mately 55-106% LD 50/30 acute dose and the similar results in dogs(17) are in distinct contrast to the results in mice in which similar doses have produced drastic effects on fertility.

Two hundred and six 12-week-old swine from non-irradiated parents, from irradiated parents, and from an irradiated boar and non-irradiated sows showed no significant difference in sensitivity to X-rays as measured by 30-day lethality. Lethality was essentially identical for each group as was survival time, hematological response, weight change, clinical signs of radiation sickness and gross changes at autopsy.

Summary. LD 50/30 values for 12-week-old offspring from non-irradiated swine, from irradiated swine, and from an irradiated boar and non-irradiated sows were not significantly different. Survival times for progeny of the different parent categories were not significantly different and were within the usual range for midlethal irradiation. Other responses were similar in each group.

1. Miller, R. W., *Pediatrics,* 1956, v18, 1.

2. Neel, J. V., Schull, W. J., Nat. Acad. Sci., Nat. Research Council, Washington, D. C., 1956.

3. Rekers, P. E., *J. Lab. Clin. Med.,* 1951, v37, 331.

4. Spalding, J. F., Hawkins, S. B., Strang, V. G., *Radiation Research,* 1958, v8, 222.

5. ——— , *ibid.,* 1958, v9, 369.

6. Russell, W. L., Proc. Nat. Acad. Sci., 1957, v43, 324.

7. McGregor, J. F., James, A. P., Newcombe, H. B., *Radiation Research,* 1960, v12, 61.

8. Woodward, K. T., McDonnel, G. M., Harris, P. S., Kirkland, W. J., Shively, J. N., *Am. J. Roent. Rad. Ther. Nuc. Med.,* 1961, v85, 179.

9. McDonnel, G. M., Claypool, H. A., Moncrief, W. H., Goldstein, J. D., Project 4-1, ITR-1428, AEC Technical Information Service, Oak Ridge, Tenn.

10. Andrews, H. L., *Radiation Research,* 1958, v9, 469.

11. Finney, D. J., Probit Analysis. 2 ed., Cambridge Univ. Press, 1952.

12. Blair, H. A., Univ. Rochester Rep. UR-442, 1956.

13. Hursh, J. B., Van Slyke, F., Casarett, G. W., Univ. Rochester Rep. UR-318, 1954.

14. Hursh, J. B., Noonan, T. R., Casarett, G. W., Van Slyke, F., *Am. J. Roent. Rad. Ther. Nucl. Med.,* 1955, v 74, 130.

15. Sacher, G. A., Argonne Nat. Lab. Rep., ANL-4163, 1948, 83.

16. Storer, J. B., *Radiation Research,* 1959, v10, 180.

17. Anderson, A. C., Schultz, F. T., *ibid.,* 1960, v12, 417.

Received October 11, 1960. P.S.E.B.M., 1961, v107.

Interaction of Histamine, Serotonin and Heparin with Hexadimethrine Bromide, a Mast Cell Fragmentor. (26521)

EUGENE T. KIMURA, PATRICK R. YOUNG AND RICHARD K. RICHARDS
Division of Experimental Therapy, Abbott Laboratories, North Chicago, Ill.

We reported(1,2) that hexadimethrine bromide (Polybrene*), an anti-heparin agent, caused disruption of tissue mast cells in the rat both *in vitro* and *in vivo*. It was suggested that histamine, serotonin and heparin, presumably released from these fragmented tissue mast cells, might perhaps be factors contributing to the symptomatology of this polymer following its injection in large doses.

* 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide, Abbott.

This present study was undertaken to explore further the possible role of these mast cell components as they affect the toxicologic response to this anti-heparin agent in mice, rats and guinea pigs. To this end, experiments were run to ascertain whether depletion or accumulation of either or both of these amines by prior pretreatment with suitable agents could alter the subsequent response of the animals to a lethal dose of this polymer.

