Changes in Blood pH in Rats After Infection with 

*Streptococcus pneumoniae*

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Received for publication 13 November 1975

Acid-base alterations in *Streptococcus pneumoniae* infection were studied in 80 male albino rats. Hematocrit and concentrations of plasma electrolytes, glucose, and total protein were also measured. At 3-h intervals throughout a 27-h study, four control and four infected rats were anesthetized with ether, and blood samples were taken. Arterial blood pH, P<sub>02</sub>, and hematocrit increased in the infected group, whereas arterial P<sub>CO2</sub>, HCO<sub>3</sub><sup>-</sup>, and venous P<sub>02</sub> decreased. Plasma K<sup>+</sup> concentration increased slightly and glucose levels decreased in the infected rats as the sepsis progressed. No significant changes were observed in venous blood pH, HCO<sub>3</sub><sup>-</sup>, and P<sub>CO2</sub>. Plasma Na<sup>+</sup>, Cl<sup>-</sup>, and total protein remained unchanged. The increase in arterial blood pH and decrease in arterial P<sub>CO2</sub> and HCO<sub>3</sub><sup>-</sup> indicated respiratory alkalosis, which was present in rats infected with *S. pneumoniae*.

A variety of biochemical and physiological changes have been reported during experimentally induced pneumococcal sepsis in rats (9), mice (4), and rabbits (11, 12). This model infection was convenient for our study, as it produces an acute fulminating sepsis with a rapid time course to death. This experimental infection is characterized by the rapid onset of fever, bacteraemia, swelling, and edema at the site of subcutaneous inoculation of the organisms, and terminal hypotension (12). By 27 h, rats become moribund and deaths begin to occur. The site of inoculation and acute nature of this experimental infection preclude any significant changes within the lungs (12) commonly associated with pneumococcal infection in clinical illness.

Data concerning blood acid-base changes during sepsis have been conflicting. It has been observed that respiratory alkalosis occurs in man and dogs during the early stages of artificially induced fevers as demonstrated by a rise in blood pH and a fall in plasma CO<sub>2</sub> (1, 6, 7). In contrast, Burger et al. (2) showed that in lethal, terminal hyperthermia in rats, there was a fall in blood pH, P<sub>CO2</sub>, and HCO<sub>3</sub><sup>-</sup> as a result of metabolic acidosis and lactic acidemia. During fever caused by bacterial sepsis in man, respiratory, metabolic, or mixed alkalosis occurred (V. E. Gilbert and M. A. Petras, Fed. Proc. 26:804, 1967). No reports have been uncovered concerning sequential changes in pH and blood gases during bacterial sepsis. The present study was designed to examine serial changes in blood acid-base balance throughout the course of fulminating pneumococcal infection in rats.

**MATERIALS AND METHODS**

Experiments were performed on 80 adult, male, Fisher-Dunning rats (180 to 220 g). The rats were randomly placed into one of two groups. Controls were subcutaneously injected with 0.1 ml of sterile saline, and an experimental group was injected subcutaneously with 10<sup>8</sup> virulent type I *Streptococcus pneumoniae*. During the experimental period the rats were starved but allowed water ad libitum.

At 3-h intervals after inoculation, four rats were randomly selected from each group and anesthetized with ether. A laparotomy was performed, and blood was drawn from the dorsal aorta and inferior vena cava into heparinized syringes. Prior to blood sampling, rectal temperatures were recorded.

Blood pH, P<sub>CO2</sub>, and P<sub>02</sub> were determined at 37 C on a Corning 160 pH/blood gas analyzer. All individual values were corrected for the increased body temperature according to Severinghaus (5, 13). Bicarbonate values were calculated from the corrected values of pH and P<sub>CO2</sub> by the Henderson-Hasselbalch equation. Arterial hematocrits were determined by the microcentrifugation technique, and total plasma protein was measured on a refractometer. A total of 0.7 cm<sup>3</sup> of arterial sample from each rat was pooled within each group for glucose and electrolyte determinations. Glucose was determined by the orthotoluidine method (14). Cl<sup>-</sup> was measured on a chloridometer (3). Na<sup>+</sup> and K<sup>+</sup> were determined on a flame spectrophotometer in which lithium was used as an internal standard (10).

