iron interfered ultimately with the successful detoxification of the endotoxin.

It has been questioned in earlier work on endotoxin-induced susceptibility to venom whether the toxic principle of the venom operating in susceptible animals is the same as that operating in the normal animal. The fact that ferrous sulfate protects against venom challenge in both groups suggests this possibility, although iron has been shown to offer nonspecific protection to a range of toxins. Further experiments are being conducted with venom fractions in an effort to separate the lethal factors in the two situations if this is possible.

Summary. 1. Incubation of ferrous sulfate with endotoxin for 6 hours at 37°C prior to injection did not alter the endotoxin-induced-hypersusceptibility-state (EIHS) to water moccasin venom in rabbits. 2. Incubation of ferrous sulfate with snake venom under the same conditions prior to injection gave significant protection against the lethal effects of the venom in endotoxin-pretreated rabbits. 3. Intravenous ferrous sulfate protected a significant number of animals against the lethal effects of snake venom when administered between the preparing endotoxin and the challenging dose of moccasin venom. 4. Prior intravenous ferrous sulfate also resulted in protection against an LD_{100} of moccasin venom in normal rabbits. 5. Finally, it was shown that administration of large, but nontoxic, doses of ferrous sulfate to endotoxin-pretreated animals resulted in the delayed death of most of the animals.

1. Gledhill, A. W., *Vet. Rev. and Annot.,* 1960, v6, 27.

2. Heilmeyer, L., Keiderling, W., Wöhler, F., *Germ. Med. Monthly,* 1959, v4, 111.

3. Heilmeyer, L., Plötner, K., in *Das Serumeisen und die Eisenmangelkrankheit,* G. Fischer, 1937.

4. Dubach, R., Callender, S. T. E., Moore, C. V., *Blood,* 1948, v3, 526.

5. Schaefer, K. H., *Klin. Wschr.,* 1940, v19, 590.

6. Schaefer, K. H., *ibid.,* 1940, v19, 979.

7. Kampschmidt, R. F., Upchurch, H. F., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 191.

8. Heilmeyer, L., Keiderling, W., Stüwe, G., in *Kupfer und Eisen als Körpereigene Wirkstoffe,* G. Fischer, 1941.

9. Janoff, A., Zweifach, B. W., *J. Exp. Med.,* 1960, v112, 23.

10. Hettche, H. O., *Klin. Wschr.,* 1939, v18, 1437.

11. Condie, R. M., Staab, E. V., Good, R. A., *J. Exp. Med.,* 1962, v115, 563.

12. Staab, E. V., Condie, R. M., Good, R. A., *ibid.,* 1962, v115, 579.

13. Shwartzman, G., *The Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance,* Paul B. Hoeber, 1937.

14. Thomas, L., Good, R. A., *J. Exp. Med.,* 1952, v96, 605.

15. Mazur, A., Green, S., Carleton, A., *J. Biol. Chem.,* 1960, v235, 595.

Received October 11, 1963. P.S.E.B.M., 1964, v115.

Comparison of Sympathetic Blocking Drugs in Prevention of Lethal Effects of Endotoxin.* (28988)

MAX H. WEIL AND KENNETH S. ALLEN

Shock Research Unit and the Department of Medicine, University of Southern California School of Medicine and the Los Angeles County Hospital, Los Angeles, California

Shock which occurs during bacteremia, particularly bacteremia caused by gram-negative enteric organisms, is an increasingly serious clinical problem. The results of therapy have been disappointing. In a clinical study based on 169 patients with bacterial shock recently reported by our group, mortality exceeded 80%(1). The development and progression of this type of shock is related to the liberation of endotoxin from the bacterial cells(2,3). Sympatholytic drugs, especially

* This investigation was supported by the American Heart Association; PHS Research Grant #H-5570 from the National Heart Institute, Public Health Service; and The John A. Hartford Foundation, Inc., New York.

phenoxybenzamine (Dibenzyline), chlorpromazine (Thorazine)† and phentolamine (Regitine),‡ are advocated for treatment. Prior administration of these agents clearly protects against local skin necrosis and systemic injury following injection of endotoxin into experimental animals(4-9). The possibility exists that these drugs are effective for treatment after endotoxin has already acted, but this has not been experimentally demonstrated. Previous studies in dogs and mice have failed to confirm that treatment with phenoxybenzamine reduces mortality, and there is evidence that it may be detrimental (10,11).

