Mutual relation of protozoans and symbiotic *Algae in Paramaecium bursaria*

II. Photosynthesis

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INTRODUCTION

The protozoan *Paramaecium bursaria* of characteristic green colour caused by endosymbiotic *Chlorella* has been the object of present investigations.

It results from Pringsheim's (1915, 1928), Parker's (1926) and Loefer's (1936) investigations that assimilation of algae plays a certain part in nutrition of a protozoan. Recent investigations in this line (Pado 1965) showed that photosynthesis plays a great role in the metabolism of *Paramaecium bursaria*. This can be clearly seen in the relation between the division rate of the organism and light conditions. It results from Wichterman's (1948) investigations that the behaviour of protozoans in darkness differs from the behaviour of those from light conditions. Protozoans from darkness swim very actively, whereas, those with algae — the green ones, direct themselves towards light and settle down on the bottom of the vessels.

Nutrition of *Paramaecium bursaria* was already studied previously by Pringsheim (1928) who established that these organisms are photoautotrophic. His results were corroborated by Pado (1965). Sterile cultures of *Paramaecium bursaria* kept in light are fully active inversely to those from dark cultures which do not reproduce at all in the same conditions.

Quantitative determination of photosynthesis in *Paramaecium bursaria* has not yet been exactly examined.

The present paper aims to investigate the gas exchange in *Paramaecium bursaria* in dependence of light conditions. The experimental material for these investigations was taken from cultures kept a) in darkness, b) in middle light intensity, c) full light intensity, thus, differing from one another both by the number and appearance of plant endosymbionts (Pado 1965).

MATERIAL AND METHOD

The clone *Paramaecium bursaria* as well as culturing conditions were described in the previous paper (Pado 1965). The microrespirometer constructed by Zurzycki (1955) and Starzecki (1961) with a reaction chamber 6 mm in diameter and working cross section of capillaries 0.044 mm² was used for measurements. 6–8 days old

cultures provided the experimental material. For every measurement 100 specimens were counted under a stereoscopic microscope and transferred by means of micropipettes on to microscopic slide glasses with small cavities. Before the protozoans were closed in the chambers of the microrespirometer they had been cleaned from bacteria in 5–6 successive washings in sterile water. They were subsequently placed in a hanging drop of water on a glass which covers the microchamber of the respirometer. The hanging drop of water was prevented from flooding over the glass surface by means of a plexiglass ring 4.5 mm in diameter and 0.4 mm high walls, this ring was fixed to the glass by means of melted paraffine. A projecting lamp 250 W sup-

Fig. 1. Example of readings of production resp. consumption of oxygen in various light intensities (data refer to protozoans from average light intensity 900 lux).

x axis — time in minutes; y axis — $\mu\text{l O}_2 \cdot 10^{-5}$

plied with 200 V current constituted the light source. The light concentrated by means of a lens passed through a 5 cm thick filter of distilled water which absorbed infra red radiation. Light intensity in the microrespirometer chambers was measured by means of a Zeiss luxmeter. Light intensity in the range from 0–12400 lux was regulated by means of a diaphragm and neutral net filters.

Measurements of gas exchange were carried out in 27° C i.e. in temperature in which *Paramecium bursaria* also was cultured.

The photosynthesis and respiration intensity were expressed in $\mu\text{l O}_2/\text{l specimen of } Paramecium \text{ bursaria} / 10 \text{ minutes}$. The presented results are mean values of 3 repetitions.

Measurements of gas exchange were taken by means of readings of O₂ consumption respectively production made every 10 minutes for 40 minutes. After a change of light conditions successive readings were taken after a 30 minutes long period of adaptation. Respiration intensity was measured before and after completing

a series of measurements of photosynthesis. The fact of identical respiration intensities in both cases gives evidence that the applied illumination does not cause a decrease of metabolic activity of the protozoan. Fig. 1 presents the results of gasometric measurements of one of the experiments in form of an example. As far as the average number of cells of the endosymbiotic *Chlorella* for one cell of the protozoan was known the intensity of gas exchange for one cell of algae could be easily determined.

