EFFECT OF L-CYSTINE ON INITIATION OF ANAEROBIC GROWTH OF *ESCHERICHIA COLI* AND *AEROBACTER AEROGENES*

LUIGI GORINI

Department of Bacteriology and Immunology, Harvard Medical School, Boston 15, Massachusetts

Received for publication March 20, 1961

ABSTRACT

GORINI, LUIGI (Harvard Medical School, Boston, Mass.). Effect of L-cystine on initiation of anaerobic growth of *Escherichia coli* and *Aerobacter aerogenes*. J. Bacteriol. **82**:305–312. 1961.—Under anaerobic conditions *Escherichia coli* and *Aerobacter aerogenes*, inoculated in a mineral-citrate-glucose medium at densities up to 10^6 bacteria per ml, exhibit a long lag, or fail to initiate growth at all. Growth is initiated rapidly if the medium is supplemented with various SH or SS compounds. Of these the most active is L-cystine, which is fully effective at 1 to 2 μM.

In a heavily seeded semisolid medium without cystine, turbidity rapidly appears at the aerobic surface and then slowly extends throughout the anaerobic region of the culture. This finding implies that a sufficiently dense anaerobically growing culture creates conditions in the medium which eliminate the requirement for cystine. The nature of this effect on the medium has not been determined, but certain possibilities (pH, pCO_2) have been eliminated.

The anaerobic cystine requirement becomes more pronounced in the presence of Cu^{++}, at concentrations far lower than those required for inhibition under aerobic conditions. While it is possible that cystine is acting by complexing the toxic metal ion, it seems more likely that L-cystine is an essential metabolite, poorly produced under anaerobic conditions, and that the marked toxicity of Cu^{++} under anaerobic conditions depends on its complexing of cystine.

Under certain conditions (e.g., high pH) mutants of *Escherichia coli* blocked in the aromatic pathway require for growth not only the five known products of this pathway, but also a sixth factor which can be supplied by any of several orthodiphenols (Davis, 1953). In this paper are described observations made during the study of this requirement. It was found that the orthodiphenol requirement disappears under anaerobic conditions, if the medium is enriched with casein hydrolyzate: in a heavily seeded tube of such a semisolid medium, turbidity appeared rapidly throughout the tube in the presence of an orthodiphenol, and up to a few millimeters from the surface in the absence of an orthodiphenol. However, in minimal medium with a quintuple aromatic supplement no growth appeared without a diphenol; and when further supplemented with an orthodiphenol, turbidity appeared first at the surface and then spread continuously for several days until it reached the bottom of the tube. It thus appeared that this mutant required for initiation of anaerobic (but not aerobic) growth some factor(s) present in casein hydrolyzate; furthermore, the extension of turbidity showed that this requirement was eliminated for a cell when the neighboring density of bacteria became high enough.

It will also be shown that the same requirement for the initiation of anaerobic growth is exhibited by wild-type *E. coli* and *A. aerogenes*; and the casein hydrolyzate can be replaced by various sulfur-containing compounds, of which L-cystine is the most active. Because strict anaerobiosis is difficult to establish, and because the initiation of growth at a slightly aerobic surface can then spread to anaerobic regions, it is hardly surprising that the results obtained with the wild types have been less regular that those obtained with the mutant which cannot initiate aerobic growth. Thus with the wild type the absence of cystine has sometimes resulted in indefinite prevention of growth under anaerobic conditions, but in some experiments the absence of cystine has caused only a lag of several hours.

MATERIALS AND METHODS

Organisms. Most of the experiments were done with wild-type *E. coli* strain W. Experiments have also been performed with a multiple aromatic auxotroph (mutant 83-1) of the same

strain (obtained from B. D. Davis), and with wild-type *E. coli* strain K12 and *A. aerogenes*.

Culture media. Minimal medium A (Davis and Mingioli, 1950) was used in all the experiments. When not otherwise indicated, glucose (0.2%) was used as carbon source. For mutant 83-1 the following growth factors (quintuple supplement) were added to the minimal medium (per ml): L-tryptophan, 10 μg; L-tyrosine, 20 μg; L-phenylalanine, 20 μg; *p*-aminobenzoic acid, 0.1 μg; *p*-hydroxybenzoic acid, 0.1 μg. A semisolid medium was prepared by addition of 0.2% agar. In some experiments the minimal medium was enriched with 0.2% enzymatic casein hydrolyzate (Sheffield NZ-Case) and 0.2% yeast extract (Difco). When not otherwise indicated, the pH of the medium was 7.

