JOURNAL OF BACTERIOLOGY, Jan., 1965
Copyright © 1965 American Society for Microbiology
Vol. 89, No. 1
Printed in U.S.A.

Cyanide Formation by *Chromobacterium violaceum*

RUTH MICHAELS[1] AND W. A. CORPE
Department of Botany, Columbia University, New York, New York

Received for publication 17 August 1964

ABSTRACT

MICHAELS, RUTH (Columbia University, New York, N.Y.), AND W. A. CORPE. Cyanide formation by *Chromobacterium violaceum*. J. Bacteriol. **89**:106–112. 1965.—The formation of cyanide by a *Chromobacterium violaceum* strain was studied with growing cultures and with nonproliferating cells grown in complex and chemically defined media. Most of the cyanide was produced during the log-phase growth of the organism, and accumulated in the culture supernatant fluid. A synergistic effect of glycine and methionine on cyanide formation in a chemically defined medium was observed, and the amount of cyanide formed was found to be dependent on the concentrations of the two substances. Cyanide formation by nonproliferating cells was stimulated by preincubation with glycine and methionine. Cyanide formation by adapted cells in the presence of glycine and methionine was stimulated by succinate, malate, or fumarate, and depressed by azide and 2,4-dinitrophenol. Methionine could be replaced by betaine, dimethylglycine, and choline.

Hydrogen cyanide is known to be a powerful respiratory poison (Fruton and Simmonds, 1959) which combines irreversibly with the ferric iron constituent of the cytochromes and inhibits oxidation of these enzymes, thereby disrupting electron transport (White et al., 1959). Other heme proteins, such as peroxidase and catalase, are inhibited by cyanide as is iron containing xanthine oxidase (White et al., 1959). These workers have also reported that certain enzymes containing copper, zinc, or other metals are inhibited by cyanide as well.

The formation of cyanide by an organism was first described in the basidiomycete *Marasmius* in 1871 by Losecke (Robbins, Rolnick, and Kavanagh, 1950), and was subsequently rereported in a species of *Pholiota* and other mushroom species (Bach, 1948; Robbins et al., 1950). Formation of cyanide by aerobic gram-negative bacteria has been known for some time (Clawson and Young, 1913; Patty, 1921), and was first reported in mesophilic *Chromobacterium violaceum* strains by Sneath (1953).

Minute quantities of cyanide have been found in various substances of animal origin such as urine, tissue extract, and gastric juice (Boxer and Rickards, 1952), and as a constituent of cyanocobalamin (vitamin B_{12}), a vitamin required by many organisms. Cyanogenic glycosides, which can be hydrolyzed to yield cyanide, were present in most of the plants investigated by Seifert (1954). It may be reasonable to assume that cyanide formation is a common event in biological systems, and that the pathway leading to its formation and subsequent metabolism is widely distributed in living organisms. The objective of the present work was to study the phenomenon of cyanide formation by bacteria in some detail, and to obtain a clearer understanding of its metabolic origin.

[1] Present address: Department of Chemistry, Wayne State University, Detroit, Mich.

MATERIALS AND METHODS

Cultures. The cultures surveyed for cyanide formation were obtained from the stock culture collection of the microbiology laboratory, Department of Botany, Barnard College, Columbia University. Strain 9 was isolated by one of us (W.A.C.) from New Jersey soil. Stock cultures of the organisms were maintained on agar slants containing glucose (1%, w/v), and peptone (Difco) (1%, w/v) at 20 C, and transferred once a month.

Media. A complex medium used routinely for the production of cyanide by growing bacteria contained 1% (w/v) peptone dissolved in distilled water and adjusted to *p*H 7.0. When solid medium was required, 2% (w/v) agar was added.