The present studies compared the prophylactic and therapeutic effects of phenoxybenzamine, chlorpromazine, and phentolamine in counteracting lethal systemic effects of endotoxin. Studies were done in mice to provide the statistical advantage of a large population. Since relatively transient benefit was observed with chlorpromazine, mortality was observed over a period of 7 days.

Materials and methods. Swiss-Webster strain male mice weighing between 20 and 25 g were housed in groups of 12 in standard metal cages on wooden chips. They were maintained in an air-conditioned rodent room at ambient temperatures ranging from 19.4 to 24.4°C. Rodent pellets§ and water were not restricted.

A single injection of *Escherichia coli* endotoxin prepared by the Boivin technic‖ was used in amounts calculated to be lethal for 90% of untreated mice (LD_{90}); this ranged from 30 to 40 mg per kilo of body weight. Drugs used for "pretreatment" and "treatment" were administered by intraperitoneal injection using sterile technics according to the following schedule: phenoxybenzamine and phentolamine, 20 mg per kilo of body weight; chlorpromazine, 25 mg per kilo of body weight; a control group received only endotoxin. In "pretreated" animals, test drugs were administered 4 hours prior to and again 15 minutes before endotoxin. In "treated" animals these agents were administered 15 minutes after and repeated at 4 hours after the injection of endotoxin.

Concentrations of the drugs were adjusted to provide essentially equal volumes ranging from 0.45 to 0.65 ml. Effects of the drugs alone, in the indicated amounts, were observed separately in otherwise untreated mice. No permanent injury resulted in 12 control animals tested with each of the 3 drugs. In these trials the injection of phenoxybenzamine and chlorpromazine produced initial excitement followed by sedation; phentolamine produced no detectable reaction.

Results. Pretreatment. Each of the 3 drugs protected against the lethal effects of a subsequent injection of bacterial endotoxin. In comparison to untreated controls, the differences at 24 hours were statistically significant in each instance (Fig. 1). This confirmed the observation of earlier investigators. However, the beneficial effect of phenoxybenzamine was temporary, for there were no survivors at the end of 7 days, an even higher mortality than in untreated controls. With chlorpromazine survival was slightly but not significantly greater than in control animals at the end of 7 days. The results with phentolamine were more impressive because of a significantly higher survival rate at the end of 7 days.

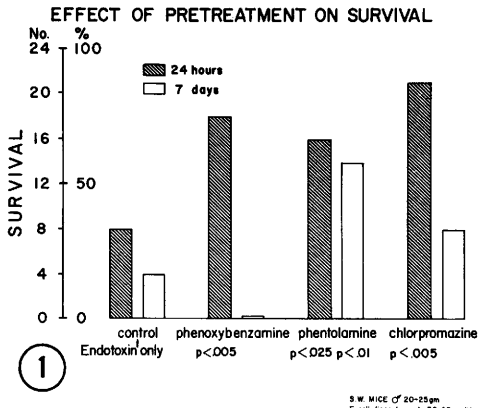
Treatment. Phenoxybenzamine was not effective when administered after endotoxin. In animals treated with chlorpromazine there was significantly greater survival at 24 hours. However, the decrease in mortality in comparison to untreated control animals was small when measured after 7 days. With phentolamine there was significantly higher survival rate at the end of 24 hours, and after 7 days (Fig. 2). These experiments on survival were recently repeated (more than 2 years after the initial studies) with an additional group of 12 Swiss-Webster mice from

