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FEVER AND SURVIVAL IN RABBITS INFECTED WITH *PASTEURELLA MULTOCIDA*

by

Linda Kay Vaughn

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Physiology)
in The University of Michigan
1977

Doctoral Committee:

Associate Professor Matthew J. Kluger, Chairman
Associate Professor Miriam R. Anver
Associate Professor Louis G. D'Alecy
Associate Professor Nancy R. Harvie
Professor Arthur J. Vander

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INTRODUCTION

There has been much conjecture about the role of fever in disease. In the seventeenth century, Sydenham stated that "fever is a mighty engine which Nature brings into the world for the conquest of her enemies" (DeKruif, 1940). Although this view remained unsupported by direct evidence, it made such sense from a teleological viewpoint, that it became very popular (Bennett and Nicastri, 1960; Schmidt, 1975). It was found that polio virus grew less well *in vitro* at 40°C. than at 37°C. (Lwoff, 1959) and that poliomyelitis could be prevented in monkeys if they were treated with hyperthermia soon after the injection of virus and before the onset of clinical signs (Wolf, 1935). It was found that high temperatures were beneficial in the treatment of neurosyphilis and gonococcal infections (Culver, 1917; Hasler and Spekter, 1936; Bennett, 1954). However, these examples are special cases and do not relate to a general role for fever in disease. In these examples, the maximally tolerated temperature of the disease causing agent is exceeded. This is not generally true in infectious diseases (Bennett and Nicastri, 1960). Also, in these examples, the fevers are not naturally occurring as a result of the disease. The high body temperatures are produced artificially by placing the animal in a hot room, injecting them with toxins, etc.

There has been one example where a naturally occuring fever has been shown to be beneficial in a mammal. In rabbits infected with some strains of pneumococcus III, inhibiting the fever with the use of barbiturates or placing the rabbit in a cold room will

cause the rabbits to survive less well than those rabbits allowed to develop a fever (Rich and McKee, 1936; Strouse, 1909; Enders et al., 1936). Again, however, this is a special case where the bacteria is killed by the temperatures reached by the fever.

Even though the belief that fever is beneficial is prevalent, the alternative view, that the reduction of fever by drugs or other means has no observable influence on the course of infection, also exists (Bennett and Nicastri, 1960). The wide use of antipyretic drugs attests to the fact that this view is widely held.

There are cases where hyperthermal treatment may be harmful and these cases are sometimes used in arguments for the thesis that fever is detrimental. Forty to seventy percent of the individuals treated with fever therapy develop herpes simplex (Schmidt, 1975). Also, rheumatic pain may be worsened by inflammatory effects of hyperthermal treatment (Ott, 1968). These are, again, cases of hyperthermal treatment and not naturally occuring fever.

In an effort to determine some generalizations about the role of fever in disease, there have been four types of experiments:

1. Attempts to correlate the fever of infected patients with their mortality rate.
2. Determinations of the physiological changes that occur with infection and fever and how these may affect survival.
3. Determination of the effects of hypo- or hyperthermal treatment on survival after the injection of endotoxin

or bacteria.

4. Determination of the role of fever in ectothermic animals and attempts to extrapolate this to the role of fever in mammals.

1. Correlation Studies

Correlations between fever and survival in clinical cases have been done (Bennett and Nicastri, 1960) but interpretation is difficult. The results seem to indicate that fever parallels the severity of infection. Since all patients were not equally infected, the effect of fever can not be determined. The use of antibiotics further complicates the interpretation.

2. Physiological Changes

In an effort to determine whether fever is beneficial, there has been much research on what physiological changes occur with fever and how these may affect survival. These changes are shown in Table 1. Some of these changes are difficult to interpret because they occur after an injection of endotoxin. It is difficult to determine if the changes were brought about by the increased temperature or some other effect of endotoxin. Other changes occurredwith fever therapy, such as changes in electrolyte balance, and it is difficult to know if they would occur with a naturally occurring fever. The survival value of some of these changes (such as changes in carbohydrate metabolism) is unclear. Other changes have more obvious value and warrant further discussion.

In frogs, fish, insects, and bats, it has been found that an increase in temperature will cause an increase in antibody

TABLE 1

PARAMETERS THAT MAY CHANGE WITH INFECTION AND FEVER

Parameter	Reference
Metabolic Rate	
Carbohydrate Metabolism	Kirstein and Bromberg, 1939 Somogyi and Kirstein, 1940 Loseki and Gunderson, 1941
Plasma Proteins	Reimann, 1932; Kopp, 1942
Plasma Copper	Beisel et al., 1974
Vitamin Requirements	Zook and Sharpless, 1938 Daun et al., 1939; Aron et al., 1946; Oshome and Farmer, 1942
Liver Function Tests	Hicks et al., 1948
Electrolyte Balance	Gordon et al., 1949 Hicks et al., 1948
Blood Flow	Kisseri and Bierman, 1933
Lymph Flow	McCarrell, 1940
Leukocyte Counts	Simon, 1936; Doan, 1938 Cohen and Warren, 1935 Habler et al., 1952
Antibody Levels	see text
Phagocytic Activity	see text
Lymphocytic Activity	see text
Plasma Iron	see text
Inflammatory Response	see text

formation (Bisset, 1948; Allen and McDaniel, 1937; Kalabuchov and Levinson, 1935). In rabbits, however, the elevation of body temperature caused a decrease in antibody levels (Ekker and O'Neal, 1932; and Hadjopoulos and Bierman, 1934) or antibody levels remained the same (Ellingson and Clark, 1942). Koomen and Morgan, (1954), showed that fever in man did not influence the anamnestic response to antigens.

Human and frog (Rana pipiens) lymphocytes showed more activation (increased uptake of radioactive thymidine) when incubated in vitro at 39°C. and 22°C. respectively, than when incubated at 37°C. or 15°C. (Ashman et al., 1976; Wright and Cooper, 1976). Also, leukocytes from humans and guinea pigs showed maximal phagocytic activity between 38°C. and 40°C.,and 39°C. and 41°C., respectively (Ellingson and Clark, 1942).

It has been shown that fever facilitates early inflammatory responses in lizards injected with bacteria (subcu). Fever leads to more marginated and tissue granulocytes, an earlier elimination of bacteremia, lower tissue bacterial counts, and a containment of the local infection (Bernheim et al., 1977). Also, fever may increase the intracellular killing power of lizard polymorphonuclearleukocytes (Bernheim, personal communication).

Finally, the change in plasma iron which is associated with infection may lead to an increased survival. The requirement for iron by bacteria increases at febrile temperatures because of a decreased ability to produce iron transport compounds (Garibaldi, 1971, 1972; Bullen et al., 1974). However, infection is associated

with a decreased absorption of iron from the gut, an increase in the amount of iron transferred to the liver, and a decreased plasma iron levels (Weinberg, 1974; Dubach et al., 1948; Kochan, 1973; Pekarek et al., 1971; Cortell and Conrad, 1967). Therefore, with a fever, a change associated with infection becomes detrimental for the growth of bacteria.

3. Effects of Hypo- or Hyperthermia

Some investigators have attempted to determine the role of fever in disease by injecting endotoxin, (the lipopolysaccharide component of the cell wall of gram-negative bacteria), subjecting the animals to hypo- or hyperthermia, and determining whether there is increased or decreased survival. Connor and Kass (1961) found that elevating ambient temperature increased the susceptability of mice to the lethal action of endotoxin. Porter and Kass (1962) found a similar effect in rats. Atwood and Kass (1964) found that in rabbits, elevating the ambient temperature to 37°C. augmented the febrile response to endotoxin, reduced the LD50 of endotoxin, and accelerated the lethal effects of endotoxin. Shaving the rabbits produced hypothermia (rectal temperatures 2.5 to 6.5°C. below normal) and the LD50 of endotoxim increased about ten-fold. These results imply that fever is harmful.

However, there are two major objections to the methods used by Kass and his associates. The first is that they used endotoxin rather than bacteria. It is certainly conceivable that the body may react differently to bacterial infection than to an

injection of endotoxin, either because the bacteria might have a longer time span of effectiveness or because of some toxic or damaging effects that bacteria have in addition to those of endotoxin. McGill et al. (1967) found that endotoxin in sera of patients with gram-negative sepsis is not associated with increased toxicity or lethality. Rolleston (1911) pointed out that the clinical picture of E. coli infection in humans does not resemble that of experimental animals given E. coli toxins. Waisbren (1964) concluded in a review on gram-negative shock and endotoxic shock that gram-negative shock is not caused by endotoxin and might be instead some type of hypersensitivity reaction. In view of these facts, the beneficial effect of hypothermia on lethality due to endotoxin may not be evidence for a beneficial effect of hypothermia on lethality due to bacterial infection.

A second criticism of Kass' work is that changes in body temperature were produced physically by shaving and/or placing the animal at high or low ambient temperatures. While such manipulations change body temperature, they do nothing to the febrile state. The animal still has an elevated hypothalamic set point and will be functioning as if it had a fever (Cooper, 1972). Since the animal's temperature is below its set point, it will be activating physiological and behavioral mechanisms to increase its temperature (shivering, vasoconstriction, changes in blood flow distribution, increased metabolic rate, and possible changes in catecholamines and cortisol). Similarly, with hyperthermia produced by a high ambient temperature, the animal will have an elevated body

temperature but this is not done actively. In fact, the animal will actively try to lower its core temperature by physiological and behavioral means. It is conceivable that the mechanisms used to increase body temperature are important in causing fever to be beneficial or harmful, instead of, or in addition to, the actual increase in temperature. Therefore, the conclusions reached by physical alteration of body temperature could be erroneous.

One article has been published which used live bacteria rather than endotoxin. Klastersky (1971) injected rabbits with pneumococcal bacteria and found that shaved rabbits at 20°C. ambient temperature had a significantly lowered mortality rate than rabbits allowed to develop a fever. He injected a dose of bacteria which was 100% lethal when antibiotics were not given. All rabbits during the experiment were given penicillin six hours after the injection of bacteria. With 1,100,000 units of penicillin, the difference in survival between shaved rabbits and controls was not significant. The addition of 500,000 more units per hour of penicillin produced a statistically significant ($p<0.02$) decrease in mortality in the shaved animals compared to the controls (31% died vs. 46% died) suggesting that an elevation in temperature is harmful. Another result of this experiment was that the suppression of fever favored the persistance of the bacteria. Eighteen hours after injection of bacteria, 100% of the hemocultures from the controls were sterile but only 75% of the shaved rabbits' hemocultures were sterile. Klastersky felt that the increased survival of the bacteria was due to the decreased

ability of the penicillin to kill the bacteria. This was based on *in vitro* tests that showed a decreased number of bacteria were killed by penicillin as temperature decreased from 40 to 35°C. However, it appears from his figures that the difference between pneumococcal survival with penicillin at 37 and 40°C. was very slight (no statistics were presented). Klastersky also used injections of 100 mg cortisol to control the temperature of the rabbit. The antipyretic effects and the increased survival were similar to the effects due to shaving the animals. Actual data were not presented in this article, only graphs of the temperature response of representative animals were shown and tables showing mortality figures. It is unknown whether antipyresis was effective in all animals because it was not stated whether all rabbits' temperatures were monitored.

There are several facets of Klastersky's work which leave the question of the benefit of fever unresolved. First, he suppressed body temperature by physical means as did Kass' group or by the use of cortisol. The disadvantages of using physical means were stated previously. The control of body temperature by the injection of cortisol is confounding since the change in mortality could be due to any of the other effects of the drug rather than the antipyretic effect. Secondly, all his experiments were done with the use of penicillin. Differences in survival could be due to the effect of temperature on the bactericidal powers of penicillin rather than an effect of fever on the defence mechanisms of the host. This does not appear too likely, however,

since animals survived better at low temperatures in spite of an increased number of positive hemocultures. However, if the products of phagocytosis were more harmful in large doses than the bacteria themselves, it is possible that killing large numbers of the bacteria in a short time might be disadvantageous. Therefore, a decreased killing ability of the penicillin could be advantageous. Furthermore, penicillin interferes with the fever producing capabilities of pneumococcus (Bennett, 1956) and therefore interferes with the experimental design. Also, it could be that an increase in temperature is generally unhealthy in itself but is beneficial in helping kill bacteria. With penicillin to take some of the bacteria killing load, then increased temperature may no longer be beneficial. In any case, it is apparent that the use of antibiotics complicates the interpretation of the experiment. A third point is that Klastersky used a gram-positive bacteria, pneumococcus. Fever due to pneumococcus can be prevented by prior injection of penicillin (Bennett, 1956). Since Klastersky used pneumococcus and penicillin, perhaps even his control animals were not allowed to develop a full fever. This may be one of the reasons that the fever only lasted about 18 hours in his experiments.

