Potentialiation of the Toxicity of Mithramycin by Bacterial Lipopolysaccharide

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The lethality for BALB/c mice of 1,3-bis(2-chloroethyl)-1-nitrosourea, cytosine arabinoside, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, hydroxyurea, mithramycin, a polymyxin-like antibiotic (SQ 21,286), polyadenylic polyuridylic acid, procarbazine, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (TIC-mustard) or uracil arabinoside administered in combination with *Escherichia coli* lipopolysaccharide (LPS) was measured. Simultaneously administered mithramycin and LPS or TIC-mustard administered 24 h after LPS synergistically killed mice. Concanavalin A potentiated the lethality of TIC-mustard but not of eight other drugs tested. Pretreatment of mice with LPS or lipid A complexed to concanavalin A rendered mice resistant to the lethal action of LPS alone or combinations of LPS and mithramycin. Mithramycin-treated mice were killed by minute amounts of LPS. Mice sensitized to LPS by mithramycin were used to detect endotoxic activity in biological materials, such as commercially available enzymes, and in a complex of lipid A with concanavalin A.

Sublethal doses of many antitumor drugs given in combination with a sublethal dose of bacterial lipopolysaccharide (LPS) result in marked lethality for the treated mice (16). Until recently, only antitumor drugs that inhibit protein or ribonucleic acid synthesis were known to potentiate the lethality of LPS. It is now recognized, however, that agents that affect deoxyribonucleic acid (DNA) synthesis, structure, and function (for example, 5-fluorouracil, 5-fluorouracil deoxyriboside, or chlorambucil [2]) can interact with LPS in vivo, leading to increased lethality for mice. Because such potentiated toxicities involving antitumor drugs and LPS may have clinical significance, their prevalence needs to be determined. Accordingly, the capability of 10 drugs to interact in vivo with LPS to enhance mouse lethality has been assessed. Most of the selected drugs have been used clinically and most affect DNA synthesis, structure or function.

Concanavalin A can substitute for LPS enhancing the lethal action of 5-fluorouracil (N. M. Marecki, S. G. Bradley, A. E. Munson, and D. C. Drummond, *Toxicol. Appl. Pharmacol.*, in press). It has been suggested therefore that a mitogen such as concanavalin A might be able to substitute for LPS in enhancing the lethality of other antitumor drugs, particularly those affecting DNA (3). Accordingly, the capability

of drugs to interact in vivo with concanavalin A has been assessed.

Of 10 antibiotics and antitumor drugs examined in this study, only mithramycin or 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (TIC-mustard), in combination with LPS, resulted in enhanced lethality for mice. Mice sensitized to LPS by mithramycin were used to detect endotoxic activity in biological materials.

MATERIALS AND METHODS

Male BALB/c mice weighing 22 to 27 g were used in all experiments. Mice were obtained from Flow Research Animals, Inc., Dublin, Va. They were allowed to adjust to their new environment for at least 1 week prior to experimentation. The mice were given free access to food (Purina Lab Chow, Purina Ralston Corp., St. Louis, Mo.) and water. All drug solutions were prepared so that 0.01 ml/g of mouse weight would provide the desired dose. All drugs and LPS injections were given simultaneously by the intraperitoneal route unless otherwise specified. The following were dissolved or suspended in sterile 0.15 M NaCl and adjusted to pH 7.0 with dilute HCl or NaOH: mithramycin (NSC-24559; Charles Pfizer & Co., Clifton, N.J.), 1,3 bis(2-chloroethyl)-1-nitrosourea (NSC-409, 962; BCNU; Merck, Sharp and Dohme, Rahway, N.J.), cytosine arabinoside (NSC-63878; The Upjohn Co., Kalamazoo, Mich.), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC-79037; CCNU), a cyclic octapeptide SQ 21,286 (antibiotic EM49; E. R.

