EFFECTS OF ALCOHOL ON THE LIFE CYCLE OF INFUSORIA.

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So many investigations have been undertaken to determine the effects of alcohol on the higher animals and with such varied results, that it is of interest to determine its effect upon the lowest and most generalized animal organisms, the Protozoa. As single-celled animals these forms cannot fail to give results of importance from the point of view of general cell-physiology, and lead to a clearer analysis of the primary effects of alcohol on metabolism. The Protozoa are particularly well fitted for cellular-physiological study, not only because as single free-living cells they lend themselves readily to experimental methods, but also because no one function predominates at the expense of the rest, and results obtained may reasonably be supposed to be due to the effects of the stimulus in question on the general metabolism of the cell.

Hunt[1] recently made some interesting experiments on the effect of small doses of alcohol on mice and guinea-pigs, and found that animals to which alcohol has been administered for some time acquire increased susceptibility to a definite poison (acetonitrile), and reached the conclusion that this increase in susceptibility is not due to a general "lowering of resistance," but is associated with a distinctly increased power of the body to break up the molecule of acetonitrile.

Calkins and Lieb[2] carried on some experiments with alcohol on *Paramecium* and found that ". . . alcohol has no effect when taken in too weak doses, and too powerful an effect when taken in over strong doses." ". . . when a medium dose is given (for example 3 parts of 1/1,000 alcohol to 2 of hay, or 1 part of

[1] Reid Hunt, "Studies on Experimental Alcoholism," Bull. No. 33, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1907.

[2] Gary N. Calkins and C. C. Lieb, "Studies on the Life-History of Protozoa,—II. The Effects of Stimuli on the Life-Cycle of *Paramecium caudatum*," *Archiv für Protistenkunde*, 1902.

1/500 alcohol to 4 parts of hay) the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series." "There is no doubt that for a time at least, alcohol will prevent death during periods of depression. . . ." ". . . there is evidence that . . . the general vitality would decrease under the constant stimulus as it does under treatment with hay infusion alone, although much more slowly." "Notwithstanding the more rapid living, the general vitality does not seem to be affected badly by the alcohol." It was chiefly to determine the latter point that this investigation was begun, and I shall outline the progress of the work to the present time.

II. Methods.

I chose *Paramecium aurelia*[1] for the main line of experiments chiefly because considerable work has already been performed on this organism ; and because it is one of the more generalized of the ciliates ; and lastly because its cosmopolitan distribution renders it a convenient form to be studied in all laboratories. It seems to me to be more desirable, in the present state of our knowledge, to learn one form thoroughly, if that is possible, rather than to distribute our energies over a broader field. As a subsidiary line, for comparison and as a check on the *Paramecium* cultures, I employed a culture of *Stylonychia mytilus*.

The general method of carrying the cultures is identical with that which has been described in detail in an earlier paper,[2] so that a brief outline at this time will suffice.

A "wild" individual was captured and placed on a depression slide in five drops of hay infusion. This infusion was made by putting about three grams of hay or grass in 200 c.c. of tap water and then raising the temperature to the boiling point. This infusion was generally used as soon as it had again attained the room temperature. It was made fresh daily as a rule. Sufficient bacteria developed to provide ample nourishment for the infusoria, and since all precautions were taken in selection of the hay, etc.,

[1] The specific name *aurelia*, instead of *caudatum*, is adopted in accordance with the data advanced by Calkins, "*Paramecium aurelia* and *Paramecium caudatum*," Biol. Stud. Pupils of W. T. Sedgwick, Chicago, 1906.

[2] Lorande Loss Woodruff, "An Experimental Study on the Life-History of Hypotrichous Infusoria," *Journal of Experimental Zoölogy*, II., 4, 1905.

it is believed that a satisfactory culture medium for comparative work was obtained. When the isolated protozoön had divided twice, producing four individuals, each was isolated on a separate depression slide and thus were started the four lines of which each culture consisted. Thus, for example, *Paramecium*, culture I, comprised four lines, *I–a*, *I–b*, *I–c* and *I–d*. These lines were thenceforth kept distinct unless one became extinct through the isolation of a weak individual or through accident, in which case the line was started again from one of the three surviving sister lines of the same culture.

The rate of division was recorded daily for each of the four lines, and at the time of record an individual from each line was isolated on a clean depression slide in five drops of hay infusion. In computing the rate of division of the culture as a whole, with which we are alone concerned, the four lines (*a*, *b*, *c*, *d*) of the culture were averaged together and this result was again averaged for five-day periods. By this method it is believed that a just conception of the rate of division of the culture was obtained, as the average of the four lines largely obliterated the fluctuations in the division rate of any one line, which may not have been of much significance, or which may have been merely due to the isolation of a weak individual. One who has carried on cultures of protozoa for considerable periods cannot fail to recognize the fact that, as is to be expected, individuals vary greatly in their general vitality, etc., and it is necessary to isolate representative individuals. It is here that the personal equation of the experimenter comes into view, and therefore it is desirable that the same person should make the daily isolations.

The culture slides were arranged in small moist chambers to prevent evaporation. As in previous experiments of this nature, the minimum and maximum temperature of the room in the vicinity of the cultures was recorded daily, as indicated by a registering thermometer. By averaging the minimum and maximum points of each day for five-day periods the results obtained are quite satisfactory for comparative work. In the experiments under consideration, as in those of previous investigators in this field, the rate of division was taken as the indication of the physiological condition of the organisms; it being generally accepted

that this is the most accurate indication of the general metabolic condition of the protoplasm of organisms which is available.

The work was started at the Thompson Biological Laboratory of Williams College in the spring of 1907; was continued at the Woods Holl Marine Biological Laboratory during the summer, and is at present being carried on at the Sheffield Biological Laboratory of Yale University.

III. Details of Control Cultures.

(*a*) *Paramecium aurelia.*

The "wild" individual with which this culture (*Paramecium* I) was started was isolated from an infusion in the Williams laboratory on May 1, 1907, and has been kept continually under observation since that time, and is at present (January 25, 1908) in the 332d generation. The average rate of division of the four lines (*I–a*, *I–b*, *I–c*, *I–d*), again averaged for each five-day period during the life of the culture up to present time, is plotted by the familiar block method. By glancing at Figs. 1 and 2 (continuous line) it will be seen that the culture started off with a division rate of just two divisions per day, and at the present time, period 54, is averaging one and three quarters divisions per day.

