one LD_{50} of Sarin and gave partial protection against 2, a uniformly fatal dose. Thus far, compounds having protective properties when injected several minutes prior to Sarin may also be effective when administered within a few minutes after the toxic material (Table II). "Prophylactic" or early "therapeutic" use of the hydroxamates intraperitoneally appears to be more effective, however, than their use subsequently. When administered after the onset of obvious signs of intoxication, the effects of these hydroxamates were negligible in terms of reversing the developing toxic manifestations in mice.

Discussion. Although there are striking hydrolytic effects of hydroxamic acid analogues *in vitro*(1-6), protection requires relatively huge doses in mice. This is of practical importance in application. Factors pertaining to optimum conditions for contact between the antidote and either free Sarin or Sarin-inhibited enzymes or both, *in vivo*, include rates of absorption and excretion, of degradation and detoxification and of diffusion from the circulation. The apparent independence of toxicity and potency ratios among candidate compounds encourages further exploration. It would appear that toxicity of an HA may limit the prospect of testing its maximum efficacy. However, the large doses required to raise the LD_{50} of Sarin significantly would appear to limit the therapeutic potential. Related compounds are being studied.

Summary. Some hydroxamic acid analogues protect mice against an LD_{50} dose of Sarin. Effective doses required in this series have been large. The efficacy of hydroxamates is variable and unrelated to toxicity.

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Received March 15, 1956. P.S.E.B.M., 1956, v92.

Host Resistance to Bacteria in Hemorrhagic Shock. VI. Effect of Endotoxin on Antibacterial Defense.* (22576)

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Transfusion for experimental hemorrhagic shock of 2 hours duration is followed by recovery. But if the shock is allowed to persist for much longer (4-5 hours) it fails to respond to transfusion. Evidence has been given that the failure of the circulation to respond to transfusion is a result of bacterial activity, which cannot be controlled because shock produces a progressively severe injury to the antibacterial mechanisms(1-6). The further observation that intravenous antibiotic therapy, given shortly after shock was induced, failed to prevent the development of irreversibility, in contrast to the success of such therapy when given before shock was induced

* Supported by contract with Office of Surgeon General, U. S. Army, and by research grant from NIH.

TABLE I. Influence of Time on the Lethality of *E. coli* Endotoxin I.V. in Normal Rabbits.*

First inj. (MSD)	Second inj. (MSD)	Second inj. at 10 min. Total	10 min. % survival	1 hr Total	1 hr % survival	4 hr Total	4 hr % survival
1	—	25	100	25	100	25	100
1/2	1/2	10	80	10	20	15	60
1/2	1/4	10	60	10	50	10	80
1/2	1/8	10	100	10	40	10	90
1/2	1/16	—	—	10	20	10	90
1/2	1/32	—	—	10	40	10	100
1/2	1/64	—	—	10	40	—	—
1/2	1/128	—	—	10	20	—	—
1/2	1/256	—	—	10	80	—	—
1/2	1/512	—	—	10	0	—	—

* MLD 100 = 0.13 mg/kg; MSD 100 = 0.05 mg/kg.

(3),† suggested that a process already in operation during the short interval between onset of shock and administration of the antibiotic was responsible for the development of irreversibility. This process was assumed to be the production of a small amount of bacterial endotoxin,‡ which eventually caused failure of transfusion because of progressive collapse of the antiendotoxic defense(3). These considerations led to experiments designed to explore (1) the relationship of time to the efficiency of the mechanisms involved in the detoxification of endotoxins; (2) the effect of antibiotics on the response to endotoxins.

Method. A purified *E. coli* endotoxin§ given intravenously was employed in all the experiments to be described. The intravenous MLD/100 (minimal lethal dose for all recipients) was 0.13 mg/kg and the intravenous MSD/100 (maximal surviving dose for all recipients) was 0.05 mg/kg for normal rabbits(6). One half MSD/100 was administered to each of a group of normal rabbits. Ten minutes later a second dose was given. The second dose, beginning with ½ MSD/100, was decreased in each successive rabbit by 50% until no effect was secured. This experiment was repeated in 2 additional groups of normal rabbits, except that the interval between the first and second dose was lengthened to one hour in one group, and to 4 hours in another group.