Bacteriological procedures. The number of viable cells inoculated was determined by plate counts. To insure uniform distribution of cells when using semisolid media, the inoculum was added to the melted medium (45 C) thoroughly mixed, and allowed to cool before incubation. Cultures were incubated at 37 C.

In liquid cultures aerobiosis was assured by shaking. Gradient anaerobiosis was obtained by leaving the culture tubes (liquid or semisolid media) standing in air. Strict anaerobiosis was achieved as follows: sterile test tubes (18-mm diameter) containing 10 ml of culture were plugged with nonabsorbent cotton. The oxygen dissolved in the medium and in the gas phase above was largely replaced by bubbling a sterile N_2 + 5% CO_2 mixture through a narrow sterile piece of glass tubing. The test tubes were then supplied with a second absorbent cotton plug containing 1 to 2 ml of "Oxsorbent" (Burrel Company, Pittsburgh) and closed with a rubber stopper sealed with Parafilm. Oxsorbent removes contaminating O_2, without removing CO_2.

In liquid cultures growth was followed by determining the optical density at 480 mμ (Lumetron). A population density of 10^8 cells/ml corresponds to an optical density of 0.050. In semisolid cultures, whenever the growth was homogeneous, it was measured as in liquid cultures. However, in most experiments a zone of maximal growth and a zone of no growth demarcated by a sharp boundary were observed in the tubes. The rate followed in these cases is the rate of downward extension of visible growth in the tube.

The duration of the lag period was calculated by extrapolation of the measured exponential growth curve, assuming a constant doubling time from the start of growth of the inoculum. The optical density of the inoculum, which was too low to be measured photometrically, was estimated from the population density established by plate count.

RESULTS

Spread of growth from aerobic to anaerobic parts of a culture. When liquid minimal medium was inoculated with wild-type *E. coli* strain W (10^6 cells/ml) and then incubated in varying volumes in tubes and flasks without shaking, it was found that the appearance of visible growth was increasingly delayed with increasing volume/surface ratio (Table 1). This finding implies that growth was initiated earlier or proceeded more rapidly in the aerobic than in the anaerobic part of the culture. In enriched medium, in contrast, the lag of growth was not dependent on the volume/surface ratio.

This phenomenon was further explored by performing similar experiments, with a similar population density, in media in which convection was prevented by the addition of dilute agar (0.2%). As expected, visible growth appeared first in the region near the surface. However, the slower growth in the depths did not appear uniformly: instead, the turbidity progressed steadily downward, with a sharp boundary at all times between the region of dense growth and the clear region below. In contrast, when oxygen was eliminated from the gas phase above the medium there was usually no growth. In some experiments growth did appear, but only after a long lag (*see below*); and it showed no preference for the surface. It therefore appeared that in this minimal medium *E. coli* exhibited an extremely long or an indefinite lag under anaerobic conditions.

The spreading turbidity could not have been due to migration of cells; for when the same semisolid medium was inoculated only on the surface the growth did not spread into the uninoculated depths. Furthermore, when the concentration of agar was increased, the time of appearance of visible growth at the aerobic surface remained the same, but the rate of spread through the anaerobic regions was decreased (Fig. 1). When the glucose concen-

TABLE 1. *Effect of volume/surface ratio on growth of Escherichia coli*

Incubation time	Minimal medium					Enriched medium				
	Volume/surface ratio*					Volume/surface ratio*				
	1.5	2	6	12	20	1.5	2	6	12	20
hr	*Optical density* × *1,000*					*Optical density* × *1,000*				
0	2	2	1	1	1	2	1	1	2	1
6	—	—	—	—	—	98	108	102	98	90
8	79	1	1	2	2	292	292	295	275	312
16	280	90	27	1	2					
20		278	108	1	1					
22			272	10	1					
23				25	1					
25				98	2					
27				268	1					
41					282					

* Milliliters/cm^2.

Minimal or enriched glucose medium inoculated with strain W (10^6 cells/ml) from a refrigerated culture in enriched medium. Incubation without shaking. Enriched medium = minimal medium + casein hydrolyzate and yeast extract (*see* Materials and Methods).

