though these two systems probably both convey information relating to the odor characteristics of pups, we believe that they may be responding to somewhat different aspects of the odor complex. Our data showing that animals sustaining cuts to the vomeronasal nerves (groups VN, VN+, and VN-OB) showed reduced licking of pups, whereas animals receiving OB cuts alone did not, support the hypothesis of Winans and Powers (*6*) that in rodents the vomeronasal system is normally activated by nonvolatile substances transmitted through the nares when the animal comes into direct contact with the odor source.

The caring of the postparturient female for her young without delay or hesitancy, as soon as they are born, may result from multiple effects of her hormones [primarily estrogen (*2*)] acting at a number of different sites in the central nervous system. For instance, the olfactory tubercle, the medial and cortical nuclei of the amygdala, and the medial preoptic region all contain receptors for estradiol (*13*). It is possible, therefore, that endogenous estradiol—which peaks just before parturition—acts simultaneously (i) on the olfactory system to change the female's attraction to pup odors, (ii) on the amygdala to reduce her neophobia (*14*), and (iii) on the preoptic region to facilitate the coordination and integration of the different caretaking behaviors (*11*).

Note added in proof: Using a technique of selectively cauterizing the vomeronasal organ (with no damage to the olfactory bulbs), A. Mayer and J. Rosenblatt have found a significant reduction in latencies to become maternal in virgin female rats of the Charles River strain.

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Fever and Reduced Iron: Their Interaction as a Host Defense Response to Bacterial Infection

Abstract. *When rabbits are infected with* Pasteurella multocida, *the concentration of iron in their plasma decreases and their rectal temperature rises. To determine whether the rise in body temperature (fever) and the fall in plasma iron may be a coordinated host defense response,* Pasteurella multocida *were grown in vitro at various temperatures and iron concentrations. At afebrile temperatures the bacteria grew equally well at low or high concentrations of iron. However, when the temperature of the bath was raised to a febrile temperature the growth of the bacteria was inhibited by the low, but not the high, iron concentrations. These data support the hypothesis that one of the mechanisms behind the adaptive (or beneficial) role of fever is the reduced ability of pathogenic bacteria to grow well at elevated temperatures in an iron-poor medium.*

Many studies have indicated that fever is a host defense mechanism during bacterial (*1, 2, 2a*) and viral (*3*) infections. Many mechanisms have been suggested as being responsible for the beneficial effects of an elevated body temperature on an organism's immune response. Among these have been an increase in leukocyte mobility (*4*), increased leukocyte killing of ingested microorganisms (*5*), and an increased production of interferon (*6*). An area that has recently received considerable attention has been that of "nutritional immunity." This term, coined by Kochan (*7*), refers to the fact that during infection the blood levels of many nutrients become altered. For example, the concentrations of iron and zinc in the serum generally decrease and the concentration of copper generally increases (*8, 9*). It has been suggested by Weinberg (*10*) and by others that the decrease in serum (or plasma) iron might reduce the growth of pathogenic microorganisms. In fact, many studies have shown that certain species of bacteria grow poorly in a medium containing low concentrations of iron and that iron supplements increase the growth of bacteria in vitro and in vivo (*8, 9*).

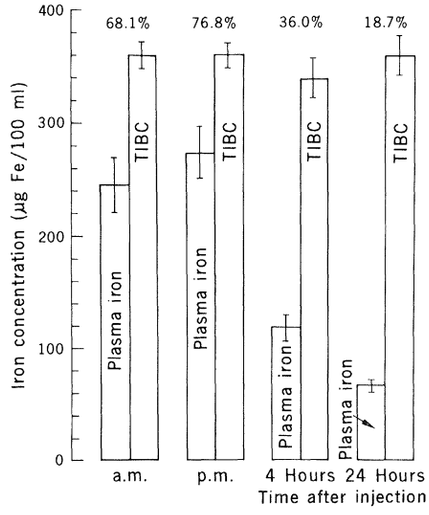

Fig. 1. Plasma iron (PI) and total iron-binding capacity (TIBC) in eight male New Zealand White rabbits. Prior to infection with live pathogenic bacteria *(Pasteurella multocida)*, the rabbits' PI concentrations averaged 247 μg per 100 ml during the morning and 275 μg per 100 ml during the afternoon hours. After infection with bacteria, the PI concentrations fell to 118 μg per 100 ml by 4 hours and to 66 μg per 100 ml by 24 hours. The TIBC did not change during this period. The percentages above each set of bar graphs indicate the percentage saturation of the plasma protein, transferrin.

