BIOLOGIA PLANTARUM (PRAHA)

10 (1) : 58—71, 1968

On the Catabolic Pathways of Sugars in Green Algae

J. DVOŘÁKOVÁ-HLADKÁ

Collection of Cultures of Autotrophic Organisms, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

Received May 18, 1967

Abstract. Respiratory metabolism of the cultures of algae *Chlorella pyrenoidosa* (82) and *Scenedesmus obliquus* (125) was investigated. One part of algae were cultivated on a mineral nutrient solution, another two parts on the solution with glucose and on the solution with glucose and yeast decoction. Individual steps of respiratory metabolism — endogenous as well as in the presence of exogenous sugars — were estimated according to the response to the following inhibitors: monoiodacetate, NaF, NaN_3, and 2,4-DNP. In the last two cases, fructose, glucose-6-phosphate and fructose-1,6-diphosphate were applied in parallel for comparison. Na-monoiodacetate was found to inhibit the respiration of both endogenous and exogenous (with glucose) substrates, NaF (concentrations up to $2.5 \cdot 10^{-2}$M) stimulated the O_2 uptake. The effect of sodium azide and 2,4-DNP depended in both strains on previous cultivation. On the basis of the results obtained, the presence of particular respiration pathways in the dissimilation of glucose is discussed. The following catabolic processes are to be considered: a) direct oxidation (with both autotrophically cultivated strains and with the *Chlorella* cultivated on glucosecontaining medium), b) the process similar to glycolysis, which, however, does not necessarily involve the enolase (it is not inhibited by NaF), c) pentosephosphate cycle (*Chlorella*), and d) glycolysis, in which both algae can operate when sugars previously phosphorylated are applied.

The present work is a continuation of a previous paper (DVOŘÁKOVÁ-HLADKÁ 1966) in which glucose was found to be an excellent growth substrate for the tested algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Glucose can serve as a source of energy as well as carbon source mainly under heterotrophic conditions (in the dark). As is seen from another paper (DVOŘÁKOVÁ-HLADKÁ 1967), the presence of exogenous glucose increases Qo_2, especially in cultures cultivated autotrophically far more than could be accounted for by the real consumption of this sugar.

Respiratory metabolism depends, among others, on the composition and amount of exogenous substrate. The light regime (FRENCH, KOHN and TANG 1934) and, generally, cultivation conditions (KANDLER 1954) affect, therefore, the algal metabolism. In addition, the different Qo_2 value in the course of developmental phases (SOROKIN, MYERS 1957) appears to be partly due to the gradually decreasing supply of endogenous substrate in dark periods. To

* *Address:* Praha 2, Viničná 5.

avoid this complication, I measured the Q_{O_2} uptake with cultures of the same age under identical conditions except for the composition of nutrient solution. The metabolism of algae cultivated under autotrophic, mixotrophic and auxotrophic conditions is being compared.

The aim of the present work is to ascertain the metabolic pathways in the presence of exogenously applied glucose.

For the investigation of metabolic pathways of sugars the conventional inhibitors were used (NaF, Na-monoiodacetate, NaN_3, 2,4-dinitrophenol) so as to be able to compare the data obtained with those from the literature. As reported by Ross et al. (1961), NaF inhibits most probably the enolase (when the catabolism of glucose proceeds via glycolysis). According to HACKETT (1960), among the inhibitors attacking thiol group monoiodacetic acid is most frequently applied to different objects and is supposed to be a rather specific inhibitor of triosephosphate dehydrogenase. NaN_3 and 2,4-DNP act as uncouplers of the macroergic and cytochrome steps of metabolism. The differences in the participation of different respiratory pathways in dependence on trophic conditions of algae were determined by the application of the two last inhibitors.

KANDLER (1955) found that monoiodacetate, NaN_3 and 2,4-DNP have a more pronounced inhibitory effect in dark than in light. He also found (KANDLER 1958) that 2,4-DNP increases endogenous respiration and that the dissimilation of endogenous substrate in the presence of 2,4-DNP stops after the addition of glucose. MOSES and SYRETT (1955) found a stimulation of endogenous respiration by NaN_3 and 2,4-DNP in mixotrophic alga *Chlorella vulgaris* cultivated on glucose. TAYLOR (1950) did not find any effect of NaN_3 and 2,4-DNP on the glucose oxidation in *Scenedesmus obliquus*.

Material and Methods

All experiments were carried out on two algae strains from the Collection of Cultures of Autotrophic Organisms of the Institute of Experimental Botany in Prague: *Chlorella pyrenoidosa* CHICK (strain 82) and *Scenedesmus obliquus* (TURP.) KRÜGER (strain 125). Both algae were cultivated on Benson's nutrient solution (DVOŘÁKOVÁ-HLADKÁ 1967). The culture cultivated autotrophically is in figures designated by the strain number and the abbreviation of the medium (e.g. 82 B, 125 B); the cultures cultivated mixotrophically are designated by initial letters of the name of substrate (e.g. BG denotes the alga cultivated on Benson's medium + 0·05 M glucose, BGY — alga cultivated on the BG medium + 80 ml of yeast decoction per 1000 ml (DVOŘÁKOVÁ-HLADKÁ, BASLEROVÁ 1957).