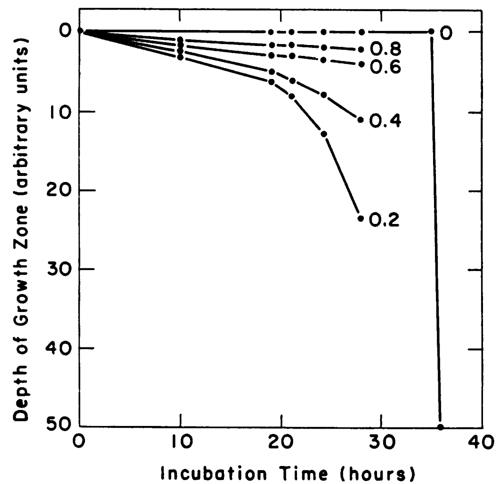

FIG. 1. *Influence of agar concentration on the rate of growth spread through the anaerobic regions of the culture. Minimal glucose semisolid medium is inoculated with strain W (10^6 cells/ml) from a stored culture in enriched medium. Then it is divided in five portions to which is added 0, 0.2, 0.4, 0.6, and 0.8% of agar. The cultures are put into 5 graduated cylinders (50-ml capacity) and incubated at 37 C. Growth spread determined visually. Abscissas: hours at 37 C. Ordinates: divisions of the graduated cylinder.*

tration was decreased, resulting in a decrease in maximal turbidity, the rate of spread of growth was also decreased (Fig. 2). A similar effect was produced by decreasing the density of the inoculum (Fig. 3). When the boundary of growth had progressed only part way down the column of semisolid medium, samples taken from the bottom, or from the region 2 to 3 cm below the boundary, yielded essentially the same population density (determined by plate count) as that originally inoculated. These results support the view that bacterial growth leads to the production of a chemical change in the medium which then by diffusion permits anaerobic growth in adjacent regions.

It seemed evident that the change responsible for growth in the depths could not be diffusion of oxygen, in view of the distance through which growth spread, the interception of diffusing oxygen by the intervening bacteria, and the fact that the rate of spread was increased by increasing the population density. Further support for this conclusion was provided by carrying out similar experiments in the presence of methylene blue (5×10^{-5} M). When the surface region became turbid, the region below became decolorized, and it remained so as the turbidity moved downward. Furthermore, when glucose was replaced as carbon source by pyruvate, lactate, or succinate, which can be used aerobically but not anaerobically, growth never spread far below the surface (Fig. 2).

It could also be shown that the spreading growth was not due to diffusion of carbon dioxide or of acid produced by heavy growth. The same failure to initiate growth in the anaerobic region, as well as the spread of growth from above, were observed when 1% sodium bicarbonate (sterilized by filtration) was added to the medium. The rate of spread was also unaffected when the shift in pH resulting from heavy growth was decreased by doubling the buffer concentration of the medium and simultaneously decreasing the glucose concentration from 0.5 to 0.2%. Under these conditions the pH did not drop, during growth, below 6.8.

Effect of added metabolites. It seemed possible that the spreading effect might be due to diffusion of one or more specific metabolites excreted by

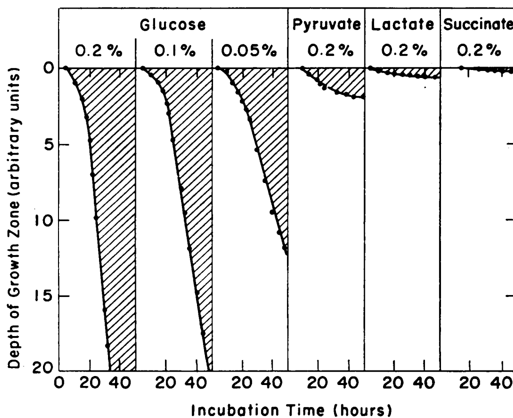

FIG. 2. *Influence of the nature of carbon source on the extent of growth spread into the depths of the culture. Rate of growth spread through the anaerobic regions as a function of glucose concentration. Minimal semisolid medium is inoculated with strain W (10^6 cells/ml) from a stored culture in enriched medium. Then it is divided in six portions to which is added: glucose, 0.2%; glucose, 0.1%; glucose, 0.05%; pyruvate, 0.2%; lactate, 0.2%; and succinate, 0.2%. The cultures are put in six graduated cylinders (20-ml capacity) and incubated at 37 C. Growth spread determined visually. Abscissas: hours at 37 C. Ordinates: divisions of the graduated cylinder.* ▨ = *Zone of growth;* □ = *zone of absence of growth.*

