FEVER AND SURVIVAL IN RABBITS INFECTED WITH *PASTEURELLA MULTOCIDA*

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SUMMARY

1. Fever and survival rate of New Zealand white rabbits, injected with two doses of live *Pasteurella multocida*, were compared to determine if the relation between fever and survival observed in reptiles is also seen in a mammal. Since it is known that fever is beneficial in infected reptiles, our experiments were viewed as an initial step in the investigation of a similar potentially beneficial effect in mammals.

2. There was a statistically significant correlation between the fever magnitude and survival. As fever increased up to 2·25 °C, the survival rate increased. Fevers above this level were associated with a decreased survival rate.

3. Antipyretic drugs were administered to half the rabbits. The drugs were ineffective in reducing the fevers produced by injections of large doses of bacteria. Rabbits infused with antipyretics had a decreased mortality rate. The decreased mortality rate may be due to some action of the drugs other than their antipyretic effect.

4. *In vitro* growth of *P. multocida* at normal (39 °C) and febrile (42 °C) temperatures was not significantly different. This suggests that the increased survival rate associated with higher fevers was not due to a direct inhibition of the growth of the bacteria by the increased temperature. It may be due to an enhancement of some aspect of the rabbits' immunological defences.

5. It remains to be determined whether fever causes a higher survival rate or whether fever is only correlated with survival rate.

INTRODUCTION

There has been much conjecture about the role of fever in disease (Bennett & Nicastri, 1960). Induced hyperthermia was once used in the treatment of poliomyelitis, neurosyphillis, and gonococcal infections (Lwoff, 1959; Wolf, 1935; Culver, 1917; Hasler & Spekter, 1936; Wagner-Jauregg, 1927). Inhibiting fever in rabbits, infected with heat sensitive strains of pneumococcus type III, decreases the survival rate (Enders & Shaffer, 1936; Rich & McKee, 1936; Strouse, 1909). Conversely, it has been found that at high ambient temperatures the susceptibility of mice, rats, and rabbits to the lethal action of endotoxin is increased (Atwood & Kass, 1964; Connor & Kass, 1961; Porter & Kass, 1962). Research on the role of fever in disease has been

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done in ectothermic animals, which regulate their temperature by behavioural means, because their temperatures can be more easily controlled. It has been shown that lizards will develop a fever in response to an injection of bacteria, that survival is correlated with the magnitude of that fever, and that drug induced antipyresis will decrease the survival rate (Vaughn, Bernheim & Kluger, 1974; Kluger, Ringler & Anver, 1975; Bernheim & Kluger, 1976). Similar results have been obtained in fish (Covert & Reynolds, 1977; Reynolds, Casterlin & Covert, 1976).

While there is considerable evidence of the beneficial effect of fever in bacterially infected lizards and fish, the relation between fever and survival in bacterially infected mammals remains unknown. The evidence suggests that the ability to develop fever evolved before the evolutionary divergence of reptiles, fish, and mammals (Kluger, Bernheim, Vaughn, Foster & D'Alecy, 1977). Although fever is beneficial in ectothermic animals, fever could be an evolutionary vestige in mammals and no longer functions to increase the ability to survive infection.

The present study was done to determine whether the correlation between fever and survival in a mammal is similar to that found in a reptile. This comparison is viewed as an initial step in the determination of the role of fever in mammals. The effects of the infusion of antipyretics on the survival rate of bacterially infected rabbits was also investigated.

METHODS

Thermocouples and catheters were implanted into the abdomens of male New Zealand white rabbits (*Oryctolagus cuniculus*) weighing 2–4 kg. Before implantation, the rabbits were injected with acepromazine, 1 ml. 10 mg/ml. I.M., and 2% lidocaine hydrochloride, 1 ml. S.C. in the middle of the back. A small incision was made through the skin of the back, and a sterile stainless steel trochar (3·2 mm o.d.) was threaded under the skin to the side of the abdominal wall and inserted into the peritoneal cavity. Thermocouples encased in sterile polyethylene tubing (P.E. 50) and sterile catheters (P.E. 160) filled with sterile, pyrogen-free, 0·9% NaCl were threaded through the trochar into the abdomen, the trochar was removed and the thermocouple and catheter 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.