The determination of the effect of particular inhibitors on the O_2 uptake was carried out by direct Warburg method in dark at 25 °C. Control samples were placed in 0·015 M phosphate buffer of pH identical with that of the inhibitor. The application interval and pH of the inhibitors were chosen individually.

NaF was applied at several different concentrations. According to JAMES (1953) it inhibits at the concentration 10^{-3}M, while lower concentrations have a rather stimulating effect on the respiration (MCNULTY and LORDS 1960). The final NaF concentrations used in the present investigation of its effect on endogenous respiration were therefore 0·25, 0·625, 1·25, 1·88, $2·5 . 10^{-2}$M, water being used as the control. The inhibitor was added to algae suspension in 0·015 M phosphate buffer (pH 6·8) 6 hours before the start of the measurement. During this interval the suspension was lighted and shaken. Then the endogenous respiration was measured for 1 hour, glucose added (final concentration 0·16 M) and the respiration measured for another hour. The inhibitor was thus proportionally diluted, its final concentration at the substrate respiration measurement being 0·21, 0·525, 1·58, and $2·1 . 10^{-2}$M (see the shift of experimental points in Figs. 3 and 4).

As reported by JAMES (1953), the effective concentration of Na-monoiodacetate is 10^{-4}M. In the present experiments, increasing concentrations were applied as follows: 0·67, 1·6, 3·3, 5·0, $6·7 . 10^{-3}$M monoiodacetic acid neutralized partly with NaOH. Water was used as a control. For the measurement manometric flasks with one sidearm were used. Because of the acidic nature of the inhibitor (SIMON and BEEVERS 1952), algae suspension was placed in 0·015 M phosphate buffer (pH 4·5). This pH seems to be most suitable for the respiration of *Chlorella* (EMERSON and GREEN 1938). The measurement interval and the final concentration of glucose added were the same as with NaF. Final concentrations of the inhibitor at the substrate respiration measurements were 0·5, 0·12, 0·25, 0·375, $5·0 . 10^{-2}$M (see Figs. 1 and 2).

According to HARTREE (1957) and HACKETT (1960), the respiration of intact cells is inhibited by NaN_3 at pH 3 to 8 with a maximum effect in acid medium. In this work a 0·015 M phosphate buffer of pH 5·3 was therefore used. Algae suspension was placed in the central well of a Warburg flask. In one group of the flasks the substrate (glucose, fructose, G-6-P or F-1,6-diP), in the other azide, was added to the central well. The respiration was measured for one hour at 10-min. intervals, then azide was added to the first group and the substrate to the second one, and the respiration measurement continued for 1 hour. The final concentration of inhibitor was $3·10^{-3}$M (KLEINZELLER et al. 1954), and that of sugars 0·16 M.

Active concentration of 2,4-dinitrophenol is $5·10^{-4}$M (KLEINZELLER et al. 1954), which was also used in this work. Central wells of the flasks were in the first group supplied with water, in the second one with 2,4-DNP. In both series the respiration substrate (glucose, fructose, G-6-P, F-1,6-diP) was placed in the sidearms. The flasks were kept in a tempered bath for 2 hours at 25 °C (all measurements were carried out at this temperature). In the first group the endogenous respiration and in the other the effect of 2,4-DNP in the absence of sugar was then measured. The sidearms were then emptied into the main parts and the respiration measured in the presence of exogenous substrate. The final sugar concentration was 0·16 M. The measurement was carried out for 1 hour. The measurements with NaN_3 and 2,4-DNP were done in parallel and pH was therefore the same, i.e. 5·3.

All results were evaluated by means of the t-test, the number of measurements being at least 8 (experiments were repeated two or three times).

In the work, the following abbreviations are used: 2,4-DNP (2,4-dinitro-

phenol), G-6-P (glucose-6-phosphate), F-1,6-diP (fructose-1,6-diphosphate), NADP (nicotinamide adenosine dinucleotidephosphate = co-dehydrogenase II), MIA (monoiodacetic acid).

Results and Discussion

Glucose is generally used as an effective growth and respiration substrate. However, the details of its catabolic pathways in certain algae under given conditions are still not known. FOGG (1953) summarized, according to a

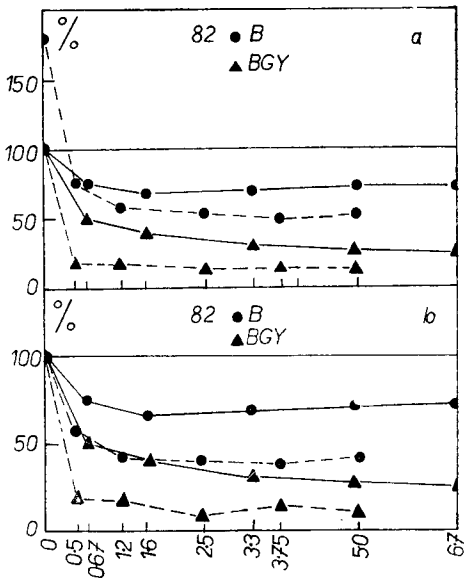


Fig. 1. The effect of concentration monoiodacetate on Q_{O_2} in *Chlorella pyrenoidosa*. ———— endogenous substrate, — — — — exogenous glucose (conc. 0.16 M). B ● — autotrophically cultivated alga, BGY ▲ — mixotrophically cultivated alga with 0.05 M glucose and yeast decoction. Abscissa: concentration of inhibitor in 10^{-3}M; Ordinate: *a* — in per cent of endogenous respiration, *b* — in per cent of substrate respiration. After adding the substrate the inhibitor becomes diluted — see methods. The shift of values due to the dilution.