the bacteria. This view was supported by the finding that the addition of a complex enrichment (casein hydrolyzate or yeast extract) not only accelerated growth but also caused it to appear just as early under anaerobic as under aerobic conditions. Similarly, as already seen in Table 1, after enrichment the time of appearance of turbidity in liquid medium exposed to the air is no longer dependent on the volume/surface ratio.

A number of known components of the enrichments were individually tested in semisolid minimal medium, at concentrations probably exceeding those that might be accumulated by wild-type *E. coli*. Of all the naturally occurring amino acids, nucleic acid bases, and vitamins tested, only L-cystine and L-cysteine were active. In the presence of either of these compounds, growth appeared throughout the depth of the culture at the same time. As is shown in Table 2, in liquid media the presence of cystine eliminated the prolonged (often indefinite) lag seen under anaerobic conditions in its absence. It is further shown in this table that a culture that eventually grew anaerobically without cystine did so, after the long lag, at a normal rate (with a generation time 30% longer than that observed under aerobic conditions). Cystine therefore affects the initiation rather than the rate of anaerobic growth.

L-Cystine and a number of related compounds were tested at a variety of concentrations. Table 3 shows that L-cystine overcame the anaerobic lag at a concentration as low as 1 μM (0.2 μg/ml). D-Cystine was about 1/5 as active, and L-cysteine and DL-homocystine 1/100 as active (on a molar basis). Other sulfur compounds tested (including thioglycolic acid) were also active, but required even higher concentrations. From these observations it appears probable that in the gradient anaerobiosis tubes the bacteria produce cystine or some closely related S—S compound, thus permitting the downward spread of growth. In strict anaerobiosis cystine must be present before growth will begin.

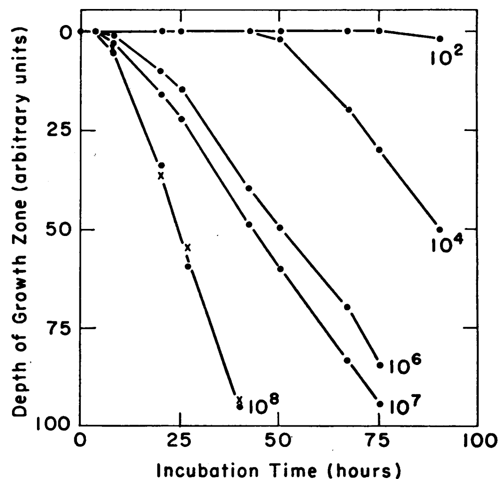

FIG. 3. *Influence of the inoculum size on the rate of growth spread through the anaerobic regions of the culture. Five portions of minimal glucose semisolid medium are inoculated with different amounts of an exponentially growing culture of strain W grown in minimal medium to bacterial densities of 10^2, 10^4, 10^6, 10^7, and 10^8 cells/ml. The cultures are put in five graduated cylinders (100-ml capacity) and incubated at 37 C. Growth spread determined visually. Abscissas: hours at 37 C. Ordinates: divisions of the graduated cylinder. Curve corresponding to inoculum size of 10^8 cells/ml.* ● = *Inoculated by the whole inoculum culture;* × = *inoculated by the pellets of the inoculum culture.*

TABLE 2. *Action of L-cystine on duration of lag, division time, and final yield of a liquid culture under anaerobic and aerobic conditions*

	Anaerobiosis; L - cystine concn:							
	0				6 μM			
Duration of lag (hr)	Indefinite			35	0	0	0	0
Division time (hr)	0	0	0	1.3	1.3	1.3	1.5	1.5
Final yield (OD max)	0	0	0	0.318	0.335	0.318	0.325	0.315
	Aerobiosis; L cystine concn:							
	0				6 μM			
Duration of lag (hr)	4	7	2	5	1	1	1	1
Division time (hr)	1	1	1	1.1	0.95	1	1.1	1
Final yield (OD max)	0.580	0.590	0.618	0.602	0.600	0.590	0.600	0.605

Minimal glucose medium was inoculated under nitrogen atmosphere with strain W (10^6 cells/ml) from an anaerobic exponentially growing culture in minimal medium. It was then distributed in tubes ± L-cystine and incubated under anaerobic or aerobic conditions. Tubes in quadruplicate.

