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Induction of Bacteriolysis by Cysteamine and Its Derivatives

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SUMMARY: Cysteamine and certain closely related derivatives induced lysis in a majority of 28 strains of *Bacillus*; cultures of all other genera tested were resistant. Forty-three additional sulphur and/or nitrogen containing compounds were unable to induce lysis. Optimum conditions for cysteamine activity included exposure of metabolizing post-log. phase organisms contained in shallow shaken layers of nutrient broth to the compound for 4-8 hr. Upon the completion of lysis, no formed cellular elements were visible by microscopy except flagella. The induction by cysteamine of a lysogenic bacteriophage or of an unusually large amount of an autolytic enzyme was not demonstrated.

Among the substances which provide some degree of protection to living cells subsequently exposed to ionizing radiation is a group of chemical compounds consisting of cysteamine (β -mercaptoethylamine; MEA) and certain closely related derivatives. A preliminary report concerning the ability of the oxidized form of cysteamine (cystamine: 2,2-dithiobis-(ethylamine)) to induce lysis of organisms of various *Bacillus* spp. has been published (Weinberg, 1957). The present paper describes tests for induction of lysis with other members of the cysteamine group and with 43 related compounds. Various hypotheses concerning the mechanism of lytic induction are considered and the results of experiments designed to test some of these hypotheses are presented.

METHODS

Organisms. The test organisms (Table 1) were obtained from the collection of the Department of Bacteriology, Indiana University, except for the culture of *Micrococcus lysodeikticus* which was kindly supplied by Dr Arthur Schade, National Institutes of Health (Bethesda, Maryland, U.S.A.). Each strain was checked for purity by the usual cytological and cultural methods and was then maintained on nutrient agar (Difco) slopes. Subcultures on fresh nutrient agar slopes were made weekly.

Culture media. The majority of the experiments were performed with organisms grown in nutrient broth (Difco). In a few tests, specified in the section on results, a chemically defined medium consisting of the following ingredients, at the molarities (M) indicated, dissolved in triple-distilled water was used: 1×10^{-2} , glucose; 1×10^{-2} , NH_4NO_3 ; 9×10^{-3} , Na_2HPO_4 ; 6×10^{-3} , KH_2PO_4 ; 3×10^{-4} , MgSO_4 ; 2×10^{-5} , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; pH value, 7.0. Ten ml.

quantities of the nutrient broth or defined liquid medium were added to 50 ml. Erlenmeyer flasks and sterilized by autoclaving.

Compounds tested for ability to induce lysis. The compounds tested for the ability to induce lysis or to affect lysis-inducing compounds are listed in Table 2. The compounds were dissolved in triple-distilled water and sterilized by autoclaving or, with the heat labile substances, by filtration through sintered glass filters. Solutions of the compounds were added aseptically to flasks of inoculated culture medium at (a) the beginning of the lag phase, or (b) during the post-log. phase. When not in use, the solutions were stored at 4°; for some tests, however, fresh solutions of the cysteamine compounds were prepared daily.

Table 1. *Susceptibility of test strains of Bacillus and other genera to induction of lysis by cysteamine*

Cysteamine (2×10^{-3} M) was added to post-log. phase cultures. Turbidity readings were made during the subsequent 24 hr. period of incubation. Of the sensitive strains, the majority of the *B. subtilis* group lysed within 4–8 hr.; the majority of the other groups required 8–24 hr. A dark pigment was produced from cysteamine by 1 sensitive and 1 resistant strain of *B. megaterium* and by 1 resistant strain of *B. cereus*.

Genus	Species	No. of strains tested	No. of resistant strains	No. of sensitive strains
<i>Bacillus</i>	<i>subtilis</i>	7	0	7
	<i>megaterium</i>	6	1	5
	<i>cereus</i>	4	1	3
	<i>macerans</i>	2	1	1
	<i>anthracis</i>	2	1	1
	<i>sphaericus</i>	1	0	1
	<i>brevis</i>	1	1	0
	<i>circulans</i>	1	1	0
	unidentified	4	3	1
<i>Sarcina</i>	<i>lutea</i>	1	1	0
<i>Staphylococcus</i>	<i>aureus</i>	1	1	0
<i>Micrococcus</i>	<i>lysodeikticus</i>	1	1	0
<i>Mycobacterium</i>	<i>avium</i>	1	1	0
<i>Escherichia</i>	<i>coli</i>	1	1	0
<i>Salmonella</i>	<i>typhimurium</i>	1	1	0

