

Influence of Body Temperature on Bacterial Growth Rates in Experimental Pneumococcal Meningitis in Rabbits

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We examined the role of fever as a host defense in experimental pneumococcal meningitis in rabbits. Twelve hours after intracisternal inoculation of an encapsulated type 3 *Streptococcus pneumoniae* strain, body temperature was manipulated by using two different anesthetic drugs: pentobarbital, which did not affect temperature, and urethane, which mitigated the febrile response to infection. Growth rates of pneumococci in cerebrospinal fluid were dramatically influenced by modification of the febrile response. Rabbits whose fever was not suppressed had mean bacterial doubling times of 2.76 ± 1.43 h. Animals with a blunted febrile response had a significantly faster mean bacterial growth rate (doubling time = 1.10 ± 0.27 h; $P < 0.02$). When the antipyretic effect of urethane was counteracted by raising the ambient temperature, animals also showed a marked reduction in pneumococcal growth rates. In vitro, the pneumococci grew well at 37°C in Trypticase soy broth (doubling time = 0.61 ± 0.05 h) and in pooled rabbit cerebrospinal fluid (doubling time = 0.85 ± 0.07 h). However, at 41°C neither medium supported growth. Thus, body temperature appears to be a critical determinant of pneumococcal growth rates in experimental meningitis, and fever could be a host defense in this disease.

Fever may exert a beneficial effect on the natural course of infectious diseases in humans and in experimental animal models (1, 15, 16). In lizards with *Aeromonas hydrophila* infection, fever enhanced local containment of the infection (2) and decreased mortality (10). Similar protective effects of fever have been demonstrated in goldfish (4). In endotherms, studies of selected bacterial infections have also suggested a protective effect of fever. Survival rates of rabbits infected with *Pasteurella multocida* increased with fever (11) and decreased when the febrile response was reduced by intrahypothalamic infusion of small quantities of salicylic acid (3, 20). In humans, fever is associated with improved outcome in spontaneous bacterial peritonitis (21).

The potential importance of body temperature in pneumococcal infections has also been recognized for many years. In 1909 Strouse (19) demonstrated that the native resistance of pigeons to infection by pneumococci was due to their high normal body temperature. In 1936 the ability of type 3 pneumococci to grow at 41°C was shown to be essential for their virulence in rabbits (6). In the same year, Rich and McGee (14) demonstrated that pneumococci could proliferate intradermally at normal rabbit temperatures but were destroyed in rabbits which developed fever. Similarly, in 1938, high body temperature was shown to reduce bacterial titers in two children with pneumococcal meningitis who were warmed with radiant energy (18).

The aim of the present study was to carefully assess the influence of body temperature on the growth rate of pneumococci in cerebrospinal fluid (CSF) of rabbits with experimental pneumococcal meningitis. With this experimental model it has been demonstrated that during the first 18 h of pneumococcal meningitis, bacteria grow logarithmically, and granulocyte-mediated host defense in CSF is ineffective (7). Our study addressed whether fever plays a role as a

nonspecific host defense early in the course of this disease. The answers could be important because, despite advances in antibiotic therapy, pneumococcal meningitis still has a very high morbidity and mortality rate (9). Furthermore, the absence of specific host defenses in CSF in the rabbit model provides the opportunity to assess the direct influence of temperature on bacterial multiplication in vivo.

MATERIALS AND METHODS

Bacterial isolate. A clinical isolate of a penicillin-sensitive, encapsulated type 3 *Streptococcus pneumoniae* strain (Clinical Microbiology Laboratory, San Francisco General Hospital) was grown on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% defibrinated sheep blood at 37°C in 5% CO₂. A standard inoculum of bacteria was stored at -70°C. Before the in vivo experiments, one aliquot was thawed and diluted in saline to yield an inoculum of approximately 5×10^5 CFU/ml.