Abdominal temperature was measured approximately every 60 sec using a multipoint temperature recorder (Honeywell Electronik, Minneapolis, U.S.A.). The rabbits were allowed 24 hr to recover from implantation and to adapt to the neck restrainers. Control temperatures were recorded during the 24 hr period beginning 24 hr after implantation.

48 hr after implantation, rabbits were injected in the marginal ear vein with live *Pasteurella multocida*, a gram-negative bacterium pathogenic to rabbits (Flatt, 1974), 3×10^{10} or 6×10^{10} organisms. The bacteria were grown on blood agar plates for 48 hr. The bacteria were then suspended in sterile, pyrogen-free, 0·9% NaCl, centrifuged, washed with the saline, recentrifuged, and resuspended in saline. The concentration of the bacteria was determined spectrophotometrically.

In vitro growth curves were determined for *Pasteurella multocida* in order to determine whether changes in survival were due to a change in the growth rate of the bacteria. Flasks containing sterile Bacto brain-heart infusion (Difco, Detroit, U.S.A.) were inoculated with bacteria from 18 hr cultures and placed in shaking water baths at 39 and 42 °C. Samples were taken periodically for 6 hr and spectrophotometric readings made. The slopes of the growth phase were compared.

Because of possible nychthemerally rhythmic variations in survival rate (Haus, Halberg, Kuhl & Lakatua, 1974) and temperature response, all injections of the bacteria were made at approximately the same time of day (14.30–15.30). Sterile, pyrogen-free, 0·9% NaCl or sterile antipyretic solution was infused I.P. into each rabbit using an infusion pump (Harvard Apparatus, Millis, Mass. U.S.A.) at a rate of 100 ml./day. The antipyretic solution was composed of sodium salicylate 3 mg/ml. and acetaminophen 10 mg/ml. dissolved in sterile, pyrogen-free, 0·9% NaCl.

The solution was passed through a metrical filter (Gelman, Ann Arbor, Mi., U.S.A.) having an average pore size of 0·20 μm to remove bacterial contaminants. Sterility was checked by inoculating blood agar plates with the solution and observing the plates for bacterial growth. The infusion began 2 hr before the injection of bacteria and was continued for 5 days. The mortality of the rabbits was monitored over this 5 day period and all rabbits which were alive at the end of this time were considered survivors. To determine the magnitude of a rabbit's fever, the average temperature over the 24 hr period beginning 6 hr after injection of bacteria was compared to the rabbit's average temperature on the control day. This 24 hr period was used because most of the deaths (76% in those rabbits injected with the high dose of bacteria) occurred within this period. The first 6 hr were excluded because the temperature during this time was unaffected by antipyretics. The fevers were then correlated with survival rate. The rabbits were grouped into fever ranges of 0·75 °C. These fever ranges were chosen because they were small enough to show the relation between fever and survival clearly. Smaller fever ranges could not be used for statistical purposes since there were too few animals in each range.

