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Bacterial Nutritional Approach to Mechanisms of Oxygen Toxicity

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ABSTRACT

GOTTLIEB, SHELDON F. (Union Carbide Corp., Tonawanda, N.Y.). Bacterial nutritional approach to mechanisms of oxygen toxicity. J. Bacteriol. 92:1021–1027. 1966.—Inhibition by oxygen of growth of the bacterium *Achromobacter* P6 was reversed by amino acid supplements. The reversal of oxygen-induced growth inhibition was not due to the presence of reducing substances in the growth medium. Oxygen primarily exerts a bacteriostatic effect. The oxygen inhibition of growth occurred over a wide *p*H range. Oxygen inhibition of growth was observed when 1-amino-2-propanol, acetate, lactate, citrate, or glucose was used as the sole source of carbon and energy. No inhibition of growth was obtained when succinate, fumarate, malate, or glutamate was used as the source of carbon and energy. Oxygen markedly depressed the respiration of P6 when 1-amino-2-propanol was the substrate. There was no depression of respiration under oxygen with succinate as substrate. P6 grown in the presence of high oxygen tensions had a higher rate of respiration under oxygen than similar air-grown cells. Chloramphenicol did not affect the rate of oxygen consumption or cause a further depression of the respiratory rate in the presence of oxygen. It is suggested that microbes may serve as a model system for studying the cellular and subcellular mechanisms of oxygen toxicity.

How oxygen damages living systems is not yet clear. Many theories have been proposed: (i) toxicity per se via free radicals or peroxides inactivating enzymes or coenzymes; (ii) promotion of interactions forming enzyme inhibitors; (iii) accumulation of CO_2 through breakdown of the CO_2-hemoglobin transport system; (iv) interference with acid-base balance; (v) oxidation of cell or organelle membrane components; (vi) endocrine disfunction; or (vii) a combination of these (6).

A prominent hypothesis on the mechanism of O_2 toxicity centers around the oxidation of essential metabolites such as coenzymes or enzymes containing sulfhydryl groups. To date, only one enzyme system has been found which, from its rate of inactivation by oxygen in vitro, seems to correlate with the time of onset of the symptoms of O_2 toxicity in the whole animal (2, 10). Increased O_2 tensions are toxic to almost all forms of life, bacteria to mammals (6), which suggests that susceptible species have a common vulnerable site and that the differences in observed pathology are due mainly to differences in tissues.

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Mechanisms of O_2 toxicity and its nutritional reversal were studied in a bacterium. The organism chosen can grow on a single organic source and inorganic nitrogen; presumably, such an organism has a larger variety of functional enzyme systems than an organism more dependent on exogenous nutrients.

MATERIALS AND METHODS

Growth experiments. The organism (*Achromobacter* species P6) chosen has been described (7). The organism was obtained by enrichment culture technique and has no growth factor requirements. Experimental basal media were prepared in double-strength solutions. Experiments were performed by adding various nutrients to concentrated media and diluting to final volume with distilled water. Experimental media were distributed in 10-ml portions to cotton-plugged test tubes (150 × 23 mm). The minimal medium employed was of the following percentage composition (grams per 100 ml of final medium): KH_2PO_4, 0.1; K_2HPO_4, 0.2; trisodium salt of *N*-hydroxyethylethylenediaminetetraacetic acid, 0.03; $MgSO_4 \cdot 7\ H_2O$, 0.01; Ca, 0.001; NH_4Cl, 0.05; trace-metal solution, 0.1 ml. The trace-metal solution contained the following (per milliliter): 3.0 mg of potassium ethylenediaminetetraacetic acid (K_2EDTA); 2.5 mg of Zn^{++}, 1.0 mg of Fe^{++}, 0.5 mg of Mn^{++}, and 0.01 mg of Cu^{++} as the sulfates; 0.5 mg of Co^{++} as the chlorides; 10^{-4} mg of

molybdenum as $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 0.02 mg of B as H_3BO_3; *p*H 6.8 to 7.0. The primary substrate used in these studies was DL-1-amino-2-propanol ("aminopropanol"), 0.5%.

