flammation. These factors have been separated chemically and identified biologically. The future will yield, as in most biological entities, greater chemical accuracy in their identification. Thus far, pyrexin and the thermostable leukocytosis factor have been brought to the state of ready crystallization.† Many of the other factors have been identified as polypeptides. Their chemical structures, however, have yet to be identified by chemists.

Conclusions. Severely injuring muscle tissue of a rabbit by mechanical means induces the formation of a leukotaxine-like substance which has essentially the biological attributes of leukotaxine as obtained from inflammatory exudates. The fractions of leukotaxine-like material induce an increase in capillary permeability and cause leukocytic migration. The isolated guinea pig intestine fails to contract when treated with these fractions, while histamine added subsequently contracts the segment. This would indicate that the leukotaxine-like fractions obtained do not appear to be histamine.

† Studies now in progress indicate the possibility that the thermostable leukocytosis factor may be adsorbed on a non-specific crystalline structure, but this will be taken up *in extenso* in a subsequent communication.

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Efficacy of 1-Ethanesulfonyl-4-Ethyl-Piperazine Hydrochloride in Treatment of Controlled Hemorrhagic Shock.* (19578)

JOS. G. STRAWITZ, WOO CHOO LEE,† E. R. SCHMIDT, AND O. SIDNEY ORTH.
From the Departments of Surgery, Anesthesiology, and Pharmacology, University of Wisconsin Medical School, Madison.

Acute hemorrhage is of frequent occurrence from industrial and highway accidents and battle casualties. While its treatment has been facilitated greatly by the ready availability of supplies of plasma and whole blood, the time required to obtain them and for the proper matching of blood still may cause such a delay that successful reversal of the pathologic state is not produced quickly enough and death ensues. The development of a drug which would be satisfactory in delaying the onset of an irreversible state consequently is highly desirable. In preliminary studies, Bovet *et al.* reported that 1-ethanesulfonyl-4-ethyl-piperazine hydrochloride (R.P. 3885) apparently is successful in protecting rats from traumatic shock produced by the Noble-Collip drum(1). Later, these workers indicated that this compound was successful in increasing the survival time of dogs subjected to "traumatic-hemorrhagic"

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† International Educational Exchange Program of The Department of State, from Severance Union Medical College, Seoul, Korea.

‡ The drug was synthesized and generously supplied to us as a pure crystalline compound by Dr. Thomas P. Carney, The Eli Lilly Co., and in a 25% solution, which was slightly amber in color, by Dr. Jacob W. Stutzman, of Smith, Kline & French, Inc.

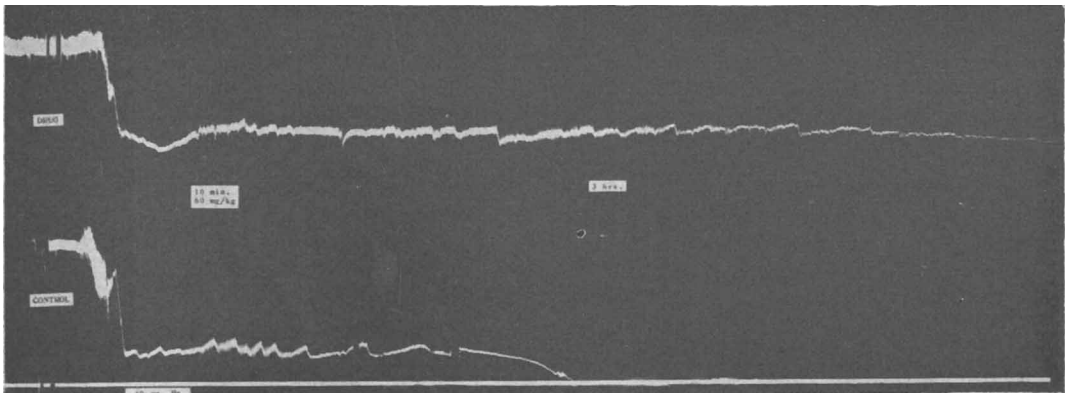

FIG. 1. Kymographic records of blood pressure from one of the paired experiments in which duration of survival was determined after production of hypotension. In the tracing indicated by "DRUG," 50 mg/kg of piperazine compound was administered intrav. in 10 min. Control animal survived for 3 hr, the treated animal 4½ hr.

shock(2). Others have reported that the drug was ineffective in the treatment of traumatic shock produced in rats(3).

