Effect of Hypothermia upon Induced Bacteremia.* (22801)

E. J. Fedor, E. R. Fisher, Sil H. Lee, W. K. Weitzel, and B. Fisher†
(With technical assistance of James Dattilo)

Departments of Surgery and Pathology, the Gibson Laboratory University of Pittsburgh and Veterans Administration Hospital, Pittsburgh, Pa.

In this laboratory observations have been made upon dogs maintained hypothermic for prolonged periods of time(1). The cause of death in some of these animals during the period of cooling and following rewarming remains unexplained. In a study by Parkins, Ben, and Vars(2), upon the effect of differential cooling in fatal ischemic shock, it was shown that a greater number of animals survived preferential cooling of the intestine than primary cooling of the liver. This led Rhoads *et al.*(3), to suggest that intestinal bacteria or their endotoxins might be a factor contributing to the poor results obtained from paramount cooling of the liver. Overton and DeBakey observed(4) that anesthesia and hypothermia, with autonomic blocking agents, was followed by a mortality of 10% when antibacterial therapy was not employed. That a bacterial factor exists in hemorrhagic shock in normothermic dogs is indicated by the experiments of Schweinburg, Frank, and Fine(5). Whether such a factor is present in the hypotension of prolonged hypothermia and subsequent rewarming has not been ascertained. The purpose of this paper is to present data showing (1) that hypothermia even after many hours does not promote a state of bacteremia, and (2) that the hypothermic animal is still capable of removing pathogenic organisms injected into its blood stream.

Methods. Healthy mongrel dogs of both sexes weighing 6.8 to 20.4 kg were used. The technic of cooling used in this laboratory has been adequately described in detail(1). Briefly, animals anesthetized with ether, were placed in a cold water bath at 1°C until the rectal temperature was 28°-29°C. Following removal from bath they were maintained at 22°-24°C in air-conditioned room. Rewarming was accomplished by placing dogs in a 40°C water bath where they were kept until rectal temperature was 28°-29°C. Following drying with towels, animals were placed in warm room where they returned to normothermic levels. Blood samples were obtained by percutaneous puncture of jugular vein. Sterile conditions were maintained during periods of sampling and culturing. To determine presence or absence of bacteremia during hypothermia and during rewarming, blood samples were obtained just prior to cooling following 6 or 12 hours of hypothermia and after 2 to 8 hours of rewarming. These samples were then incubated 48 hours at 37°C in brain heart infusion for determination of aerobic growth and chopped meat broth for anaerobic growth. Transfers were made from thioglycolate broth to blood agar plates and Levine E.M.B. plates and observed for growth and identification of organisms. Transfers from chopped meat broth to blood agar plates were incubated in environment of CO_2 at 37°C. The effect of hypothermia and rewarming on bacterial clearance was determined by intravenous injection of saline suspension of 24 hour culture of *E. coli* and *A. aerogenes* which had been prepared previously from cultures of stools of the animals. All dogs were inoculated with 5-50 billion bacteria. The number of organisms introduced was determined by nephelometry. The first sample of blood, 2 cc, was taken 10 minutes after injection and mixed with liquid agar, plated in duplicate and incubated at 37°C. Succeeding samples were drawn at 6, 12, 18, and 24 hours. Following withdrawal of the 24 hour sample, rewarming of the animal was instituted. Additional 2 cc samples were taken and plated 6, 12, and 24 hours after removal of animal from the warm water

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† John and Mary Markle Scholar.

TABLE I. Blood Clearance of 5-50 Billion Bacteria Injected into 5 Normothermic Dogs.

No. of colonies/ml of blood				
10 min.	6 hr	12 hr	18 hr	24 hr
2,750	9	3	7	0
5,025	0	1	3	0
2,750	23	3	4	0
4,775	5	4	3	0
5,770	4	2	3	0

bath. After a 48 hour incubation period at 37°C, bacterial colony counts were made on a Quebec-Colony Counter. An average of the number of colonies on duplicate plates was made and results expressed as number of colonies per cc of blood.

Results. Twelve animals were cooled either 6 or 12 hours and then rewarmed. All of these animals maintained the sterility of their blood both during the period of hypothermia and following rewarming. In spite of this, 2 of the 6 animals cooled for 12 hours died 5 and 8 hours following rewarming.

To determine the effect of hypothermia and rewarming on the capacity to destroy bacteria, 5 normothermic and 13 hypothermic dogs were inoculated with 5-50 billion bacteria (*E. coli* and *A. aerogenes*). The normothermic dogs (Table I) revealed no ill effects and completely cleared their blood streams of bacteria in 24 hours. Hypothermic animals were divided into 2 groups (Table II). Group I was injected with bacteria when their temperatures were stabilized at 23 ± 0.6°C. Group II was injected 6 hours after hypothermia had been maintained at 23 ± 0.6°C. In the first group all animals except one had positive blood cultures after 24 hours of hypothermia. Following rewarming all animals in this group demonstrated negative blood cultures within 24 hours. Three of the dogs died 12, 42, and 44 hours following rewarming. All of these animals had negative blood cultures before death and at autopsy no apparent cause of death could be ascertained. In the second group, where bacteria had been injected after 6 hours of hypothermia at 23°C the ability to clear organisms appears to be about the same as in the first group. One animal in this group died during hypothermia although a blood culture taken previous to death revealed no growth. Three other dogs died following rewarming. Only one of these demonstrated a bacteremia. In this animal, however, there was a decline from 7000 colonies per cc to 3 colonies per cc during the course of the experiment.