Inocula and conditions of incubation. Flasks of nutrient broth or defined liquid medium were inoculated with organisms from the agar slopes and were shaken in a New Brunswick shaker (160 strokes/min.) at 37°. After 16 hr., the cultures were diluted in fresh nutrient broth or defined medium so that 1 ml. of the diluted culture contained approximately 2×10^6 viable organisms. One-twentieth ml. portions of the diluted culture were added to the flasks to be used in the actual experiments. The experimental flasks were placed in the shaker and incubated at 37° for periods from 1 to 6 days.

Determination of bacterial multiplication and lysis. The organisms of the inocula were observed to multiply and, in some cases, subsequently to lyse by turbidimetric measurements made with the Junior Coleman spectrophotometer model 6A (at 660 m μ). To confirm multiplication and subsequent lysis

Table 2. *Compounds tested for ability to induce lysis of Bacillus subtilis strain Marburg*

Each compound was added to 16 hr. nutrient broth cultures to obtain a final concentration of 1×10^{-3} M. Turbidity readings were made at 16 hr. and at appropriate intervals up to 40 hr. of incubation.

I. Substituted ethanes

Ethyl amine	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{NH}_2$
Ethylene diamine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$
Thio ethane	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{SH}$
Cysteamine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SH}$
Taurine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SO}_3\text{H}$
Ethanol	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{OH}$
Ethylene chlorohydrin	$\text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$
Ethanol amine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$
β -Mercaptoethanol	$\text{HS} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$
Acetic acid	$\text{CH}_3 \cdot \text{COOH}$

II. Substituted acetic acids

Thiolacetic acid	$\text{CH}_3 \cdot \text{COSH}$
Thioglycollic acid	$\text{HS} \cdot \text{CH}_2 \cdot \text{COOH}$
Sulphoacetic acid	$\text{HO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SO}_3\text{H}$
Glycine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{COOH}$

III. Substituted propionic acids

L-Alanine	$\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
DL-Serine	$\text{HO} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
β -Alanine	$\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$
Mercaptopropionic acid	$\text{HS} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$
L-Cysteine	$\text{HS} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
L-Cysteic acid	$\text{HO}_2\text{S} \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
D-Penicillamine	$\text{HS} \cdot \text{C}(\text{CH}_3)_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$

IV. Mono- and di- thio ethers

Thiodiethanol	$(\text{HO} \cdot \text{CH}_2 \cdot \text{CH}_2)_2\text{S}$
Mercaptosuccinic acid	$(\text{HOOC} \cdot \text{CH}_2)_2\text{S}$
Aminoethyl sulphide	$(\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2)_2\text{S}$
Cystamine	$(\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{S})_2$
L-Cystine	$(\text{HOOC} \cdot (\text{NH}_2)\text{CH} \cdot \text{CH}_2 \cdot \text{S})_2$
L-Djenkolic acid	$(\text{HOOC} \cdot (\text{NH}_2)\text{CH} \cdot \text{CH}_2 \cdot \text{S})_2\text{CH}_2$

V. Substituted butyric acids

DL-Homocysteine	$\text{HS} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
DL-Methionine	$\text{H}_3\text{C} \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$

VI. Isothiuronium compounds

Aminoethyl isothiuronium bromide (AET)	$(\text{Br} \cdot \text{H}_3\text{N})(\text{HN}:)\text{C} \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{HN}_2$
S, γ -Aminopropyl isothiuronium bromide (APT)	$(\text{Br} \cdot \text{H}_3\text{N})(\text{HN}:)\text{C} \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{HN}_2$

Table 2 (cont.).