For stationary-system growth experiments, petri dishes containing solidified (2.0% agar) growth medium or test tubes containing liquid growth medium were put in desiccators after inoculation; the desiccators were flushed five times with the experimental atmosphere by alternate evacuation of the desiccator atmosphere by means of a vacuum pump and refilling to 1 atm with the appropriate gas.

The constant gas-flow system consisted of four manifolds, each having 58 outlets. Each outlet had a needle valve, to control the flow of gas to individual tubes. The incoming gas passed through a double humidifying system to decrease the evaporation in the experimental tubes, then through the manifold to the individual outlets. The gas was dispersed in the individual growth tubes by means of a 10-mm glass immersion filter (medium porosity) inserted into a two-hole rubber stopper. The second hole contained a right-angle tube which permitted the gas to escape, and the gas flow was monitored by means of a flow meter. At the time of inoculation, each tube received 0.1 ml of a 1:10 aqueous dilution of a sterile silicone antifoam agent (SAG 470, Union Carbide Corp.). The antifoam was autoclaved at 121 C for 30 min.

Carbon dioxide-free air (20% O_2–80% N_2) was prepared by adding the appropriate amounts of liquid, O_2 and N_2 (Driox) to evacuated compressed gas cylinders. To assure further the absence of CO_2 from the gas mixture, a molecular sieve (Linde 5A) column (3 m × 38.1 mm copper tubing) was inserted before the humidifiers of the constant gas-flow system described above. The humidifiers contained freshly boiled, acidified, distilled water. Before use, the entire gas-conducting system was purged for 3 hr with 100% N_2 at a rate of 800 ml/min to remove traces of CO_2 from the line.

Inocula for stationary-system experiments were prepared by inoculating 5 ml of synthetic medium and incubating at 34 C. After 23 hr of incubation, 1 ml was transferred to another 5 ml of synthetic medium, and incubated for 24 hr at 34 C. One drop of a 1:1,000 dilution of the resulting culture was used to inoculate the experimental tubes. Inocula for the constant gas-flow system were prepared as follows. A 5-ml amount of sterile basal medium containing 0.5% aminopropanol was inoculated from the stock culture and permitted to grow at 34 C while being continuously purged with humidified air at 20 ml/min. After 24 hr, 0.1 ml of the resultant culture was inoculated into fresh sterile medium. After 24 hr of incubation under these conditions, the organisms were centrifuged at 2,500 rev/min, resuspended in 10 ml of sterile, substrate-free basal medium, and recentrifuged. The pellet was suspended in sterile, substrate-free basal medium to give a reading between 25 and 30 on a Klett-Summerson colorimeter (no. 66 red filter); 0.05 ml of this suspension was used to inoculate the experimental tubes. Growth was measured turbidimetrically with this colorimeter.

Respiratory experiments. Respiration was measured by conventional Warburg techniques (15). Cultures of P6 were grown in 250 ml of synthetic medium containing either 0.5% DL-1-amino-2-propanol or 0.5% Na_2 succinate$\cdot 6H_2O$ as sole carbon and energy source, either under a continuous air or O_2-CO_2 purge of 200 ml/min. With succinate as substrate, 0.1% tris(hydroxymethyl)aminomethane (Tris) and 2-amino-2-hydroxymethyl-1,3-propanediol (Fisher Scientific Co., Pittsburgh, Pa.) were added to the medium as a buffer. Cells in the logarithmic phase of growth were harvested by centrifugation for 10 min at 5,000 rev/min at 2 C. The cells were washed once with 0.03 M phosphate buffer (*p*H 7.0) and resuspended in phosphate buffer.

Protein was determined by the biuret method. The biuret reagent consisted of: KI, 0.1%; Na_2 tartrate$\cdot 2H_2O$, 0.9%; $CuSO_4\cdot 5H_2O$, 0.3%; and NaOH, 20%. To a sample of protein, distilled water was added to dilute to 4.5 ml; 1.5 ml of biuret reagent was added and thoroughly mixed. To determine bacterial protein, the mixture was incubated at 50 to 55 C for 30 min. The standard curve was prepared from dehydrated sheep-blood serum (Mayer and Myles Laboratories). Protein in the range of 1,000 to 6,000 μg could be determined precisely. Optical density was read from a Klett-Summerson colorimeter equipped with a green filter.