Squibb & Sons, Princeton, N.J.), hydroxyurea (NSC-32065; Squibb), polyadenylic polyuridylic acid (Sigma Chemical Co., St. Louis, Mo.), procarbazine (NSC-77213; Hoffmann-LaRoche Inc., Nutley, N.J.), 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (NSC-82196; TIC-mustard), and uracil arabinoside (Upjohn). Bacterial LPS (Difco Laboratories, Detroit, Mich.) was suspended in 0.15 M NaCl.

Lipid A was extracted from *Escherichia coli* 0127:B8 lipopolysaccharide (Boivin method; Difco) by a procedure adapted from the method of Galanos et al. (7). The absence of 2-keto-3-deoxyoctonate, as confirmed by the thiobarbiturate assay of Waravdekar and Saslaw (19), was used to establish that hydrolysis was complete. Complexes of lipid A and bovine serum albumin (Sigma) or concanavalin A (grade IV; Sigma) were prepared by methods described by Galanos et al. (8). Immediately before use, the complexes were suspended in distilled water and dispersed with sonication.

The mean lethal dose of mithramycin or LPS was determined by interpolation of probit plots. Tests for parallelism of dose-effect curves, and calculations of potency ratios and confidence intervals, were also done by the method of Litchfield and Wilcoxon (10). The significance of differences between the proportion of dead mice in a test group and the control group was evaluated by the chi-square test. The acceptable level of significance was $P < 0.05$.

The biological materials selected for assay of endotoxin activity or contaminating LPS were: L-asparaginase (NSC-109229; Merck, Sharp and Dohme), bovine serum albumin (crystallized; Sigma), α -chymotrypsin (crystallized three times, type II; Sigma), concanavalin A (grades III and IV; Sigma), pronase (grade B; Calbiochem, San Diego, Calif.), trypsin (crystallized two times, type III; Sigma), and complexes of concanavalin A (grade IV) with myristic acid, myristoyl choline chloride, *N,N*-dimethyl myristamide (all from Sigma), or (β - γ -dimyristoyl-L- α -lecithin (Calbiochem).

RESULTS

The lethality of 10 drugs, administered simultaneously with *E. coli* LPS was measured (Table 1). Only mithramycin in combination with LPS markedly enhanced lethality when administered simultaneously, cytosine arabinoside or procarbazine in combination with LPS somewhat enhanced lethality.

TIC-mustard, administered 24 h after LPS, markedly enhanced mouse lethality (Table 2). Administration of BCNU, CCNU, hydroxyurea or the polymyxin-like antibiotic SQ 21,286 (13) 24 h before or after LPS, at the doses reported in Table 1, did not lead to enhanced mouse lethality. The moderate enhanced lethality noted with a simultaneous combination of procarbazine and LPS was also observed when LPS was administered 24 h before procarbazine (6 mice dead of 20 given both agents versus 0 mice

TABLE 1. Ability of selected drugs to enhance the lethal action of lipopolysaccharide administered simultaneously^a

Drug	LPS	No. of mice	% Dead
BCNU (40 mg/kg)	None	20	0
BCNU (40 mg/kg)	2 mg/kg	10	0
Cytosine arabinoside (1,000 mg/kg)	None	10	0
Cytosine arabinoside (1,000 mg/kg)	2 mg/kg	10	40
CCNU (200 mg/kg)	None	20	10
CCNU (200 mg/kg)	2 mg/kg	10	0
Hydroxyurea (4,000 mg/kg)	None	10	0
Hydroxyurea (4,000 mg/kg)	2 mg/kg	10	0
Mithramycin (1.0 mg/kg)	None	40	7
Mithramycin (1.0 mg/kg)	2 mg/kg	20	100
Poly(AU) (5 mg/kg)	None	15	0
Poly(AU) (5 mg/kg)	2 mg/kg	10	0
Procarbazine (500 mg/kg)	None	10	0
Procarbazine (500 mg/kg)	2 mg/kg	10	20
SQ 21,286 (25 mg/kg)	None	35	20
SQ 21,286 (25 mg/kg)	2 mg/kg	30	13
TIC-mustard (200 mg/kg)	None	70	7
TIC-mustard (200 mg/kg)	2 mg/kg	40	12
Uracil arabinoside (1,000 mg/kg)	None	10	0
Uracil arabinoside (1,000 mg/kg)	2 mg/kg	10	0

^a Male BALB/c mice were injected ip. Abbreviations: Poly(AU), polyadenylic polyuridylic acid; BCNU, 1,3 bis(2-chloroethyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; SQ 21,286, an octapeptide antibiotic; TIC-mustard, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide. The LPS was from *E. coli* 026:B6 (B); lethality was scored after 2 weeks.