VII. Pyrrolidine, thiazole, thiazoline, and thiazolidine compounds

L-Proline	$\overbrace{\text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH} \cdot \text{CH}(\text{COOH}) \cdot \text{CH}_2}$
2-Aminothiazole	$\overbrace{\text{S} \cdot \text{C}(\text{NH}_2) : \text{N} \cdot \text{CH} : \text{CH}}$
Rhodanine	$\overbrace{\text{S} \cdot \text{C}(:\text{S}) \cdot \text{NH} \cdot \text{C}(:\text{O}) \cdot \text{CH}_2}$
2-Methyl thiazoline	$\overbrace{\text{S} \cdot \text{C}(\text{CH}_3) : \text{N} \cdot \text{CH}_2 \cdot \text{CH}_2}$
2-Mercapto thiazoline	$\overbrace{\text{S} \cdot \text{C}(\text{SH}) : \text{N} \cdot \text{CH}_2 \cdot \text{CH}_2}$
Thiazolidine-4-carboxylic acid	$\overbrace{\text{S} \cdot \text{CH} : \text{N} \cdot \text{CH}(\text{COOH}) \cdot \text{CH}_2}$
2,4-Thiazolidine dione	$\overbrace{\text{S} \cdot \text{C}(:\text{O}) \cdot \text{NH} \cdot \text{C}(:\text{O}) \cdot \text{CH}_2}$
2-Thiazolidine thione	$\overbrace{\text{S} \cdot \text{C}(:\text{S}) \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2}$

VIII. Miscellaneous compounds

Biotin
 1,2-Diaminocyclohexane tetraacetic acid
 Ethylene diamine tetraacetic acid
 Glutathione
 Nitrogen mustard
 Pantetheine
 Putrescine
 Thiamine
 Trypsin

as indicated by the increase and decrease in turbidity, plate counts of viable organisms and microscopic examinations were made of the cultures at appropriate intervals. The microscopic examinations included: Gram staining, cell-wall stain (Dyar, 1947), observation of wet mounts by phase microscopy, and photographs of organisms under electron microscopy.

Extraction of possible cysteamine-induced lytic principles. Organisms exposed to cysteamine added to the medium at the time of inoculation were ruptured by sonic oscillation or by ballotini in the Mickle tissue disintegrator at 6 hr., 10 hr., and 14 hr. after inoculation. Extracts of organisms not exposed to cysteamine were similarly prepared at the same times. The culture filtrates from 6, 10, and 14 hr. cysteamine-exposed and unexposed organisms were saved. Some cultures containing cysteamine were permitted to lyse and the lysates were saved for testing. In some cases, the culture filtrates and lysates were dialysed in a rocking dialyser against running tap water at 4° for periods from 3 hr. to 4 days and the materials within the bag were saved for testing. The cell-free extracts, culture filtrates, lysates, and dialysis residue were stored at -20° when not in use.

Assay systems for detection of possible lytic principles. In general, organisms grown in nutrient broth for 16 hr. were used to detect lytic activity. The organisms were either kept in their culture medium or were removed by centrifugation and resuspended in the test solutions. In a few tests, 6 and 10 hr. crops of organisms were used. In experiments designed to study tem-

perature effects, 16 hr. crops of organisms either retained in their culture medium or centrifuged and resuspended in $\text{M}/30$ phosphate buffer (pH 8.0) were heated at various temperatures for 10 min. immediately before being used in the assay system. On occasion, assay organisms were grown in the defined medium rather than in nutrient broth and were then either retained in their culture medium or were resuspended in staled nutrient broth. In addition to intact organisms, isolated cell walls were used as an assay tool. The method of cell-wall extraction and purification used was that of Salton & Horne (1951).

RESULTS

Compounds which induced lysis of Bacillus subtilis strain Marburg

Of the 48 compounds listed in Table 2, only 5 were able to induce lysis under the conditions of the experiments (Table 1). The active substances included cysteamine and 4 closely related derivatives; the structural formulae of these compounds are given in Fig. 1. It should be noted that when aminoethyl isothiuronium bromide (AET) and S- γ -aminopropyl isothiuronium bromide

Fig. 1. Structural formulae of the compounds which induce lysis.

(APT) are dissolved in neutral aqueous solutions, an intra-transguanylation occurs so that the active compounds are believed to be 2-mercaptoethylguanidine and 3-mercaptopropylguanidine, respectively (Shapira, Doherty & Burnett, 1957).