A chemically defined glutamate salts medium used contained the following ingredients (per liter of distilled water): L-glutamic acid, 4.413 g; KH_2PO_4, 1.36 g; $Na_2HPO_4 \cdot 7H_2O$, 2.13 g; $MgSO_4 \cdot 7H_2O$, 0.20 g; and $FeCl_3 \cdot 6H_2O$, 0.005 g. The *p*H was adjusted to 7.0 with NaOH. When other carbon or nitrogen sources were tested, glutamate was omitted. All amino acids and other supplements used were of chemically pure quality. Other individual amino acids tested as carbon and nitrogen sources for growth and for cyanide formation were DL-alanine, DL-aspartate, glycine, L-histidine, L-arginine, L-hydroxyproline, DL-

methionine, L-tryptophan, DL-phenylalanine, DL-threonine, L-tyrosine, DL-valine, L-lysine, L-leucine, and L-isoleucine.

Inoculum flask. A cotton-stoppered Erlenmeyer flask (125 ml) containing 25 ml of peptone solution or glutamate salts medium was inoculated from a 24-hr agar-slant culture, and incubated at 30 C for 12 to 16 hr on a rotary shaker.

Experimental flasks. Unless specified otherwise, rubber-stoppered Erlenmeyer flasks (500 ml), each containing 75 ml of peptone solution or glutamate salts medium appropriately supplemented, were inoculated with 1 ml of inoculum, and incubated at 30 C on a rotary shaker for the time specified. The glutamate salts cultures were supplemented toward the end of the log phase with sterile, neutral, distilled water solutions of the following compounds. Unless otherwise specified, the compounds were added to give a final concentration of 10 μmoles/ml. The compounds tested were: L-aspartate, DL-alanine, glycine, L-glutamate, DL-methionine, L-tryptophan, L-hydroxyproline, L-histidine, L-ornithine, L-arginine, L-serine, L-citrulline, L-tyrosine, L-cysteine, L-glutamine, sarcosine HCl, ammonium carbonate, formamide (15 μg/ml), glycamine, ethanolamine, glycine amide, formimino-glycine, *N*-acetyglycine, glycine methyl ester, glycine ethyl ester, guanine, xanthine, adenine, uric acid, guanidino-acetic acid, creatine, creatinine, choline, betaine, formate, and formaldehyde (50 μg/ml). Yeast extract (0.5 mg/ml), vitamin B_{12} (1 μg/ml), and folic acid (1 μg/ml) were also tested in this system.

Substances used to replace methionine (final concentration of 1 μmole/ml) in cyanide formation by nonproliferating cells were *S*-methyl-cysteine, *N*,*N*-dimethylglycine, methyl methionine sulfonium chloride, glycine ethyl ester, and methyl mercaptan.

Nonproliferating cells. The cells were grown in test flasks containing glutamate salts medium. At the end of the incubation period, the cells were harvested by centrifugation, washed twice with a sterile solution of basal salts, and suspended in rubber-stoppered Erlenmeyer flasks (500 ml) containing 75 ml of "suspension medium," a solution of basal salts to which substrates had been added as specified in Results. The suspended cells were then incubated with shaking for an additional 6 hr. In experiments where nonproliferating cells were used, absorbancy of the suspension was measured before and after incubation as a check on cell growth. Absorbancy measurements were made in a Bausch & Lomb Spectronic-20 colorimeter at 600 mμ.

Adapted cells. Glycine (5 μmoles/ml) and methionine (1 μmole/ml), or equimolar quantities of potential substitutes of these substances, were added aseptically to 7-hr-old cultures growing in glutamate salts test flasks. The flasks were then incubated with shaking for an additional 2 hr before harvesting by centrifugation, and used as described above.

Qualitative and quantitative estimation of cyanide. The alkaline picrate test and the copper sulfide tests gave positive reactions for cyanide when performed on both standard cyanide solution and culture distillates. Aqueous solutions of potassium cyanide (Fisher Scientific Co., Pittsburgh, Pa.; reagent grade) were standardized by the Liebig silver nitrate titration method described by Kolthoff and Sandell (1952). Cyanide was estimated in culture distillates with colorimetric methods. A good linear response was obtained with both the Aldridge (1944) and the Epstein (1947) procedures. Direct quantitative determination of cyanide in the culture supernatant liquid was not possible with the colorimetric procedures, because of development of nonspecific color by noncyanide constituents. The Aldridge test was used routinely for making quantitative estimations.