Methods. To deplete most of the tissues of the rat of their histamine, 5 injections of

polymyxin B sulfate† were given intraperitoneally over a 3-day period according to the method of Parratt and West(3). Animals weighing between 150-180 g were injected with a single dose of 2.5 mg/kg of polymyxin B on the 1st day, 2 doses of 5 mg/kg on the 2nd day and 2 doses of 7.5 mg/kg on the 3rd day. On the fourth day, the animals were divided into groups of 10 and injected intravenously with various dose levels of hexadimethrine. Controls were run concurrently on saline treated rats here and in all subsequent tests; all survivors were observed for 1-2 weeks.

An intraperitoneal injection of reserpine, 10 mg/kg, was given to rats (160-180 g) to deplete most of their tissues of their serotonin (4). Hexadimethrine in graded doses was given to groups of these rats 24 hours after reserpinization in order to establish its LD_{50} following this pretreatment.

Rats weighing between 90-110 g were injected intraperitoneally twice daily for 5 days with compound 48/80 to deplete the tissue mast cells of the rat of their histamine, heparin and possibly serotonin(4,5). The initial dose on the 1st day was 100 μg/rat; the doses were then increased by increments of 100 μg each day to reach a dose of 500 μg by the 5th day. On the 6th day, the animals were divided into groups of 10 and intravenous LD_{50} determined for hexadimethrine.

To simulate, in part, the pharmacologic responses of the animals toward 48/80, groups of rats were pretreated with both polymyxin B sulfate and reserpine prior to hexadimethrine. Pretreatment regimens for both drugs were as described previously, except that one less injection of polymyxin was given to these animals.

To test the possibility of histamine potentiation by hexadimethrine, groups of mice weighing between 20-22 g were pretreated for 1 hour with either 2 or 6 mM/kg of imidazole, subcutaneously. Animals were then divided into groups of 20 and given hexadimethrine, intraperitoneally, to establish its comparative LD_{50} following this pretreatment regimen to inhibit diamine oxidase, *i.e.*, histaminase. Thus, several fixed doses of the inhibitor were pitted against the dose-mortality curve of hexadimethrine.

A similar pretreatment regimen was followed in mice using semicarbazide, another diamine oxidase inhibitor. Doses of 12.5 and 50 mg/kg were given subcutaneously to groups of mice 30 minutes prior to hexadimethrine given intraperitoneally at various dose levels.

As an ancillary study, a second series of mice was pretreated further with 10 and 25 mg/kg of chlorcyclizine hydrochloride, orally, 1 hour prior to imidazole. The rationale underlying this multi-pretreatment schedule stemmed from the hypothesis that the antihistaminic agent would perhaps counteract the histamine released by hexadimethrine and protected from inactivation by imidazole. Thus, a subsequent injection of hexadimethrine would perhaps be able to exert its effects without the histamine component entering the picture.

Using a specie more susceptible to histamine, groups of guinea pigs, 200-300 g, were pretreated, intraperitoneally, with 10 and 24 mg/kg of chlorcyclizine, 90 minutes prior to being pitted against the dose-mortality curve of hexadimethrine, intravenously. A second series of animals was pretreated for 4 hours with 15 mg/kg of the antihistaminic, intraperitoneally, then challenged with a lethal dose (22 mg/kg) of hexadimethrine, intravenously.

As an ancillary experiment, mice (20-22 g) in groups of 10 were pretreated intravenously with varying doses of either heparin sodium (150 U.S.P. units/mg) or with "inactivated" heparin‡ to compare their prophylactic potencies against the anti-heparin agent given at a fixed dose of 56 mg/kg, intravenously, equivalent to 2 $\times$ LD_{50}.

The molecular weight of the hexadimethrine used in this study was 6000.

Male Scientific strain mice and male Holtzman rats were used in all these studies. The

† Burroughs Wellcome & Co., Tuckahoe, N. Y.

‡ Batch #1582-82, "inactivated" by boiling in 0.08 N HCl for 3 hours and containing only 2.5-3 U.S.P. units/mg.

TABLE I. Effect of Depletion of Tissue Amines on Hexadimethrine Bromide Toxicity in Rats, I.V.