number of different papers, that hexoses can act as direct respiration substrate. They take part in subsequent metabolic processes involving i) glycolysis, ii) oxidation of glycolytic products. The respiration is mediated by enzymes of flavoprotein nature resistent to cyanide. EMERSON (1926) assumes, on the other hand, that the stimulation of respiration by glucose is mediated by the cytochrome system. Thus far, the cyanide-resistent enzyme system, catalyzing the endogenous respiration, is not known. REAZIN (1956) proved that the respiration of algae *Ochromonas* corresponds with the EMP pathway with a direct

oxidation. Similarly, OAKS (1962) found an oxidation of glucose via glycolysis and pentosephosphate cycle in *Chlorella ellipsoidea*. This leads to the conclusion that algae can operate by various systems according to the internal conditions (e.g. development of trophic factors).

I tried to solve this problem using inhibitors of specific enzymatic reactions.

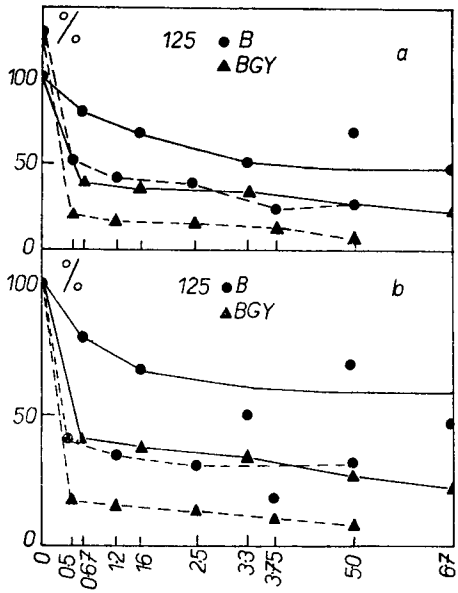


Fig. 2. Q_{O_2} in *Scenedesmus obliquus* at different concentrations of monoiodacetate. Otherwise indicated as in Fig. 1.

Monoiodacetate inhibits endogenous as well as substrate respiration of both algae. In *Chlorella* (Fig. 1) the inhibitory effect slightly increases with the rising concentration up to $1{\cdot}6 \cdot 10^{-3}$M, further increase of the concentration to $6{\cdot}7 \cdot 10^{-3}$ does not affect the inhibition. The concentration $1{\cdot}6 \cdot 10^{-3}$M seems thus to be sufficient to block the systems attacked by the inhibitor. Especially the systems operating in the consumption of exogenous substrate or activated by its presence are more sensitive to monoiodacetate than endogenous systems; after the addition of exogenous substrate the inhibition increases (even as compared with endogenous respiration — see Fig. 1a). Endogenous respiration of algae cultivated mixotrophically on glucose is also more inhibited by monoiodacetate. A similar situation is in *Scenedesmus* (Fig. 2). The inhibitory effect, however, seems to increase up to the highest concentration used ($6{\cdot}7 \cdot 10^{-3}$M). No differences were found between particular concentrations. Significant differences are only between the concentrations differing by one order of magnitu de($0{\cdot}67$ and $6{\cdot}7 \cdot 10^{-3}$M). An inexplicable decrease of the inhibition of endogenous respiration occurred at the concentration $5{\cdot}0 \cdot 10^{-3}$M. The decrease was perceptible also in the substrate respiration. It occurred regularly and significantly in all the three experiments repeated (a total of eight measurements).

In either case the percentages of inhibition in autotrophic and mixotrophic variants do not coincide in the range of concentrations used (i.e. the inhibition of BGY variant at the concentration 0·5 is higher than that of B variant at 6·7).

Na-monoiodacetate inhibits triosephosphate dehydrogenase which acts in the glycolysis of glucose but does not occur in the pentose cycle (JAMES 1953).

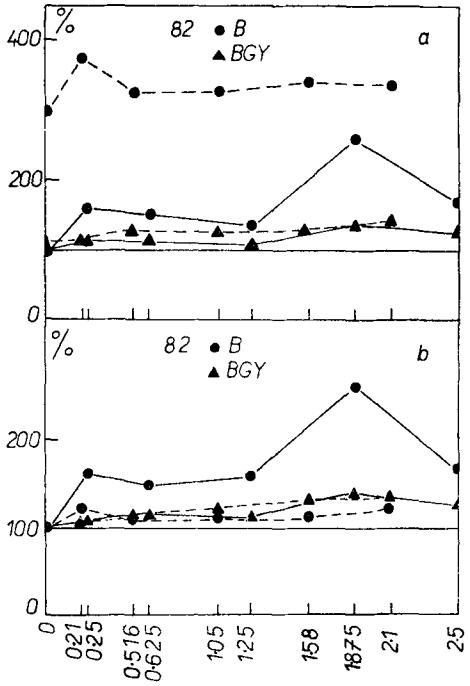


Fig. 3. Q_{O_2} in *Chlorella pyrenoidosa* at different concentrations of NaF. Concentration of inhibitor in 10^{-2}M. Otherwise indicated as in Fig. 1.

