July 1945

THE EFFECTS OF OXYGEN, CARBON DIOXIDE, AND PRESSURE ON GROWTH IN CHILOMONAS PARAMECIUM AND TETRAHYMENA GELEII FURGASON

D.M. Pace
University of Nebraska-Lincoln

R.L. Ireland
University of Nebraska-Lincoln

The Effects of Oxygen, Carbon Dioxide, and Pressure on Growth In *Chilomonas paramecium* and *Tetrahymena geleii* Furgason

D. M. Pace and R. L. Ireland

Published in *Journal of General Physiology* 28:6 (July 1945), pp. 547–557.

THE EFFECTS OF OXYGEN, CARBON DIOXIDE, AND PRESSURE ON GROWTH IN CHILOMONAS PARAMECIUM AND TETRAHYMENA GELEII FURGASON*

BY D. M. PACE AND R. L. IRELAND

(*From the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln*)

(Received for publication, March 23, 1945)

The effects of oxygen, carbon dioxide, and pressure on animal organisms have been widely studied, but most of the investigations in this field concern chiefly the effects of extreme, especially high, atmospheric pressures on blood changes, respiration, length of life, etc., in Vertebrates.

Very few detailed observations have been made on the relation between oxygen tension or carbon dioxide tension and growth in Protozoa. The few investigations that have been carried out are concerned primarily with the effect of these gases on respiration although some observations have been made on the relation of aerobic and anaerobic conditions and growth in unicellular forms.

Lwoff (1932) found that *Glaucoma piriformis* does not grow under anaerobic conditions, and Hall (1933) found that *Colpidium campylum* does not grow as well under anaerobic as under aerobic conditions. Jahn (1936) observed that *Chilomonas paramecium* grew best in unaerated culture flasks but that *Glaucoma piriformis* grew best in aerated flasks. The results of Phelps (1936) give support to those of Lwoff and Jahn on *Glaucoma piriformis*. Rottier (1936) maintained that inadequte amounts of oxygen limit the growth of *Polytoma uvella* and Reich (1936) claimed that oxygen concentration is very important in cultures of *Amoeba*.

Except for the work of Mast and Pace (1933) on the effects of carbon dioxide on growth in *Chilomonas paramecium* in inorganic solution, no observations have been made on the effect of various oxygen or carbon dioxide tensions on growth in unicellular forms. In the following pages, data concerned with the effects of different concentrations of these gases as well as the effects of total atmospheric pressure on unicellular organisms are presented.

Material and Methods

Two very different species were used in these investigations; a colorless flagellate, *Chilomonas paramecium* and a small ciliate, *Tetrahymena geleii* Furgason.[1] The for-

* These investigations were partly supported by a grant obtained from The Society of Sigma Xi.

[1] *Tetrahymena geleii* Furgason, used in this work, was kindly furnished us by Professor George W. Kidder of Brown University. There is some controversy as to terminology, but in this report the genus will be referred to as *Tetrahymena*.

mer is plant-like and the latter animal-like in characteristics. Both species can be grown in sterile culture. The chilomonads were cultured in a solution of Na-acetate, 125 mg; NH_4Cl, 46 mg.; $(NH_4)_2SO_4$, 10 mg.; K_2HPO_4, 20 mg.; $CaCl_2$ 1 mg.; $MgCl_2$, 1 mg.; thiamine hydrochloride, 0.001 mg.; $FeCl_3$, 0.001 mg.; and redistilled water to make 1 liter. The H ion concentration was adjusted to pH 6.8. This solution will be referred to as the acetate-ammonium or ac-am solution. Tetrahymenas were grown in a 2 per cent proteose-peptone solution, the H ion concentration of which was pH 6.8. Therefore no adjustment was necessary.

