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Anaerobic Replacement of Carbon Dioxide.*

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Previous reports[1] on the heterotrophic replacement of carbon dioxide in bacteria have dealt exclusively with aerobic metabolism, largely because the compounds substituting for CO_2 were known to occur in the normal aerobic metabolism of bacteria. Further research in this field, however, reveals first that organisms normally requiring CO_2 when growing aerobically require it anaerobically, and secondly, that the same compounds replace CO_2 under anaerobic conditions. The purpose of the present communication is to report on the anaerobic replacement of carbon dioxide and to compare the function of these compounds under aerobic and anaerobic conditions.

Methods. The methods used were essentially those employed in previous work.[1] The organisms used in these experiments were *Escherichia coli* and *Aerobacter aerogenes*. The inoculum consisted of a 24-hour culture grown in 10 ml of the basal medium consisting of 0.8% K_2HPO_4, 0.4% $(NH_4)_2SO_4$, 0.8% glucose and 10% tap water, final pH adjusted to 6.8. The compounds tested were added before autoclaving except in the case of oxalacetic acid. A solution of the sodium salt was sterilized by filtration and added aseptically.

Cylinder nitrogen was passed over hot reduced copper gauze to remove oxygen and then through a CO_2-absorbing train before passing through the cultures. The reaction flask contained 25 ml of basal medium to which 2% of inoculum was added aseptically. Growth was measured by turbidimetric readings on a Klett-Summerson photoelectric colorimeter with a 660 mμ light filter. All readings were made after 12 hours incubation at 37°C. Sterile, uninoculated media were used as blanks.

Experimental. Neither *E. coli* nor *A. aerogenes* will grow anaerobically in the absence of CO_2. Atmospheric CO_2 can be replaced by $NaHCO_3$; these results are in accord with Gladstone *et al.*[2] Since no growth takes place in the absence of CO_2, the conclusion may be drawn that CO_2 has a definite function under anaerobic conditions.

The compounds and the extent to which they replace CO_2 are listed in Table I. The results are essentially similar to those obtained aerobically with the exception of oxalacetic acid which most effectively replaces CO_2 in the complete absence of oxygen. It is, therefore, possible that oxalacetic acid is a key compound in the anaerobic metabolism of the cells.

α-Ketoglutaric acid (and glutamic acid) substitutes anaerobically to a greater extent than any of the other compounds, except oxalacetate. Similar results were obtained aerobically. These facts again suggest a possible further fixation over and above the Wood and Werkman reaction. Such a fixation reaction would in normal metabolism (in the presence of CO_2) yield C_5 acids which are more essential to the cell. Indirect evidence for this has been obtained. More abundant growth is obtained with bicarbonate and succinic, malic or fumaric acid than with any of these acids alone (Table II).

A number of compounds metabolically related to those of Table II will also replace CO_2 anaerobically (Table III).

In the case of *A. aerogenes*, the function of citric acid under anaerobic conditions should be stressed. Whereas, the effect is comparatively small aerobically, in the absence of O_2 the organism uses this acid very effectively

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TABLE I.
Effect of Addition of Various Compounds on Anaerobic Growth of *E. coli* in the Absence of CO_2.

C_3-Compounds	Growth	C_4-Compounds	Growth	C_5-Compounds	Growth	C_6-Compounds	Growth
dl-Alanine	6	Succinic Acid	30	α-Ketoglutaric Acid	85	Citric Acid	9
Pyruvic Acid	4	Fumaric Acid	40	Glutamic Acid	200	cis-Aconitic Acid	25
		Malic Acid	35				
		Oxalacetic Acid	220				
		Aspartic Acid	62				

All compounds were used in 2-mM quantities. Growth is indicated by turbidimetric readings on the photoelectric colorimeter.
Control experiments give readings from 3 to 9.
Total volume, 25.5 cc. Incubation time, 12 hr. Temp., 37°C

TABLE II.
Effect of Added Carbonate on the Growth of *E. coli* in the Presence of C_4 Compounds.

Compound	With $NaHCO_3$	Without $NaHCO_3$
Succinic	55	30
Fumaric	65	40
Malic	60	45

All of the compounds including $NaHCO_3$ were added in 2-mM quantities. The extent of growth is indicated by turbidimetric readings on the photoelectric colorimeter.
Control gave readings from 3 to 9.
Total volume, 25.5 cc. Incubation time, 12 hr. Temp., 37°C.

in the place of CO_2 (Table IV). *E. coli* will not utilize citric acid under the above conditions.

The fact that the compounds normally occurring in the Krebs cycle replace CO_2 anaerobically suggests a possible function of this cycle in the absence as well as in the presence of oxygen. However, it should be noted that the existence of this cycle has not been demonstrated in bacterial respiration.

It is generally considered that the tricarboxylic acid cycle is an aerobic mechanism by which various substrates can be oxidized to CO_2 and H_2O, to yield energy to the system. Since the final products of anaerobiosis are not generally the same as in the presence of O_2, some other function of this cycle must be proposed. Since fat, protein, and carbohydrate metabolism have many points in common in the form of identical intermediate products and since the compounds supplied in the absence of CO_2 are some of the interconvertible intermediate products, it is possible that by amination, transamination, and other reactions the various compounds are directly utilized for the synthesis of proteins. On such a basis the utilization of CO_2 to form these compounds which in turn are used for cellular material can then be explained.

Summary. A number of compounds have been found which will replace CO_2, necessary for the anaerobic metabolism of *Escherichia coli* and *Aerobacter aerogenes*. The C_5 compounds appear to replace CO_2 more effectively than other compounds tested with the exception of oxalacetic acid and citric acid, the latter in the case of *A. aerogenes* only. The

TABLE III.
Effect of Glutamine, Asparagine, Arginine, and Proline on Anaerobic Growth of *E. coli* in the Absence of CO_2.

Compound	Glutamine	Asparagine	Arginine	Proline	Control
Growth	125	40	55	30	5

The extent of growth is indicated by turbidimetric readings on the photoelectric colorimeter. Compounds were added in 2-mM quantities.
Total volume, 25.5 cc. Incubation time, 12 hr. Temp., 37°C.

TABLE IV.
Replacement of CO_2 by Citric Acid Under Aerobic and Anaerobic Conditions.

Organism	Citric Acid	
	Nitrogen with O_2 and CO_2 removed	Air with CO_2 removed
Escherichia coli	9	4
Aerobacter aerogenes	180	50

The extent of growth is indicated by turbidimetric readings on the photoelectric colorimeter. 2-mM quantities of citric acid were used.
Total volume, 25.5 cc. Incubation time, 12 hr. Temp., 37°C.

fact that oxalacetic by-passes the CO_2 requirement to such an extent indicates that this acid may function as the chief substrate for protein synthesis under anaerobic conditions.

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Magnesium and Hyaluronidase Inhibitor of Blood Serum.

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Numerous reports[1-12] have established the presence of a hyaluronidase inhibitor in the blood sera of several species. The nature of the inhibitor has not been elucidated: but evidence has been presented that it is not a specific antibody;[2,13-16] nor an enzyme specifically attacking hyaluronidase.[6,9,12] The

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