The bacteriostatic concentration of each of the cysteamine compounds is indicated in Fig. 2 and the concentrations which induced lysis are presented in Fig. 3. It is apparent that sub-bacteriostatic concentrations were quite

Fig. 2

Fig. 3

Fig. 2. Ability of various concentrations of the cysteamine compounds (added at the time of inoculation) to prevent growth of *Bacillus subtilis* strain Marburg in nutrient broth. Growth in the absence of the compounds was equivalent to that obtained in the presence of a concentration of 1×10^{-4} M. $\square$ — $\square$, APT; $\bullet$ — $\bullet$, AET; $\times$ — $\times$, cysteamine; $\circ$ — $\circ$, cysteamine; $\circ$ — $\circ$, amino ethyl sulphide.

Fig. 3. Ability of sub-bacteriostatic concentrations of the cysteamine compounds (added to 16 hr. nutrient broth cultures of *Bacillus subtilis*) to induce lysis. The readings were made at 24 hr. $\square$ — $\square$, APT; $\bullet$ — $\bullet$, AET; $\times$ — $\times$, cysteamine; $\circ$ — $\circ$, cysteamine; $\circ$ — $\circ$, amino ethyl sulphide.

effective in induction of lysis. The time required for induction of lysis by various concentrations of cysteamine is given in Fig. 4. It may be noted that lysis occurred mainly between 4 and 8 hr. after the addition of the compound to post-log.-phase cultures. In the presence of suboptimal concentrations of cysteamine, a small amount of lysis occurred in the subsequent 16 hr. period; no additional cysteamine-induced lysis occurred thereafter.

The spectrum of bacterial sensitivity to induction of lysis by cysteamine

Of the test bacterial strains listed in Table 1, it may be observed that the majority of the strains of *Bacillus* especially within the species *Bacillus subtilis*, were susceptible to lytic induction. Attempts were made to induce lysis of strains of other genera by cysteamine by: (1) increasing the concentration of cysteamine to as much as 1×10^{-2} M; (2) adjusting the pH value of post-log.-phase cultures to 8.5; (3) extending the time of contact between cysteamine and the strains to 6 days. These attempts to induce lysis were unsuccessful.

*Physical and chemical environmental requirements for induction of lysis
of Bacillus subtilis strain Marburg by cysteamine*

Despite the fact that sub-bacteriostatic concentrations of cysteamine induced lysis, such concentrations could be included in the culture medium at the time of inoculation without harm to the organisms of the inoculum. The organisms proceeded to complete the lag and logarithmic growth phases

Fig. 4

Fig. 5

Fig. 4. Ability of various concentrations of cysteamine (added to 16 hr. nutrient broth cultures) to induce lysis in *Bacillus subtilis*. In the absence of the compound, cultures decreased in turbidity to 70% of the 16 hr. turbidity at 40 hr.

Fig. 5. Effect of changing the pH value of 16 hr. nutrient broth cultures of *Bacillus subtilis* on the ability of cysteamine (added at the time of the pH adjustment) to induce lysis. The pH value of the 16 hr. cultures before adjustment was 8.7. ○—○, no cysteamine; ●—●, 1×10^{-3} M cysteamine.

of growth as rapidly as organisms in plain nutrient broth. The post-log. phase in each case was reached in approximately 10 hr.; in the cultures containing cysteamine, lysis occurred between 14 and 18 hr. Organisms in the control cultures retained their structural integrity for 6 days, at which time they were discarded. Cysteamine and aminoethyl sulphide could also be added either at the time of inoculation or during the post-log. period to obtain lysis, whereas the two isothiuronium compounds (AET, AEP) induced lysis only when they were added to post-log. phase cultures.

The influence of various physical and chemical environmental factors on induction of lysis by cysteamine is indicated in Table 3 and in Fig. 5. The most efficient conditions for such lysis included growth and maintenance of the cultures in nutrient broth in shaken shallow layers at 37° without alteration of the normal post-log. pH value of 8.7. Because of a deficiency of available manganese in nutrient broth (Weinberg, 1955) such cultures could not sporulate. Under conditions where sporulation occurs, as in nutrient agar, lysis induced by cysteamine required approximately 24–28 hr. rather than 4–8 hr. (Weinberg, 1957). It is probable that germination of the spores followed by

logarithmic growth was required before the organisms became competent to lyse.