TABLE 2. Effect of sequence of administration on the enhanced lethality of TIC-mustard with lipopolysaccharide^a

TIC-mustard	LPS (day 0)	No. of mice	% Dead after 4 days
200 mg/kg	None	70	7
400 mg/kg	None	12	100
None	2 mg/kg	40	5
200 mg/kg (day 0)	2 mg/kg	40	12
200 mg/kg (day -1)	2 mg/kg	20	10
200 mg/kg (day +1)	2 mg/kg	40	100

^a Male BALB/c mice were injected ip at the time indicated. TIC-mustard, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide. The LPS was from *E. coli* 026:B6 (B).

dead of 10 treated with either agent alone).

TIC-mustard, administered 24 h after concanavalin A, markedly enhanced mouse lethality

ity (Table 3). None of the other test drugs interacted in vivo with concanavalin A to enhance mouse lethality.

The combination of mithramycin and LPS was selected for further study. Death due to mithramycin alone, LPS alone, or the combination usually occurred within 24 h after challenge. The mean lethal dose on day 4 for mithramycin alone was 1.7 mg/kg (95% confidence limits, 1.6 to 1.9), and for *E. coli* 026:B6 Boivin LPS was 11.2 mg/kg (95% confidence limits, 9.9 to 13.4). Administration of mithramycin with 2 mg of LPS per kg resulted in a 19-fold reduction in the mean lethal dose of the drug on day 4 (Fig. 1). Administration of LPS with 0.25 mg of mithramycin per kg resulted in a 100-fold reduction in the mean lethal dose of LPS on day 5 (Fig. 2). The mean lethal dose of mice administered 0.5 mg of mithramycin per kg was less than 10 μ g of LPS per kg and that for mice administered 1 mg of mithramycin per kg was less than 1 μ g of LPS per kg. The dose-effect curves for the combinations were parallel to those for mithramycin alone and LPS alone, indicating that the lethal action of the combination did not involve a new toxic mechanism. An isobologram based upon the 50% lethal doses of combinations of mithramycin and LPS established that this in vivo interaction was markedly synergistic (Fig. 3).

TABLE 3. Ability of concanavalin A to enhance the lethal action of selected drugs^a

Drug (day 1)	Concavalin A (day 0)	No. of mice	% Dead by day 14
BCNU (40 mg/kg)	None	10	0
BCNU (40 mg/kg)	5 mg/kg	10	10
CCNU (200 mg/kg)	None	10	0
CCNU (200 mg/kg)	5 mg/kg	10	10
Cytosine arabinoside (1,000 mg/kg)	None	10	0
Cytosine arabinoside (1,000 mg/kg)	5 mg/kg	10	0
Hydroxyurea (4,000 mg/kg)	None	10	0
Hydroxyurea (4,000 mg/kg)	5 mg/kg	10	0
Mithramycin (0.5 mg/kg)	None	10	0
Mithramycin (0.5 mg/kg)	5 mg/kg	10	0
Procarbazine (500 mg/kg)	None	10	0
Procarbazine (500 mg/kg)	5 mg/kg	10	0
SQ 21,286 (25 mg/kg)	None	10	0
SQ 21,286 (25 mg/kg)	5 mg/kg	10	0
TIC-mustard (200 mg/kg)	None	20	0
TIC-mustard (200 mg/kg)	5 mg/kg	20	55
Vincristine (1 mg/kg)	None	20	0
Vincristine (1 mg/kg)	5 mg/kg	20	0

^a Male BALB/c mice were injected ip at the time indicated.

