PROLONGED HYPOTHERMIA IN EXPERIMENTAL PNEUMOCOCCAL PERITONITIS [1]

By B. EISEMAN, W. G. MALETTE, R. S. WOTKYNS, W. B. SUMMERS, AND J. L. TONG

(From the Department of Surgery, University of Colorado School of Medicine and Denver Veterans Administration Hospital, Denver, Colo.)

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The recent use of generalized body hypothermia as an adjunct to anesthesia has prompted the application of this technique to the management of various types of infections. The isolated case reports on the use of hypothermia in clinical infections have attributed varying degrees of success to the use of this agent, but these studies in general have not been adequately controlled, nor has the mechanism of such benefit been properly investigated (1–9).

The rationale for the use of chronic hypothermia in the management of infectious processes rests upon the fact that the metabolism of bacteria pathogenic to man is reduced at temperature levels below that of normal body temperature. Reduction of the temperature of the animal might retard bacterial growth with possible benefit to the host. Such benefit would occur only if host resistance is depressed less than is bacterial growth.

The effect of cold on the resistance to infection was first noted in 1878 by Pasteur, Joubert, and Chamberland (10), who found that immersion of an unanesthetized fowl in cold water increased the susceptibility of the animal to subsequent anthrax infection. Thereafter numerous studies were carried out on the effect of body chilling (11) or the local application of cold (12) on host resistance to infection. Other studies have been reported on the *in vitro* alteration of phagocytosis by reduced temperatures (11, 13–18).

The purpose of this experiment is to determine the effect of prolonged (24-hour) generalized body hypothermia on the course of experimental peritonitis in anesthetized mice. It is emphasized that these animals were subjected to hypothermia after narcosis. This resulted in a metabolic situation very different from that obtained by the techniques used in other investigations (10–13) of chilling the unanesthetized animal. Another metabolic and immunologic difference of prime importance is that the animals in this study underwent prolonged generalized body hypothermia, not mere local cooling as used in some experiments (12).

MATERIALS AND METHODS

A total of 329, six to eight-week old (25 to 30-gram) Webster strain white mice [2] with type III pneumococcal peritonitis were included in this study. Of these, 160 animals were kept in a hypothermic state for 24 hours, while 130 animals served as simultaneous normothermic controls. Thirty-nine animals with experimental peritonitis received anesthesia according to the same schedule as the hypothermic group but were not cooled. Experimental runs were made on groups of 20 animals at a time, half being selected at random as controls.

The organisms [3] were maintained at a standard virulence by weekly mouse passage. The inoculum was prepared by plating on blood agar for 24 hours at 37° C. This plate was placed in a refrigerator (4° C.) until ready for sub-culture. In no instance did this period exceed 6 days. Prior to use 1 to 3 of the smoother mucoid colonies were inoculated into 15 to 20 ml. of trypticase soy broth in a *25* by *150*-mm. test tube and incubated at 37° C. for 18 hours. At the end of this period there was obvious diffuse turbidity. The inoculum was drawn into a 1-ml. dry tuberculin syringe and 0.5 ml. injected intraperitoneally through a No. 26 hypodermic needle into each animal.

The *in vitro* growth of pneumococci at varying temperatures was measured by determining the turbidity of a broth culture at hourly intervals on a Beckman B. spectrophotometer at 470 λ. A preliminary absorption curve was plotted on uninoculated broth. Growth was plotted as the reciprocal of turbidity. Cultures were maintained in a constant temperature incubator set either at 37.5° or 19° C.

After all of the animals had been inoculated they were arbitrarily divided into a control and hypothermic group—

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[2] Obtained from Colorado Serum Company, Denver, Colorado.

[3] *D. pneumoniae III* originally obtained from the National Institutes of Health.

thus producing simultaneous paired experiments in each run. Animals in each group were identified by alcoholic-picric acid solution skin markings.

The control animals were placed in separate jars containing adequate food and water at room temperature and observed at 6-hour intervals.

The hypothermic animals were placed in similar jars for 2 hours following inoculation, following which each animal received a subcutaneous abdominal wall injection of 0.25 to 0.30 ml. of 1.0 per cent saline solution of sodium pentobarbital (Nembutal®, Abbott). This inoculum provided 0.1 mg. of pentobarbital per gram of body weight.

After 30 minutes the animals were unconscious and were placed on a metal pan in the cold room at 4° C. for 6 to 8 minutes. The hypothermic animals were then immediately placed in a constant temperature box[4] adjusted to 19.5° C.