RESULTS

Thermal responses

The temperature response of rabbits infused with saline and injected with two dose levels of live *Pasteurella multocida* are shown in Fig. 1. This graph characterizes the febrile response over a 4·5 day period and provides a comparison between the responses to different doses of bacteria. They also provide data from which the effectiveness of antipyretics could be determined. The average fever during a 24 hr period which began 6 hr after injection, was 1·9 °C when the lower dose of bacteria, and 1·6 °C when the higher dose of bacteria, was injected. This difference is statistically significant ($P < 0.02$).
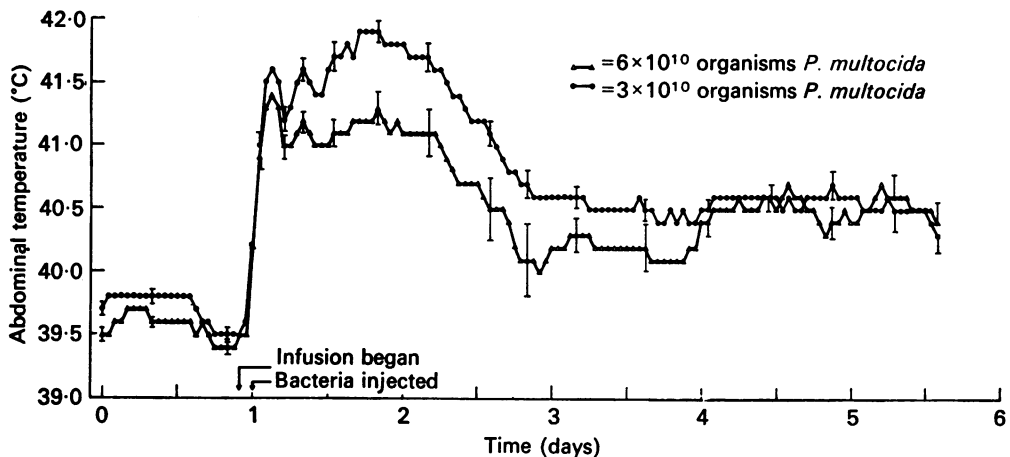

Fig. 1. Average abdominal temperatures of rabbits before and after injection of two doses of *P. multocida*. Vertical bars represent ± 1 s.e. Thirty rabbits were injected with 3×10^{10} organisms and forty-six rabbits with 6×10^{10} organisms.

The effectiveness of the antipyretics can be seen in Fig. 2. The difference between the temperatures of rabbits infused with antipyretics compared to those infused with saline is plotted for both doses of bacteria. When 3×10^{10} organisms were injected, the temperature was lower for most of the time when antipyretics were infused. There was a decrease of 0·73 °C ($P < 0.001$, Student's t test) during the 24 hr period beginning 6 hr after injection. When 6×10^{10} organisms were injected, however,

there was only a 0·32 °C decrease in temperature during the same 24 hr period ($P < 0.005$, Student's t test). While this decrease is statistically significant, the magnitude of change is small.

Antipyretic toxicity

Neither acetaminophen, 1 g/day, nor sodium salicylate, 300 mg/day, had significant antipyretic actions in rabbits injected with 6×10^{-10} organisms of *P. multocida*. The average fever of ten rabbits infused with acetaminophen was 0·13 °C higher than the average fever of ten rabbits infused with saline ($P < 0.80$, Student's t test). The average fever of eleven rabbits infused with salicylate was 0·12 °C higher than the average fever of twelve rabbits infused with saline ($P < 0.50$, Student's t test).
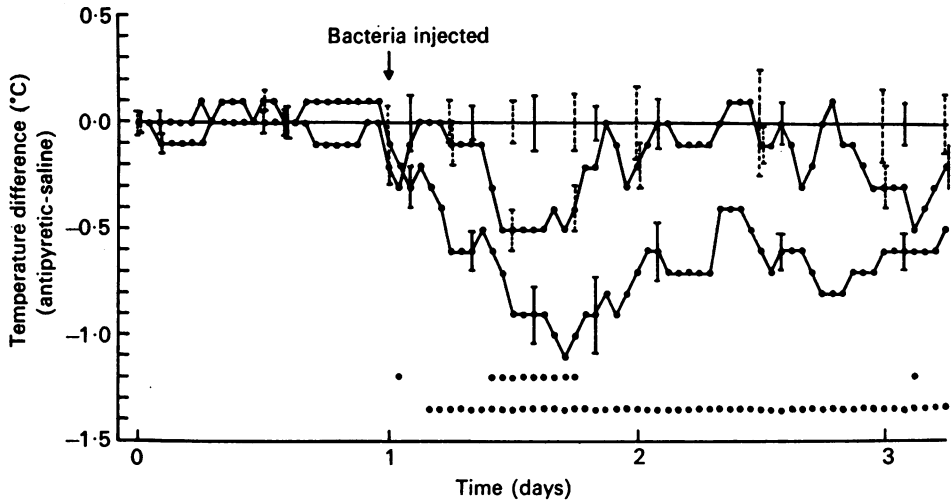