Quantitative cyanide recovery. Cyanide was recovered by the Ruchhoft method (Ludzack, Moore, and Ruchhoft, 1954). The culture supernatant fluid was diluted to 250 ml with distilled water, and acidified to a *p*H of approximately 2.5 with a 15% (w/v) tartaric acid solution in distilled water. The volume of tartaric acid required was determined on a sample of the supernatant liquid. After acidification, the distillation flask was immediately attached to a distillation apparatus, and 210 ml of distillate were collected in 40 ml of 2% (w/v) sodium hydroxide. The distillate was diluted to a known volume, and cyanide was estimated colorimetrically as described above. Distillation of known quantities of cyanide from peptone or basal salts-amino acid solution by this method gave a recovery error of less than 5%. More than 90% of both standard cyanide and cyanide elaborated by the culture was distilled within the first 50 ml.

Volatile cyanide collecting in the air space above the test flasks was recovered by gently blowing air through the culture into a 20% (w/v) sodium hydroxide solution. Cyanide was estimated as described above.

Preparation of cyanide derivatives. Zelinsky and Stadnikoff (1908) suggested a modification of the Strecker synthesis of alanine from acetaldehyde and HCN. This method was adapted to preparation of alanine in small amounts. A 40-mg amount of ammonium chloride was dissolved in 3 ml of distilled water, and mixed with a solution of 33 mg of acetaldehyde in ether. An aqueous solution of 50 mg of KCN was added by drops, and the mixture was stirred for 3 hr at room temperature. At the end of this period, the ether layer was discarded, and 7 ml of concentrated HCl were added to the aqueous layer. The resulting solution was refluxed for 1 hr, and then evaporated on a steam bath. Alanine was identified in the residue by one-dimensional paper chromatography with three solvent systems: butanol-acetic acid-water, 12:3:5 (Smith, 1960); butanol-formic acid-water, 77:11:12 (Blass, 1960); and phenol-water, 4:1, buffered at *p*H 12.0 (McFarren, 1951).

Cyanocobalamin (vitamin B_{12}) was prepared

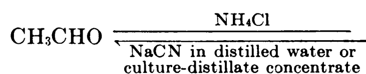
from hydroxocobalamin (vitamin B_{12b}) by the method of Veer et al. (1950). These were identified by their unique absorption peaks. The absorption spectrum of vitamin B_{12} was obtained with a solution of 40 μg of vitamin B_{12} per ml in 0.001 N HCl. The absorption spectrum of vitamin B_{12b} was obtained by allowing the vitamin B_{12} solution to stand in diffuse light for 90 min. The absorption spectrum of vitamin B_{12} was obtained a second time by the addition of 0.1 mg of KCN per ml of vitamin B_{12b} solution adjusted to pH 6.0 with dilute alkali.

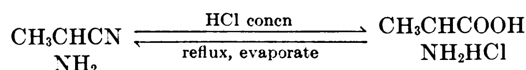
RESULTS

Survey of bacterial species for cyanide formation. A number of bacteria, mostly gram-negative aerobes, were tested for their ability to form cyanide in various peptone-containing liquid and agar media using the alkaline picrate test (Feigl, 1946). Among the strains of *C. violaceum* tested, four were found to be cyanide-positive within 24 hr, and nine were cyanide-negative. Strains of *C. lividum*, described as psychrophilic species by Sneath (1960), were cyanide-negative. Two strains of *Pseudomonas chlororaphis* and two strains of *P. aureofaciens* gave a positive test within 48 hr. The other organisms tested were cyanide-negative. Species tested were *P. fluorescens*, *P. aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*.

Identification of cyanide. Proof that the substance in the culture distillates was cyanide was obtained by the preparation of two specific derivatives.

