Respiratory Function of Blood of Prairie Dogs.* (29440)

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In the course of studies of responses of several species of small mammals to hypoxia, it was observed that the prairie dog showed a comparatively marked resistance to low oxygen tensions. As a consequence, studies were made of the respiratory function of the blood of this species in order to offer some evidence for the explanation of hypoxic tolerance.

Natural history of the prairie dog. The prairie dog has lived on the great plains of the United States since Pleistocene times. It was an important source of food to the American Indian. The prairie dog was first brought to the attention of zoologists by a specimen obtained by the Lewis and Clark expedition of 1804-1806. They called it a "barking squirrel" which is a more appropriate name than the present one of "prairie dog." Taxonomists place them in the family Sciuridae. Prairie dogs are not true hibernators. They appear to maintain a fairly constant body temperature of 37.5°C in the laboratory throughout the year. The species used in these studies is *Cynomys ludovicianus ludovicianus* (Ord).

Specimens used in these studies were obtained from Western Texas. All were adult males ranging in weight from 985-1310 g. They were kept in animal quarters at room temperatures between 20°C and 24°C and gained in weight throughout the winter months. They were fed timothy hay, oats, lettuce, carrots and Purina rat chow. They also became quite docile.

Gas transport functions of the blood involve primarily measurements of oxygen capacity, position of the oxygen dissociation curve, buffering capacity, carbon dioxide dissociation curve, effect of changes in pH on the oxygen dissociation curve (Bohr effect) and effect of oxygenation of blood on the carbon dioxide dissociation curve (Haldane effect). These studies were made on the blood of prairie dogs. Results of some measurements on the morphology of the blood are also reported.

Methods. Blood samples were drawn by direct cardiac puncture. Animals were tranquilized by a small injection of Nembutal given intraperitoneally. Minimal amounts of Heparin were used as an anticoagulant. Heparin (Organon) was diluted with an equal volume of isotonic saline and syringes rinsed with this solution. All excess solution was ejected from the syringes before cardiac puncture.

The pH of Heparin and saline solutions was measured routinely and found neutral. Blood samples were kept in ice water until transferred to tonometers. All tonometric analyses were completed within 2 hours of blood withdrawal.

Blood equilibration was carried out by methods previously described(1). Both oxygen and carbon dioxide were measured by use of 0.5 ml pipettes and the procedure used for these combined analyses has been described by Van Slyke and Neill(2). One additional procedure has been found useful. The fluid capacity of each Van Slyke pipette was calibrated by weighing mercury held by the pipette between the lower marking on the pipette and the delivery tip. After blood was delivered to the Van Slyke apparatus, the remaining volume was extruded into a volumetric flask (50 ml) containing Drabkin's reagent. The pipette volume up to the lower mark was rinsed several times with this reagent. This solution was used for determination of total hemoglobin. This procedure has been described previously(3) and the factor of 1.34 times the grams percent of hemoglobin was used to give total oxygen capacity of hemoglobin. Total oxygen capacity on one sample of blood was also determined by saturation of the blood with a pO_2 of 200 mm Hg. The 2 methods employed for determination of oxygen capacity agreed within the errors of the analytical methods. Measurement of oxygen capacity on the same sam-

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ple of blood as used in the Van Slyke analysis has certain obvious advantages.

Gas used in tonometric equilibration was delivered from large cylinders of mixed gases which had been analyzed in our own laboratory. Gases were completely saturated with water vapor at the temperature of equilibration. This becomes very important when small samples of blood are equilibrated in open tonometers.

In the study of the Bohr effect, samples for determination of pH of the equilibrated blood were drawn from tonometers directly into an Astrup micro pH meter and readings were taken immediately without change in temperature or exposure to air. Equilibrations of all samples were for 20 mintes at 37°C.

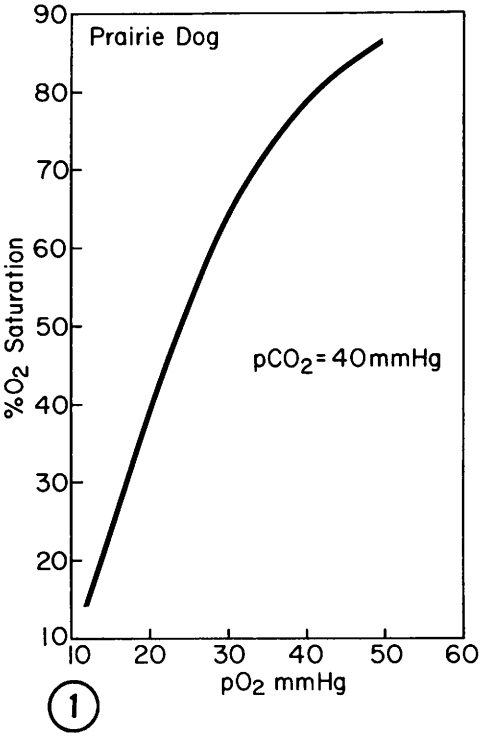
Analyses of hemoglobin, hematocrit, red blood count, and plasma protein were also made. Hematocrit values were determined by use of a micro-hematocrit centrifuge. Blood counts were made in the orthodox method of dilution and counting on a Neubauer slide. Hemoglobin values were determined by use of an Evelyn photocolorimeter and hemoglobin was read as metcyanhemoglobin. The indices calculated by these methods are shown in Table II.