Pretreatment drugs*	Hexadimethrine bromide LD_{50} in mg/kg (95% C.L.) Controls (A)	Pretreated (B)	Toxicity ratio $= \frac{B}{A}$ (95% C.L.)
48/80	19.5 (17.4-21.8)	26.0 (22.0-30.0)	1.33† (1.11-1.60)
Polymyxin B	20.0 (17.2-23.2)	24.5 (20.4-29.4)	1.23 (.98-1.53)
Reserpine	20.0 (17.3-22.8)	23.0 (21.7-24.4)	1.15 (1.00-1.32)
Polymyxin B + reserpine	22.8 (20.5-25.3)	18.5 (14.5-23.7)	1.23‡ (.95-1.59)

* See text for dosage regimen. † Significantly different. ‡ Toxicity ratio of $\frac{A}{B}$.

method of Litchfield and Wilcoxon(6) was used for all statistical calculations.

Results. Table I shows that pretreatment of rats with either polymyxin B or with reserpine failed to alter significantly the subsequent LD_{50} values for hexadimethrine obtained in these same animals. Toxicity ratios were 1.23 and 1.15 for polymyxin B and for reserpine pretreatments, respectively. However, pretreatment with 48/80, which is reported to deplete both amines, significantly decreased the toxicity of hexadimethrine given subsequent to this prophylaxis. The intravenous LD_{50} for hexadimethrine controls in this series of experiments was 19.5 mg/kg while LD_{50} value for 48/80 pretreated animals given the anti-heparin agent was 26 mg/kg.

Concomitant pretreatment with polymyxin B and reserpine did not, however, simulate the prophylactic response obtained with 48/80 pretreatment alone. On the contrary, there was increased sensitivity towards hexadimethrine in these animals when it was given after the dual chemical assault by these 2 pretreatment agents. The LD_{50} value for hexadimethrine in this series of experiment was 22.8 mg/kg while that for the multi-pretreated group was 18.5 mg/kg. That this dual pretreatment regimen may have yet shown a subtle protective effect was inferred from several observations. There was absence of the characteristic edema of the extremities as induced by hexadimethrine; onset of death was delayed up to 7 days in the pretreated group while deaths occurred during the second through the fourth days in the control groups, albeit final mortality ratio was greater with the pretreatment.

The data in Table II show that subcutaneous pretreatment of mice with imidazole at a dose level of 2 mM/kg, equivalent to one-sixth of its LD_{50} (and to LD_0), did not materially affect hexadimethrine bromide toxicity. Increasing the pretreatment dose to 6 mM/kg, equivalent to one-half LD_{50} (and to $LD_{0.5}$), significantly increased the sensitivity of the animals toward a subsequent dose of the anti-heparin agent. Thus, control animals showed a LD_{50} value of 46 mg/kg while the LD_{50} value for imidazole pretreated mice was about one-half, *i.e.*, 22.5 mg/kg. Pretreatment of mice with semicarbazide, another diamine oxidase inhibitor, at dose levels of 12.5 and 50 mg/kg did not alter the dose mortality curve of hexadimethrine.

Pretreatment of mice with 10 and 25 mg/kg of chlorcyclizine, equivalent to about 1/25 and 1/10 of its LD_{50}, caused a slight antagonistic action towards the toxicogenic effect of imidazole on hexadimethrine. This was more evident with the lower dose of the antihistaminic wherein pretreatment with 10 mg/kg caused a slight decrease in the imidazole induced toxicity of hexadimethrine from a value of 22.5 mg/kg to 34 mg/kg. However, the magnitude of this protective effect was not pronounced.

Again, intraperitoneal pretreatment for a period of 90 minutes with 10 and 24 mg/kg of chlorcyclizine did not decrease hexadimethrine toxicity in guinea pigs. Rather, an indication of additive toxicity was evident at the higher dose of chlorcyclizine. Furthermore, intraperitoneal pretreatment for 4 hours with 15 mg/kg of chlorcyclizine had no protective effect against a lethal intravenous dose of hexadimethrine.

