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Studies on the physiology of the euglenoid flagellates.

VII. The effect of salts of certain organic acids on growth of *Euglena gracilis* Klebs.

By

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The literature concerning the effect of salts of organic acids on growth of *Euglena gracilis* and other species of *Euglena* has been thoroughly reviewed and extended by Dusi (1933, 1933 a). In the present experiments the strain of organisms and the methods of culture and enumeration are the same as have been used previously. The medium used was:

NH_4NO_3	.50 gm.
KH_2PO_4	.50 gm.
$MgSO_4$	.25 gm.
Hydrolyzed casein	5.00 gm.
Distilled water	1000.00 cc.

Nine cc. of the stock medium was measured into each of a number of Pyrex test tubes. Then 1cc. of a M/160 or N/160 solution of the salt to be tested was added before autoclaving. After autoclaving, the tubes were inoculated with .9 cc. of a stock culture grown in the standard medium. The organisms were allowed to grow for 7.5 to 14 days and were then killed by heating to 60°, and counts were made in the usual manner.

Four series of experiments were performed. Series I and II were started at the same time from the same stock solution and

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culture. The initial concentration of organisms was 3 thousand per cc., and the concentration of tested salts was M/160. Series I was placed in a rack by a north window. Series II was placed in the dark in a closed box and was maintained at the same room temperature (about 21° C) as series I. Series I was killed after 8 days, and series II after 14 days. Series III and IV were similar to series I and II respectively, except that the concentration of salt was N/160 and the initial concentration of organisms was 5 thousand per cc. Series II was killed after 7.5 day and series IV after 14.0 days.

The results of these four series of experiments are shown in Table 1. The initial p_H was 6.7 ± .1 in all cases. The final con-

Table 1.

Salt tested	Series I $x_0 = 3$ t = 8.0			Series II $x_0 = 3$ t = 14.0			Series III $x_0 = 5$ t = 7.5			Series IV $x_0 = 5$ t = 14.0		
	Daylight			Dark			Daylight			Dark		
	x	D	Final p_H	x	D	Final p_H	x	D	Final p_H	x	D	Final p_H
Control (NaCl)	69	.39	6.7	6.3	.05	6.7	100	.40	6.7	11.1	.06	6.7
Na-formate	66	.38	7.0	5.7	.03	7.0	95	.39	7.0	7.0	.02	7.0
Na-acetate	234	.54	7.3	159.0	.26	7.5	320	.55	7.6	200.0	.26	7.6
Na-butyrate	351	.59	7.3	174.0	.28	7.5	425	.59	7.5	280.0	.29	7.6
Na-tartrate	—	—	—	—	—	—	102	.40	6.7	11.5	.06	6.7
Na-lactate	90	.42	6.7	39.0	.17	6.7	140	.44	6.7	110.0	.22	7.0
Na-citrate	—	—	—	—	—	—	102	.40	6.8	11.0	.06	6.8
Na-oxalate	99	.44	6.9	6.0	.04	6.7	150	.45	6.7	11.0	.06	6.7
Na-succinate	30	.29	7.0	9.9	.08	7.0	80	.37	6.3	13.8	.08	6.3
	Concentration of salt = M/160 Initial p_H = 6.7 ± .1						Concentration of salt = N/160 Initial p_H = 6.7 ± .1					

centrations (x), the division rates (D, computed as in the preceding paper), and the final p_H values are shown in the table. The p_H changes which occurred during growth were relatively small (.3 unit) except in the case of acetate and butyrate. As the growth rate does not change greatly with such small p_H changes in the range p_H 6.7—7.0 (JAHN, 1931), these changes are not believed to be important in the present experiments. The larger changes (to p_H 7.6) in acetate and butyrate may have produced a slight effect on the values of D. However, such an effect could not be great enough to change them appreciably.

A further analysis of these data is shown in Table 2. Here the value of D for chloride has been subtracted from the value of

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Light	Series	Conc.	Values of △D for
Daylight	I	M/160	butyrate +.20 > acetate +.15 > oxalate +.05 > lactate +.03 >
	III	N/160	butyrate +.19 > acetate +.15 > oxalate +.05 > lactate +.04 >
Dark	II	M/160	butyrate +.23 > acetate +.21 > lactate +.12 > succinate +.03 >
	IV	N/160	butyrate +.23 > acetate +.20 > lactate +.16 > succinate +.02 >

D for each of the other salts so that the values given (△D) are a measure of the amount of acceleration or deceleration which occurred. That is, $\triangle D = D_{NaR} - D_{NaCl}$, where NaR denotes the sodium salt of the tested compound. It is seen from this table that the values of D are in the following order in the light: butyrate > acetate > oxalate > lactate > tartrate, citrate, chloride, formate > succinate. In the dark this order is changed to: butyrate > acetate > lactate > succinate > tartrate, citrate, chloride, oxalate > formate.

In general, these results are in agreement with those of DUSI (1933) who found that both acetate and butyrate allowed very rapid growth in the dark. The present experiments show that both are very good, but that butyrate allows more rapid growth than acetate. Proprionate was also used, and it was found that the D values obtained were almost identical with those found for acetate. However, since there was some doubt as to the purity of the sample of proprionate used, the results are not included in the above data.

Oxalate and lactate, which were reported by LWOFF and DUSI as being incapable of replacing acetate in the dark, were here found to give a considerable acceleration in the light, oxalate giving $^1/_3$ and lactate giving $^1/_4$ to $^1/_5$ that found for acetate. Also, lactate was found to give a considerable acceleration (△D = .12 and .16) in the dark. Superficially these results do not seem to be in complete agreement with those of LWOFF and DUSI, but the difference is probably explainable on the basis of the criteria used. The data of table 1 show that a very definite acceleration was obtained in both light and darkness, while the data of LWOFF and DUSI show that lactate was not able to serve as a source of carbon in the dark, when four successful serial transfers were taken as the criterion.

ble 2.

Na compounds used					
		chloride 0 ,		formate 0 >	succinate —.10
tartrate 0 ,	citrate 0 ,	chloride 0 ,		formate 0 >	succinate —.03
		chloride 0 ,	oxalate 0 >	formate —.02	
tartrate 0 ,	citrate 0 ,	chloride 0 ,	oxalate 0 >	formate —.04	

Two possible explanations may be considered: 1. The acceleration shown in the present data is not indefinitely continuous. 2. The growth rate in the dark (.17—.22 divisions/day/organism) is low enough to cause the loss of cultures by dilution with the serial transfer method. In the present case the sample of sodium lactate used was an Eimer and Amend c. p. product. Lactate has also been reported as a suitable source of carbon for the colorless organisms *Prototheca zopfii* (Bond, 1932), *Polytoma uvella* (Lwoff, 1932; Bond, 1933) and *Colpidium campylum* (Bond, 1933), but was found inadequate for *Haematococcus pluvialis* or *Chlamydomonas algaeformis* (Lwoff, 1932). In the present experiments oxalate had no measureable effect on growth in the dark, and this is in agreement with the experiments of Lwoff and Dusi.

The results obtained with succinate make an interesting comparison with the above results with lactate. Succinate was found to produce a small but definite acceleration in the dark. However, in the light it was found to be toxic with an $\triangle D$ value of —.10 in M/160 concontration and —.03 in N/160 concentration. An explanation of this result is not evident. Succinate was found by Lwoff and Dusi (1931) to be unsuitable as a carbon source in the dark.

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