similar to that observed in the chicken except for the rapid uptake which gave some evidence of uptake by the cells in the peripheral circulation.

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Effect of Chlorpromazine and Dibenzyline on Bacterial Toxins. (22562)

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Various bacterial toxins, particularly the endotoxins from "coliform" bacteria and the exotoxin of *Clostridium perfringens,* have been considered important factors in the pathogenesis of the decompensated phase of prolonged hypotension (irreversible shock) (1-3). Premedication with a number of pharmacologic agents particularly chlorpromazine and dibenzyline, has been shown to decrease the mortality in the shock syndrome, whereas the same treatment initiated after the onset of shock has been ineffective(4-6). The mechanism of protection has not been elucidated. Chlorpromazine recently has also been reported to offer protection against "endotoxemia" in man and experimental animals and to be useful in the management of tetanus (7-10). The effect of either chlorpromazine or dibenzyline in attenuating lethal shock might be the result of direct action on bacterial toxins or alteration of the host response to them. A study of the effects of chlorpromazine and dibenzyline in animals injected with a variety of bacterial toxins, including those which may play a role in the pathogenesis of irreversible shock, was undertaken and is the basis of this report.

Methods and materials. White Swiss mice, Bagg Strain, each weighing 16-18 g were used. Food and water were readily available to the animals throughout the experiments. The characteristics of the toxins used are listed in Table I. Dosages of the various toxins to range from an LD_{40} to an LD_{70} were estimated by preliminary titrations. All toxins were diluted in 0.9% NaCl solution and, if not sterile, sterilized by filtration through sintered glass. The intravenous route of administration was used with the toxin from *S. dysenteriae,* the subcutaneous route with the tetanus toxin, and the intraperitoneal route with the other toxins. Chlorpromazine and dibenzyline‡ were obtained as sterile solutions. The therapeutic regimens generally corresponded to those reported to be effective in the shock syndrome. The dose of chlorpromazine was 33.5 μg per injection (2 mg/kg) while that of dibenzyline was

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TABLE I. Sources of Toxin Used in This Study.

Organism	Type of toxin	Obtained from	Chemical composition	Extraction procedure
Cl. botulinum	Neurotoxin	Wright(11)	Protein	Acid precipitation of culture filtrate
Cl. perfringens	Culture filtrate	Lederle Lab.	?	Culture filtrate
Cl. tetani	Neurotoxin	Anonymous	Protein	Ammonium sulfate precipitation of autolysed culture
A. aerogenes	Endotoxin	Noyes(12)	Lipopolysaccharide	Trichloracetic acid extraction followed by ethanol fractionation
E. coli	"	" (12)	"	
Past. pestis	Murine toxin	Ajl(13)	Protein	Ammonium sulfate fractionation of autolysed culture followed by electrophoretic separation
S. typhosa	Endotoxin	Webster(14)	Lipopolysaccharide	Trichloracetic acid extraction
S. dysenteriae	Neurotoxin	Branham(15)	Protein	Autolysed cells

17 μg (1 mg/kg). These dosages were contained in 0.5 ml of sterile 0.9% saline solution. Both were administered intraperitoneally. The mice were premedicated 24 hours and 2 hours before administration of toxin in most experiments. Treatment was repeated at 24-hour intervals in most studies until termination of the experiment. All experiments were continued until no additional deaths occurred. Control mice were treated with saline solution according to the same schedule. *In vitro* experiments were carried out to determine if either chlorpromazine or dibenzyline exerted direct "antitoxic" effects. Dibenzyline was mixed with the *S. dysenteriae* neurotoxin to give a dibenzyline concentration of 100 μg/ml or 33 μg/ml with approximately an LD_{70} of toxin/ml. Control mixtures were diluted with saline solution. The mixtures were incubated at 37°C for 6 hours. Aliquants were removed immediately after mixing and during incubation for assay of toxicity in mice.

The therapeutic effectiveness of dibenzyline against *S. dysenteriae* toxin was studied by treating groups of mice either 3 or 6 hours after administration of toxin. The effectiveness of chlorpromazine against *E. coli* endotoxin was similarly studied at 2, 4, and 6 hours after injection of toxin.

Statistical evaluation of the differences in mortality between experimental and control animals, using the method of Chi square, was made. Most of the experiments were repeated at least twice. These individual experiments were combined for statistical evaluation.

