Biological Laboratory, University College, New York University and Osborn Zoological Laboratory, Yale University.

Studies on the physiology of the euglenoid flagellates.

V. The effect of certain carbohydrates on the growth of *Euglena gracilis* Klebs.

By

Theo. L. Jahn[1]).

Since the demonstration by Zumstein (1900) that *Euglena gracilis* is capable of purely saprophytic nutrition, the question of utilization of particular organic substances has attracted the attention of several workers. It is generally agreed that *E. gracilis* is capable of growing in solutions containing protein decomposition products even when photosynthesis is prevented, but the ability of the organism to utilize various carbohydrates has been disputed. The results that have been obtained are by no means uniform, and in some cases exactly opposite, although apparently valid, results have been obtained by different investigators. For this reason it was considered advisable to attack the problem with the quantitative methods employed in a previous paper (Jahn, 1931) and to endeavor to explain, if possible, the divergent results of previous workers.

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[1]) National Research Fellow in Zoölogy.

Historical survey.

A study of the nutrition of *E. gracilis* was first undertaken by ZUMSTEIN (1900) who demonstrated that, although *E. gracilis* ordinarily lives by mixotrophic methods, it is also a facultative autotrophic as well as a facultative heterotrophic organism. He found that it was able to grow in the light in synthetic inorganic media and that is also grew very well in peptone medium in the dark. By adding various carbohydrates to his synthetic medium, he attempted to determine the ability of *E. gracilis* to utilize the substances in question. He obtained good cultures with dextrose, average cultures with maltose, lactose, and raffinose, relatively poor cultures with levulose, and poor cultures with sucrose.

TERNETZ (1912) investigated the acceleration produced by dextrose, mannit, sucrose, and maltose. She found that dextrose produced a far greater acceleration than the others and that mannit produced a slightly higher acceleration than sucrose and maltose. However, in a duplicate experiment with dextrose, the acceleration attained was less than with mannit and only very slightly higher than with sucrose.

PRINGSHEIM (1912), working with bacteriologically pure cultures of *E. gracilis*, was unable to find any acceleration of growth by dextrose in either peptone or inorganic media, in either light or darkness. He found that in some cases dextrose appeared to be injurious in that it inhibited growth of the flagellates. His work stands in direct contradiction to that of ZUMSTEIN (1900) and TERNETZ (1912).

MAINX (1924, 1928) found that when photosynthesis was prevented, *E. gracilis* showed only a doubtful growth in an inorganic medium to which dextrose had been added. No growth whatever was obtained with sucrose, mannit, or glycerol. He also found that cells devoid of paramylum could build paramylum in the dark from dextrose, but not from sucrose, mannit, or glycerol.

It is apparent that, at the beginning of the present investigation, the ability of *E. gracilis* to utilize various carbohydrates was certainly questionable. Some of the experimental evidence seemed to indicate that dextrose can be utilized (ZUMSTEIN, 1900; TERNETZ, 1912; MAINX, 1928); whereas other results suggested that dextrose produces a deceleration (TERNETZ, 1912; PRINGSHEIM, 1912). None of these investigators was able to detect an acceleration of growth

in cultures containing sucrose or glycerol; mannit, however, was reported by TERNETZ (1912) to be usable, while negative results were obtained by MAINX (1928).

The literature concerning the effect of substances other than carbohydrates on *E. gracilis* has been thoroughly reviewed and considerably extended by DUSI (1933).

Material and methods.

The bacteria-free strain of *E. gracilis* Klebs used in these experiments was the same one obtained from Professor E. G. PRINGSHEIM of the German University of Prague and used in previous papers of this series (JAHN, 1931, 1933). The methods of culture and of determining the amount of growth are also the same as those described previously (JAHN, 1931, 1929), and the cultures, unless otherwise noted, were maintained under conditions of constant temperature (28,3° C) and light.

