APPLIED MICROBIOLOGY
Vol. 12, No. 3, p. 234-239 May, 1964

Induction of Lambda-Bacteriophage in *Escherichia coli* as a Screening Test for Potential Antitumor Agents[1]

BERNARD HEINEMANN AND ALMA J. HOWARD

Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York

Received for publication 9 January 1964

ABSTRACT

HEINEMANN, BERNARD (Bristol Laboratories, Syracuse, N.Y.), AND ALMA J. HOWARD. Induction of lambda-bacteriophage in *Escherichia coli* as a screening test for potential antitumor agents. Appl. Microbiol. **12**:234–239. 1964.— A simple, rapid, quantitative test procedure to measure induction of phage production in lysogenic *Escherichia coli* K-12 (λ) was described. This test was used in a study of 209 substances, including antibiotics, pyrimirines, purines, alkylating agents, thiols, amino acids, vitamins, and miscellaneous compounds. Minimal inducing concentrations for the 26 (12.5% of total tested) substances found to be effective inducing agents, as well as a listing of the inactive compounds, are presented. Since 21 of the 26 active agents reportedly have antineoplastic activity in rodent tumor systems, it was concluded that the induction test may provide a useful screen for the detection of potentially useful antitumor compounds.

A number of chemical and physical agents are known to induce bacteriophage development in lysogenic bacteria. Lwoff (1953) and, later, Geissler (1962) suggested that a correlation exists between the inducing activity of such agents and their mutagenic and carcinogenic properties. Lein, Heinemann, and Gourevitch (1962) observed that the capability of antibiotics to induce lambda-phage formation in lysogenic *Escherichia coli* K-12 correlated with their ability to inhibit development of transplanted tumors in animals. Further evidence for such correlation was recently obtained by Endo et al. (1963) in experiments with mitomycin C and related compounds, nitrogen mustard and derivatives, and the antitumor antibiotic, carcinophilin.

In the present report, we describe in detail the quantitative method used by Lein et al. (1962) for selecting agents capable of inducing phage production in lysogenic *E. coli* K-12 (λ). A total of 209 substances, including antibiotics, pyrimidines, purines, alkylating agents, thiols, amino acids, vitamins, and miscellaneous compounds, were tested for inducing activity. The minimal inducing concentrations for the effective inducing agents, as well as a listing of the inactive compounds, are presented. Findings in this test system are considered in relation to results obtained in experimental animal tumor systems.

[1] Presented in part at the 63rd Annual Meeting of the American Society for Microbiology, Cleveland, Ohio, 7 May 1963.

MATERIALS AND METHODS

Microorganisms. The lysogenic bacterium *E. coli* K-12 (λ) and the streptomycin-resistant indicator culture *E. coli* W3001 were obtained from J. Lederberg. The lysogenic culture was maintained on nutrient agar (Difco nutrient broth, dehydrated, 0.8%; and Difco agar, 1.8%), and the indicator culture was maintained on nutrient agar containing streptomycin (100 μg/ml).

Culture preparation. E. coli K-12 (λ) was grown at 37 C in a synthetic broth (KH_2PO_4, 0.3%; K_2HPO_4, 0.73%; $MgSO \cdot 7H_2O$, 0.012%; NH_4Cl, 0.1%; and filter-sterilized dextrose, 0.4%). The culture was harvested during the logarithmic phase of growth and diluted with induction broth (KH_2PO_4, 0.3%; NaCl, 0.05%; NH_4Cl, 0.1%; Na_2HPO_4, 0.6%; filter-sterilized dextrose, 0.4%; and filter-sterilized $MgSO_4 \cdot 7H_2O$, 0.041%) to 10^5 cells per ml. Cell concentration was established by measuring the optical density of the suspension with a Bausch & Lomb Spectronic-20 colorimeter (530 mμ).

E. coli W3001 was grown in nutrient broth (Difco nutrient broth, dehydrated, 0.8%; and NaCl, 0.5%) for 18 hr at 37 C and used without dilution.