In these experiments, cultures were kept in large tightly sealed desiccators, which were connected by means of high pressure tubing to a vacuum pump and the cylinders containing the different gases. The connections were such that complete evacuation could be obtained and the desired gas or mixture of gases added without disconnecting the apparatus. In preparing cultures, 2000 ± 200 chilomonads or tetrahymenas from a flourishing culture were added to each 70 ml. of fresh ac-am or proteose-peptone solution, respectively, in 125 ml. pyrex Erlenmeyer flasks. These were placed in desiccators. In order to test the effects of different gases of known concentration, the desiccators were evacuated by means of a Cenco pressovac pump, and the gas or mixture was added. The temperature was held at 25 ± 1° C. for 4 days. The cultures were then removed, the density of the population calculated, and transfers were made to fresh solutions. The organisms were counted by diluting the culture with fresh ac-am solution (in thickly populated cultures the dilution was 1:100; otherwise, it varied according to density); then 0.1 ml. portions of the diluted culture fluid were added to Columbia dishes and the chilomonads counted under the dissecting microscope by drawing them into a micropipette and then discarding them. Six to ten counts were made on each culture. Very little variation was found.

RESULTS

The Effect of O_2 Tension on Growth

Since any variation in atmospheric pressure results in a proportionate variation in oxygen tension, results obtained from experiments designed to ascertain the effect of reduced pressures merit attention only if the O_2 pressure is held constant. Consequently, the first experiments in these investigations were concerned with the optimum O_2 concentration and the range of O_2 concentrations in which *Chilomonas* and *Tetrahymena* continue to grow and reproduce. In each test, four 125 ml. Erlenmeyer flasks containing 70 ml. ac-am or proteose-peptone solution were used for each different 0_2 concentration; then 2000 ± 200 chilomonads or tetrahymenas were added to each flask. The concentration of O_2 varied from 0.5 mm. to pure O_2 at atmospheric pressure (738 ± 3 mm.). The total pressure was kept constant for all the tests. This was done by evacuating the desiccator to approximately 3 mm. and adding the desired amounts of O_2 and CO_2 plus N_2 to atmospheric pressure. The temperature was held at 25 ± 1° C. After 4 days, the number of organisms in each culture was calculated and recorded. This test was repeated and the average results for both tests are given in Table I, Experiment A. The experiment was then repeated and the averages for both tests are given in Experiment B.

The average results of both experiments are shown in order to emphasize the similarities in maximum numbers produced. Obviously, *Chilomonas* does not grow well in high concentrations of O_2. At 600 mm. O_2 pressure there were comparatively few chilomonads found, and they died out on the first transfer. It can be concluded, then, that very little, if any, growth occurs in *Chilomonas* exposed to O_2 tensions above 500 mm. The optimum O_2 tension is approximately 75 mm. at which an average of 302,950 chilomonads per ml. was found for all tests. *Chilomonas* grew well even in O_2 tensions as low as 0.5 mm. This is in contrast to results obtained for *Tetrahymena*, which did not grow at these low O_2 concentrations. However, at 10 mm. O_2 pressure it grew very well and as the O_2 tension increased above this, maximum density of population increased until in pure O_2 495,900 tetrahymenas were found in each milliliter of solution. These two species have very different respiratory systems. Notwithstanding, both will live under conditions in which they are exposed to a rather wide range of O_2 concentrations. *Chilomonas*, however, is killed at high O_2 tensions, while *Tetrahymena* grows best at high O_2 tensions. An interesting observation was

TABLE I

The Effect of Oxygen in Various Concentrations on Growth in Chilomonas paramecium and Tetrahymena geleii

To each 70 ml. of culture solution, 2000 ± 200 organisms were added. Atmospheric pressure, 738 ± 3 mm.; temperature, 25 ± 1° C.

Concentration of O_2 at atmospheric pressure	No. of organisms per ml. after 4 days					
	Chilomonas paramecium			*Tetrahymena geleii*		
	Experiment A	Experiment B	Total average	Experiment A	Experiment B	Total average
mm. Hg						
0.5	102,400	98,000	100,200	13,000*	All dead	—
10	227,900	193,000	210,450	146,700	132,000	139,350
25	248,200	198,500	223,100	111,500	158,000	134,750
50	250,000	209,000	228,500	121,000	157,000	139,000
75	317,100	288,800	302,950	173,120	163,000	168,000
150	248,500	210,000	228,750	176,310	170,000	173,150
300	233,400	218,000	225,700	208,000	196,000	202,350
400	230,500	202,000	216,200	365,000	315,000	340,000
500	157,800	98,500	128,150	317,500	325,600	321,550
600	500*	600*	—	432,000	397,000	414,500
700	—	—	—	409,000	422,000	415,500
739 (Pure O_2)	—	—	—	527,000	464,000	495,900

* Died after first transfer.

noted with regard to chilomonads grown in extremely low O_2 concentrations (*i.e.*, when the gaseous environment consisted almost entirely of N_2). Under these conditions they became very fragile and disintegrated unless great care was taken when preparing the organisms for counting.