Attempts were made to suppress lysis in nutrient broth by increasing the molar strength of the environment with high concentrations of sucrose or glucose. As is indicated in Table 3, high concentrations of these carbohydrates caused both control and cysteamine-exposed organisms to lyse. Lower concentrations retarded the induction of lysis by cysteamine. The formation

Table 3. *Effect of environmental conditions on ability of cysteamine to induce lysis of post-log. phase assay organisms of Bacillus subtilis strain Marburg*

The organisms were grown in shaken shallow layers of nutrient broth (except in E) at 37° for 16 hr. Cysteamine (1×10^{-3} M) was added at 16 hr. and incubation was continued under the various test conditions for a subsequent period of 48 hr.

Condition during 48 hr. post-log. phase		Rapidity and extent of lysis	
		Control organisms	Cysteamine-exposed organisms
(A) Amount of aeration	(1) Shaken shallow layer	None	Rapid, complete
	(2) Aspirated air through deep layer	None	Slow, partial
	(3) Compressed air through deep layer	None	Slow, partial
	(4) Stationary shallow layer	Slow, partial	Slow, complete
(B) Temperature	(1) 80°	None	None
	(2) 58°	Rapid, complete	Rapid, complete
	(3) 37°	None	Rapid, complete
	(4) 25°	None	Slow, complete
	(5) 2°	None	None
(C) pH value (see Figure 5)	(1) 8-9	None	Rapid, complete
	(2) 7-8	None	Slow, partial
	(3) 6-7	None	Slow, slight
(D) Assay organisms grown in nutrient broth	(1) Resuspended in nutrient broth filtrate	None	Rapid, complete
	(2) Resuspended in defined medium filtrate (adjusted to pH 8.0)	None	Slow, partial
	(3) Resuspended in M/80 PO ₄ buffer (pH 8.0)	Slow, partial	Slow, complete
(E) Assay organisms grown in defined medium	(1) Resuspended in nutrient broth filtrate	None	Rapid, complete
	(2) Resuspended in defined medium filtrate (adjusted to pH 8.0)	None	None
(F) Increase in molar strength	(1) 1.4 M-sucrose or glucose	Immediate, complete	Immediate, complete
	(2) 0.7 M-sucrose or glucose	Rapid, partial	Rapid, complete
	(3) 0.14 M-sucrose or glucose	None	Slow, complete

of protoplasts was not observed even when bovine serum albumin was added to the lysing preparations that were being examined by phase microscopy.

Of interest are the observations that a few of the 43 compounds which did not induce lysis partially suppressed the activity of cysteamine. The cysteamine-suppressing compounds included D- or L-cysteine, DL-cystine, D-penicillamine, thiazolidine thione and thiazolidine-4-carboxylic acid. The last compound, an antimetabolite of proline, was the most efficient suppressor of cysteamine (Fig. 6); L-proline had no effect on the activity of thiazolidine-4-carboxylic acid.

Fig. 6. Ability of thiazolidine-4-carboxylic acid and L-cysteine to suppress lysis-induction activity of cysteamine in *Bacillus subtilis*. The analogue and cysteamine were added at 16 hr. and the readings were made at 24 hr.

Various inorganic ions were tested for their effect on cysteamine-induced lysis. Cu^{++} slightly accelerated lysis; Mg^{++} and MoO_4^- were inactive; Fe^{++} , Mn^{++} , and Zn^{++} slightly delayed lysis; Co^{++} and Ni^{++} totally inhibited the cysteamine effect. In each case a concentration equimolar to cysteamine was used; since some of the metallic ions are bacteriostatic in these concentrations, both cysteamine and the ions were added to post-log. cultures rather than to fresh cultures.

Other requirements for cysteamine-induced lysis included: (1) metabolizing organisms rather than organisms inactivated by exposure to 80° for 10 min. or isolated cell walls; (2) the continual presence of cysteamine (or a compound synthesized by cysteamine-exposed organisms) in the culture filtrate. When at any time during the period required for lysis to occur, the cysteamine-exposed organisms were removed from their culture medium and resuspended in normal culture filtrate of the same age, lysis was considerably slower and often incomplete.