TABLE II. Effect of Imidazole and Chlorcyclizine Pretreatments on Hexadimethrine Bromide Toxicity.

Pretreatment drugs	mM/kg, s.c.	mg/kg	Hexadimethrine bromide LD_{50} in mg/kg (95% C.L.) Controls (A)	Pretreated (B)	Toxicity ratio $= \frac{A}{B}$ (95% C.L.)
A. In mice:				(Intraperitoneal)	
Imidazole	2		46.0 (42.8-49.5)	41.0 (37.3-45.1)	1.1 (1.0-1.3)
"	6*		46.0 (42.8-49.5)	22.5 (19.1-26.6)	2.0† (1.7-2.4)
Chlorcyclizine + imidazole	6	10-oral	48.0 (45.7-50.4)	34.0 (31.5-36.7)	1.4 (1.3-1.6)
Chlorcyclizine + imidazole	6	25-oral	48.0 (45.3-50.9)	25.0 (22.7-27.5)	1.9† (1.7-2.1)
B. In guinea pigs:				(Intravenous)	
Chlorcyclizine		10‡	18.8 (18.1-19.6)	18.5 (17.7-19.4)	1.0 (.9-1.1)
"		24	18.8 (18.1-19.6)	14.8 (13.4-16.3)	1.3 (1.2-1.4)

* Non-lethal *per se* and equivalent to about $LD_{0.5}$. LD_{50} and 95% C.L. of imidazole determined to be 11 mM/kg (10.2-11.8).

† Significantly different.

‡ Intraper. dose which protects against about 25 lethal doses of histamine, intravenously.

Table III shows that "inactivated" heparin, containing only 2.5-3 U.S.P. units/mg of heparin, was only half as potent as "regular" heparin sodium (150 U.S.P. units/mg) as a prophylactic agent against hexadimethrine given at a dose level of twice its LD_{50}.

None of the comparative LD_{50} slopes showed any significant deviation from parallelism (Tables I and II).

Discussion. The failure of combined pretreatment with polymyxin and reserpine to decrease hexadimethrine toxicity may be explained on the basis of additive toxicity from these 2 pretreatment drugs. Reserpinization of rats already subjected to polymyxin pretreatment caused a further debilitation in these animals. In contrast, pretreatment with a single drug depleting both amines, namely, 48/80, actually increases the resistance against hexadimethrine.

A relatively high dose of imidazole was required to exert significant effect on hexadimethrine toxicity (Table II). That the resultant toxicity ratio was only of the order of 2.0 would be in accord with previously documented findings that histamine does not play a major role in rodents as it does in some other species.

TABLE III. Comparative PD_{50} Values of Heparin Sodium and "Inactivated" Heparin *vs* 2 × LD_{50} of Hexadimethrine Bromide, I.V. (56 mg/kg) in Mice.

Pretreatment drug	Calculated PD_{50}, I.V., (mg/kg) *vs* 2 × LD_{50} hexadimethrine, I.V. (56 mg/kg)
Heparin sodium	52
"Inactivated" heparin	90

Results obtained with imidazole and semicarbazide pretreatment would tend to support the data of Angelakos and Loew(7) that of a large series of histaminase inhibitors, only imidazole was capable of significantly potentiating histamine toxicity in mice.

The finding that an antihistaminic was of little or no value against hexadimethrine toxicity in guinea pigs and mice substantiated our earlier findings(1,2) that pretreatment with antihistaminic agents was able only partially to alter the character of the hypotensive response to hexadimethrine in dogs. MacIntosh and Paton(8) have pointed out that although antihistaminic drugs are able to antagonize the circulatory effect of histamine liberators, their antagonism is only of a limited nature.