The Effect of Reduced Pressure on Growth

In preliminary tests which were carried out to ascertain the lowest pressure under which these organisms would grow, it was found that *Chilomonas* grew best under slightly reduced pressures. However, in these tests the oxygen concentration was not held constant. The results of the experiments reported above show that *Chilomonas* grew best in O_2 tensions lower than those found at atmospheric pressure. On the other hand, in preliminary tests, it was found that growth in *Tetrahymena* was retarded when pressure was reduced. The results of the experiments reported above for this organism show that the same is true when the oxygen tension is reduced. Could this increase in growth in *Chilomonas*, or retardation of growth in *Tetrahymena*, found when the pressure is reduced, be due entirely to lowered O_2 tension? The following tests were carried out in an attempt to answer this question.

The same procedure was followed as in precedmg tests except that the O_2 tension was held at 50 mm. pressure in all tests. This particular pressure was selected for two reasons; first, both *Chilomonas* and *Tetrahymena* grow well at this concentration and, secondly, it is low enough to allow for considerable variation in pressure. The total pressures were varied from 50 mm. to atmospheric pressure, but the partial pressure of O_2 was always 50 mm. Thus, at 50 mm. total pressure, there was pure O_2 in the desiccator. The desiccators containing the cultures were kept at a fairly constant temperature, 25 $\pm$ 1° C., for 4 days when the number of organisms in the cultures was ascertained. The results are presented in Table II. They indicate that in *Chilomonas* there is no significant difference in growth over a wide range of pressures as long as the oxygen concentration is held constant. This answers the question that arose during the preliminary tests; *i.e.*, the question pertaining to the increase in growth observed when total pressure was reduced without regard to changes in O_2 concentration. The increase in growth was apparently due to the low oxygen tension and not to the low pressure. In *Tetrahymena*, however, there is a significant increase in maximum numbers as the total pressure decreases. The optimum pressure seems to be at about 500 mm., since 280,000 organisms per ml. were produced as compared to 148,000 per ml. at atmospheric pressure. With further reduction in pressure, growth decreased until at 50 mm. an average of only 93,000 organisms per ml. was produced. The O_2 tension used was far below the optimum for *Tetrahymena*, and therefore it can be concluded that the increase in growth was caused by some low pressure effect on the mechanism concerned with growth.

The Effect of CO_2 Tension on Growth

In most of the experiments previously reported the CO_2 was held constant at the concentration usually found at atmospheric pressures (approximately 0.03 per cent). Mast and Pace (1933) claimed that CO_2 is utilized by *Chilomonas* in the formation of starch, *etc.*, and maintained that when it is omitted from the surrounding air, the rate of growth is considerably reduced. Jahn (1936) confirmed the observations of Mast and Pace but found that in *Glaucoma piriformis* there was no difference in growth either in the presence or absence of CO_2. In the following experiments, the effect of carbon dioxide on growth was ascertained in *Chilomonas* and *Tetrahymena*. The O_2 concentration was the same for all cultures (150 mm.) and the total pressure was always atmospheric (738 mm.). The same procedure was followed as in previous experiments. The concentration of CO_2 ranged from that found in atmosphere (0.22 mm.) to 400 mm. CO_2. The results are presented in Table III. This table shows that *Chilomonas* grew well in any of the concentrations used and that as the concentration of CO_2 was increased from zero, the growth increased to a maximum at 100 mm. CO_2 and then decreased. In *Tetrahymena*, growth was best in cultures exposed to CO_2-free air. They did not live when exposed to CO_2 tensions much above 122 mm.; *i.e.*, they died out after the first or second transfer in the higher concentrations. Could these results have been influenced by the change in H ion concentration?