Experimental model. The rabbit model of pneumococcal meningitis described by Dacey and Sande was used (5). Anesthetized rabbits were placed in stereotactic frames which allowed puncture of the cisterna magna with a 25-gauge, 3.5-in. (ca. 8.4-cm) spinal needle (Becton Dickinson, Rutherford, N.J.). Infection was produced by intracisternal inoculation of 0.3 ml of the inoculum, equal to approximately 10^5 CFU of *S. pneumoniae*.

Modulation of body temperature. Twelve hours after infection, the rabbits were again anesthetized. One group of eight rabbits received pentobarbital, which did not significantly affect body temperature. A second group of 13 rabbits received a slow intravenous infusion of 1.75 mg of urethane (ethyl carbamate; Sigma Chemical Co., St. Louis, Mo.) per kg, which interfered with the animal's ability to thermoregulate. This led to a reduction of body temperature in rabbits kept at room temperature ($n = 7$), while rabbits kept at a higher (35.5°C) ambient temperature in a walk-in incubator remained febrile ($n = 6$). Body temperatures were monitored

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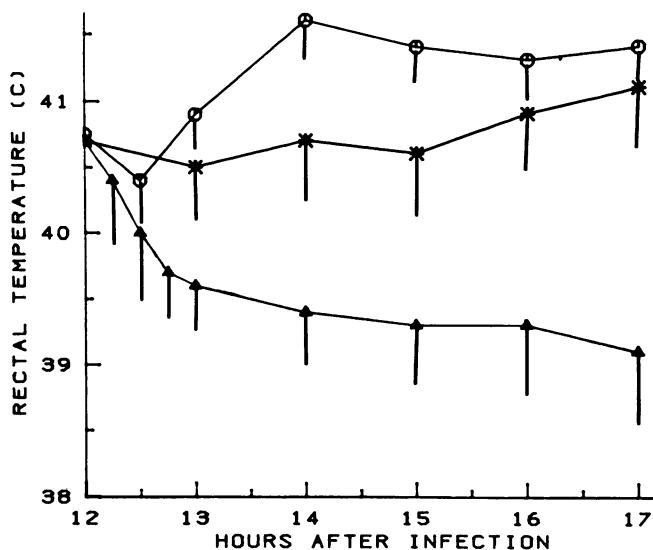


FIG. 1. Mean \pm SD rectal temperatures over time in the three experimental groups of rabbits with pneumococcal meningitis. Symbols: *, rabbits receiving pentobarbital at room temperature; \blacktriangle , rabbits receiving urethane at room temperature; \circ , rabbits receiving urethane at elevated (35.5°C) ambient temperature.

every 30 min by placing a digital thermometer (VWR Scientific, San Francisco, Calif.) 8 cm into the rabbits' rectums. The mean temperature over the course of the experiment was calculated for each rabbit. The group mean was then calculated by averaging the mean body temperature for all of the rabbits in each group.

Bacterial growth rate in vivo. After the body temperature of the experimental animals had been modulated by modification of either the anesthetic or the ambient temperature, a total of five CSF samples were collected from the cisterna magna at 1- to 2-h intervals. Bacterial titers in CSF were determined by quantitatively subculturing the samples. Bacterial doubling times were calculated by least-squares regression. In three animals receiving pentobarbital and in three animals receiving urethane, CSF leukocyte counts were determined in the first and last CSF samples with a Neubauer hemacytometer (American Optical, Buffalo, N.Y.).

Bacterial growth rates in vitro. To determine the direct effects of the anesthetics on bacterial growth, in vitro growth curves were generated in media containing various concentrations of either urethane (0.02 to 20 mg/ml) or pentobarbital (0.25 to 250 $\mu\text{g/ml}$). An inoculum of 10^4 CFU of pneumococci was added to 3-ml portions of Trypticase soy broth containing the anesthetics. These samples were incubated at 37°C in 5% CO_2 and quantitatively subcultured at 1-h intervals. Bacterial log-phase doubling times were calculated by least-squares regression. To determine the influence of temperature on growth rates of the pneumococcal strain in vitro, an inoculum of 10^4 CFU was added to 3 ml of Trypticase soy broth (TSB) or pooled rabbit CSF. Samples were incubated at 37, 39, and 41°C , and bacterial doubling times were determined as described above.