I shall not attempt to analyze the division rate of the culture in relation to the life cycle of *Paramecium* at the present time, as in the experiments under consideration it is of interest solely as the "control."

(*b*) *Stylonychia mytilus.*

This culture (*Stylonychia* I) was started with a "wild" individual found in an infusion in the Yale laboratory on October 23, 1907, since which time it has been under daily observation and has been subjected to the same method and treatment as the *Paramecium* culture. It is at present (January 25, 1908) in the 165th generation. The rate of division is plotted in Fig. 3.

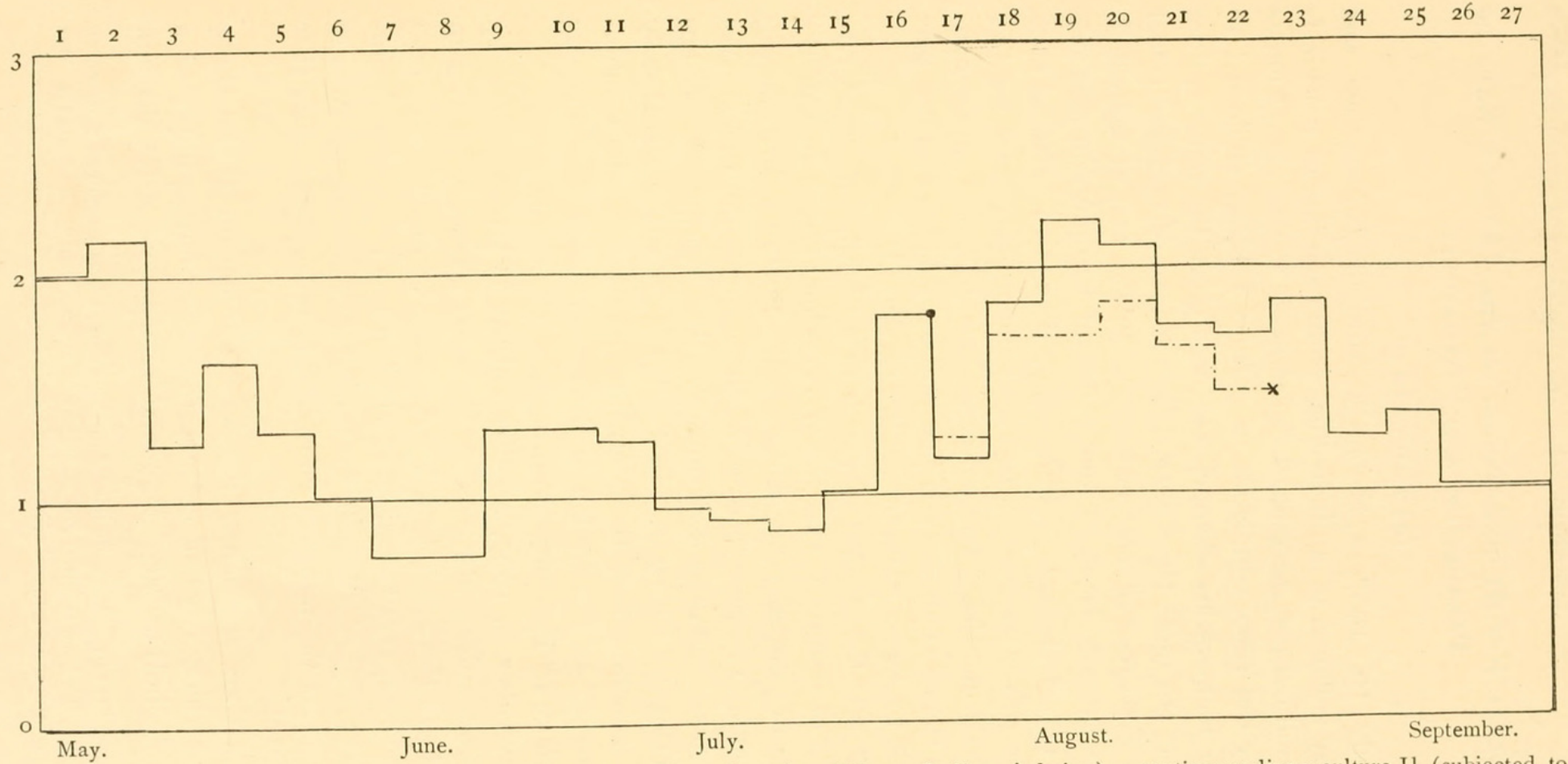

FIG. 1. Life-history of *Paramecium aurelia* (periods 1 through 27). Culture I (hay infusion) = continuous line; culture I^1 (subjected to 1/2,500 alcohol) = · - - - · line. ● = point of isolation of culture I^1 from culture I. × = point at which culture I^1 was discontinued. The figures above the diagram represent the five-day periods for which the rate of division is averaged. The figures at the left represent the average number of divisions per day of the four lines.

IV. General Effect of Alcohol on the Division Rate of Paramecium and Stylonychia.

(*a*) *Paramecium.*

On July 19, 1907, a second culture of *Paramecium* was started by isolation of an individual from each line of culture I. This second culture, designated *Paramecium* I^1, was carried under identically the same conditions as culture I, being subjected to the same temperature changes, etc., and given the same culture medium at the same time, except that instead of each individual receiving five drops of hay infusion (as in the case of culture I, the control), each received four drops of hay infusion and one drop of 1/500 alcohol. There were, then, two cultures each consisting of four lines which had been under observation for two and one half months. The only apparent difference in the conditions of the two cultures was that one was subjected to one part of alcohol to 2,500 parts of culture medium.[1]

The effect of the alcohol was seen in a slightly increased rate of division of culture I^1 (see Fig. 1, ·-·-· line) above that of the control during the first five-day period. But after that, during the remaining twenty-five days of the experiment, the alcohol caused a decrease in the division rate. This culture was discontinued on August 19, 1907. In Table I is given the actual daily record of generations of the control culture and of the alcoholized culture, as an illustration of the general method of keeping the records of all the cultures throughout the work.