Mouse body temperatures were recorded by means of plastic covered No. 24 gauge constantin wire thermocouples previously greased with petroleum jelly and inserted 2 to 3.5 cm. into the rectum of each animal. Each electrode led into a constant recording Brown potentiometer (Model Y 153 × 60 PA a-x-iv) via an electric-clock-operated selector switch which serially recorded the temperature of each animal and of the constant temperature box at 10-minute intervals.

Rectal temperatures reached a constant level of 21° to 24° C. within 30 minutes after the animals were placed in the constant temperature box. When an animal began to emerge from anesthesia its body temperature was noted to rise gradually.

Subsequent additional anesthesia was administered according to the following dosage schedule, as required to maintain narcosis: 0.05 ml. of a 1 per cent pentobarbital saline solution was given subcutaneously into the back, at not less than 20-minute intervals. Two to six such inoculations were variously required over the first 3 hours of hypothermia in order to obtain adequate narcosis. Individual variation was wide and did not correlate with body weight.

[4] Manufactured by Precision Scientific Co., Chicago Ill., No. 9040.

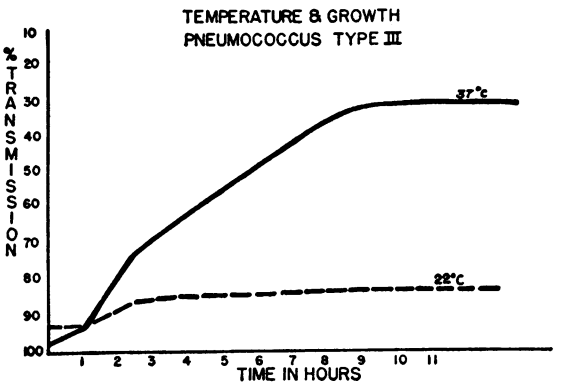

FIG. 1. EFFECT OF HYPOTHERMIA ON *in vitro* GROWTH OF PNEUMOCOCCI III

If, at the end of 3 to 5 hours following the original anesthesia, the animal appeared to be emerging from anesthesia (response to pinching of tail or ear), 0.20 to 0.25 ml. of a 1 to 3 per cent saline phenobarbital solution was given subcutaneously in the back. This combination of anesthesias in the presence of hypothermia served to maintain narcosis for 24 hours.

Twenty-four hours after the induction of hypothermia the animals were removed from the constant temperature box and placed in glass jars at room temperature and allowed to warm spontaneously. In 1 to 4 hours the animals awoke, were running about their cages, and body temperatures returned to normal (37.2° C.).

In each instance where a control animal died during the first 24 hours following inoculation or where a hypothermic animal died within 48 hours following inocu-

TABLE I

Survival following experimental peritonitis—All deaths included *

Experiment number	12 Hours		24 Hours		36 Hours		48 Hours		60 Hours		72 Hours		84 Hours		96 Hours	
	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
15 (10 H & 10 C)	9	10	5	9	4	4	3	0	3	0	0	0	0	0	0	0
16 (9 H & 10 C)	9	10	7	10	6	2	4	2	2	0	0	0	0	0	0	0
17 (20 H & 20 C)	15	20	14	13	14	0	13	0	12	0	9	0	7	0	0	0
18 (15 H & 20 C)	12	20	10	18	10	13	10	1	10	0	8	0	5	0	2	0
19 (10 H & 10 C)	10	10	8	10	8	9	7	1	7	0	5	0	2	0	0	0
20 (20 H & 20 C)	20	20	18	16	12	11	11	0	11	0	9	0	4	0	0	0
21 (20 H & 10 C)	20	10	13	10	12	6	9	2	8	2	1	2	0	2	0	2
22 (20 H & 10 C)	20	10	7	10	7	10	6	1	6	1	6	0	3	0	0	0
23 (20 H & 10 C)	20	10	11	5	9	4	5	0	3	0	1	0	0	0	0	0
24 (20 H & 10 C)	20	10	11	9	10	6	9	0	6	0	0	0	0	0	0	0
Total No. Living	155	130	104	110	92	65	77	7	68	3	39	2	21	2	2	2
Total No. Dead	9	0	60	20	72	65	87	123	96	127	125	128	143	128	162	128

* H = Hypothermia.
C = Control.