TABLE II

The Effect of Reduced Pressure on Growth in Chilomonas paramecium and Tetrahymena geleii

To each 70 ml. of culture solution, 2000 ± 200 organisms were added. Each figure represents the average for 3 tests. Atmospheric pressure, 740 mm.; O_2 concentration, 50 mm.; temperature, 25 ± 1° C.

Pressure	No. of organisms per ml. after 4 days	
	Chilomonas paramecium	*Tetrahymena geleii*
mm. Hg		
50	195,000	93,300
100	190,000	—
200	180,800	118,700
300	185,100	122,000
400	185,300	214,000
500	192,000	280,000
600	202,100	204,000
740	191,000	148,000

The H ion concentration at the optimum CO_2 tension (100 mm. CO_2) is approximately pH 6.9. Mast and Pace (1938) found that the optimum H ion concentration for *Chilomonas* when grown in ac-am solution is pH 6.8.

It has been observed repeatedly that cultures of chilomonads become more alkaline as the population increases, but that when the CO_2 concentration is increased sufficiently, the solution becomes (or remains) acid. However, the

TABLE III

The Effect of Carbon Dioxide in Various Concentrations at Atmospheric Pressure on Growth in Chilomonas paramecium and Tetrahymena geleii

To each 70 ml. of culture solution, 2000 ± 200 organisms were added. O_2 concentration, 150 mm.; pH, average H ion concentration of solutions after 4 days (pH varied by ± 0.1); temperature, 25 ± 1° C.

Concentration of CO_2 at atmospheric pressure	No. of organisms per ml. after 4 days							
	Chilomonas paramecium				*Tetrahymena geleii*			
	Experiment A	Experiment B	pH	Total average	Experiment A	Experiment B	pH	Total average
mm. Hg								
0	92,000	87,000	7.5	89,000 ±8,000	172,000	143,000	7.3	157,500 ±17,000
0.2	225,000	200,000	7.4	212,500 ±14,000	155,000	125,000	7.2	140,000 ±19,000
2	216,400	214,500	7.2	215,000 ±12,000	140,700	129,000	6.9	134,500 ±11,000
12.2	454,000	358,000	7.3	406,000 ±40,000	136,300	115,500	6.7	126,000 ±4,000
50	698,000	570,000	7.1	634,000 ±32,000	159,800	101,000	6.8	130,000 ±7,500
100	654,000	678,200	6.9	668,600 ±30,000	131,000	109,000	6.3	120,000 ±6,000
122	574,700	519,000	6.9	546,000 ±28,000	137,000	99,000	6.4	118,000 ±4,800
244	325,000	219,500	6.2	272,000 ±16,000	59,700 (Died out, 2nd transfer)	36,500	6.2	—
400	228,700	207,500	6.0	216,600 ±9,000	34,900 (Died out, first transfer)	27,000	6.0	—

increased acidity produced by the addition of CO_2 had no significant effect on the results obtained in the experiment. This was proved by means of tests carried out to ascertain the extent to which the H ion concentration influenced the results. Several cultures of *Chilomonas* and *Tetrahymena* were set up at the same time and in the same way as those of the other tests. They were kept in air at ordinary atmospheric pressure but the H ion concentration was held at the same pH value as those of other cultures which were exposed to CO_2. The results are given in Table IV.

These results show that there is an increase in growth in *Chilomonas* as the H ion concentration increases (from pH 7.2–6.9) even in solutions to which HCl is added (to hold the pH on the acid side of neutrality). The increase, however, is hardly significant when compared to the increase in growth observed when the H ion concentration increases due to high CO_2 tensions. In other words, under atmospheric conditions, the average maximum number of chilomonads produced at pH 7.2 was 200,000 per ml. while at pH 6.9 (with HCl added) the maximum was 231,000. However, when the change in pH was caused by the addition of CO_2, the maximum at pH 7.2 (2 mm. CO_2 pressure) was 215,000 per ml. and at 6.9 (100 ml. CO_2 pressure), 668,600 chilomonads per ml. Even at pH 6.2, many more chilomonads were produced in cultures exposed to CO_2 than in those which were not exposed to CO_2. In *Tetrahymena*, when the H ion concentration was increased by adding HCl, the growth maximum decreased from an average of 148,000 per ml. at pH 7.2 to 115,200 at pH 6.2. Indications are that the organisms would continue to grow and reproduce indefinitely at this H ion concentration. If the CO_2 tension is raised enough (to 244 mm.) to increase the H ion concentration to pH 6.2, all the tetrahymenas died (Table III). Death was due to the high CO_2 concentration and only partly, if at all, to the comparatively high H ion concentration; in fact, *Tetrahymena* will live and grow in much higher H ion concentrations than this.

