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PROTOZOA NEED CARBON DIOXIDE FOR GROWTH*

OTTO RAHN

Laboratory of Bacteriology, Cornell University, Ithaca, New York

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On page 113 of this volume, the statement of Valley and Rettger that all bacteria need carbon dioxide for growth had been shown to apply to young as well as old cells. Although most bacteria produce CO_2 by respiration or fermentation, it is possible by the right technique to remove it as rapidly as it is produced, and under these circumstances, bacteria cannot multiply.

This study has now been extended to one alga and two protozoa, and it was possible to demonstrate that both types of organisms must have CO_2 for multiplication. With algae, as chlorophyll plants, this may not seem surprising, but the cultures were kept dark, in a medium containing glucose and citric acid. In this medium, the species tested, an unidentified *Chlorella* (No. 11 of the Cornell Laboratory of Plant Physiology) grew well in complete darkness to large numbers, producing chlorophyll. The alga obtained the growth energy, and perhaps the building material, from the organic matter; it lived essentially like a bacterium.

The method of testing the CO_2 requirement was the same as with bacteria. The alga was grown in pure culture in tiny drops of medium hanging from a sterilized cover-glass which was sealed airtight to a microculture slide by a ring of vaseline. At the bottom of the cavity was a small drop of 2.5 per cent KOH for the culture without CO_2, or of 2.5 per cent KCl for the control with a normal CO_2 content of the atmosphere.

Table 1 shows a typical experiment. With a heavy inoculum—100

TABLE 1

MULTIPLICATION OF CHLORELLA WITH AND WITHOUT CO_2

Numbers indicate cells per droplet.

	CO_2	No CO_2	CO_2	No CO_2	CO_2	No CO_2
Start	100	100	30	40	14	4
2 days	360	350	69	22	9	4
6 days	>800	>800	490	120	63	4
11 days	moldy	lost	dried	250	149	3

cells of this fairly large Chlorella in 2 mg. of medium is quite a large initial population—the CO_2 was produced more rapidly than it could be removed, and there is no difference between test and control. With the smaller inoculum of 30 to 40 cells per drop, CO_2 removal made an impression by causing a very definite delay, and slower multiplication. Very small numbers of cells could not get started at all, and the cells never multiplied in 11 days. This checks well with the behavior of bacteria.

Two pure cultures of flagellates were tested in the same way, *Astasia longa* and *Polytomella caeca,* which I owe to the kindness of Professor R. P. Hall of Columbia University. These animals thrive well in a solution of 1.00 per cent tryptone plus 0.05 per cent KH_2PO_4. They were tested in hanging droplets, but the KOH and KCl solutions had to be reduced to 1.00 per cent because of the weak osmotic pressure of the medium.

TABLE 2

MULTIPLICATION OF PROTOZOA WITH AND WITHOUT CO_2

Numbers indicate motile cells per droplet.

		CO_2	No CO_2	CO_2	No CO_2
Polytomella	Start	250	163	14	11
	1 day	Very many	165	66	19
	2 days	620	163	101	16
	3 days	Very many	46	—	3
	6 days	175	0	98	0
Polytomella	Start	225	153	11	11
	1 day	300	164	38	19
	2 days	285	71	48	16
	3 days	44	5	27	3
	6 days	Bacteria	0	27	0
Astasia	Start	13	29	5	2
	1 day	27	32	7	2
	2 days	33	27	15	2
	3 days	54	33	28	2
	6 days	85	33	140	2
Astasia	Start	30	20	5	5
	3 days	60	25	10	7
	6 days	100	21	65	5
	11 days	270	10	183	4*

*Dead.

Typical results in Table 2 show that it is quite possible to remove CO_2 as rapidly as it is produced. Since both organisms are motile, at times even rapidly motile, the counts of the individuals show considerable fluctuation. Yet, there is no doubt that all cultures with

CO_2 grew, and those without it did not. Only motile cells were counted, because it seemed impossible to ascertain which of the non-motile cells were still alive. These protozoa seem to respond more promptly to CO_2 removal than algae or bacteria, be it that their demand is greater, or that their rate of respiration per mg. body weight is smaller. *Polytomella* is quite sensitive and dies easily. *Astasia* is more resistant.

The rôle of the CO_2 is not known. Valley and Rettger observed with bacteria a slight decrease in viability when CO_2 was withheld for one or two days. The sensitive *Polytomella* dies without CO_2 after three to six days, but *Astasia* may be motile after 11 days.

Summary

Pure cultures of an alga (*Chlorella*) grown in the dark with organic food, and of two protozoa (*Polytomella* and *Astasia*) increased in number in hanging drop cultures in a normal atmosphere, but did not multiply when the carbon dioxide of the air was removed by alkali. The reason for the necessity of carbon dioxide for multiplication is unknown.