Fig. 2. Difference in average abdominal temperature of rabbits infused with anti-pyretics and those infused with saline before and after injection of the low (filled circles, $n = 30$ antipyretics, thirty salines) and high dose of bacteria (open circles, $n = 51$ antipyretics, forty-six salines). The temperatures of rabbits infused with saline are considered 0·0 °C and the relative temperatures of rabbits infused with anti-pyretics are plotted. Vertical bars represent ± 1 S.E. Filled bars indicate S.E. of rabbits injected with the low dose of bacteria and dotted bars indicate S.E. of rabbits injected with the high dose of bacteria. When bars are near each other, the one to the right is the S.E. of rabbits infused with antipyretics. Hours when the difference in temperature between antipyretics and salines are statistically significant ($P < 0.05$, Student's t test) are indicated by open and filled circles on the bottom of the graph (high and low dose respectively).

Since the temperature was not significantly changed when each drug was given singly, any difference in mortality between rabbits infused with the antipyretic or with saline could be due to side effects of the drug. There was no difference in mortality between rabbits when sodium salicylate, 300 mg/day, was infused. Four of eleven rabbits infused with salicylate died and three of twelve rabbits infused with saline died ($P < 0.40$, Fisher exact probability test). There was also no difference in the mortality rate when acetaminophen, 1 g/day was infused. Nine of twelve rabbits infused with acetaminophen died and eleven of thirteen rabbits infused with saline

died ($P < 0.40$, Fisher exact probability test). This does not rule out the possibility that both drugs together may have some toxic side effects. The fever and survival studies seem to indicate that the antipyretics were not toxic.

Fever and survival

The principal question being investigated was whether a relation exists between the febrile response and survival. Rabbits were grouped into four fever ranges for statistical purposes (based on the average fever during a 24 hr period beginning 6 hr after injection of bacteria) and the fever plotted against survival rate. The results for both the injection of 6×10^{10} and 3×10^{10} organisms of *P. multocida* in rabbits which were infused with saline are shown in Fig. 3. The pattern is the same for rabbits injected with either dose of bacteria. Survival increased as fever increased up to a
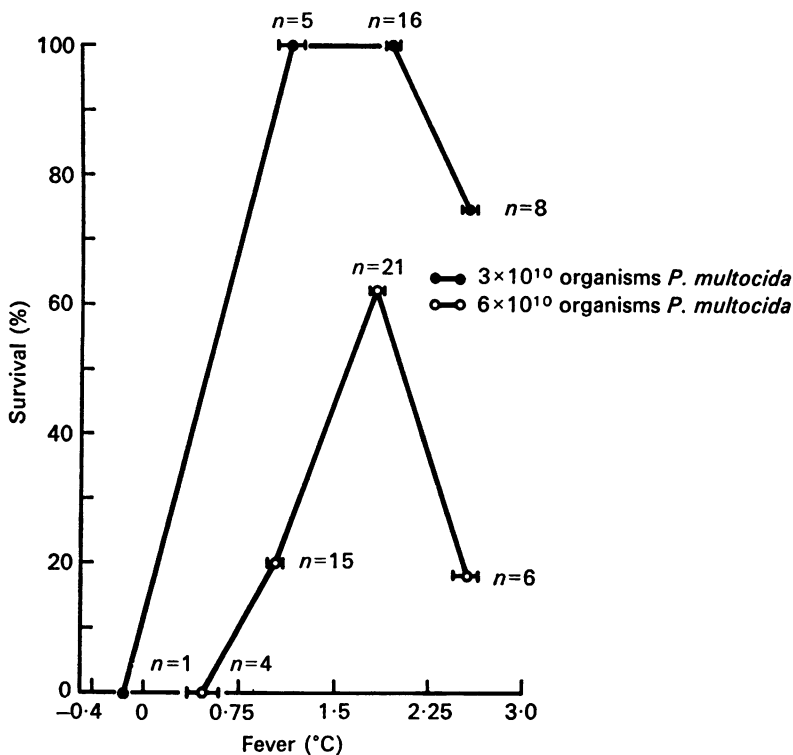