Preparation of test materials. Compounds were dissolved in water or, when necessary, in solvents. The highest final concentrations of solvents that could be employed without effect in the induction test were as follows: ethyl alcohol, 8%; acetone, 8%; and dimethylacetamide, 2%. Agents were tested at final concentrations up to 1.0 mg/ml when sufficiently soluble and nontoxic for bacterial cells.

Although every effort was made to insure stability of the compounds prior to and during the test period, it is conceivable that instability, particularly among the antibiotics, may have influenced test results.

Induction test. A 0.8-ml amount of diluted *E. coli* K-12 (λ) cell suspension was added to 0.2 ml of test sample. Tubes were then incubated in a water bath at 37 C for 1 hr (induction period) with brief shaking every 15 min. A 9-ml amount of nutrient broth (warmed to 37 C) was then added to each tube and mixed thoroughly. Tubes were then placed in a shaker water bath at 37 C for 2.25 hr (incubation period). The shaker had 1.5-in. stroke amplitude and was run at the rate of 100 strokes per min. At the end of the incubation period, mature phage were measured by the soft agar layer technique, and the number of *E. coli* K-12 (λ) colony formers was determined.

Plaque counts. The dilutions required for enumerating plaque formers were made in nutrient broth at the end of the incubation period. Efforts were directed toward obtaining a desired optimum of 20 to 300 plaques per plate. A 1-ml amount of an appropriate test sample dilution and 2 drops (from a 1-ml pipette) of *E. coli* W3001 suspension were added to a tube maintained at 48 C and containing 3 ml of melted soft agar (Difco nutrient broth, dehydrated, 0.8%; NaCl, 0.5%; and Difco agar, 0.5%). After mixing, the soft agar was overlaid upon the surface of a base layer of warmed nutrient agar in petri dishes. The base layer agar, dispensed in 20-ml amounts, contained 0.5% NaCl and streptomycin (50 mg/liter) to suppress growth of *E. coli* K-12 (λ). In addition, a dye mixture in a concentration of 0.6%, prepared by combining 500 ml of FD & C red #2 (2 mg/ml) and 70 ml of FD & C blue #1 (2 mg/ml) was added to the base layer agar to increase contrast between plaques and background. Room temperature was maintained at 30 C during the overlay process. After solidification, plates were quickly transferred to an incubator (37 C). Best results were obtained when metal covers with absorbent liners were used to reduce surface moisture. Determination of plaque numbers from duplicate plates was made after overnight incubation.

Cell counts. Dilutions for enumeration of cells that survived to the end of the incubation period were made in sterile physiological saline. Portions (0.1 ml) of each of the appropriate test dilutions were placed on the surface of nutrient agar plates and uniformly distributed with a sterile glass rod. After overnight incubation at 37 C, colonies on duplicate plates were counted.

Controls. A negative control (0.2 ml of induction broth) and a known active material (mitomycin C, 0.2 ml of a 0.25 μg/ml solution) were included in each test.

Expressing activity. The induction index is a measure of the extent to which a substance can induce lysogenic bacteria and is defined as the ratio of the number of plaque-forming phage in the test sample (T) to that of the negative control (C). The minimal inducing concentration (lowest concentration that increases the induction index at least tenfold over the negative control) of active agents was determined by conducting dose-response titrations.

Evidence of significant growth stimulation or toxicity to *E. coli* K-12 (λ) was obtained by examination of bacterial cell counts.

The designation "known antineoplastic agent" was assigned on the basis of animal data reported in the literature and must be qualified in that results of such tests are highly dependent on the particular tumor strain and host employed.

TABLE 1. *Minimal inducing concentration of antibiotics for Escherichia coli K-12* (λ)

Antineoplastic agent	Minimal inducing concn
	μg/ml
Azaserine	0.01
Carzinostatin	0.1
Gancidin A	0.1
Griseolutein A and B (mixture)	10.0
Mitomycin C	0.01
Phleomycin	0.2
Pluramycin A	2.5
Porfiromycin	0.05
Streptonigrin	0.05
Streptozotocin	1.0
Xanthomycin*	1.5

* Antineoplastic activity not reported for xanthomycin.