FIG. 1. Potentiation of mithramycin toxicity for mice by *E. coli* LPS. The lethal responses of BALB/c mice to various doses of mithramycin with no LPS (●), 0.1 mg of LPS per kg (◐) or 2 mg of LPS per kg (○) are plotted on a probit scale.

FIG. 2. Potentiation of *E. coli* LPS toxicity for mice by mithramycin. The lethal responses of BALB/c mice to various doses of LPS with no mithramycin (●), 0.1 mg of mithramycin per kg (◐), or 0.25 mg of mithramycin per kg (○) are plotted on a probit scale.

Enhanced lethality of combinations of mithramycin and LPS occurred when the two agents were administered simultaneously but not when 24 h separated the two challenges (Table 4). The enhanced lethality of combinations of mithramycin and LPS was not affected by the route of administration of mithramycin. LPS given ip or intravenous (iv) rendered the mice hyperreactive to mithramycin administered ip or iv (Table 5).

The capability of five different preparations of bacterial LPS to interact in vivo with mi-

FIG. 3. Synergistic lethality in mice of combinations of mithramycin with *E. coli* LPS. The solid line connects mean lethal dose values for various combinations; the dashed line indicates the response expected for additive effects.

TABLE 4. Effect of sequence of administration on the enhanced lethality of mithramycin with LPS^a

Mithramycin (0.5 mg/kg)	LPS (day 0)	No. of mice	% Dead by day 4
Day 0	None	20	5
None	10 µg/kg	10	0
Day 0	10 µg/kg	20	80
Day -1	10 µg/kg	24	0
Day +1	10 µg/kg	20	0

^a Male BALB/c mice were injected ip at the time indicated. The LPS was from *E. coli* 026:B6 (B).

TABLE 5. Effect of route of administration on the enhanced lethality of mithramycin and LPS^a

Mithramycin		Route for LPS (0.1 mg/kg)	No. of mice	% Dead by day 4
Dose	Route			
0.5 mg/kg	ip	iv	10	100
0.5 mg/kg	ip	ip	10	100
0.5 mg/kg	iv	iv	10	100
0.5 mg/kg	iv	ip	10	100

^a Male BALB/c mice were injected with mithramycin and *E. coli* 026:B6 (Boivin) LPS simultaneously by the routes indicated. None of these agents alone, at the routes and doses indicated, killed any of the treated mice.

thramycin was tested. Four of the preparations were from *E. coli*; two of these were prepared by the Boivin technique. The other three LPS preparations were obtained by the Westphal

technique. All five LPS preparations, given in combination with 0.5 mg of mithramycin per kg, resulted in enhanced lethality. LPS from *E. coli* 026:B6, whether obtained by the Boivin technique or the Westphal technique, was not as potent as LPS from *E. coli* 0127:B8 and *E. coli* 0111:B4 (Table 6).

Mice were administered *E. coli* 026:B6 (B) LPS daily for 4 days to render them resistant to a challenge of LPS that kills normal mice. Mice pretreated with LPS were significantly protected from the lethal action of mithramycin in combination with LPS (Table 7). Similarly, mice administered lipid A complexed to concanavalin A daily for 4 days were rendered

TABLE 6. Specificity of the *in vivo* lethal interaction between mithramycin and bacterial lipopolysaccharide^a

Mithramycin	LPS (0.1 mg/kg)	No. of mice	% Dead
0.5 mg/kg	None	30	0
0.5 mg/kg	<i>E. coli</i> 0127:B8 (B)	10	100
0.5 mg/kg	<i>E. coli</i> 026:B6 (B)	10	60
0.5 mg/kg	<i>E. coli</i> 026:B6 (W)	10	70
0.5 mg/kg	<i>E. coli</i> 0111:B4 (W)	10	100
0.5 mg/kg	<i>S. typhosa</i> 0901 (W)	10	100
0.25 mg/kg	<i>E. coli</i> 0127:B8 (B)	10	50
0.25 mg/kg	<i>E. coli</i> 026:B6 (B)	10	0
0.25 mg/kg	<i>E. coli</i> 026:B6 (W)	10	0
0.25 mg/kg	<i>E. coli</i> 0111:B4 (W)	10	100
0.25 mg/kg	<i>S. typhosa</i> 0901 (W)	10	100

^a Mithramycin and LPS were administered simultaneously by the ip route to male mice. (B) and (W) refer to Boivin and Westphal preparations, respectively. A dose of 1 mg of *E. coli* 026:B6 (B)/kg killed 1 of 10 treated mice; this dose of the other four LPS preparations did not kill any of the treated mice (10 mice per group). Lethality was scored on day 4.