In order to calculate the photosynthetic efficiency of chlorophyll a and b, chlorophyll was extracted from the endosymbionts. Protozoans were, thus, cultured in mass in 0 lux (darkness), 900 lux and 2700 lux. A determined number of protozoans present in a determined volume of nutrient solution were thoroughly centrifuged and chlorophyll was extracted from the obtained sediment by means of acetone. The extracted pigments were subsequently transferred into diethylic ether. Every time a constant volume of 30 ml was obtained by adding solution. In case of need the solution was densified in nitrogen atmosphere. The chlorophyll absorption was determined by means of a spectrophotometer Uvispek Hilger at wave length 662 and 644 nm.

The content of chlorophylls in the examined solution was calculated according to the formula given by Smith and Benitz (1955):

$$\text{Chlorophyll a (mg/l)} = 10.1 \cdot D_{662} - 1.01 \cdot D_{644}$$

$$\text{Chlorophyll b (mg/l)} = 16.4 \cdot D_{644} - 2.57 \cdot D_{662}$$

where D — denotes the extinction for a given wave length of a 1 cm thick layer.

RESULTS

The present investigations have shown that photosynthetic and respiration activity in *Paramaecium bursaria* attain various values in dependence on light conditions the material is cultured in. Respiration of a protozoan in darkness is expressed by the sum of this process both for the protozoan and its symbiotic algae.

A reduced number of algae (Pado 1965) is characteristic of *Paramaecium bursaria* cultured in darkness. The average number of algae for protozoan cultured in darkness equals about 110 cells, whereas in 900 lux this number increases to 421 cells and in 2700 lux even to 1030 cells.

Figure 2 presents light curves for protozoans cultured in various light conditions. It can be seen from the light curves for protozoans cultured in darkness that only consumption of oxygen was observed within the whole range of examined light intensities. This consumption is somewhat lower in high light intensities, what means that there exists a very low photosynthesis which does not attain the compensation point however the small number of algae for a cell of the host cannot cause such a high photosynthetic production which would compensate for the respiratory consumption of oxygen. The small number of algae in these protozoans influences the considerably lower respiration rate of the whole symbiotic complex. Light curves for protozoans from average light intensity — 900 lux and intensive light 2700 lux are

qualitatively similar. In both cases a true photosynthetic production was stated in high light intensity. Both these series show higher respiration values than protozoans from dark conditions which results from a much higher number of algae for one cell of protozoan.

The compensation point for both these series lies in an approximate range of light intensity within 1200 and 1500 lux. The saturation point presenting the smallest light intensity necessary to obtain the maximum photosynthetic intensity, is

Fig. 2. Light curves for *Paramaecium bursaria* cultured in various light intensities
x axis — light intensity in lux; y axis — photosynthesis and respiration expressed in $\times 10^{-5} \mu\text{l O}_2 / 1 \text{ Paramaecium bursaria} / 10 \text{ min.}$
1 — protozoans from darkness (0 lux); 2 — protozoans from middle light intensity (900 lux); 3 — protozoans from high light intensity (2700 lux)

another characteristic point of the light curve. The saturation point for protozoans from high light intensity is attained at 7000 lux, whereas, protozoans cultured previously in average light intensity reach the saturation point at about 12 000 lux.

Protozoans cultured in various light conditions show great differences in respiration. E.g. the average respiration rate in *Paramaecium bursaria* from intensive light equals $77.5 \cdot 10^{-5} \mu\text{l O}_2 / 10 \text{ minutes}$, whereas, from darkness only $20.7 \cdot 10^{-5}$

$\mu\text{l O}_2/10$ minutes. The dependence of respiration rate in relation to the means number of algae — different in various culture conditions — is shown in fig.3. As it results from the diagram there is a direct proportionality between the respiration rate and the number of algae. The respiration rate of a protozoan free from any algae may be calculated by extrapolating the obtained curve.

Its value is $17.5 \cdot 10^{-5} \mu\text{l O}_2/10$ minutes. When the average number of algae for one protozoan is known the light curve can be calculated for one cell of the plant endosymbiont. The results presented in fig. 5 show that the algae present in

Fig. 3. Intensity of respiration process in *Paramaecium bursaria* in dependence on the number of endosymbiotic algae for 1 host cell

x axis — mean number of algae/1 *Paramaecium bursaria*; y axis — respiration rate expressed in $\times 10^{-5} \mu\text{l O}_2/\text{Paramaecium bursaria}$

a protozoan from average and high light intensities assimilate with equal intensity, whereas, the photosynthetic activity with reference to the algae from darkness is considerably lower.