Therefore, experiments on the effects of hypo- or hyperthermia on survival after an injection of endotoxin or bacteria have not answered the question of the effect of fever or antipyresis on survival in bacterially infected animals.

4. Fever in Ectothermic Animals

Because of the ease with which the temperature of ectothermic animals can be controlled, definitive research determining whether fever is beneficial to bacterially infected animals has been done in reptiles and fish. Vaughn et al. (1974) showed that lizards, Dipsosaurus dorsalis, will develop a fever of 40 to 42°C. in response to an injection of dead bacteria (Aeromonas hydrophila) and Kluger et al. (1975) showed that survival of bacterially infected lizards is related to the magnitude of the fever that they develop. Lizards, D. dorsalis, whose normal preferred body temperature is 38°C. were placed in temperature controlled environments of 34, 36, 38, 40, and 42°C. and injected with live A. hydrophila. The survival rate was dramatically correlated with body temperature. 75% of the lizards with a body temperature of 42°C. survived, but only 25% of the lizards with a body temperature of 38°C. survived, and none of the lizards with a temperature of 34°C. survived. It was also shown that drug-induced antipyresis will decrease survival of lizards infected with bacteria (Bernheim and Kluger, 1976). When 12 lizards were injected with bacteria and sodium salicylate, the 5 animals who increased their body temperature at least 0.6°C survived a week but the 7 lizards that did not develop a fever died within 3 days. Therefore, preventing D. dorsalis from developing a fever after an injection of A. hydrophila either by drug-induced antipyresis or by behaviorally preventing a fever increases the mortality rate.

Similar results have been obtained with fish. When teleost

fish are injected with live or dead bacteria, they will develop a fever (Reynolds et al., 1976; Reynolds and Covert, 1977) and this fever increases the fish chance of surviving infection with A. hydrophila (Covert and Reynolds, 1976).

In conclusion, while there is considerable evidence which suggests that fever is beneficial in bacterially infected lizards and fish, the question of the relationship between fever and survival in bacterially infected mammals remains unanswered.

The present study was done in attempt to determine if there is a correlation between fever and survival in mammals. The effects of drug-induced antipyresis on the survival rate of bacterially infected rabbits was also investigated.

In order to investigate these questions, New Zealand white rabbits (Oryctolagus cuniculus)were injected with various doses of Pasteurella multocida, a bacterium known to be pathogenic to rabbits. After the bacteria were injected, the resultant fevers were correlated with survival rate. The effects of antipyretics on survival were investigated by comparing the relationship between fever and survival in rabbits infused with either nontoxic doses of antipyretics or saline.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits (Oryctolagus cuniculus) obtained from Langshaw Farms (Agusta, Michigan) and weighing 2-4 kg were used in these experiments. The rabbits were housed in standard stainless steel cages (45x60x40 cm) and fed Purina Rabbit Chow and water ad libitum. The rooms containing the rabbits were lighted from 0600 to 1800 and were dark from 1800 to 0600. Only rabbits that did not show clinical signs of pasteurellosis such as rhinitis, conjuctivitis, abscesses, or sneezing were used.

General Protocol

Most experiments involved the recording of the rabbits' abdominal temperatures, the injection of bacteria, the infusion of antipyretic drugs or saline, and the monitoring of survival rate.

Implantation of Catheters and Thermocouples. In order to record abdominal temperatures and infuse drugs intraperitoneally, thermocouples and catheters were implanted in the abdomen of the rabbits. To accomplish this, rabbits were given an i.m. injection of 1cc acepromazine (10 mg/ml) (a tranquilizer) and a mid-dorsal subcu. injection of 1 cc lidocaine HCl (20 mg/ml) (a local anesthetic). A small incision was made through the skin of the mid-dorsum, and a sterile stainless steel trochar (3.2 mm outside diameter) was threaded under the skin to the side of the abdominal wall and inserted into the abdomen. Thermocouples encased in sterile polyethylene tubing (P.E. 50) and sterile

catheters (P.E.160) filled with sterile, pyrogen-free saline, were threaded through the trochar into the abdomen, the trochar removed, and the thermocouple and cathether were sutured to the skin of the back. A neck restrainer similar to that used by Hampton (1973) prevented the rabbits from destroying the catheters and thermocouples while still allowing the rabbits considerable mobility and free access to food and water. The rabbits were allowed 24 hours to recover from implantation and to adapt to the neck restrainers.

Temperature Measurement. Abdominal temperature was recorded approximately every minute using a Honeywell Electronik 112 multipoint temperature recorder. Twenty-four hours of control temperature data were collected beginning 24 hours after implantation. Temperature data for the experimental period were collected for over 4 1/2 days after the injection of bacteria except where noted.

The temperatures of the rabbits were calculated by inspecting the 60 temperature points per hour and determining average hourly temperatures for each rabbit. The temperatures of the 24 hours of the control day were averaged to determine the baseline temperature. To determine the rabbits' fevers, the average temperature of the 24 hours beginning 6 hours after injection of bacteria was calculated. The baseline temperature of each rabbit was then subtracted from this value and the result was considered the rabbit's fever in all cases except where noted. This time period was used because most of the mortality (76%) occurred withing this time period. The first 6 hours was excluded since the degree of

fever during this time period was unaffected by the infusion of antipyretic drugs.

Preparation of Bacteria for Injection. A stock culture of Pasteurella multocida, a gram-negative bacterium pathogenic to rabbits (Flatt, 1974), obtained from American Type Culture (#7228) was maintained in Bacto Stock Culture Agar (Difco Laboratory) and stored at room temperature. Two days before needed for an experiment, 10% sheep blood, trypticase soy agar plates were inoculated with the bacteria. The inoculated plates were stored at 37°C. for 48 hours. The bacteria were then suspended in sterile, pyrogen-free, 0.9% sodium chloride, centrifuged, washed with the saline, recentrifuged, and resuspended in saline. The concentration of bacteria was determined spectrophotometrically, using a standard curve determined from bacteria whose concentrations were determined by McFarland Barium Sulfate turbidity tubes. In order to prevent possible diurnal variations in survival (see for example, Haus et al., 1974) and temperature response, injections of the bacteria were made at approximately the same time of day in each experiment (1430-1530 h.).

Infusion of Antipyretic Solution or Saline. Each rabbit was infused via a Harvard infusion pump with either sterile, pyrogen-free 0.9% sodium chloride solution or a sterile solution of an antipyretic drug dissolved in saline. In order to lessen the effect of variations in the number of bacteria injected from week to week, half of the rabbits were infused with control saline solution and half received the antipyretic solution. Infusions

began two hours prior to injection of the bacteria. The sterility of the antipyretic solution was ensured by passing the solution through a sterile Gelman Metricel filter (pore size 0.20 microns) into a sterile glass flask. The solution was stored at 4°C. for not over a week in sterile bottles. The solution was infused using sterile plastic syringes and sterile needles.

Sterilization. Glass, metal, and filtering equipment were sterilized by autoclaving in a Castle Thermatic 60 at 121°C. for 30 minutes. Syringes, catheters, and thermocouples were gas sterilized.

Survival Rate. Mortality was monitored for 4 1/2 days after the injection of the bacteria. When an animal was dead, the abdomen was inspected and the location of the thermocouple and catheter noted. If the thermocouple or catheter were not in the abdomen, the data from the rabbit were not used. All animals which survived the 4 1/2 days after injection of bacteria were considered survivors.

Specific Experimental Protocols

Proper Dose of Antipyretic. Various doses of sodium salicylate, acetaminophen, and a combination of sodium salicylate and acetaminophen were infused into rabbits that were injected with different doses of bacteria. The rabbits were prepared as described above. In each experiment, half of the rabbits received a dose of antipyretic and half received a corresponding volume of saline. Rabbits were injected intravenously with bacteria. The fever of

the rabbits infused with antipyretics was compared with the fever of the rabbits infused with saline.

Three doses of sodium salicylate were used: 300 mg/day, 500 mg/day, and 600 mg/day. The 300 and 600 mg/day doses were infused in saline at a rate of 100 ml/day. The 500 mg/day dose was infused in saline at a rate of 4 ml/day. One dose of acetaminophen was used: 1000 mg/day. A combination of 1000 mg/day acetaminophen and 300 mg/day sodium salicylate was also used.

To test whether 300 mg/day sodium salicylate or 1000 mg/day acetaminophen were toxic, $6x10^{10}$ organisms of P. multocida were injected and the mortality rate was compared between rabbits infused with each drug singly and rabbits infused with saline. Since the temperature was not significantly changed when each drug was given singly, any difference in mortality between rabbits infused with the drug or with saline could be attributed to side effects of the drug. The rabbits were prepared as described above and mortality was monitored for 4 1/2 days after the injection of bacteria.

Dose of Bacteria. Several doses of P. multocida ($1x10^{10}$, $3x10^{10}$, and $6x10^{10}$ organisms) were injected using the procedures described in the general protocol section in order to determine a dose that would kill about 50% of the rabbits and to provide a dose at which antipyretics would be effective.

Bacterial Characteristics. A biochemical profile was run by the diagnostic laboratory of the Unit for Laboratory Animal Medicine to confirm that the bacterium being used was P. multocida.

To determine if there is a difference in the ability of

P. multocida to grow at febrile and control temperatures, in vitro growth curves were done. Sterile flasks containing sterile Bacto-Brain Heart Infusion were inoculated with P. multocida from the stock cultures. The inoculated broth was incubated for 18 hours at 37°C. At the end of this time, 0.5 ml of this broth was placed into sterile tubes containing 9.5 ml of sterile brain heart infusion. The tubes were then placed in shaking water baths at 39 or 42°C. Samples of the broth were taken periodically for 6 hours and the turbidity determined spectrophotometrically (700μ).

Fever and Survival. To determine the correlation between fever and survival, rabbits were prepared using the procedures described in the general protocol section. Rabbits were injected via the marginal ear vein with $3x10^{10}$ or $6x10^{10}$ organisms of P. multocida and infused with antipyretics or saline. The fever and mortality rate were determined as described above and the correlation between them plotted for both the rabbits receiving saline and those receiving antipyretics. For statistical analysis, the rabbits were grouped into fever ranges. It was found that fever ranges of 0.75°C. showed the relationship between fever and survival clearly and provided a large enough sample size in each group so that Chi squared tests could be done on the relationship in rabbits infused with antipyretics. In order for Chi squared tests to be done on the data from rabbits infused with saline, animals had to be grouped into fever ranges of 1.5°C., but to show the relationship more clearly, plots were done with 0.75°C. fever ranges. Probit analysis (see statistics section)

was also done on the data to determine if the amount of fever developed was a good predictor of survival rate.

Chi squared tests and Probit anaysis were done on the above data to determine if there were differences in the survival rate between rabbits infused with saline and those infused with antipyretics.

Blood Pressure. The rabbits were tranquilized with a 1 ml injection of acepromazine intramuscularly and 20 minutes later, anesthetized with sodium pentobarbital (15 mg/kg) via the marginal ear vein. The right carotid artery was isolated and a sterile polyvinyl catheter (PB3, outside diameter 0.62mm) was inserted into the aortic arch. The catheter was secured to the musculature of the neck and threaded under the skin and brought through the small incision in the mid-dorsum which was used for abdominal implantation. Furacin (a topical antibacterial ointment) was placed on the wound, and the incision sutured closed. The rabbit was prepared for abdominal infusion and temperature measurement and injection of bacteria as described in the general protocol section.