If the consumption of glucose proceeds via glycolysis, the process should be inhibited by monoiodacetate. Glycolysis itself is not attached to the oxygen consumption but can be followed by other aerobic oxidative systems. According to the results obtained with monoiodacetate we can say that endogenous respiratory systems of both the algae involve a system which is inhibited by monoiodacetic acid. Similarly, the systems mediating the oxidation of exogenous glucose at its long-term application during the cultivation (BGY — the inhibition always higher) as well as during the respiration measurements, involve apparently an intermediate with SH-group, probably triosephosphate dehydrogenase. In both cases the systems appear to be identical. They are activated by exogenously applied glucose (DVOŘÁKOVÁ-HLADKÁ 1967). From this we can assume that glucose is an endogenous respiration substrate, its catabolism providing at least 30 to 40% of the electrons transported to oxygen.

NaF is another inhibitor of glycolysis. Its effect implies the presence of enolase in the respiration process. The results are given in Figs. 3. and 4. In neither case an inhibition was found; on the contrary, endogenous respiration of Chlorella is significantly stimulated. The absence of inhibition cannot be accounted for by low concentrations of the inhibitor (McNULTY, LORDS 1960)

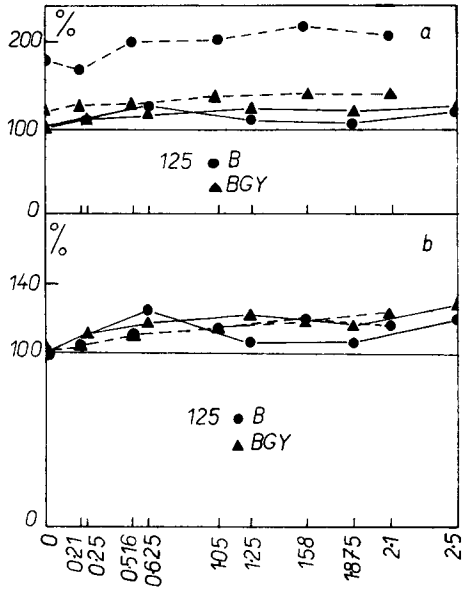


Fig. 4. Q_{O_2} in Scenedesmus obliquus at different concentrations of NaF (see Fig. 3). Otherwise indicated as in Fig. 1.

since between the individual variants there is no significant difference (save for 82B variant — concentration $1{\cdot}875 \cdot 10^{-2}$M). Even at a ten times higher concentration of the inhibitor there is no inhibitory tendency (neither the unspecific one, e.g. inhibition of other phosphorylation processes). The increase of respiration may be due to salt effect because the same increase may be brought about by equimolar ($0{\cdot}67$ and $0{\cdot}5 \cdot 10^{-2}$M) NaCl used as a control in this experiment. The effect is demonstrated in the course of oxygen consumption in autotrophically cultivated *Chlorella* (Fig. 5).

NaN_3 and 2,4-DNP have a similar effect as to the blocking of oxidative phosphorylation (KLEINZELLER et al. 1954).

The application of NaN_3 enabled us to explore another part of aerobic dissimilation of glucose in which NADP — cytochrome system is employed. This oxidative system involving active Fe^{2+} and Fe^{3+} ions is inhibited by azide, in contrast to the oxidases of a flavine nature. The effect of 2,4-DNP, uncoupling the macroergic metabolism, is manifested by increased Q_{O_2} in the system where the macroergic phosphate formation is a controlling factor of the respiration rate. The Q_{O_2} increase was observed in *Saccharomyces cerevisiae* by STOPPANI et al. (1961) who proved, by means of it, the different metabolism of glucose and pyruvic acid.

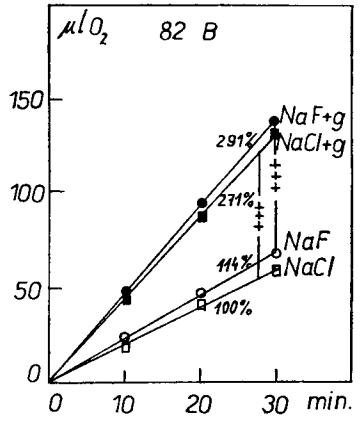


Fig. 5. Q_{O_2} in *Chlorella pyrenoidosa* during 6-hour action of NaCl and NaF. Abscissa: time in minutes; Ordinate: µl O_2. ○ — effect of NaF on endogenous respiration, □ — the same with NaCl, ● — effect of NaF on substrate (glucose) respiration, ■ — the same with NaCl.

