Ascorbic acid content (in mg/100 g of the fruit pulp) of healthy and infected guava fruit

	Ascorbic acid content							% loss in ascorbic acid after 10 days of incubation
	Days of incubation							
	0	2	4	6	8	10	12	
'Safeda'								
Healthy	305.2	296.0	282.5	260.5	230.0	203.5	190.0	33.3
Infected	–	250.0	177.5	92.0	30.5	–	–	100.0
'Apple coloured'								
Healthy	372.2	349.5	317.5	276.0	238.0	222.0	200.0	40.3
Infected	–	298.0	200.0	195.5	69.5	39.5	–	89.2

Myrothecium verrucaria. Similar oxidation of ascorbic acid during fungal infection is known in the case of rusts [5,6].

It therefore seems probable that the decline in the ascorbic acid content may be due to the production of certain ascorbic acid degenerating enzymes, either by the fungus itself or by the host-parasite interaction as postulated by GHOSH et al. [3]. The comparatively rapid decline in ascorbic acid in the infected tissues may also be attributed to the increased respiration in the infected tissues which may induce rapid oxidation of ascorbic acid. Such an increase in respiration rate under pathogenesis is known to be incited by many fungi – especially powdery mildews and rusts [7–9].

Zusammenfassung. Der Ascorbinsäuregehalt nimmt in reifen Guavafrüchten nach Infektion durch *Botryodiploida theobromae* rasch ab, was wahrscheinlich auf enzymatischer Oxydation der Ascorbinsäure beruht.

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Studies on the Growth of *Prymnesium parvum* Carter (Chrysomonadina) and on the Formation of its Toxin (Prymnesin)

Prymnesium parvum forms a number of toxic principles which are responsible for its ichthyotoxic, hemolytic, and cytotoxic activities [1–3]. Likewise, prymnesin, the purified toxin of the phytoflagellate, inhibits the contraction of the guinea-pig ileum, evoked by a variety of smooth muscle stimulants [4].

For the purification of the toxin, the separation of its components and the study of their chemical properties, large quantities of *P. parvum* are required. ULITZUR and SHILO [5] have grown *P. parvum* on a medium developed by DROOP [6], and have harvested the cells after a period of 30–40 days. In our laboratory, the phytoflagellate has been cultivated routinely at 22 °C in artificial sea-water, enriched with 0.3% (w/v) oxoid liver infusion. Under these conditions, maximum yield of cells and toxin is obtained already within about 20 days (Table, example 1).

Recently a new medium has been developed, containing inorganic salts and 0.5 *M* glycerol [7]. This medium was originally designed to allow the growth of *P. parvum* in the dark, but if the culture was exposed to continuous illumination, the new medium also shortened the growth period considerably [7]. We have found that the results can be further improved by combination of glycerol with amino acids. So far, we have tried glycine, serine and α-alanine (Table). Thus, addition of D,L-serine (example 2) produces an almost threefold increase in cell density and toxin yield as compared with the basal medium (example 1). Examples 2 and 5 also show that, regardless of the conditions of illumination, D,L-serine and D,L-alanine give nearly the same number of cells and comparable yields of crude toxin, while glycine (example 4) is only $^1/_3$ as effective as the other 2 amino acids. Even supplementation of glycine with methionine did not improve growth above the level attained with glycine alone, i.e. the mixture of these 2 amino acids did not simulate the effect of serine. In order to test the stereospecificity of the stimulatory agents, we have examined the L-, the D- and the D,L-forms of α-alanine. All 3 gave approximately the same final yield of cells (Figure). On the other hand, β-alanine did not accelerate growth during the first 7 days,

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Growth of *Prymnesium parvum* and yield of toxin in various media

