Effect of different amino acids on the sporulation of *C. falcatum* on the sugarcane juice of resistant variety

Cane variety	Total nitrogen content present in 100 ml of juice (mg)	Amount of total nitrogen adjusted to 18 mg/100 ml juice by substituting	Sporulation (mean of 3 replicates) 1×10^8
Co. 550 (resistant)	12.80	–	21.70
		DL-Alanine	22.50
		L-Arginine	23.25
		L-Asparagine	29.82
		L-Cysteine	18.11
		L-Glutamic acid	31.00
		Glycine	21.25
		L-Histidine HCl	22.00
		DL-Isoleucine	26.12
		DL-Leucine	24.00
		L-Lysine HCl	22.32
		DL-Methionine	17.10
		DL-Phenylalanine	20.55
		L-Proline	21.25
		DL-Serine	21.00
		DL-Threonine	21.75
		DL-Tryptophan	23.55
		DL-Valine	24.95
		S. Em.	± 0.0352
		C.D. at 5%	0.0884
		C.D. at 1%	0.1159
Co. 608 (susceptible)	22.50	–	14.85

Results and discussion. It was found, in general, that the sporulation was more in the juice agar of resistant variety as compared with that of the susceptible variety. The juice of resistant variety had significantly lesser content of total nitrogen than that of the susceptible ones. There was a significant increase in sporulation in the cane juice agar of resistant variety due to the addition of various amino acids (Table). Further, the addition of L-asparagine and L-glutamic acid have remarkably increased the sporulation. This confirms the earlier observation that the nitrogen content in juice, particularly amino nitrogen, plays an important role in supporting the sporulation of the fungus and also the resistance of the cane towards the disease. The addition of L-cysteine and DL-methionine significantly decreased the sporulation. The sporulation was poor in the cane juice agar of susceptible variety due to the presence of high total-nitrogen content present in the juice[8].

Zusammenfassung. Die Sporenbildung von *Colletotrichum falcatum* Went. wird bei der Kultur auf Zuckerrohrsaft durch den Zusatz von Asparagin und Glutaminsäure begünstigt, während Zystein und Methionin die Sporenbildung herabsetzen.

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Regulation of Host and Symbiont Population Size in *Paramecium bursaria*

The ciliate protozoan, *Paramecium bursaria* maintains within its cytoplasm several hundred green algal cells of a *Chlorella* species. Perpetuation of this association has been explained by proposing a steady state in number of algae per protozoan cell[1-3]. This paper examines this proposal by comparing population growth of the 2 partners, alga and protozoan, throughout the growth cycle of cultures maintained in light and in darkness. Evidence is also presented bearing on the existence and nature of regulatory mechanisms for maintaining such a proposed steady state.

Materials and methods. The chlorella-bearing strain of *P. bursaria*[4] was grown in a baked lettuce infusion[5] modified by the addition of 3 m*M* phosphate buffer adjusted to pH 7.2 and inoculated with *Enterobacter* (Aerobacter) *aerogenes*. Stock cultures were maintained in continuous light of 150 ft-c produced by cool white fluorescent tubes. The temperature of stock and experimental cultures was 25 ± 1 °C. Experimental cultures (50 ml in 125 ml cotton-plugged Erlenmeyer flasks) were provided with a diurnal regimen of 20 h light + 4 h darkness. The concentration of protozoa in each experimental culture was determined by averaging the number counted in three 0.1 ml samples of animals immobilized by adding a bacteriological loopful of 10% aqueous formaldehyde. Populations of endogenous algae in the protozoan samples were determined by liberating the endoplasmic algae. This was accomplished by forcibly ejecting the animal cells in a stream from a 2.0 ml standard glass hypodermic syringe with a No. 27 needle. Algal counts were then made with a hemacytometer. Endogenous algal cell population is expressed either as cell population per millilitre of culture; or the algal cell population divided by the prozoan cell population in a particular sample yields the 'symbiont index' representing the mean algal cell population per paramecium cell.