RESULTS

To determine whether O_2 inhibited the growth of this organism and whether this toxicity could be mitigated nutritionally, growth of P6 on solidified basal medium enriched with 0.5% aminopropanol was compared with growth on nutrient agar in an air and oxygen environment. Under air, the organism grew well on both media within 24 hr, but under O_2 the organism grew only in the nutrient agar. When the organism previously incubated under O_2 was reincubated under air, the organism grew. Oxygen inhibition of growth also could be obtained in a liquid system (thereby permitting quantitation); inhibition was more pronounced with a dilute inoculum. In the stationary system, 0.8 atm was the minimal inhibitory pressure. Nutrient broth, protective against O_2 toxicity in a liquid system (Table 1), was replaceable by purified "vitamin-free" casein hydrolysate (Nutritional Biochemicals Corp., Cleveland, Ohio) or an amino acid mixture [TC Amino Acids L (Difco), 100 times]. The effect of these protective substances was synergistic and not additive; i.e., the amount of growth under oxygen obtained in the presence of nutrient supplementation was three to five times greater than what would be expected from the sum of the growth under oxygen in nutrient medium alone and substrate-enriched medium alone. This protection was probably not attributable to reducing substances (Table 2); cysteine and ascorbic acid inhibited the growth of P6 in an air environment,

TABLE 1. *Protection of P6 against oxygen by nutrients after 8 hr of incubation*[a]

	Expt	Air		Oxygen	
		No addition	1-NH_2-2-propanol (0.5%)	No addition	1-NH_2-2-propanol (0.5%)
I	No addition	1	80	0	12
	Nutrient broth, 5 mg/100 ml	3	88	3	52
	Nutrient broth, 10.0 mg/100 ml	5	105	5	67
II	No addition	2	135	2	25
	Casein hydrolysate, 1.0 mg/100 ml	2	133	2	122
	Casein hydrolysate, 10.0 mg/100 ml	5	135	5	125
III	No addition	0	95	0	37
	TC Amino Acids L (× 100), 0.05 ml/100 ml	0	102	0	95

[a] Data expressed in Klett units.

TABLE 2. *Effect of reducing substances on protecting P6 against toxic effects of 100% oxygen after 48 hr of incubation*[a]

Addition	Amt	Air		Oxygen	
		No addition	1-amino-2-propanol	No addition	1-amino-2-propanol
	mg/100 ml				
None	—	3	119	4	23
Cysteine[b]	1.0	5	118	4	18
	5.0	3	3	3	2
	10.0	3	3	3	2
Ascorbic acid[b]	1.0	3	116	3	37
	5.0	3	103	3	44
	10.0	3	100	3	41

[a] Data expressed in Klett units.
[b] Added aseptically at time of inoculation.

cysteine being more toxic. Under O_2, cysteine was still toxic; ascorbate may have afforded partial protection, but this protection appeared unrelated to its reducing powers, since protection did not increase with increasing ascorbate.

The nutrients may have protected P6 from harm by high O_2 tensions by (i) supplying a substance which bypassed a blocked important anabolic reaction, or (ii) the added nutrients locally reducing the O_2 tension in the growth medium, either by stimulating endogenous metabolism or by providing readily oxidizable substrates. In either case, the O_2 concentration of the medium, especially at the bottom of the tube where the rate of gas diffusion was slowest, would be reduced enough to permit normal growth. In these stationary-system experiments, the gas was so supplied as to make it difficult to distinguish between these two hypotheses. If a uniform oxygen tension could be maintained throughout the growth medium, the gas-diffusion limitation would be removed, thus providing a means for distinguishing between a metabolic reversal of O_2 toxicity as opposed to a local reduction of the O_2 tension. To circumvent the difficulties of gas diffusion found in the stationary system, the desideratum was a system in which the appropriate gas mixture was dispersed uniformly throughout the growth medium. With the constant gas-flow system, O_2 was markedly inhibitory, and this inhibition was annulled by addition of an amino acid mixture to the growth medium (Table 3). The O_2 inhibition occurred over a wide *p*H range; the limiting *p*H value for the growth of P6 in air was in the range of 5.5 to 6.0. The surfactant (added to decrease foaming) appeared to enhance the growth of P6 in an air environment; alone, it did not protect P6 against O_2 inhibition. An apparent inhibition in air by amino acids was due to a decrease in *p*H of the medium brought about by the aseptic addition of the very acidic amino acid solution as ascertained by *p*H measurement of sterile, uninoculated media; as in the petri-dish experiments, subjecting O_2-exposed, inoculated media to air resulted in rapid and luxuriant growth.