Discussion. Although animals maintained hypothermic for long periods of time did not clear bacteria from the blood as efficiently as the normothermic dogs, phagocytosis was not abolished. Samples of blood from hypothermic dogs, 6 hours subsequent to injection of bacteria, indicate a 99% phagocytosis in all but one animal. In all instances, the 12, 18, and 24 hour samples from cooled animals showed the blood to be free of 99% of the initially injected bacteria. Recently it has

TABLE II. Blood Clearance of 5-50 Billion Bacteria Injected into 2 Groups of Dogs during Hypothermia and after Rewarming.

	No. of colonies/ml of blood								
	During hypothermia					After rewarming			
	10 min.	6 hr	12 hr	18 hr	24 hr	6 hr	12 hr	24 hr	Remarks
Group I	7,900	3	3	7	2	0	0	0	Died
	4,650	2	0	3	0	0	0	0	Survived
	3,775	13	9	6	19	5	4	0	"
	4,450	12	11	1	105	50	5	0	Died
	12,500	4	23	20	14	3	0		"
Group II	2,800	2	0						Died
	9,500	21	50	10	7	3	0		"
	7,000	1,000	27	20	14	7	3		"
	3,000	15	9	3	3	0	0	0	Survived
	9,200	42	60	13	4	2	0	0	"
	8,500	42	13	5	1	0	0	0	"
	8,000	2	2	6	0	0	0		Died

Group I: Bacteria inj. when temp. stabilized at 23.0 ± 0.6°C. Group II: Bacteria inj. 6 hr after hypothermia at 23.0 ± 0.6°C.

been demonstrated that there is an 86% reduction in the leucocyte count in dogs cooled to the range of 18°-26°C for a hundred minutes(6) and that the leukopenia disappears within a half hour of rewarming(7). *In vitro* studies of temperature relations in phagocytosis by Harmon and associates(8) showed that the phagocytic powers of the polymorphonuclear leucocytes decreased with lowered temperatures. Since the experiments in this report encompass the time interval reported by Helmsworth(6), a leukopenia should be expected at the time of the injection of the bacteria. It would thus seem that the paucity of leucocytes and the decline of the phagocytic powers of the remaining leucocytes could not account for the 99% removal of the injected bacteria as seen here. It is reasonable to assume that the reticulo-endothelial system of the liver and spleen assumes the major role in phagocytosis under these conditions. Manwaring(9) and Cannon(10), on a basis of blood flow, availability of macrophages and leucocytes, and mechanical conditions favoring filtration, felt that the liver and spleen were the principal phagocytic centers. Fisher *et al.*(11) showed that there was a progressive decrease in hepatic blood flow as the hours of hypothermia were extended and that on rewarming there was a prompt return to control levels. The removal of bacteria from the circulation as reported here would seem to parallel these changes in blood flow. A trapping and intermittent release of contaminated blood as well as the decrease in hepatic blood flow may account for the incomplete phagocytosis which occurs during the hypothermia. It would seem that it is impossible to relate the cause of death in rewarming following prolonged hypothermia to a bacterial factor or at least a bacteremia. Although 7 out of 12 animals into whose circulation bacteria were injected died after being rewarmed, in only one of these was there a positive blood culture.

Conclusions. 1. Dogs cooled to 23°C 6 or 12 hours and then rewarmed, maintained sterility of blood during cooling and following rewarming, suggesting that the gastrointestinal tract and other bacterial foci maintained their integrity for at least 12 hours of hypothermia. 2. Hypothermic and normothermic animals intravenously inoculated with bacterial suspension were able to rid blood within 6 hours of 99% of the injected pathogens. Within 24 hours after injection of cultures of *A. aerogenes* and *E. coli*, normothermic animals cleared the blood completely of bacteria while dogs maintained hypothermic did not. However, within 12-24 hours after rewarming only 1 of 11 hypothermic animals had a bacteremia. 3. Inability to survive following prolonged hypothermia does not seem to be related to bacteremia.

1. Fisher, B., Russ, C., Fedor, E. J., Wilde, R., Engstrom, P., Happel, J., and Prendergast, P., *A. M. A. Arch. Surg.*, 1955, v71, 431.

2. Parkins, W. M., Ben, M., and Vars, H. M., *Surgery*, 1955, v38, 38.

3. Rhoads, J., Varn, W., Parkins, W. M., Ben, M., and Vars, H. M., *Surg. Clin. N. A.*, 1955, v35, 1585.

4. Overton, R. C., and DeBakey, M. E., *Ann. Surg.*, 1956, v143, 439.

5. Schweinburg, F. B., Frank, H. A., and Fine, J., *Am. J. Physiol.*, 1954, v179, 532.

6. Helmsworth, J. A., Stiles, W. J., and Elstun, W., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 474.

7. Villalobos, T. J., Adelson, E., and Brila, T. G., *ibid.*, 1955, v89, 192.

8. Harmon, D. R., Zarafonetis, C., and Clark, P. F., *J. Bact.*, 1946, v52, 337.

9. Manwaring, W. H., and Fritschen, W., *J. Immunol.*, 1923, v8, 83.

10. Cannon, P. R., Sullivan, F. L., and Neckermann, E. F., *J. Exp. Med.*, 1932, v55, 121.

11. Fisher, B., Fedor, E. J., Lee, S. H., Weitzel, W. K., Selker, R., and Russ, C., *Surgery*, 1956.

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