of the embryos and placentae was observed following administration of high doses of ethionine to pregnant rats. A similar dose of methionine had no apparent effect by itself while a combination of methionine with ethionine resulted in about a 50% reduction in the resorptive effect of ethionine. The sequential destruction of the tissues of placenta and embryo associated with the ethionine-induced resorption is described.

1. Waddington, C. H., Perry, M., *J. Embryol. Exp. Morph.*, 1958, v6, 365.

2. Herrmann, H., Konigsberg, U. R., Curry, M. F., *J. Exp. Zool.*, 1955, v128, 359.

3. Stekol, J. A., Weiss, K., *J. Biol. Chem.*, 1950, v185, 577.

4. Levine, M., Tarver, H., *ibid.*, 1951, v192, 835.

5. Koch-Weser, D., Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 34.

6. Stein, R. J., Kent, G., Barka, T., Popper, H., *ibid.*, 1960, v103, 210.

7. Lee, C. M., Wiseman, J. T., Kaplan, S. A., Warkany, J., *Arch. Path.*, 1955, v59, 232.

Received July 6, 1960. P.S.E.B.M., 1960, v105.

Adrenergic Inhibition and Lethal Effect of Bacterial Endotoxin in Mice. (26019)

R. A. MCLEAN AND L. JOE BERRY

Smith Kline and French Labs., Philadelphia, and Bryn Mawr College, Bryn Mawr, Pa.

Participation of epinephrine or related substances in the sequelae of bacterial endotoxin administration has been suggested(1-3). Adrenergic blocking agents such as phenoxybenzamine HCl have been used against traumatic shock with some success in laboratory animals, though with less in man. They have been largely ineffective against bacterial endotoxin. Recently several new ways of affecting sympathetic reactivity have been described. The following study tests the ability of some of the presently available types of adrenergic inhibition to alter lethal effects of injected epinephrine and of bacterial endotoxin administration.

Methods. Survival after intraperitoneal epinephrine was determined by the technic of Loew and Micetich(4) and results were evaluated by the method of Litchfield and Wilcoxon(5). Data from selected dose levels were used for comparison in Table I. High doses of epinephrine with low expected survivals were used when protection was anticipated and the reverse when sensitization was indicated. The significance of difference between results from these selected doses was judged by the Rank Correlation method of Wilcoxon(6).* Doses of synthetic l-epinephrine bitartrate were calculated as mg/kg free base. The blocking agents were given as salts, also on a mg/kg basis. Difco crystalline lipopolysaccharide from *E. coli* was used in the endotoxin studies. It was dissolved in pyrogen-free saline and given intraperitoneally on the basis of total mg/mouse (not/kg). Average weight of the male CF-1 mice used in this study was about 20 g. For reference purposes 2 SD_{50}, or 50% survival, doses were determined one hour after epinephrine in normal untreated male CF-1 mice. These were 8.0 (5.41-11.8) and 7.8 (6.45-9.44) mg/kg i.p. respectively. Another series received an intraperitoneal saline blank (0.1 cc/10 g of mouse) 24 hours before epinephrine. SD_{50} here was 7.6 (6.39-9.04) mg/kg i.p. Obviously, the susceptibility of the animals to epinephrine was essentially unchanged. We did find that mice were adversely affected if one intraperitoneal injection followed soon after another. For short term testing we used subcutaneous or oral premedication followed by intraperitoneal epinephrine.

Results. A. *Effects on lethal action of injected epinephrine.* It is recognized that lethal actions of toxic doses of epinephrine are very different from circulatory collapse and shock. However, as a preliminary step in our study we tested the ability of the several

* Kindly computed by Dr. Irving Sher of Smith Kline and French Labs.

TABLE I. Protective Effects of Various Forms of Adrenergic Inhibition against the Lethal Action of Injected Epinephrine.

Condition	Pretreatment	Dose, mg/kg	Pretreat. time, hr	Epin. i.p., mg/kg	1 hr survival living/total and % Test		Control*		Effect†
Peripheral adrenergic									
Excitation									
Block	Phenoxybenzamine	5 p.o.	1	12	10/10	100	2/10	20	+
Sensitization	Cocaine HCl	9 s.c.	1	4	8/20	40	19/20	95	—
Inhibition									
Block	3,4-dichloroisoproterenol	10 "	1	9	4/20	20	5/20	25	—
"Sympatholysis"	TM 10	10 "	1	12	4/20	20	2/10	20	0
	"	10 "	24	12	6/10	60	7/20	35	+
Catechol amine content									
Release	Reserpine	10 i.p.	24	12	7/10	70	7/20	35	+
Accumulation	Iproniazid	100 "	24	3	10/10	100	19/20	95	0

* Physiological saline blanks substituted for pretreatment with the adrenergic inhibitors.
† Effect: +, protected; —, more vulnerable; 0, no effect.

forms of adrenergic inhibition to oppose the acute toxicity of injected epinephrine.