The present report deals with a preliminary survey of the efficacy of 1-ethanesulfonyl-4-ethyl-piperazine hydrochloride‡ in two types of experiments, in which acute hypotension was produced by controlled hemorrhage in dogs. In the first series of experiments the time of survival was determined in paired dogs, one of which served as the control while the other was treated with the drug. In the second type of experiment similar procedures were followed, but the blood of each dog was reinfused and its recovery was sought.

Method. Healthy mongrel dogs maintained on a standard *ad libitum* diet of dried dog biscuits were paired as nearly as possible as to sex, age, general breed, and weight. Unconsciousness was produced with 30 mg of pentobarbital sodium per kg, given intravenously in about 3 minutes. Endotracheal intubation was employed in most of the dogs so that a free airway was maintained at all times. Two mg heparin per kg also was given intravenously to aid in preventing clotting while arterial blood pressure was determined from the femoral artery with a mercury manometer and recorded graphically. The opposite femoral artery was cannulated for bleeding and connected to a receiving flask containing a preservative solution consisting of 1.38% sodium citrate, 1.58% dextrose, and 0.5% citric acid. The hypotensive state was produced essentially in one stage, a modification of the technic of Wiggers(4), by permitting blood to flow rapidly and simultaneously into an individual container for each dog of the pair until a hypotension of 40 mm Hg was attained within approximately 5 minutes. Small volumes of blood were withdrawn subsequently to maintain the initial hypotensive level. The first of the 2 general types of experiments was performed on 13 pairs of dogs. After blood was withdrawn to produce the hypotension, one animal of the pair was treated intravenously with 50 to 100 mg of the drug per kg while the other served as the control. The time of survival then was determined for each animal. In a second series of 17 pairs of dogs, hypotension again was produced and one animal of each pair was treated within the first 30 minutes or after 90 minutes. Some 150 to 180 minutes after the onset of hypotension, the withdrawn blood was reinfused into the respective treated and control animals and their recovery was determined. During the course of the experiments specific gravity was measured by the copper sulfate method. Postmortem examinations were performed on most of the animals which succumbed.

Results. A record of an experiment in which length of survival was tested is given in Fig. 1. The drug was injected intravenously after the first 10 minutes of hypotension and the blood pressure was maintained at a level that did not exceed 40 mm Hg. At the end of

TABLE I. 7 experiments Nov. 17 to Dec. 21, 1951 in which, after production of a hypotension of approximately 40 mm Hg, length of survival of dogs was determined. 50 mg/kg piperazine was administered within 30 min of production of hypotension. No transfusion was given these animals.

	Control animals Survival time,		Treated animals Survival time,	
	hr	min	hr	min
	1	45	4	25
	2	56	2	53
	2	44	2	5
	2	50	4	28
	3	25	8	41
	2	45	1	10*
	1	53	4	20
Avg	2	37	4	0

* Toxic reaction to drug.
$t = 1.5$ with 12 degrees of freedom.

TABLE II. 6 Experiments of Jan. 2-14, 1952 in Which Duration of Survival Was Determined When Piperazine Was Given 90 Min after Production of the Hypotensive State. No blood was administered to this group of animals.

	Control animals Survival time,			Treated animals Survival time,	
	hr	min	Dose, mg/kg	hr	min
	5	12	50	8	6
	2	50	60	2	25
	2	52	50	2	30
	3	0	50	3	55
	3	50	50	2	35
	2	55	50	2	45
Avg time of survival	3	24		3	42

3 hours the control animal died, whereas the treated dog lived for 4 hours and 30 minutes.

The data from a series of 7 pairs of animals in which the drug was injected within 30 minutes after the production of hypotension are given in Table I. The survival time was measured from the beginning of hypotension until death. In Exp. 6 the treated animal died in the extraordinarily short time of one hour and 10 minutes. Since there was no instance for any of the control animals of such a brief time of survival, it seems quite possible that toxicity of the drug was responsible. The average survival time for the treated animals was 4 hours and for the control group 2.6 hours. Statistical analysis of these figures reveals that the difference in survival time is significant since, by the Fischer formula, $t = 1.5$, for 12 degrees of freedom.

