Perphenazine (Trilafon®) and Chlorpromazine in Experimental Shock.* (24278)

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Reduction of mortality in animals subjected to hemorrhage has been classically associated with sympathectomy(1) or pretreatment with drugs(2,3,4) which prevent compensatory response of excessive and prolonged vasoconstriction. Bacterial, metabolic, and other factors have been implicated(5,6,7,8) in development of shock due to hemorrhage or trauma, but a causal relationship between these factors and shock is as yet incompletely established or controversial. It has been recently demonstrated that clinically useful ganglionic blocking agents possess protective action when administered prophylactically in experimental shock(9,10). The effectiveness of chlorpromazine(11,12,13,14,15,16) in similar studies may be at least partly attributable to its adrenergic blocking activity. Other possible mechanisms of protection, not involving autonomic blockade, have not been clarified at this time. In this report, the protective action of perphenazine (PPZ), a new phenothiazine derivative(17,18) was compared with that of chlorpromazine (CPZ) in hemorrhage shock in dogs and traumatic shock in rats.

Methods. Traumatic shock. Female Carworth rats (110-140 g) were subjected to tumbling trauma by the Noble-Collip procedure(19). The drum was revolved at 40 rpm for 600 revolutions. Drugs were administered intramuscularly 15 min before exposure to trauma. Animals which were dead on removal from the drum were not included in any calculations. The ratio of rats which had survived for more than 24 hours to those which were alive on removal from the drum was calculated for each treatment and dose group. Untreated control animals were used with each dose group and survival of these never exceeded 35%. *Hemorrhage shock* was produced in 73 healthy mongrel dogs (7-15 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). Bleeding was allowed from femoral artery to elevated reservoir bottle (2). The experiments were conducted during July, August, and September, in air-conditioned room, at 68-70°F. The entire procedure, including all equipment and operative technic, was performed under aseptic conditions. Blood pressure was recorded from a side-arm in the bleeding-line by mercury manometer. The ipsilateral femoral vein was isolated for direct drug administration. Immediately following vessel exposure all dogs received heparin (5 mg/kg, iv) and additional 30 mg injected into the reservoir bottle. Following arrangement of femoral arterial bleeding system, the animals were allowed to bleed into the maximally elevated reservoir bottle until the blood column height had equilibrated with the blood pressure. Two dogs were simultaneously prepared in this fashion, one receiving drug treatment (doses expressed in terms of free base) and the other acting as untreated control. Immediately after equilibration, a test drug was slowly administered, iv, and 15 min allowed for stabilization of blood pressure. The response to drug was a gradual decline in pressure not exceeding 15-30 mm Hg. During this drug-induced hypotensive phase there was a slight automatic reinfusion of blood back into the animal. Hemorrhage was then simultaneously initiated in both dogs by gradual lowering of reservoir bottles for 7-10 min to attain desired blood pressure level of 30 mm Hg. Constancy of

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® TRILAFON is trade name of Schering's perphenazine (1-(2-hydroxyethyl)-4-[3-(2-chloro-10-phenothiazinyl) propyl] piperazine dihydrochloride).

TABLE I. Effects of Perphenazine and Chlorpromazine on Survival of Rats Exposed to Traumatic Shock (Noble-Collip Drum).*

Drug	Dose, mg/kg (intramusc.)	Survival ratio, No. survivors/No. tested	
			%
Controls	—	32/103	31
PPZ	1.5	23/26	88
	.8	7/7	100
	.5	3/4	75
	.4	5/9	55
	.2	3/9	33
CPZ	1.5	22/26	85
	.8	9/9	100
	.4	8/8	100
	.2	9/12	75

* 600 rotations at 40 r.p.m.