The blood pressure was monitored using a Grass polygraph. The blood pressure catheter was connected to a Statham pressure transducer (either model #P23Dc, P23Db, or P23Gb) which was connected to a Grass preamplifier (model 7P1E). Mean and pulsatile blood pressure was monitored continuously for the entire experiment.

The difference in blood pressure before and after injection of bacteria (using the 24 hour period beginning 6 hours after

injection) was determined and its statistical significance analyzed using the paired t-test. The correlation between the blood pressure response and the febrile response was made.

Statistics

The statistical significance of the data described in this thesis were evaluated using the Student's t-test, the paired t-test, one way analysis of variance, chi squared test, Fisher exact probability test, and Probit analysis. The first four test are common standard statistical test and need not be described here.

The Fisher exact probability test determines whether two populations have an equal proportion in two non-overlapping classes (e.g. whether the proportion of antipyretic rabbits dying is equal to the proportion of saline rabbits dying). The advantage of this test is that smaller sample sizes are required than with the Chi squared test (Siegel, 1956).

Probit analysis is a statistical test which determines a regression equation from which the probability of an event (in this case a rabbit surviving a bacterial infection) can be predicted by some independent variables (in this case the amount of fever developed by a rabbit and the drug that was infused). If the coefficients for the independent variables in the regression equation are significantly different from zero (Probit tests this also), one can say that the variables significantly affect survival. The advantage of this test is that actual fevers can be used (no need for grouping into fever ranges) and the effect of two independent variables can be tested at the same time (fever and antipyretic drugs) (Natrella).

RESULTS

A. Thermal Responses

The temperature response of rabbits when Pasteurella multocida is injected is shown in figure 1. One dose of dead bacteria ($6x10^{10}$ organisms) and three doses of live bacteria ($1x10^{10}$, $3x10^{10}$, and $6x10^{10}$ organisms) were injected. This was done in an effort to characterize the febrile response over a longer period of time than had previously been published, to provide data which would allow comparisons between different doses of bacteria, to allow a comparison of the febrile response with dead and live bacteria, and to provide control data from which the effectiveness of antipyretic therapy could be determined. There are several points of interest.

1) The first six hours after the injection of bacteria are nearly identical regardless of which dose of live or dead bacteria was injected.

2) The fever is bimodal or trimodal. (See table 2) The first peak occurs 3 hours after injection of all doses. The first trough occurs 5 to 6 hours after injection. The time at which the second peak occurs varies with the dose of bacteria and occurs from 8 to 13 hours after injection. When the high and medium dose of bacteria are injected, there appears to be a third peak 17 to 20 hours after injection. When the average temperatures at the peak are compared to the average temperatures at the preceeding trough, this third peak is statistically significant ($p<.001$, Student's t-test) when the medium dose is injected but not stat-

Figure 1. Average abdominal temperatures of rabbits before and after injection of 3 doses of live and one dose of dead P. multocida. Vertical bars represent ± one SE. Open circles represent 30 rabbits injected with $3x10^{10}$ organisms live P. multocida. Triangles represent 46 rabbits injected with $6x10^{10}$ organisms live P. multocida. Squares represent 8 rabbits injected with $1x10^{10}$ organisms live P. multocida. Closed circles represent 4 rabbits injected with $6x10^{10}$ organisms dead P. multocida.

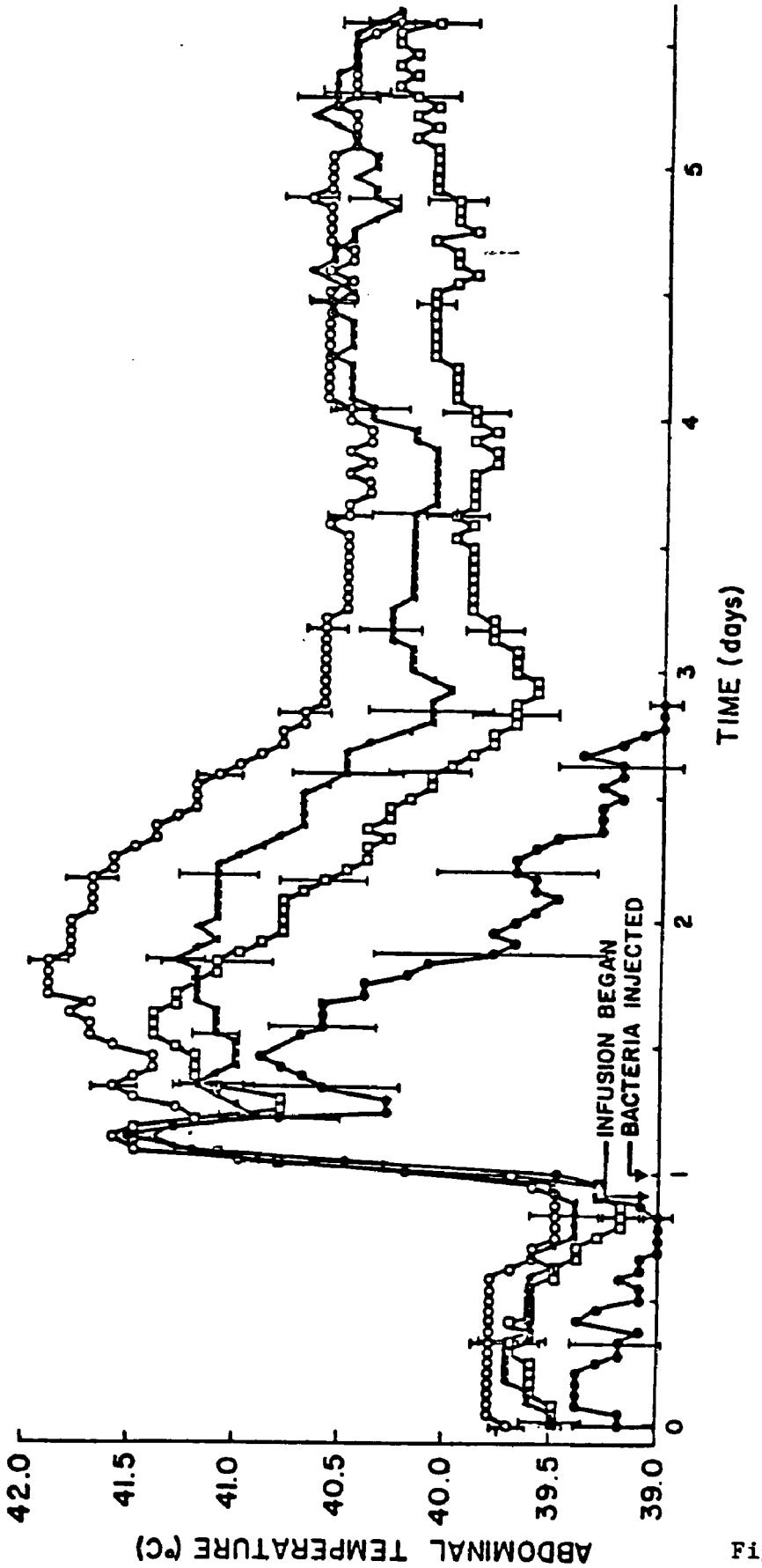

ABDOMINAL TEMPERATURE (°C)
42.0
41.5
41.0
40.5
40.0
39.5
39.0
0
1
2
3
4
5
TIME (days)
INFUSION BEGAN
BACTERIA INJECTED

Figure 1

TABLE 2

FEBRILE CHARACTERISTICS

Bacterial Dose (organisms)	Fever (°C. ± SD)	Peak 1 (hrs. post injection)	Peak 2 (hrs. post injection)	Peak 3 (hrs. post injection)	Fever Duration (days)
$1x10^{10}$ live	1.55 ± .52	3	13	-	> 4.5
$3x10^{10}$ live	1.92 ± .63	3	8	17	> 4.5
$6x10^{10}$ live	1.55 ± .63	3	8	20	> 4.5
$6x10^{10}$ dead	.97 ± .53	3	11	-	1.5

istically significant with the high dose ($p>.05$, Student's t-test).

3) The magnitudes of the fevers differed with the dose of bacteria. (See table 2) The 24 hour average fever beginning 6 hours after injection was 1.92°C. when the medium dose of bacteria was injected and 1.55°C. when the low or high dose was injected. The difference in the three means were statistically significant ($p<.05$, one way analysis of variance).

The 24 hour average fever of those animals injected with $6x10^{10}$ live bacteria was significantly greater than those injected with $6x10^{10}$ dead bacteria ($p<.05$, Student's t-test).

4) The duration of the fevers differed. When the dead bacteria were injected, the rabbits' temperatures were back to baseline levels in 30 to 36 hours. The temperatures of the rabbits which were injected with live bacteria were still elevated four and one half days after injection. (See figure 1)

B. Antipyretic Therapy

The appropriate dose of an antipyretic drug when various doses of bacteria were injected had to be determined before the effect of antipyretic therapy on survival could be tested.

The effectiveness of sodium salicylate alone was first tested. (See table 3). There was no difference ($p>.80$, Student's t-test) in the average fever after injection of $6x10^{10}$ organisms of P. multocida between 11 rabbits infused with 300 mg sodium salicylate per day and 12 rabbits infused with saline. When the dose of

TABLE 3

EFFECT OF ANTIPYRETICS

Dose of Antipyretic (mg/day)	Dose of Bacteria (organisms)	Effect
300 (Na salicylate)	$6x10^{10}$	decreased fever 0.0°C.
500 (Na salicylate)	$1x10^{10}$	decreased fever 0.1°C.
600 (Na salicylate)	$3x10^{10}$	increased mortality
1000 (acetaminophen)	$6x10^{10}$	increased fever 0.1°C.
300 (Na salicylate)+ 1000 (acetaminophen	$6x10^{10}$	decreased fever 0.3°C.
300 (Na salicylate)+ 1000 (acetaminophen)	$3x10^{10}$	decreased fever 0.7°C.

bacteria was decreased to $1x10^{10}$ organisms and the dose of salicylate was increased to 500 mg/day, there was still no significant difference in the fever between animals infused with salicylate and those infused with saline (n=10 salicylates, 8 salines, $p>.50$, Student's t-test). It was not possible to increase further the dose of salicylate since when 600 mg/day were infused and $3x10^{10}$ organisms of bacteria were injected, the mortality response indicated that the salicylates caused an increased number of rabbits to die because of toxic side effects. Seven out of seven rabbits infused with salicylates died but only three out of seven rabbits infused with saline died ($p<.035$, Fisher exact probability test). Therefore, it was concluded that sodium salicylate alone was not an effective antipyretic.

The effectiveness of acetaminophen alone in reducing a fever was also determined. Rabbits infused with 1000 mg/day acetaminophen (the maximum amount that could be infused) had a 0.13°C. higher average fever after injection of $6x10^{10}$ organisms of P. multocida than rabbits infused with saline ($p>.50$, Student's t-test, n=10 acetaminophens, 10 salines).

The effectiveness of both sodium salicylate and acetaminophen together can be seen in figure 2. 300 mg/day sodium salicylate and 1000 mg/day acetaminophen were infused. The difference between the temperatures of rabbits infused with antipyretics compared to those infused with saline is plotted for both those rabbits receiving $6x10^{10}$ and $3x10^{10}$ organisms. It can be seen that when $3x10^{10}$ organisms are injected, the temperature is statis-

Figure 2. Difference in average abdominal temperature of rabbits infused with antipyretics and those infused with saline before and after injection of $3x10^{10}$ (closed circles, n= 30 antipyretics, 30 saline) and $6x10^{10}$ organisms (open circles, n=51 antipyretics, 46 saline). The temperature of rabbits infused with saline are considered 0.0°C. and the relative temperatures of rabbits infused with antipyretics are plotted. Vertical bars represent $\pm$ 1 SE. Solid bars indicate SE of rabbits injected with $3x10^{10}$ organisms and dotted bars represent SE of rabbits injected with $6x10^{10}$ organisms. When bars are close, the one to the right is the SE of rabbits infused with antipyretics. Hours when the difference in temperature between antipyretics and salines are statistically significant ($p<.05$, Student's t-test) are indicated by open and closed circles on the bottom of the graph ($6x10^{10}$ and $3x10^{10}$ organisms respectively).