The Strecker synthesis was performed as described in Materials and Methods. Alanine was obtained by use of either standard NaCN solution or a concentrate of culture distillate containing an equivalent amount of cyanide as determined by the Aldridge test. When distilled water or a concentrate of distillate from a 3% (w/v) peptone solution were used instead, no alanine was obtained.





Cyanocobalamin was prepared from hydroxocobalamin as described above. The characteristic ultraviolet and visible absorption spectrum of cyanocobalamin was regenerated by the addition of a standard KCN solution, or a culture-distillate concentrate containing an equivalent amount of cyanide as determined by the Aldridge test (Table 1).

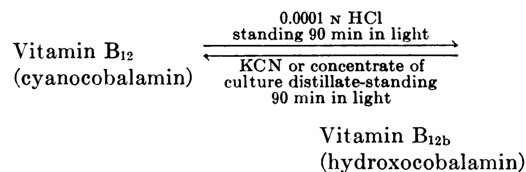


TABLE 1. *Preparation of vitamin B_{12} from vitamin B_{12b}*

Vitamin	Absorption maximum (mμ)	
	Present work*	Veer et al. (1950), Wijmenga, Veer, and Lens (1950)
B_{12}	544	544
	410†	410
	360	359
	320	323
	306	306
	278	278
B_{12b}	525	525
	351	351
	275	275

* Concentrate of distillate and standard solution of KCN.
† Poor.

Formation of cyanide in complex media. C. violaceum strain 9, a vigorous cyanide producer, was selected for detailed study. About 98% of the cyanide produced by the organism was found in the culture supernatant fluid. Distillation of a suspension of washed cells in distilled water yielded no cyanide, and the air space above the culture gave only 1.9% of the total cyanide contained in the culture supernatant liquid, after 24 hr of incubation.

The time-course production of cyanide in 1% (w/v) peptone solution is shown in Fig. 1. The bulk of the cyanide was produced during the log phase of the organism, and the greatest yield of cyanide per viable cell occurred near the end of the log phase. No larger cyanide yields were found in 1-, 3-, and 8-day-old cultures. Cultures maintained good viability for at least 72 hr after maximal yield of cyanide had been attained. The addition of 20 μg/ml of cyanide to a culture at 0 and 6 hr after inoculation did not depress total cell yield. When the concentration of peptone was lowered to less than 1% (w/v), a reduction in cyanide yield and cell growth occurred. When peptone concentration of the production medium was raised to 3% (w/v), a 13% increase in cyanide yield and a 40% increase in cell growth was observed.

Formation of cyanide in chemically defined media. A few individual amino acids supported growth of the organism when used as sole carbon and nitrogen source, and small amounts of cyanide were produced (Table 2). Other common amino acids listed in Materials and Methods, including glycine and methionine, did not support growth. Glutamate served particularly well as a carbon and nitrogen source for growth, and routinely gave rise to some cyanide. A medium containing glucose or succinate as carbon source with ammonium sulfate as nitrogen source also allowed accumulation of small amounts of cyanide.

The glutamate concentrate of 4.41 mg/ml used in the growth medium permitted a cell yield of 3×10^9 cells per ml by the end of the log phase, which occurred after 8 hr under conditions described above. Most of the exogenous glutamate had been consumed by the end of the log phase; stimulation of cyanide formation by supplements added aseptically at that time might be expected to reflect potential cyanide precursors. Most of the 45 individual nitrogenous compounds added did not increase cyanide yield (Table 3). Guanine, alanine, glycine, and methionine, however, increased cyanide yield to more than 3 μg/ml of supernatant liquid. When glycine and methionine were added together, cyanide formation increased more than eightfold. None of the other substances or mixtures tested gave a cyanide yield of this magnitude.