Previous experiments(4,9,10) have shown that a pretreatment regimen with 48/80, polymyxin and reserpine can deplete most of the tissues of the rat of their histamine and/or serotonin. Our previous reports(1,2) showed hexadimethrine capable of fragmenting tissue mast cells in the rat both *in vivo* and *in vitro*. If the symptomatology ob-

served in animals given high doses of this polymer is due, in part, to the action to these amines, then animals previously depleted of these amines would be less sensitive to the action of a drug which presumptively acted *via* this pathway. Such may be the case with hexadimethrine.

Summary. Animals partially depleted of their histamine and serotonin by pretreatment with 48/80 were rendered less sensitive to the toxic effects of hexadimethrine. Conversely, pretreatment of mice with imidazole increased the toxic effects of a subsequent injection of hexadimethrine. These results, coupled to our previous findings(1,2) suggest that some of the responses to hexadimethrine in certain laboratory animals may be mediated *via* histamine, serotonin and heparin release. It is probable that there are other factors which may be concerned in the over-all toxicologic and pharmacologic picture of this antiheparin agent.

1. Kimura, E. T., Young, P. R., Stein, R. J., Richards, R. K., *Tox. & Appl. Pharmacol.*, 1959, v1, 185.
2. Kimura, E. T., Young, P. R., Ebert, D. M., *ibid.*, 1959, v1, 560.
3. Parratt, J. R., West, G. B., *J. Physiol.*, 1957, v137, 179.
4. West, G. B., *Int. Arch. Allergy*, 1957, v10, 257.
5. Riley, J. F., West, G. B., *Arch. int. Pharmacodyn.*, 1955, v102, 304.
6. Litchfield, J. T., Jr., Wilcoxon, F., *J. Pharmacol. & Exp. Therap.*, 1949, v96, 99.
7. Angelakos, E. T., Loew, E. R., *ibid.*, 1957, v119, 444.
8. MacIntosh, F. C., Paton, W. D. M., *J. Physiol.*, 1949, v109, 190.
9. Telford, J. M., West, G. B., *J. Pharm. & Pharmacol.*, 1960, v12, 254.
10. Bhattacharya, B. K., Lewis, G. P., *Brit. J. Pharmacol.*, 1956, v11, 411.

Received December 5, 1960. P.S.E.B.M., 1961, v107.

Diphosphopyridine Nucleotide Binding Effect of Sickle Cell Erythrocytes On *in vitro* Growth of *Hemophilus influenzae.** (26522)

JOHN LOUIS SEVER† (Introduced by David Yi-Yung Hsia)
Chicago Children's Memorial Hospital and Department Microbiology, Northwestern University Medical School, Chicago, Ill.

The recent demonstration that erythrocytes from patients with sickle cell anemia fail adequately to support *in vitro* growth of certain hemophilic bacteria(1) suggests the existence of an unidentified inhibitor in these erythrocytes. The present investigations demonstrate and identify the inhibitory system.

Materials and methods. Blood from 8 patients with sickle cell anemia, 6 patients with sickle cell trait, and 5 normal controls was collected under sterile conditions in standard (A.C.D.) anticoagulant. The blood cells were immediately washed 3 times with sterile 0.85% saline solution. The washed cells were resuspended in physiological saline to a hematocrit value of 40% and incorporated in culture media.

Trypticase soy agar and broth (BBL) was employed for agar plates and broth cultures. Erythrocyte suspensions were added to the cultures in a concentration of 4%.

Lyophilized cultures from the American Type Culture Collection, Washington, D.C., were used: *Hemophilus influenzae* type *A* (9006); *H. influenzae* type *B* (9334); *Diplococcus pneumoniae* type *III* (6303); and *Streptococcus salivarius* (9756). Hospital laboratory cultures of *H. influenzae* type *A; H. influenzae* type *B*; *Salmonella montevideo*; *S. oranienberg*; and *S. bareilly* were also employed.

Inocula were prepared from 24 hour broth cultures. A single 0.04 ml drop of a 1:200 di-

* Aided by a grant from Otho S. A. Sprague Memorial Inst. Laboratories, Children's Memorial Hospital.

† Present address: Nat. Inst. Health, Bethesda, Md.