Results. Populations of both the ciliate host and its endosymbiont algae are capable of reproducing in continuous darkness on the bacterized lettuce medium used in this study (Figures 1a and 1b, lower curve), confirming the results of SIEGEL and others[1,6,7]. The rate of increase and total yield of endogenous chlorella populations is greatly enhanced by light (Figure 1a, upper 2 curves). On the other hand, growth of host protozoan populations is also enhanced by light (Figure 1b, upper 2 curves). Since paramecia devoid of algae are unaffected by light, these results establish that algal photosynthesis is directly

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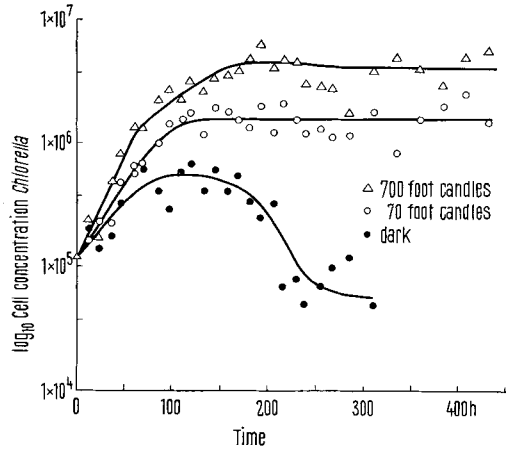

Fig. 1a. The effect of light of 2 intensities, 70 ft-c and 700 ft-c, on growth of endogenous *Chlorella* populations. Cell populations are expressed as $\log_{10}$ number of cells/ml.

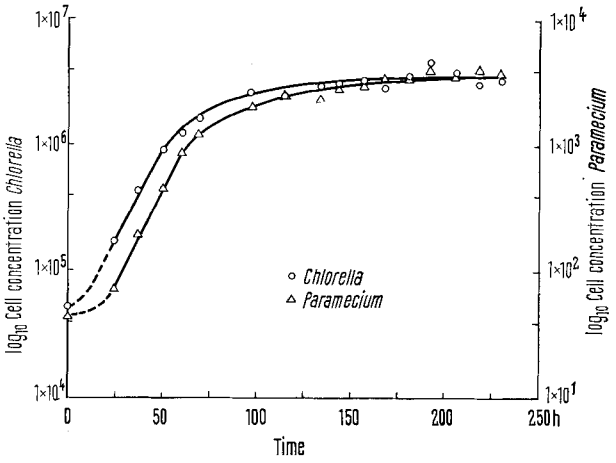

Fig. 2. Precise growth coordination of host and symbiont populations with low bacterial food. Light intensity 1000 ft-c.

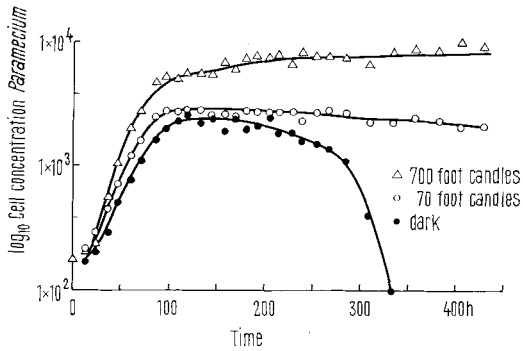

Fig. 1b. The effect of light of 2 intensities, 70 ft-c and 700 ft-c, on growth of host *Paramecium* populations. Cell populations are expressed as $\log_{10}$ number of cells/ml.