TABLE 7. Effect of pretreatment with LPS on the enhanced lethality of mithramycin in combination with LPS^a

Mithramycin	LPS	No. of mice	% Dead	
			Normal	LPS-pretreated
1 mg/kg	None	10	10	0
2 mg/kg	None	10	70	40
1 mg/kg	0.01 mg/kg	10	100	0
0.1 mg/kg	2 mg/kg	10	50	0
None	8 mg/kg	10	40	0

^a Male BALB/c mice were injected ip; mice pretreated with *E. coli* LPS 026:B6 (B) were given 1 mg/kg on day -5, 2 mg/kg on days -4 and -3, and 4 mg/kg on day -2. The mice were challenged on day 0 and lethality was scored after 1 week.

resistant to a challenge of LPS or a combination of mithramycin and LPS or lipid A-concanavalin A complex (Table 8).

The mithramycin-treated mouse was used as an indicator of endotoxic activity in several commercial enzyme preparations and other biological materials. Of the four enzymes tested, only pronase contained endotoxic activity as assayed by both the mouse-lethality assay (Table 9) and by gelation of the *Limulus* amoebocyte lysate (E-TOXATE, Sigma). Of the five concanavalin A complexes tested, only two possessed endotoxic activity as assayed both by the mouse lethality assay and by gelation of *Limulus* amoebocyte lysate. The two complexes possessed several magnitudes less activity than LPS and the myristic acid-concanavalin A complex was less than one-tenth as active as the lipid A-concanavalin A complex (Table 9).

DISCUSSION

Of the 10 drugs and antibiotics examined in this study, six affect DNA synthesis, structure, or function (BCNU, CCNU, cytosine arabinoside, hydroxyurea, procarbazine, and TIC-mustard) (4, 15, 20). Mithramycin is a potent inhibitor of ribonucleic acid synthesis (21). Only mithramycin of these 10 agents interacts in vivo with LPS to enhance lethality markedly when administered in simultaneous combinations. TIC-mustard enhances lethality for mice when LPS is given before the antitumor drug. Accordingly, existence of synergistic toxicities involving an antitumor drug and LPS cannot be ruled out unless sequence of administration of the two agents is examined.

The diversity of the adverse effects of combinations of selected antitumor drugs and LPS may be a factor in the failure of physicians to clinically recognize synergistic toxicity as a

problem. Moreover, when physicians are dealing with a seriously ill patient, there is a tendency to explain puzzling symptoms or episodes as the manifestation of the patient's grave state of health. Because a wide assortment of gram-negative bacteria can serve as the LPS source (11) and because bacteremia and fevers of unknown etiology occur frequently in patients with neoplastic disease (9), the clinical investigator should be aware of the possible additional hazard associated with chemotherapy in patients with concurrent bacterial infections. Moreover, it is conceivable that a protocol

TABLE 9. Detection of endotoxin activity in diverse biological materials by using mithramycin-treated mice as the indicator^a

Test material	Dose	No. of mice	% Dead	<i>Limulus</i> assay
L-Asparaginase	10 mg/kg	10	0	—
Bovine serum albumin	5 mg/kg	10	0	—
Chymotrypsin	5 mg/kg	10	10	—
Concanavalin A, III	5 mg/kg	10	0	—
	50 mg/kg	10	20	±
Concanavalin A, IV	50 mg/kg	10	0	—
Pronase	5 mg/kg	10	20	±
	10 mg/kg	10	100	+
Trypsin	100 mg/kg	10	0	—
Lipid A-concanavalin A	5 mg/kg	10	100	+
Myristic acid-concanavalin A	50 mg/kg	10	20	+
Myristoyl choline-concanavalin A	50 mg/kg	10	0	—
Dimethyl myristamide-concanavalin A	50 mg/kg	10	0	—
Dimyristoyl lecithin-concanavalin A	50 mg/kg	6	0	—