The results of this first phase of our investigation are summarized in Table I. On the left are types of adrenergic influence which are thought to be produced and on the right, survival under these conditions. Saline blanks were run for control. The marked protection by phenoxybenzamine and reciprocal activity of cocaine parallel the well-known autonomic effects of similar doses of these drugs as demonstrated by blood pressure and nictitating membrane responses in anesthetized cats and dogs. Blockade of the peripheral adrenergic excitatory mechanism in the first case and sensitization in the second appear to be indicated. Site of action here is probably the vascular smooth muscle.

An opposite condition is produced by the 3, 4-dichloro analog of isoproterenol.† This substance has been shown to block inhibitory adrenergic responses(7). It also blocks the cardiac stimulant effect of adrenergic substances(8). This latter response is unaffected by blockers of excitatory adrenergic receptors, for example, by phenoxybenzamine. 3,4-Dichloroisoproterenol may well interfere with some of the metabolic processes initiated by epinephrine and which are related to vasodilation rather than vasoconstriction. Ten mg/kg of 3,4-dichloroisoproterenol were given subcutaneously to the mice one hour before the intraperitoneal epinephrine. From the results obtained in various species by the authors cited above such a procedure should establish the characteristic effect of this compound. It did not protect against 9 mg/kg of epinephrine. In fact, 25% of the control animals survived and only 20% of the treated animals were able to withstand the epinephrine. This difference is not significant in the rank correlation test. To check whether this difference was real, a larger number of mice were used and dose response curves were calculated for animals treated as described above and for parallel control groups dosed with saline. These lines were not parallel but this was not surprising. The ED_{50} for saline controls was 6.5 (5.0-8.4) mg/kg epinephrine i.p., while comparable figures for animals pretreated with 3,4-dichloroisoproterenol was 4.3 (2.9-6.3) mg/kg. This difference is significant ($p = 0.074$).* Clearly, pretreatment with this type of peripheral adrenergic blocker has reduced ability of the mice to survive acute lethal effects of injected epinephrine.

A unique type of adrenergic inhibition was recently described by Exley(9). 2,6-Xylyloxyethyltrimethylammonium bromide, or TM 10, was shown to produce some form of blockade of the terminal sympathetic nerve endings. An interference with enzymes on the metabolic pathway to epinephrine and norepinephrine has been suggested. Whatever the mode of action, the net result appears

† Generously supplied by Dr. I. H. Slater, Eli Lilly Co., Indianapolis, Ind.

TABLE II. Protective Effects of Various Forms of Adrenergic Inhibition against Lethal Action of Injected Endotoxin.

Condition	Pretreatment	Dose, mg/kg	Pretreat. time, hr	Lot No.*	48 hr SD_{50} of endotoxin, mg i.p.; values in parentheses are 95% confidence limits		Effect at p .05
					Test	Control†	
Peripheral adrenergic							
Excitation							
Block	Phenoxybenzamine	5 p.o.	1	β	.56 (.42-.74)	.47 (.39-.58)	0
Sensitization	Cocaine HCl	9 s.c.	1	γ	.38 (.32-.46)	.31 (.24-.40)	0 ?
Inhibition							
Block	3,4-dichloroisoproterenol	2 × 10 s.c.	1 & 18	γ	.55 (.47-.64)	.29 (.26-.33)	+
"Sympatholysis"	TM 10	10 s.c.	1	γ	.31 (.24-.41)	.27 (.21-.36)	0
Catechol amine content							
Release	Reserpine	10 i.p.	24	β	.37 (.27-.50)	.47 (.39-.58)	− ?
Accumulation	Iproniazid	100 "	24	β	.44 (.38-.51)	.47 (.39-.58)	0 ?

* Endotoxin lot number.
† Physiological saline blanks substituted for pretreatment with the adrenergic inhibitors.

to be reduced sympathetic reactivity. The term sympatholytic would seem to fit this compound. A dose of 10 mg/kg subcutaneously, which should be effective according to Exley's results, failed to offer protection when given one hour before the challenging dose of epinephrine. If given 24 hours ahead more treated animals survived than did saline controls. However, the difference was not significant by the rank correlation method. If active at all TM 10 is certainly far less effective than phenoxybenzamine in this test.

