

showed a 70 percent reduction of the $1,25\text{-(OH)}_2\text{D}$ increase that occurred in the lactating rats with sham operations. These results indicate that PTH may control $1,25\text{-(OH)}_2\text{D}$ synthesis during lactation, as well as in other situations observed previously (5, 6), although it is possible that the high phosphate concentration in the PTX group (Table 1), as well as in the group that had been weaned 2 days previously (Fig. 1) may also serve to depress the hormone. In addition, these findings do not rule out still other regulators because the PTX group retained concentrations of $1,25\text{-(OH)}_2\text{D}_3$ that were twice that of the nonlactating group.

Since Bouillon *et al.* (19) have demonstrated that the serum vitamin D-binding protein, which transports $1,25\text{-(OH)}_2\text{D}$ in the blood, does not increase in either pregnancy or lactation in the rat, the observed changes in circulating $1,25\text{-(OH)}_2\text{D}$ (Fig. 1) could not be the result of increased plasma carrier protein synthesis. We propose that the increases in $1,25\text{-(OH)}_2\text{D}$ in pregnancy and lactation are produced by increased biosynthesis of the hormone as catalyzed by the renal 25-hydroxyvitamin D-1-hydroxylase. This enzyme is probably stimulated by augmented calcium requirements, with the parathyroid glands acting as an intermediary to sense low serum calcium and elaborate PTH which in turn increases the activity of the 1-hydroxylase. Other evidence suggests that prolactin can also influence the 1-hydroxylase (20) and circulating $1,25\text{-(OH)}_2\text{D}$ (9), at least in birds. Further, bromocriptine, a drug that inhibits prolactin secretion, can drastically reduce $1,25\text{-(OH)}_2\text{D}$ concentrations in early lactation in rats, an effect that is reversed by administration of prolactin (21). Since serum prolactin concentrations are maximum at 2 days of lactation in rats (22), it is therefore probable that any effect of prolactin on $1,25\text{-(OH)}_2\text{D}$ occurs during early lactation and that PTH is primarily responsible for the increased $1,25\text{-(OH)}_2\text{D}$ in late lactation. A synergistic affect of PTH and prolactin is also conceivable. The physiological function of the elevated $1,25\text{-(OH)}_2\text{D}$ concentration in lactation is probably to maintain a normal serum calcium level, since vitamin D-deprived lactating rats with reduced $1,25\text{-(OH)}_2\text{D}$ concentrations develop severe hypocalcemia (11). This action of $1,25\text{-(OH)}_2\text{D}$ may be by intestinal absorption of calcium, although direct evidence for this in lactating rats is not yet available.

In mammalian pregnancy the humoral activator of the 1-hydroxylase is also obscure, although PTH (1) and $1,25\text{-(OH)}_2\text{D}$ (18) are both increased near term in human pregnancy, suggesting that PTH is again functioning to stimulate $1,25\text{-(OH)}_2\text{D}$ formation. It is interesting that neither PTH (1) nor $1,25\text{-(OH)}_2\text{D}$ (23) are increased in women with well-established lactation (6 weeks postpartum)—again pointing to the inextricable association of PTH and increased $1,25\text{-(OH)}_2\text{D}$ in the calcium alterations of health and disease.

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Dark Anaerobic Dinitrogen Fixation by a Photosynthetic Microorganism

Abstract. *Photosynthetic purple bacteria can grow with dinitrogen gas as the sole nitrogen source under anaerobic conditions with light as the energy source. The bacterium Rhodospseudomonas capsulata can fix nitrogen in darkness with alternative energy conversion systems, namely, anaerobic sugar fermentation and aerobic respiration at low oxygen tension. Although growth on dinitrogen is optimal under photosynthetic conditions, the results show that reduction of dinitrogen is not obligatorily coupled to activity of the photosynthetic apparatus.*

The earliest studies on dinitrogen (N_2) fixation by photosynthetic purple bacteria capable of growing in several alternative modes indicated that N_2 reduction occurred only under anaerobic photosynthetic conditions (1, 2). No growth was observed under an atmosphere of air with N_2 as sole nitrogen source (2), presumably because of the inhibitory effects of molecular oxygen on nitrogenase synthesis and activity (3). A recent investigation on the characteristics of N_2 fixation by the purple bacterium *Rhodospseudomonas capsulata* (4) suggested

that in this organism N_2 fixation is dependent on the availability of a reductant uniquely produced by the photosynthetic apparatus. We report experiments demonstrating that *R. capsulata* can grow on N_2 as sole nitrogen source anaerobically in darkness; thus, a "photoreductant" is not obligatory.