Presence of formed elements in the lysate

As lysis proceeded to completion, the majority of organisms (as determined by successive stained preparations) first became Gram-negative and then

disappeared. When organisms in lysing cultures were observed by phase microscopy, the organisms appeared to become less dense before they disappeared. No formed elements were detected in lysates by phase microscopy. Cell-wall stains of partially lysed cultures revealed circular 'ghosts' which were not evident in completely lysed preparations. Similarly, circular ghosts were seen in electron photomicrographs of partially-lysed cultures. Electron photomicrographs of normal cultures contained only intact organisms while those of completely lysed cultures contained no formed elements except flagella. Seemingly completely lysed cultures were, however, not sterile; plate counts to detect viable organisms indicated that a few hundred organisms/ml. lysate survived exposure to cysteamine. It is noteworthy that these few surviving organisms were not able to multiply in filtrates of cysteamine-lysed cultures, whereas survivors of populations lysed by other means (such as high concentrations of sucrose) were usually able to undergo logarithmic growth in their homologous culture media when incubation was continued.

The ability of dialysed lysates, culture filtrates, and fresh nutrient broth to induce lysis (in the absence of cysteamine) of normal and heated assay organisms with and without added Co^{++}

An extensive series of experiments was performed to determine whether cysteamine-exposed organisms produced a bacteriophage or an unusually large quantity of an autolytic enzyme. Tests for bacteriophage production consisted of: (a) adding successive crops of post-log. normal organisms to lysates, and (b) the addition of lysates to normal organisms of various ages in nutrient broth and on nutrient agar. To detect the presence of autolytic enzymes, similar experiments were performed with soluble extracts of normal and cysteamine-exposed crops of organisms of various ages which had been ruptured by sonic oscillation and by Mickle disintegration. No evidence of lytic activity in any of these preparations was obtained except in those containing residual cysteamine. When cysteamine was present in the minimum concentration required for lysis of the first crop of organisms, subsequent crops were not lysed in the particular sample. When a twofold, threefold, fourfold, or fivefold concentration of cysteamine was contained in the original sample, two, three, four or five but not six crops of post-log. organisms, respectively, could be added successively to the sample and be completely lysed.

To remove residual cysteamine from cell-free extracts or from lysates, the samples were dialysed in a rocking dialyser against running tap water at 4° for periods from 3 hr. to 4 days. Samples of culture filtrate and fresh nutrient broth were likewise dialysed for 2 days. Dialysed cell-free extracts possessed no lytic activity for normal post-log. assay organisms but lysates from cysteamine-exposed organisms did possess lytic activity when dialysed for at least 2 days. Normal culture filtrate and fresh nutrient broth which had been dialysed for 2 days similarly acquired lytic activity, although they were less efficient than dialysed lysates. The time required for lysis of 50 % of crops of assay organisms added to the various samples is summarized in Table 4. It may

be seen that as the residual cysteamine was removed from lysates by dialysis up to 24 hr. the lysates became somewhat less active but that activity was significantly greater in lysates dialysed for 48 hr. With some lysates, a period of dialysis of 3 days was required to obtain maximum activity; no additional activity of any sample was gained by a 4th day of dialysis.

Table 4. *Effect of dialysis of test samples and of heat and Co⁺⁺ activation on rapidity of lysis of post-log. phase assay organisms of Bacillus subtilis*

Test samples	Unheated organisms	Organisms exposed to 58° for 10 min.	Unheated organisms exposed to 2×10^{-3} M Co ⁺⁺ during tests
(A) Post-log. phase culture filtrates from which normal organisms were removed			
	Time for 50 % lysis (hr.)		
(1) Non-dialysed	No lysis	1.3	2.4
(2) Non-dialysed plus 8×10^{-4} M-cysteamine	4.0	0.6	6.5
(3) Dialysed 48 hr.	12.0	1.2	3.0
(B) Post-log. phase lysate from cysteamine-exposed cultures			
(1) Non-dialysed (contained slight amount of residual cysteamine)	13.0	1.2	3.0
(2) Dialysed 3 hr.	15.0	1.0	3.0
(3) Dialysed 6 hr.	16.0	1.1	3.0
(4) Dialysed 24 hr.	16.5	1.0	3.0
(5) Dialysed 48 hr.	8.4	0.5	2.6
(C) Fresh nutrient broth			
(1) Non-dialysed	No lysis	No lysis	6.5
(2) Dialysed 48 hr.	24.0	2.1	4.5