Oxygen inhibition of P6 was not limited to the use of aminopropanol as the sole source of carbon and energy; growth was delayed when acetate, lactate, citrate, or glucose was used as the sole source of carbon and energy in an O_2 environment. These substances supported excellent growth of the organisms within 24 hr in air. The oxygen inhibition of growth of P6 observed with these substrates also was reversed by nutritional enrichment with the amino acid mixture. However, unlike growth with 1-amino-2-propanol, each of these substrates in an O_2 atmosphere sup-

TABLE 3. *Effect of pH and amino acids on the growth of P6 in the presence of oxygen*[a]

Medium	pH	24 hr								48 to 72 hr			
		Air				100% Oxygen				100 % Oxygen			
		A	B	C	D	A	B	C	D	A	B	C	D
Basal	7.0	0	0	0	0	0	0	0	0	0	0	0	0
Basal + 0.5% 1-amino-2-propanol	7.0	240	315	210	254	0	0	0	0	0	0	119	188
	6.5	168	242	97	93	0	0	0	0	0	0	75	75
	6.0	107	114	0	0	0	0	0	0	0	0	0	0
	5.5	0	0	0	0	0	0	0	0	0	0	0	0
	5.0	0	0	0	0	0	0	0	0	0	0	0	0

[a] A, no addition; B, SAG 470, 0.1 ml of a 1:10 dilution per tube; C, amino acid mixture, 0.5 ml/100 ml; D, B + C. Growth expressed in Klett units.

ported growth even in the absence of nutrient supplementation, although growth was somewhat delayed. Growth was not inhibited when succinate, fumarate, malate, or glutamate was the sole source of carbon and energy.

The addition of 0.05% CO_2 to the oxygen did not result in growth equivalent to that in air in a similar time interval. In the presence of surfactant and CO_2, growth (29 Klett units at 48 hr) occurred 24 to 48 hr earlier than in the presence of O_2-CO_2 without surfactant (196 Klett units at 72 to 96 hr); the relative time of onset of growth, and not the relative amount of growth, was important. (This growth appeared in the presence of 1-amino-2-propanol, 0.5%.) Growth-curve experiments revealed that, under increased O_2 tensions, even in the presence of CO_2, the log phase was prolonged compared with that in air. Sterile media purged with air, 99.95% O_2-0.05% CO_2, or 100% O_2 did not show changes in *p*H. In the presence of 1-amino-2-propanol, P6 grew in air devoid of CO_2 (with no addition, 276 Klett units at 44 hr; in presence of surfactant, 283 Klett units at 44 hr); however, the time of onset of growth was delayed by a few hours in comparison with air (with no addition, 195 Klett units at 20 hr; in presence of surfactant, 271 Klett units at 20 hr). Growth was not prevented as it was under 100% O_2, and the absence of CO_2 did not delay the appearance of growth as long as did the O_2-CO_2 mixture [44 hr in absence of CO_2 compared with 72 to 96 hr (no addition) and 48 hr (with SAG 470) with the O_2-CO_2 mixture]. Surfactant did not manifest growth-enhancing effects in the presence of CO_2-free air (283 and 276 Klett units in the presence and absence, respectively, of surfactant).

As expected (Fig. 1A), 100% O_2 depressed the rate of O_2 consumption of P6 (aminopropanol the substrate) in the presence or absence of chloramphenicol. Since P6 can use the amino nitrogen of aminopropanol as a sole source of nitrogen for growth, it was necessary to incorporate a growth inhibitor in these studies to see how this respiratory depression was related to growth. Chloramphenicol was chosen, because it inhibits synthesis of cellular proteins while having little direct effect on oxidative processes (Wisseman et al., Federation Proc. **12**:466, 1953). In the short time period of these experiments, growth did not appear to affect rate of respiration; high concentrations of chloramphenicol neither affected the rate of O_2 consumption nor acted synergistically with O_2 in depressing respiration.