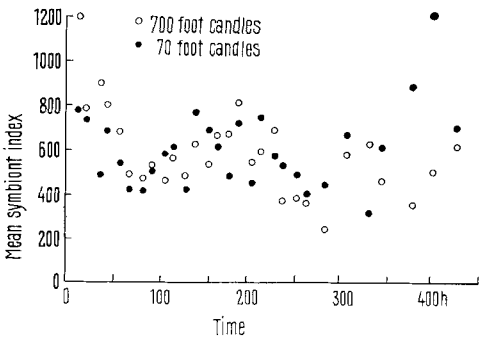

Fig. 3. Mean symbiont index (algal cell population/protozoan cell) as a function of light intensity and time.

or indirectly stimulating reproduction of the protozoan host cells. Other experiments demonstrate how close the correlation of population growth by the two partners can be. In these experiments, the animal hosts presumably were made even more nutritionally dependent on their plant symbionts by further decreasing live bacterial food. This was accomplished by adding to the medium streptomycin (15 μg/ml) and penicillin (400 U/ml). Figure 2 shows the closely parallel growth curves of populations of the 2 partners under these conditions of low bacterial food and light of 1000 ft-c.

Further evidence for regulation is presented in Figure 3 in which the mean symbiont index is plotted against time for high and low light intensities. Statistical analysis has shown that the 2 sets of points for the stationary phase of the 2 cultures (60–230 h) will fit a common regression line. Also, there is no consistent trend in symbiont index with time. Thus it is clear that in this experiment the mean symbiont index is independent of light intensity and time, suggesting internal regulation of some sort.

Discussion. KARAKASHIAN[1] proposed that in *P. bursaria* there exists a steady state in symbiont index. The experiments reported here confirm the proposal and show how close the correspondence between the growth curves of host and symbiont populations can be. PADO[3] found that *P. bursaria* cultures, kept for long periods in the dark on bacterial food and maintaining a low and constant symbiont index, when brought into the light demonstrate what appears to be a temporary loss of regulation, the algae increasing in numbers much faster than their protozoan hosts early in this period of adaptation to the light. In the second phase of the adaptation period, the algal growth rate oscillates around it's final stable growth rate which coincides with the protozoan growth rate. Finally, the reproductive rates of host and symbiont become equal and stable. One gets the impression from the algal growth oscillations and the light independence of the symbiont index that the number of algae per protozoan cell is being regulated by the host as suggested by SIEGEL[7]. Basically, host elaborated regulation could be either by means of direct inhibition of algal cell division or by frequent removal of surplus algae. Egestion[7] and digestion[8] of algal cells by host protozoa have been observed and are possible causes of such regulation now under investigation[9–11].

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[9] The author's thanks to Dr. R. SIEGEL for kindly supplying the culture of *P. bursaria* and for advice and encouragement.

[10] Much of the experimental work reported here was carried out at The Barnes Botanical Laboratories, The University of Chicago.

[11] Supported by grants from the Society of Sigma Xi and the American Medical Association.

Zusammenfassung. Es hat sich gezeigt, dass eine nahe Wechselbeziehung zwischen der Vermehrungsgeschwindigkeit der symbiotischen *Chloralla*-Bevölkerung im *Paramecium bursaria* und der Vermehrungsgeschwindigkeit des Wirts besteht, wenn man diese Kultur der Lichteinwirkung aussetzt. Die durchschnittliche (mittlere) Anzahl von Algenzellen pro Protozoenzelle ist in Massenkulturen von Lichtstärke und Einwirkungszeit unabhängig.

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On the Presence of Multivesicular Bodies in *Leishmania donovani*

Single membrane limited cytoplasmic bodies containing small vesicles enclosed in a smooth membrane, called multivesicular bodies, are often found in the cytoplasm of many cellular types in metazoan animals (Hruban and Rechcigl[1]). The matrix of multivesicular bodies shows the activity of acid phosphatase (Moe et al.[2]). The role of these bodies in lytic processes has been demonstrated by Biberfeld et al.[3] and by Smith and Farquhar[4]. It was suggested that multivesicular bodies arise by sequestration of the vesicles formed in the Golgi complex (Biberfeld et al.[3]; Ericsson and Glinsmann[5]; Smith and Farquhar[4]).