The standard medium adopted for this series of experiments was as follows:

NH_4NO_3	.50 gm.
KH_2PO_4	.50 gm.
$MgSO_4$	.25 gm.
NaCl	.10 gm.
Distilled water	100.00 gm.
Normal NaOH to standard p_H value.	

The substance to be tested for growth acceleration was made up as a .25 % solution in the standard medium. This standard medium is the same as that used previously, except that ammonium nitrate is substituted for potassium nitrate. This substitution was made because it has been demonstrated by MAINX (1928) and DUSI (1930) that ammonium ions are more easily utilized by *E. gracilis* than nitrate ions. Four tubes, each containing 10 cc. of the substance to be tested, were inoculated which 1 cc. of the stock culture in each series. The initial number of organisms per cc. (hereafter refferred to as the "concentration") was determined for two extra tubes. These were averaged, and the average was taken as the initial concentration for all tubes in the series. At the end of the experiment the concentration was determined for three or four tubes of each substance. These were then averaged to obtain the values tabulated.

Experimental results.

Series I.

In this series glycerol and a number of carbohydrates were tested for their power to accelerate growth in media containing no other organic substances. The initial concentration (x_0) was .1 thousand per cc., the initial and final p_H values were 6,3 $\pm$ .1 in all cases, and the concentrations of organisms were determined at the end of ten days. The stock culture used for inoculations was a ten day old culture in the standard medium. The strain of organisms had been growing for several weeks in inorganic media and had undergone several transplants previous to inoculation of the stock culture. The results of series I are given in table 1. Benedict's test for reducing sugars was negative at the end of the experiment in solutions containing sucrose or starch.

Table 1.

Medium	Series I		Series II		
	Concentration at end of ten days (x)	x/x_0	Concentration at end of nine days (x)	Encystment	x/x_0
Standard	1.2	12.0	9.0	—	12.0
Standard + dextrose	1.9	19.0	6.6	$\pm$	8.8
Standard + levulose	1.8	18.0	5.5	+	7.3
Standard + galactose	.5	5.0	3.9	+	5.2
Standard + sucrose	1.8	18.0	5.0	+	6.3
Standard + maltose	1.8	18.0	2.8	+	3.7
Standard + lactose	1.4	14.0	4.5	+	6.0
Standard + xylose	1.8	18.0	4.1	+	5.5
Standard + arabinose	.3	3.0	4.0	+	5.5
Standard + dextrin	1.3	13.0	9.5	—	12.7
Standard + soluble starch	1.5	15.0	6.6	$\pm$	8.8
Standard + glycerol	1.3	13.0	8.6	—	11.5
	Initial conc. $(x_0) = .1$		Initial conc. $(x_0) = .75$		

Key to amount of encystment: — no encystment, $\pm$ approximately 40 % encysted, + over 80 % encysted.

Series II.

This series was similar to series I, except that the stock culture was twenty-two days old, the initial concentration was .75 thousand per cc., and the concentrations were determined at the end of nine days. The results are shown in table I. At the end of the experiment, Benedict's test was negative in the solutions containing sucrose or starch.

Since the culture used to inoculate the tubes of series I was ten days old, and that used for series II was twenty-two days old, the inocula in the two cases were not strictly comparable. The stock culture used for series I was composed of rapidly dividing organisms, whereas that of series II was an older culture in which a few encysted forms were noticed at the time inoculation. Therefore, the culture used in series II may be considered to be in a precystic condition in that some of the cells were already encysted and others were probably on the verge of encystment.

Series III.

The stock culture used for this series was made by an inoculation from an unused twelve day old control culture of series II into 200 cc. of fresh medium. At the and of six days, all the organisms in this stock culture were in the motile stage, and rapid multiplication was taking place. Inoculations were made as in the preceding series. The initial concentration was .9 thousand per cc., and the final concentrations were determined at the end of nine days. The initial and final p_H values where $6.7 \pm .1$ in all cases. The medium was adjusted p_H 6.7 in this case, since it had by this time been shown (JAHN, 1931) that growth of *E. gracilis* is more rapid at p_H 6.7 than at p_H 6.3. Benedict's test was negative at the end of the experiment for tubes containing sucrose or starch.