A very different type of adrenergic influence is produced by reserpine. We found little or no evidence of peripheral adrenergic blockade when this alkaloid was tested in cats and dogs as noted above. At the same time it markedly reduces excitability of the peripheral vasculature and is widely used in human hypertension. Recently a number of investigators have attributed its inhibition of vascular excitability to release of catechol amines such as epinephrine and norepinephrine from associated chromaffin tissue. In our series of survival studies the effectiveness of this type of adrenergic inhibition was evaluated against acute lethal effects of epinephrine injected into mice. The results are included in Table I. Reserpine increased an expected survival of only 35% after 12 mg/kg of epinephrine to 70%. This difference is significant. In the series of over a hundred mice from which these data were taken more than half the animals survived at all dose levels up to a dose of 30 mg/kg of i.p. epinephrine. In another experiment an SD_{50} of 198 (158-247) mg/kg of epinephrine was obtained about 6 hours after an intraperitoneal dose of 10 mg/kg of reserpine. This is nearly 25 times as high as the control. Clearly, reserpine provided some form of protection in our experiments. In contrast, Luduena *et al.* (10) found no protective effect in rats after pretreatment with reserpine 1 mg/kg, but their pretreatment time was only 3 hours. Failure of iproniazid to influence toxicity is apparent in the table. With a graded series of epinephrine doses an SD_{50} of 8.2 (6.7-10.0) mg/kg of epinephrine was obtained 24 hours after intraperitoneal administration of iproniazid 100 mg/kg. This is almost the same as the normal and saline control SD's mentioned earlier. The implication is that the protective effect of reserpine does not result from release of catechol amines. Possibly the direct smooth muscle depression described by Gillis and Lewis(11) may be responsible for the observed protection. The findings are at variance with results of Borowitz and North(12) who found augmented toxicity on the part of epinephrine after iproniazid pretreatments. They stress the importance of a slow, prolonged administration of epinephine. They also used 24 hour survival. These 2 factors may explain the difference between their findings and ours.

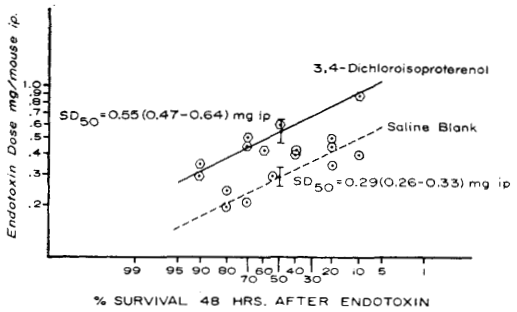

FIG. 1. Dose-response curves including SD_{50} doses and their 95% confidence limits.

B. *Survival after bacterial endotoxin administration.* Experiments with adrenergic blocking drugs such as phenoxybenzamine have offered little promise of clinical usefulness when employed against bacterial endotoxins. Some prolongation of survival time has been obtained, but lethal outcome was not materially altered. It was therefore of considerable interest to us to test these newer forms of adrenergic inhibition against pure crystalline endotoxin. Doses and pretreatment times used were considered adequate to establish the particular type of block or inhibition characteristic of the compound being studied. The results of this series of tests are summarized in Table II.

Blockade of the excitatory adrenergic receptors by phenoxybenzamine has not provided a degree of protection which is significant at p 0.05. Like results were obtained after sensitization of these receptors with cocaine. Somewhat higher doses were required to produce a 50% mortality in each case and low order protective effects are possible. A useful degree of protection is not apparent.

In the case of the next compound, 3,4-dichloroisoproterenol, however, survival was significantly increased. This substance blocks peripheral adrenergic inhibition.

The sympatholytic compound TM 10 seems to offer no protection, at least as it was used in our experiment. Additional tests with 24 hour pretreatment time were also negative. Catechol amine release by reserpine, or accumulation after iproniazid, did not improve survival.

Discussion. The principal point in the foregoing account where questions might be raised is in the matter of dosage and pretreatment time. In the case of phenoxybenzamine and cocaine the obvious results in Table I verify their effectiveness at doses and times employed. Their peripheral site of action in the vascular smooth muscle excitatory system does not seem to be germane to the problem of protection against endotoxin.

The other substances affecting excitatory mechanisms also failed to offer protection. Thus TM 10, which should reduce responsiveness of terminal sympathetic nerve endings, did not increase survival significantly. The dose used was reported by Exley(9) to cause marked relaxation of the nictitating membrane in cats. The effect came on within an hour and lasted more than 24 hours. Reserpine, by depleting catechol amines from chromaffin tissue, reduces overall reactivity of efferent sympatho-adrenal nervous pathways. It did not protect the mice against endotoxin. Six and a half mg/kg of reserpine largely depleted rabbits' ears of catechol amines in 48 hours according to Burn and Rand(13). Shimamoto *et al.*(14) report significant protection of rabbits against *Shigella* endotoxin by doses of reserpine ranging from 1.0 to 5.0 mg/kg. Our dose in Table II is high but smaller doses (1, 3 or 5 mg/kg) also failed to protect. The deleterious effect of the 10 mg/kg dose suggested in Table II may result from fluid loss and poor alimentation. Finally the reciprocal effect of catechol amine accumulation brought about by monoamine oxidase inhibition on the part of iproniazid also had no protective action. The dose of 100 mg/kg should allow a considerable build-up of epinephrine and norepinephrine in 24 hours according to Pletscher(15).