Cells grown in the presence of 99.95% O_2 plus 0.05% CO_2 (Fig. 1B) had a depressed respiration in air or O_2 as compared with the respiration of air-grown cells under air (compare H and I of Fig. 1B with D of Fig. 1A), the rate of respiration of O_2-grown cells in air being approximately 50% of that of air-grown cells. Air-grown cells under oxygen (F of Fig. 1A) showed a greater degree (71%) of respiratory depression compared with the control grown in air (F versus D in Fig. 1A) than did the corresponding oxygen-grown cells under oxygen (I of Fig. 1B) show compared with the respective control grown in air (H of Fig. 1B, 24%). In an O_2 environment, the rate of respiration of O_2-grown cells (I of Fig. 1B) was greater than the rate of respiration of air-grown cells (F of Fig. 1A).

High tensions of O_2 had no discernible effect on the respiration of P6 when succinate served as the oxidizable substrate (Fig. 1C).

DISCUSSION

In 1911, Moore and Williams (11) reported that O_2 inhibited growth of a wide variety of aerobic and facultatively aerobic bacteria. Not all bacteria were similarly affected. Even within a given genus, the response of individual species to

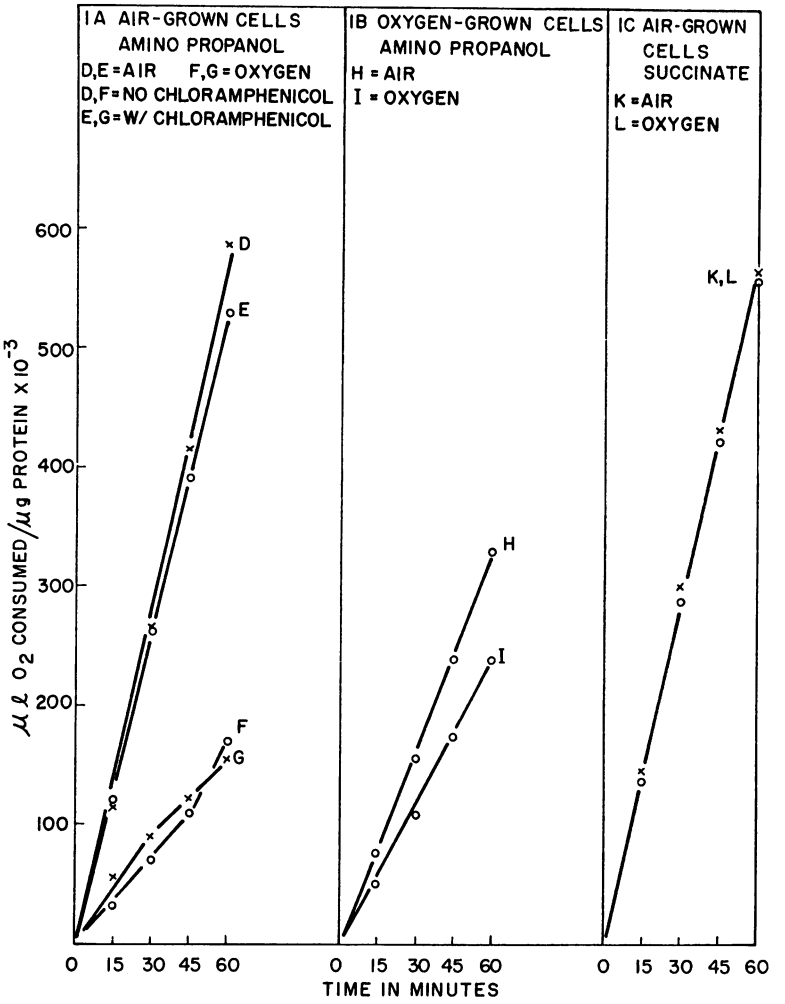

FIG. 1. *Effect of oxygen on the respiration of P6. (A) Cells grown on aminopropanol in an air environment. Warburg vessels contained 1.0 ml of cells (433 μg of protein) suspended in either 0.03 M phosphate buffer or 0.03 M phosphate buffer containing 500 μg of chloramphenicol per ml, and 1.0 ml of aminopropanol (10.0 μM) dissolved in 0.03 M phosphate buffer; 0.2 ml of 10% KOH was in the center well. Rates of respiration are expressed as microliters of O_2 per microgram of protein per minute in the 15- to 60-min period: $D = 7.0 \times 10^{-3}$, $F = 2.0 \times 10^{-3}$. Per cent depression = 70. These values have been corrected for the endogenous respiration. (B) Cells grown on aminopropanol in an oxygen environment. Warburg vessels were the same as in A, except chloramphenicol was not employed and 1.0 ml of cell suspension was equivalent to 650 μg of protein. Rates of respiration (corrected for the endogenous, expressed as in A) were: $H = 3.85 \times 10^{-3}$, $I = 2.91 \times 10^{-3}$. Per cent depression = 24. (C) Cells grown on succinate in an air environment. Warburg vessels contained 1.0 ml of cells (633 μg of protein) suspended in 0.03 M phosphate buffer, 0.5 ml of succinate (10.0 μM), 0.5 ml of 0.03 M Tris, and 0.2 ml of 10% KOH in the center well. Rate of respiration (corrected for the endogenous) was 9.6×10^{-3} μliters of O_2 per μg of protein per min. The pH of all reaction vessels was in the range of 6.8 to 7.0. Temperature was held constant at 34 ± 0.05 C. The flasks were "gassed" for 15 min while in the water bath.*