Fig. 3. Correlation between the magnitude of fever (calculated from the 24 hr period beginning 6 hours after injection of bacteria) and the survival rate of rabbits infused with saline and injected with either 3×10^{10} or 6×10^{10} organisms of *P. multocida*. Rabbits were grouped into fever ranges of 0·75 °C and the average fever in each fever range was plotted. Horizontal bars indicate $\pm$ S.E.

2·25 °C fever. Above this level of fever, survival decreased. When 6×10^{10} organisms were injected, those rabbits with an average 24 hr fever greater than 1·5 °C showed a greater survival rate than those with less than 1·5 °C fever ($P < 0.02$, χ^2 test). When 3×10^{10} organisms were injected only three animals died and the difference in survival rate was not significantly different, although the pattern of survival was the same as in rabbits injected with the larger dose of bacteria.

The relation between fever and survival is similar in rabbits which were infused with antipyretics and injected with either dose of bacteria. These results are shown in Fig. 4. Again, as fever increased, survival increased. This is statistically significant with the high dose of bacteria ($P < 0.02$, χ^2 test). There were no rabbits with a fever of greater than 2·25 °C and there was no concommitant decrease in survival. The results were not statistically significant with the low dose of bacteria.

When Probit analysis was done on the data, it was found that the size of the fever was a statistically significant factor in predicting the probability of a rabbit's survival ($P < 0.01$, Probit method of analysis).
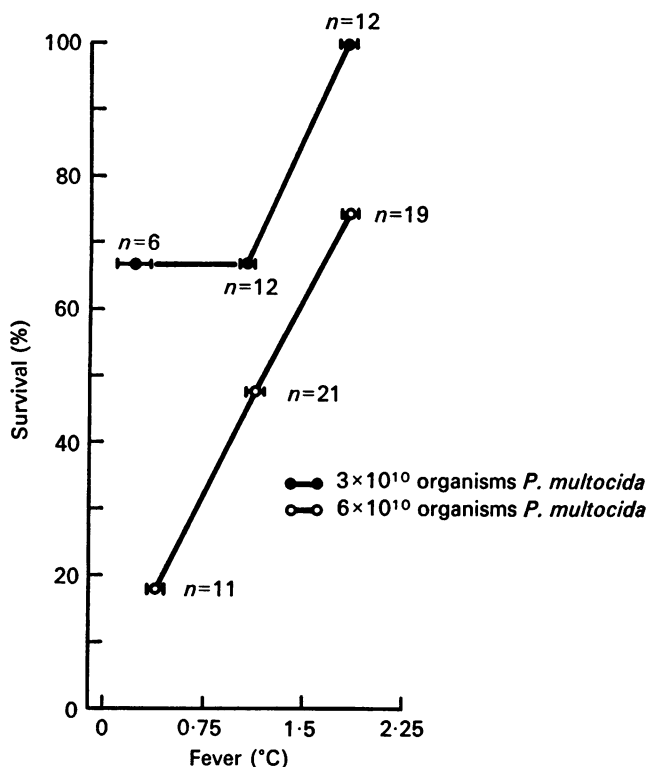

Fig. 4. Correlation between the magnitude of fever and the survival rate of rabbits infused with antipyretics and injected with either 3×10^{10} or 6×10^{10} organisms of *P. multocida*. Fevers were calculated and plotted as in Fig. 3. Horizontal bars indicate ±1 s.e.