Results. Table I shows first, that 11% (6 of 55) of normal rabbits were killed by the second dose of toxin given 10 minutes after the first dose; and secondly, that 67% (47 of 70) were killed when the second dose was given one hour after the first safe dose. Even with a second dose as little as 1/128 of MSD/100, most of the recipients died. When the second dose was given 4 hours after the first, the mortality rate was 18% (10 of 55 rabbits). The survival time of most of these recipients was about the same (3-24 hours) as of those receiving an MLD/100. Some that survived showed varying degrees of transient

† In our original experiments (1952) on dogs, penicillin, neomycin and the tetracyclines were effective when given prior to shock. In 1955 they were ineffective, except when given to dogs just prior to delivery to our laboratory from the dealer's kennels, and promptly submitted to the shock procedure, *i.e.*, before they could acquire the now resistant intestinal flora in our animal quarters. Prophylactic antibiotic therapy was, however, successful in rat experiments performed in 1955(7). This latter observation has been confirmed by Shorr and Baez(8).

‡ Evidence has been given for the presence of bacterial activity in the tissues of dogs in hemorrhagic shock, in spite of the absence of positive blood or tissue cultures (except Clostridia) until a few minutes after death, and that this bacterial activity is responsible for development of irreversibility to transfusion(1). There is also evidence that bacterial invasion from the intestine is a continuous process in the normal as well as the shocked animal(7,9,10). Moreover there is reason to believe that endotoxins can be absorbed from the intestinal canal(6). Recently a leucotoxin which is neutralized by Properdin has been identified in the blood of the rabbit in hemorrhagic shock(11).

§ We are grateful to Dr. A. Braude of the University of Texas Medical School for a generous supply of this material.

TABLE II. Effect of Preloading the RE System with Trypan Blue* on Toxicity of *E. coli* Endotoxin.

Normal rabbits			Hemorrhagic shock rabbits†		
Dose of endotoxin I.V. (mg)‡	No. rabbits	No. survived	Dose of endotoxin I.V. (mg)§	No. rabbits	No. survived
.05	1	0	.00000012	1	0
.025	4	0	.00000006	3	0
.0125	5	0	.00000003	5	1
.0063	5	1	.000000015	4	3
.0032	5	5	.000000007	3	3

* 10 cc of an 0.1% solution 3 times a wk for 7 wk.

† Hemorrhagic shock of 2 hr duration, produced by a technic described elsewhere (Schweinburg, F. B., Frank, H. A. and Fine, J., *Am. J. Physiol.*, 1954, v179, 523; and Schweinburg, F. B. and Fine, J., PROC. SOC. EXP. BIOL. & MED., 1955, v88, 589).

‡ .05 mg was upper limit of a uniformly non-lethal dose of a standardized *E. coli* endotoxin in normal rabbits.

§ Endotoxin given 4 hr after transfusion. .00000012 mg was upper limit of a uniformly non-lethal dose of a standardized *E. coli* endotoxin when given to rabbits 4 hr after transfusion for hemorrhagic shock of 2 hr duration.

muscular weakness and anorexia. Those that died showed progressive muscular weakness beginning within a few hours. They developed progressive hypotension, oliguria or anuria, and pallor of the mucous membranes. Gross post-mortem examination disclosed nothing more than scattered petechial hemorrhages. and a small amount of blood in the gut. There was no evidence of the Shwartzman reaction.

Comment. These results demonstrate that a small dose of endotoxin quickly induces a hypersensitivity to more endotoxin, but that substantial recovery of resistance to endotoxin is already present 4 hours after the initial dose. This is the interval required for blood clearance of a dose of endotoxin injected intravenously, and for maximal uptake by the RE system(12). That the RE system inactivates endotoxin is indicated by the observation (Table II) that the sensitivity of rabbits preloaded with Trypan Blue was much higher than in comparable rabbits not given the dye. Since shock injures the RE system(13), failure of detoxification can explain a persisting and even an increasing hypersensitivity to endotoxin during shock. That the death resulting from the second dose of endotoxin is due to the release and exposure to still more endotoxin is shown by the results of experiments (Table III) in which antibiotics (100,000 units of penicillin and 50 mg of Streptomycin given intravenously) were administered with the initial dose of endotoxin. In this situation, the second dose, given one hour later, proved to be virtually harmless, for the mortality rate fell from 67% to 4% (2 of 46 animals). Since the antibiotics protect by preventing the generation of more endotoxin‖, one might expect the

TABLE III. Coli Endotoxin I.V. Penicillin, streptomycin I.V. with first dose of endotoxin. Second inj. of endotoxin one hr later.

1st inj. of endotoxin	2nd inj. of endotoxin	No. of rabbits		
——— MSD ———		Total	Dead	Survived
1	—	25	—	25
1/2	1/2	10	1	9
1/2	1/4	10	0	10
1/2	1/8	5	0	5
1/2	1/16	6	0	6
1/2	1/32	5	0	5
1/2	1/64	5	0	5
1/2	1/128	5	1 (72 hr)	4
1/2	1/256			

MSD 100 = 0.05 mg/kg.