high O_2 tensions differed. That different species responded differently to O_2 was confirmed for mycobacteria by Gottlieb et al. (8). Hence, it was suggested (6, 8) that responsiveness to high O_2 tensions might be an additional character for differentiating bacteria. The data presented may also serve to distinguish this *Achromobacter* species from other members of the genus.

The data presented here support the idea that O_2 toxicity can represent a disturbance of metabolism, because this toxicity can be reversed nutritionally. Hyperoxia was bacteriostatic rather than

bactericidal (although it may have killed some of the members of the bacterial population), since, in both the "stationary" and "constant" gas-flow systems, oxygen-exposed, inoculated media, when incubated in air, manifested rapid and luxuriant growth. These data are also interpreted to mean that it was unlikely that oxygen reacted with components in the media to produce significant concentrations of toxic substances. Had this occurred, there should not have been subsequent rapid growth on incubation under air.

The respirometric results correlated well with data obtained from growth studies. O_2 inhibition of both growth and respiration of P6 was obtained with aminopropanol as substrate, and there was no measurable oxygen inhibition of either growth or respiration of P6 when succinate was substituted for aminopropanol as the source of carbon and energy. Sanders et al. (12) reported succinate to be protective to rats exposed to oxygen under high pressure. Oxygen depression of respiration may account in part for inhibition of growth of P6 on a synthetic medium under increased O_2 tensions. The respiratory system injured by O_2 is not known; the observations of Chance et al. (2) on oxygen inhibition of energy transfer may be applicable to the O_2 inhibition of growth and respiration of P6.

The observation that P6 grown under O_2 had a greater rate of respiration in oxygen than P6 similarly grown in air suggests that cells grown under O_2 are adaptively more resistant to O_2 damage. Presumably, the mechanism of O_2 resistance could be valuable in search for agents counteracting oxygen toxicity in man. In addition, such information may be useful in enhancing microbial sensitivity to O_2 for use in hyperbaric oxygen therapy (6).

The mechanism whereby the amino acids manifested protection must await identification of the active components of the amino acid mixture. Recent studies have shown that intraperitoneal administration of various amino acids delays the convulsant manifestations of oxygen toxicity in mammals (4, 16). It is conceivable that the amino acids supply intermediates for maintaining nicotinamide adenine dinucleotide (NAD) in a reduced state (2). Whether O_2 inhibition of growth of other microbes can be reversed nutritionally is unknown.