Considering that algae present in protozoans from dark conditions are not only smaller in size but also have less intensive colour in comparison with *Chlorella* from protozoans from light cultures (Pado 1965) it may be supposed that the low photosynthetic activity in this algae is connected with a small amount of chlorophyll.

Fig. 4. Chlorophyll $a+b$ content calculated for one protozoan and one cell of algae *Chlorella*
 x axis — light intensity from which the material derived; y axis — chlorophyll $a+b$ content
 1 — Chlorophyll $a+b$ for 1 cell of *Chlorella* in $g \times 10^{-12}$; 2 — Chlorophyll $a+b$ for 1 cell *Paramecium bursaria* in $g \times 10^{-9}$

Fig. 5. Light curves of algae *Chlorella* from various light conditions (0 lux, 900 lux, 2700 lux)
 x axis — light intensity in lux during measurements of photosynthesis; y axis — photosynthetic activity of *Chlorella*
 expressed in $x \cdot 10^{-7} \mu l O_2 / \text{cell} / 10 \text{ minutes}$
 1 — algae from darkness (0 lux); 2 — algae from average light conditions (900 lux); 3 — algae from high light
 intensity (2700 lux)

Corroboration of this hypothesis was based on measurements of the content of chlorophyll $a+b$. Fig. 4 presents the content of chlorophyll $a+b$ calculated for one protozoan and one cell of *Chlorella*. As it results from the diagram the amount of chlorophyll for one paramecium increases proportionally with the increase of light intensity in which the paramecia are cultured.

The calculation of chlorophyll for one cell of *Chlorella* from average and high light intensities gives almost comparable amounts of chlorophyll, whereas, this

Fig. 6. Photosynthetic production of O_2 in dependence on light intensity calculated for mg of chlorophyll $a+b$

x axis — light intensity in lux; y axis — the amount of efused O_2 in $\mu l/mg$ chlorophyll $a+b/10$ min.

1 — material from dark cultures (0 lux); 2 — material from cultures in average light intensity (900 lux); 3 — material from cultures in high light intensity (2700 lux)

amount is considerably lower in algae from dark conditions. A successive calculation of photosynthetic intensity for one unit of chlorophyll $a+b$ which expresses the photosynthetic efficiency of chlorophylls is presented in fig. 6. The efficiency of chlorophyll in paramecia from light cultures is much lower and lowest values are obtained for organisms from highest light intensity.

DISCUSSION

As it is known that solid organic aliment is the basic nutrition of *Paramecium bursaria* already Pringsheim (1928) established that photosynthesis can be at additional source of nutrition.

It results from previous investigations of the author (1965) that the influence of photosynthesis is clearly seen in the dependence of the growth rate on light intensity in which the culture is kept. On the grounds of mutual relations between the animal organism and the algae, conclusions concerning the role of photosynthesis for the paramecium can be drawn. The influence of photosynthesis may be removed by placing the protozoan culture in darkness. In such a situation the paramecium is deprived of any nutrient substances produced by the algae and, moreover, the growth of algae proceeds in a heterotrophic way at the cost of organic compounds drawn from the host.

Under such unfavourable conditions the protozoan reduces the number of endosymbionts to a great extent but does not get completely rid of them. The rest of algae lives at the cost of the protozoan for a long time and retains its potential developmental possibilities. Due to these developmental possibilities a synthesis of chlorophyll and an active division leading to a quick reproduction of endosymbionts (Parker 1926; Pado 1965) take place when placed into light.

It results from the obtained data that photosynthetic properties of protozoans depend on light conditions the examined material is cultured in, and to be more precise, they are connected with the number of algae calculated for one cell of the protozoan and on the amount of chlorophyll.

Establishment of a true photosynthetic production (fig. 2) should be specially stressed. The production of organic substances by algae in paramecia from light of 900 and 2700 lux is high enough to satisfy the requirements for nutrition of both symbiotic components. These results are corroborated by previous observations, (Pado 1965) in which the author stated that *Paramecium bursaria* can be a photoautotrophic organism in sterile conditions.