On November 6, 1907, another culture (I^2) was started by isolation from culture I and given the same treatment as that to which culture I^1 was subjected. A glance at Fig. 2 (. -·-·- line) shows, however, that the effect of alcohol was this time a stimulation of the rate of division, for during the first thirty days of the experiment the rate was considerably more rapid than that of the control.

There is then, comparing the results of culture I^1 and those of the first thirty days of culture I^2, a clear-cut example of a stimulus (alcohol) causing a general retardation of the division

[1] Various amounts of alcohol were tried and the strength here employed was chosen as the one giving the best result. All strengths of alcohol which were tried at this period produced the same general effect.

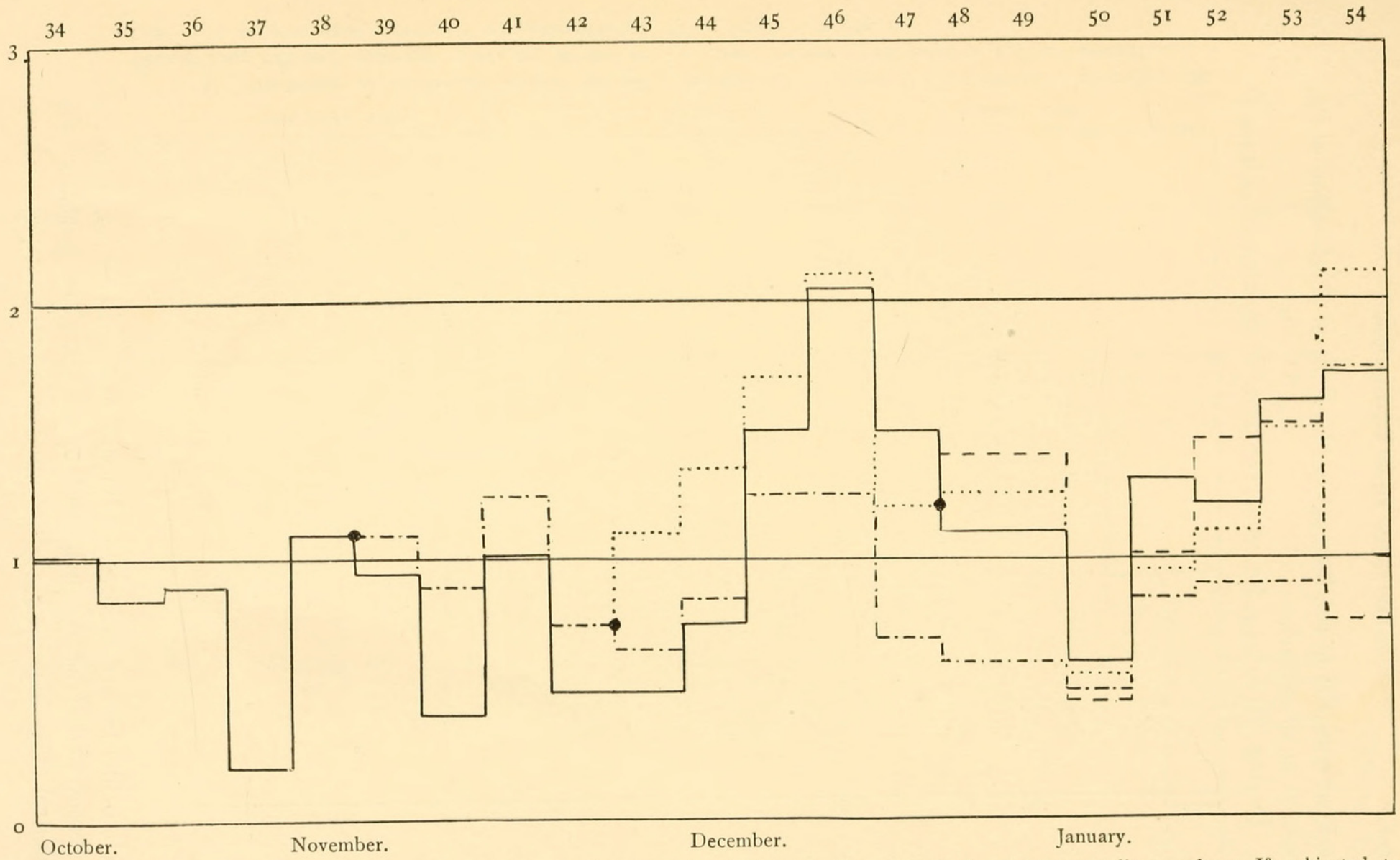

FIG. 2. Life-history of *Paramecium aurelia* (periods 34 through 54). Culture I (hay infusion) = continuous line; culture I^{2} subjected to 1/2,500 alcohol = - - - - - - line; culture I^{2a} subjected to 2/2,500 alcohol = · · · · line; culture $I^{2a'}$ subjected to 4/2,500 alcohol = - - - - - line. ● = points of isolation of the respective alcoholized cultures. Other details as in Fig. 1.

rate at one part of the life cycle and a general acceleration of the rate at another part.