The one case of clear cut protection in Table II is that of 3,4-dichloroisoproterenol. Fig. 1 represents a dose-response curve obtained with mice pretreated with this compound, 10 mg/kg SC, 1 hour before and 18 hours after graded endotoxin doses. This is a very interesting finding. Moran and Perkins(8) reported that this substance blocked cardiac effects of epinephrine. These may be metabolic effects rather than muscular excitant effects involving receptors according to

Mohme-Lundholm(16). Carbohydrate metabolism may be involved.

Summary. 1) Protective effects of various types of adrenergic inhibition have been tested against lethal action of injected bacterial endotoxin in mice. 2) Preliminary experiments substantiated abilities of phenoxybenzamine to block lethal effect of injected epinephrine and of cocaine to augment its toxicity. 3,4-Dichloroisoproterenol also increased lethal effect of epinephrine. TM 10, or 2,6-xylyloxyethyltrimethylammonium bromide had no effect acutely but offered low order of protection when given 24 hours before epinephrine. Reserpine also protected when given 24 hours in advance. Iproniazid was without effect. 3) Against bacterial endotoxin from *E. coli* the only compound to give significant protection was 3,4-dichloroisoproterenol. This substance has been shown by previous workers to block inhibitor and vasodilator effects of epinephrine and related catechol amines. Such effects are probably metabolic in nature and may concern carbohydrate metabolism.

1 Boquet, P., Izard, Y., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 254.

2. Thomas, L., *J. Exp. Med.,* 1956, v104, 865.

3. Zweifach, B. W., Nagler, A. L., Thomas, L., *ibid.,* 1956, v104, 881.

4. Loew, E. R., Micetich, A., *J. Pharm. Exp. Therap.,* 1948, v93, 434.

5. Litchfield, J. T., Jr., Wilcoxon, F., *ibid.,* 1949, v96, 99.

6. Wilcoxon, F., *Some Rapid Approximate Statistical Procedures,* Am. Cyanamid Co., N. Y., 1949.

7. Powell, C. E., Slater, I. H., *J. Pharm. Exp. Therap.,* 1958, v122, 480.

8. Moran, N. C., Perkins, M. E., *ibid.,* 1958, v124, 223.

9. Exley, K. A., *Brit. J. Pharmacol.,* 1957, v12, 297.

10. Luduena, F. P., O'Malley, E., Oyen, I. H., *Arch. Internat. Pharm. Ther.,* 1959, v122, 111.

11. Gillis, C. N., Lewis, J. J., *J. Pharm. Pharmacol.,* 1956, v8, 606.

12. Borowitz, J. L., North, W. C., *Science,* 1959, v130, 710.

13. Burn, J. H., Rand, M. J., *Brit. Med. J.,* 1958, v1, 903.

14. Shimamoto, T., Incue, M., Konishi, T., Iwahara, S., *Arch. Internat. Pharm. Ther.,* 1959, v121, 342.

15. Pletscher, A., *Experientia,* 1958, v14, 73.

16. Mohme-Lundholm, E., *Acta Physiol. Scand.,* 1953, v29, Suppl. 108.

Received May 23, 1960. P.S.E.B.M., 1960, v105.

Placental Transfer and Fetal Tissue Uptake of Mg^{28} in the Rabbit.* (26020)

JERRY K. AIKAWA AND PAUL D. BRUNS
(With the technical assistance of Jacqueline Z. Reardon, Dale R. Harms and Robert O. Linder)
Departments of Medicine and Obstetrics and Gynecology, University of Colorado Medical Center, Denver

The factors regulating the metabolism of magnesium are obscure and very little is known about the placental transfer of this ion or its uptake in the fetal tissues. The purpose of the present study in rabbits was to investigate, by use of a radioactive isotope of magnesium (Mg^{28}), the maternal-to-fetal transfer of magnesium and uptake of the isotope by various maternal and fetal tissues.

* This work was supported in part by contract between U. S. Atomic Energy Comm. and the University and in part by a grant-in-aid from Playtex Park Research Inst.

Material and methods. Pregnant domestic albino rabbits, between 28 and 30 days of gestation, were anesthetized with sodium pentobarbital given intravenously in a dosage of 40 mg/kg of body weight. After a tracheotomy had been performed, the carotid artery was cannulated and an indwelling polyethylene catheter, connected to a reservoir of heparin, was inserted.

Mg^{28} was then injected into the marginal vein of the ear as a solution of magnesium sulfate, prepared according to a method previ-