Comparison of survival between rabbits infused with saline and antipyretics

A comparison of the survival rate between rabbits infused with saline or antipyretics was done to determine if fever causes an increase in survival or if fever is merely correlated with an increased survival rate. When the high dose of bacteria was injected, 37 % of the rabbits infused with saline survived and 51 % of the rabbits infused with antipyretics survived. This difference is statistically significant ($P < 0.05$, Probit method of analysis).

A comparison of the survival rate between rabbits infused with antipyretics and saline and injected with 3×10^{10} organisms of bacteria appeared to show that with

this dose, more rabbits infused with antipyretics died (20%) than rabbits infused with saline (10%), but this difference was not statistically significant ($P < 0.20$, Fisher exact probability test).

In vitro growth of 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. There was essentially no difference in the growth rate between 39 and 42 °C (0·069 u./hr at 39 °C and 0·073 u./hr at 42 °C).

DISCUSSION

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 animals infected with bacteria. The specific mechanisms responsible for increased survival with increased fever (up to 2·25 °C in this study), remain unknown.

The possibility that the increased temperature had a direct effect on the growth of the bacteria is counter-indicated since the bacteria used in this experiment grew equally well in brain heart infusion at febrile (42 °C) and control (39 °C) temperatures.

The febrile temperature may have had some, as yet undetermined, indirect effect on the growth of the bacteria. A possible factor is the iron requirement of bacteria: at febrile temperatures, some species of bacteria have an increased requirement for iron because they have a decreased ability to produce iron transport compounds (Bullen, Rogers & Griffiths, 1974; Garibaldi, 1972). Also, during infection, there is less plasma iron available because of decreased absorption of iron from the gut and an increased amount of iron transferred to the liver (Cortell & Conrad, 1967; Kochan, 1973; Pekarek & Beisel, 1971; Weinberg, 1974). At a time when bacterial requirements for iron are increased, there is a decreased amount of available iron. The effects of increased temperature on the ability of bacteria to obtain adequate amounts of iron has been implicated as a survival mechanism in lizards injected with *A. hydrophila* (Grieger & Kluger, 1978). Therefore, the viability and growth of the bacteria may be affected by an indirect action of fever.

In reptiles, an increased temperature causes an increased migration of leukocytes to the site of infection (Bernheim, Bodel, Askenase & Atkins, 1977). This response 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 temperature on the rabbit. Very high body temperatures can be harmful even in healthy rabbits (Adolph, 1947).

These experiments have shown a correlation between fever and survival but there remains some uncertainty about the causal nature of the relation. If fever causes an increase in survival, then antipyresis should produce a decrease in survival. When the higher dose of bacteria was injected, treatment with antipyretics did not produce substantial antipyresis (0·3 °C), however, and the antipyretics produced an increase in survival rate. When the smaller dose of bacteria was injected, greater antipyresis resulted and antipyretic drugs did not cause an increase in survival. Rabbits infused with antipyretics had two times the mortality rate of rabbits infused with saline (this difference was not statistically significant).

Some side effect of the sodium salicylate or acetaminophen, not related to their effect on fever, may have caused the increased survival of the rabbits. Aspirin has been shown to inhibit the vascular reactivity to substances released by endotoxins (catecholamines, histamine, seratonin, bradykinin) and to inhibit edema (Hinshaw, Solomon, Erdos, Reins & Gunter, 1967). Inhibiting the inflammatory responses to this high dose of bacteria could increase 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. Fever may be merely correlated with some other aspect of the response to infection which increases the survival rate. However, 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 relation between fever and survival.

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