To explore the catabolic pathways of exogenous glucose more in detail, the effect of both the inhibitors on previously phosphorylated sugar was investigated. The pairs: glucose G-6-P and fructose F-1,6-diP were therefore applied in parallel.

Table 1 gives the results with the two inhibitors and with all substrates used, in *Chlorella* precultivated in different ways. The O_2 consumption is

Table 1

Effect of inhibitors on respiration in the presence of different substrates in *Chlorella pyrenoidosa* (82). Expressive values are significant with respect to the values of endogenous respiration (first column) or of uninhibited substrate respiration (second column).

Inhibitor	NaN_3		2,4-DNP	
Substrate	% endog.	% subst.	% endog.	% subst.
82 B endogenous	**224.5**		**129.5**	
glucose	**193**	**147**	**135**	109·5
G-6-P	81·8	**64**	95	**74.5**
fructose	**190**	**194**	**125**	**152**
F-1,6-diP	**281.5**	**149**	**333**	**175**
82 BG endogenous	**134.5**		**70.6**	
glucose	183	**179**	**174**	**170**
G-6-P	90·5	88	99	91·5
fructose	**44**	**25.5**	**73.5**	**69**
F-1,6-diP	119	**70**	74	**124**
82 BGY endogenous	**83**		**128**	
fructose	**67.5**	**60**	130·5	**129**
F-1,6-diP	108	111	95	91·5

given partly in per cent of endogenous respiration, and partly in per cent of substrate respiration in the absence of an inhibitor.

In autotrophically cultivated *Chlorella* (82B), both inhibitors act only in the presence of glucose phosphorylated to the first degree. Other substrates stimulate Qo_2. The non-phosphorylated sugars seem thus to be metabolized by a process different from glycolysis. The catabolism of endogenous substrates does not proceed via the pathway involving G-6-P, either (except in the case of a direct conversion of the pool polysaccharide into G-1-P and further to G-6-P without the intermediate step of non-phosphorylated glucose).

Table 2

Effect of inhibitors on respiration in the presence of different substrates in *Scenedesmus obliquus* (125). Expressive values are significant with respect to the values of endogenous respiration (first column) or of uninhibited substrate respiration (second column).

Inhibitor	NaN_3		2,4-DNP	
Substrate	% endog.	% subst.	% endog.	% subst.
125 B endogenous	**150**		**136**	
glucose	124·5	91	136	98
G-6-P	**156**	**11,8**	**64,5**	**48,7**
fructose	**208**	**166**	**203,5**	**158**
F-1,6-diP	**144**	**56**	**226**	85
125 BG endogenous	**63,7**		**160**	
glucose	**67**	**62**	**67,2**	117
G-6-P	**49,7**	**32**	**49,7**	**43,7**
fructose	**78**	**44**	**226,5**	**147**
F-1,6-diP	**52,5**	**28**	**133**	75
125 BGY endogenous	78·5		**144,5**	
fructose	**25**	**14,6**	**207**	**147**
F-1,6-diP	**25,5**	**22**	**165**	105

The endogenous respiration of mixotrophically cultivated algae is inhibited only by 2,4-DNP. The exogenous fructose catabolism involves the steps which can be inhibited by both the inhibitors. On the other hand, the respiration in the presence of glucose is stimulated by the two inhibitors whereas in the presence of phosphorylated sugars the inhibitors are almost ineffective. The only exception is 2,4-DNP in the presence of F-1,6-diP which increases endogenous respiration of BG variant. The metabolic pathways of the two isomeric sugars are thus different. The regularly observed stimulation of the respiration in the presence of NaN_3 is in keeping with the data of MOSES and SYRETT (1955) on the respiration of *Chlorella vulgaris* in the presence of glucose. This may be due to uncoupling the oxidative phosphorylation similar to that of 2,4-DNP, or to the substitution of Fe-systems in the presence of NaN_3 by other terminal oxidases.

There are differences between the comparable data in the mixotrophic cultures BG and BGY. In BGY culture the respiration is inhibited by NaN_3