Glycine and certain substances related to it were added as supplements to glutamate cultures. The cyanide yield was more than doubled when glycine was replaced by glycine methyl ester (Table 4). Methionine was not supplied to these cultures. The other substances, containing different groups on either the methyl or carboxyl

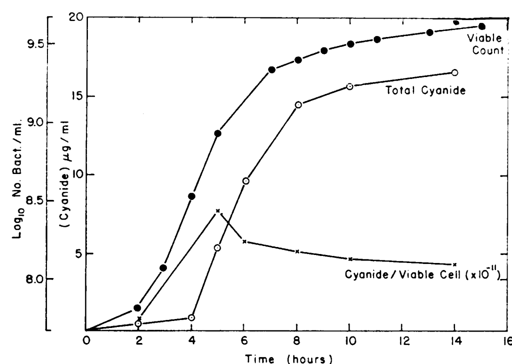


FIG. 1. *Time-course production of cyanide by Chromobacterium violaceum, in 1% (w/v) peptone medium, in relation to growth.*

TABLE 2. *Cyanide formation by Chromobacterium violaceum on various media**

Medium	Time of incubation	Relative growth absorbance at 600 mμ (× 10)	Amt of cyanide per ml of culture
	hr		*μg*
Single amino acids in salt solution* (25 μmoles/ml)			
L-Glutamic acid	48	.360	4.0
DL-Alanine	48	.245	3.8
L-Histidine	48	.275	2.6
DL-Aspartic acid	48	.090	0.0
L-Hydroxyproline	48	.035	0.0
L-Arginine	48	.025	0.0
Other common amino acids†	48	No growth	0.0
Glucose-ammonium-salts solution	40	.155	0.8
Peptone (1%)	16	.245	17.0

* The inoculum for these experiments consisted of cells grown on peptone-glucose-agar washed three times with sterile distilled water.

† Other amino acids tested listed in Materials and Methods.

carbon of glycine, did not increase cyanide yield beyond that obtained in the control culture of glutamate salts medium.

The addition of glycine, at a concentration of 5 μmoles/ml, and methionine, at a concentration of 1 μmole/ml, gave a yield of cyanide of 0.71 μmoles/ml. When smaller quantities of glycine and methionine were used, cyanide yield fell sharply. Greater quantities of glycine and methionine did not increase cyanide yield.

Time-course production of cyanide in glutamate salts medium, supplemented at 7.5 hr with glycine and methionine, is shown in Fig. 2. Cyanide yield increased from 2 μg/ml of supernatant liquid present at the time of supplementation to 3.5 μg/ml 2 hr after supplementation. The yield then increased sharply during the next 8 hr to 16 μg/ml. A 57-hr-old culture contained 21 μg/ml of cyanide, but no further increase was observed after that time. After the addition of glycine and methionine, there was essentially no increase or decrease in cell numbers as shown by colony counts.

Cyanide formation by nonproliferating cells. Supplementation of the growth medium with glycine and methionine for 2 hr before centrifugation and washing of the cells substantially increased cyanide yield by cells (Table 5). Addition of succinate to the buffered cell suspension containing glycine and methionine doubled cyanide yield, but did not cause further

TABLE 3. *Effect of the addition of various compounds to glutamate cultures of Chromobacterium violaceum on cyanide formation**

Addition	Amt of cyanide per ml of supernatant liquid
	µg
Distilled water	2.4
Individual nitrogenous substances	
L-Glutamic acid	2.4
DL-Alanine	5.0
Glycine	4.9
DL-Methionine	6.5
Guanine	3.6
25 other individual substances†	3.0
Mixtures of nitrogenous substances	
Glycine and methionine	21.0
Alanine and methionine	4.9
Serine and methionine	2.0
Arginine, glycine, and methionine	21.0
B_{12}, glycine, and methionine	20.0
Glycine, methionine, and sarcosine	3.6
Yeast extract (0.5 mg/ml), or B_{12} (1 µg/ml), or folic acid (1 µg/ml)	1.0–3.0

* Compounds were added after 7 hr to glutamate-salts cultures to give a final concentration of 10 µmoles per ml, unless otherwise indicated. Incubation was continued for 14 hr; then cyanide was determined.

† Listed in Materials and Methods.