The results of series III are shown in table 2. It is to be noted that growth in dextrose and dextrin showed no significant difference from that of the control, whereas sucrose and especially starch showed accelerations, and all other substances showed a decelerative effect.

These results, in general, do not agree with those of either series I or series II. Among the differences, it may be pointed out that starch showed and accelaration of almost 200 % in series III and only 25 % in series I. Glycerol showed no effect in series I, but a deceleration of 60 % in series III. Levulose, maltose, lactose, and xylose, substances which produced an acceleration in series I, produced a decelerative effect in series III as they had done in series II, whereas arabinose and galactose showed a decelerative effect in all three series. It may also be noted that in some of the cultures (dextrose, levulose, lactose, galactose, and maltose) the organisms were approximately 50 % larger than the average normal size under autotrophic conditions, and that in others (those contai-

Table 2.
(Results of series III.)

Medium	Concentration at end of nine days (x)	x/x_0	Size of organisms	Encystment
Standard only	11.0	12.0	*	none
Standard + dextrose	10.3	11.4	+	slight
Standard + levulose	8.0	8.8	+	slight
Standard + galactose	7.2	8.0	+	moderate
Standard + sucrose	16.7	18.5	—	none
Standard + maltose	7.0	7.7	+	moderate
Standard + lactose	9.3	10.3	+	slight
Standard + xylose	5.0	5.5	*	none
Standard + arabinose	4.2	4.6	*	none
Standard + dextrin	10.8	12 0	*	none
Standard + soluble starch	31.0	34.4	—	none
Standard + glycerol	4.5	5.0	—	none

Initial concentration (x_0) = .9. Key to size of organisms: * average normal size under autotrophic conditions, + approximately 50 % larger than average normal size, — approximately 30 % smaller than average normal size.

ning succrose, starch, and glycerol) the organisms were approximately 30 % below normal in size. Slight encystment (less than 20 %) occured in dextrose, levulose, and lactose, and a moderate amount of encystment (20—50 %) occurred in galactose and maltose. In no case did more than 50 % encystment occur, as was the case in series II.

The inocula of series I and series III were both obtained from young rapidly dividing stock cultures, ten and six days old respectively. The initial concentration was approximately the same, and temperature and intensity of light were the same in both series. The only environmental difference between the cultures of the two series was that of hydrogen ion concentration. The p_H value of the tubes of series I was 6.3, and that of series III was 6.7. It seemed possible, therefore, that the differences between these two series might have been due to the difference in hydrogen ion concentration. If the carbohydrates which caused no deceleration in the two series are compared from this viewpoint, three types of results are obtained. For one group of carbohydrates (dextrose, levulose, maltose, lactose, and xylose), a rise in p_H was accompanied by a decrease in the amount of acceleration produced. In the case of soluble starch, however, a rise in p_H was accompanied by an increase in acceleration. A third type of result is exhibited by sucrose and dextrin which showed no change in the effect produced

Table 3.

Carbohydrate	$\frac{(x/x_0) \text{ of carbohydrate}}{x/x_0 \text{ of control}}$		Effect of change of pH
	Series I pH 6.3	Series III pH 6.7	
Dextrose	19/12 = 1.7	11/12 = .9	Show a decrease in the amount of acceleration with rise in pH
Levulose	18/12 = 1.5	8.8/12 = .7	
Maltose	18/12 = 1.5	7.7/12 = .6	
Lactose	14/12 = 1.2	10.3/12 = .9	
Xylose	18/12 = 1.5	5.5/12 = .5	
Starch	15/12 = 1.3	34.4/12 = 2.9	Shows an increase in the amount of acceleration with rise in pH
Sucrose	18/12 = 1.5	18.5/12 = 1.5	Show no change in effect on growth rate with rise in pH
Dextrin	23/12 = 1.1	12/12 = 1.0	