Garibaldi (*11*) has shown for *Salmonella typhimurium* and Kochan (*9*) has shown for *Escherichia coli* that the ability to produce iron-transport compounds (siderophores) is diminished by small elevations in temperature and, as a result, it has been suggested that the reduction in serum iron, coupled with an elevation in body temperature (fever) is a coordinated host defense mechanism (*9-11*). To further support this contention, recent evidence indicates that the leuko-

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cyte-derived protein which is responsible for the decrease in serum iron (leukocyte endogenous mediator) and the development of fever (endogenous or leukocytic pyrogen) is the same compound (*12*).

In studies involving reptiles *(Dipsosaurus dorsalis)* it has been shown that during infection with *Aeromonas hydrophila* these lizards develop a fever (body temperature goes from about 38° to 41°C) and this fever increases their survival rate (*1, 13*). During these infections the plasma iron concentrations decrease (*14*). When cells of *A. hydrophila* were grown in vitro there was little difference in the rate of growth at the normal (38°C) and febrile (41°C) temperatures when the iron concentrations of the growth media were maintained at physiological levels. However, a reduction in the available iron levels by precipitation (with $MgCO_3$) or by chelation (with desferrioxamine B-sulphate) led to marked reductions in the growth rate of the bacteria at 41°C but ot at 38°C. These data were interpreted to support the hypothesis that one of the mechanisms behind fever's adaptive function is to decrease the ability of pathogenic bacteria to sequester sufficient iron for normal growth.

To investigate the effects of fever on the survival rate of mammals, we infected New Zealand rabbits with live *Pasteurella multocida* (a common rabbit pathogen) and correlated their resultant fevers with the rabbits' survival rates (*2a, 15*). The results demonstrated that there is a positive correlation between the fever and survival rates for fevers of up to 2.25°C (or as body temperature increased from about 39.50° to 41.75°C).

In the present study we measured the plasma iron (PI) concentration and total iron-binding capacity (TIBC) in rabbits before they were infected with *P. multocida* and during infection. We found that their PI concentrations decreased from an average of 261 μg of iron per 100 ml of plasma to 66 μg per 100 ml within 24 hours. On the basis of these results, we grew these bacteria at various temperatures and in different concentrations of iron and found that these bacteria do not grow well at febrile temperatures in an iron-poor medium. These results support the hypothesis that a fever coupled with a reduction in PI may be part of a coordinated host defense response.

Male New Zealand White rabbits *(Oryctolagus cuniculus)* weighing 2.3 to 3.2 kg were used in these experiments. The rabbits were maintained on a photoperiod of 12 hours of light and 12 hours of darkness, and were given unlimited access to food (Tekland rabbit chow) and water. During experimentation each rabbit was restrained in specially designed holders.

Body temperature was recorded by inserting a thermocouple probe 10 cm into each animal's rectum. Temperature data were collected on a multipoint recorder (Honeywell Electronik 112) which printed each animal's rectal temperature approximately every 30 seconds.

The bacterial strain used in this study was *Pasteurella multocida* (American Type Culture Collection No. 7228). The bacteria were grown on sheep blood agar plates for 48 hours at 37°C. The cells were suspended in sterile 0.9 percent pyrogen-free sodium chloride (saline), washed twice by centrifugation, and resuspended in saline. A concentration of 1×10^{10} bacteria per milliter was prepared by comparing visual turbidity with McFarland barium sulfate standards.

Plasma iron concentrations and TIBC were determined from 5-ml blood samples that were drawn from marginal ear veins and collected in heparinized polystyrene tubes. Blood was drawn from the rabbits on day 1 at 1000 hours and day 2 at 1400 hours. On day 3 the rabbits were injected intravenously with 1 ml of 1×10^{10} organisms *(P. multocida)* per milliter at 1000 hours. Blood was drawn 4 hours (day 3 at 1400 hours) and 24 hours (day 4 at 1000 hours) after injection. All blood samples taken in the morning were processed immediately; the afternoon samples were centrifuged and the plasma was refrigerated until it was processed the next morning. The PI samples were prepared according to the method of Fernandez (*16*). For details of the iron determinations see (*17*).

Growth curves were completed to determine the effects of iron concentration and temperature on *P. multocida* in vitro (*18*).

