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(Osborn Zoölogical Laboratory, Yale University.)

Studies on the physiology of the euglenoid flagellates.

VI. The effects of temperature and of acetate on *Euglena gracilis* cultures in the dark.

By

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(With 2 figures in the text.)

The literature on the effect of temperature on *Euglena* has been reviewed previously (Jahn, 1933), and in the present study only one paper seems to be pertinent: that of Zumstein (1900) who found that growth of *Euglena gracilis* was 500 times as great at 24° C as at 16° C in a period of eight days when cultures were exposed to daylight.

The present study is an attempt to determine the temperature-growth relationships of *E. gracilis* Klebs when cultured in the dark. In view of the results obtained by previous workers on the effect of acetate compounds on the growth of flagellates, two groups of experiments were performed, one with and one without an acetate compound. The literature concerning the effect of acetate on various species of *Euglena* has been reviewed by Lwoff (1932) and Dusi (1933, 1933 a), and our knowledge of the subject has been greatly increased by the work of these two investigators. It was found that none of the species of *Euglena* (*E. deses*, *E. gracilis*, *E. stellata*, *E. klebsii*, *E. anabaena*, or *E. pisciformis*) was capable of continued growth in the dark in media of protein decomposition products. However, if sodium acetate were added *E. gracilis* grew very

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rapidly, but all of the others failed to grow. The present experiments show the temperature relationships of cultures of *E. gracilis* maintained in the dark, with and without acetate.

Material and methods.

The bacteria-free strain of *E. gracilis* KLEBS used in this study was obtained from Prof. E. G. PRINGSHEIM of the German University of Prague, and is the same as was used in previous papers of this series. The culture medium used was as follows:

NH_4NO_3	.50 gm.
KH_2PO_4	.50 gm.
$MgSO_4$	.25 gm.
NaCl	.10 gm.
Hydrolyzed casein	5.00 gm.
Distilled water	1000.00 cc.
Normal NaOH or HCl to the desired p_H value.	

Equal amounts (always 9 cc.) of the medium were measured directly into 16 × 150 mm. thin walled Pyrex test tubes by means of a SCHELLBACH side-arm burette. The tubes were autoclaved and stored in a cool place until used.

Stock cultures were grown in flasks, and before being used the stock culture (one for each series) was well shaken. Inocula of .9 cc. were then taken with a 12-inch graduated 1 cc. pipette. The initial number of organisms in a random sample of inoculated tubes from each series was determined by a counting method described previously (JAHN, 1929), and the average of these counts was assumed to be the initial number in all the tubes of that series. At the end of each series of experiments, the organisms were killed by heating to 60° C, and final counts were made on all tubes by the same method.

Experimental results.

Series I and II were performed with the standard medium as given above. In series I the initial concentration was 10.8 thousand per cc., and the duration of the experiment was 21 days. In series II the initial concentration was 3.3 thousand per cc., and the duration of the experiment was 42 days. The relative amounts of growth (x/x_0, x being the final number and x_0 the initial number) at the temperatures used in the two series are shown in Fig. 1. In each series four tubes were maintained at each temperature,

and the x/x_0 value for each temperature is the mean value of the four tubes. It is seen that the greatest amount of division took place at 10° C. In both series a number of the cells at temperatures of 15° C and above were non-motile. In series I about 90 % of the non-motile forms were division cysts, and in series II only about 25 % of the non-motile forms were encysted. The dash line in Fig. 1 shows the approximate percentage of non-motile (encysted and not encysted) organisms which was about the same in the two series. The general trend of the two curves is the same except at 23° C where series I shows a lower growth rate than series II. This difference might have been caused by the higher percentage of encysted forsm in series I or by the same factor which determined the percentage of encystment, and at present this factor is unknown. The initial and final p_H values in these two series were 6.7 ± .1 in all cases.