RESULTS

Up to the time of preparation of this manuscript, 209 substances had been evaluated for inducing activity. Several of the antibiotics were obtained from the Bristol Laboratories screening program and most of the chemicals through commercial channels. The sources of all other compounds are indicated in Acknowledgments.

Table 1 gives the minimal inducing concentrations of 11 antibiotics for *E. coli* K-12 (λ). Ten are considered to be antineoplastic agents; no information is available regarding the antitumor activity of xanthomycin. Inducing activity had previously been reported for three of these antibiotics: Gots, Bird, and Mudd (1955) found that L-azaserine was capable of inducing lysogenic bacteria; Otsuji et al. (1959) and Levine and Borthwick (1963) reported similar results for mitomycin C and streptonigrin, respectively.

Table 2 lists 29 known antineoplastic antibiotics which were ineffective as inducers of *E. coli* K-12 (λ) at the highest concentration tested.

Table 3 lists a group of 69 antibiotics which have not

TABLE 2. *Known antineoplastic antibiotics ineffective as inducers for Escherichia coli K-12* (λ)

Antibiotic	Maximal concn tested	Antibiotic	Maximal concn tested
	μg/ml		μg/ml
Aburamycin	500	Carzinocidin	500
Actinobolin sulfate	1	Clavacin	1
Actinogan	1,000	Cycloheximide	1,000
Actinomycin C	100	6-Diazo-5-oxo-L-norleucine	5
Actinomycin D	10	M5-18903	10
Alazopeptin	1	Melanomycin	100
Amicetin	500	Netropsin sulfate	1
Angustmycin	500	Psicofuranine	500
NSC A-649	100	Puromycin dihydrochloride	100
Actinoleukin-like	1	Pyridomycin hydrochloride	10
Aureolic acid	100	Rimocidin	500
Ayamycin A_2	100	Sarkomycin	1,000
Calvacin	100	Spiramycin	100
3-Carboxy-2,4-pentadienal lactol	10	Streptovitacin A	500
		Toyocamycin	500

been tested in experimental tumor systems or which have shown only negative or borderline activity in such systems. All were found to be ineffective as inducers of *E. coli* K-12 (λ) at the highest concentration tested.

Ten alkylating agents, all of which are known antineoplastic agents, are shown in Table 4. Six were effective inducers, and four were not. Although inducing activity with tris (1-aziridinyl)-phosphine oxide (TEPA) and tris (1-aziridinyl)-phosphine sulfide (thio-TEPA) could be demonstrated, results were highly variable, possibly due to the unstable nature of these materials. Lwoff and Jacob (1952) reported inducing effects with 1,2:3,4-diepoxybutane and 2,4,6-tris(1-aziridinyl)-*s*-triazine (TEM); Jacob (1952) obtained similar results with nitrogen mustard.

Table 5 lists 14 thiols and thiol derivatives. Four sulfhydryl compounds, D,L-cysteine, D,L-homocysteine, β-mercaptoethylamine, and D,L-penicillamine, and the only known antineoplastic agent in this group, S-carbamyl-L-cysteine, were found to be effective inducers. Although Lwoff and Siminovitch (1952) had reported inducing effects for thiomalic acid and reduced glutathione with a lysogenic strain of *Bacillus megaterium*, neither these compounds nor two others that contain a sulfhydryl group, β-mercaptoethanol and sodium thioglycolate, were active in the *E. coli* K-12 (λ) system. Inducing effects were not observed with compounds such as S-benzyl-L-cysteine, L-cysteic acid, cystine, D,L-homocysteic acid and S-methyl-L-cysteine, all of which lack a sulfhydryl group.