TABLE 4. *Effect of the addition of glycine-related compounds to glutamate cultures of Chromobacterium violaceum on cyanide formation**

Substance added	Amt of cyanide per ml of supernatant liquid
	µg
Distilled water (control)	2.4
Glycine	4.9
Glycamine	2.1
Ethanolamine	2.1
Glycine amide	2.8
Formiminoglycine	2.1
N-acetylglycine	2.1
Glycine methyl ester	10.8
Sarcosine	0.0
Betaine	2.6
Glycine ethyl ester	5.6

* Compounds were added after 7 hr to glutamate-salts cultures to give a final concentration of 10 µmoles/ml. Incubation was continued for 14 hr; then cyanide was determined.

cell growth. Succinate could be replaced with malate or fumarate in this system. The addition of malonate with succinate in the complete system decreased cyanide formation to a level below that obtained without the addition of a four-carbon compound.

The addition of 200 µg/ml of sodium azide or 2,4-dinitrophenol to the complete system, containing glycine, methionine, and succinate in buffer, reduced cyanide yield to 3.0 and 1.5 µg/ml, respectively; 600 µg/ml of sodium azide or 400 µg/ml of 2,4-dinitrophenol did not depress cyanide yield further.

Attempts were made to replace methionine with various potential methyl donors. Betaine,

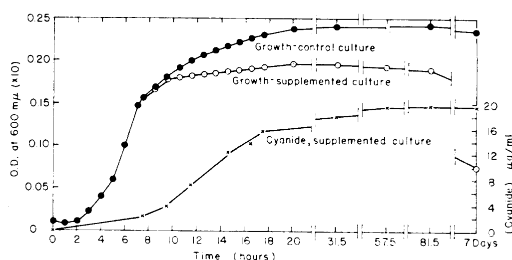


FIG. 2. *Time-course production of cyanide by Chromobacterium violaceum in glycine-methionine supplemented glutamate mineral salts medium. The 7.5-hr culture was supplemented with a sterile neutral solution of glycine and methionine in 5 ml of water to give a final concentration of 5 and 1 µmole per ml of medium, respectively; 5 ml of water were added to the control culture.*

TABLE 5. *Cyanide formation by nonproliferating cells of Chromobacterium violaceum*

System*	Amt of cyanide per ml of supernatant liquid
	µg
Nonadapted cells in mineral salts solution + glycine + methionine	2.6
Adapted cells in mineral salts solution, no substrates	0.0
Adapted cells in mineral salts solution + glycine + methionine	5.9
Adapted cells in mineral salts solution + glycine + methionine + succinate	11.0

* System: washed adapted and nonadapted cells from 150 ml of glutamate salts culture were suspended in 75 ml of basal salts solution (*p*H 7.0) with glycine (5 µmoles/ml), methionine (1 µmole/ml), and succinate (28.4 µmoles/ml) where indicated. The suspensions were incubated on a shaker at 30 C for 6 hr, and cyanide was determined.

dimethylglycine, and choline, when added individually with glycine, gave yields of cyanide higher than those obtained with methionine (Table 6).

DISCUSSION

At the outset of these experiments, it was considered important to establish clearly that the substance responsible for the positive alkaline picrate test was cyanide ion. The formation of cyanide by microorganisms had previously been detected by smell, by the formation of Prussian blue, or by other traditional, but equivocal, qualitative tests. The Aldridge and Epstein tests, used for quantitative estimation, involve the formation of colored complexes with characteristic absorption maxima. However, it was conceivable that substances other than cyanide might, under certain conditions, give similar spectra; therefore, preparation of the two derivatives described furnished additional proof that the substance under consideration was cyanide.