this pressure level was self-regulatory(2). Initial bleeding volume (IBV) was recorded, and subsequent readings were made at 10 minute intervals, or less. The amount of blood which slowly accumulated subsequent to the IBV reading is the secondary bleeding volume (SBV). The IBV plus the SBV is maximal bleeding volume (MBV). When the compensatory action necessary to maintain the set blood pressure began to fail there was an automatic reinfusion of blood (ARV) back into the animal. Criteria for termination of experiment were: 1) The ARV having reached 25% of the MBV, or 2) survival of animal for 8 hours at hypotensive level of 30 mm Hg. It has been our experience that less severe criteria for termination of experiment were inadequate for demonstration of a uniformly high mortality rate in the control group. Termination proceedings were instituted by rapid raising of reservoir bottle so that all shed blood was returned to the animal, and all but approximately 6 inches of residual line-volume was slowly forced into the artery under air pressure. When stabilized, blood pressure was recorded for the last time, and usually ranged from 70-80 mm Hg. The femoral arterial cannula was removed, the artery ligated and the incision closed with skin clips after dusting with sulfadiazine powder. An animal was considered a survivor of hemorrhage shock if it was alive after 96 hours. Survivors or dogs which died during experiment demonstrated varying degrees of "typical shock" pathology, namely, intestinal and endocardial hemorrhage and liver engorgement.

Results. Statistical analysis of data in Tables I, II, and III demonstrated the following similarities or distinctions ($p<0.05$) between drug-treated and control groups:

Table I. Traumatic shock. Survival ratio of PPZ-treated rats was significantly greater than that of controls at doses between 0.5 and 1.5 mg/kg. The same protection was observed in the CPZ-treated groups, but CPZ was more effective than PPZ at doses below 0.5 mg/kg. It also was observed that CPZ had lesser "tranquilizing" potency than PPZ(17) since no sedation was apparent at doses of 0.4 to 0.2 mg/kg of CPZ; whereas, animals treated with 0.2 mg/kg of PPZ still showed some motor depression, not associated with the induced shock.

Table II. Hemorrhage shock. Survival ratio of PPZ-treated dogs (1 mg/kg) was significantly greater than that found for controls; whereas, the ratio for CPZ-treated dogs (1 mg/kg) was not. However, the ratio for the combined drug-treated groups (9/32) was significantly greater than that of controls. There was no significant difference in survival between CPZ and PPZ-treated groups. There was no difference in average MBV for either drug-treated or control groups. Average dur-

TABLE II. Effect of Perphenazine and Chlorpromazine on Bleeding Volume, Hypotension Time,* and Survival of Dogs Exposed to Hemorrhage Shock.

Drug, mg/kg (intrav.)	Avg max bleeding vol (ml/kg) ± S.E.		Avg hypotension time (min.) ± S.E.		Survival ratio, No. survivors/No. tested	
						%
Controls	58.1	1.3	134.9	16.3	1/41	2
PPZ (1.0)	57.2	2.1	248.1	31.3	6/16	37
CPZ (1.0)	53.1	2.1	281.6	32.0	3/16	19

* Duration of induced hypotension (30 mm Hg) before satisfying criteria for termination of experiment.

TABLE III. Effect of Perphenazine and Chlorpromazine on Bleeding Volumes at Various Stages of Exposure of Dogs to Hemorrhage Shock.

Drug, mg/kg (intrav.)	Avg bleeding vol (ml/kg ± S.E.)						Time to attain MBV (min. ± S.E.)	
	IBV		SBV		MBV			
Controls	45.4	1.2	12.8	1.1	58.1	1.3	39.6	3.8
PPZ (1.0)	33.6	1.9	22.9	1.8	57.2	2.1	89.2	13.5
CPZ (1.0)	33.1	1.9	19.9	1.8	53.1	2.1	83.6	12.7

ation of hypotension (maintained at 30 mm Hg) before criteria of shock were satisfied, was similar for drug-treated groups but significantly different from that found for control animals. This also was indicative of the protective action of the drugs. At a dose of 0.5 mg/kg, 1 out of 5 (20%) PPZ-treated dogs survived. At a dose of 3 mg/kg, 3 out of 7 (43%) CPZ-treated dogs survived. Although these studies are considered incomplete because of the few animals involved, it does indicate that significant protection can be achieved with CPZ.

Table III. The average IBV or SBV values were similar for both drug-treated groups but differed significantly from those values found for the controls. This also was true in regard to the time it took for the IBV to reach the MBV.