TABLE IV

The Relation between Carbon Dioxide and H Ion Concentration and Growth in Chilomonas paramecium and Tetrahymena geleii

To each 70 ml. of culture solution, 2000 ± 200 organisms were added. Temperature, 25 ± 1° C.

H ion concentration	No. of organisms per ml. after 4 days			
	Chilomonas paramecium		*Tetrahymena geleii*	
	pH decreased by adding CO_2 (Table III)	pH decreased by adding HCl	pH decreased by adding CO_2 (Table III)	pH decreased by adding HCl
pH				
7.2	215,000	200,000	140,000	148,000
6.9	668,600	231,000	134,500	142,000
6.2	272,000	197,000	Died out	115,200

At frequent intervals, organisms were taken from the cultures at random and examined. Tests for starch and fat were made on chilomonads by means of diluted Lugol's solution (1 part in 20 parts water) and sudan III, respectively. Numerous specimens and their contents were traced by means of a camera lucida. Outlines of chilomonads so treated and drawn are given in Fig. 1.

Some of these representative specimens were grown in ac-am solution at ordinary atmospheric pressure while others were grown in ac-am solution with the optimum O_2 and CO_2 concentration for *Chilomonas.* The starch content was much greater in chilomonads grown under high CO_2 pressures, and the starch grains were much larger than in the organisms grown in air at atmospheric pressure. The total starch produced by all the chilomonads (per unit volume) grown under optimum concentrations of CO_2 and O_2 was at least 4 to 5 times as great as that produced by all the chilomonads (per unit volume) grown in atmospheric air. Fat formation seemed to be at a minimum under these conditions. In some individuals grown in CO_2 and O_2 at optimum concentrations,

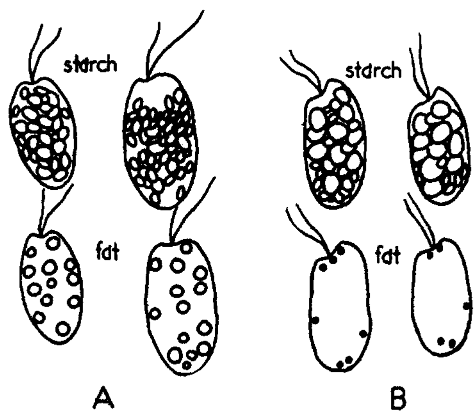

FIG. 1. Effect of high CO_2 concentration on starch and fat content in *Chilomonas.* The chilomonads were selected at random and treated with Lugol's solution and sudan III. Outlines in upper sketches represent starch grains; those in lower sketches represent fat globules. *A*, camera lucida outlines of specimens cultured in ac-am solution in air at atmospheric pressure (737 mm.); *B*, chilomonads cultured in ac-am solution in O_2 at 75 mm., CO_2 at 122 mm., and N_2 at 540 mm. pressure (total pressure = 737 mm.).

no fat could be found. The low fat content is probably the result of the combined action of CO_2 and O_2, for it has been observed that chilomonads grown in solutions exposed to high O_2 tensions also contain very little fat.

In Fig. 2, several camera lucida sketches of *Tetrahymena* are shown. They were made from organisms which had been exposed to the same conditions as the chilomonads shown in Fig. 1. Numerous specimens were taken at random and stained with sudan III.