Investigators reporting cyanide formation in fungi (Bach, 1948; Robbins et al., 1950) believed it was produced as the result of an autolytic process. Cyanide-producing *Pseudomonas* species were reported to yield most of the cyanide after 24 hr (Lorck, 1948) as was true of the *Pseudomona* species examined in the present work. *C. violaceum* produced the bulk of the cyanide during active growth. Dying cultures, presumably undergoing autolysis, did not produce more cyanide. Viability and total cell yield of the organism was not impaired by accumulated cyanide, or by the addition of cyanide to the growth medium in quantities normally produced by the organism. Preliminary experiments showed that strain 9 of *C. violaceum* was by no means unique in its surprisingly high resistance to cyanide, as this phenomenon was also encountered with a number of other aerobic bacteria and fungi tested (Michaels, *unpublished data*). The reasons for this phenomenon have not been sufficiently explored, but a number of explanations have been considered. Cyanide may react with medium components rendering it ineffective as an inhibitor, or the CN radical might be unable to permeate the cell. The existence of a mechanism of detoxification or of a functional cyanide-resistant aerobic pathway of respiratory metabolism also appear to be reasonable explanations of the phenomenon.

Strain 9, as well as other *Chromobacterium* species examined, contain cytochrome *c* in quantities readily detectable in a hand spectroscope (Corpe, *unpublished data*).

Whether cyanide is present in the culture supernatant liquid of *Chromobacterium* as its ion, or is released from unstable compounds during distillation, has not been determined. The material behaves in the manner of simple cyanide salts, because the first 50 ml of distillate contained all but a trace of the cyanide, whereas, with most complex cyanides, good recovery would only be obtained after refluxing the distillation mixture for 1 hr or more according to Ludzack et al. (1954). Of the total cyanide produced, 1 to 2% appeared in the air space above the neutral or slightly alkaline culture. This was taken as partial evidence that cyanide existed as a salt, because the air space above neutral solutions of pure KCN also gave a positive test for cyanide ion.

The earlier work on cyanide formation by fungi (Lebeau and Dickson, 1953) or *Pseudomonas* (Lorck, 1948) contributed little to an understanding of the metabolic origin of cyanide. Lorck's (1948) study, however, suggested the possible importance of glycine as a metabolic precursor. In the present work, a synergistic effect of glycine and methionine on cyanide formation in a chemically defined medium was established. A far greater amount of cyanide was produced when glycine and methionine were added together rather than separately. Results have shown that neither glycine nor methionine alone or together supports growth of the organism. Growth did not occur when a utilizable carbon source like succinate was also present; this suggests that glycine or methionine fails to supply nitrogen for growth. In contrast, glutamate

TABLE 6. *Replacement of methionine in cyanide formation by nonproliferating cells of Chromobacterium violaceum**

Substance added (1 μmole/ml)	Amt of cyanide per ml of supernatant liquid
	μg
DL-Methionine	11.0
L-Methionine	11.5
Formate	9.2
Betaine	13.7
S-methyl-cysteine	6.4
N,*N*-dimethylglycine	15.0
Methyl methionine sulfonium chloride	4.9
Glycine ethyl ester	3.8
Choline	14.4
Methyl mercaptan	1.8

* Adapted cells were suspended in phosphate buffer (pH 7.0) with glycine (5 μmoles), succinate (28.4 μmoles/ml), and indicated substance, and then incubated on a shaker for 7 hr at 30 C.

could be used both as carbon and nitrogen source for the production of cyanide. Cyanide was also produced in glucose-ammonium salts solution, indicating the pathway leading to cyanide is constitutive in these organisms. Cyanide produced by glutamate-grown cells was increased by preincubation with glycine and methionine which suggested either a total increase in the level of enzymes associated with cyanide formation, or a stimulation in activity of enzymes already present. Stimulation of cyanide formation by succinate and other four-carbon acids indicated the process requires energy made available through an oxidation of these substrates. This view is reinforced in view of the depressing effect on formation by azide and 2,4-dinitrophenol which are known to block oxidative phosphorylation (Fruton and Simmonds, 1959).

The function of methionine, which is required in smaller amounts than glycine for maximal cyanide yield, is not entirely clear but, because it could be replaced by betaine, dimethylglycine and choline donation of methyl group to the system is suggested (Fruton and Simmonds, 1959). Glycine methyl ester gave good cyanide yields in growing cultures in the absence of methionine; this suggests that methyl group might arise from -O-CH3 as well as from -S-CH3 and -N-CH3 linkages. Sarcosine (*N*-methylglycine) did not replace glycine; in fact, its presence depressed cyanide yield in growing cultures.