Rhodospseudomonas capsulata grows anaerobically in darkness on N_2 in a completely defined medium with fructose as the sole source of carbon, energy, and reducing power (Fig. 1). The doubling time is approximately 12 hours,

a growth rate about half that observed with an ammonium salt as the nitrogen source (5). The control experiment of Fig. 1 shows that, when N_2 is replaced by argon, growth does not occur. Anaerobic dark growth under the conditions specified is dependent on an unusual fermentation process in which an accessory oxidant is required (6). In this instance the oxidant is dimethyl sulfoxide (DMSO), which is reduced to dimethyl sulfide as growth proceeds (6). In experiments of the kind shown in Fig. 1, growth ceases upon exhaustion of DMSO; cells can be repeatedly subcultured anaerobically in darkness if DMSO and fructose are provided. We have

shown earlier that trimethylamine- N -oxide (TMAO) also can function as the accessory oxidant (5). Although N_2 fixation is demonstrable with the TMAO system under certain conditions, high concentrations of the amine oxide inhibit growth on N_2 and for this reason we used DMSO as the accessory electron acceptor.

Table 1 lists in vivo nitrogenase activities (as measured by the acetylene reduction technique) of *R. capsulata* cells grown anaerobically either in light or in darkness on various nitrogen sources. Cells cultured with ammonia as sole nitrogen source had undetectable nitrogenase activities. When *R. capsulata* was

grown photosynthetically on glutamate or N_2 as the nitrogen source, however, acetylene reduction activities were maximal. With such cells, measurable activities were observed only when the incubations were carried out in the light. In contrast, *R. capsulata* cells grown anaerobically in darkness (with fructose as carbon and energy source) on N_2 or glutamate were capable of reducing acetylene when incubated in either the light or the dark. Although illumination had a stimulatory effect, fermentatively grown cells expectedly showed significant rates of acetylene reduction in darkness. The well-known inhibitory effect of ammonia on nitrogenase activity of photosynthetic bacteria (7) is observed with cells of *R. capsulata* grown in both the photosynthetic and fermentative modes (Table 1).

Thus, the energy and reducing power necessary for N_2 fixation by certain purple photosynthetic bacteria can be derived from either photosynthetic or dark anaerobic metabolism. A third alternative is furnished by dark aerobic respiratory metabolism, provided that the oxygen tension is maintained below inhibitory concentrations. Siefert (8) has reported that *R. acidophila* as well as several other purple bacteria can grow on N_2 under dark "microaerophilic" conditions, and we have confirmed this with *R. capsulata*. The capacity of *R. capsulata* to fix N_2 under photosynthetic, respiratory, or fermentative conditions suggests that this organism may be of special value for further studies on bioenergetic aspects of nitrogenase activity.

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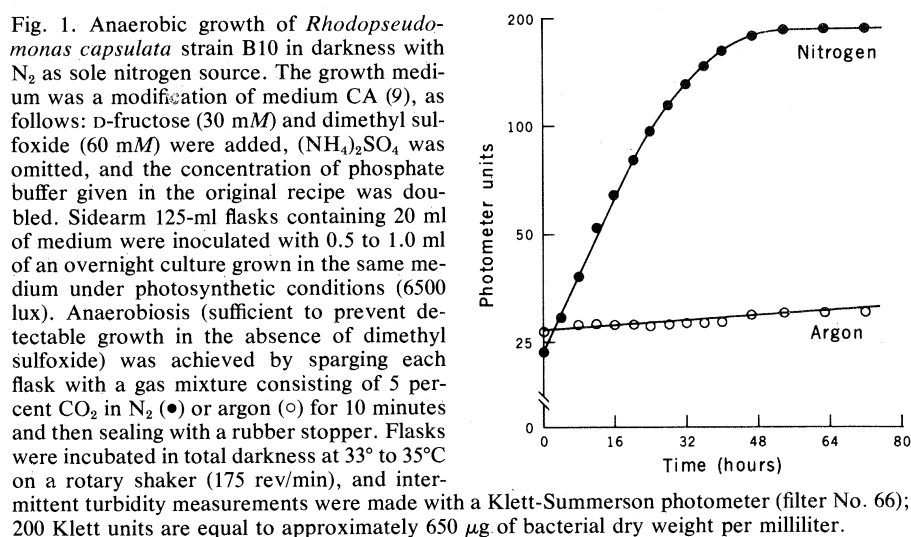