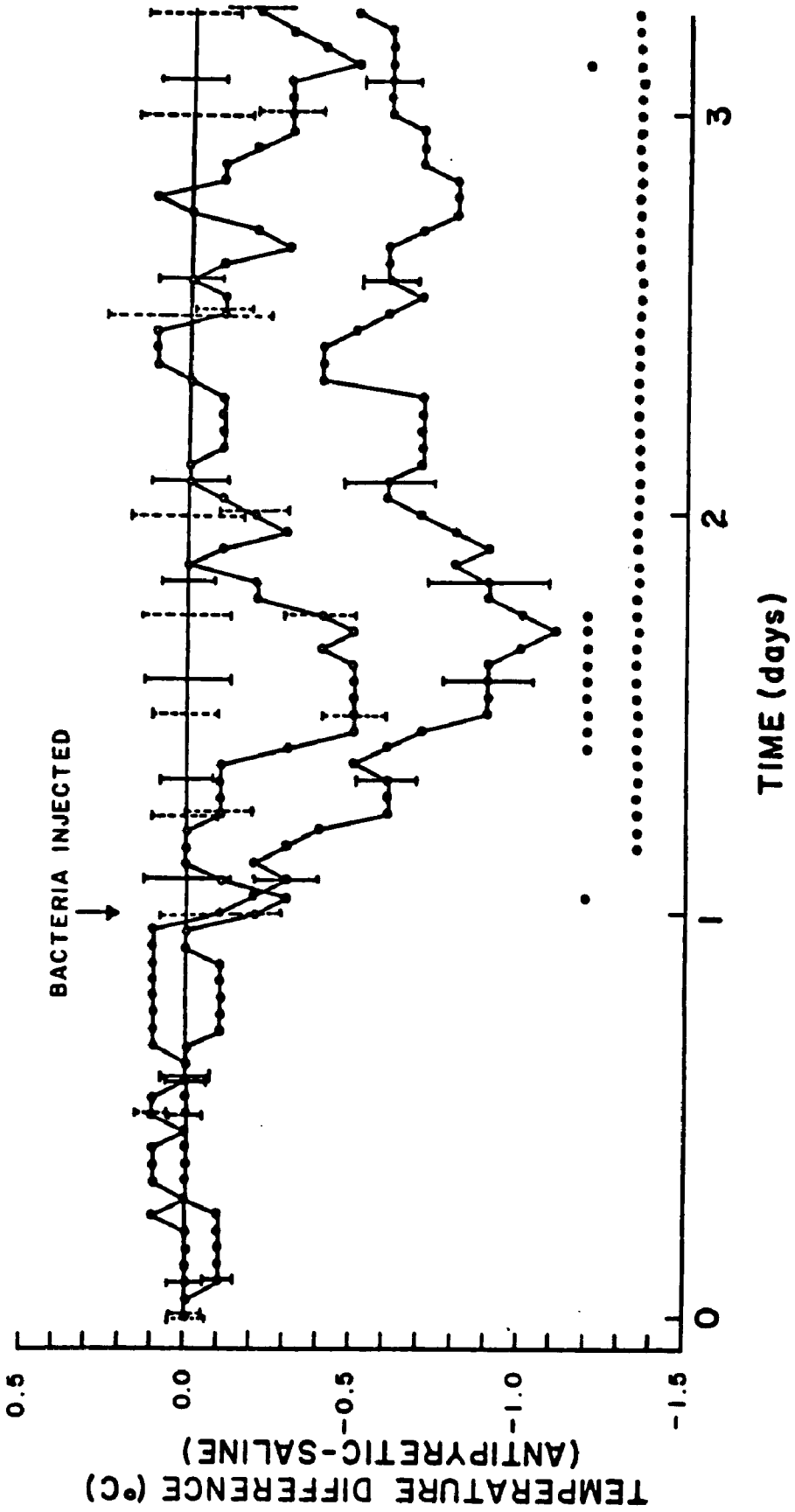

TEMPERATURE DIFFERENCE (°C)
(ANTIPYRETIC-SALINE)
0.5
0.0
-0.5
-1.0
-1.5
0
1
2
3
TIME (days)
BACTERIA INJECTED

Figure 2

tically lower when antipyretics are infused. The temperature becomes statistically lower the fifth hour after injection of bacteria. There is a decrease of 0.73°C. ($p<.001$, Student's t-test) during the 24 hour period beginning 6 hours after injection. When $6x10^{10}$ organisms were injected, however, there was only a 0.32°C. decrease in temperature during the same 24 hour period when antipyretics were infused ($p<.005$, Student's t-test). While this is statistically significant, the magnitude of change is small. Also, the statistical significance is accounted for by only a 9 hour period.

The combination of antipyretic drugs was also effective in changing the distribution of rabbits with high and low fevers. A histogram of the number of rabbits in different fever ranges is shown in figure 3. There is a statistically significant difference in the number of rabbits with a low (<1.5°C.) or high (>1.5°C.) fever, depending on whether antipyretics or saline was infused ($p<.05$, Chi squared test).

To test whether 300 mg/day sodium salicylate or 1000 mg/day acetaminophen were toxic, the mortality rate after $6x10^{10}$ organisms of P. multocida were injected when each drug was given without the other was compared to the mortality rate after injection of bacteria when saline was infused. Since the temperature was not significantly changed when each drug was given alone, any difference in mortality between rabbits infused with the drug or with saline could be attributed to side effects of the drug. There was no difference in mortality between rabbits

Figure 3. Histogram illustrating the number of animals infused with saline or antipyretics and injected with $6x10^{10}$ organisms of P. multocida in fever ranges of 0.5°C. The fevers are calculated from the 24 hour period beginning 6 hours after injection of bacteria. Diagonal bars represent rabbits infused with antipyretics. Dotted bars represent rabbits infused with saline.

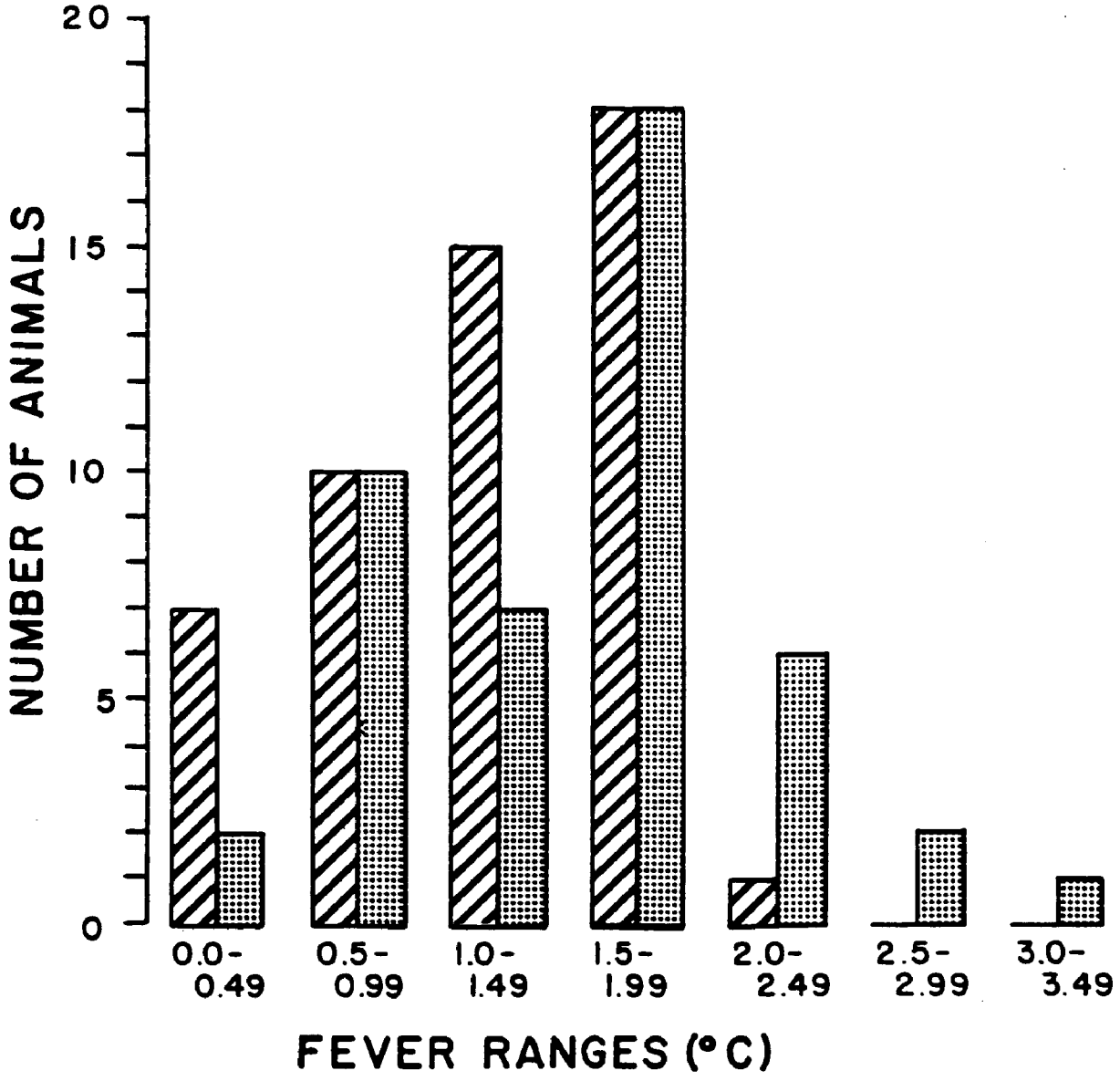

20
15
10
5
0
NUMBER OF ANIMALS
0.0-
0.49
0.5-
0.99
1.0-
1.49
1.5-
1.99
2.0-
2.49
2.5-
2.99
3.0-
3.49
FEVER RANGES (°C)

Figure 3

when 300 mg/day sodium salicylate were infused. Four out of eleven rabbits infused with salicylate died and three out of twelve rabbits infused with saline died ($p>.40$, Fisher exact probability test). There was also no difference in the mortality rate when 1000 mg/day acetaminophen were infused. Nine out of twelve rabbits infused with acetaminophen died and eleven out of thirteen rabbits infused with saline died ($p>.40$, Fisher exact probability test). This does not rule out the possibility thay they may have toxic effects when both are infused together, however.

C. Correlation Between Fever and Survival

The principal question being investigated was whether a relationship exists between the febrile response and survival. Rabbits were grouped into four fever ranges for statistical purposes (using the rabbits' average 24 hour fever beginning 6 hours after injection of bacteria) and the fever plotted against survival rate. The results for both the injection of $6x10^{10}$ and $3x10^{10}$ organisms of P. multocida in rabbits which were infused with saline are shown in figure 4. The pattern is the same for both doses of bacteria. Survival increased as fever increased up to a 2.25°C. fever. Above this level of fever, survival decreased. When $6x10^{10}$ organisms were injected, those rabbits with greater than 1.5°C. fever showed a statistically significant better survival rate than those with less than a 1.5°C. fever ($p<.02$, Chi squared test).

The relationship between fever and survival is similar in rabbits which were infused with antipyretics and injected with

Figure 4. Correlation between the magnitude of fever (calculated from the 24 hour period beginning 6 hours after injection of bacteria) and the survival rate of rabbits infused with saline and injected with either $3x10^{10}$ or $6x10^{10}$ organisms of P. multocida. Rabbits were grouped into fever ranges of 0.75°C. and the average fever in each fever range was plotted. Horizontal bars indicate $\pm$ 1 SE.