During period 45, however, the rate of division of culture I^2

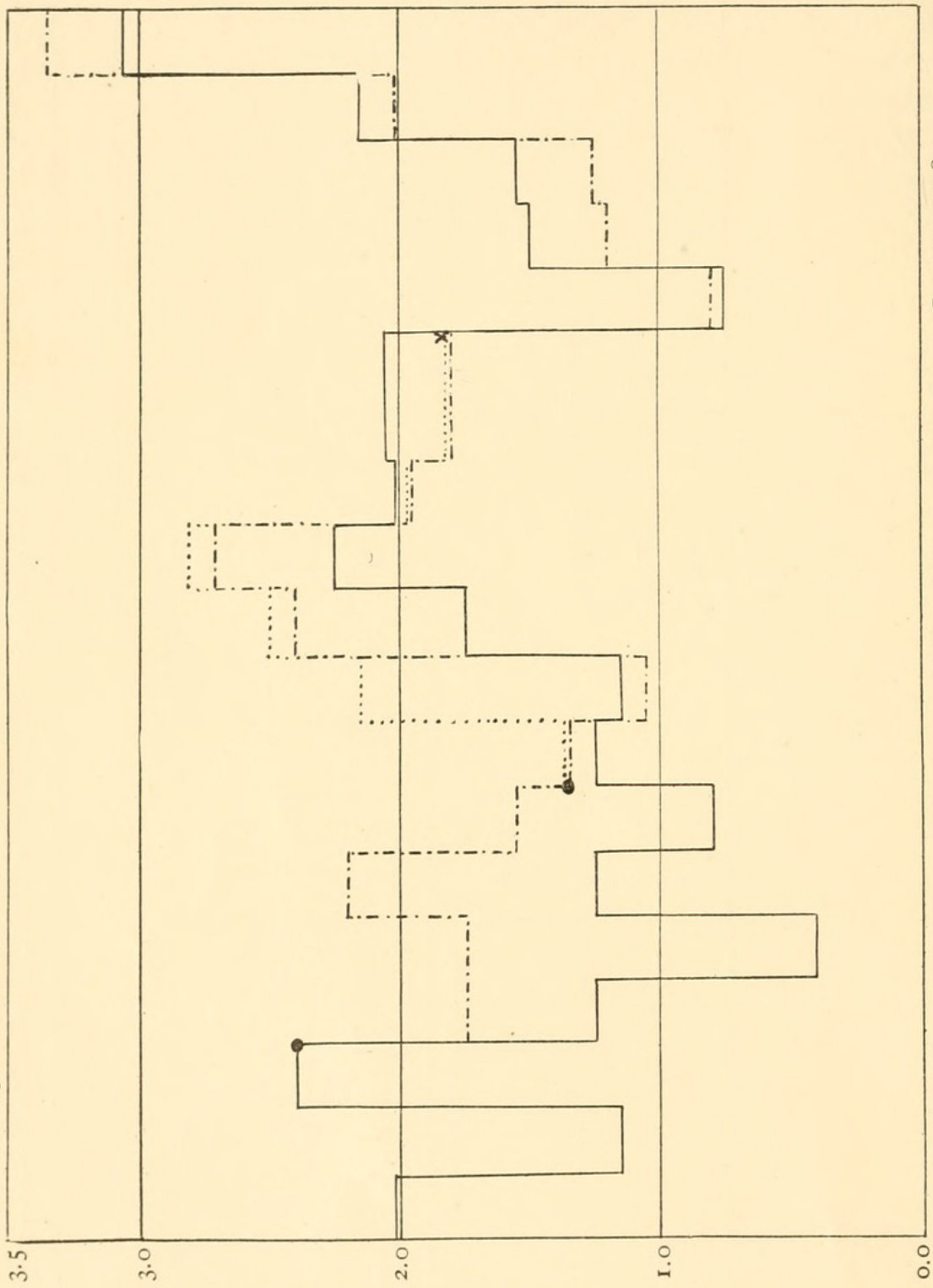

FIG. 3. Life-history of *Stylonychia mytilus* (periods 1 through 19). Culture I (hay infusion, control) = continuous line; culture I^1 subjected to 1/2,500 alcohol = · · · · · line; culture I^{1a} subjected to 2/2,500 alcohol = · · · · line; × = point at which culture $I^{1a'}$ was discontinued. Other details as in Fig. 1.

fell below that of the control (I) and has remained so up to the present period when it increased to the same rate as that of the control (Fig. 2, periods 45 through 54). It was suspected, how-

PARAMECIUM I.

Date.	July.													August.																		
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	71/76	71/77	67/76	65/75	66/72	66/71	70/75
Line *a* generations	100	101	101	103	103	b 106	107	110	111	113	115	117	120	121	124	126	128	130	132	134	137	138	141	143	145	147	148	150	151	153	155
" *b* "	99	100	101	102	104	106	107	110	111	113	115	118	120	122	124	126	129	130	133	135	138	139	142	144	146	148	149	151	152	154	156
" *c* "	97	98	98	100	100	102	104	107	108	110	112	115	117	119	121	123	126	127	127	a 134	138	139	142	144	146	147	149	152	153	155	156
" *d* "	100	101	101	103	105	107	108	110	111	114	116	119	121	123	125	127	129	131	133	135	138	138	140	142	144	146	147	150	151	153	155

PARAMECIUM I^1.

Date.	July.													August.																		
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	71/76	71/77	67/76	65/75	66/72	66/71	70/75
Line *a* from *I–a*.	100	101	101	104	104	106	108	110	111	113	115	117	119	120	122	123	125	127	129	131	133	134	135	138	139	141	143	146	147	149	151
" *b* " *I–b*.	99	100	101	103	104	105	107	107	108	110	112	113	115	116	118	120	122	123	125	127	130	131	133	135	137	139	140	143	144	146	147
" *c* " *I–c*.	97	98	99	100	101	103	105	107	108	111	113	115	117	118	120	122	124	124	125	127	130	131	134	136	138	140	141	144	145	147	149
" *d* " *I–d*.	100	101	102	104	105	107	108	110	111	113	115	117	119	120	122	124	126	127	129	130	133	133	135	137	139	139	140	140	140	141	141

TABLE I.

Daily Record of Paramecium Cultures I and I^1 from July 19, 1907, to August 18, 1907.

ever, that the accelerating effect of a given strength of alcohol would not be continuous, so at period 43 another culture was started line by line from culture I^2 in identically the same way except that the amount of alcohol administered was doubled, each individual receiving one drop of 2/500 alcohol and four drops of hay infusion. The rate of division of this series, designated culture I^{2a}, at once greatly increased (see Fig. 2, ... line) and kept considerably above the control during the first twenty days of the experiment. During the succeeding periods it fluctuated above and below the control and at the present time is dividing considerably faster than the control culture (Fig. 2 . . . . line).