The fact that utilization of the dicarboxylic acids is not inhibited by O_2 stands in contrast to O_2 inhibition of citrate utilization. This may be considered presumptive evidence for a functional di- and tricarboxylic acid cycle in this organism, the dicarboxylic acid cycle being more O_2-resistant. Sensitivity of the tricarboxylic acid cycle enzymes to O_2 has been reported in both mammalian and botanical systems (1, 3, 9). Since tricarboxylic acid cycle enzymes contain sulfhydryl groups and have been shown to be inhibited by O_2, it would appear likely that the inhibition of growth of P6 is due to the oxidation of sulfhydryl-containing enzymes. Recently, Thomas et al. (14) proposed a mechanism for the toxic action of oxygen based on a direct oxidation by O_2 of the dithiol moiety of α-lipoic acid, which interferes with the normal functioning of pyruvic oxidase and α-oxoglutarate dehydrogenase. This hypothesis would tend to fit the observations presented here that O_2 inhibited utilization of acetate, lactate, glucose, and citrate but not that of succinate, malate, or fumarate. The absence of O_2 inhibition of the dicarboxylic acids may be explained on the basis of a metabolic bypass of the O_2-inhibited enzymes. The absence of glutamate inhibition by O_2 could possibly be explained by postulating the existence of a γ-aminobutyrate (GABA) shunt as suggested by Wood and Watson (16) for mammalian systems. Chance et al. (2) offer an alternate suggestion. GABA may be providing adequate succinate concentrations and thereby provide electrons and high energy intermediates for maintaining NAD in a reduced state. It is unlikely that the observed inhibition of growth is due to hydrogen peroxide, since P6 produces catalase. This organism was initially described as catalase-negative. Retests during these experiments showed that P6 has an active catalase. The reasons for the discrepancy between the initial and recent observations are unknown. The organism used in these studies was reconstituted from a lyophilized culture that had been refrigerated for 4 years. It is not known whether this storage procedure affects catalase production. Dickens (3) showed that susceptibility of tissues to oxygen poisoning is not related to their catalase content.

Conceivably, growth inhibition by O_2 was not due to O_2, but rather to the absence of CO_2. Had CO_2 lack been the sole explanation, then addition of CO_2 to O_2 at the concentration almost equivalent to that of air should have reversed the toxicity and produced luxuriant growth (similar to that under air) within 24 hr; this did not occur. If absence of CO_2 was the only factor in O_2 inhibition of growth, then the organism should not have grown in CO_2-free air. The onset of growth in CO_2-free air was delayed by a few hours, but not as long as the growth in the O_2-CO_2 mixture or as in 100% O_2. Hence, increased O_2 tensions, not lack of CO_2, was responsible for the growth inhibition.

The effectiveness of CO_2 overcoming the O_2 inhibition of growth was enhanced by the presence of a surface-active agent. The surfactant did not

manifest growth-promoting properties in the absence of CO_2 (100% O_2 or CO_2-free air). The mechanism whereby CO_2 and surfactant reversed the toxic oxygen effects is unknown. CO_2 may be required for metabolic purposes or maintaining the "permeability tone" of the cell membrane (5, 13, 16), or both. Surfactant may be involved in removal of any surface capsule or slime material; the removal of a permeability barrier would result in more efficient access of the cell surface for exchange of nutrients, gases, and wastes.

It is premature to attempt extrapolation from these bacterial experiments to the mechanism of oxygen toxicity in higher forms of life. However, an understanding of the mechanism of nutritional reversal of oxygen toxicity in microbes may result in new insights into reversing or preventing O_2 toxicity in man, as well as providing fundamental information which may be useful for understanding disease states such as retrolental fibroplasia, respiratory distress syndrome of the newborn, and convulsive disorders. In addition, studies on the mechanism of oxygen toxicity in microbes may yield new insights into ways of selectively enhancing the phenomenon of microbial oxygen toxicity, and thereby permit its use for the differential therapy of various infections and diseases caused by aerobic microorganisms [oxygen under pressure is being used for the treatment of various infections caused by anaerobic bacteria (6)]. Experimentation on the biochemical effects of gaseous environments may add new depth to the study of intermediary metabolism with all its attendant ramifications. On the basis of the data presented in this communication, it seems that P6 may serve as a model system for the cellular study of oxygen toxicity.

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