Statistical analysis. Results were expressed as the mean \pm standard deviation (SD). Comparisons between different experimental groups were performed by Student's *t* test.

RESULTS

Twelve hours after intracisternal inoculation of *S. pneumoniae*, all of the rabbits were infected and had higher body

temperatures ($40.7 \pm 0.5^{\circ}\text{C}$) than uninfected control rabbits ($39.2 \pm 0.3^{\circ}\text{C}$; $P < 0.0001$). After pentobarbital anesthesia, body temperature remained elevated throughout the experimental period (the mean temperature of all animals in the group over the course of the experiment was $40.9 \pm 0.3^{\circ}\text{C}$) (Fig. 1). With urethane anesthesia, the body temperature of rabbits kept at room temperature fell (group mean, $39.4 \pm 0.5^{\circ}\text{C}$), whereas rabbits kept at elevated ambient temperature remained febrile throughout the experimental period (group mean, $41.1 \pm 0.1^{\circ}\text{C}$) (Fig. 1). Leukocyte counts in CSF were $1,075/\text{mm}^3$ (range, 430 to 2,000) 12 h after infection and were not affected by the type of anesthesia.

The body temperature in the different experimental groups directly influenced the bacterial doubling time of pneumococci in CSF (Table 1). In the group with reduced body temperature (urethane at room temperature), bacterial doubling times were 1.10 ± 0.27 h. In contrast, in both groups with elevated body temperatures, bacterial growth in CSF was significantly reduced; mean doubling times were 2.76 ± 1.43 h in animals receiving pentobarbital ($P < 0.02$ versus afebrile animals), whereas bacterial titers in CSF declined in four of six rabbits kept at elevated ambient temperature. In the latter group, we found measurable doubling times in only two rabbits (1.86 and 9.21 h).

In two rabbits, the reaction of body temperature to the anesthetics was atypical. Results in these two rabbits confirmed the role of body temperature in determining pneumococcal growth in CSF. In one animal receiving pentobarbital, the body temperature decreased (mean, 39.7°C), and the doubling time (1.19 h) was similar to the mean doubling time in the group of rabbits with comparable body temperature (urethane at room temperature). The second rabbit receiving urethane at room temperature had a persistently elevated temperature (40.8°C), and the bacterial doubling time (2.23 h) in this rabbit was similar to that in the febrile animals receiving pentobarbital.

When absolute changes in bacterial titers (as another measure of bacterial growth) over the 6-h experimental period were plotted against the average temperature for each experimental animal, we again found a significant correlation between temperature and bacterial growth ($r = -0.703$; $P < 0.001$) (Fig. 2).

Additional experiments addressed the influence of anesthetics and temperature on the growth rate of the experimental pneumococcal strain in vitro. Pentobarbital at concentrations ranging from 0.25 to 250 $\mu\text{g/ml}$ did not influence the doubling time of the pneumococcus in TSB at 37°C (doubling time ranged from 0.52 to 0.61 h). Likewise, the addition of urethane in concentrations ranging from 0.02 to 2 mg/ml did not affect bacterial growth in vitro (doubling time, 0.59 to 0.64 h), whereas a higher concentration of urethane (20

TABLE 1. Mean body temperature and bacterial doubling times in rabbits with experimental pneumococcal meningitis

Anesthetic (no. of rabbits)	Ambient temp ($^{\circ}\text{C}$)	Rectal temp ($^{\circ}\text{C}$ mean \pm SD)	Bacterial doubling time (h, mean \pm SD)
Urethane (6) ^a	21	39.4 ± 0.5^b	1.10 ± 0.27^c
Pentobarbital (7) ^a	21	40.9 ± 0.3^b	2.76 ± 1.43^c
Urethane (6)	35	41.1 ± 0.1	— ^d

^a One animal in each of these groups was excluded from the analysis because of atypical temperature response to anesthesia (see text).

^b $P < 0.01$.

^c $P < 0.02$.

^d Only two animals in this group had measurable bacterial growth (doubling times of 1.86 and 9.21 h).

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