Table 6 lists 20 purines, pyrimidines, and related com-

TABLE 3. *Antibiotics for which antineoplastic activity has not been demonstrated and which are not inducers for Escherichia coli K-12* (λ)

Antibiotic	Maximal concn tested	Antibiotic	Maximal concn tested
	μg/ml		μg/ml
Althiomycin	1	Narbomycin	500
Amphomycin sodium	500	Neomycin sulfate	0.1
Amphotericin B	500	Novobiocin	500
Antibiotique 362	1	Nucleocidin	10
Ascosin	500	Nybomycin	100
Aspartocin	500	Nystatin	500
Ayfactin	500	Paromomycin	1
Azalomycin B	500	Penicillin F	10
Azalomycin F	10	Penicillin G	100
Bamicetin	10	Penicillin K	100
Blasticidin S	10	Penicillin V	100
Caerulomycin	500	Penicillin X	10
Candicidin	100	Phalamycin	500
Candidin	500	Pyoluteorin	1
Carbomycin hydrochloride	10	Quinocycline complex	1
Celesticetin salicylate	500	Ramycin	10
Cephalosporin P	100	Rifomycin B	10
Chartreusin	100	Ristocetin A	10
Chloramphenicol	1	Ristocetin B	10
Cinnamycin	500	Staphylomycin	10
Colistin hydrochloride	0.1	Streptolydigin	100
Cycloserine	0.1	Streptomycin-SO_4	1
Enteromycin	10	Streptovaricin	10
Erythromycin	1	Synnematin B	10
Esperin R	500	Taitomycin	500
Etamycin	10	Telomycin	100
Fervenulin	10	Terreic acid	0.1
Fungichromin	500	Tertiomycins A and B	10
Gramicidin J	1	Tetracycline hydrochloride	1
Hygromycin	500	Trichomycin	100
Kanamycin sulfate	1	Tylosin	100
LA-7017	100	Ustilagic acid	100
Leucomycin	10	Vancomycin	10
Mycorhodin	100	Violacetin hydrochloride	10
		Viomycin	10

TABLE 4. *Effectiveness of alkylating agents with antineoplastic activity as inducers of Escherichia coli K-12* (λ)

Agent	Minimal inducing concn	Maximal concn tested
	μg/ml	μg/ml
Inducers		
1,2:3,4-Diepoxybutane	7.5	—
2,2′-Dichloro-*N*-methyldiethylamine (nitrogen mustard)	0.5	—
2,2′-Dichloro-diethylamine	500	—
2,4,6-tris(1-aziridinyl)-*s*-triazine	25	—
tris(1-aziridinyl)-phosphine oxide	Variable	—
tris(1-aziridinyl)-phosphine sulfide	Variable	—
Noninducers		
Cytoxan	—	500
D-Sarcolysin	—	500
Methane sulfonic acid nonamethylene ester	—	500
Methane sulfonic acid tetramethylene ester	—	500

TABLE 5. *Effectiveness of some thiols and thiol derivatives as inducers of Escherichia coli K-12* (λ)

Agent	Minimal inducing concn	Maximal concn tested
	μg/ml	μg/ml
Inducers		
D,L-Cysteine	5.0	—
D,L-Homocysteine	125.0	—
β-Mercaptoethylamine	50.0	—
D,L-Penicillamine	100.0	—
S-carbamyl-L-cysteine*	75.0	—
Noninducers		
S-benzyl-L-cysteine	—	100
L-Cysteic acid	—	100
Cystine	—	100
Glutathione	—	500
D,L-Homocysteic acid	—	100
β-Mercaptoethanol	—	0.1†
S-methyl-L-cysteine	—	100
Sodium thioglycolate	—	500
Thiomalic acid	—	1,000

* Known antineoplastic agent.
† Per cent.

pounds, all of which were ineffective as inducers. Six of the purines and three of the pyrimidines are considered to be antineoplastic agents.

Table 7 lists nine amino acids and six vitamins which do not have antineoplastic activity and which were noninducers.