A considerably small number of algae in a paramecium from dark cultures is of very small importance for photosynthesis but at the same time their respiration is not very intensive either. The assumed intensity of oxygen consumption for *Paramecium bursaria* free of any algae should be $17.5 \cdot 10^{-5} \mu\text{l O}_2/10 \text{ minutes}$ (fig. 3), whereas, a protozoan from average light intensity containing 421 algae respire with intensity $44.0 \cdot 10^{-5} \mu\text{l O}_2/10 \text{ minutes}$. The quoted data are comparable with results obtained by Cunningham and Kirk (1942) who examined the respiration processes in *Paramecium caudatum* and stated that their respiration intensity equals $40 \cdot 10^{-5} \mu\text{l O}_2/10 \text{ minutes}/1 \text{ Paramecium caudatum}$. This higher respiration rate of *Paramecium caudatum* in relation to *Paramecium bursaria* free from any algae is justified by the fact that *Paramecium caudatum* are bigger in size.

It results from the obtained data that the respiration activity of algae is very high in relation to the respiration of a host cell (*Paramecium bursaria* free from algae

$17.5 \cdot 10^{-5}$ $\mu\text{l O}_2/10$ minutes, with a great number of algae $77.5 \cdot 10^{-5}$ $\mu\text{l O}_2/10$ minutes).

Thus, in darkness algae are the factor accelerating the drop of organic substances. In the light of these findings the reduction of the number of algae in dark cultures (Pado 1965) is quite justified and comprehensible.

The form of symbiosis in *Paramaecium bursaria* is very similar to that in alcyonacean corals where an endosymbiosis of an animal organism (a coral) and a plant organism (zooxanthellae) is observed. Yonge and Nicholls (1931) examined coral reefs and forwarded the hypothesis that zooxanthellae are supplied with CO_2 and P and N salts by the host, whereas, corals receive additional amounts of O_2 and other products secreted by algae as a result of photosynthesis of algae.

Muscatine and Hand (1958) determined photosynthesis in corals quantitatively. They stated that in favourable conditions compensation point of photosynthesis in examined corals was exceeded.

Intensity of true photosynthesis depends on the species of the corals as well as on the content of chlorophyll and caroten pigments in symbiotic algae. Photosynthetic activity of chlorophyll in examined corals varies from 0.015–0.023 $\text{mg O}_2/\text{min/mg}$ of chlorophyll *a*. When calculating the results of the present paper in similar units following values will be obtained for protozoans: from high light intensity 0.121, from average 0.226 and from darkness 0.762 $\text{mg O}_2/\text{min/mg}$ chlorophyll *a+b*.

These values are about 10^x higher than values obtained for corals by Burkholder and Burkholder (1960) but comparable with the efficiency of diatoms determined by Talling as 0.5 $\text{mg O}_2/\text{min/mg}$ of chlorophyll *a*.

Oorschot (1955) examined the photosynthesis of a chlorophyll unit *a+b* for cells of non symbiotic *Chlorella*. In a free living *Chlorella* this activity is in the saturation point (according to table 6) as follows:

$\mu\text{l O}_2/\text{min}/\mu\text{g}$ of chlorophyll (<i>a+b</i>)	
20°	0.076–0.121
30°	0.084–0.101
40°	0.110–0.154

The above given results are also comparable with the results obtained in the present investigations for the endosymbiotic *Chlorella*.

As it results from diagram 6 the light curves referred to a unit of chlorophylls are convergent in the range of low intensities of light but differ greatly in the range of high intensities. This result is in agreement with Emerson's (1929) data. He showed for a *Chlorella* suspension that photosynthesis does not depend on the concentration of chlorophyll in low light intensity, whereas, in high light intensity such a dependence exists.

Lichenes are another symbiotic organism where a symbiosis of fungi and algae takes place. The algae living in symbiosis with fungi assimilate CO_2 from the atmosphere and secrete organic substances which constitute a nutrition common for algae

and fungi. In lichens algae live in low light intensities at the cost of organic substances which are absorbed from the substratum (Quispel 1959) by the fungi. In high light intensities photosynthesis increases and after the compensation point has been exceeded the lichen become quite self nourishing. It is characteristic of the photosynthetic curves obtained for lichens that the compensation point is attained only in rather high light intensities lying in the range 800—2000 lux (Stälfelt 1939). This is because of an active respiration of fungi — thus a component which does not take part in photosynthesis. Light curves obtained in present investigations show that the compensation point lies also in relatively high light intensities 1200—1500 lux and this fact is explained by a high respiration rate of protozoans.