TABLE II

Survival following experimental peritonitis—Anesthesia and accidental deaths excluded *

Experiment number	12 Hours H	12 Hours C	24 Hours H	24 Hours C	36 Hours H	36 Hours C	48 Hours H	48 Hours C	60 Hours H	60 Hours C	72 Hours H	72 Hours C	84 Hours H	84 Hours C	96 Hours H	96 Hours C	Hypothermia anesthesia and accidental deaths 12 Hours	Hypothermia anesthesia and accidental deaths 24 Hours	Remarks
15 (9 H & 10 C)	9	10	5	9	4	4	3	0	3	0	0	0	0	0	0	0	1	0	
16 (9 H & 10 C)	9	10	7	10	6	2	4	2	2	0	0	0	0	0	0	0	0	0	
17 (15 H & 20 C)	15	20	14	13	14	0	13	0	12	0	9	0	7	0	0	0	5	0	
18 (12 H & 20 C)	12	20	10	18	10	13	10	1	10	0	8	0	5	0	2	0	3	0	
19 (9 H & 10 C)	9	10	8	10	8	9	7	1	7	0	5	0	2	0	0	0	0	1	
20 (19 H & 20 C)	19	20	18	16	12	11	11	0	11	0	9	0	4	0	0	0	0	1	
21 (16 H & 10 C)	16	10	13	10	12	6	9	2	8	2	1	2	0	2	0	2	0	4	2 control mice continued to live
22 (7 H & 10 C)	7	10	7	10	7	10	6	1	6	1	6	0	3	0	0	0	0	13	13 hypothermic mice accidentally drowned
23 (13 H & 10 C)	13	10	11	5	9	4	5	0	3	0	1	0	0	0	0	0	0	7	California Webster strain of mice used
24 (14 H & 10 C)	14	10	11	9	10	6	9	0	6	0	0	0	0	0	0	0	0—	6	California Webster strain of mice used
																	Total 9	32	Grand Total of 41 deaths due to technical difficulties
Total No. Dead	0	0	19	20	31	65	46	123	55	127	84	128	102	128	121	128			
Total No. Living	123	130	104	110	92	65	77	7	68	3	39	2	21	2	2	2			
Std. Deviation	0	0	4.23	3.74	3.1	4.17	3.49	.82	3.49	.67	3.90	.63	2.55	.63	.63	.63			
χ^2			1.89×10–4		16.5		93.27		85.24		40.15		19.89		0.203				
P	†		.99		<.001		<.001		<.001		<.001		<.001		.50–.70				

* H = Hypothermia C = Control.

† χ^2 need be only 10.82 for P = .001.

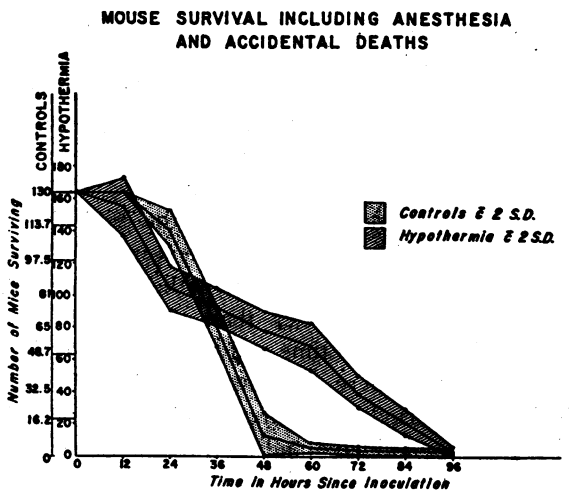

FIG. 2. SURVIVAL FOLLOWING EXPERIMENTAL PERITONITIS—ALL DEATHS INCLUDED

Shaded area represents 2 S.D. on each side of mean.

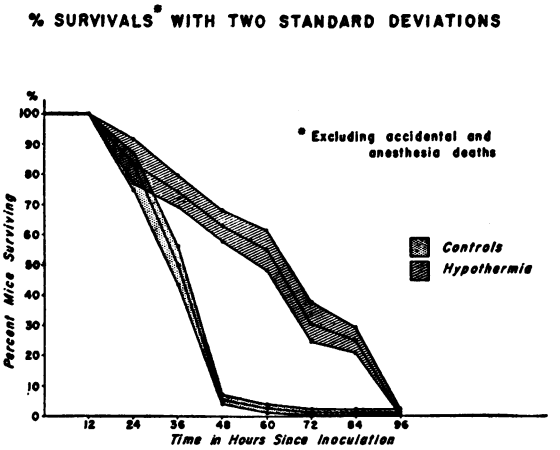

FIG. 3. PERCENTAGE SURVIVALS—EXCLUDING ANESTHESIA AND ACCIDENTAL DEATHS

Shaded area represents 2 S.D. on each side of mean.

lation an autopsy was performed and the cause of death ascertained. This obviated misinterpretation of deaths due to peritonitis from those due to accidental causes or from overdose of anesthesia.