Samples of all the growth media were tested on the atomic absorption spectrophotometer to determine their iron content before they were inoculated with bacteria. Growth medium prepared in the usual way had an iron content of 79 μg per 100 ml, which approximates the plasma iron concentration in rabbits injected 24 hours previously with *P. multocida*. To approximate the iron concentrations in uninfected rabbits and in rabbits injected 4 hours previously with *P. multocida*, we added a sterile solution of ferric ammonium citrate (1 g per 1000 ml) to the growth medium to obtain iron concentrations of 266 μg and 139 μg per 100 ml, just before it was inoculated with

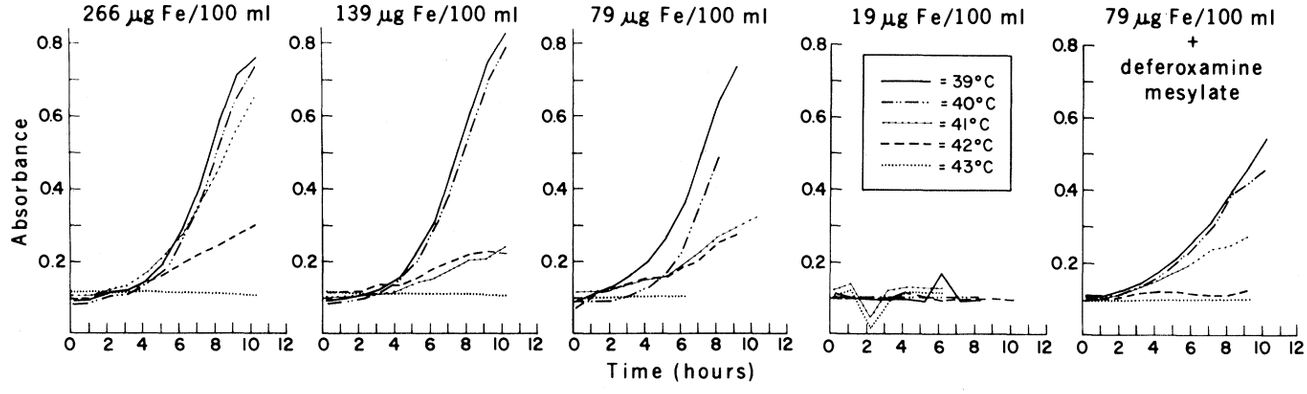

Fig. 2. Growth of *Pasteurella multocida* (measured as absorbance on a spectrophotometer) at temperatures of 39° to 43°C and in iron concentrations of 19 to 266 μg of iron per 100 ml of growth medium. At an iron concentration corresponding to the normal plasma concentrations of iron in uninfected rabbits (266 μg per 100 ml), the bacteria grew well at 39°, 40°, and 41°C. When the bacteria were grown in a medium containing concentrations of iron corresponding to that which occurs in rabbits during infection (139 or 79 μg per 100 ml), the growth of the bacteria was not inhibited at the afebrile temperatures of 39° or 40°C, but was depressed at the febrile temperature of 41°C. When the iron concentration was reduced to subphysiological levels (19 μg per 100 ml) the bacteria did not grow at any temperature. The addition of the iron chelator deferoxamine mesylate to the growth medium depressed the growth of the bacteria at all temperatures, but clearly had a greater effect at the febrile temperatures of 41° and 42°C.

P. multocida. The subphysiological iron concentration of 19 μg per 100 ml was obtained by adding 15 g of $MgCO_3$ (to precipitate most of the iron) to 100 ml of the growth medium, stirring for 5 minutes, letting the solution stand for 15 minutes, centrifuging for 10 minutes at 2500*g*, and collecting the supernatant. This procedure was completed prior to sterilization of the growth medium. Another growth medium was prepared by adding 50 mg of deferoxamine mesylate (CIBA), an iron chelating agent, to 100 ml of the normally prepared growth medium. The deferoxamine mesylate was added to the growth medium just prior to inoculation with bacteria.

Prior to infection, the PI of eight rabbits averaged 247 μg of iron per 100 ml of plasma (68.1 percent saturation) in the morning and 275 μg per 100 ml (76.8 percent saturation) in the afternoon. After injection with bacteria, PI concentrations decreased to 118 μg per 100 ml (36.0 percent saturation) by 4 hours and to 66 μg per 100 ml (18.7 percent saturation) by 24 hours (Fig. 1). The TIBC did not change appreciably and remained at approximately 360 μg of iron per 100 ml of plasma.

