(Fig. 1), but maintained normal pressure during the second phase of shock. The effect of phenylbutazone in this respect was similar to that of 20 mg/kg indomethacin.

The use of nonsteroidal anti-inflammatory drugs has a beneficial effect in various forms of experimental shock(1,2,12), but their mode of action in the area of the splanchnic circulation may be different.

Summary. Pretreatment of dogs with 20 mg/kg of indomethacin blocked some of the hemodynamic effects of endotoxin. In the treated animals i.v. injection of the LD_{80} dose of endotoxin (*E. coli*) caused a significantly smaller transient fall in the first phase of shock than in the control group. After the initial brief fall, the arterial blood pressure stayed at normal level for the duration of the experiment in dogs that received indomethacin. This was in contrast to the characteristic decline of arterial pressure in the untreated dogs in the second phase of shock. Administration of indomethacin lowered the rectal temperature and portal venous pressure of dogs. The results were discussed and compared to those obtained with other anti-inflammatory agents.

The assistance of Mr. L. L. Tague and Mr. W. J. Graham is greatly appreciated. Indomethacin was donated by Drs. C. G. Van Arman and K. C. Mezey of Merck and Co. Inc., kallikrein by Professor E. Werle.

1. Massion, W. H., Erdös, E. G., J. Oklahoma State Med. Assn., 1966, v59, 467.
2. Hinshaw, L. B., Solomon, L. A., Erdös, E. G., Reins, D. A., Gunter, B. J., J. Pharmacol. Exp. Therap., in press.
3. Northover, B. J., Subramanian, G., J. Path. Bact., 1962, v83, 463.
4. Garattini, S., Dukes, M. N. G., ed., Nonsteroidal anti-inflammatory drugs. 1964 Excerpta Med., Amsterdam.
5. Winter, C. A., Risley, E. A., Nuss, G. W., J. Pharm. Exp. Therap., 1963, v141, 369.
6. MacLean, L. D., Weil, M. H., Circulation Res., 1956, v4, 546.
7. Gilbert, R. P., Physiol. Rev., 1960, v40, 245.
8. Hinshaw, L. B., Emerson, T. E., Jr., Iampietro, P. F., Brake, C. M., Am. J. Physiol., 1962, v203, 600.
9. Erdös, E. G., Adv. Pharmacol., 1966, v4, 1.
10. Erdös, E. G., Back, N., Sicuteri, F., ed., Hypotensive Peptides. Springer-Verlag, New York, 1966, pp1-660.
11. Meyer, A., Werle, E., in Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des exokrinen Pankreas, Heinkel, K., Schön, H., eds., Schattauer-Verlag, Stuttgart, 1964, 299.
12. Lecomte, J., Acta Allergol., 1959, v14, 408.

Received March 20, 1967. P.S.E.B.M., 1967, v125.

Methyl 5 (or 4)-(3,3-Dimethyl-1-Triazeno)imidazole-4 (or 5)-Carboxylate (NSC 87982): Mouse Tissue Concentrations as Determined by Microbiological Assay.* (32240)

DALE E. HUNT AND ROBERT F. PITTILLO
Kettering-Meyer Laboratories (Affiliated with Sloan-Kettering Institute for Cancer Research, New York), Southern Research Institute, Birmingham, Ala.

Several triazenoimidazoles have been shown to have antitumor activity in experimental neoplasms(1,2). Although lacking noteworthy antitumor activity, a new member of this group of compounds, methyl 5 (or 4)-(3,3-dimethyl-1-triazeno)imidazole-4 (or 5)-carboxylate (designated NSC 87982 by the Cancer Chemotherapy National Service Center, National Cancer Institute), has been reported to have broad spectrum antimicrobial activity *in vitro*, and therapeutic activity against experimental infections of *Staphylococcus aureus* in mice has been demonstrated(3,4). The knowledge that this compound is being considered for clinical trial prompted the development of an assay procedure applicable to the determination of concentrations

* This work was supported by the Cancer Chemotherapy National Service Center, Nat. Cancer Inst., under Nat. Inst. Health Contract PH43-65-594.

of NSC 87982 in the tissues of mice and other biological materials.

Materials and methods. To find an appropriate assay microorganism, a large and diverse spectrum (over 300 species) of microorganisms, including bacteria, yeasts, filamentous fungi, and algae, was tested for sensitivity to NSC 87982 using previously described cultural procedures and methods(5).