During the early part of culture I^{2a}, there was again reason to believe that the increase in the division rate would not be permanent, so still another culture ($I^{2a'}$) was isolated from culture I^{2a} (during period 48), and was treated with double the amount of alcohol to which the parent culture was subjected, that is, with one drop of 4/500 alcohol and four drops of hay infusion. The result was still again an initial increase in the rate of division, though of shorter duration, followed by fluctuations above and below the control series (Fig. 2, - - - - line).

In view of the fact that culture I^1 showed a practically uniform depression of the division rate when subjected to alcohol, it was thought that possibly the depression effect which secondarily appeared in cultures I^2, I^{2a}, and $I^{2a'}$, might be due to the fact that the culture as a whole again had attained a period in the life cycle when alcohol had a depressing effect on cell division and, therefore, that the falling off of the rate of division of the organisms, after being a certain length of time subjected to the alcohol, was not due to the animals becoming accustomed to the alcohol. To test this point another culture (I^3) was isolated from the control (I) and carried for three periods of five days each on one drop of 1/500 alcohol and four drops of hay infusion. During this time the division rate of culture I^3 was consistently accelerated and as greatly as that of culture $I^{2a'}$ which was receiving four times as much alcohol, thus showing that the results obtained in the alcohol experiments were due to "acclimatization," and not to the fact that the organisms were in a period of the cycle characterized by a changed susceptibility to alcohol.

(*b*) *Stylonychia.*

On November 6, 1907, a second culture of *Stylonychia* (culture I^1) was started line by line from culture I in exactly the same manner as *Paramecium* I^1 was started from *Paramecium* I. The treatment which followed was identically the same as that already described for the *Paramecium* experiments — culture I^1 being subjected to one part of alcohol to 2,500 parts of culture medium. The effect of this treatment is shown in Fig. 3, beginning at period 4. It will be noted that the division rate of the alcohol treated series (· - · - · line) was very much more rapid than that of the control (continuous line) during the first five five-day periods of the experiment. It fell slightly below the control during the next period, but during the following two periods of the experiment it was again far more rapid than the control. Thus we find in the case of *Stylonychia* that the treatment with alcohol of the strength employed produced, as in the *Paramecium* (culture I^2) experiment, stimulation for about the first month of the work. The plotted curve shows also that from this point on the rate of division of this series fluctuated above and below the control and at the present period it is again exceeding that of the control culture.

On November 26, 1907, culture I^{1a} was started from culture I, and was thenceforth treated with double the amount of alcohol (one drop of 2/500 alcohol and four drops of hay infusion) to which the parent culture was subjected. The result, again similar to that of the corresponding *Paramecium* culture, shows an increased division rate for several five-day periods of the culture subjected to the increased amount of alcohol. (Cf. Fig. 3, period 8 through 14, · · · · · line.) Again the stimulating effect was not continuous, but instead, as in the previously described experiments, the division rate of the alcohol-treated line finally fell below that of the control and remained below until the experiment was discontinued on December 31, 1907 ; though a new culture isolated from this culture and stimulated with double the amount of alcohol showed an increased division rate at first, and later decreased division rate as compared with that of the control culture.

V. Does Alcohol Cause a General Lowering of Resistance to Changes in the Environment?

Experiments to determine if treatment with alcohol will cause a general "lowering of resistance" to inimical changes in the environment are in progress, and the experiments described in this paper outline the method which is being employed in such a study.

For this work certain of the previously described cultures are being used, viz.,

Paramecium.

I = hay infusion.
I^2 = hay infusion + 1/2,500 alcohol.
I^{2a} = hay infusion + 2/2,500 alcohol.
$I^{2a'}$ = hay infusion + 4/2,500 alcohol.

Stylonychia.

I = hay infusion.
I^1 = hay infusion + 1/2,500 alcohol.
I^2 = hay infusion + 2/2,500 alcohol.

On December 21, 1907, an individual was isolated from each line of the first three *Paramecium* cultures and from each line of the three *Stylonychia* cultures, and treated in identically the same way as the culture from which they were taken except that each was subjected to one part of copper sulphate in 1,250,000 parts of culture medium.[1] For example, the culture I^2_c isolated from *Paramecium* I^2 received daily three drops of hay infusion plus one drop of 1/500 alcohol plus one drop of 1/250,000 copper sulphate, *i. e.*, it received one drop of 1/250,000 copper sulphate in place of one of the drops of hay infusion received by culture I^2.

The six cultures isolated were then as follows:

Paramecium.

I_c = hay infusion + 1/1,250,000 $CuSO_4$.
I^2_c = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.
I^{2a}_c = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.

Stylonychia.

I_c = hay infusion + 1/1,250,000 $CuSO_4$.
I^1_c = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.
I^2_c = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.

The results of these experiments are plotted in Figs. 4–7. These curves show that, in the experiments on *Paramecium*, the alcohol-treated cultures (Figs. 5, 6) died out under the administration of copper sulphate during the fifth period of experimentation, that

[1] Various solutions of copper sulphate were tried and the one employed was selected because it appeared to be the maximum strength which all the cultures could withstand.

is, after being subjected to copper sulphate for twenty-three days, whereas the culture which had never been subjected to alcohol survived under the copper sulphate treatment until discontinued (Fig. 4). A closer analysis of the curves shows that during the earlier periods of the experiment the division rate of the organisms treated with 1/2,500 alcohol was somewhat less affected by the copper sulphate treatment than the division rate of the non-