RESULTS

The comparison of pneumococcal growth at 37.5° C. and at 21° C. over a 24-hour period is recorded in Figure 1. It is evident that hypothermia markedly diminishes the rate of bacterial growth.

The dose of pneumococci chosen for this series of experiments purposely was adjusted to cause death in the test animals within a 4-day period. Long-term survivals therefore did not occur. The number of survivors in both the control and hypothermic group is indicated in Table I and Figure 2, which includes all animals included in the study. The statistical analysis of even these raw data indicates hypothermia delays the progress of the course of the infection.

Table II and Figure 3 record the survival rates of the animals, excluding the 41 animals who died from the anesthesia or by some technical accident. Thirteen of these deaths were due to accidental drowning in the constant temperature box (Experiment No. 22). In each case included in this group of 41 animals, postmortem examination confirmed that the animals did not die from pneumococcal peritonitis. These data are shown in Table II. Statistical analysis of these data shows an even more prominent survival of the hypothermic animals, since the accidental and anesthetic deaths

TABLE III

Survival following experimental peritonitis—Excluding anesthesia and accidental deaths with control values moved ahead to end of period of hypothermia

Time (*hrs.*)		Hypothermia		Controls			
Hypothermia	Controls	Survival	Standard deviation	Survival	Standard deviation	x^2	P
0		123	0				
12		123	0				
24	0	104	4.23	130	0	5.67	<0.02
36	12	92	3.10	130	0	16.27	< .001
48	24	77	3.49	110	3.74	19.48	< .001
60	36	68	3.49	65	4.17	0.704	<0.5
72	48	39	3.90	7	0.82	19.05	< .001
84	60	21	2.55	3	0.67	5.07	>0.02
96	72	2	0.63	2	0.63	.0195	0.9

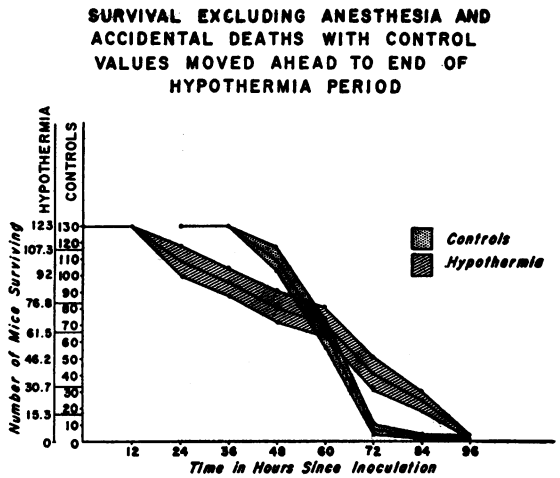

FIG. 4. PERCENTAGE SURVIVALS WITH CONTROL MICE MOVED AHEAD TO END OF PERIOD OF HYPOTHERMIA (24 HOURS)

Shaded area represents 2 S.D. each side of mean.

were all from the hypothermic group. It is evident that the hypothermic animals survive longer than the controls and that this survival is statistically significant.

The period of hypothermia was 24 hours and the foregoing data indicate that hypothermia retarded the progress of infection. In order to determine whether hypothermia did more than merely retard the infection during the period of cooling, animal survivals were plotted with the control animal data moved ahead 24 hours so that determination of survival would begin simultaneously with the hypothermic animals as the latter were removed from their cool environment and began to warm spontaneously (Table III and Figure 4). Such a maneuver weighs survival in favor of the controls, since 19 of the 123 (15 per cent) of the hypothermic animals had expired during anesthesia. None of these animals died because of an obvious accident and in none could postmortem examination definitely prove that infection was not the cause of death, so that these animals were considered to have died from the infection. By the 48th hour survivals among the hypothermic animals were greater than the controls, and this difference is of statistical significance ($p < 0.001$) from this point to the 96th hour when all animals of both groups expired. These data make it evident that the hypothermic animals withstood infection better than the controls as measured by survival time and that this protection was more than mere retardation of the infection during the period of cooling. When the period of cooling is eliminated and both hypothermic and control groups compared from the time of their normothermic conditions, the animals previously cooled outlive their controls.