Fig. 1. Anaerobic growth of *Rhodospseudomonas capsulata* strain B10 in darkness with N_2 as sole nitrogen source. The growth medium was a modification of medium CA (9), as follows: D-fructose (30 mM) and dimethyl sulfoxide (60 mM) were added, $(NH_4)_2SO_4$ was omitted, and the concentration of phosphate buffer given in the original recipe was doubled. Sidearm 125-ml flasks containing 20 ml of medium were inoculated with 0.5 to 1.0 ml of an overnight culture grown in the same medium under photosynthetic conditions (6500 lux). Anaerobiosis (sufficient to prevent detectable growth in the absence of dimethyl sulfoxide) was achieved by sparging each flask with a gas mixture consisting of 5 percent CO_2 in N_2 (●) or argon (○) for 10 minutes and then sealing with a rubber stopper. Flasks were incubated in total darkness at 33° to 35°C on a rotary shaker (175 rev/min), and intermittent turbidity measurements were made with a Klett-Summerson photometer (filter No. 66); 200 Klett units are equal to approximately 650 μg of bacterial dry weight per milliliter.

Table 1. Nitrogenase activities of *Rhodospseudomonas capsulata* (strain B10) cells grown under various nutritional conditions. Photosynthetically grown cells were cultivated anaerobically at saturating light intensity (6500 lux) provided by incandescent lamps, on DL-malate as the carbon source. The composition of media with different nitrogen sources was as follows: $(NH_4)_2SO_4$ (7.5 mM), as specified for medium RCVB in (10); L-glutamate (15 mM), ammonium sulfate being replaced by sodium L-glutamate; N_2 , RCVB medium minus ammonium sulfate. For growth on N_2 , 20 ml of medium were placed in a 125-ml flask and the system was made anaerobic with 5 percent CO_2 in N_2 (legend of Fig. 1). For dark fermentative growth, cells were cultivated on fructose plus dimethyl sulfoxide (Fig. 1); where indicated, N_2 was replaced as the sole nitrogen source with either 15 mM glutamate or 7.5 mM $(NH_4)_2SO_4$. Cells in the late logarithmic phase were collected anaerobically under argon, concentrated, and resuspended in the respective growth media used, but lacking a combined nitrogen source. Portions (1 ml) of cell suspension were transferred anaerobically to 8.5-ml serum vials that were flushed with 100 percent argon. After injection of 0.5 ml (at standard pressure and temperature) of acetylene [and where indicated, $(NH_4)_2SO_4$ solution to give a concentration of 7.5 mM] through the vial cap, the vials were incubated at 35°C for 2 hours with intermittent shaking either under saturating illumination or in total darkness. Ethylene in the gas atmospheres was then estimated with a Hewlett-Packard model 402 gas chromatograph fitted with a Porapak Q column (operating at 80°C) and flame ionization detector. Rates of acetylene reduction were linear for at least 3 hours under the assay conditions used. Growth rates [expressed as mass doubling time (hours)] in light and dark, respectively, were: with N_2 , 3.5 and 12 to 14; with glutamate, 3.0 and 8.0; and with $(NH_4)_2SO_4$, 2.0 and 6.0.

Growth conditions and nitrogen source	Nitrogenase activity*			
	In light		In darkness	
	- NH_4^+	+ NH_4^+	- NH_4^+	+ NH_4^+
Light				
N_2	3.5	0.87	<0.001	
Glutamate	3.5	1.50	<0.001	
$(NH_4)_2SO_4$	<0.001			
Dark†				
N_2	0.88	0.25	0.20	<0.001
Glutamate	0.18	<0.001	0.02	<0.001
$(NH_4)_2SO_4$	<0.001		<0.001	

*Micromoles of ethylene per hour per milligram of cells (dry weight). †Anaerobic.