TABLE 3. *Relative activity of various sulfur-containing compounds for initiation of anaerobic growth*

Compounds	Concn (μM)						
	1,000	500	100	50	10	5	1
L-Cystine	+	+	+	+	+	+	+
D-Cystine	+	+	+	+	+	−	−
Oxidized glutathione	+	+	+	+	+	−	−
DL-Homocystine	+	+	+	−	−	−	−
L-Cystathione	−	−	−	−	−	−	−
L-Cysteine	+	+	+	−	−	−	−
Reduced glutathione	+	+	+	−	−	−	−
Thioglycolic acid	+	−	−	−	−	−	−
Disodium sulfide	+	+	−	−	−	−	−

Minimal glucose semisolid medium, inoculated with strain W (10^6 cells/ml) from an overnight culture grown in minimal medium, was distributed in tubes containing the substances under examination.

The test is positive (+) when growth takes place uniformly throughout the whole culture. It is negative (−) when growth appears first at the surface and successively spreads through the anaerobic regions of the culture.

Conditions for demonstration of anaerobic requirement for cystine. Different strains of *E. coli* and *A. aerogenes* were compared for their anaerobic requirement for cystine. All the strains exhibited a delay in anaerobic growth when cystine was absent. However, the strains showed considerable differences. With *A. aerogenes*, the growth lag in the absence of cystine is only about 10 times longer than in its presence, with *E. coli* strain K12 about 20 times, and with *E. coli* strain W it may last indefinitely (Table 2). Table 2 also shows that even with strain W the appearance of visible growth may occasionally occur after a few days. In the cases in which the lag lasts for an indefinite length of time it was possible to show that we were dealing with a dormant rather than a dead culture. Even after 1 week at 37 C growth resumed after admitting air to these cultures. Viable counts performed on samples taken after 4 days showed no substantial difference from the number of cells present at the time of inoculation. It was found that the anaerobic requirement for cystine was more readily demonstrable if an inoculum grown

TABLE 4. *Inhibition of anaerobic growth by Cu^{++}*

Cu^{++} (μeq/liter)	0	1	10	40
	Lag (hr)			
Anaerobiosis	48	Indefinite	Indefinite	Indefinite
Aerobiosis	1	1	1	1

Minimal glucose medium prepared with glass distilled water was inoculated with strain W (10^6 cells/ml) from an exponentially growing aerobic culture, then distributed in tubes containing the calculated amount of $CuSO_4$. Incubation under aerobic or anaerobic conditions.

grown aerobic culture, the lag in anaerobic growth was only 2 days in medium prepared using glass distilled water and lasted indefinitely when 1 μM $CuSO_4$ was added. If the same culture was grown aerobically, even 40 times more Cu^{++} had no appreciable effect on the lag. Furthermore, it could be shown that the minimal amount of Cu^{++} that affected aerobic growth (50 μM) was also bactericidal. In contrast, under anaerobic conditions the low concentration of Cu^{++} required for indefinite prolongation of lag was not bactericidal.

This effect of Cu^{++} can be reversed by cystine. The ability of cystine to overcome the inhibition of anaerobic growth by Cu^{++} was compared with that of related SS and SH compounds such as L-cysteine and DL-homocystine. Table 5 shows that cystine is more effective than the other compounds. This finding corroborates the results concerning the effect of L-cystine and other SS and SH compounds summarized in Table 3. It should be stressed that cystine proved to be more effective than cysteine even though the latter is known to be a better complexing agent for heavy metals.