Since the autolytic enzyme of post-log.-phase organisms of *Bacillus subtilis* studied by Strange & Dark (1957) was activated by exposure to 58° and by the presence of high concentrations of Co⁺⁺, it seemed of interest to include a study of the effect of these two conditions in lysis of *B. subtilis* by the test samples. Heat activation was accomplished by heating the assay organisms (in a small volume of pH 8.0 phosphate buffer) for 10 min. at 58° before suspension of the organisms in the various test samples. The actual tests were then performed at 37°, although the results were similar when the tests were conducted at 58°. Activation by Co⁺⁺ was tested by adding to the test samples a solution of CoSO₄·7H₂O to obtain a final concentration of Co⁺⁺ of 2×10^{-3} M.

It can be observed in Table 4 that exposure of the normal post-log. phase assay organisms to 58° markedly accelerated lysis in all samples. As was true of non-heated organisms, organisms exposed to cysteamine or to the 48 hr. dialysed lysate lysed most rapidly. The presence of Co⁺⁺ did not accelerate lysis of heated organisms but did significantly hasten the lysis of non-heated organisms with one exception. The single exception was that of the culture filtrate containing cysteamine and it is believed that the addition of Co⁺⁺ to this sample neutralized the cysteamine by forming an inactive chelate. As

evidence of chelate formation, solutions containing cysteamine and Co^{++} developed a pink colour. Lysis eventually did occur in these samples presumably because of the excess Co^{++} .

DISCUSSION

Lytic phenomena in the genus *Bacillus* have been reported quite frequently within the past 3 years (Greenberg & Halvorson, 1955; Ivánovics & Alföldi, 1957; Nomura & Hosoda, 1956; Norris, 1957; Strange & Dark, 1957). The present observations are similar to those of the majority of the previous reports in that (a) post-log. phase rather than younger crops of organisms are most readily lysed, (b) there is considerable strain and species specificity, (c) a complex medium usually is required, and (d) most of the formed elements of the organism are destroyed during lysis.

The present observations differ from those of the previous studies on lysis of organisms of *Bacillus* sp. in that the assay organisms themselves actively participate in the lytic process. Organisms inactivated by heat do not lyse nor are isolated cell walls destroyed by cysteamine. In order to lyse, the assay organisms must be maintained at 37° in staled broth in the presence of the cysteamine compounds for several hours.

Induction of lysis of strains of *Bacillus* spp. by the cysteamine compounds is somewhat comparable to induction of lysis of various bacteria of different genera by glycine (Cowles, 1946). With glycine, post-log. phase metabolizing assay organisms are required, and 3–20 hr. are needed for the appearance of visible lysis. As with cysteamine, the cultures do not become completely sterile. Although glycine has a much broader bacteriolytic spectrum than has the cysteamine compounds, it is much weaker in activity. Concentrations of glycine as great as molar are required, whereas in the present experiments, active compounds induced lysis optimally at concentrations as low as 10^{-4}M .

An interesting feature of induction of lysis by cysteamine is that post-log. phase organisms which are used as inoculum for fresh cultures do not lyse within 4–8 hr. in the presence of the compound in fresh broth. Rather, the organisms proceed through the normal phases of growth and give rise to progeny which do lyse within 4–8 hr. in the post-log. phase provided cysteamine has not been removed from the environment. Apparently, fresh nutrient broth lacks factors possessed by staled broth that are required for cysteamine lysis. An alternative hypothesis is that fresh broth permits the post-log. phase organisms to begin logarithmic growth before 4 hr. has elapsed and they thus temporarily escape the usual fate of post-log. phase organisms exposed to cysteamine.

The requirement for nutrient broth rather than the defined medium for the exhibition of cysteamine activity is analogous to the situation reported by Ivánovics & Alföldi (1957) in which induction of lysis of *Bacillus megaterium* by thioglycollate or ultraviolet light occurred only when the organisms were grown in a complex medium. Perhaps cell walls are synthesized differently in complex media and simpler defined nutritive environments (cf. Litwac & Pramer, 1956). In the present study, however, organisms grown in the

defined medium did lyse in the presence of cysteamine when they were suspended in staled nutrient broth. It is of interest to note that the intra-transguanylation product of AET (i.e. 2-mercaptoethylguanidine) was found to be considerably more active in protecting bone marrow cells from X-irradiation when the cells were suspended in plasma rather than in a chemically defined medium or in saline (Smith, 1957).