**RESULTS**

Six hours after inoculation with 10<sup>8</sup> *S. pneumoniae*, average rectal temperatures had in-
increased in the infected rats from 38.5 to 39.1°C (Fig. 1) and remained higher than those of controls through 27 h ($P < 0.05$). At 9 h postinoculation, the mean arterial pH in the infected group was increased over the control value ($0.06 \pm 0.01$ unit) and remained elevated throughout the study. The pH values were significantly higher than those of controls at 21 and 27 h (Fig. 2). In contrast, the arterial $P_{CO_2}$ and $HCO_3^-$ levels in the infected rats were significantly lower than those in controls at 9 h and remained low through 27 h (Fig. 2). Venous blood $P_{O_2}$ values were lower in the experimental rats than in controls at 6 h and became significantly lower at 18 h ($P < 0.05$) (Fig. 2). On the other hand, arterial $P_{O_2}$ levels in the infected rats were elevated at 6 h and remained above controls through 27 h.

When comparison was made between control and infected groups at corresponding time intervals, the infected rats showed the following changes. Between 6 and 27 h postchallenge, hematocrit increased (Fig. 1), and plasma K+ concentration, which averaged 3.4 meq/liter in controls, was elevated slightly at 15 h in the infected rats. This difference became more apparent between 21 and 27 h, at which time an average concentration of 5 meq/liter was reached in the infected group. Plasma glucose concentration began to decrease at 9 h and the difference of glucose levels between control and infected rats became progressively greater as time continued. By 24 h postinfection, plasma glucose levels were 50% of the control values. Although the mean values for $P_{CO_2}$ and $HCO_3^-$ in the venous blood of infected rats were lower than in the blood of the corresponding controls, and venous pH in infected rats was generally higher than in the control group, differences were not significant. No changes were observed in plasma Na+, Cl−, and total protein.

**DISCUSSION**

After the rats were infected with *S. pneumoniae*, the increased arterial blood pH and decreased $P_{CO_2}$ and $HCO_3^-$ demonstrated that respiratory alkalosis was predominant despite the undoubted presence of other apparent physiological and metabolic changes, which led toward a rapid septic death (4, 9, 11, 12). Respiratory alkalosis was probably due to fever and its induced hyperventilation (8). Changes in blood pH, $P_{O_2}$, and $HCO_3^-$ were noted at 9 h and at subsequent intervals. By 9 h, the infected rats developed a significantly elevated body temperature. Other signs of the infection were not obvious until 12 to 15 h postinfection, when rats became less active and developed roughened coats. Gilbert and Petras (Fed. Proc. 26:804, 1967) reported respiratory or mixed alkalosis in man with infection-induced fever. Likewise, respiratory alkalosis results from hyperthermia in dogs and man (1, 7). This model infection was not accompanied by pulmonary lesions that could have prevented or impaired exchange of gases in the lungs. Although unknown metabolic processes were involved in the rat febrile from pneumococcal infection, it appeared that respiratory alkalosis was responsible for blood pH changes.

Induction of severe hyperthermia in normal
rats produced metabolic acidosis as a terminal event (2). It should be noted, however, that the body temperature of the rats with induced hyperthermia (2) was raised to values 2.0 to 3.0°C higher than those presently observed during *S. pneumoniae* infection and, hence, considerably greater acute tissue injury may have occurred in the rats with extreme hyperthermia. This possible explanation for the observed differences between studies is strengthened by the fact that lactic acidemia was already well advanced when blood gas measurements were first made in rats with induced hyperthermia (2). The septic process was sufficiently rapid in the present study to preclude the appearance of any terminal evidence for metabolic acidosis.

Venous $P_{O_2}$ was lowered in the experimentally infected rats, particularly after 18 h. This finding may be the result of increased tissue extraction of $O_2$ decreased blood flow, or both. Although blood pressure and cardiac output were not measured in the present study, these values decreased in rabbits with lethal pneumococcal infection (12). The measured increase in arterial $P_{O_2}$ in the infected rats may be of little physiological significance, since the physico-chemical effect of elevated body temperature in the infected animal increases the partial pressure of $O_2$ in the blood. Although pooled blood samples prevent any statistical analysis, both an increase in plasma $K^+$ and a decrease in plasma glucose have been reported in mice with pneumococcal sepsis (4).

The hematocrit increased shortly before death, between 24 and 27 h postinfection. The increase in hematocrit may be caused by hemoconcentration due to decreased water consumption in infected animals or to a possible increase in capillary permeability allowing plasma to leak into the interstitial space, or both. Also, dehydration due to fever, hyperventilation and accumulation of fluid in subcutaneous tissues about the area of bacterial inoculation may contribute to the phenomenon of hemoconcentration.

ACKNOWLEDGMENTS

We thank Jack Condon and Clair Meske for their technical assistance, and Kenneth Zielmanski for chemical analysis.

LITERATURE CITED