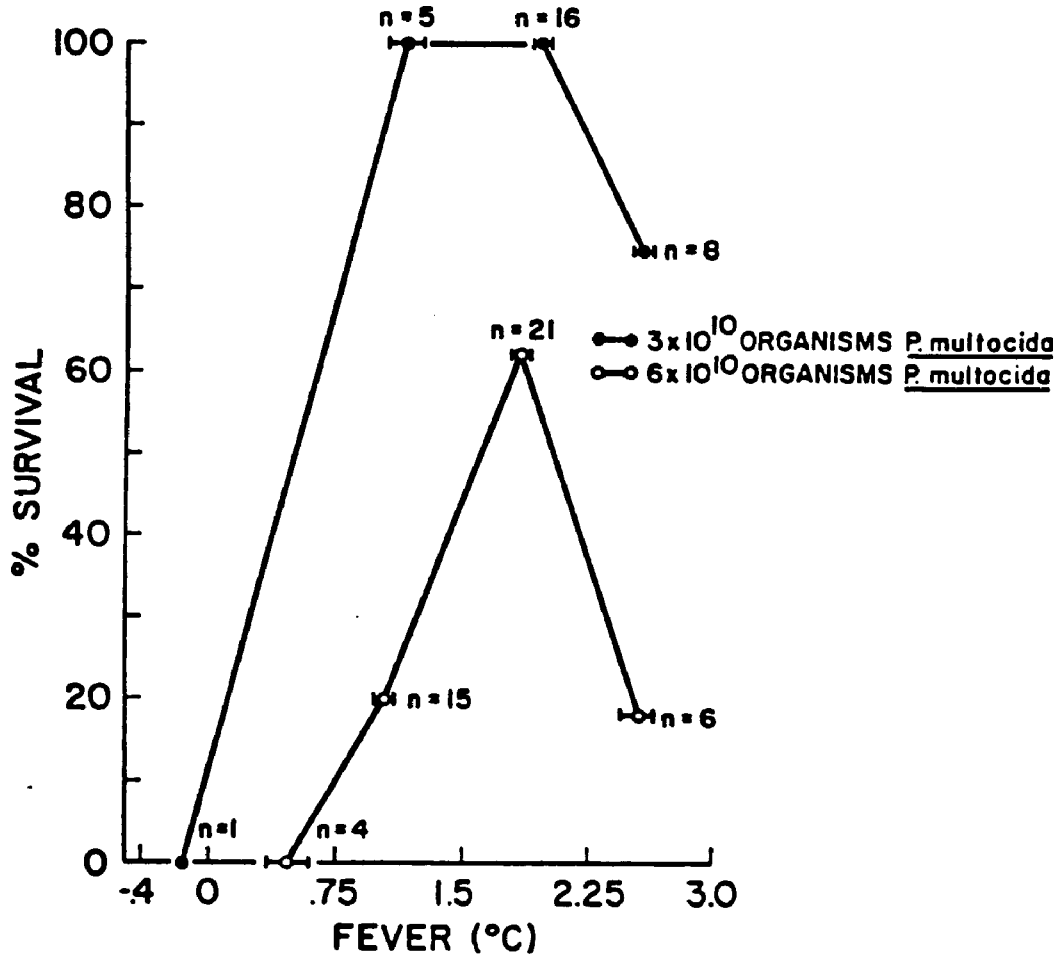

n = 5
n = 16
n = 8
n = 21
n = 15
n = 6
n = 1
n = 4
100
80
60
40
20
0
% SURVIVAL
-.4
0
.75
1.5
2.25
3.0
FEVER (°C)
3 x 10^10 ORGANISMS P. multocida
6 x 10^10 ORGANISMS P. multocida

Figure 4

$6x10^{10}$ and $3x10^{10}$ organisms of P. multocida. The results are shown in figure 5. Again, as fever increased, survival increased. This is statistically significant with the high dose of bacteria ($p<.02$, Chi squared test). There were no rabbits with a fever of greater than 2.25°C. and there was no concommitant decrease in survival.

When Probit analysis was done on the data, it was found that the amount of fever was a statistically significant factor in predicting the probability of a rabbit's survival ($p<.01$, Probit analysis).

D. Comparison of Survival Between Rabbits Infused with Saline and Antipyretics

A comparison of the survival rate between rabbits infused with saline and those infused with antipyretics was done in an attempt to determine if fever causes an increase in survival or if fever is merely correlated with an increased survival rate. The results are shown on figures 6 and 7. When $6x10^{10}$ organisms of bacteria were injected, 37% of the rabbits infused with saline survived and 51% of the rabbits infused with antipyretics survived (figure 6). This difference is not statistically significant using the Chi squared test ($p>.10$). Within any given fever range, there was also no statistically significant increase in survival with antipyretics ($p>.3$, $p>.05$, $p>.4$ for the fever ranges 0 - 0.74°C., 0.75 - 1.49°C., and 1.50 - 2.24°C. respectively). However, using Probit analysis, when the effect

Figure 5. Correlation between the magnitude of fever and the survival rate of rabbits infused with antipyretics and injected with either $3x10^{10}$ or $6x10^{10}$ organisms of P. multocida. Fevers were calculated and plotted as in figure 4. Horizontal bars indicate $\pm$ 1 SE.

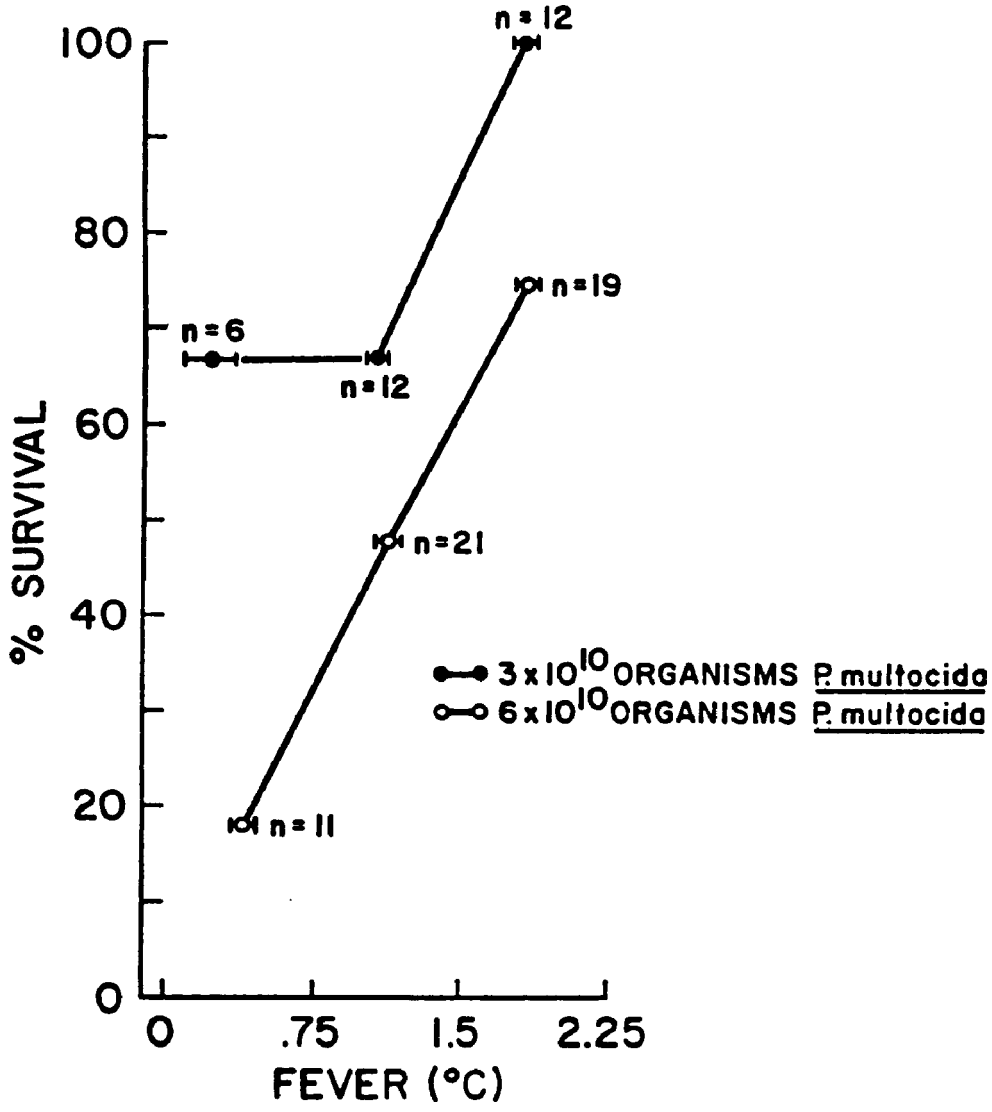

100
80
60
40
20
0
% SURVIVAL
0
.75
1.5
2.25
FEVER (°C)
n = 12
n = 19
n = 6
n = 12
n = 21
n = 11
3 x 10^10 ORGANISMS P. multocida
6 x 10^10 ORGANISMS P. multocida

Figure 5

Figure 6. Comparison of the correlation between survival rate and fever magnitude of rabbits injected with $6x10^{10}$ organisms of P. multocida and infused with antipyretics or saline. Horizontal bars indicate $\pm$ 1 SE. Solid circles indicate rabbits infused with antipyretics. Open circles indicate rabbits infused with saline.

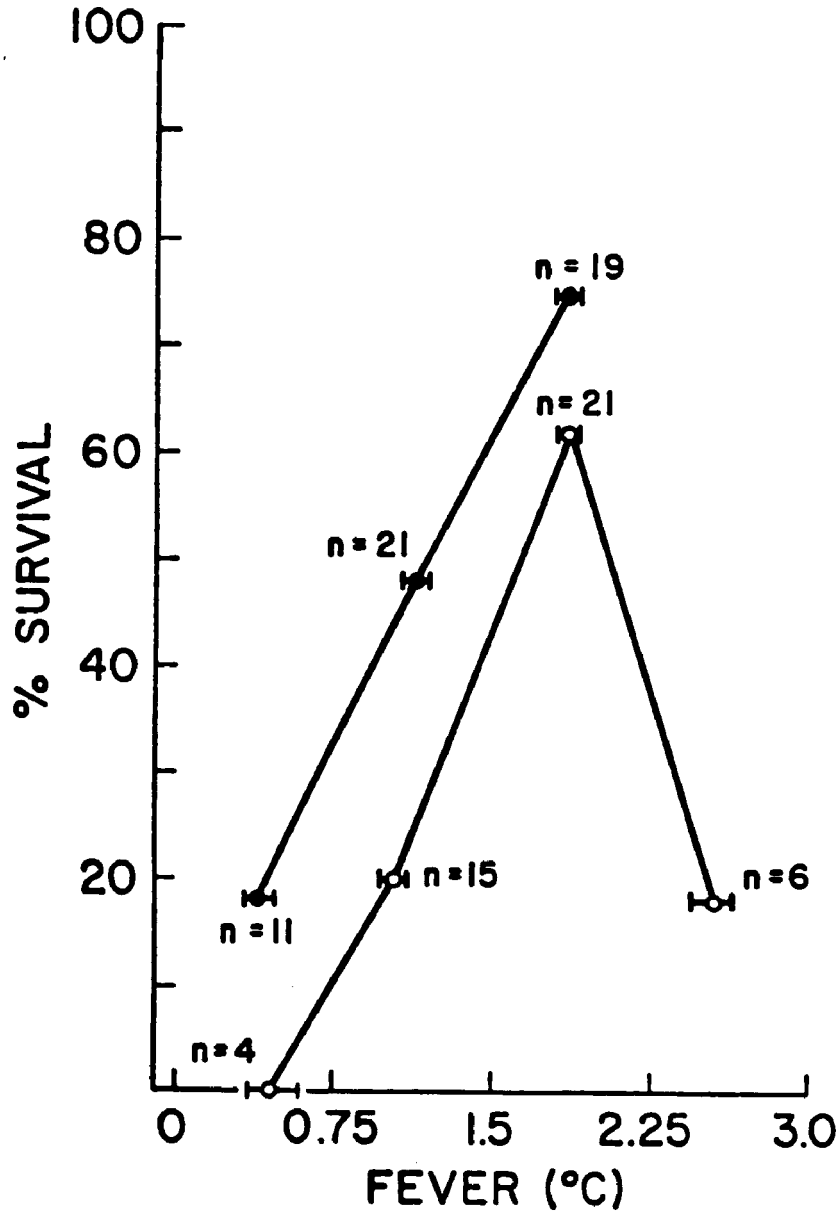

100
80
60
40
20
% SURVIVAL
n = 19
n = 21
n = 21
n = 11
n = 15
n = 6
n = 4
0
0.75
1.5
2.25
3.0
FEVER (°C)

Figure 6

of temperature on survival was held constant, the use of antipyretics was found to be associated with an increased survival rate ($p < .05$).