A strain of *Escherichia coli* ATCC 11303, >10-fold resistant to 8-azaguanine and designated *E. coli*/AZG, was selected as the assay microorganism. It was maintained on chemically defined agar slants (1.5% agar, 0.1% NH_4Cl, 0.73% K_2HPO_4, 0.3% KH_2PO_4, 0.012% $MgSO_4$, and 0.4% glucose) containing 1 mg 8-azaguanine/ml of medium. For inoculation of assay plates, broth cultures of *E. coli*/AZG were grown for 18 hours at 37°C in the glucose-salts medium containing 1 mg azaguanine/ml. Cells from this culture were washed twice by centrifugation in 0.85% NaCl (saline), resuspended in saline, and adjusted to 20% light transmittance in a Bausch and Lomb Spectronic 20 Colorimeter equipped with a 660 mμ interference filter. Such suspensions contain approximately 1.0×10^8 cells/ml. This suspension was diluted 1:100 with saline, and 10 ml of this diluted suspension was added to one liter of cooled (50-55°C) glucose-salts agar medium. Five ml of the inoculated medium was added to petri dishes containing 8 ml of congealed sterile medium.

A stock solution of NSC 87982 was prepared in sterile saline. This was appropriately diluted so that when 0.08 ml of the various dilutions was added to filter paper discs (1.27 cm diameter, No. 740-E, Schleicher and Schuell Co., Keene, N. H.) the following concentrations were obtained (μg NSC 87982/disc): 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, and 0.06.

An additional stock solution of NSC 87982 was prepared in which the drug was dissolved in mouse blood and diluted with saline. A third stock solution was prepared in which the compound was dissolved in and diluted with modified Eagle's medium(6). The filter-paper discs were impregnated with 0.08 ml of the various solutions containing graded concentrations of drug. These discs were placed on the surface of each seeded agar plate and pressed down securely with flamed forceps. All plates and drug concentrations/disc were prepared in triplicate. Each individual plate contained a maximum of 3 discs: 2 discs contained individually either experimental samples or standard curve solutions of different concentrations. A third control disc, containing an empirically selected concentration (0.2 μg NSC 87982/disc) of drug, was added to each plate to allow for correction of plate-to-plate variation in zone sizes. All plates were incubated at 37°C for 18-20 hours simultaneously with plates which contained discs impregnated with undiluted, 2-fold and 4-fold dilutions of blood or saline homogenates of tissues from animals which received NSC 87982. Tissue homogenates were prepared by grinding weighed portions (approximately 1 g) of the respective tissues in Teflon pestle tissue grinders (A. H. Thomas Co., Philadelphia, Pa.) containing 2 ml saline. The resulting zones of inhibition on the triplicate standard plates were measured and correction factors applied where necessary as previously described(7). A standard curve was constructed by plotting the corrected mean diameters of these zones against drug concentrations on semi-logarithmic paper. A straight line was constructed through the points thus obtained by the method of least squares. To determine the concentration of NSC 87982 in the blood of mice following constant perfusion, intraperitoneal (i.p.), intravenous (i.v.), or *per os* (p.o.) administration of drug, BDF_1 mice (mixed sexes, 18-22 g) were used. Single LD_{10} or ½ LD_{10} doses of drug were administered in 0.2 ml of saline. Samples of blood from treated animals were obtained by repeated cardiac puncture in anesthetized mice or replicate entry into the postorbital sinus.

Results and discussion. From the initial screening for an appropriate assay microorganism, *E. coli*/AZG was selected because of its sensitivity to the inhibitor, dosage response, and rapid growth rate. Fig. 1 shows a representative, standard assay curve obtained with NSC 87982 dissolved in heparinized mouse blood. As little as 0.06 μg of