TABLE 5. *Reversal of Cu^{++} inhibition by L-cystine and related compounds*

Compound	Concn (μM)			
	0	1	10	100
	Lag (hr)			
L-Cystine	Indefinite	1	1	1
L-Cysteine	Indefinite	Indefinite	1	1
DL-Homocystine	Indefinite	Indefinite	Indefinite	1

The test compounds, along with 1 μM $CuSO_4$, were added to minimal glucose medium, prepared with glass distilled water. After inoculation with strain W (10^6 cells/ml) from an exponentially growing anaerobic culture, the tubes were incubated under anaerobic conditions.

anaerobically was used instead of one grown aerobically. Subsequently it was found that the duration of the anaerobic lag without cystine was constantly shortened to 1 to 2 days if glass redistilled instead of the usual distilled water was used for preparing media and rinsing glassware.[1] This prompted us to study the effect of adding Cu^{++}, which could reasonably be a contaminant in the usual distilled water. Since it is known that Cu^{++} is a good complexing agent for SH compounds, its presence in traces might enhance the cystine requirement.

It was established that 1 μM Cu^{++} was sufficient to prevent initiation of anaerobic growth no matter what strain was used or what the previous growth conditions of the inoculum. Table 4 shows that, using an inoculum from an exponentially

[1] Results obtained in our laboratory in New York proved difficult to reproduce at the Marine Biological Laboratories in Woods Hole. The differences were subsequently found to be due to the different distilled water used in the two laboratories.

Cystine requirement for growth of mutant 83-1. It was known (Davis, 1953) that mutant 83-1, a multiple aromatic auxotroph, requires traces (10^{-5} to 10^{-6} M) of an orthodiphenol (pyrocatechol or any compound carrying the orthodiphenol group) in addition to the usual aromatic quintuple supplement. On the basis of considerations discussed elsewhere in regard to another organism requiring orthodiphenol (Gorini and Lord, 1956) it was suspected that orthodiphenol was only needed for aerobic growth, but definitive evidence has always been lacking until the cystine require-

ment for anaerobic growth of this mutant was studied.

A batch of semisolid medium containing quintuple supplement but without pyrocatechol and cystine was seeded with mutant 83-1. The medium was dispensed into four test tubes containing (1) no addition, (2) 10^{-5} M pyrocatechol, (3) 10^{-6} M cystine, (4) 10^{-5} M pyrocatechol + 10^{-6} M cystine. No growth was observed in tube (1). Uniform growth was observed only in the presence of both factors (tube 4). In the presence of merely one factor, growth started exclusively in the corresponding region: at the aerobic surface with pyrocatechol (tube 2); or in the anaerobic deep parts of the tube with cystine (tube 3). Subsequently, growth spread down in tube 2 and up in tube 3.

An experiment analogous to the one described in Table 2 was also performed on the mutant. The medium used was liquid medium with quintuple supplement, absence of pyrocatechol, and with or without 10^{-6} M cystine. Growth occurred practically without lag in the presence of cystine, whereas no growth was observed after 1 week in its absence.

The interpretation of these results is that 83-1 requires pyrocatechol under aerobic conditions and cystine under anaerobiosis, and that these requirements are independent of each other. Seeded in tubes under gradient anaerobiosis mutant 83-1 is able to provide the cystine requirement for growth in the deep part of the culture if it is allowed to grow at the surface. This behavior is not dissimilar to that of the wild type with the only difference that aerobic growth of wild type requires only air and that of mutant 83-1 requires also the presence of an orthodiphenol.

DISCUSSION

The results of the preceding experiments show that in minimal medium (mineral salts-glucose) initiation of growth of *E. coli* or *A. aerogenes* under conditions of strict anaerobiosis may be delayed for an indefinite length of time even with the inocula as large as 10^6 cells/ml. Casein hydrolyzate contains a factor which permits the rapid initiation of anaerobic growth. Of the known compounds in casein hydrolyzate, only L-cystine insures anaerobic growth without lag in minimal medium, and it is effective in concentrations as low as 1 to 2 μM.

A solid culture in minimal medium seeded uniformly, and thus under conditions of gradient anaerobiosis, will grow uniformly only if 1 μM at least of L-cystine is present. In the absence of L-cystine, growth occurs first at the surface exposed to air and subsequently spreads to the anaerobic region. Using mutant 83-1, with which aerobic surface growth can be prevented by omitting any orthodiphenol from the medium, it can be shown that in the absence of orthodiphenol growth can occur only in the anaerobic region and is strictly dependent upon the presence of cystine. These facts indicate that *E. coli* growing in minimal medium secretes either cystine or some substance with a similar action. The experimental results indicate that this substance is likely to be an SS compound.