It is the purpose of this paper to report on the presence of multivesicular bodies in *Leishmania donovani*, a parasitic protozoan belonging to the family Trypanosomatidae. The relation of the multivesicular bodies of *Leishmania* to Golgi complex will also be discussed.

L. donovani was kept in vitro for several years in diphasic medium prepared according to Tobie et al.[6]. For electron microscopy the cells were fixed for 5 h at 4 °C in 3% glutaraldehyde buffered with *s*-collidine. The cells were postfixed in 4% unbuffered osmium tetroxide solution for additional 14 h at the same temperature. After dehydration in ethanol, the cells were embedded in Epon 812. The sections were stained with lead citrate.

All cells present in the culture medium were in the leptomonad form. For general ultrastructural data of these cells, see original papers by Rudzińska et al.[7] and Djaczenko et al.[8]. The Golgi complex of *Leishmania* was most frequently situated in a juxtanuclear position (Figure 1). It was composed of parallel arrays of flattened sacs and vacuoles. Some of them were slightly distended and contained accumulations of small smooth-walled vesicles with watery content. The amount of vesicles per single Golgi complex was variable. Multivesicular bodies of *L. donovani* (Figures 2 and 3) were limited by a single membrane 95 A thick. The whole interior of the body was tightly packed with vesicles having similar morphological properties as the vesicles seen in the Golgi complex. Some of the vesicles had the tendency to dissolve in the matrix of the body. In some instances the

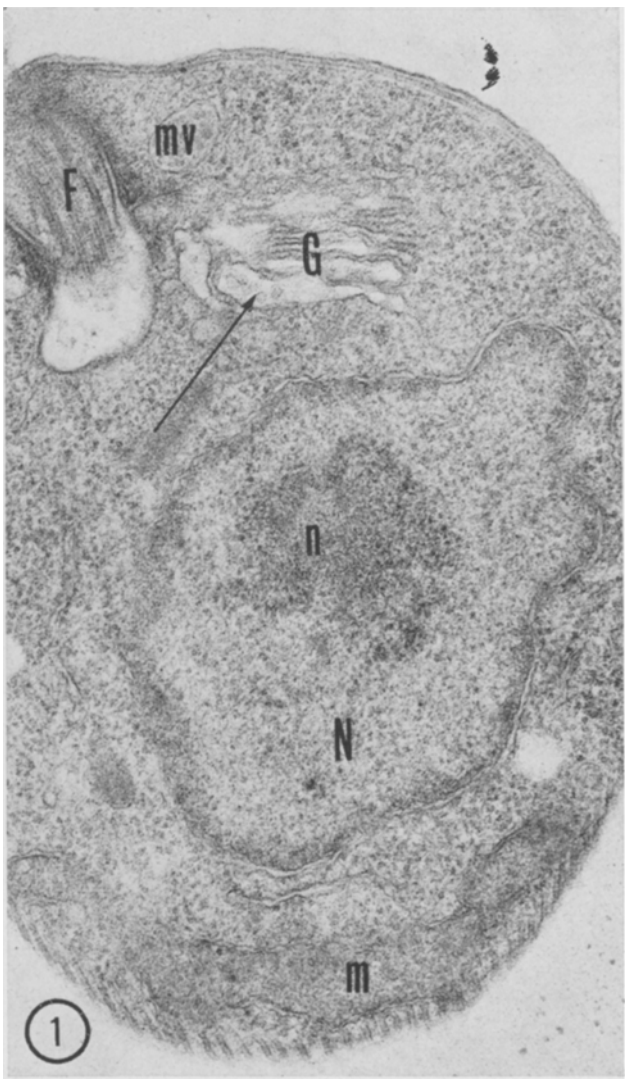

Fig. 1. Golgi complex (G) contains in one of its sacs small smooth vesicles (arrow). Multivesicular body (mv) is seen in the proximity of the Golgi complex. The nucleus (N) contains prominent nucleolus (n). At the cellular periphery is visible a mitochondrion (m). A part of the flagellum is marked (F). × 36,000.

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