Hyperventilation-Induced Hemolysis in the Dog (33635)

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Canine blood hemolyzes *in vitro* when CO_2 is depleted and pH is 7.8 or higher (1). Hemolysis occurs only after at least 30 min exposure of the cells to high pH. Preliminary data also indicate that hemolysis occurs *in vivo* when dogs hyperventilate during heat exposure. Only terminal pH values and a qualitative measure of hemolysis were obtained in the heat studies so that a quantitative relationship could not be established between pH and hemolysis. In more recent studies in which anesthetized dogs were artifically ventilated it was possible to collect blood periodically for determination of pH and hemolysis. The present paper presents the results of these studies.

Methods. Healthy, male mixed-breed dogs were anesthetized (pentobarbital, 30 mg/kg) and prepared with a tracheal cannula, and femoral venous and arterial catheters. The tracheal cannula was connected to a Harvard type respirator pump. Respiratory frequency was adjusted to simulate rates exhibited by unanesthetized dogs during heat exposure (2, 3). Stroke volume was maintained at 240 ml.

After a control period of 30 min with a "normal" rate of breathing (approx. 14/min) the frequency was increased to about 340 "breaths"/min for 90 min. Small infusions of 0.66 *M* THAM were used to help maintain blood pH at high levels. During control runs respiratory frequency was maintained at a "normal" rate for 120 min. During the control period the normal rate was essentially one which maintained blood (arterial) pH at 7.35–7.40. Blood (arterial) was taken into a heparinized syringe at the end of the control period and at 30, 60, and 90 min of the experimental period. Care was taken to prevent loss of CO_2 or contamination of the sample with air. Blood pCO_2 and pH were measured to the nearest 0.1 mmHg, 0.01 pH unit with an Instrumentation Laboratory blood pH/gas analyzer (model 113). Only blood pH will be reported here. Hemoglobin was measured spectrophotometrically using the reagent of Drabkin and Austin (4) dissolved in 0.9% NaCl to prevent precipitation of plasma proteins. Blood samples were prediluted 1:40 with 0.5% Triton X-100 (Rohm and Haas) in 0.9% NaCl. Triton X-100 produced hemolysis without interfering with the cyanmethemoglobin reaction. Plasma samples were not diluted. Hemolytic fraction (HF) was derived by dividing the concentration of hemoglobin in each plasma sample by the concentration of hemoglobin in the corresponding sample of whole blood.

Fifty dogs were run of which 26 were hyperventilated. Nine of the hyperventilated animals were also exposed to heat (43.3°) while the remainder were hyperventilated at 26.6°. Rectal temperature was monitored with a thermistor probe inserted 15 cm into the colon.

Results. Figure 1 shows that the erythrocytes of the dog hemolyze *in vivo* when blood pH is 7.7 or higher. We have included data from *in vitro* experiments (1) in Fig. 1 also. These data indicate that hemolysis *in vitro* occurs at a higher pH than *in vivo* (7.8 vs 7.7). However, pH changes *in vitro* occurred much more rapidly than *in vivo* so that a simple plot of pH vs HF does not show the time the blood was exposed to a given pH. Our earlier study (1) indicated that hemolysis of canine blood was time-dependent as well as pH-dependent. This observation is further strengthened by data shown in Fig. 1. This plot shows that even though pH of the 30-min samples is high (most around 7.8) the amount of hemolysis is not extensive and does not occur in a large percentage of the samples from that time period. Samples maintained for longer periods of time (60 and 90 min) at essentially the same or a slightly higher pH exhibit more hemolysis in