by the carbohydrate, with a rise in the p_H value of the medium. The results of such a comparison are shown in table 3. It may be worthy of note that all of the carbohydrates in the first group have a strong reducing action on BENEDICT'S solution; whereas, of the three substances comprising the other two groups, starch and sucrose do not reduce BENEDICT'S solution, and dextrin exerts only a slight reducing action. These results suggest that a change in p_H may modify the effect produced by various carbohydrates on growth of *E. gracilis*, and that the change in effect may vary with the carbohydrates used. In order to test this possibility series VI—VII were performed, and these will be discussed later.

Also, a series of experiments was started under conditions which decreased photosynthesis. It had previously been pointed out by MAINX (1928) that the total exclusion of light is objectionable in that photodynamic reactions other than photosynthesis may be affected, and that the total exclusion of carbon dioxide also may be criticized in that the hydrogen ion concentration of the medium may be modified and that there may be a specific effect of the carbonate ion other than that of promoting photosynthesis. Therefore, the organisms were grown in light of a lower intensity in the hope that the accelerative effects of the carbohydrates would be accentuated and that negative results, if obtained, would not be subject to the criticisms resulting from the total exclusion of light or of carbon dioxide. The results are described in series IV.

Series IV.

This series was similar to series III, except that the organisms were maintained at room temperature (approximately 20° C) under a constant light intensity that was approximately one-eighth of that used in the preceding series. The stock culture used in this series was an eight day old culture started from an unused control of series II, as was the stock culture of series III. The initial concentration of organisms was 1.2 thousands per cc., and the final concentrations were determined at the end of twelve days. The initial and final p_H values were 6.7 $\pm$ .1 in all cases. The results are shown in table 3. BENEDICT'S test was negative at the end of the experiment for solutions containing sucrose and starch.

One of the most striking differences between this series and the preceding one is that, in spite of the fact that the initial inoculation was higher in series IV than in series III, the control of series IV showed a concentration of only 3.0, whereas that of series III was 11.0. Otherwise the results of this series are somewhat similar to those of the preceding series, since most of the final concentrations in the two series are comparable. However, tubes containing some of the substances showed a significantly lower amount of growth in series IV than in series III; these were sucrose, galactose, arabinose, and glycerol. It is to be noted that galactose showed an acceleration over the control, whereas it had shown a deceleration in all previous experiments. Arabinose, however, continued to show a deceleration in this series. Glycerol showed a deceleration as in the preceding series. Another significant difference is that dextrin, a substance which had no effect in all previous experiments, here showed a large acceleration over the control. It was also noted that fewer carbohydrate cultures contained large forms (dextrose, lactose, maltose, and dextrin, as compared to dextrose, levulose, lactose, galactose, and maltose in series III), while the cells in the cultures containing arabinose, starch, and glycerol were smaller than normal (cf. sucrose, starch, and glycerol in series III). Slight encystment (below 20 %) occurred only in tubes containing dextrose.

A comparison of the results of series III and IV shows that the intensity of light may modify considerably the accelerating effect of carbohydrates when added to inorganic media. In series IV, galactose, which had failed to accelerate growth in any of the preceding series, produced an acceleration in light of low intensity.

Table 4.
(Results of series IV.)

Medium	Concentration at end of twelve days (x)	x/x_0	Size of organisms	Encystment
Standard only	3.0	2.5	—	none
Standard + dextrose	9.3	7.7	+	slight
Standard + levulose	8.4	6.6	*	none
Standard + galactose	4.8	4.0	*	none
Standard + sucrose	9.6	8.0	*	none
Standard + maltose	7.1	5.9	+	none
Standard + lactose	8.2	6.8	+	none
Standard + xylose	4.0	3.3	*	none
Standard + arabinose	2.4	2.0	—	none
Standard + dextrin	11.9	9.2	+	none
Standard + soluble starch	30.0	25.0	—	none
Standard + glycerol	2.3	1.9	—	none

Initial concentration (x_0) = 1.2. Key to size of organisms: * average normal size under autotrophic conditions, + approximately 50% larger than normal size, — approximately 30% smaller than average normal size.