Results. Dibenzyline and chlorpromazine were each effective in decreasing the mortality from various toxins. However, the 2 agents were not effective against the same toxins (Table II). Premedication and treatment with dibenzyline afforded protection to mice injected with toxins of *S. dysenteriae, P. pestis,* and *Cl. tetani.* The effectiveness of dibenzyline on endotoxin derived from various species of the enterobacteriaceae was variable. It afforded significant protection against endotoxin derived from a strain of *A. aerogenes,* offered no protection against endotoxin derived from a strain of *E. coli,* and increased the mortality from endotoxin prepared in a similar fashion from *S. typhosa.* Dibenzyline offered no protection to animals injected with toxins derived from *Cl. botulinum* or *Cl. perfringens.* Chlorpromazine afforded protection against the endotoxins of *E. coli* and *S. typhosa,* but was not protective in a single experiment using endotoxin derived from *A. aerogenes.* Chlorpromazine was very effective in 2 groups of mice injected with endotoxin isolated from a strain of *S. typhosa* ($P = 0.001$). However, in a third experiment using the same *S. typhosa* toxin, an increased mortality occurred in the chlorpromazine-treated group of animals (Table II). Chlorpromazine had no protective effect against the other toxins studied. The administration of chlorpromazine actually increased the mortality rate in mice injected with *Cl. perfringens* toxin (Table II). The possibility that the protective effects of these drugs were the result of direct interaction of toxin and drug was studied *in vitro.* The lethality of the Shiga neurotoxin was diminished after 6 hours

of incubation with dibenzyline (Table III). It was also noted that a precipitate formed when dibenzyline was mixed with the *S. dysenteriae* neurotoxin. The precipitate was resuspended immediately prior to injection.

Administration of chlorpromazine and *E. coli* endotoxin to mice immediately following mixing or after 6 hours of incubation decreased mortality rate (Table III). A direct antitoxic action of chlorpromazine could not be assessed because it was effective even when administered after the endotoxin. The effectiveness of these drugs administered at intervals after injection was compared to their effectiveness following administration prior to toxin. Chlorpromazine administered simultaneously with, or 2 hours after, the injection of an LD_{80} of *E. coli* endotoxin afforded marked protection (0 hours—$P<0.001$, 2 hours—$P<0.01$) (Fig. 1). There was a suggestion of protection at 4 hours ($P<0.20$). Dibenzyline administered either 3 or 6 hours after *S. dysenteriae* toxin offered no protection.

Discussion. Pretreatment of mice with chlorpromazine or dibenzyline conferred protection against a variety of bacterial toxins. Protection might have been the result of alteration in the vascular response of the host to the bacterial toxins. Both chlorpromazine

TABLE II. Mortality* of Mice from Bacterial Toxins following Premedication with Chlorpromazine or Dibenzyline.

	Chlorpromazine					Dibenzyline				
Source of toxin	Treated	%	Controls	%	P‡	Treated	%	Controls	%	P
S. dysenteriae	64/ 89*	72	60/ 83	72		50/100	50	68/100	68	<.01
	47/100	47	44/100	44						
A. aerogenes	18/101	18	25/ 90	28		9/ 98	9	20/100	20	
						26/107	24	40/100	40	<.001
						27/ 50	54	36/ 50	72	
						40/ 60	68	48/ 80	60	
						46/ 50	92	49/ 50	98	
E. coli	78/100	78	97/104	93	<.001	67/100	67	75/100	75	
	67/100	67	96/107	90		74/100	74	78/100	78	
						10/ 50	20	18/ 50	36	
S. typhosa	40/100	40	72/100	72		69/104	66	50/105	48	
	60/100	60	48/100	48	<.01	61/ 97	63	53/100	53	<.01
	87/102	85	99/101	98		23/ 50	46	21/ 50	42	
P. pestis	26/100	26	19/100	19		88/100	88	96/100	96	<.05
	77/ 93	83	82/ 93	88		87/100	87	92/100	92	
Cl. botulinum	46/100	46	36/100	36		90/102	88	92/102	90	
Cl. perfringens	46/100	46	33/100	33		67/102	66	66/102	65	
	48/100	48	40/100	40	<.05†	65/100	65	54/100	54	
Cl. tetani	71/100	71	44/100	44		93/100	93	99/100	99	<.01
	82/100	82	98/100	98		69/ 99	70	82/ 97	85	

* Dead/injected.
† Mortality increased.
‡ Probability values computed from all data for each toxin. Values less than >.05 have been omitted.

TABLE III. Mortality from Bacterial Toxins following Incubation *In Vitro* with Chlorpromazine or Dibenzyline.

			Mortality* after incubation *in vitro*			
Source of toxin	Agent	(μg/mouse)	0 hr	P	6 hr	P
S. dysenteriae	Dibenzyline	100	36/40		23/40	
	Saline	—	38/40	>.50	37/40	<.001
E. coli	Chlorpromazine	100	3/23		0/23	
		33	17/58		9/58	
	Saline	—	39/58	<.001	31/58	<.001

* Dead/injected.