TABLE 6. *Purines, pyrimidines, and derivatives found ineffective as inducers of Escherichia coli K-12* (λ)

Agent	Maximal concn tested
	μg/ml
Purines	
Known antineoplastic activity	
8-Azaguanine	100
6-Chloropurine	100
6-Mercaptopurine	100
6-Mercaptopurine ribonucleoside	500
Purine	100
Thioguanine	100
Antineoplastic activity not demonstrated	
Adenine	1,000
Adenosine	1,000
Guanine	100
Guanosine	1,000
Guanylic acid	1,000
Hypoxanthine	1,000
Xanthine	100
Pyrimidines	
Known antineoplastic activity	
4-Aminopyrazole (3,4-D) pyrimidine	100
5-Fluorodeoxyuridine	1
5-Fluorouracil	0.1
Antineoplastic activity not demonstrated	
5-Bromodeoxyuridine	500
5-Iodo-2′-deoxyuridine	500
Thymine	1,000
Uracil	100

TABLE 7. *Amino acids and vitamins ineffective as inducers of Escherichia coli K-12* (λ) *and as inhibitors in rodent tumor systems*

Agent	Maximal concn tested
	μg/ml
Amino acids.	
L-Arginine	1,000
L-Aspartic acid	600
L-Histidine	1,000
D,L-Isoleucine	1,000
D,L-Methionine	500
L-Ornithine	1,000
D,L-Phenylalanine	1,000
D,L-Threonine	1,000
D,L-Valine	1,000
Vitamins	
L-Ascorbic acid	10
D-Biotin	1,000
Nicotinamide	1,000
d-Pentothenic acid, Ca salt	1,000
Pyridoxine hydrochloride	1,000
Thiamine hydrochloride	1,000

Four miscellaneous compounds found to be effective as inducers of *E. coli* K-12 (λ) and which are known antineoplastic agents are shown in Table 8. Ben-Gurion (1962) recently reported that aminopterin is capable of inducing lysogenic bacteria; Lwoff and Jacob (1952) had found hydrogen peroxide to be active.

Table 9 lists 20 miscellaneous antineoplastic agents ineffective as inducers.

Table 10 lists 17 miscellaneous noninducing compounds for which antineoplastic activity has not been demonstrated. The four compounds known to be active in radiation protection tests were tested, because β-mercaptoethylamine and cysteine, both effective radiation protectants, had been found to be effective inducers.

DISCUSSION

There appears to be a correlation between a compound's capability to induce phage production in lysogenic *E. coli*

TABLE 8. *Effectiveness of four miscellaneous compounds with known antineoplastic activity as inducers of Escherichia coli K-12* (λ)

Compound	Minimal inducing concn
	μg/ml
Aminopterin	100
1,4-Bis(3-bromopropionyl) piperazine	500
Vincaleukoblastine sulfate	500
H_2O_2	0.001*

* Per cent (v/v).

TABLE 9. *Miscellaneous antineoplastic agents found ineffective as inducers of Escherichia coli K-12* (λ)

Agent	Maximal concn tested
	μg/ml
Amethopterin	1,000
2-Amino-4-arsenosophenol HCl	10
1-Aminocyclopentane carboxylic acid	500
6-Aminonicotinamide	1,000
1 H-benzotriazole	100
Cortisone	500
Dichloroamethopterin	500
2,4-Dinitrophenol	100
D,L-Ethionine	100
Ethyl carbamate	500
Hydrocortisone	500
Hydroxyurea	1,000
N-methylformamide	1*
N-methyl *N*′-nitro-*N*-nitrosoguanidine	10
Potassium arsenite	500
Pyrogallol	10
Terephthalanilide, 2-Cl-4′,4″-bis (2-imidazolin-2-yl)-	10
Terephthalanilide, 2-nitro-4′,4″-bis (2-imidazolin-2-yl)-,dihydrochloride	10
Terephthalanilide, 4′,4″-bis (2-imidazolin-2-yl)-	1
2-Thiophenealanine	500

* Per cent.

K-12 (λ) and its ability to inhibit development of transplanted tumors in rodents. Of the 26 substances found to be effective inducers, tumor activity data were not available for one of the antibiotics, and 4 compounds (all possessing a sulfhydryl group) failed to demonstrate antitumor activity. The remaining 21 compounds, comprising 10 antibiotics, 6 alkylating agents, 1 thiol compound, and 4 miscellaneous chemicals, were all known antineoplastic agents. Although this is evidence for positive correlation, the induction test apparently detects only certain classes of antitumor activity, since 62 compounds with carcinostatic activity demonstrated no inducing effects. Included among the carcinostatic compounds found ineffective as inducing agents were some of the antibiotics, alkylating agents, miscellaneous chemicals, and all of the tumor-inhibitory purines and pyrimidines tested.