Acknowledgments

This investigation was supported in part by Public Health Service Research grant AI-04280 from the National Institute of Allergy and Infectious Diseases.

Literature Cited

ALDRIDGE, W. N. 1944. Estimation of microquantities of cyanide. Analyst **69**:262–265.

BACH, E. 1948. On hydrocyanic acid formation in mushrooms. Physiol. Plantarum **1**:387–389.

BLASS, J. P. 1960. The simple monosubstituted guanidines of mammian brain. Biochem. J. **77**:484–489.

BOXER, G. E., AND J. C. RICKARDS. 1952. Studies on the metabolism of the carbon of cyanide and of thiocyanate. Arch. Biochem. Biophys. **39**:7-26.

CLAWSON, B. J., AND C. C. YOUNG. 1913. Preliminary report on the production of hydrocyanic acid by bacteria. J. Biol. Chem. **15**:419–422.

EPSTEIN, J. 1947. Estimation of microquantities of cyanide. Anal. Chem. **19**:272–274.

FEIGL, F. 1946. Qualitative analysis by spot tests. Elsevier Publishing Co., Amsterdam.

FRUTON, J. S., AND S. SIMMONDS. 1959. General biochemistry. John Wiley & Sons, Inc., New York.

KOLTHOFF, I. M., AND E. B. SANDELL. 1952. Textbook of quantitative inorganic analysis. The Macmillan Co., New York.

LEBEAU, J. B., AND J. G. DICKSON. 1953. Preliminary report on production of hydrogen cyanide by a snow mold pathogen. Phytopathology **43**:581–582.

LORCK, H. 1948. Production of hydrocyanic acid by bacteria. Physiol. Plantarum **1**:142–146.

LUDZACK, F. J., W. A. MOORE, AND C. C. RUCHHOFT. 1954. Determination of cyanide in water and waste samples. Anal. Chem. **26**:1784–1797.

MCFARREN, E. F. 1951. Buffered filter paper chromatography of the amino acids. Anal. Chem. **23**:168–174.

PATTY, F. A. 1921. The production of hydrocyanic acid by *Bacillus pyocyaneous*. J. Infect. Dis. **29**:73–77.

ROBBINS, W. J., A. ROLNICK, AND F. KAVANAGH. 1950. Production of HCN by cultures of a basidiomycete. Mycologia **42**:161–166.

SEIFERT, P. 1954. Blausaure-Verbindungen, p. 676–688. *In* K. Paech and M. V. Tracey [ed.], Moderne Methoden der Pflanzenanalyse, vol. 4. Springer, Berlin.

SMITH, I. (ED.) 1960. Chromatographic and electrophoretic techniques. Interscience Publishers, New York.

SNEATH, P. H. A. 1953. Fatal infection by *Chromobacterium violaceum*. Lancet **265**:276.

SNEATH, P. H. A. 1960. A study of the bacterial genus *Chromobacterium*. Iowa State J. Sci. **34**: 243–500.

VEER, W. L. C., J. H. EDELHAUSEN, H. G. WIJMENGA, AND J. LENS. 1950. Vitamin B_{12}. I. The relation between vitamins B_{12} and B_{12b}. Biochim. Biophys. Acta **6**:225–228.

WHITE, A., P. HANDLER, E. L. SMITH, AND D. W. STETTEN, JR. 1959. Principles of biochemistry. McGraw-Hill Book Co., Inc., New York.

WIJMENGA, H. G., W. L. C. VEER, AND J. LENS. 1950. Vitamin B_{12}. II. The influence of HCN on some factors of the vitamin B_{12} group. Biochim. Biophys. Acta **6**:229–236.

ZELINSKY, N., AND G. STADNIKOFF. 1908. Ein Beitrag der Synthese des alanins und der a-aminobuttersaure. Chem. Ber. **41**:2061–2063.