Discussion. It may be concluded that control dogs bled more initially (IBV) than did the treated dogs, and subsequently bled less than test dogs in the SBV period. Treated animals bled more slowly and steadily but no less *in toto* (MBV) than did the controls. This pattern of hemorrhage as to rate or volumes (IBV and SBV) in the drug-treated dogs may be attributed, for the most part, to prevention of rapid and severe vasoconstriction(2,3). It appeared that the adrenergic blocking action of PPZ or CPZ(18) was manifested to a moderate, but not marked, degree since the drug-treated dogs were able to bleed the same amount as did the control animals. Other, as yet undefined, mechanisms of the "anti-shock" action of these compounds also may be operating. However, at this time the evidence of a great increase in circulating epinephrine and norepinephrine following hemorrhage(20,21) appears to favor the concept of protection, by adrenergic blockade of excessive vasoconstriction and its pathologic sequelae. As has been reported for CPZ(22), the protective action of PPZ in preventing death following severe hemorrhage or trauma may be of clinical use in preventing untoward reactions to "stress situations."

Summary. 1) Pretreatment of rats with perphenazine or chlorpromazine (0.5 - 1.5 mg/kg, im) afforded significant protection against lethal effects of Noble-Collip drum trauma. Chlorpromazine was still effective at doses of 0.2 to 0.4 mg/kg, whereas perphenazine was not protective in this dose range. Pretreatment of dogs with perphenazine (1 mg/kg, iv) significantly protected against lethal effects of hemorrhage under aseptic conditions. Chlorpromazine did not display significant protection at 1 mg/kg, but it did appear to be effective at 3 mg/kg in a limited study. Both drug treated groups (1 mg/kg) bled the same maximal volume as did the controls but at much slower rates when systolic pressure was maintained at 30 mm Hg. 2) Reduction of mortality in pretreated animals subjected to experimental shock may be due, at least in part, to adrenergic blocking activity of the test compounds.

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Fibril Formation in Stationary Cultures of Mouse Lung Cells. (24279)

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It has recently been reported(1) that a strain 929 of L fibroblasts agitated in a serum-free medium gave rise to the formation of fibers. This fiber material was shown to be digested by collagenase, partially degraded by trypsin, and resistant to alpha amylase and hyaluronidase. The observations reported here are cited to bear out this report using a different cell line in a non-agitated medium, to present new information regarding the period of appearance and disappearance of the fibrils, and to present photographic evidence of the cell fibril complex.

Materials and methods. The line of cells used in this report was isolated from lung tissue of new-born Swiss mice, NIH strain. The original isolation was made using trypsin dispersed cultures prepared by a procedure similar to that of Youngner(2). Prior to their growth on a serum-free medium the cells were in their 110th passage on a medium consisting of 10% horse serum and 90% medium #199 (3). Penicillin and streptomycin were added in a final concentration of 50 units each per ml. Morphologically the cells appeared fibroblast-like, although small numbers of epithelioid cells were in evidence. The cells were routinely passed every 4 days by trypsinizing, centrifuging, resuspending, and inoculating, (hemocytometer count) 3 x 10^5 cells per ml in T-30 flasks, the total volume being 5 ml.

During the course of adapting these cells to a serum-free medium consisting of 99% medium #199, 1% Difco Bactopeptone, and 100 mg% glucose (BPD) it was noticed that a floating layer of cells was formed. These cells were separate from the cell layer which had attached to the glass surface, and appeared in the early passages after 5 to 8 days of incubation at 37°C. The floating cells were seen as discrete patches often 3-4 mm in diameter. Photomicrographs of this material were obtained by inverting and reinverting the T-30 flasks so that the unattached cells adhered temporarily to the glass surface opposite the normal cell layer. In this way the natural configuration of the material could be studied. Portions of the material were then removed and tested with several enzymatic preparations. Results of these tests showed that the material was resistant to digestion by hyaluronidase and trypsin but susceptible to digestion with collagenase.

Fig. 1 shows a typical growth of mouse lung cells on a glass surface using a 10% horse serum medium. The cells form an even sheet which is extremely difficult to dislodge physically. Fig. 2 illustrates the tangled mass of