An Effect of Yeast Extract on the Growth of *Chlorella*

Chlorella vulgaris (Pearsall's strain) grows well on glucose in darkness[1]. Recently, in this laboratory, we tried to grow this alga on glucose in darkness in an atmosphere free from carbon dioxide. The attempt was unsuccessful; presumably carbon dioxide fixation is essential in at least one of the reactions necessary for growth. Since it has been reported that the requirement for carbon dioxide by *Neisseria gonorrhoeae* can be replaced by a supplement of yeast extract[2], the effect of adding yeast extract to the *Chlorella* cultures was investigated.

Pure cultures of *Chlorella* were grown in 50-ml. Erlenmeyer flasks in a mineral salts medium[3] containing 0·5 per cent glucose and 'Difco Bacto' yeast extract in the required concentration. The flasks contained a centre well, in which sodium hydroxide and filter paper were placed to remove carbon dioxide, and were closed by vaccine stoppers. The cultures were shaken at 25° C. in darkness. Growth was estimated by counting the cells four days after inoculation; it was expressed as a percentage of the growth in the cultures with glucose, no yeast extract and an atmosphere of air.

It was found that the addition of yeast extract did, in fact, allow some growth in a carbon dioxide-free atmosphere (Fig. 1); but the more striking effect of yeast extract was the inhibition of growth in an atmosphere of air. The growth of *Chlorella* on glucose in darkness, in air, was reduced by one-half by yeast extract in a final concentration of only 0·01 per cent. The inhibition was just as pronounced when the yeast extract was sterilized by Seitz filter and added to the autoclaved medium. The constituent of the yeast extract which inhibits growth is unknown. Organic acids inhibit the growth of *Chlorella* by combining with iron[4]. The possibility that a constituent of yeast extract acted in this way was tested by adding additional iron to the medium, but it had no effect on the inhibition.

It is of interest that a related, but colourless, alga, *Prototheca zopfii*, requires thiamine for growth[5] and, in fact, grows well in the *Chlorella* mineral medium with the addition of 1 per cent glucose and 1 per cent yeast extract. Removal of carbon dioxide from the atmosphere has little effect on the growth of *Prototheca* in this medium or when 10^{-5} *M* thiamine is substituted for the yeast extract (Table 1). This suggests either that *Prototheca* has a much lower carbon dioxide requirement for growth than *Chlorella*, or that thiamine can replace this requirement. However, the addition of 10^{-5} *M* thiamine to *Chlorella* cultures did not allow growth in a carbon dioxide-free atmosphere.

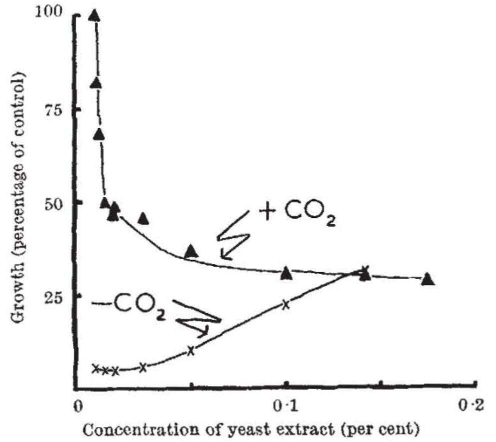

Fig. 1. The effect of yeast extract on the growth of *Chlorella vulgaris*. All cultures contained 0·5 per cent glucose and were shaken in darkness. The carbon dioxide-free cultures contained sodium hydroxide in centre wells of the flasks. Growth is expressed as a percentage of that in the flask with glucose, no yeast extract and an atmosphere of air

Table 1. Growth of *Chlorella* and *Prototheca* with and without Carbon Dioxide in the Atmosphere

	Cells/c.mm.			
	Chlorella vulgaris (Pearsall's strain)		*Prototheca zopfii* (Beijerinck's strain)	
	+ CO_2	− CO_2	+ CO_2	− CO_2
No addition	12,320 (100%)	2,960 (24%)	—	—
1 per cent yeast extract	4,176 (34%)	3,430 (28%)	12,400 (100%)	12,501 (101%)
10^{-5} Thiamine	20,760 (168%)	3,135 (25%)	10,310 (83%)	11,500 (93%)
Inoculum	863 (7%)		79 (0·6%)	

Each flask contained 10 ml. of *Chlorella* mineral medium with 1 per cent glucose added and the other additions indicated. After inoculation the flasks were shaken in the dark at 25° C. in a Warburg tank. The carbon dioxide-free cultures contained sodium hydroxide in centre wells.

I wish to acknowledge the receipt of a maintenance grant from the Agricultural Research Council.

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March 3.

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Isolation of *l*-Quinic Acid in Citrus Fruit

The organic acids in citrus juices are chiefly citric and malic, although tartaric and oxalic acids also have been reported in grapefruit[1]. In our laboratory, succinic acid was found in frozen mature oranges. Wolf[2] isolated citric, malic and an unidentified acid from citrus fruit. Sinclair and Eny[3] reported that in the peel malic acid predominates while citric and oxalic acids occur in smaller amounts.

In a study of organic acids in citrus fruit using the gradient elution technique on a 'Dowex-1' column, an unknown acid with an R_{malic} of 0·56 was detected in both the peel and juice. This acid alone or with authentic quinic acid was eluted from the column in the same fractions as quinic acid, and gave a positive test with the Cartwright and Roberts[4] reagent. The presence of *l*-quinic acid has been reported in apples[5] and peaches[6].

The quinic acid was first isolated from 1,600 gm. of immature Valencia orange peel using an ion-exchange resin, 'Dowex-1 × 8', of 200–400 mesh packed in a 2·3 cm. × 25 cm. column. The macerated peel was extracted three times with 60 per cent ethanol which was then evaporated under reduced pressure. The remaining aqueous solution was treated with charcoal, filtered, and first passed through a column of 'Dowex-50' ion-exchange resin and then through a column of 'Dowex-1' in the acetate form. This column was washed with distilled water, and the acid was eluted according to the gradient elution method of Hulme and Wooltorton[7] with a slight