Likewise, dextrose, levulose, maltose, lactose, and xylose all showed a definite accelerative effect in series IV, whereas in series III deceleration was apparent. In this case the differences may be due to the difference in temperature as well as in light intensity. However, in view of the results of BECKWITH (1929), it seems that light intensity alone might be sufficient to account for all the differences between series III and IV. BECKWITH, working with six species of *Chlorella* and one species each of *Chlorococcus* and *Mannochloris*, tested the ability of these organisms to grow in solutions containing inorganic salts and various amino-acids and carbohydrates. He found that the results obtained with various sugars were by no means uniform for the group, and that some sugars which accelerated growth of certain species in the dark exerted a depressant effect upon the same species when grown in the light under otherwise similar conditions. Also, some sugars which accelerated growth in the light exerted an inhibitory effect upon the same species in the dark.

Series V—VIII.

Series V and VI were performed to test the possibility shown in table 3 that the p_H may determine the effect of carbohydrates, and the x/x_0 values for the data are shown in table 5, together with two extra series at p_H 6.1 (VII) and 6.9 (VIII). All of the

Table 5.
(Values of x/x_0 for series V—VIII.)

Series	V		VI		VII	VIII
pH value	6.1	6.9	6.1	6.9	6.1	6.9
Control	66.2	76.1	5.0	13.3	15.3	13.3
Dextrose	51.5	50.0	4.0*	8.3*	4.3*	6.9*
Levulose	44.6	47.7	—	—	5.7*	6.0*
Sucrose	82.3	88.5	5.8	10.6	13.7	15.2
Dextrin	61.5	73.8	3.5*	9.1*	8.3*	17.7
Starch	76.1	93.1	16.0	41.0	28.2	39.2
Initial conc. (x_0)	1.3		.6		.9	.7
Duration	8 days		15 days		11 days	15 days
Stock medium	Standard + .05 % casein peptone		Standard		Standard	Standard

organisms used for inoculations for these series were from quite young stock cultures (series V, 3 days old; series VI, 6 days old; VII, 4 days old; VIII, 5 days old). The stock culture used for series V contained .05 % casein peptone; therefore the tubes of series V contained .005 % casein peptone due to the introduction of a .9 cc. inoculum into each tube. The other series were grown in the standard medium which was titrated to p_H 6.1 and 6.9.

In these series of experiments it is to be noted that there are no large differences in the amount of acceleration comparable to those shown in table 3 which could be accredited to differences in p_H. With dextrose and levulose decleration occurred in all cases, and encystment occurred in all except series V which contained a small amount of peptone. Sucrose showed a deceleration in series VI, and also in series VII at p_H 6.1, and an acceleration in series V (p_H 6.1 and 6.9) in series VIII, and in series VI at p_H 6.1. However, the accleration in the last case is hardly significant. Dextrin shows a deceleration in series V—VII, and an acceleration in series VIII. Starch shows a large acceleration in all cases. Benedict's test in the media containing sucrose or starch was negative at the end of the experiment. Encystment in table 5 is denoted by an asterisk. In general, the results of these series can not be fitted into the simple scheme shown in table 3. Therefore, it is believed that factors other than p_H are operative in determining the effect of carbohydrates on growth.

Discussion.

