakteriengiften auf Ziliaten. Ctrbl. f. Bakt. Bd. 86 p. 494—500.

— (1924): Gereinigte Zucht von freilebenden Amöben, Flagellaten und Ciliaten. Arch. f. Protistenk. Bd. 49 p. 287—296.

Parpart, A. K. (1928): The bacteriological sterilization of Paramecium. Biol. Bull. Bd. 55 p. 113—120.

Peters, R. A. (1920): Nutrition of Protozoa. The growth of Paramecium in sterile culture medium. Journ. Physiol. Vol. 53 (Proc. Physiol. Soc. p. cviii).

— (1921 a): Nutrition of Protozoa. 2. The carbon and nitrogen compounds needed for the growth of Paramecium (Colpidium). Ibid. Vol. 54 p. 1—11.

— (1921 b): The substances needed for the growth of a pure culture of Colpidium colpoda. Ibid. Vol. 55 p. 1—32.

— (1929): Observations on the oxygen consumption of Colpidium colpoda. Ibid. Vol. 68 (Proc. Physiol. Soc. p. ii—iii).

Phelps, A. (1934): Studies on the nutrition of Paramecium. Arch. f. Protistenk. Bd. 82 p. 134—163.

— (1935): Growth of protozoa in pure culture. I. Effect upon the growth curve of the age of the inoculum and of the amount of the inoculum. Journ. Exp. Zool. Vol. 70 p. 109—130.

Pruthi, H. S. (1926): On the hydrogen-ion concentration of hay infusions, with special reference to its influence on the protozoan sequence. Brit. Journ. Exp. Biol. Vol. 4 p. 292—300.

Sandon, H. (1932): The Food of Protozoa. Publications of the faculty of Science 187 pp. Cairo.

Saunders, J. T. (1924): The trichocysts of Paramecium. Biol. Reviews Vol. 1 p. 249—269.