Chromatographic analysis of the free amino acid pool of *E. coli*, either growing exponentially or in the stationary phase, has shown that cystine is absent or barely detectable (J. Mandelstam, *unpublished results*). Thus the amount of cystine which may leak out of the cells is exceedingly small. This would explain why the spreading of growth from the surface to the bottom of a column of agar 20-cm long requires as much as 40 hr. Moreover, if one considers the lability of cystine under reducing conditions, it is not surprising that experiments done to determine whether growth could pass from the upper region of a culture, separated by a layer of unseeded agar medium 1 cm in depth, to the lower region of the same culture showed that growth did not proceed past the unseeded layer.

Since aerobic growth occurs without addition of cystine, and since cystine is normally produced during growth, carefully controlled conditions have to be set up to realize an absolute cystine deficiency. The inoculum must not be too large (10^6 cells/ml or less), contact with air should be minimized during the inoculation technique, and anaerobiosis must be strict during the incubation of the culture. The inoculum itself is derived from an anaerobic culture and this eliminates any problem of adaptation to anaerobic growth. The anaerobic requirement of cystine is most dramatically demonstrated using mutant 83-1, which requires a specific factor for either one of the two growth conditions: orthodiphenol for aerobiosis and cystine for anaerobiosis. Since, in absence of orthodiphenols, growth of this mutant is prevented even if traces of oxygen are left in

the anaerobic culture, the cystine requirement is more regularly demonstrated and the prevention of growth is indefinitely prolonged.

It is well known that heavy metal ions are good complexing agents for SH compounds. Since different results were obtained with different supplies of distilled water, and since we are dealing with amounts of cystine of the same order of magnitude as the heavy metal contaminants that one could expect in commonly prepared media, we have studied the toxicity to bacterial growth of heavy metal ions at those concentrations. Cu^{++} (a common contaminant) proved to be much more toxic in anaerobiosis than in aerobiosis. A concentration of cupric ions lower than 10^{-5} M is innocuous to aerobic growth, whereas 10^{-6} M is sufficient to secure prevention of anaerobic growth with constant reproducibility and for an indefinite period of time. This inhibition is reversible by readmission of air or by addition of complexing agents of Cu^{++}. Among cysteine, homocystine, and cystine, the last is the most efficient. These results support the idea that Cu^{++} at these concentrations inhibits bacterial growth indirectly, by removal of cystine, and that cystine itself is an essential factor and not merely a detoxifying agent. For it is unlikely that a direct interaction between SS compounds and Cu^{++} ions is as specific as we have found and, in addition, one would expect that the SH group would react more readily than the SS group.

At present the reason for this requirement of cystine is obscure. Since it has been found (Mazia and Zimmerman, 1958) that SS compounds are required for the formation of the mitotic apparatus and for division in higher organisms, we may speculate that a similar requirement is needed in bacterial cell division. In anaerobiosis it may be difficult for a nongrowing cell to establish a high enough SS/SH ratio to permit initiation of division.

In studies in this laboratory on the induction of a transport system for citrate in *E. coli*, which appears only under anaerobic conditions, reproducible results in minimal medium were only obtained when cystine was added to the medium (B. D. Davis, *personal communication*). It seems possible that the use of cystine will prove generally useful in studies of facultative anaerobes in simple media.

ACKNOWLEDGMENTS

This work was performed at New York University, Department of Pharmacology, College of Medicine, New York City, aided by a grant (G1662) from the National Science Foundation.

I would like to acknowledge the valuable assistance of B. D. Davis in the preparation of this manuscript.

LITERATURE CITED

DAVIS, B. D. 1953. Recent applications of genetic methods to the study of intermediary metabolism in microorganisms. Intern. Congr. Microbiol. 6th Congr. (Rome), p. 23–37.

DAVIS, B. D., AND E. S. MINGIOLI. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **50**:17–28.

GORINI, L., AND R. LORD. 1956. Nécessité des orthodiphénols pour la croissance de *Coccus P* (*Sarcina Sp.*) Biochim. et Biophys. Acta **19**:84–90.

MAZIA, D., AND A. M. ZIMMERMAN. 1958. SH compounds in mitosis. II. The effect of mercaptoethanol on the structure of the mytotic apparatus in sea urchin eggs. Exptl. Cell Research **15**:138–153.