‖ The protective action of antibiotics in shock or against endotoxin cannot be due to other than their antibiotic properties for several reasons: 1. Penicillin has no known pharmacologic activity apart from its antibiotic action. 2. Except for the acceleration of growth from long-term administration in food, these compounds, in so far as they do exert any non-antibacterial properties, act adversely rather than protectively. 3. As will be shown below, Bacitracin and Polymyxin B, when given orally, protect even though they are wholly non-absorbable. Finally, it is improbable that such widely diverse chemical compounds could exert a uniformly protective non-antibacterial pharmacologic effect.

TABLE IV. Effect of Antibiotic Therapy on Survival Rate of Normal and Shocked Rabbits Subjected to Their Respective MLDs (100) of Bacterial Toxin or Multiples Thereof.

Exp. No.	Antibiotic*	Toxin: Dosage (MLD)	Toxin: Time relative to antibiotic therapy	Normal: No. of rabbits	Normal: Survival (%)	Shock: No. of rabbits	Shock: Survival (%)
	None	1	—	20	0	20	0
I	Pen & S	1	4 hr after	10	60	20	45
		1½	"	10	50	20	50
		2	"	10	50	10	50
II	"	1	Simultaneously	20	55	20	45
		1½	"	10	60	20	45
		2	"	20	60	10	50
		3	"	10	0	—	—
III	"	1	"	20	65	10	40
		1½	"	20	60	10	60
		2	"	20	60	10	50
IV	B & P or N	1	Immediately after 4th dose or 6 hr later in 2 hr shocked dog	10	50	15	60
		1½	*Idem*	10	50	15	35
		2	"	10	50	10	40

* Pen = penicillin; S = streptomycin; B = bacitracin; P = polymyxin; N = neomycin.

timing of the antibiotics to condition the results. To determine the effect of timing and to disclose the source of the additional endotoxin, the following experiments were undertaken.

Method. Rabbits of the same breed and approximate weight (2-3 kg) were variously exposed to the *E. coli* endotoxin and to antibiotics as follows: *Group I*: Penicillin G. 100,000 U and Streptomycin (50 mg) were given intravenously to normal rabbits, and to rabbits immediately after transfusion for hemorrhagic shock of 2 hours duration. Four hours later one MLD/100 of endotoxin was administered intravenously to each rabbit in both groups. This dose, as stated, was 10^{-1} mg for normal rabbits. For rabbits 4 hours after transfusion for hemorrhagic shock of 2 hours duration the MLD/100 was 10^{-6} mg (6). The experiment was repeated with multiples of the respective MLDs/100. *Group II*: The procedure was the same as in Group I, except that the antibiotics were injected into one ear vein at the time the endotoxin was injected into the other. *Group III*: The procedure was the same as in Group II, except that the antibiotics and the endotoxin were injected together, after mixing and standing at 4°C for 4 hours. *Group IV*: Bacitracin (10,000 U) and Polymyxin (100,000 U) or Neomycin (100,000 μg) were fed daily by gavage for 4 successive days to 2 groups of normal rabbits. About one hour after the fourth dose each rabbit in one group received one MLD/100 endotoxin (10^{-1} mg) intravenously. The rabbits in the other group were put into hemorrhagic shock for 2 hours, and then transfused. Four hours later, each of these received one MLD/100 endotoxin (10^{-6} mg). *Group V*: Normal rabbits were given one MLD/100 of endotoxin, and the antibiotics employed in Group I were given 30, 60, or 90 minutes *afterward* instead of before or simultaneous with the endotoxin. All animals were observed until they were fully recovered, or until death, in which case a gross postmortem examination was made.

Results (Tables IV and V). Without anti-

TABLE V. Penicillin (100,000 Units) and Streptomycin (50 mg) I.V. Simultaneous with or at Various Intervals after a Single MLD/100 Dose of *E. coli* Endotoxin.

	No. normal rabbits	% survived
Simultaneously	50	50
10 min. after endotoxin	10	10
30 " " "	10	0
1 hr " "	6	0
1½ " " "	4	0
2 " " "	6	0

biotic therapy the expected survival rate of both the normal and shocked rabbits would have been 0%(6). The average survival rate proved to be about 50% in Groups I, II, III, and IV, *i.e.*, in those in which antibiotics were given *prior to or simultaneous with* the administration of endotoxin (Table IV). In Group V, in which the antibiotic was given 30 or more minutes *after* the endotoxin, the survival rate was 0% (Table V).