^a Male BALB/c mice were injected ip with the indicated dose of the test material and 0.5 mg of mithramycin per kg. None of these agents alone at the doses indicated killed any of the treated mice. Lethality was scored on day 4. Gelation of the *Limulus* amoebocyte lysate (E-TOXATE, Sigma) is denoted by (+), increased viscosity by (±), and no change by (—).

TABLE 8. Effect of pretreatment with lipid A complexed to concanavalin A on the enhanced lethality of mithramycin in combination with LPS^a

Mithramycin	Endotoxin source	No. of mice	% Dead	
			Normal	Pretreated
1 mg/kg	None	10	0	0
None	Lipopolysaccharide (1 mg/kg)	10	0	0
None	Lipopolysaccharide (10 mg/kg)	10	70	0
0.5 mg/kg	Lipopolysaccharide (1 mg/kg)	10	100	0
0.5 mg/kg	Lipid A-concanavalin A (20 mg/kg)	10	100	0
None	Lipid A-concanavalin A (20 mg/kg)	10	0	0

^a Male BALB/c mice were injected ip; mice pretreated with a lipid A-concanavalin A complex were given 10 mg/kg on day -5, 20 mg/kg on days -4 and -3, and 40 mg/kg on day -2. The mice were challenged on day 0 and lethality was scored after 4 days. The LPS was from *E. coli* 026:B6 (Boivin preparation).

examining drug toxicity in animals treated with LPS may be more predictive of adverse effects in patients than a protocol examining drug toxicity in a variety of normal animals.

Death of mice given TIC-mustard and LPS or concanavalin A at doses that are sublethal individually is probably due to the toxic action of TIC-mustard. LPS or concanavalin A may cause lymphoid cells to become more uniformly susceptible to the lethal action of the alkylating agent (6).

Death of mice given mithramycin and LPS at doses that are sublethal individually is probably due to the toxic action of LPS. In general, inhibitors of protein or ribonucleic acid synthesis render mice hyperreactive to LPS (18). Bates and Margolin (1) have established that LPS induces potentially lethal renal failure in the mouse.

It has been demonstrated that lipid A is the component of LPS responsible for mitogenicity (13) and pyrogenicity (8). In addition, we suggest that lipid A is responsible for the synergistic interaction of LPS and antitumor drugs.

The mithramycin-treated mouse is a sensitive indicator for endotoxic activity in complex materials. The specificity of the lethal effect can be assessed by rendering the mice resistant to LPS by pretreatment. The mithramycin-treated mouse is comparable to the actinomycin D-treated mouse (5, 13) for detecting minute amounts of endotoxin. We were able to detect endotoxic activity in a complex of concanavalin A and myristic acid by using the mithramycin-treated mouse as the indicator. It is not known whether this endotoxic activity is due to contamination of the myristic acid or to reconstitution of a legitimate active site of the endotoxin molecule. Rosenstreich et al. (17) have demonstrated that *N*-palmitoyl D-glucosamine possesses at least one attribute of endotoxin, mitogenicity for B-cells. A study of the biological properties of fatty acids complexed to a protein carrier may make it possible to develop structure-function relationships for bacterial LPS.