Of interest are reports that cystamine is metabolized to a dark coloured compound by extracts of mammalian amine oxidases (Cavallini, De Marco & Mondovi, 1957; Bergeret & Blaschko, 1957). In the present study, one of the sensitive and two of the resistant strains of *Bacillus* spp. likewise produced a dark pigmented compound from cysteamine and from cystamine. Unpublished observations in this laboratory indicate that cysteamine is neither deaminated nor desulphurated by intact organisms or by cell-free extracts of sensitive strains.

Possible mechanisms whereby the cysteamine compounds induce lysis of sensitive strains include the following:

A. *Activation of a normal 'lysozyme-like' autolytic enzyme.* Autolytic enzyme molecules are present in large amount in post-log. phase organisms of *Bacillus* spp. (Richmond, 1957; Strange & Dark, 1957). This enzyme presumably facilitates the emergence of the spore through the vegetative cell wall. In the present study, spores did not develop because of the lack of sufficient Mn^{++} (Weinberg, 1955) but the organisms did possess an autolytic mechanism as demonstrated by their rapid autolysis in the presence of Co^{++} or when heated to 58° . Possible activation of this autolytic mechanism by the cysteamine compounds might be comparable to the activation of egg-white lysozyme by ethylene diamine tetraacetic acid (Repaske, 1956).

B. *Suppression of new cell-wall synthesis.* If continual synthesis of new cell-wall material by post-log. phase organisms is necessary to balance the normal autolytic mechanism, then a suppressor of such synthesis would indirectly cause the organisms to lyse. It is known that cell-wall synthesis may be suppressed by (a) penicillin and (b) a deficiency of a constituent of cell walls such as α,ϵ -diaminopimelic acid in the nutrient for certain auxotrophs (McQuillen, 1958). Unlike penicillin, cysteamine and related compounds are not active during logarithmic growth; moreover, Co^{++} neutralizes rather than enhances these compounds, presumably by formation of an inactive chelate. Conceivably, such compounds, rather than an unknown analogue, might be incorporated into cell walls of post-log. phase organisms of sensitive strains with the result that such walls could not maintain their structural integrity.

C. *Binding of metallic ions in cell walls.* Kozloff & Lute (1957) presented evidence that cell walls of *Escherichia coli* contain zinc atoms. It is possible that metallic ions are present in the walls of *Bacillus* and that post-log. phase organisms depend on such ions to maintain the structural integrity of the walls to a greater extent than do younger cells. However, numerous metal-binding agents other than cysteamine and its derivatives have been found in this laboratory (unpublished observations) to be unable to induce lysis of post-log. phase organisms of *Bacillus* spp.

D. *Binding a metallic ion inhibitor of lysis.* Autolysis of *Streptococcus faecalis* has been observed to be suppressed by Mn^{++} (MacLeod, 1951; van Eys & Pearson, 1954). In the present study, lysates, culture filtrates and fresh nutrient broth gained autolytic activity by being dialysed for 1–2 days. It is conceivable that such dialyses remove metallic ions that are tightly bound to non-dialysable molecules in the preparations, and that such metallic ions are suppressors of autolytic activity of post-log. phase organisms of *Bacillus*. Thus, dialysed preparations might induce lysis in the absence of, for example, cysteamine, whereas in non-dialysed materials, lysis would occur only if the active compounds herein reported combined with certain metallic ions of these materials.

E. *Suppression of a protease that might inhibit lysis.* Experiments performed in this laboratory (to be published) indicate that cysteamine and its derivatives suppress the auto-protease activity of extracts of sensitive organisms. If protease activity is needed to inhibit normal autolysis of post-log. phase organisms then a suppressor of such activity would indirectly induce lysis. Obviously much additional investigation is needed to establish the relationship, if any, among the biochemical mechanisms whereby cysteamine and similar compounds protect cells against radiation damage, suppress protease activity, and induce lysis of post-log. phase organisms of sensitive strains of *Bacillus*.

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