A comparison of the survival rate between rabbits infused with antipyretics and saline and injected with 3×10^{10} organisms of bacteria shows that with this dose, there was no statistically significant difference between the survival rate. Unlike when the higher dose of bacteria was injected, however, more rabbits infused with antipyretics died than rabbits infused with saline. Ten percent of the rabbits infused with saline died but 20% of the rabbits infused with antipyretics died.

E. Bacterial Characteristics

A biochemical profile of the bacteria was done in an effort to characterize and identify the bacteria. The results are shown in table 4. The results show that the bacteria being used in these experiments was a nitrate negative strain of *Pasteurella multocida* (Cowan and Steel, 1966).

In an attempt to determine if there is a difference in the ability of *P. multocida* to grow at febrile and control temperatures, *in vitro* growth curves were done. The results are shown in figure 8. There was essentially no difference in the growth rates (determined by the slopes of the curves) between 39 and 42°C. (0.069 units/hr. at 39°C. and 0.073 units/hr. at 42°C.).

Figure 7. Comparison of the correlation between survival rate and fever magnitude of rabbits injected with $3x10^{10}$ organisms of P. multocida and infused with antipyretics or saline. Horizontal bars indicate $\pm$ 1 SE.

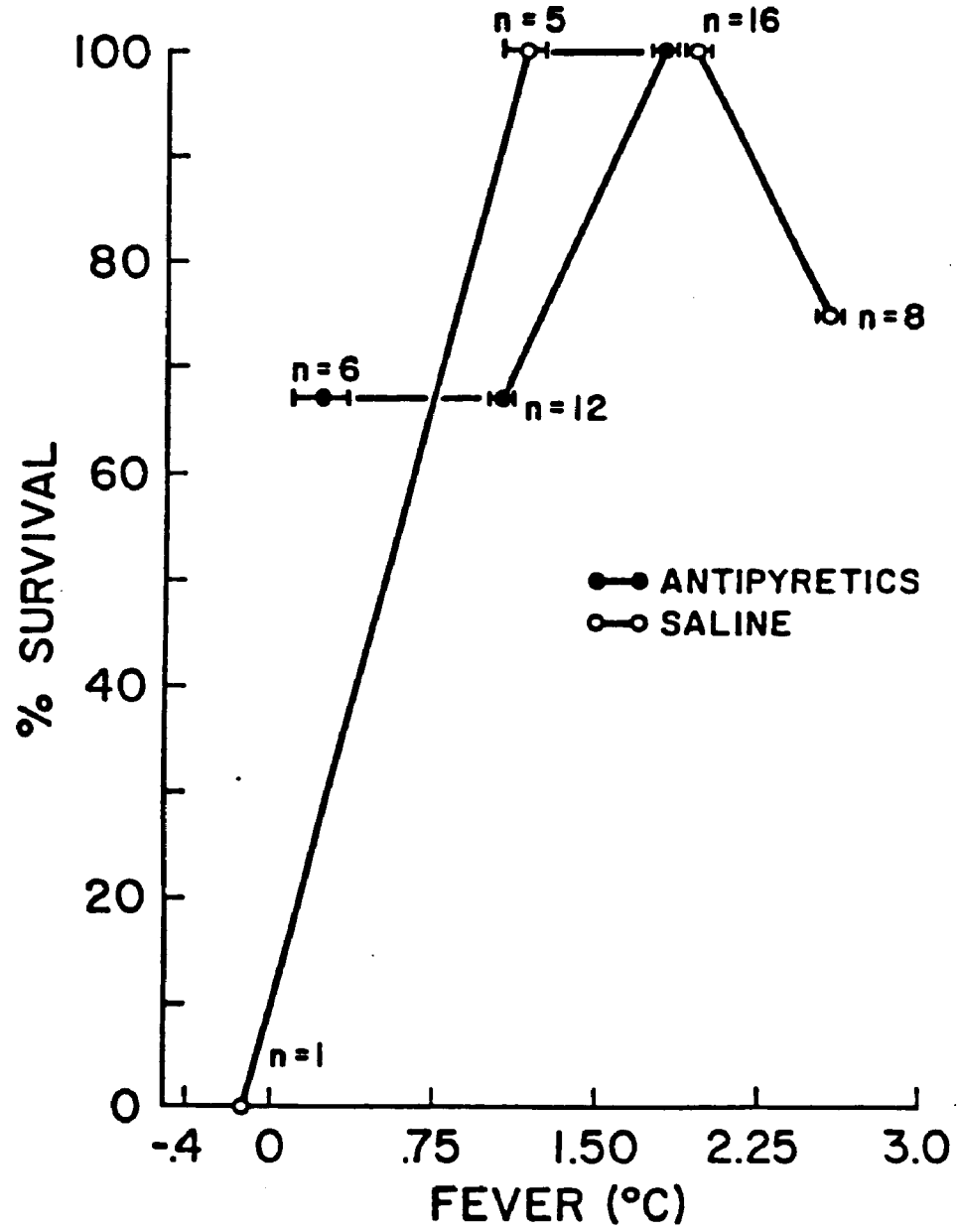

n = 5
n = 16
100
80
n = 8
n = 6
n = 12
60
% SURVIVAL
ANTIPYRETICS
SALINE
40
20
n = 1
0
-.4
0
.75
1.50
2.25
3.0
FEVER (°C)

Figure 7

TABLE 4

BIOCHEMICAL PROFILE OF *PASTEURELLA MULTOCIDA* *

Test		Result
TSI (Triple Sugar Iron Agar)	(Alkaline to Acid)	A/A
Motility (Temp.)		-
H_2S		-
Indole		+
VP (Voges-Proskauer)		-
Citrate		-
ONPG (ortho-nitrophenyl-B-D-galactopyranoside)		-
Urea		-
Lysine		-
Ornithine		-
Adonitol		-
Sorbitol		+
Arginine		-
Nitrate		-
Oxidase		+
Catalase		+
KCN		+
Dextrose		+
Mannose		+
Lactose		-
Sucrose		+
Phenylalanine		-
Inositol		-
Nucleic Acid		-
MacConkey		-
Mannitol		+
Urease		-

* Performed by the diagnostic laboratory of the Unit for Laboratory Animal Medicine

Figure 8. Comparison of the growth rate of P. multocida at 39°C. (control temperature) and 42°C. (febrile temperature).

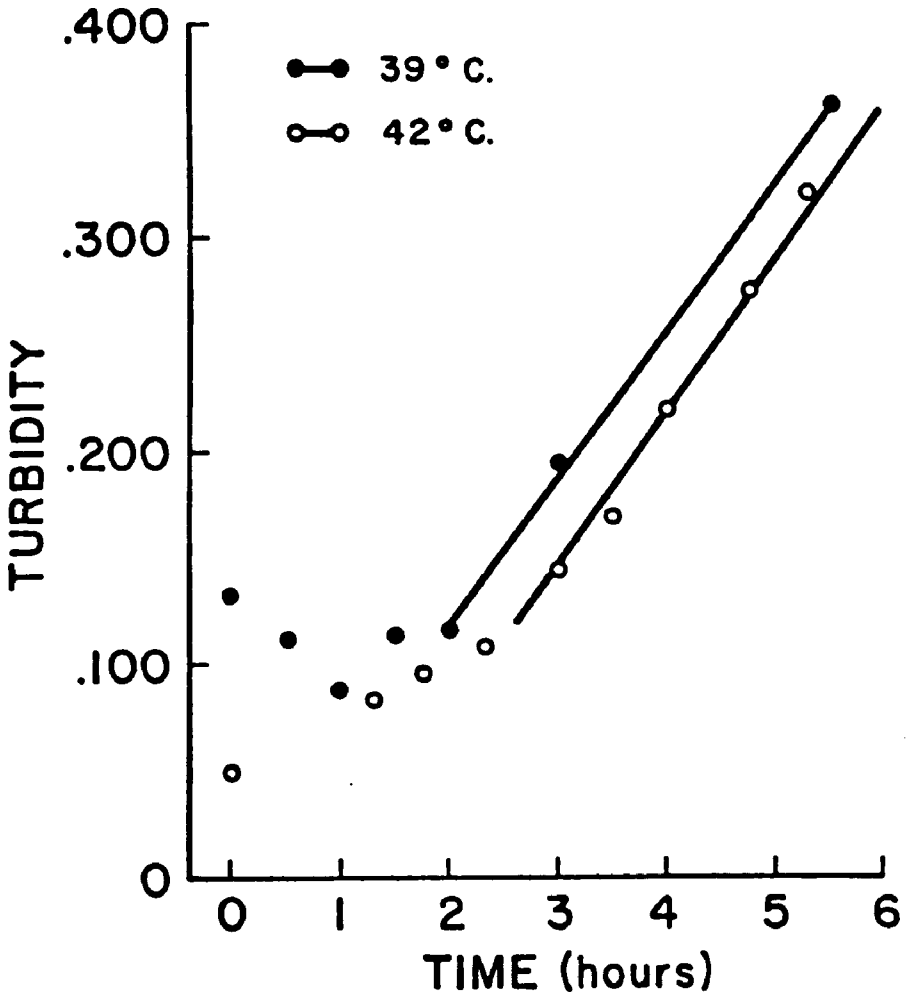


Figure 8

F. Blood Pressure Response

Blood pressure was monitored in some rabbits in order to investigate and quantify the effect of the injection of bacteria on blood pressure and to determine if there were a relationship between the magnitude of the fever and the change in blood pressure.

The results are shown in figure 9. There was an average drop in blood pressure of 9.67 mm Hg (1 SD = 9.35, n=6) over the 24 hour period beginning 6 hours after the injection of bacteria. With this small sample size, there appears to be no correlation between the magnitude of the fever and the change in blood pressure with an injection of bacteria.

Figure 9. Correlation between the magnitude of fever and the change in blood pressure of rabbits infused with saline and injected with $6x10^{10}$ organisms of P. multocida. Vertical and horizontal bars indicate $\pm$ 1 SE.