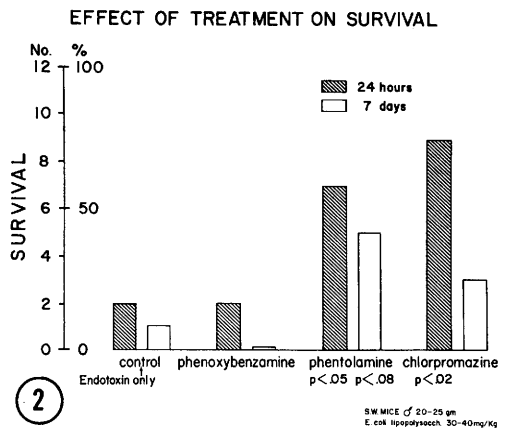
† Phenoxybenzamine hydrochloride was generously supplied as Dibenzyline, and chlorpromazine hydrochloride as Thorazine by Smith, Kline and French Laboratories of Philadelphia.

‡ Phentolamine methanesulfonate was generously supplied as Regitine by Ciba Pharmaceutical Co., Summit, N. J.

§ Supplied by Golden State Mills, Downey, Calif.

‖ *E. coli* lipopolysaccharide 0127:2B supplied by Difco Laboratories, Inc., Detroit, Mich.





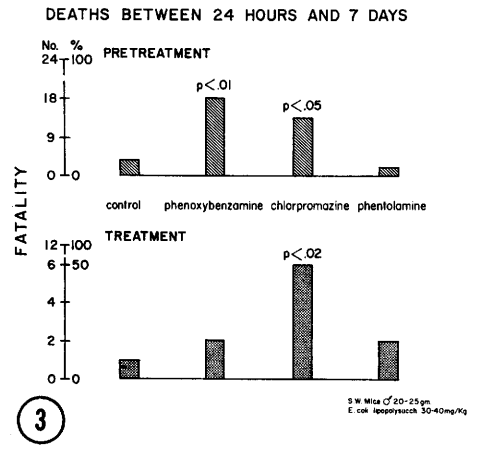


FIG. 1. Effect of pretreatment on survival. Each of the drugs produces a significant increase in survival although the benefit observed with phenoxybenzamine and, to a lesser extent, chlorpromazine is transient.

FIG. 2. Effect of treatment on survival. Phenoxybenzamine is ineffective. The impressive effect of chlorpromazine is relatively transient.

a different substrain. The earlier findings were confirmed. In Fig. 3, the mortalities between 24 hours and 7 days are graphically shown. Phenoxybenzamine and chlorpromazine have a more transient beneficial effect. The number of fatalities occurring with these drugs after 24 hours is significantly higher than in the control group or in animals treated with phentolamine.

Discussion. In the dog, rabbit, mouse, guinea pig and rat, the circulatory defect caused by bacterial endotoxin is characterized by arteriolar constriction with resulting ischemic injury. These effects have been experimentally demonstrated in the mesentery of the mouse, rat and guinea pig, and in the transilluminated ear of the rabbit(12). On the other hand, arteriolar dilatation and venous engorgement are observed in vessels that supply connective tissues and voluntary muscle in mice, and in the splanchnic veins in the mouse, rat and dog(13,14). The ultimate importance of vasoconstriction in the progression of endotoxin shock is not clear. Because of the prophylactic value of sympatholytic drugs of the type used in the present study, the lethal effect of endotoxin has been attributed to sympathetic overactivity. This conclusion is open to question, not only because of the differences in therapeutic action herein reported but also because the pharmacologic action of these drugs is far from settled.

Phenoxybenzamine and phentolamine block the effect of the adrenal medullary hormones, and block vasoconstriction acting through the adrenergic mediators of the sympathetic postganglionic nerves. While both drugs cause transient vasodilatation when injected into arteries of skin and muscle, phenoxybenzamine has an initial constrictive effect on the vessels. Chlorpromazine acts primarily as a central nervous system depressant, and incidentally as a mild adrenergic blocking agent. Clearly, the pharmacological actions of these

FIG. 3. A comparison between number of deaths observed at 24 hr and 7 days. Significantly greater mortality between the 1st and 7th day in the case of phenoxybenzamine and chlorpromazine indicate more transient action of these drugs in comparison to phentolamine.

3 drugs are dissimilar, and the differences observed in relation to endotoxin are not surprising.