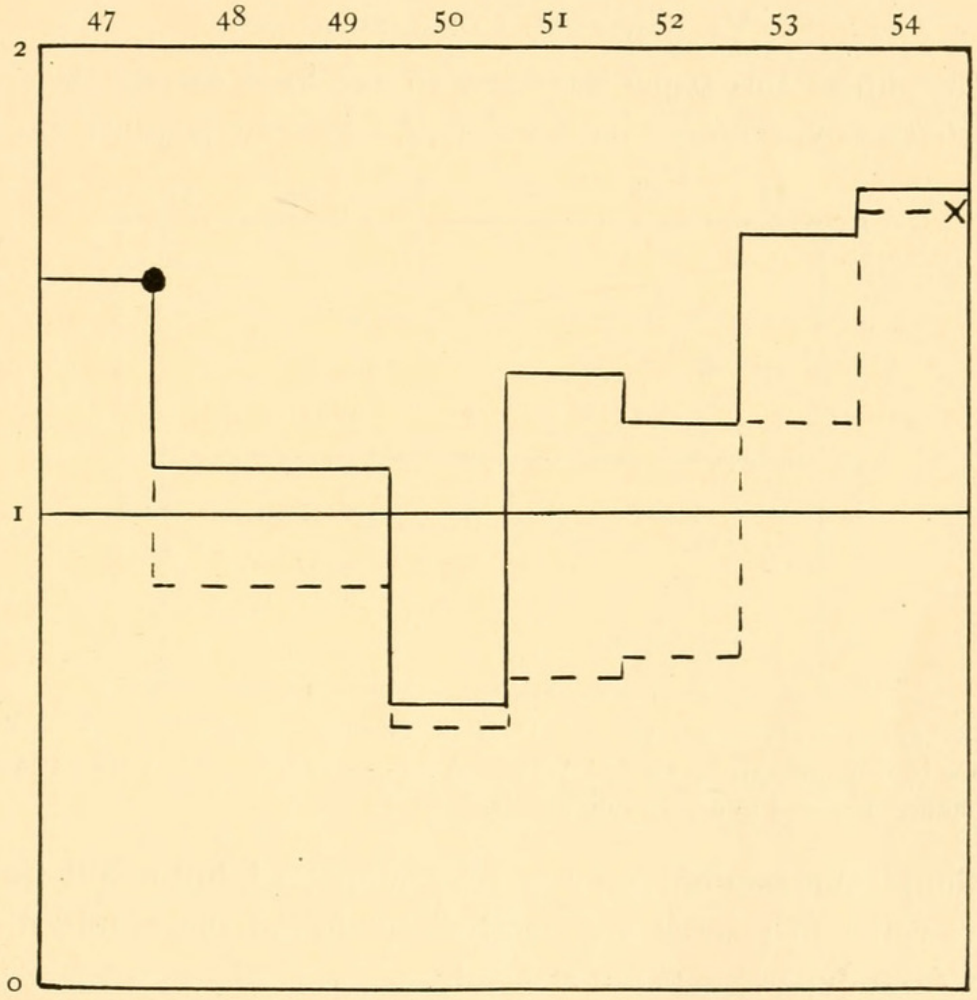

FIG. 4. *Paramecium.* Culture I (hay infusion, control) periods 47 through 54 = continuous line; culture I_c subjected to $CuSO_4$ = - - - - - line. × = point at which culture I_c was discontinued. Other details as in Fig. 1.

alcoholized line, but this was merely temporary. The lines subjected to the greater amount of alcohol (Fig. 6), which were averaging a higher rate of division than the lines treated with the less amount (Fig. 5), were more susceptible to copper sulphate from the beginning than either the non-alcoholized line (Fig. 4) or the line on the less amount of alcohol, and finally died out.

The copper sulphate experiments on the *Stylonychia* culture were carried on for only ten days, but increased susceptibly to copper sulphate is shown by the alcoholized lines (cf. Fig. 7).

The division rate of the three *Paramecium* cultures and the three *Stylonychia* cultures subjected to copper sulphate shows distincly that the organisms which have been treated with alcohol are less resistant to copper sulphate.

VI. General Considerations.

The aim of this paper has been to set forth merely the facts which the experiments have revealed. Several possible causes

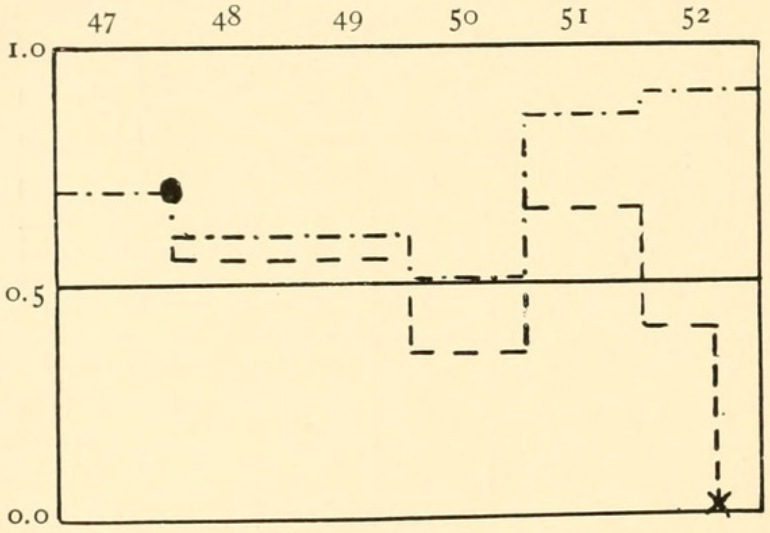

Fig. 5. *Paramecium.* Culture I^2 (hay infusion + alcohol) = · · · · · line; culture $I\frac{2}{c}$ (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

of the phenomena observed may be suggested, but it will be of more value to reserve a general discussion of these until more data from experiments are at hand.