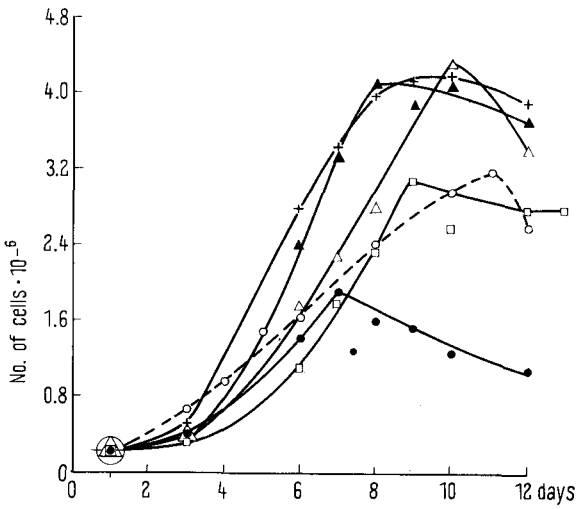
Medium	Condition of illumination	Growth period for maximum population density (days)	Maximum No. of cells/ml ($\cdot 10^{-6}$)	Crude toxin[a] mg/l of culture medium	Crude toxin[a] μg/cell ($\cdot 10^6$)	Activity of toxin H_{50}[b] (μg/ml)	Activity of toxin LD_{50}[c] (μg/fish)
1. 30% seawater[d] + liver extract	12 h light 12 h dark	21–25	4.5	23	5	0.6	65
2. Basal medium[e] + 0.5 *M* glycerol + 0.05 *M* D,L-serine	12 h light 12 h dark	10	12.2	60	5	not tested	70
3. Basal medium + 0.5 *M* glycerol + 0.05 *M* D,L-serine	24 h dark	10[f]	2	29	14.5[g]	not tested	200
4. Basal medium + 0.5 *M* glycerol + 0.05 *M* glycine	24 h light	12	4.5	26	5.7	not tested	55
5. Basal medium + 0.5 *M* glycerol + 0.05 *M* D,L-alanine	24 h light	10	15	80	5.3	0.5	65

[a] Prepared according to F. BERGMANN et al.[11]. [b] Concentration of toxin which caused 50% hemolysis of rabbit erythrocytes. Method of K. REICH et al.[12]. [c] Concentration of toxin injected intraperitoneally, which caused 50% mortality in minnows of *Gambusia affinis* of 300 mg body weight. For method see footnote [a]. [d] K. REICH et al.[13]. [e] See reference [7]. [f] This culture continued to grow over a long period (more than 20 days). [g] The high values for the weight of crude toxin is due to the formation of inactive material, extractable by methanol.

but subsequently kept the cell number at a high level by preventing lysis. The latter process is responsible for the decline of cell density, which appears in the Figure in the curve of the control culture.

It has been shown previously[8] that exposure to continuous light promotes the growth of the phytoflagellate, but diminishes the yield of toxin, while alternating periods of light and darkness favour its accumulation. The yield of prymnesin was highest when the organism was protected from light for 8–12 h before collection of the cells. The beneficial effect of the dark periods was explained by the photosensitivity of the toxin[9]. However, as shown in the Table, example 5, in a medium containing D,L-alanine and glycerol, it is possible to expose the organism to continuous illumination without impairing the overall yield of toxin. Also the cells can be collected directly, without being previously kept in the dark. We can now obtain $15 \cdot 10^6$ cells/ml within 10 days by growing the *P. parvum* in 1.5 l flasks, containing 1 l of the salt-glycerol-D,L-alanine medium. The yield is thus 3 times higher than the one obtained previously within 20 days.

Experiments are being undertaken at present to determine whether the toxin, formed by the new rapid growth method, is identical with the prymnesin formed in the earlier slow process.



Growth curves of *Prymnesium parvum* in basal medium[7] with 0.5 *M* glycerol, under continuous illumination at 20 °C. All amino acids were 0.05 *M*. ●—● = control, note decline of cell number after the seventh day; ○---○ = with glycine; △—△ = with D-α-alanine; ▲—▲ = with L-α-alanine; +—+ = with D,L-α-alanine; □—□ = with β-alanine. Note that all amino acids delay the decline of cell density, as compared with the control.

Résumé. *Prymnesium parvum* Carter atteint sa taille maximale en 9–10 jours et produit une grande quantité de toxine, quand il est cultivé à la lumière dans un milieu comprenant des sels, du glycérol et certains acides aminés (sérine ou α-alanine).

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