In the choice of medications for management of bacterial shock, "pretreatment" must, of course, be considered entirely apart from treatment. The practical importance of this distinction has been commented upon previously. Although "pretreatment" with phenoxybenzamine has been shown to protect rabbits and mice against the lethal effects of endotoxin(4,5), Abernathy(10) failed to show that treatment with the drug increased survival in animals shocked with endotoxin. In support of this earlier work, Weil and Miller(11) demonstrated that phenoxybenzamine did not increase survival in endotoxin-shocked dogs. In fact their studies indicated that the treated animals succumbed earlier than control animals. On the other hand, Noyes and associates(4) have shown that chlorpromazine provides significant protection against the lethal effects of endotoxin when administered either prior to or up to 2 hours after the endotoxin, but no differentiation was made between early and late mortalities.

More significant therapeutic benefit might have been obtained in the present work with phentolamine and chlorpromazine if additional injections of the drug had been given at a later time. On the other hand, the best results were obtained with phentolamine, which is known to have the shortest duration of action. Yet the longest acting drug, phenoxybenzamine, was ineffective. The question of optimal dosage also arises. In planning this study the decision was made to compare prophylactic and therapeutic effects. Within this framework we used amounts of each drug that were shown to have significant prophylactic value. While therapeutic results might be improved with larger or smaller amounts of drug than those effective for prophylaxis, to our knowledge there is no experimental basis for this assumption.

Clarification of fundamental pharmacodynamic differences between phenoxybenzamine, chlorpromazine and phentolamine may help to improve understanding of the mechanisms of endotoxin action and its appropriate treatment. The present work would also call to the attention of workers in the field the potential value of phentolamine for treatment of endotoxin shock.

Summary and conclusions. The prophylactic and therapeutic effects of phenoxybenzamine, chlorpromazine, and phentolamine against the lethal effects of *Escherichia coli* endotoxin in mice were compared. Mortality was observed at 24 hours and 7 days to clarify the value of these agents for "long-term" protection. Phenoxybenzamine, which has been recommended for therapy in clinical cases of bacterial shock, appears to be effective for prophylaxis but has no proven value for treatment in experimental animals. The beneficial effects of chlorpromazine are such that they delay rather than prevent the fatal reaction to bacterial endotoxin. However, the more lasting effectiveness of phentolamine in the treatment of reaction to endotoxin deserves further intensive study.

1. Shubin, H., Weil, M. H., *J. Am. Med. Assn.,* 1963, v184, 220.

2. Burrows, W., *Ann. Rev. Microbiol.,* 1951, v5, 181.

3. Gilbert, R. P., *Physiol. Rev.,* 1960, v40, 245.

4. Noyes, H. E., Sanford, J. P., Nelson, R. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 617.

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

6. Lasker, S. E., Fox, C. L., Jr., *Clin. Res.,* 1960, v8, 31.

7. Lillehei, R. C., Longerbeam, J. K., Rosenberg, J. C., in *Shock, an International Symposium,* Stockholm, 27-30 June, 1961. K. D. Bock, ed., Springer Verlag, Berlin, 1962, 106.

8. Lillehei, R. C., MacLean, L. D., *Arch. Surg.,* 1959, v78, 464.

9. Zucker, G., Levine, J., *Arch. Int. Med.,* 1959, v104, 607.

10. Abernathy, R. S., Ph.D. Thesis, Univ. of Minnesota, 1957.

11. Weil, M. H., Miller, B. S., *J. Lab. Clin. Med.,* 1961, v57, 683.

12. Delaunay, A., Boquet, R., Lebrun, J., Lehoult, Y., Delaunay, M., *J. Physiol.* (Paris), 1948, v40, 89.

13. Algire, G. H., Legallais, F. Y., Parks, H. D., *J. Nat. Cancer Inst.,* 1947, v8, 53.

14. Algire, G. H., Legallais, F. Y., Anderson, B. F., *ibid.,* 1952, v12, 1279.

Received October 16, 1963. P.S.E.B.M., 1964, v115.