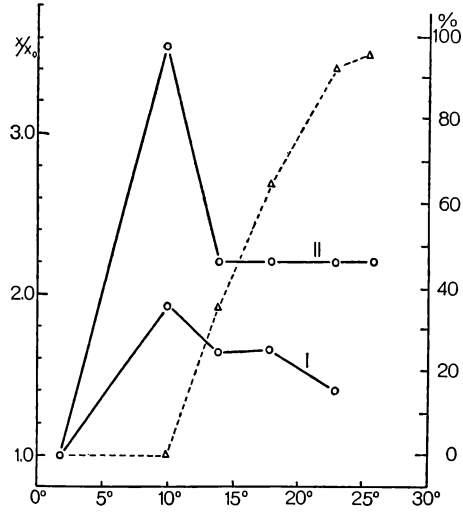

Fig. 1.

Series III was performed with the standard medium plus 3 grams of sodium acetate per liter. The initial number of organisms was 3.24 thousand per cc., and the duration of the experiment was 13 days. The final numbers in thousands per cc. at different temperatures are shown below.

Temperature	2°	10°	15°	18°	23°	26°	30°
Final numbers	3.3	18	28	36	130	80	30

The results of this series, together with the results of the two preceding series are shown in Fig. 2. In this figure it is assumed that growth is approximately logarithmic for the duration of the experiment. On this assumption, velocity constants for the logarithmic equation have been computed, and these are plotted against temperature. The equation is the same as that used previously (Jahn, 1933 a): $D = 1/t \ln x/x_0$ where t is time, x the number at time t, x_0 the initial number, and D the velocity constant. In this equation D is also the average division rate per organism per day. This method

of expressing results allows the comparison of the average division rates rather than the relative amounts of growth for the time period considered. Since x and x_0 may be measured with an accuracy of 5 or 10 %, depending inversely upon the square root of their values, the value of 1/t times, the logarithm of their ratio is probably accurate in most cases to one unit in the second decimal place, and this might be considerably increased where large values of x (over 50 thousand per cc.) are involved. Since x_0 is always small it is the greatest source of error in computing values of D. However, since it is always the same in all tubes of a given series it is a constant error which does not affect the relative differences in D values within any one series. This method also allows widely different x/x_0 values to be denoted on the same graph by fractions of their logarithms.

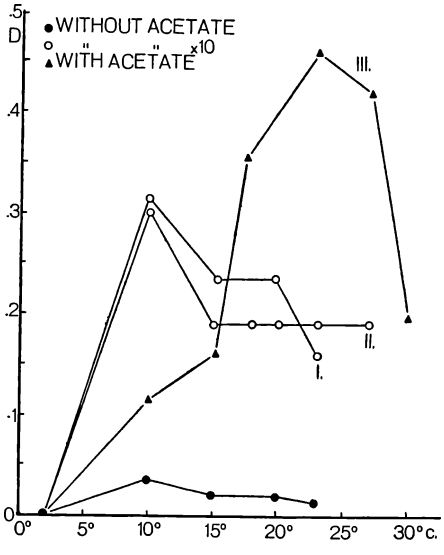

Fig. 2.

It is seen from Fig. 3 that the highest division rate in series III occurred at 23° C and that the division rate was lower above and below this optimum. The comparison with series I and II is interesting in that the optimum is shifted from 10° C to 23° C by the addition of acetate and in that the actual division rate is enormously increased from an optimum of .030 (series II) —.031 (series I) at 10° C without acetate to an optimum of .47 with acetate at 23° C — an increase of over 15 times. Comparison of the same series at 23° C shows a division rate of .016 (series I) and .019 (series II) without acetate and .47 with acetate — an increase of 24—28 times. In series III the point at 10° C is somewhat higher than one would expect if the curve were smooth, and this can easily be explained as the summation of the optimum at 10° with the otherwise "normal" position of the point. Whether or not this is true has not been determined.

The initial p_H value in this series was 6.7 in all cases. However, the final p_H values, especially in the cases where rapid growth

occurred, were decidedly more alkaline. The final p_H values were as follows: 2° C, 6.7; 10° C, 6.9; 15° C, 7.0; 18° C, 7.5; 23° C, 7.8; 26° C, 7.6; 30 C, 7.0. All values are accurate to ± .1 unit. This large change in p_H with growth in acetate media is probably due to the selective utilization of the acetate ion in preference to the sodium ion. Similar results have been reported with ammonium and nitrate ions by MAINX (1928) and have been explained in the same manner. The oxidation product of acetate is probably carbonate, and this would readily render the cultures alkaline. Accurate measurements of the p_H changes in acetate cultures of *Chilomonas paramecium* have been made previously (JAHN, 1935).