Despite the apparent drawback offered by the failure of the induction test to detect all tumor-inhibitory substances, the finding that 80% of the inducing agents have antitumor activity indicates that the test could be used to screen for antineoplastic agents. The induction test offers a number of advantages, since, in contrast to screening systems that involve inhibition of animal tumors, it is rapid, inexpensive, and requires very small quantities of the test agent.

At the present time, the test is being utilized successfully to screen fermentation broths of actinomycetes. It has also shown utility as an assay procedure for following extraction and isolation of active agents from such broths.

TABLE 10. *Miscellaneous compounds for which antineoplastic activity has not been demonstrated and which were ineffective as inducers of Escherichia coli K-12* (λ)

Compound	Maximal concn tested
	μg/ml
Betaine	1,000
Carbamyl phosphate	500
Copper sulfate	100
L-Glutamine	1,000
1,5-Naphthalene disulfonic acid, disodium salt	200
β-Naphthalene sulfonic acid	1,000
β-Propiolactone	0.01*
Ribonuclease	500
Sulfathiazole	100
Radiation protection compounds	
2-Aminoethylisothiouronium bromide, HBr	500
ρ-Aminopropiophenone	500
Ammonium dithiocarbamate	500
Histamine diphosphate	500
Plant growth hormones	
o-Chlorophenoxyacetic acid	100
Malic acid hydrazide	100
α-Naphthalane acetamide	100
β-Naphoxyacetic acid	100

* Per cent.

ACKNOWLEDGMENTS

This study was supported by Cancer Chemotherapy National Service Center contract no. SA-43-ph-4362, National Cancer Institute, U.S. Public Health Service.

The technical assistance of N. Heck, S. Abrams, J. Hollister, and P. Watson, Jr., is gratefully acknowledged.

We are grateful to the following individuals and organizations for substances used in this study: K. Aiso of Chiba University, Japan, for gancidin A and violacetin-HCl; Chas. Pfizer & Co. Inc., Brooklyn, N.Y., for streptonigrin, netropsin-SO_4, carbomycin-HCl, quinocycline complex, and rimocidin; Merck Sharpe and Dohme, Philadelphia, Pa., for fungichromin; The Upjohn Co., Kalamazoo, Mich., for streptozotocin, amicetin, cycloheximide, psicofuranine, celesticetin salicylate, fervenulin, novobiocin, streptolydigin and streptovaricin; H. Umezawa of National Institutes of Health, Tokyo, Japan, for griseolutein A and B, 3-carboxy-2,4-pentadienal lactol, pyridomycin-HCl, blasticidin S, tertiomycins A and B, pluramycin A, and enteromycin; Shionogi Research Laboratories, Amazasaki, Japan, for aburamycin and toyocamycin; Parke, Davis and Co., Detroit, Mich., for actinobolin-SO_4, bamicetin, paromomycin, viomycin, and chloramphenicol; Banyu Pharmaceutical Co., Tokyo, Japan, for actinomycin C and colistin-HCl; Lederle Laboratories, Pearl River, N.Y., for alazopeptin, aspartocin, nucleocidin, and Thio-TEPA; Y. Sumiki of University of Tokyo, Tokyo, Japan, for angustmycin; Dr. Ishida of Tohoku University, Japan, for ayamycin A_2; Y. Harada of Kyowa Fermentation Industry, Tokyo, Japan, for carzinocidin; Eli Lilly & Co., Indianapolis, Ind., for M5-18903, tylosin, cycloserine, erythromycin, hygromycin, pencillin V, and vancomycin; T. Hata and Y. Sano of Kitasato Institute for Infectious Diseases, Tokyo, Japan, for melanomycin and leucomycin; E. R. Squibb and Co., New Brunswick, N.J., for amphotericin B and nystatin; SIFA Laboratories, Paris, France, for antibiotique 362; Takeda Pharmaceutical Industries, Osaka, Japan, for pyoluteorin; P. VanDijck of Louvain University, Belgium, for ramycin and staphylomycin; Michigan State Health Department, East Lansing for synnematin B and calvacin; Taito Co., Japan, for trichomycin; Prairie Regional Laboratories, Canada, for ustilagic acid; Commercial Solvents, Terre Haute, Ind., for ascosin; Abbott Laboratories, North Chicago, Ill., for aureolic acid and ristocetins A and B; Sankyo Co., Japan, for azalomycins B and F; National Research Council of Canada for caerulomycin; S. B. Penick and Co., New York, N.Y., for candicidin; U.S. Department of Agriculture, Peoria, Ill., for cinnamycin; Dr. Otani of Osaka University Japan, for gramicidin J; Lapetit, Milan, Italy, for LA-7017 and rifomycin B; New York Botanical Garden, Bronx, for nybomycin; New York State Health Department, Albany, for phalamycin; H. Yamaguchi of Nippon Kayaku Co., Tokyo, Japan, for althiomycin; Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J., for candidin; Ciba,