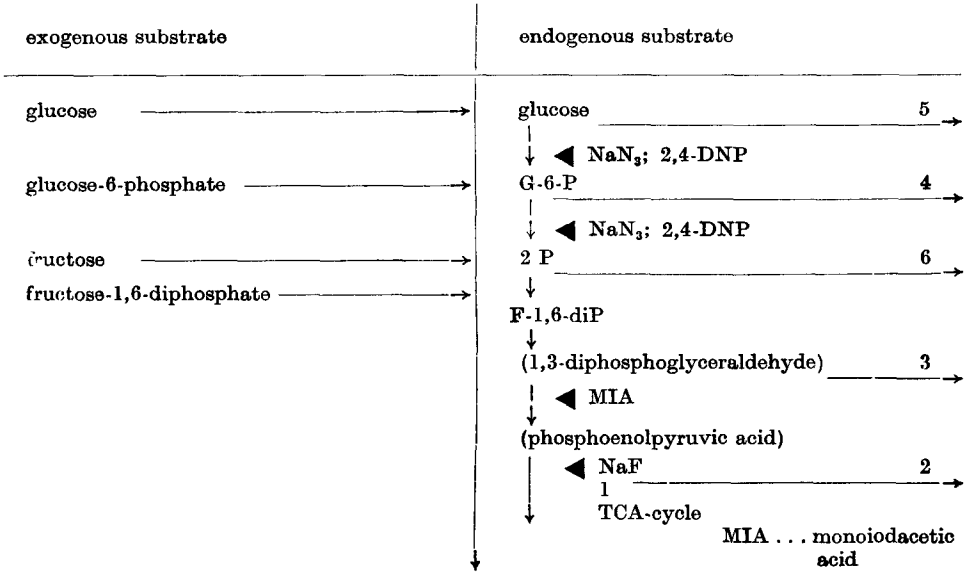
but not by 2,4-DNP. Similarly, Qo_2 is decreased by NaN_3 in the presence of fructose; 2,4-DNP inhibits only the respiration of phosphorylated fructose. The yeast may have saturated the alga with co-factors of different enzymes (e.g. vitamins B_1, B_2 etc.) so that other systems could contribute to the process. These systems could be suppressed in the absence of an autotrophic source, especially in the mixotrophic BG culture with a high growth energy (DVOŘÁKOVÁ-HLADKÁ 1966).

The results of analogous experiments with *Scenedesmus* are presented in Table 2. When comparing the results regarding the respiration with the same substrate without inhibition, we find that both inhibitors affect the respiration of both phosphorylated sugars in the autotrophically cultivated alga. We can again assume that the alga can metabolize non-phosphorylated sugars by a process different from glycolysis. With phosphorylated sugars, this process is missing (see the effect of monoiodacetate). The oxidation pathways of endogenous as well as of both exogenous non-phosphorylated sugars are thus obviously different.

With both mixotrophic variants (BG, BGY) the respiration of Scenedesmus is inhibited by sodium azide independent of the presence of yeast extract and the type of substrate. The effect of 2,4-DNP on the culture is not unambiguous. It can, therefore, be assumed that primary as well as secondary phosphorylation of endogenous and both exogenous substrates, too, may occur in mixotrophically cultivated *Scenedesmus*.

According to TAYLOR (1950) neither NaN_3 nor 2,4-DNP affect the glucose oxidation in *Scenedesmus obliquus*. He used a higher concentration of sodium azide (1·5 ×) and lower concentration of 2,4-DNP (5 ×).

The scheme of glycolysis, involving only the steps included in the experiments, gives a number of six possible catabolic pathways of sugars applied to our two algae:



On the basis of the inhibition experiments the following pathways are to be considered:

1) Classical glycolysis, possibly linked to Krebs cycle
2) Glycolysis to enolase step (NaF does not inhibit — possible role of carboxylation and joining TCA-cycle by means of other acetyl sources — TING, DUGGER 1965)
3) Glycolysis to triosephosphate dehydrogenase step; monoiodacetate does not inhibit (possible joining the pentosephosphate cycle)
4) G-6-P is not inhibited by NaN_3 and 2,4-DNP; corresponds to pentosephosphate cycle
5) Direct oxidation of glucose is not inhibited by NaN_3 and 2,4-DNP, either (REAZIN 1956)
6) Fructose pathway, presumably similar to (5)

The substrate that have not been applied exogenously are in brackets.

The results of all experiments with inhibitors are summarized in Table 3. For both the algae the last column gives the pathway that can be derived from the preceding scheme according to the results.

As can be seen from Table 3, different cultivation causes greater differences than the appurtenance to different species. A substantial difference in cultiva-

Table 3

Respiration pathways deduced from the scheme according to the inhibition of respiration in the presence of different substrates.

Substrate	82 *Chlorella pyrenoidosa*					125 *Scenedesmus obliquus*				
Inhibitor	NaN_3	2,4 DNP	mono-iod-aceta-te	NaF	Path-way	NaN_3	2,4 DNP	mono-iod-aceta-te	NaF	Path-way
B										
endogenous	—	—	+	—	5/2	—	—	+	—	5/2
glucose	—	—	+	—	5/2	—	—	+	—	5/2
G-6-P	+	+	0	0	1	+	+	0	0	1
fructose	—	—	0	0	6	—	—	0	0	6
F-1,6-diP	—	—	0	0	1	+	+	0	0	?
BG										
endogenous	—	+	0	0	1 ?	+	—	0	0	1,2
glucose	—	—	0	0	5 ?	+	+	0	0	1,2
G-6-P	—	—	0	0	4	+	+	0	0	1
fructose	+	+	0	0	1	+	—	0	0	1 ?
F-1,6-diP	—	—	0	0	1	+	+	0	0	?
BGY										
endogenous	+	—	+	—	2 ?	+	—	+	—	2 ?
glucose	0	0	+	—	2 ?	0	0	+	—	2
G-6-P	0	0	0	0		0	0	0	0	
fructose	+	—	0	0	1 ?	+	—	0	0	1
F-1,6-diP	—	+	0	0	?	+	—	0	0	?