In the acetate cultures (series III) no encystment was found at 2° C. At 10° approximately 90 % of the organisms formed division-cysts; at 15° only about 40 % were in the form of division-cysts. However, at 18° and 23° about 50 % of the organisms were in the form of thin walled cysts or palmella stages, and at 26° about 90 % and at 30° almost 100 % of the organisms were in this condition. The palmella stages were very similar in appearance to those found in old cultures in the light except that they were a cream-yellow instead of green.

Experiments similar to the above were attempted with *E. deses*. However, it was found that this species would not grow in the dark in casein peptone or in casein peptone plus acetate. This necessity of light for the growth of *E. deses* has also been noted by DUSI (1933 a).

Discussion.

The first group of experiments in the present paper are very interesting in view of the studies of LWOFF and DUSI (1929, 1931) and DUSI (1933, 1933 a) who found that with well hydrolyzed peptones, comparable to the hydrolyzed casein used by the writer, *E. gracilis* when grown in the dark was not indefinitely transplantable unless acetate were present. At the division rate found in the present experiments without acetate (an average of less than one division in three weeks at the optimum of 10° C) it would require more than two years for a 12 cc. culture inoculated with one drop (the inoculum used by LWOFF and DUSI) to become as concentrated as the parent culture had been. In order for cultures to be indefinitely transplantable the newly inoculated culture must, on the average, become as concentrated as the parent culture before the next inoculation is made. Therefore, such cultures could not

be maintained indefinitely unless inoculations were less frequent than once every two years. If the temperature were 15° C or higher the division rate would be still lower, and encystment would probably make transfer very difficult. In a case of this sort it might be the shortness of time between transfers and not the ability of the organism to grow that causes the loss of the strain through continual dilution. In the case of the present organisms, however, it is not known how long division rate of .02 divisions/day/organism can be continued. The only quantitative data bearing on the problem is that afforded by series I and II. In these experiments it is seen that the division rate for the first three weeks (series I) is somewhat higher than that for the first six weeks (series II). This seems to indicate that division might cease after a longer period of time.

A shift in the optimal temperature, comparable to the one described above, must also occur under other conditions. The optimal temperature, which in the present case seems to depend upon the presence or absence of acetate, might also depend upon the presence or absence of light. Although no experimental data is available the optimal temperature for *E. gracilis* in the light in a casein peptone medium is probably about 25° C, and it can be grown for more than a year in this medium at a temperature of 28—29° C. This is in agreement with the experiments of ZUMSTEIN (1900). The difference between this optimum in the light and the 10° C optimum in the dark is explainable on the basis of a change in the type of metabolism from saprozoic plus autotrophic to pure saprozoic. The change of optimum in the dark from 10° C to 23° C must be due to a change from a diet of casein peptone to one of casein peptone plus acetate. It is obvious, therefore, that the optimal temperature for growth depends not only upon the specific characters of the organism but also on the chemical and physical conditions of the experiment. Inasmuch as growth is the summation of innumerable metabolic processes, and as some of these processes may be affected by various factors independently of the others, this relationship between optimal temperature and environmental factors is to be expected.

Summary.

1. *E. gracilis* was grown in bacteria-free culture in the dark in a medium of hydrolyzed casein peptone with and without Na-acetate at different temperatures.

2. Without acetate the maximal rate of division (.031 divisions/day/organism) was attained at 10° C. Above this temperature an increasingly large number of organisms became encysted or otherwise non-motile. Above 23° C the non-motile forms comprised 90—95 % of the total.

3. When Na-acetate was present (3 gm./liter) the division rate was enormously increased (maximum = .47 divisions/day/organisms), and the optimal temperature was found to be about 23° C. Division-cysts were formed at 10° and 15°, and palmella stages were formed at 18° and above.

4. *E. deses* could not be grown in the dark in casein peptone or in casein peptone and acetate.

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