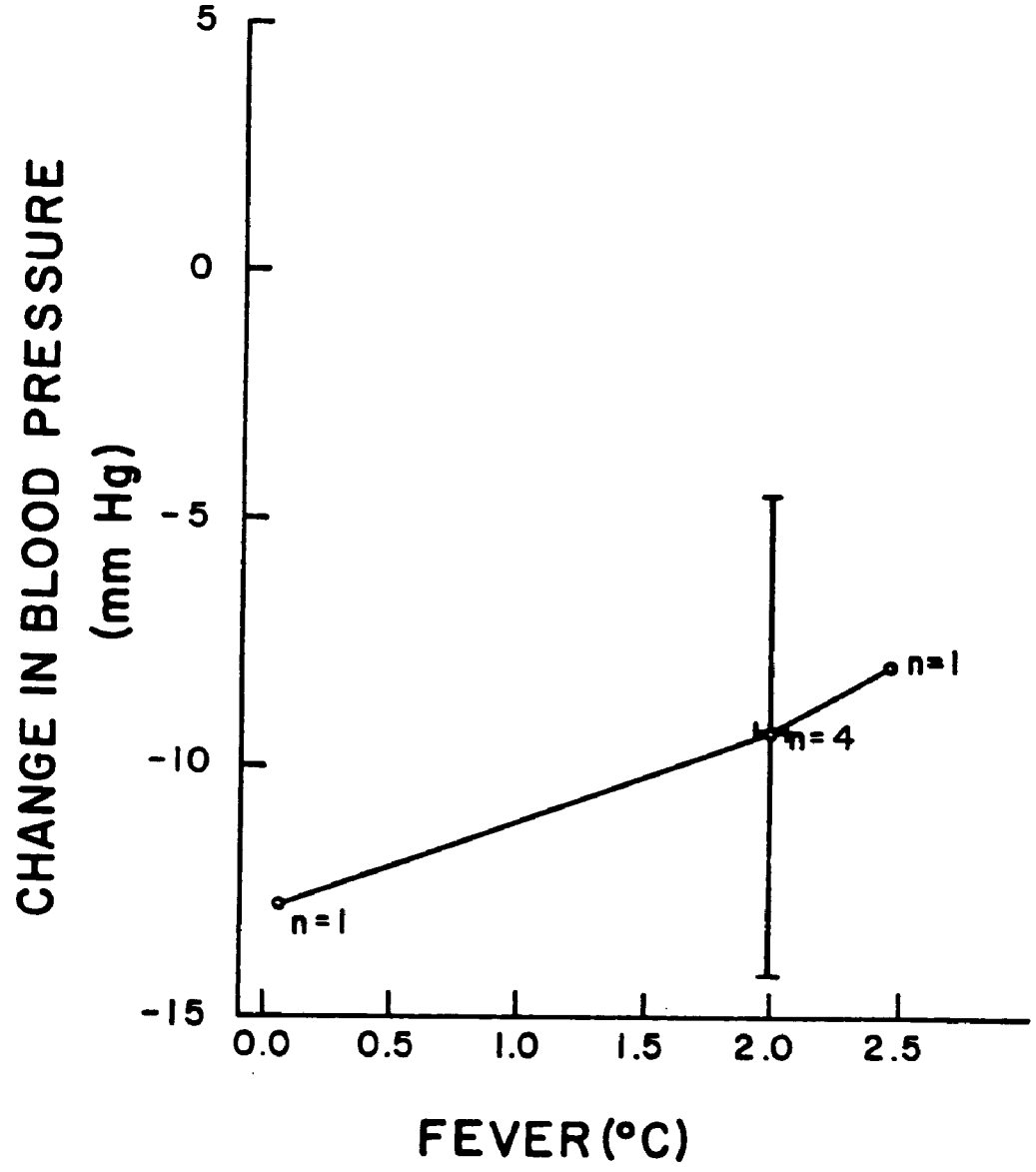

5
0
-5
-10
-15
CHANGE IN BLOOD PRESSURE
(mm Hg)
n=1
n=4
n=1
0.0
0.5
1.0
1.5
2.0
2.5
FEVER (°C)

Figure 9

DISCUSSION

A. Thermal Responses

The febrile response of rabbits after injection of bacteria can be interpreted if a dose response curve relating the resultant fever to the dose of bacteria injected is hypothesized (see figure 10). The dose response curve would logically show an increase in fever as the dose of bacteria increased until a plateau phase at the maximal level of fever that can be tolerated by the animal is reached. Then, as the number of bacteria increases and the animal's ability to thermoregulate is affected, the fever would decrease. This hypothesis is supported by the data obtained from the injection of $1x10^{10}$, $3x10^{10}$ and $6x10^{10}$ organisms of P. multocida. The maximal fever was produced by the medium dose of bacteria. The fact that moribund patients with fulminating infections may have low or absent fevers also supports this view (Bennett and Nicastri, 1960).

The febrile response during the first six hours after injection of bacteria was nearly identical in all rabbits whether they were injected with live or dead bacteria and whether they were infused with antipyretics or saline. This suggests that the fever producing mechanisms are maximally stimulated by the lowest dose used during this time period and that the fever is at its plateau level.

The fevers were less affected by antipyretics during the first few hours than during later hours. The rabbits were pretreated with antipyretics (infusion began 2 hours prior to

Figure 10. Hypothetical curve relating fever to the dose of bacteria injected.

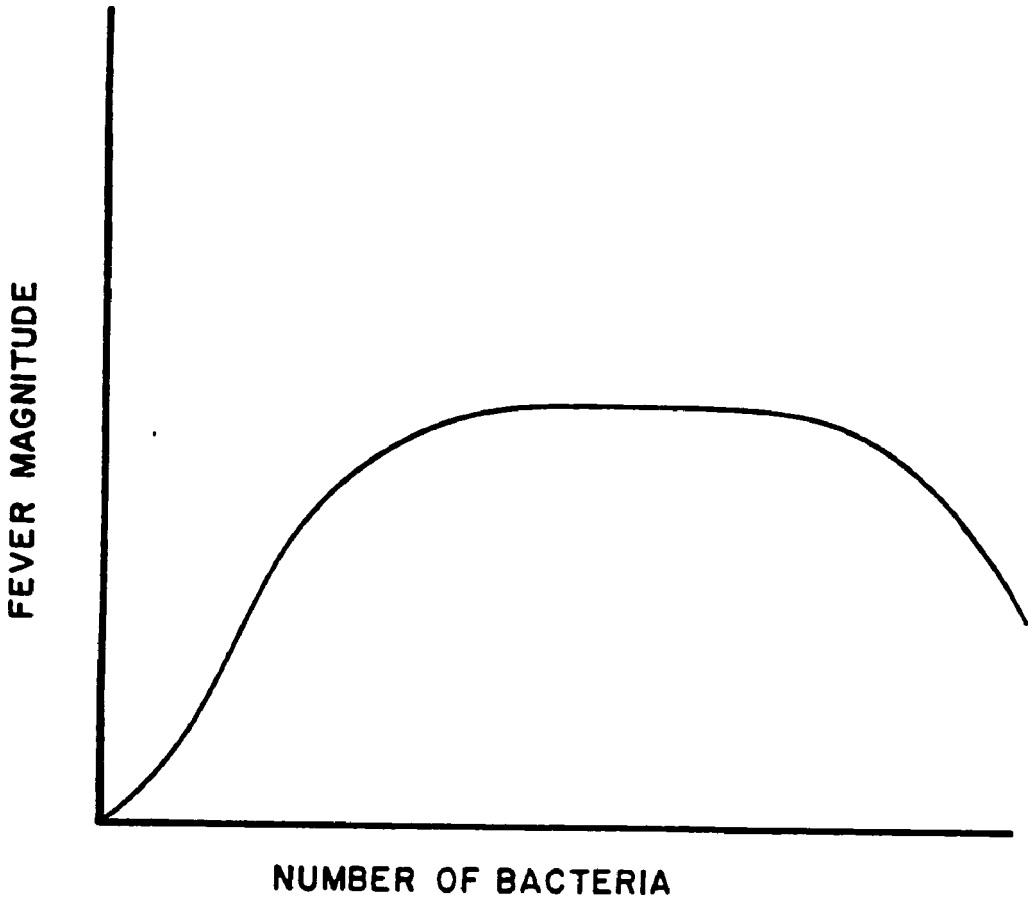


Figure 10

injection of bacteria) so this was probably not due to insufficient levels of available antipyretics. Again, it is possible that since the fever was at a plateau level, the pyrogenic stimuli exceeded the minimal amount necessary to produce the maximal fever and a reduction in the amount of pyrogenic stimuli would still produce a maximal fever.

It has previously been reported that a rabbit's fever is bimodal (Atkins and Freedman, 1963 Snell, 1971 Bennett and Cluff, 1957). Initially it was thought that the first peak was due to a direct effect of endotoxin on hypothalamic centers responsible for producing a fever and that the second peak was due to endogenous pyrogen (Bennett and Cluff, 1957). (Endogenous pyrogen is a small molecular weight protein produced by stimulated leukocytes. The pyrogen circulates to the hypothalamus and is believed to be the mediator responsible for stimulating the production of fever.) It is now believed that endogenous pyrogen is responsible for both peaks, the second peak being caused by a second wave of endogenous pyrogen production (Snell, 1971). Alternatively, D'Alecy and Kluger (1975) have hypothesized that in birds there may be competing mechanisms at work in the initial phase of fever. They hypothesized that there may be two factors influencing the temperature response to fever: an endogenous pyrogen and an endogenous cryogen. Peaks and troughs in the febrile response may be a result of the competition between these two substances.

The results in this study seem to show that there may be more than two peaks when high doses of bacteria are given. Whether

this third peak is due to a third wave of endogenous pyrogen release, competition between endogenous pyrogen and endogenous cryogen, an instability of the new set point, or the inability of a sick animal to maintain its temperature at its febrile temperatures is unknown.

B. Antipyretic Therapy

The results of this study demonstrated that salicylate was ineffective in producing antipyresis when the doses of bacteria used in this study were injected into rabbits. There are conflicting reports in the literature on the effectiveness of salicylates in rabbits, some stating that salicylates are ineffective (Van Miert et al., 1972; Cranston et al., 1970; Cranston, et al., 1975) and others stating that salicylates are effective (Cooper et al., 1968; Bodel et al., 1973; Gander et al., 1967).

The results of this study also demonstrate that acetaminophen is not effective in producing antipyresis when the doses of bacteria used in this study are injected into rabbits.

The discovery that acetaminophen and salicylate in combination can produce antipyresis even when fairly large doses of bacteria are injected is interesting and potentially very useful since using each antipyretic drug alone does not produce antipyresis.

Why the two drugs work better together than alone is an interesting question. It does allow a greater total amount of antipyretic to be infused since more of each drug alone can not

be infused without producing toxic side effects. It is possible that the antipyresis produced by the combination of drugs is simply additive. It is also possible that the increased effectiveness is due to synergism between the two drugs. In order to consider this possibility, it is necessary to discuss the mechanisms of inhibition of prostaglandin synthesis since it is believed that antipyretics work in this manner (Vane, 1971). The oxygenase enzyme involved in prostaglandin synthesis has three binding sites: a site for binding with oxygen, substrate (fatty acid), and activator (Lands, 1976). The activator is a hydroperoxide that binds to the enzyme and causes an accelerated reaction velocity. The activator is also a product of the reaction and therefore is involved in a positive feedback system which leads to more formation of activator and increased reaction velocity (Lands et al., 1976; Cook and Lands, 1975; Lands, 1976). Salicylates bind to the substrate site on the enzyme and cause irreversible inhibition of the reaction by producing an inactive form of the enzyme (Lands, 1976). Acetaminophen causes reversible inhibition of the reaction by binding to the activator site (Lands, 1976). Interestingly, this inhibition is not linear; as acetaminophen concentration increases, the inhibition increases logarithmically. It could be therefore, that the sodium salicylate may not inhibit the reaction enough to affect the amount of fever but it may sufficiently reduce the production of activator substance to the point that the effectiveness of acetaminophen inhibition is increased. Together

prostaglandin synthesis is reduced to the level where fever is attenuated.

With increased doses of bacteria, the antipyresis became less effective. This was not a suprising finding but it did make the determination of the effect of fever on survival more difficult. At doses of bacteria where antipyretics were fairly effective ($3x10^{10}$ organisms), the percentage of rabbits which died was so small that the sample size needed for statistical significance was prohibitive. Even at a ratio of two antipyretic rabbits dying for every one saline rabbit dying, if only 20% and 10% of the rabbits die, 200 rabbits would be required before the difference in mortality rate would be statistically significant using the Chi squared test. Therefore, the dose of bacteria had to be increase to $6x10^{10}$ organisms. At this dose, the antipyretics were only very mildly effective.

C. Correlation between Fever and Survival

The correlation between fever and survival found in this study is similar to that found in reptiles and fish and supports the hypothesis that fever is beneficial to bacterially infected animals. The specific mechanisms responsible for increased survival with increased fever (up to 2.25°C. in this study), remain unknown.

It is doubtful that the increased temperature had a direct effect on the growth of the bacteria. The bacteria used in this experiment grew equally well at febrile (42°C.) and control

(39°C.) levels.

It is possible that the febrile temperature had an indirect effect that inhibited the growth of the bacteria in vivo. One possibility is the increased requirement for iron by bacteria at febrile temperature (see Introduction).