SUMMARY

The role of photosynthesis of green endosymbionts *Chlorella* in nourishing of the whole symbiotic organism *Paramecium bursaria* has been determined in the present paper. It has been established that the rate of photosynthesis and respiration of *Paramecium bursaria* attain various values in dependence on conditions of light the examined material comes from. This stands in close connection with the amount of algae for one cell of protozoan.

The obtained results permitted to draw photosynthetic curves for *Paramecium bursaria* from various light intensities. Similar photosynthetic curves have been drawn with reference to cells of the algae *Chlorella* from various light conditions and photosynthetic efficiency has been calculated for a unit of chlorophyll $a+b$. It proves that highest photosynthetic efficiency is demonstrated by algae from average light intensity (900 lux) and not from the highest one (2700 lux); whereas, photosynthetic efficiency of chlorophyll $a+b$ unit is highest for protozoans from dark conditions and lowest for those from high light intensity.

The existence of true photosynthesis in paramecia cultured in the middle and high light intensities should be specially stressed. This explains at the same time why the complex *Paramecium bursaria* can be a photoautotrophic organism.

It results from the obtained data that the respiration rate in algae is very high in comparison with that of the host cell. Consequently the existence of mechanisms regulating the number of algae in dependence on light conditions seems more comprehensible as algae are a factor accelerating the consumption of organic substances.

When comparing the obtained results with investigations on photosynthesis of endosymbiotic animals such as alcyonacyan corals — it can be stated that the efficiency of the photosynthetic apparatus in *Paramecium bursaria* is considerably higher.

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Wzajemna współzależność pierwotniaków i symbiotycznych glonów u *Paramecium bursaria*

II. Fotosynteza

Streszczenie

W niniejszej pracy określono rolę fotosyntezy zielonych endosymbiontów z rodzaju *Chlorella* dla odżywiania się całego układu symbiotycznego pierwotniak — glon. Stwierdzono, że aktywność fotosyntezy i oddychania u *Paramecium bursaria* osiąga różne wartości, zależne od tego, z jakich warunków światła pochodzi badany materiał, gdyż to pozostaje w bezpośrednim związku z ilością glonów przypadających na komórkę pierwotniaka.

W wyniku uzyskanych danych możliwe było wykreślenie krzywych świetlnych fotosyntezy dla pierwotniaków *Paramecium bursaria* z różnych natężeń światła. Podobne krzywe świetlne fotosyntezy podano odnosząc je do komórek glonów *Chlorella* z różnych natężeń światła, oraz przeliczono sprawność fotosyntetyczną jednostki chlorofilu $a+b$. Okazuje się, że najwyższą sprawność fotosyntetyczną wykazują glony ze średniego światła (900 lux), a nie najsilniejszego (2700 lux). Sprawność fotosyntetyczna jednostki chlorofilu $a+b$ jest natomiast najwyższą dla pierwotniaków z ciemności a najniższą ze światła silnego.

Na specjalne podkreślenie zasługuje tu fakt istnienia rzeczywistej produkcji fotosyntetycznej (u pierwotniaków ze światła średniego 900 lux i światła silnego 2700 lux). Jest to zarazem wytłumaczeniem dlaczego kompleks *Paramaecium bursaria* może być organizmem fotoautotroficznym.

Z uzyskanych danych wynika też, że aktywność oddechowa glonów jest bardzo wysoka w porównaniu z oddychaniem komórki gospodarza. W związku z tym bardziej zrozumiałe staje się istnienie mechanizmów regulacji liczby glonów w zależności od warunków świetlnych, gdyż glony są czynnikiem bardzo przyspieszającym zużywanie substancji organicznych.

Porównując uzyskane wyniki z badaniami nad fotosyntezą zwierząt endosymbiotycznych, jakimi są korale alcyonacyanowe, można stwierdzić, że sprawność aparatu fotosyntetycznego u *Paramaecium bursaria* jest znacznie wyższa.