The results of the experiments here presented, altough superficially contradictory when the results of the various series are compared, are, in general, quite consisteat within each series. Four tubes containing the substance to be tested were inoculated for each set of oxperiments, and the final concentrations seldom varied more than ten per cent from the mean. It is believed, therefore, that the results are valid and that the differences noted are due to differences of material (age of stock culture) or to different conditions of the experiments (temperature, intesity of light, hydrogen ion concentration). The experiments of previous workers (ZUMSTEIN, 1900; TERNETZ, 1912; PRINGSHEIM, 1912; and MAINX, 1928) have in many cases seemed directly contradictory and have given rise to much controversy concerning the ability of Euglena gracilis to utilize various substances. All of these experiments may be shown to be, in all probability, perfectly valid, in spite of the seemingly contradictory results. Inasmuch as similar contradictory results have been obtained again in strictly comparable experiments, and since previous workers did not always give definite information in regard to the age of the stock culture used, the amount of growth that actually occurred, or the p_H of the medium, it seems that the contradictions may be due to differences in any of these factors. Also, some of the experiments of previous investigators were performed under conditions of constant light (PRINGSHEIM, 1912; MAINX, 1928) and the others were performed in daylight. In view of the differences obtained in series III and IV, experiments carried out in different intensities of light can not be considered strictly comparable.

In comparing the results of series I and II it is seen that dextrose, levulose, sucrose, maltose, xylose, and starch, all substances which seemingly induced acceleration in series I, apparently induced deceleration and encystment in series II. Dextrin and glycerol, substances which produced no significant accleration in series I, produced no significant effect in series II in that neither acceleration, deceleration, nor encystment occurred. Galactose and arabinose, however, produced a deceleration in both cases. These results seem to indicate that, in general, carbohydrates which accelerate division in actively growing cultures may also accelerate encystment (which probably involves a concomitant deceleration of division rate) in cultures which are in a precystic condition. It has already been noted (MAINX, 1928) that, in *E. gracilis*, the accu-

mulation of reserve materials seems to be a factor in inducing encystment. This suggestion is offered as a possible explanation of the results of these two series of experiments, in which the factors seem to be identical in all respects except age of the stock culture used for inoculations.

Volkonsky (1930) has shown that the kind and amount of reserve material formed by *Polytoma uvella* varies with the type of medium, the rate of growth, and the age of the culture. He has also demonstrated that *P. uvella*, if transferred to a medium containing ammonium acetate, will build starch as the dominant type of reserve material. As the age of the culture increases, fat droplets, characteristic of "senescence", appear and become more numerous than the starch granules. Volkonsky's observations thus establish the fact that, in *P. uvella*, definite physiological changes occur as the culture grows older. The observations of Mainx (1928) on the formation of haematochrome indicate that similar changes my take place in *E. gracilis*. It seems probable, therefore, that the different results of series I and II may be dependent upon partially known, or totally unknown, relationships between age of the culture and accumulation of reserve materials, between reserve materials and encystment, between encystment and rate of division in the presence of certain compounds, or between type and amount of reserve material and the division rate.

Since in all series media containing sucrose and soluble starch failed to give a positive Benedict's test at the end of the experiments, it seems as if these substances were not hydrolyzed into reducing sugars. The results of these experiments indicate, therefore, that *E. gracilis* does not secrete sucrase, or amylase, and that sucrose and suluble starch are probably taken into the cell unchanged.

Summary.

1. The effect of various carbohydrates upon the growth of *E. gracilis* in bacteria-free culture has been measured quantitatively.

2. *E. gracilis* when transferred from an inorganic medium to one containing a carbohydrate in addition to inorganic substances, may undergo encystment or an acceleration or deceleration of division rate. This effect varies with the carbohydrate used, and results indicate that it may also be influenced by:

1. The physiological condition of the stock cultures from which transfers are made.

2. The intensity of light, and perhaps temperature.

3. Perhaps to some extent by the p_H of the medium.

The exact nature of the influence of these factors remains a matter for future investigation.

3. It is demonstrated that *E. gracilis* probably does not secrete sucrase or amylase.

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