In a series of 6 pairs of animals the drug was administered after 90 minutes of hypotension. The average survival time for the two groups was essentially the same, since that for the treated animals was 3.7 hours while for the controls it was 3.4 hours. The data are given in Table II and indicate that when treatment is delayed until this late in the hypotensive period, survival time is not prolonged.

A graphic record of the blood pressure from an experiment in which there was reinfusion of blood is given in Fig. 2. Intravenous injection of the piperazine was begun within the first 10 minutes of hypotension and 10 to 15 minutes was taken for its infusion. The blood pressure level in the treated animal subsequently stabilized at 20 mm Hg. This effect

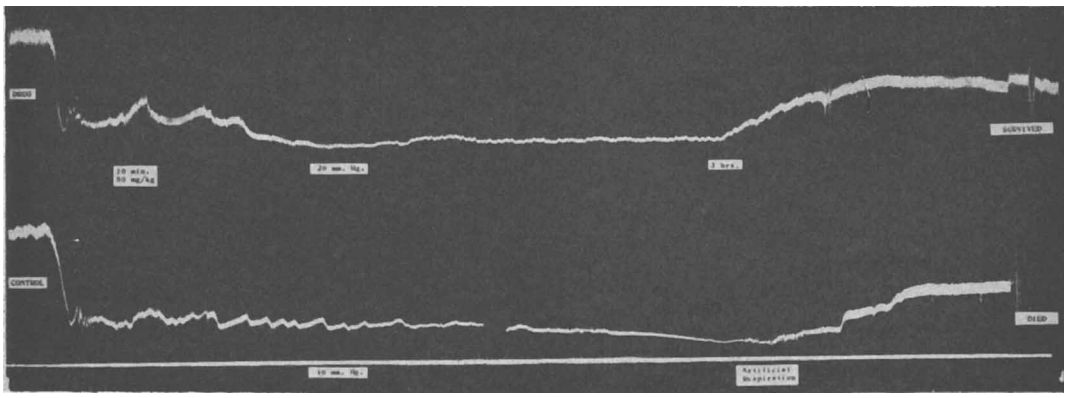

FIG. 2. A record from paired experiments in which administration of 50 mg/kg of the piperazine drug to one of the dogs was started 10 min after hypotension was established by acute hemorrhage. Blood was reinfused into each animal after 3 hr. Control animal died 8 hr later but the treated (drug) animal survived.

TABLE III. 12 Experiments Conducted Between Jan. 16 and Feb. 18, 1952 in Which There Was Reinfusion into Each Animal of Each Pair of the Blood Withdrawn. Administration of the drug was made intravenously within 30 min of production of the hypotension.

Time of reinfusion for each dog, hr	min	Control animal: Initial mean B.P., mm Hg	Result	Piperazine treated animal: Initial mean B.P., mm Hg	Dose, mg/kg	Result
1	25	180	D*	160	50	S*
3		155	D	145	50	S
2	40	150	S	140	50	S
2	5	150	D	120	75	S
2	50	160	D	140	100	S
2	20	140	D	130	100	S
2	50	170	D	130	100	S
2	35	130	D	120	100	S
3		140	D	130	100	S
1	20	120	D	140	100	D
3		135	D	140	100	S
3		160	S	140	100	S
	Survival†		17%			92%

* D = died; S = survived.
† Statistical analysis by the "t" test reveals p = .0003.

TABLE IV. 5 Experiments Performed Feb. 20-29, 1952 in Which the Blood Withdrawn to Produce Hypotension Was Reinfused into the Pairs of Animals at the Times Indicated. Administration of the piperazine drug was made at 90 min.

Time of reinfusion for each dog, hr	min	Control animal: Initial mean B.P., mm Hg	Result	Piperazine treated animal: Initial mean B.P., mm Hg	Dose, mg/kg	Result
3	10	145	D*	150		
2	28	165	D	150		
2	30	140	S	130	75	D
2	35	140	S	140		
2	32	140	D	140		

* D = died; S = survived.

occurred shortly after injection of the drug and indicates that the piperazine compound may impose an added increment on the hypotension resulting from hemorrhage. The slow rate employed for its administration was an attempt to minimize this action. During the same period the blood pressure of the control animal was maintained at 40 mm Hg. At the end of 3 hours of hypotension, each animal had its withdrawn blood reinfused. The control animal was deemed to be in a very critical condition at this time and artificial respiration was administered during the first 30 minutes of reinfusion. Both animals regained a blood pressure level which was somewhat lower than their initial normal levels. The treated animal survived while the control one died 8 hours after the reinfusion of its blood.