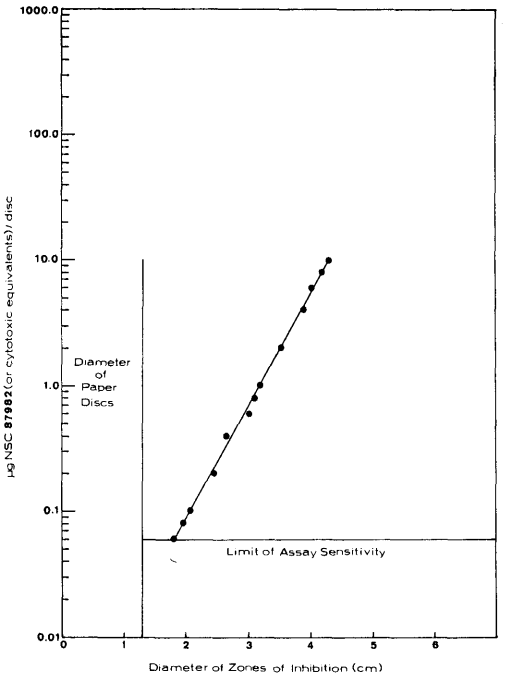

FIG. 1. Standard inhibition curve for microbiological assay of methyl 5 (or 4)-(3, 3-dimethyl-1-triazeno) imidazole-4 (or 5)-carboxylate (NSC 87982) in the blood of mice. Assay microorganism: *Escherichia coli* ATCC 11303 >10-fold resistant to 8-azaguanine. Standard curve: Constructed by method of least squares. Index of precision: 0.038.

NSC 87982/disc (0.75 μg/ml) could be detected and a linear dose response was obtained. Essentially identical results were obtained when saline or modified Eagle's medium was used as a diluent. The index of precision (λ) of the curve is 0.038 (λ = standard deviation of the points on the curve/slope of assay curve in millimeters between any two points which represent a 10-fold difference in the concentration of the assay compound), which indicates a precise assay technique(8).

The utility of this assay is revealed in Figs. 2 and 3 which show the concentrations of NSC 87982 (or cytotoxic equivalents) detected in the blood and other tissues of mice which were treated with this compound.

The marked sensitivity of *E. coli*/AZG to NSC 87982 currently cannot be explained. Azaguanine is known to inhibit the synthesis of purines(9) and protein(10). NSC 87982 also inhibits the synthesis of protein;† however, it is difficult to correlate these observations with the sensitivity of *E. coli*/AZG to NSC 87982. Nevertheless, a precise assay has been developed and its utility demonstrated for determining concentrations of NSC 87982 (or cytotoxic equivalents) in the tissues of mice.

† Unpublished observations.

Summary. Using a strain of *Escherichia coli* ATCC 9637 resistant to 8-azaguanine, a microbiological assay has been developed for a new broad spectrum antimicrobial compound, methyl 5(or 4)-(3,3-dimethyl-1-tri-

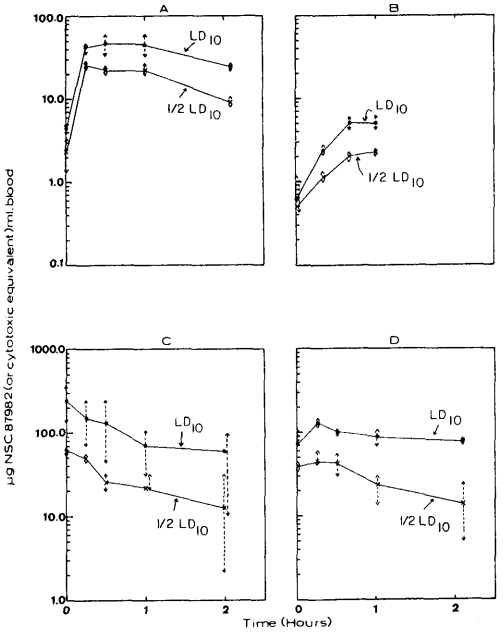

FIG. 2. Concentrations of methyl 5 (or 4)-(3, 3-dimethyl-1-triazeno) imidazole-4 (or 5)-carboxylate (NSC 87982) detected in the blood of mice by microbiological assay following administration of this drug by various routes. Assay microorganism: *Escherichia coli* ATCC 11303>10-fold resistant to 8-azaguanine. **A**. Route of drug administration: *Per Os*. Samples of blood: Obtained by replicate cardiac puncture. Doses of drug: LD_{10} single dose (198 mg/kg) and ½ LD_{10} (99 mg/kg). **B**. Route of drug administration: Constant perfusion intraperitoneally. Samples of blood: Obtained by replicate entry into postorbital sinus. Doses of drug: 3.3 mg/kg/min and 1.65 mg/kg/min (equivalent to a single LD_{10} and ½ LD_{10}, respectively, after 60 min perfusion with drug). **C**. Route of drug administration: Intravenous. Samples of blood: Obtained by replicate cardiac puncture. Doses of drug: LD_{10} single dose (198 mg/kg) and ½ LD_{10} (99 mg/kg). **D**. Route of drug administration: Intraperitoneal. Samples of blood: Obtained by replicate cardiac puncture. Doses of drug: LD_{10} single dose (198 mg/kg) and ½ LD_{10} (99 mg/kg).