Basel, Switzerland, for narbomycin; J. Schmutz of A. Wander S. A., Berne, Switzerland, for terephthalanilides; Cancer Chemotherapy National Service Center, Washington, D.C., for L-azaserine, 6-diazo-5-oxo-L-norleucine, puromycin-di-HCl, streptovitacin A, 1,2:3,4-diepoxybutane, nitrogen mustard, TEM, TEPA, cytoxan, D-sarcolysin, nonamethylene ester of methane sulfonic acid, Myleran, 8-azaguanine, 6-chloropurine, purine, thioguanine, 5-fluorodeoxyuridine, 5-fluorouracil, 1,4-bis (3-bromopropionyl) piperazine, vincaleukoblastine-SO_4, amethopterin, 2-amino-4-arsenosophenol HCl, 1-aminocyclopentane carboxylic acid, 1 H-benzotriazole, 2,4-dinitrophenol, D,L-ethionine, ethyl carbamate, *N*-methylformamide, potassium arsenite, pyrogallol, hydroxyurea, 2-thiophenealanine, and dichloroamethopterin.

LITERATURE CITED

BEN-GURION, R. 1962. On the induction of *Escherichia coli* K-12 (λ) by aminopterin. Biochem. Biophys. Res. Commun. **8**:456–460.

ENDO, H., M. ISHIZAWA, T. KAMIYA, AND SONODA. 1963. Relation between tumouricidal and prophage-inducing action. Nature **198**:258–260.

GEISSLER, E. 1962. Lysogenie and cancerogenese. Neoplasma **9**:113–121.

GOTS, J. S., J. BIRD, AND S. MUDD. 1955. L-azaserine as an inducing agent for the development of phage in the lysogenic *Escherichia coli*, K-12. Biochim. Biophys. Acta **17**:449–450.

JACOB, F. 1952. Production de bactériophages par action de la méthyl-bis (chloroéthyl) amine sur des bactéries lysogènes. Compt. Rend. **234**:2238–2240.

LEIN, J., B. HEINEMANN, AND A. GOUREVITCH. 1962. Induction of lysogenic bacteria as a method of detecting potential antitumor agents. Nature **196**:783–784.

LEVINE, M., AND M. BORTHWICK. 1963. Induction of phage production in lysgenic bacteria with streptonigrin. Bacteriol. Proc., p. 153.

LWOFF, A., AND F. JACOB. 1952. Induction de la production de bactériophages et d'une colicine par les peroxydes, les éthylèneimines et les halogénoalcoylamines. Compt. Rend. **234**:2308–2310.

LWOFF, A., AND L. SIMINOVITCH. 1952. L'induction du développement du prophage par les substances réductrices. Ann. Inst. Pasteur **82**:676–689.

LWOFF, A. 1953. Lysogeny. Bacteriol. Rev. **17**:269–337.

OTSUJI, N., M. SEKIGUCHI, T. IIJIMA, AND Y. TAKAGI. 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin. Nature **184**:1079–1080.