Tetrahymenas grown in pure oxygen show a decided increase in maximum numbers produced per unit volume (Table I). This is equivalent to approximately 3 times the maximum in atmospheric air. However, the organisms in pure O_2, as illustrated in Fig. 2, are much smaller than those in ordinary atmosphere. This is due to the greater rate of reproduction. Those grown in high CO_2 concentration were very small; sometimes less than one-fourth the volume of those in atmosphere. In other words, there was comparatively little growth in these organisms. In fact, it is quite likely that if the cultures had been continued under these high CO_2 pressures, they soon would have died out. The tetrahymenas grown in air at atmospheric pressure contained a comparatively large number of fat globules which stain a deep orange-red with sudan III. These globules were located only at the anterior end of the organ-

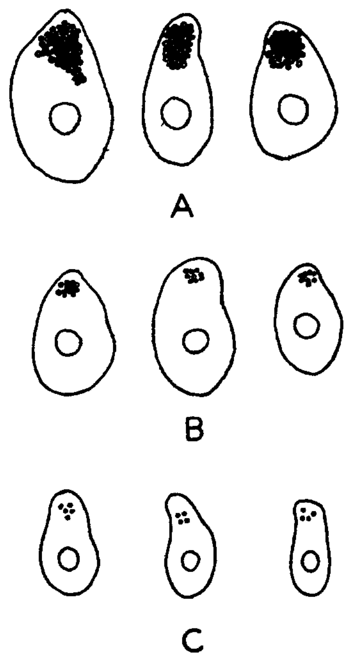

FIG. 2. Effect of CO_2 and O_2 on *Tetrahymena geleii.* Camera lucida sketches of organisms taken at random and stained with sudan III. Small circles represent fat globules. *A*, specimens from 2 per cent proteose-peptone solution in air at atmospheric pressure (737 mm.); *B*, specimens from solutions in pure O_2 (737 mm.); *C*, specimens from solutions in O_2 at 75 mm., CO_2 at 122 mm., and N_2 at 540 mm. pressure (total pressure = 737 mm.)

isms. In pure O_2 and in high CO_2 plus O_2, the fat content decreased considerably. It is evident that the decrease in each condition was not caused by the same factor or factors. In pure O_2, the organisms divided very rapidly, oxidative metabolism was high, and fatty oxidation was stimulated. In high CO_2 plus O_2, the tetrahymenas have evidently lost most of their power of synthesis, and thus very little protein, carbohydrate, or fat is formed.

Chilomonas, when grown in a favorable environment, contains a large quantity of stored food material in the form of starch granules and neutral fat. The presence of starch indicates that at least part of its metabolism is similar to that found in plants. That its metabolic behavior is peculiar, compared to other animal cells, is now well known. Mast and Pace (1933) maintained that it produces starch, fat, and protein in a wholly inorganic medium (with one part of CO_2 added to 5 parts air at atmospheric pressure). Mast, Pace, and Mast (1936) obtained relatively low values for the respiratory quotient of *Chilomonas* which, they suggest, might be due to the reabsorption of the CO_2 which the chilomonads form during respiration and which may then be used in the resynthesis of starch. Hutchins (1941), however, disputes this claim and suggests that these low R.Q. values are due to incomplete absorption of the CO_2 produced in the Warburg flasks which were used for ascertaining respiration rate.

In the investigations reported here, the results indicate that *Chilomonas* differs considerably in its metabolism from the ciliate, *Tetrahymena*. The latter will not live in extremely high concentrations of CO_2 in which *Chilomonas* flourishes even when the carbon dioxide pressure reaches 400 mm. On the other hand, *Tetrahymena* grows well in high O_2 tensions and maximum growth is found in pure O_2 at atmospheric pressure (or possibly higher). *Chilomonas* does not grow well in O_2 concentrations above 500 mm. and its maximum is at 75 mm. O_2 (approximately one-half that of atmospheric air). Furthermore, it has been observed that in the higher CO_2 concentrations chilomonads not only show an increase in growth but also show an increase in the quantity of starch produced, and in the size of the individual starch granules which are extremely large compared to those produced in chilomonads grown in atmospheric air. Undoubtedly the increased synthesis of starch is enhanced by the presence of CO_2. This is further evidence in support of the contention of Mast and Pace, that chilomonads utilize CO_2 in the synthesis of starch.