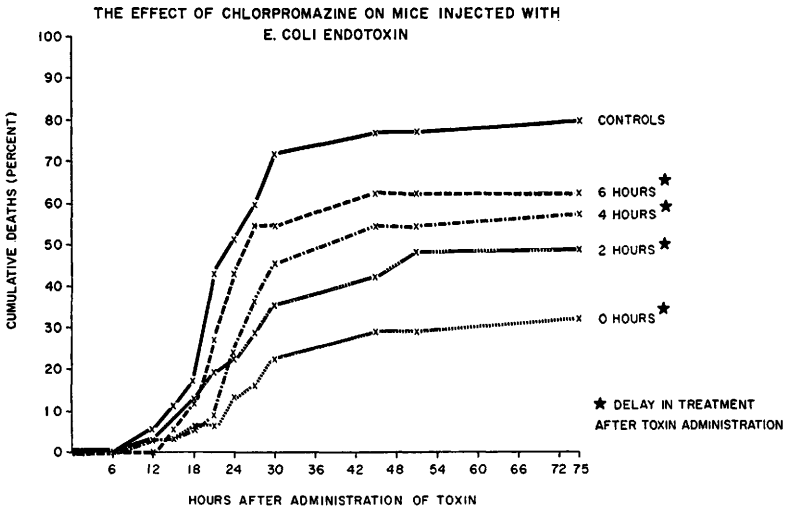

FIG. 1.

and dibenzyline have potent adrenolytic actions. Chlorpromazine may also have a central nervous system action, in that blood pressure can be lowered by amounts which do not block the vasopressor effects of epinephrine. The injection of minute doses intracisternally into rhesus monkeys caused a fall of blood pressure and blockade of the carotid pressor reflex, whereas the pressor response to the systemic injection of adrenaline remained unaffected(16). However, a direct action on the toxin cannot be eliminated, since inactivation of one bacterial toxin occurred upon incubation *in vitro*.

The protective effect of these agents in hemorrhagic or traumatic shock may be the result of alterations in the response to bacterial toxins. Both chlorpromazine and dibenzyline were effective against various endotoxins, but their effectiveness was not uniform. Dibenzyline failed to protect against endotoxin prepared from a strain of *E. coli* or *S. typhosa* when it did afford protection against endotoxin prepared in a similar fashion from a strain of *A. aerogenes*. Neither agent afforded any protection against the toxin of *Cl. perfringens*. A common antitoxic action seems unlikely as the mechanism of protection by chlorpromazine and dibenzyline in hemorrhagic shock, because of the discrepancies noted in their effectiveness against those toxins considered as possible factors in the pathogenesis of irreversible shock. Separate mechanisms of action by chlorpromazine and dibenzyline also were suggested by a series of experiments on their protective effect in hemorrhagic shock following partial or complete abdominal evisceration(17). Protection by one agent and not the other should not have been observed if their modes or sites of action were similar.

The effectiveness of chlorpromazine and dibenzyline in various specific bacterial toxemias should be stressed, in addition to any interrelationships between chlorpromazine, dibenzyline, bacterial toxins, and shock. Dibenzyline decreased mortality in animals injected with Shiga neurotoxin, plague toxin, and tetanus toxin. Chlorpromazine was primarily effective against the endotoxins of Gram-negative bacteria, including *S. typhosa*. Chlorpromazine not only was effective when administered before injection of endotoxin, but was also very effective when treatment was delayed until several hours after injection of endotoxin. The mechanism of the protection against endotoxin derived from the enterobacteriaceae is not known. However, epinephrine has been demonstrated to increase susceptibility of the host to endotoxin(18). The adrenolytic action of chlorpromazine may account for some of the protective effect, though dibenzyline, which has a similar action, would also be expected to be effective

and was not.

Summary and conclusions. 1. Chlorpromazine and dibenzyline were tested in mice for their ability to afford protection against a variety of bacterial toxins. 2. Premedication and treatment with chlorpromazine significantly decreased the mortality from endotoxins derived from *Escherichia coli* and *Salmonella typhosa*. Similar treatment with dibenzyline decreased the mortality from toxins derived from *Shigella dysenteriae, Aerobacter aerogenes, Pasteurella pestis* and *Clostridium tetani*. 3. Chlorpromazine afforded significant protection when administered 2 hours following the injection of endotoxin derived from *Escherichia coli*. Premedication was not necessary. 4. Premedication with chlorpromazine increased the mortality from the toxin of *Clostridium perfringens*, likewise premedication with dibenzyline increased the mortality from the endotoxin derived from *Salmonella typhosa*.

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Concentration of ACTH in Cavernous Sinus and Peripheral Arterial Blood in the Dog.* (22563)

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Collection of effluent blood from the pituitary has been impractical because of its position in the skull and the early divergence of cerebral venous drainage into many channels. Since blood drains directly from the pituitary into the cavernous sinus, at least in some species, samples of blood from this location, although contaminated by blood from the ophthalmic veins, would be expected to contain a high concentration of pituitary hormones (1). The present paper describes a simple and reliable technic for obtaining blood from the

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