The results from a series of 12 pairs of animals in which the drug was injected within the first 30 minutes of hypotension are given in Table III. The withdrawn blood was reinfused after 80 to 180 minutes of hypotension. These results are considered to be highly significant since statistical analysis by the "t" test reveals p = .0003.

Data from a series of 5 pairs of animals in which administration of the piperazine was withheld until there had been 90 minutes of hypotension are given in Table IV. Reinfusion of the withdrawn blood was instituted at 2½ to 3 hours. The mortality of 100% in the treated animals indicates that, after such a prolonged hypotensive period, no benefit obtains from administration of the drug. Two of the control animals of this group obviously had not reached an irreversible state since they survived the hypotensive period.

Postmortem examinations revealed the usual morphologic changes characteristic of hemorrhagic shock. Changes in specific gravity likewise followed the characteristic pat-

tern previously reported.

Discussion. Rigidly controlled experiments suggest that within definite limits 1-ethanesulfonyl-4-ethyl-piperazine administered intravenously is effective in prolonging the survival time and decreasing the mortality in dogs subjected to extreme hemorrhagic shock. The protective action of the drug occurred when it was administered in a dosage of 50 to 100 mg/kg within the first 30 minutes of hypotension. No protection was evident when hypotension was present for 90 minutes before the drug was injected.

The procedures used varied somewhat from others previously employed in testing a drug for the treatment of hemorrhagic shock(5). Preliminary experiments suggested that a 1½ to 2 hour period of hypotension did not consistently produce a state of irreversible shock. The period of hypotension was therefore prolonged to as much as 3 hours with an average of 2 hours and 30 minutes. To obviate variations of seasons of the year, weather, and technic, all experiments were done on paired dogs, so that a control was run simultaneously with the experimental animal.

The pharmacodynamic actions and toxicity of 1-ethanesulfonyl-4-ethyl-piperazine were not determined in these experiments although it was determined that larger doses could be injected much more rapidly in normotensive dogs. Thus, a definitely increased toxicity was present in some of the hypotensive animals. Evidence of this was the pronounced drop in blood pressure which resulted soon after administration of the drug for which compensation did not occur for approximately 30 minutes (Fig. 2). In several animals compensation never occurred and death ensued rapidly (Dog 6, Table I). During injection of the drug a rather marked increase in respiratory rate and depth usually was evident.

Summary. 1. When dogs which had been subjected to severe hemorrhage were treated within 30 minutes with 50 to 100 mg/kg of 1-ethanesulfonyl-4-ethyl-piperazine administered intravenously, survival time was prolonged or mortality decreased in comparison to untreated control animals. 2. No protective action was evident when treatment was withheld until there had been 90 minutes of hypotension. 3. The piperazine compound is definitely toxic and was considered responsible for death of 3 of 30 dogs in which it was used for treatment. 4. The drug imposed a decided hypotensive factor on the acute 40 mm Hg state produced deliberately by controlled hemorrhage.

Dr. William Young, Chief of Surgery at the Veterans Administration Hospital, Madison, helped institute work on this project.

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Platelets: VII. Shortened "Platelet Survival Time" and Development of Platelet Agglutinins Following Multiple Platelet Transfusions.* (19579)

MARIO STEFANINI,† WILLIAM DAMESHEK, AND EDWARD ADELSON.‡
(With technical assistance of Louise Désy and Lucy Salomon.)
From the Ziskind Laboratories, Joseph H. Pratt and New England Center Hospitals, and Department of Medicine, Tufts College Medical School, Boston.

In the course of studies on the fate of platelets injected into patients with various types of thrombocytopenia(1,2), it was observed that the survival time became shorter

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† Damon Runyon Senior Clinical Research Fellow.
‡ Damon Runyon Clinical Research Fellow.