SUMMARY

1. The effects of O_2, CO_2, and pressure were studied in two very different species of protozoa, a flagellate, *Chilomonas paramecium*, grown in acetate-ammonium solution and a ciliate, *Tetrahymena geleii*, grown in 2 per cent proteose-peptone solution.

2. *Chilomonas* and *Tetrahymena* live and reproduce in solutions exposed to a wide range of O_2 concentrations, but *Chilomonas* is killed at high O_2 tensions in which *Tetrahymena* grows best. The optimum O_2 concentration for *Chilomonas* is about 75 mm. pressure but it lives and reproduces in O_2 tensions as low as 0.5 mm. while *Tetrahymena* fails to grow in concentrations below 10 mm. O_2 pressure.

3. With a constant O_2 tension of 50 mm. pressure, it was found that there is no significant variation in growth in *Chilomonas* between 50 mm. and 740 mm. total pressure. In *Tetrahymena*, however, under the same conditions, an optimum total pressure was found at about 500 mm. and growth is comparatively poor at 50 mm. total pressure.

4. *Tetrahymena* does not live very long in CO_2 tensions over 122 mm., although *Chilomonas* grows as well at 400 mm. CO_2 as in air at atmospheric pressure (0.2 mm. CO_2). *Tetrahymena* grows best in an environment minus CO_2, but the optimum for *Chilomonas* is 100 mm. CO_2 at which pressure an average of 668,600 $\pm$ 30,000 organisms per ml. was produced (temperature, 25 $\pm$ 1° C.).

5. Chilomonads grown in high CO_2 concentrations (*e.g.*, 122 mm.) produce larger starch granules and more starch than those grown in ordinary air at atmospheric pressure.

6. In solutions exposed to 75 mm. O_2 tension (optimum) and 122 mm. CO_2 plus 540 mm. N_2 pressure, chilomonads contain very little, if any, fat. This phenomenon seems to be due to the action of CO_2 on the mechanisms concerned with fat production.

7. In *Tetrahymena* exposed to pure O_2, there is very little fat compared to those grown in atmospheric air. This may be due to the greater oxidation of fat in the higher O_2 concentrations.

8. Further evidence is presented in support of the contention that *Chilomonas* utilizes CO_2 in the production of starch.

LITERATURE CITED

Hall, R. P., 1933, Growth of *Colpidium campylum* with reference to oxygen relationships, *Anat. Rec.*, **57**, suppl., 95.

Hutchins, J. O., 1941, The effect of the age of the culture on the rate of oxygen consumption and the respiratory quotient of *Chilomonas paramecium*, *J. Cell. and Comp. Physiol.*, **17**, 321.

Jahn, T. L., 1936, Effect of aeration and lack of CO_2 on growth of bacteria-free cultures of protozoa, *Proc. Soc. Exp. Biol. and Med.*, **33**, 494.

Lwoff, A., 1932, Recherches biochimiques sur la nutrition des protozoaires. Le pourvoir synthèse, Monograph de l'Institut Pasteur, Paris.

Mast, S. O., and Pace, D. M., 1933, Synthesis from inorganic compounds of starch, fats, proteins, and protoplasm in the colorless animal, *Chilomonas paramecium*, *Protoplasm*, **20**, 326.

Mast, S. O., and Pace, D. M., 1938, The relation between the hydrogen ion concentration of the culture medium and the rate of reproduction in *Chilomonas paramecium*, *J. Exp. Zool.*, **79**, 429.

Mast, S. O., Pace, D. M., and Mast, L. R., 1936, The effect of sulfur on the rate of respiration and on the respiratory quotient in *Chilomonas paramecium*, *J. Cell. and Comp. Physiol.*, **8**, 125.

Phelps, A., 1936, Growth of Protozoa in pure culture. II. Effect upon the growth curve of different concentrations of nutrient materials, *J. Exp. Zool.*, **72**, 479.

Reich, K., 1936, Studies on the physiology of Amoeba. I. The relation between nutrient solution, zone of growth, and density of population, *Physiol. Zool.*, **9**, 254.

Rottier, P. B., 1936, Recherches sur les courbes de croissance de *Polytoma uvella*. L'influence de l'oxygenation, *Comp. rend. Soc. biol.*, **122**, 65.