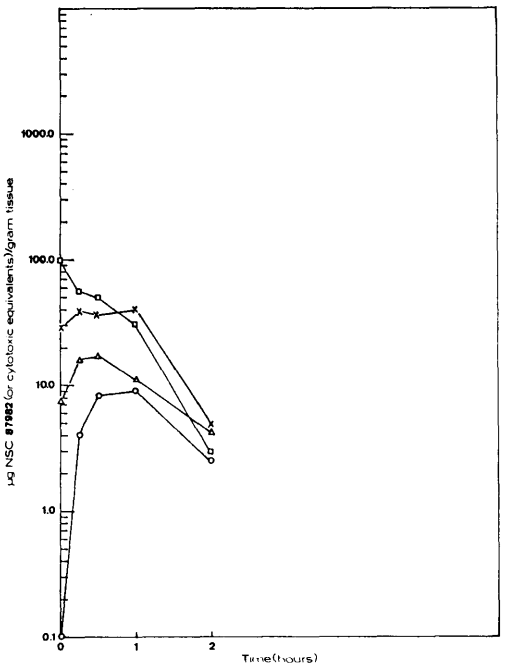

FIG. 3. Concentrations of methyl 5 (or 4)-(3, 3-dimethyl-1-triazeno) imidazole-4 (or 5)-carboxylate (NSC 87982) detected in various tissues of mice by microbiological assay following single LD_{10} (198 mg/kg) intraperitoneal dose of drug. Each point represents the levels of NSC 87982 detected in the pooled tissues from 10 mice. □ = Liver, × = Kidney, △ = Lung, ○ = Brain.

azeno)-imidazole-4(or 5)-carboxylate (NSC 87982). As little as 0.75 μg of NSC 87982/ml of sample can be detected. The applicability of this assay has been demonstrated for the estimation of concentrations of this drug in various tissues of mice.

The technical assistance of Mrs. C. Woolley, Miss B. G. Tate, Mrs. E. Wyatt, Mrs. B. Cummings, and Mrs. M. McCarty is gratefully acknowledged. Generous amounts of NSC 87982 were supplied by Dr. Y. Fulmer Shealy.

1. Shealy, Y. F., Montgomery, J. A., Laster, W. R., Jr., Biochem. Pharmacol., 1962, v11, 674.
2. Shealy, Y. F., Krauth, C. A., Nature, 1966, v210, 208.
3. Shealy, Y. F., Krauth, C. A., Hunt, D. E., Pittillo, R. F., J. Pharmaceut. Sci., 1967, v561, 147.
4. Pittillo, R. F., Hunt, D. E., Appl. Microbiol., in press.
5. Hunt, D. E., Pittillo, R. F., Antimicrobial Agents & Chemother., 1965, 1966, 710.
6. Dixon, G. J., Dulmadge, E. A., Schabel, F. M., Jr., Cancer Chemother. Rep., 1966, v50, 247.
7. Pittillo, R. F., Hunt, D. E., Proc. Soc. Exp. Biol. & Med., 1967, v124, 217.
8. Bliss, C. I., The Statistics of Bioassay, Academic Press, Inc., New York, 1952, 472.
9. Brockman, R. W., Bennett, L. L., Jr., Simpson, M. S., Wilson, A. R., Thompson, J. R., Skipper, H. E., Cancer Reseach, 1959, v19, 856.
10. Chantrenne, H., Devreux, S., Nature, 1958, v181, 1737.

Received March 23, 1967. P.S.E.B.M., 1967, v125.

Hamster Hyperimmune Ascites as a Source of Antibody. (32241)

WILLIAM S. COLLINS, II (Introduced by S. B. Formal)
Department of Viral and Rickettsial Diseases, 406th Medical Laboratory, U. S. Army Medical Command, Japan

Ascitic fluid from immunized hamsters as a source of high titer antibody has been reported(1,2). Ascites from immunized mice are extensively used as a source of antibody (3-6) and have been examined in some detail. Hamsters offer several advantages over mice; they are larger, easier to handle, and offer a possibility of greater volumes of ascites per animal. Where the hamster is the animal of choice for virus isolation and propagation, the use of hamster ascitic fluid offers a source of large volumes of homologous antibody.

Success in eliciting ascites production in hamsters has not been uniform in this laboratory. Numerous personal communications have indicated that other investigators have had the same problems. This report is concerned with factors affecting ascites production, antibody content of Syrian hamster ascites and possible causes of observed phenomena due to extensive cross reactions be-