+ ... inhibition, — ... no effect of inhibitor, 0 ... was not performed, x/y ... alternative possibilities, ? ... questionable pathway

tion conditions is especially between the two mixotrophic variants of *Chlorella*, which is, probably, due to the amount of auxotrophic components in the culture cultivated in the presence of yeast extract. It can be assumed that autotrophic (B) and mixotrophic (BG) cultures are deficient in these components (I mentioned the possibility of riboflavine deficiency in earlier work 1967) and that they adapt themselves by the formation of other catabolic pathways. As to the adaptation, *Chlorella* is obviously more pliant than *Scenedesmus*, which is not able to adapt to the changed conditions and retains identical pathways in both mixotrophic variants. The different reactivity of both algae has been similarly mentioned in my earlier work (1967).

The fructose metabolism remains still a problem. As a certain argument could probably serve the finding of TAYLOR (1950) on *Scenedesmus obliquus*. He claims that in this alga fructose is not absorbed nor forms a reserve polysaccharide, otherwise similar to starch.

The algae reacted to the applied sugars. However, this cannot be considered as a proof that the sugars were at all admitted by the cell, and in which form.

Conclusions

From the above results it can be concluded:

1) In view of the significant inhibition by monoiodacetate it can be assumed that glucose can serve as endogenous as well as exogenous respiration substrate.
2) Both algae can direct the glucose catabolism according to trophic conditions. Likewise, in the presence of the inhibitor of a particular metabolic pathway another one can be applied which is otherwise negligible. The following catabolic pathways are essentially involved: a) direct oxidation (especially both autotrophically cultivated algae and 82 BG), b) the pathway similar to glycolysis which does not involve the enolase, being not inhibited by NaF, c) pentosephosphate cycle (*Chlorella*) and d) glycolysis, in which both algae can operate when sugar previously phosphorylated is applied.
3) Both algae are similar in catabolism of all substrates at autotrophic cultivation; they differ in the response to the mixotrophic cultivation without supply of auxotrophic sources (yeast extract). *Chlorella* is in this regard obviously more pretentious than *Scenedesmus*.
4) The metabolism of fructose and its phosphorylated derivative in both algae is not satisfactorily clear.

References

DVOŘÁKOVÁ-HLADKÁ, J.: Utilization of organic substrates during mixotrophic and heterotrophic cultivation of *Algae*. — Biol. Plant. **8** : 354—361, 1966.

DVOŘÁKOVÁ-HLADKÁ, J.: The role of sugars in the respiration of differently cultivated green *Algae*. — Biol. Plant. **9** : 340—353, 1967.

DVOŘÁKOVÁ-HLADKÁ, J., BASLEROVÁ, M.: Nahrazení peptonu Witte odvarem z droždí při kultivaci *Chlorella vulgaris, Ch. protothecoides* a *Ch. xanthella.* [The replacement of Witte-pepton by yeast decoct.] — Čs. Biol. **6** : 232—237, 1957.

EMERSON, R.: The effect of certain respiratory inhibitors on the respiration of *Chlorella.* — J. Gen. Physiol. **10** : 469—477, 1926.

EMERSON, R., and GREEN, L.: Effect of hydrogen ion concentration on *Chlorella* photosynthesis. — Plant Physiol. **13** : 157—168, 1938.

FOGG, E. G.: The Metabolism of Algae. — London Methuen 1953.

FRENCH, C. S., KOHN, H. J. and TANG, P. S.: Temperature characteristics for the metabolism of *Chlorella* II. The role of respiration of cultures of Chlorella pyrenoidosa as a function of time and of temperature. — J. gen. Physiol. **18** : 193—207, 1934.

HACKETT, D. P.: Respiratory inhibitors. In: RUHLAND, W. (ed.): Handb. d. Pflanzenphysiol. **XII/2**: 23—41. — Springer-Verlag, Göttingen; Berlin, Heidelberg - 1960.

HARTREE, E. F.: Cytochrome in higher Plants. — Adv. Enzymol. **18** : 1—64, 1957.

JAMES, W. O.: Plant Respiration. — Oxford, Clarendon Press 1953.

KANDLER, O.: Über die Beziehungen zwischen Phosphathaushalt und Photosynthese II. Gesteigerter Glucoseeinbau im Licht als Indikator einer lichtabhängigen Phosphorilierung. — Ztschr. Naturforsch. **9b** : 625—644, 1954.

KANDLER, O.: Über die Beziehung zwischen Phosphathaushalt und Photosynthese. III. Hemmungsanalyse der lichtabhängigen Phosphorylierung. — Ztschr. Naturforsch. **10b** : 38—46, 1955.

KANDLER, O.: The effect of 2,4-dinitrofenol on respiration, oxidative assimilation and photosynthesis in *Chlorella.* — Physiol. Plant. **11** : 675—684, 1958.

KLEINZELLER, A., MÁLEK, J., VRBA, R.: Manometrické metody a jejich použití v biologii a biochemii. [Manometrical methods and their application in biology and biochemistry.] — Stát. zdrav. nakl., Praha 1954.

MCNULTY, I. B., LORDS, J. L.: Possible explanation of fluoride induced respiration in *Chlorella pyrenoidosa.* — Science **132** : 1553—1554, 1960.