Also, it has been found recently that in reptiles, an increased temperature causes both an increased migration of leukocytes to the site of infection (Bernheim et al., 1977) and an increased intracellular killing power of these leukocytes (Bernheim, personal communication). These responses may also occur in rabbits and lead to an increased survival with increased fever.

The decrease in survival associated with fevers of 2.25°C. and above may be due to a harmful effect of the high temperatures on the rabbit. High body temperatures can be harmful in healthy rabbits (Adolph, 1947) and infected rabbits may be more affected by the circulatory collapse and pulmonary edema associated with high temperatures (Frankel, 1959).

D. Comparison of Survival between Rabbits Infused with Saline and Those Infused with Antipyretics

Although these experiments have shown a correlation between fever and survival, they have not answered the question of whether fever causes an increase in survival of bacterially infected rabbits. It was hoped that the relationship between survival in rabbits infused with antipyretics and saline would

shed light on this question.

Theoretically, if fever was the only variable affecting survival, and fever caused an increase in survival, then giving antipyretics would cause a decrease in fever and, therefore, a decrease in survival. At any given febrile temperature, however, survival would be the same in those animals that were infused with saline and those infused with antipyretics. The total number of antipyretic treated rabbits surviving would be less because there would be more animals in the lower fever ranges which are associated with lower survival rates. The ideal curve relating fever and survival with the infusion of antipyretics and saline is shown in figure 11.

The results that were obtained were different from this idealized curve. With the injection of the high dose of bacteria, the curve relating fever and survival in rabbits infused with antipyretics is shifted to the left or upward from the curve in rabbits infused with saline. When the low dose of bacteria was injected, there were too few animals to compare the relationship between fever and survival in rabbits infused with saline and antipyretics.

There is a theoretical distinction between whether the curve is shifted to the left or shifted upward. An upward shift in the curve is consistent with the hypothesis that fever causes an increase in survival. A given fever causes

Figure 11. Idealized curve relating fever and survival rate in rabbits infused with antipyretics or saline and injected with bacteria.

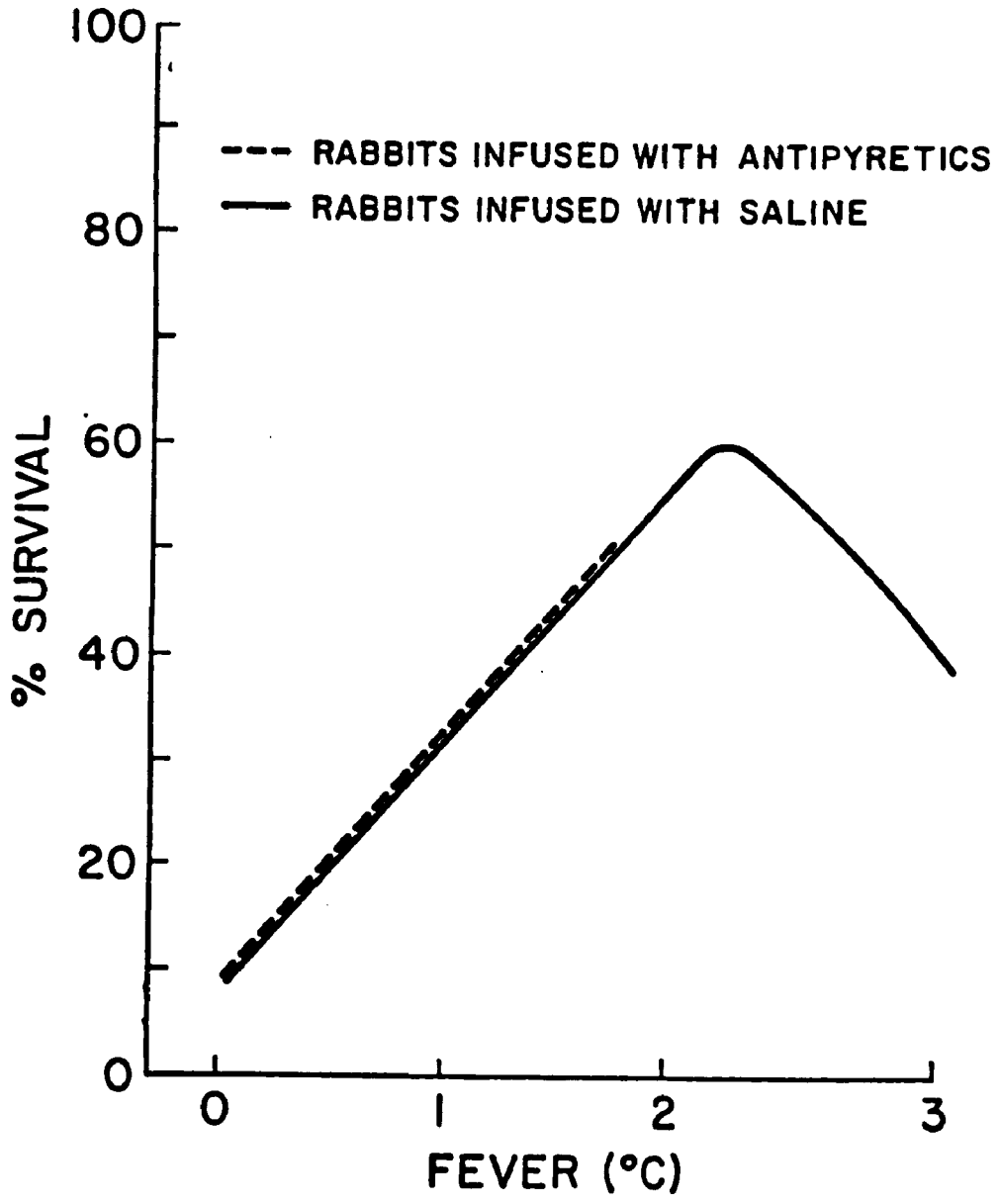

100
80
60
40
20
0
% SURVIVAL
RABBITS INFUSED WITH ANTIPYRETICS
RABBITS INFUSED WITH SALINE
0
1
2
3
FEVER (°C)

Figure 11

a specific survival rate. If the antipyretics had some beneficial side effects, the survival rate would increase over the rate dictated by the fever and the curve would shift upward. However, if the curve is shifted to the left, it suggests that fever may be unimportant for survival. A given fever may be associated with a group of animals which have a specific mortality rate which is based on some other factor besides fever. Giving antipyretics will lower the temperatures of these animals but not change the mortality rate if fever is unimportant. This will shift the curve to the left.

It is not possible to distinguish between the two possibilities by inspection of the curves. Therefore, it is impor-tnat to consider the various reasons that the curves are different.

The survival of the rabbits may have been influenced by some side effect of the sodium salicylate or acetaminophen not related to their effect on fever. One might have expected the drugs to have harmful side effects. It could be, however, that with this very high dose of bacteria, the inflammatory responses may be so severe that they are harmful. The anti-inflammatory actions of sodium salicylate then may be beneficial. The fact that the rabbits infused with antipyretic drugs did not survive better than the rabbits infused with saline when injected with the lower dose of bacteria supports this hypothesis since the inflammatory actions would have been less in this lower dose group. It could be, of course, some other side effect of the drug which may influence the

survival rate.

It is also possible that the general health of each rabbit before the bacterial infection may affect the rabbit's ability to generate a fever and its chances of surviving the infection. Although all of the rabbits used in this study came from one animal supplier, were exposed to similar environmental stressors, and were superficially healthy prior to experimentation, there still remains a chance that those rabbits which developed the higher fevers may have been an initially healthier population. If this were the case, giving the rabbits antipyretics would lower their fevers but not affect their chances for survival.

It is also possible that some other aspect of infection could cause an increase inboth survival rate and the magnitude of fever. An example of this might be an increased amount of phagocytosis in some rabbits. The increased amount of phagocytosis could increase survival by decreasing the number of circulating bacteria and also increase the magnitude of the fever since phagocytosis stimulates the release of endogenous pyrogen. Again, if fever was merely correlated with increased fever due to increased phagocytosis, the fever of the rabbits given antipyretics would be lower but the related survival rate would be similar to the fever that they would have had without the antipyretics and thus the curve relating fever and survival would be shifted to the left.

The blood pressure data, although only preliminary, show that the decrease in blood pressure after injection of

bacteria is not correlated with fever. Assuming that animals that were initially healthier would be better able to eliminate the bacteria and therefore would have less of a blood pressure drop after injection of bacteria, the blood pressure response might show a correlation with initial health of the animal. Since fever was not correlated with blood pressure, the results don't support the hypothesis that the animals with higher fevers were healthier.

The results that were obtained from the injection of three doses of bacteria tend to dispute the idea that the fevers were merely a result of the health of the animals. The dose response curve (figure 10 and table 2) shows that both the sickest (injected with $6x10^{10}$ organisms) and the healthiest (injected with $1x10^{10}$ organisms) developed the least fever. If the curve relating fever and survival relected the health of the animals, the mortality rate of those animals with low fevers would tend to be moderate, being a mix of sick and healthy animals. This was not the case. The lower fevers were associated with the lowest survival rate.

The correlation between fever and survival found in this study is a first step in the investigation of whether fever is beneficial for the survival of bacterially infected mammals. The results of the infusion of antipyretic drugs demonstrate that more effective antipyresis and the elimination of side effects are necessary before this hypothesis can be proven. Hypothalamic injections of antipyretic drugs could provide the answer.

The correlation demonstrated in this study is similar to that found in reptiles and provides a stimulus for further study on the causal nature of the relationship between fever and survival in mammals.

If later studies demonstrate that fever has a survival value in mammals, then the widespread use of antipyretic drugs and the use of physical means (e.g. sponge baths) to lower a patient's fever may not be advisable unless the fever is of life threatening magnitude.

SUMMARY AND CONCLUSIONS

This research was designed to determine what relationship exists between the survival rate and the magnitude of fever developed after an injection of Pasteurella multocida into male New Zealand white rabbits. In addition, in an effort to determine if fever causes a change in survival rate, the survival rate of those rabbits infused with antipyretics was compared to the survival rate of those rabbits infused with saline.

Rabbits (Oryctolagus cuniculus) were implanted with abdominal thermocouples and catheters. Abdominal temperature was measured every 60 seconds. Control temperature data were collected during the 24 hour period beginning 24 hours after implantation. Forty-eight hours after implantation, rabbits were injected with live Pasteurella multocida ($6x10^{10}$ or $3x10^{10}$ organisms, i.v.), a gram-negative bacterium pathogenic to rabbits. Each rabbit was infused with either 100 ml/day sterile, pyrogen-free, 0.9% saline or 100 ml/day sterile antipyretic solution, composed of 3 mg/ml sodium salicylate and 10 mg/ml acetaminophen. The mortality and the temperature response were monitored for 5 days after injection of the bacteria. The rabbits fever over the 24 hour period beginning 6 hours after injection of bacteria was correlated with survival rate.

It was found that survival increased as fever increased up to 2.25°C. in both the rabbits infused with antipyretics and the rabbits infused with saline. Above a 2.25°C. fever, survival decreased. When the lower dose of bacteria was injected, 20%

of the rabbits infused with antipyretics died compared to 10% of the rabbits infused with saline. When the higher dose of bacteria was injected, 49% of the rabbits infused with antipyretics died compared to 63% of the rabbits infused with saline.

In vitro growth curves were done for P. multocida at 39°C. and 42°C. It was found that the bacteria grew equally well at both temperatures ensuring that the differences in survival rate were not due to a direct temperature effect on the bacteria.

The blood pressure response of the rabbits after injection of bacteria was monitored in an attempt to determine whether there was a difference between rabbits with a high and low fever. An average decrease of 9.7 mm Hg was found and there was no correlation with the temperature response.

The results demonstrate that there is a correlation between fever and survival in rabbits injected with Pasteurella multocida. Those rabbits with a higher fever had a higher survival rate. It remains to be determined, however, whether fever causes a higher survival rate or whether fever is only correlated with a higher survival rate.

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