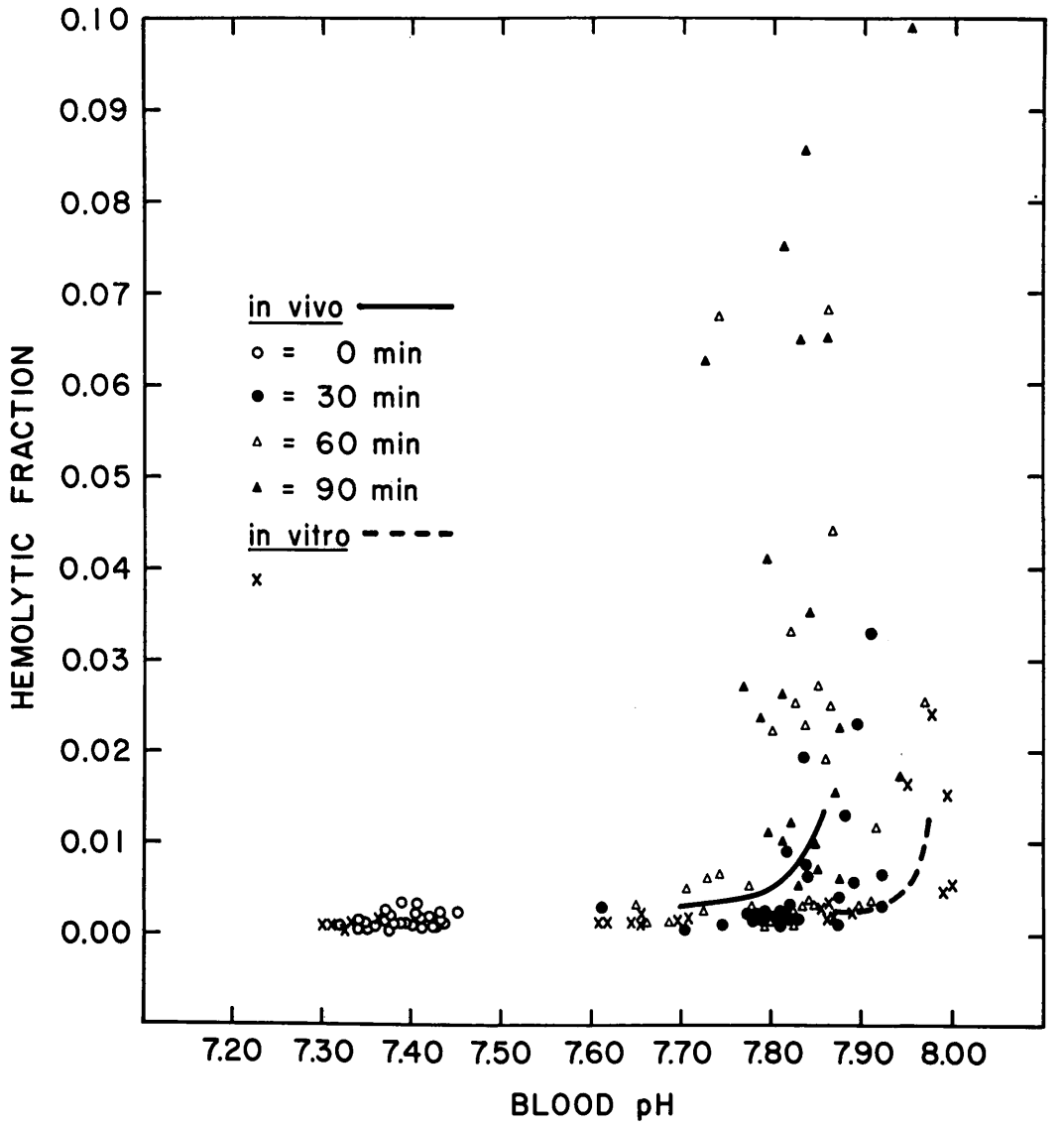

FIG. 1. The relationship of hemolysis to blood pH in dogs: data from *in vitro* experiments are shown for comparative purposes.

more samples. Table I shows these percentages and extent of hemolysis at each time period. The relationship of body temperature, pH, and time to degree of hemolysis is shown in Table II. Table II indicates that hemolysis occurs with time as long as pH is high and regardles of whether body temperature is rising or falling.

Discussion. The results of this study corroborate and extend the conclusion reached

TABLE I. The Relationship of Hemolysis of Canine Erythrocytes to Time.[a]

Time (min):	30	60	90
Samples with hemolysis (%)	35	58	85
Extent of hemolysis (HF)	.0139	.0269	.0334

[a] Sample was considered to exhibit hemolysis if hemolytic fraction (HF) value at 30, 60, and 90 min was above highest control (0 min) value (HF = .004).

TABLE II. Degree of Hemolysis in Canine Blood as a Function of Time of Exposure, Body Temperature, and Blood pH.

Time (min):	0[a]	30[b]		60[b]		90[b]	
Exposure temp. (°):	26.6	26.6	43.3	26.6	43.3	26.6	43.3
Body temp. (°)	37.96	36.11	38.52	34.99	39.72	34.04	40.19
Blood pH	7.39	7.83	7.81	7.81	7.77	7.84	7.82
HF[c]	.0015	.0071	.0045	.0208	.0087	.0341	.0180

[a] Values are means of 26 animals.

[b] Nine animals were exposed to heat (43.3°) and 17 were exposed to a neutral temperature (26.6°).

[c] HF = hemolytic fraction.

earlier (1) that canine erythrocytes hemolyze when blood pH is increased. The *in vitro* study showed that hemolysis would occur in canine erythrocytes when CO_2 was depleted and pH was 7.8 or higher. Quantitative evidence of *in vivo* hemolysis in the dog was lacking. The present study showed that *in vivo* hemolysis occurs when blood pH is increased to 7.7, or higher, via hyperventilation and maintained at that level for 30 min or longer. In addition, this study showed that a high body temperature was not required for hemolysis to occur *in vivo*. In fact, it would appear that more hemolysis occurred when body temperature was low. Body temperature of dogs hyperventilated at 26.6° fell as low as 34°. However, the animals exposed to heat always had a lower blood pH than the animals maintained at 26.6° ambient. Our earlier study showed that *in vitro* hemolysis was enhanced when the temperature of the blood was increased from 36 to 42°. It is possible that hemolysis would have been less in the cooled dogs reported here if pH had been maintained at the same level as in the heated dogs.

The mechanisms of pH-induced hemolysis remain open to speculation. These results only indicate that the mechanisms suggested (1) (i.e., direct effect of pH on cell membrane or disturbance of "ion pumps") are probably still valid.

Summary. Anesthetized dogs were hyperventilated via a respirator pump to increase blood pH to high levels. Canine erythrocytes hemolyze *in vivo* when blood pH is maintained at a high level (7.7 or higher) for 30 min or longer. Hemolysis occurs whether body temperature of the dog is rising or falling.

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