Calkins and Lieb, as already mentioned, found that alcohol in medium doses, *e. g.*, one part of alcohol in 2,500 parts of culture medium, acted as a continued stimulus to the division rate of *Paramecium.* The results of my experiments obtained up to the present time fail to show such a marked uniformity of effect from alcohol treatment, as a depression of the rate of division, followed by fluctuations above and below the control, was the result in *Paramecium* I^2 and in all the succeeding cultures of both species, after an initial stimulation of the division rate of a longer

or shorter duration. Since these experiments were conducted with the same general method as that employed by these authors, except that I have carried four lines instead of one line of each alcohol culture and therefore have the average rate of division, the cause of this variation in the results is not apparent. It seems to be clear, however, that alcohol in optimum amounts does usually cause an increase in the rate of division for a certain length of time, and then a falling off of the rate. Whether the

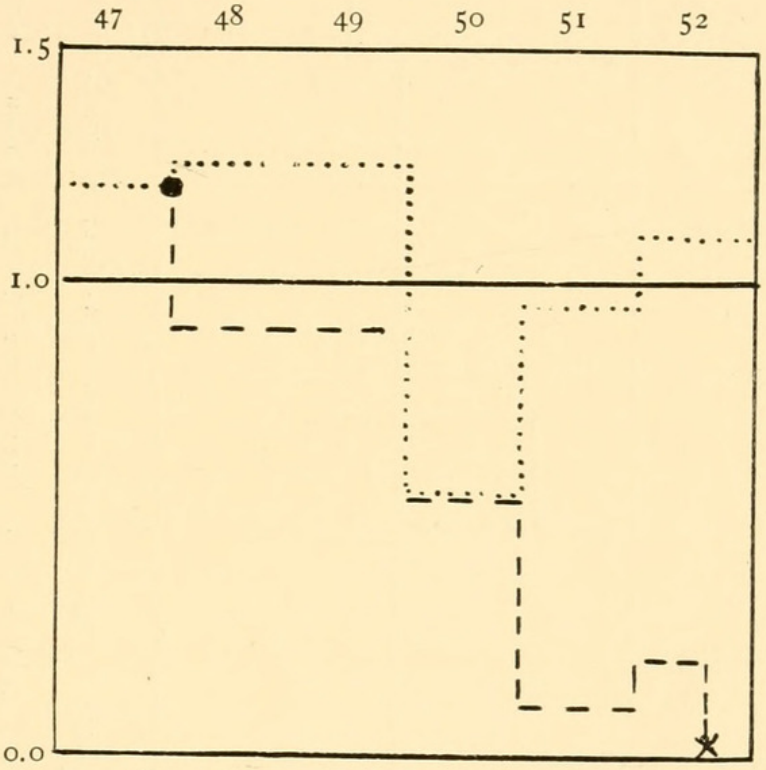

FIG. 6. *Paramecium.* Culture I^{2a} (hay infusion + alcohol) = ····· line; culture $I^{\frac{2a}{c}}$ (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

continued stimulation with alcohol will cause a prolongation of the cycle beyond the normal one of the non-alcoholized lines, as found by Calkins and Lieb, remains to be seen.

It is generally accepted at the present time that alcohol has a tendency to prevent the oxidation of other material in the body by its own oxidation — thus alcohol is a "food" rather than a "drug." Without attempting to consider in detail the question of alcohol as a "food" in relation to the metabolism of the protozoa, about which too little is known, I believe that the effect which alcohol exerts on the division rate of infusoria is not to be

interpreted as an increase in the available food supply of the organisms or as a substitute therefor. Any increase in the number of bacteria in the alcohol cultures due to an effect of alcohol on the reproduction of the bacteria can, I believe, be disregarded

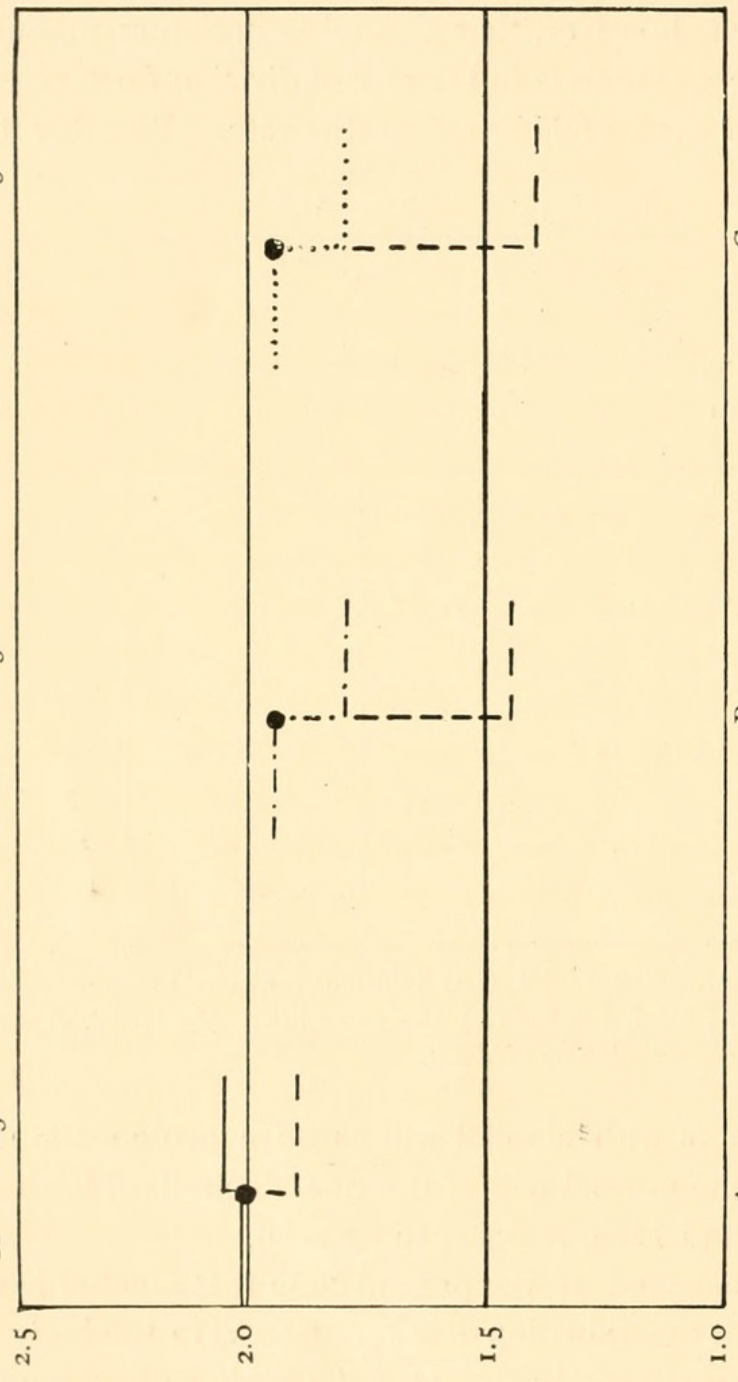

FIG. 7. *Stylonychia.* A. Culture I (hay infusion) = continuous line; culture I_c subjected to $CuSO_4$ = - - - - line.