Prior to injection with live bacteria, the rectal temperatures of the eight rabbits averaged 39.70° ± 0.17°C (standard error). Rectal temperature increased to 40.85°C ± 0.29°C by 4 hours after injection and was still somewhat elevated by 24 hours after injection (40.9° ± 0.30°C).

To determine whether an increase in temperature coupled with a reduction in iron concentration decreases the growth rate of *P. multocida*, we grew the bacteria in vitro at afebrile (39° and 40°C) and febrile (41°, 42°, and 43°C) temperatures at various concentrations of iron from subnormal (19 μg of iron per 100 ml) to normal concentrations encountered during infection (79 and 139 μg per 100 ml), to normal concentrations in uninfected rabbits (266 μg per 100 ml). In addition, we determined the effects of deferoxamine mesylate on these bacteria at various temperatures (Fig. 2).

The addition of deferoxamine mesylate to the growth medium slightly inhibited the growth of the bacteria at 39° and 40°C, severely attenuated the growth at 41°C, and completely prevented the growth at 42° and 43°C. The addition of this iron chelator did not reduce the concentration of iron in the medium but did reduce the availability of the iron to the growing bacteria.

These results indicate that in response to infection, PI decreases and body temperature rises; they also support the hypothesis that very small changes in temperature, corresponding to a moderate fever, coupled with a reduction in PI may be part of a coordinated host defense response. In vivo, the growth of many microorganisms is inhibited in large part by their inability to obtain adequate amounts of iron, because virtually all PI is bound to transferrin [see review in (*19*)]. The virulence of many pathogenic bacteria seems to be related to their ability to obtain adequate amounts of iron from the host's iron-binding proteins (*20*). As such, the increase in survival rate (*1-3*) in febrile organisms might be related, in part, to the synergistic effects of temperature and reduced iron on the growth of the pathogenic microorganisms.

If these results can be extrapolated to organisms pathogenic to human beings, then the use of drugs to reduce or attenuate moderate fevers during bacterial infections may limit to a certain extent the host defense response. It is also possible that the addition of excess iron to our diet (through fortified foods and minerals, for example) increases the growth potential of pathogenic bacteria and harms some individuals who already have adequate iron stores. In fact, these data along with those of Sword (*21*) and others (*9-11, 14*), raise the possibility that drugs such as deferoxamine mesylate or other iron chelators could have therapeutic value during certain bacterial infections.

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17. For the PI determinations, equal parts of plasma and trichloroacetic acid (1.22*M*) were mixed in polystyrene culture tubes, heated at 90°C for 15 minutes (to denature the protein molecules), and centrifuged for 10 minutes at 2500*g*. The supernatant was saved for testing. The samples for the TIBC (the potential amount of iron that the plasma transferrin molecules can bind) determinations were prepared as follows. Two milliliters of a solution containing 6 μg of iron per milliliter [derived from Iron Stock solution I [see (*16*)] was added to 1 ml of plasma, mixed for 30 seconds, and allowed to stand for 5 minutes with occasional mixing. $MgCO_3$ (380 mg) was then added to the sample (to precipitate the unbound iron), mixed for 15 minutes, and allowed to stand for 15 minutes. The sample was centrifuged at 2500*g* for 10 minutes and the supernatant was then used as in the PI sample preparation. Iron concentration was determined on an atomic absorption spectrophotometer (Varian AA-375). Four absorbance values were obtained per sample with each value being determined over a 3-second period. Sample concentrations were determined by comparison of absorbance values to a standard curve. The standards were prepared according to the method of Fernandez (*16*). The percentage saturation of transferrin was calculated as: (PI/TIBC) × 100.
18. The bacteria were incubated for 44 to 48 hours at 37°C on 10 percent sheep blood agar plates, suspended in saline, centrifuged, and resuspended in saline. One hundred milliliters of the growth medium, brain heart infusion (Difco), were inoculated with 2 ml of the bacterial suspension. The medium was then placed in a temperature-controlled shaking bath (Forma Scientific) set at 39°, 40°, 41°, 42°, or 43° ± 0.2°C. Samples were taken after the first 15 minutes and every hour thereafter. Estimates of bacterial growth were determined by turbidity (absorbance) on a spectrophotometer (Beckman DB) set at 700 nm.
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Mineral Salt: A Source of Costly Energy?

The report by Wick and Isaacs (*1*) on the energy that can be obtained from salt and brine deposits through osmotic conversion neglects some basic economic problems associated with these energy sources: the costs of mitigating the environmental impact, the costs of the physical plant and maintenance, and mining

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