MOSES, V., SYRETT, P. J.: The endogenous respiration of microorganisms. — J. Bact. **70** : 201 t-o 203, 1955.

OAKS, A.: Influence of glucose and light on pyruvate metabolism by starved cells of *Chlorella ellipsoidea.* — Plant Physiol. **37** : 316—322, 1962.

REAZIN, G. H., JR.: The metabolism of glucose by the Alga *Ochromonas malhamensis.* — Plant. Physiol. **31** : 299—303, 1956.

ROOS, C. W., WIEBE, H. H., MILLER, G. W.: Effect of fluoride on glucose catabolism in plant leaves. — Plant Physiol. **37** : 305—309, 1962.

SIMON, E. W., BEEVERS, H.: The effect of pH on the biological activities of weak acids and bases. I. The most usual relationship between pH and activity. — New Phytol. **51** : 163—167, 1952.

SOROKIN, C., MYERS, J.: Time course of respiration during the life cycle of *Chlorella* cells. — J. Gen. Physiol. **40** : 579—592, 1957.

STOPPANI, A. V. M., RAMOS, E. H., WINDUCZYNSKI, I., BENNUN, A., DE PAHN, E. M.: Acción del 2,4-dinitrofenol sobre oxidación de substratos endogénos y exógenos por la levadura Saccharomyces cerevisiae. [The effect of 2,4-dinitrophenol on the oxidation of endogenous and exogenous substrates in the yeast *Saccharomyces cerevisiae.*] — Symp. on the Use of Radioisotopes in Anim. Biol. and Med. Sci., Mexico City, 1961.

TAYLOR, F. J.: Oxidative Assimilation of Glucose by *Scenedesmus quadricauda.* — J. expt. Bot. **1** : 302—321, 1950.

TING, I. P., DUGGER, W. M.: Transhydrogenation in root tissue: Mediation by carbon dioxide. — Science **150** : 28, 1965.

[J. DVOŘÁKOVÁ-HLADKÁ, Ústav experimentální botaniky ČSAV Praha, oddělení Sbírka kultur autotrofních organismů: **K otázce cest katabolismu cukrů u zelených řas.** — Biol. Plant. **10** : 58 až 71, 1968.

Byl sledován respirační metabolismus u kultur řas *Chlorella pyrenoidosa* (82) a *Scenedesmus obliquus* (125), kultivovaných jednak na minerálním živném roztoku, jednak na témže roztoku s glukosou a na roztoku s glukosou a odvarem z kvasnic. Jednotlivé stupně respiračního metabolismu endogenního i v přítomnosti exogenně aplikovaných cukrů byly stanoveny podle reakce na inhibitory: monojodacetát, NaF, Na-azid a 2,4 DNP. Pro srovnání byly paralelně aplikovány

při posledních dvou inhibitorech fruktosa a fosforylované cukry glukoso-6-fosfát a fruktoso-1,6--difosfát. Na-monojodacetát inhibuje dýchání jak endogenního, tak exogenního (s glukosou) substrátu. NaF (v konc. až 2,5 . 10^{-2}м) příjem O_2 zvyšuje. Vliv azidu a 2,4 DNP je u obou řas určen předběžnou kultivací. Na základě toho je v práci diskutováno o možné účasti jednotlivých respiračních cest v dissimilaci glukosy. V zásadě je možno počítat s těmito cestami katabolismu: a) přímá oxidace (zvláště obě autotrofně kultivované řasy a *Chlorella* kultivovaná na půdě s glukosou), b) cesta, podobná glykolyse, která však nutně nezahrnuke enolázu (neinhibuje NaF), c) pentofosfátovým cyklem (*Chlorella*) a obě řasy d) glykolyticky jsou schopny katabolovat jen již fosforylovaný cukr.

Й. Дворжакова-Гладка, Институт экспериментальной ботаники ЧСАН, отделение Коллекции культур автотрофных организмов: **К вопросу путей катаболизма сахаров у зеленых водорослей.** — Biol. Plant. **10**: 58—71, 1968.

В настоящей работе я хотела установить, какими путями метаболизируется глюкоза, которая является хорошим субстратом для роста и активирует дыхание при добавлении извне. Для определения отдельных звеньев я применила обычные ингибиторы. Натрий-монойодацетат ингибирует дыхание как эндогенного так и экзогенного субстрата. Фтористый натрий (в концентрации до 2,5 . 10^{-2}м) повышает потребление O_2. Влияние азида и 2,4 DNP у обеих водорослей зависит от предшествующей культивации. На основании этого в работе обсуждается возможное участие отдельных дыхательных путей в процессе диссимиляции глюкозы. В принципе можно считать осуществимыми следующие пути катаболизма сахаров: а) прямое окисление (особенно у обеих автотрофно культивируемых водорослей и у *Chlorella* культивированной на среде с глюкозой), б) путь подобный гликолизу но не обязательно включающий энолазу (не ингибируется NaF), в) пентозофосфатным циклом (*Chlorella*) и обе водоросли способны г) гликолитически метаболизировать лишь фосфорилированный сахар.