Comment. The results of the Group I, II, and III experiments confirm those of numerous investigators(14-19), who noted a considerable decrease in the lethal power of certain bacterial toxins as a result of prior administration of certain sulfonamides or antibiotics. No explicit view of the way these agents affect the action of toxin is offered in most of these reports, but several investigators(18,19) assert that the antibiotics exert an anti-endotoxic action *in vivo*. Our Group III experiments exclude a direct effect of the antibiotics on the endotoxin. Our Group IV experiments, in which non-absorbable antibiotics (Bacitracin and Polymyxin B) introduced into the intestine were as effective as absorbable antibiotics given by the same route (Neomycin¶) or intravenously, exclude both a direct and an indirect effect upon the endotoxin. Therefore, the antibiotics act only by suppressing bacterial activity. The Group IV experiments demonstrate further that the bacteria which are the source of the additional toxin are to an important degree, if not entirely, those invading from the gastrointestinal tract.

It follows that a lethal dose of test endotoxin is not necessarily lethal *per se*, and that death involves an effect consequent to injury by the endotoxin to the antibacterial defense mechanisms, with the subsequent elaboration of more bacterial toxin. This additional dose, added to the test dose, constitutes the lethal dose. From the results of the experiments with multiples of the MLD/100 it would appear that the additional dose may equal as much as 1 MLD 100 of the test endotoxin, because the antibiotics achieved the same degree of protection with test doses up to 2 MLD/100. In the first 4 groups of experiments the antibiotics were immediately available to shut off the generation of more endotoxin than was administered to the animal. In the Group V experiments the antibiotics were not immediately available, and proved completely ineffective. The effect of this difference in timing is strikingly analogous to the effect of the difference in timing of antibiotic therapy in hemorrhagic shock. We have attributed the failure of antibiotic therapy applied soon *after* the induction of hemorrhagic shock, in contrast to its effectiveness if applied *prior* to shock, to the interim production of a lethal dose of bacterial toxin (3). This assumes that the antibacterial functions begin to fail very shortly after the induction of shock, and that the toxin which can develop in the interval between the onset of shock and the administration of antibiotic constitutes a lethal dose eventually, because of the rapid and enormous rise in sensitivity to the toxin, which cannot be neutralized or excreted. The results of the Group V experiments, as contrasted to those of Groups I-IV, suggest that a similar process operates when a lethal dose of endotoxin is given; *i.e.* that the antibacterial defense mechanisms are as promptly and as severely damaged by a large dose of endotoxin as by hemorrhagic shock, and that exposure to an otherwise insignificant amount of endotoxin shortly thereafter is sufficient to produce irreversible shock as a result of increasing sensitivity to endotoxin. Presumably a dose of endotoxin is lethal because it permanently paralyzes the recuperative powers of the detoxifying system, whereas a sublethal dose does not prevent their recovery after a certain interval unless an additional, though relatively trivial, dose is added in the interim.

This investigation was done with one endotoxin only. The results obtained may not hold for other endotoxins. But because there are no identifiable distinguishing features among the various endotoxins, we may assume that their pathological effects are similar.

Summary and conclusions. 1. The mortality rate from one intravenous MLD/100 of

¶ A very small fraction of neomycin is absorbed.

endotoxin was reduced from 100% to 40-60% by antibiotic therapy given prior to or at the time of injecting the endotoxin. This was true even if the antibiotic was given orally and was completely non-absorbable. Therefore, endotoxins from intraintestinal bacteria are involved in the death from an intravenous MLD/100 of endotoxin. Data are given to show that antibiotics act solely as antibacterial agents, and not as anti-endotoxic agents. 2. A sublethal intravenous dose of endotoxin promptly induced in rabbits an increased sensitivity to a supplementary fractional dose of endotoxin so that a second dose given one hour later increased the mortality from zero to 67%, even though the total of both doses, if given as a single initial dose, was not lethal. Recovery of nearly normal resistance to the second dose of endotoxin required about 4 hours. Antibiotics given with the initial dose rendered the second dose harmless; but the same antibiotics given with the second dose did not prevent death. Therefore, it is inferred that death from the second dose involved the production of additional endotoxin. 3. Reasons are given for inferring that similar phenomena operate in hemorrhagic shock and account for the development of irreversibility to transfusion.

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Received April 9, 1956. P.S.E.B.M., 1956, v92.

Serial Propagation of Adenoviruses (APC) in Monkey Kidney Tissue Cultures. (22577)

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Production of adenovirus (APC) vaccines suitable for general usage, is dependent on the serial propagation of virus in cultures of acceptable non-malignant tissue. Since monkey kidney tissue cultures demonstrated cytopathogenic effects and supported limited growth of adenoviruses(1), it seemed possible that with further adaptation, these agents might be induced to grow satisfactorily in such cultures. This report describes the procedures used in establishing monkey kidney passage lines of 7 serotypes (1 through 7) of