B. Culture I^1 (hay infusion + alcohol) = . - . - . - line; culture I^1_c (hay infusion + alcohol + $CuSO_4$) = - - - -line.

C. Culture I^{1a} (hay infusion + alcohol) = ... line; culture I^{1a}_c (hay infusion + alcohol + $CuSO_4$) = - - - - line.

because the control cultures show that bacteria develop in the freshly made hay infusion far more rapidly than they are consumed by the animals under the conditions of the experiment. If it be assumed that alcohol serves in a limited sense as a food

in that it is more easily oxidized than the products of the bacteria, and therefore the alcohol-treated lines are able to assimilate more food, I think the fact that the increased division rate of the alcoholized lines is not permanent, but gradually declines and falls below the division rate of the control, suggests that the effect of the alcohol must be more subtle. Again the decrease in the rate of cell division due to alcohol at the early period of the cycle is not so readily explained on the assumption that alcohol is a "food" for the organisms in question.

It is obvious that the movements of the organisms in the culture medium is considerably more rapid, as a rule, when treated with alcohol. This might suggest, since food is largely received through vortex currents passing down the peristome, that more food is thus secured; but I believe that this suggestion is answered negatively by the fact that alcohol does not have a consistently accelerating action.

It might be suspected that an osmotic change brought about by the strength of alcohol used would be sufficient to influence the division rate of the infusoria, but the osmotic change is so exceedingly small that there is no reason to believe, from what is known of the effects of the phenomenon on the cleavage of eggs, etc., that any effect in this case is to be attributed to it.

As far as the experiments go I believe that they indicate that alcohol has a stimulating effect on some aspect of metabolism — possibly, as Calkins has suggested in this connection, on the secreting activities of the protoplasm. I think the evidence derived from the experiments justifies the idea that, in the case of the forms studied, alcohol supplies no "energy," so to speak, but stimulates the liberation of the "initial of potential" with which the organism is endowed.

In other words, we are justified in looking upon the protozoan cell as possessing a certain amount of metabolic energy, or it might be termed "division energy." In the normal course of the cycle, this is gradually expended in reaching the number of generations, more or less, for which the individual is endowed; but when alcohol, for example, is encountered in the environment this tends, directly or indirectly, toward a more rapid liberation of the division energy with the result that

multiplication is more rapid and more generations, for a certain length of time, are produced. But this stimulation of reproduction is not permanent, and in fact the division rate falls temporarily below the normal for the culture, as is shown by the rate of division of the control. Consequently the actual number of generations attained in the cycle is but slightly affected. From this point of view the alcohol has an effect on the individual cell of the cycle — but not on the cycle as a whole. That is, it influences the rate of reproduction but does not affect the number of generations which otherwise would be attained. This assumption will explain possibly the opposite effects produced by alcohol on the cultures at different periods of the cycle. Figs. 1 and 2 show that when the division rate is rapid the alcohol has a general depressing effect — and this may be due to the fact that the maximum division energy is being expended already, whereas when the division rate is on the decline, then the alcohol "stimulates" temporarily, and a greater number of bipartitions occur in a given period, than is the case in the control culture.

It is a point of considerable interest that alcohol produces opposite effects on the division rate at different points in the cycle, and this shows the danger of drawing conclusions from experiments on short cultures or on individuals about the ancestry of which little or nothing is known, which have been isolated merely from stock cultures. The same point is illustrated by some previously published experiments with the salts of potassium [1] — in which it was found that the dibasic potassium phosphate caused an acceleration of the rate of division during the early part of the cycle, and a retardation of the rate during the later part of the cycle. It is to be noted, however, that alcohol caused a retardation of the rate during the early part of the cycle of the *Paramecium aurelia* culture, whereas K_2HPO_4 caused an acceleration of the rate during the early part of the cycle of the *Oxytricha fallax* culture.

To draw any general conclusions from the experiments on copper sulphate at the present time would be hazardous as the results obtained, though definite, are insufficient. The data

[1] Woodruff, *loc. cit.*, pp. 617–619, Diagram IX.

show that organisms which are subjected for long periods to small amounts of alcohol, and which have attained a greater number of generations than the non-alcohol series, are more susceptible to copper sulphate; and that the lines which were subjected to the greater strength of alcohol are more susceptible to the copper sulphate than the series treated with the less strength. This shows clearly that alcohol in such amounts which may be said to be "beneficial" from the standpoint of cell metabolism, since more cell divisions have occurred, nevertheless renders the cells more susceptible to the "injurious" effects of copper sulphate. In what way this is brought about is not evident from the results obtained to date. It seems improbable that we are justified in assuming that the alcohol has caused a general "lowering of resistance" in view of the fact that the general effect of the alcohol is to increase cell division. The results suggest that probably alcohol exerts some specific effect on the metabolism of the organism, or possibly as has been suggested, for example, effects some change in the permeability of the cell membrane to copper sulphate.

VII. Summary.

The experiments briefly recorded were conducted for considerable periods on two species of Protozoa, whose status in the life-cycle was known through long cultures, and on a sufficiently large number of individuals to afford reliable averages. It is believed, therefore, that the results obtained show the general effect of alcohol on the division rate and, therefore, on the metabolism of the forms studied, when subjected to a practically constant environment.

The evidence brought forward shows that:

1. Minute doses of alcohol will decrease the rate of division at one period of the life cycle and increase it at another period of the life cycle.

2. When alcohol increases the division rate, the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control, followed by fluctuations above and below the rate of the control.

3. An increase (doubling) of the amount of alcohol adminis-

tered, however, will again cause a more rapid cell division for a limited period. But again the effect is not constant since the rate of division falls below the control, and is followed by fluctuation above and below the division rate of the control. Up to the present time the amount of alcohol has been increased (doubled) three times, always with the same result.

4. Treatment with alcohol lowers the resistance of the organisms to copper sulphate.

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