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LITERATURE CITED

1. Bates, H. M., and S. Margolin. 1968. Endotoxin-induced inhibition of renal function in the mouse. *Proc. Soc. Exp. Biol. Med.* **129**:642-646.
2. Bradley, S. G. 1974. Enhanced toxicity for mice of combinations of *Escherichia coli* lipopolysaccharide with antitumor drugs. *Dev. Ind. Microbiol.* **15**:368-376.
3. Bradley, S. G., N. M. Marecki, J. S. Bond, A. E. Munson, and D. T. John. 1974. Enhanced cytotoxicity in mice of combinations of concanavalin A and selected antitumor drugs, p. 291-307. *In* T. K. Chowdhury and A. K. Weiss (ed.), *Concanavalin A*. Plenum Publishing Corp., New York.
4. Dawson, M. 1972. Cellular pharmacology, p. 317. Charles C Thomas, Springfield, Ill.
5. Dowling, J. N., and H. A. Feldman. 1970. Quantitative biological assay of bacterial endotoxins. *Proc. Soc. Exp. Biol. Med.* **134**:861-864.
6. Eaves, A. C., and W. R. Bruce. 1972. Endotoxin-induced sensitivity of hematopoietic stem cells to chemotherapeutic agents. *Ser. Haematol.* **5**:64-72.
7. Galanos, C., O. Lüderitz, and O. Westphal. 1969. A new method for the extraction of R lipopolysaccharides. *Eur. J. Biochem.* **9**:245-249.
8. Galanos, C., E. T. Reitschel, O. Lüderitz, O. Westphal, Y. B. Kim, and D. W. Watson. 1972. Biological activities of lipid A complexed with bovine serum albumin. *Eur. J. Biochem.* **31**:230-233.
9. Klastersky, J., D. Daneau, and A. Verherst. 1972. Causes of death in patients with cancer. *Eur. J. Cancer* **8**:149-154.
10. Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**:99-113.
11. Marecki, N. M., and S. G. Bradley. 1973. Enhanced toxicity for mice of combinations of bacterial endotoxin with antitumor drugs. *Antimicrob. Agents Chemother.* **3**:599-606.
12. Meyers, E., F. E. Pansy, H. I. Basch, R. J. McRipley, D. S. Slusarchyk, S. F. Graham, and W. H. Trejo. 1973. EM49 a new peptide antibiotic. III. Biological characterization *in vitro* and *in vivo*. *J. Antibiot.* **26**:457-462.
13. Peavy, D. L., J. W. Shands, Jr., W. H. Adler, and R. T. Smith. 1973. Mitogenicity of bacterial endotoxins: characterization of the mitogenic principle. *J. Immunol.* **111**:352-357.
14. Pieroni, R. E., E. J. Broderick, A. Bundeally, and L. Levine. 1970. A simple method for the quantitation of submicrogram amounts of bacterial endotoxin. *Proc. Soc. Exp. Biol. Med.* **133**:790-794.
15. Rose, W. C. 1973. Interaction of bacterial toxins in the toxicity of chemotherapeutic agents. *CRC Crit. Rev. Toxicol.* **3**:159-209.
16. Rose, W. C., S. G. Bradley, and I. P. Lee. 1972. Potentiation of the toxicity of several antitumor agents by *Salmonella typhosa* endotoxin. *Toxicol. Appl. Pharmacol.* **23**:102-111.
17. Rosenstreich, D. L., J. Asselineau, S. E. Mergenhagen, and A. Nowotny. 1974. A synthetic glycolipid with B-cell mitogenic activity. *J. Exp. Med.* **140**:1404-1409.
18. Seyberth, H. W., H. Schmidt-Gayk, and E. Hackenthal. 1972. Toxicity clearance and distribution of endotoxin in mice as influenced by actinomycin D, cycloheximide, α -amanitin and lead acetate. *Toxicon* **10**:491-500.
19. Waravdekar, V. S., and L. D. Saslaw. 1959. A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde-thiobarbiturate acid reaction. *J. Biol. Chem.* **234**:1945-1950.
20. Yarbro, J. W., B. J. Kennedy, and C. P. Barnum. 1965. Hydroxyurea. Inhibition of DNA synthesis in ascites tumor. *Proc. Nat. Acad. Sci. U.S.A.* **53**:1033-1035.
21. Yarbro, J. W., B. J. Kennedy, and C. P. Barnum. 1966. Mithramycin. Inhibitor of ribonucleic acid synthesis. *Cancer Res.* **26**:36-39.