The control animals given a course of anesthesia similar to the hypothermic animals, but not cooled, expired at a rate similar to the unanesthetized normothermic controls (Table IV and Figure 5). This indicates that the anesthesia did not affect the course of the infection. It was necessary to maintain the anesthetized mice in a carefully warmed room, for it was found that the animals' body temperature dropped to hypothermic levels on relatively short exposure to a cool environment.

DISCUSSION

According to these data hypothermia is associated with prolongation of survival following experimental pneumococcal peritonitis. Cooling depresses *in vitro* bacterial growth and it is to be expected that a similar effect occurs in the infected hypothermic animal. Of importance, as far as ultimate clinical value however, is the relative effect of hypothermia on bacterial growth compared to any change in host immunologic response. Should hypothermia hold back bacterial growth

TABLE IV

Infected warm anesthetized mice compared with infected controls

Time in hours	0 Hours		12 Hours		24 Hours		36 Hours		48 Hours		60 Hours	
	C	A	C	A	C	A	C	A	C	A	C	A
Number Living	130	39	130	39	116	32	65	21	3	1	2	0
Number Dead	0	0	0	0	20	7	65	18	127	38	128	39
% Living	100	100	100	100	80.8	82.0	50.0	53.8	5.4	2.6	2.3	00.0
% Dead	00.0	00.0	00.0	00.0	19.2	18.0	50.0	46.2	94.5	97.4	97.7	100.0

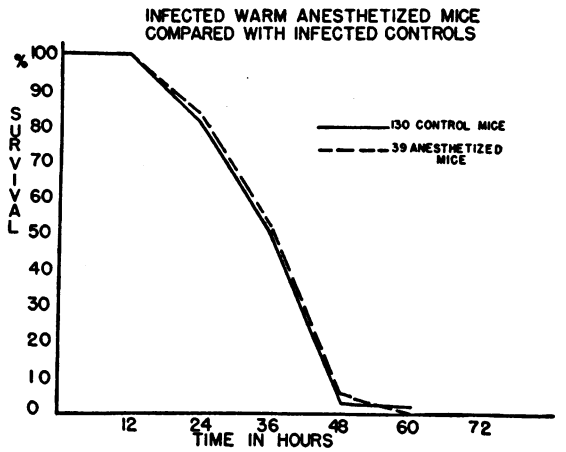

FIG. 5. SURVIVAL OF NORMOTHERMIC CONTROLS COMPARED TO NORMOTHERMIC ANIMALS GIVEN ANESTHESIA IN A SCHEDULE SIMILAR TO THE HYPOTHERMIC GROUP

Shaded area represents 2 S.D. on each side of mean.

and immunologic response at the same rate, no host benefit will be attained in the subject not otherwise treated with bactericidal substances. Thus, if unaltered bacterial growth is resumed with the return of normothermia, and bacterial defense mechanisms have been depressed to a similar degree during the period of cooling, no increased host survival can be anticipated.

Animal survival following infection is determined both by the characteristics of bacterial growth and the immunologic reaction of the host. Since the hypothermic animals in this study had a longer survival than could be accounted for merely by depression of bacterial growth during the period of cooling, it can be concluded that diminution in host resistance is less than reduction in the rate of bacterial growth.

In this study we were unable to determine the absolute nature of the degree of alteration in the immunologic response of the hypothermic animal, since only the combined effect of infection and host resistance has been measured. Studies are currently in progress in which the effect of hypothermia on the host resistance factors, such as the phagocytic index, are being analyzed.

By experimental design no long-term animal survivals have resulted from these studies, but length of survival appears to be a valid relative measurement of bacterial and host response to hypothermia.

SUMMARY AND CONCLUSIONS

1. The effect of hypothermia at 19° C. for a 24-hour period on anesthetized mice with type III pneumococcal peritonitis has been described.

2. There is a statistically significant longer period of survival in the hypothermic animal than in the controls. Such survival is greater than can be accounted for merely on the basis of inhibition of bacterial growth during the actual period of hypothermia.

3. It is suggested that under the conditions of these experiments that bacterial multiplication is inhibited at a rate greater than is diminution of host immunologic response with subsequent beneficial effects on the length of host survival.

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