Plasma protein was determined by use of a Bausch and Lomb refractometer designed for this purpose.

In study of the Bohr effect, 2 or more oxygen saturation points were determined at several pH values. Gas tensions were selected to give values in the proximity of 50% saturation to increase accuracy of this procedure. pH values were varied by varying the CO_2 concentrations of the equilibrating gases. These points were plotted logarithmically and a straight line drawn through them. The intersection with the 50% saturation line was used to determine the pO_2 at this saturation and pH.

The Haldane effect was determined by equilibration of similar samples of blood at a pCO_2 of 40 mm Hg, in one case with CO_2 in nitrogen and in the other with CO_2 at a pO_2 of 280 mm Hg.

Results. The position of the oxygen dissociation curve of blood from 8 male prairie dogs is shown in Fig. 1. The curve is drawn through the average values obtained. No at-



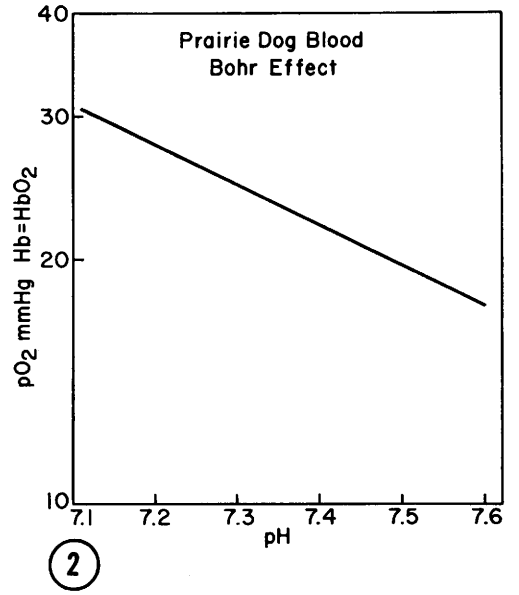


FIG. 1. Oxygen dissociation curve of prairie dog blood at pCO_2 of 40 mm HG.

FIG. 2. Effect of pH on oxygen saturation of prairie dog blood (Bohr effect).

tempt was made to determine percentage saturation below 15% or above 85%. Values for T_{50} (pO_2 in mm Hg required to half saturate blood, *i.e.*, where Hb = HbO_2) were found to be 24.1 mm Hg with a standard deviation of 1.5 mm Hg at a pCO_2 of 40 mm Hg.

Characteristics of the blood of 8 prairie dogs are summarized in Table II.

Fig. 2 shows the effect of pH on the position of the oxygen dissociation curve of blood from 5 prairie dogs. This curve was constructed by determining the partial pressure of oxygen required to half saturate the blood with oxygen. Variations in pH were obtained by equilibrating blood with gas mixtures containing various concentration of carbon dioxide and oxygen. A range of pH from 7.1-7.6 was obtained. The Bohr effect can be expressed by the formula:

$$\text{Log} \cdot \Delta pO_2 \text{ at } T_{50}/\Delta pH.$$

The blood of the prairie dog was found to have a value of 0.51. The significance of this value has been discussed by Hilpert *et al*(4).

The Haldane effect is shown in Table I. The "standard bicarbonate" is shown as the vol. % of CO_2 contained in reduced and oxygenated blood at a pCO_2 of 40 mm Hg. The difference in the standard bicarbonate represented the Haldane effect.

Discussion. Many suggestions have been offered to explain the position of the oxygen dissociation curve but most of them have been concerned with tolerance to low oxygen pressures. Schmidt-Nielsen and Larimer(5) investigated the position of the dissociation curve in 16 adult mammals and found that

TABLE I. "Standard Bicarbonate" Content and Haldane Effect in the Blood of 5 Male Prairie Dogs.

Prairie dog No.	Oxygen capacity, vol %	Vol % CO_2 (pCO_2 = 40 mm) of blood		Δ vol %
		Oxygenated	Reduced	
1	18.3	44.4	51.3	6.9
2	20.2	36.0	42.6	6.6
3	21.4	36.5	43.1	6.6
4	17.3	35.0	41.7	6.7
5	18.0	42.6	49.3	6.7

TABLE II. Blood Constituents of 6 Male Prairie Dogs Ranging in Weight from 985 to 1310 g.

Hematocrit (% cells)	47.34 ± 3.61*
Hemoglobin (g/100 ml)	15.11 ± 1.18
R. B. C. (millions/m^3)	9.50 ± .73
M. C. V. (μ^3)	49.8 ± .25
M. C. Hb ($\mu\mu g$)	15.91 ± .22
Plasma protein (g/100 ml)	6.82 ± .12

* ± S.D.

the partial pressure of oxygen required to half saturate blood was closely correlated with body weight. We have found the same correlation when similar species were studied, with the exception of the prairie dog, and certain inhabitants of high altitude.

The viscacha, a rodent inhabiting the high Andes has blood with a significantly greater affinity for oxygen than its lowland relative, the rabbit(6). The prairie dog, another rodent, appears to have blood with a higher affinity for oxygen than its close relative, the gray squirrel (unpublished data). The prairie dog is a burrowing animal and lives much of the time in burrows several feet below the surface of the ground. It's quarters are confined and while there appears to be no information as to the gas composition of burrows, it seems likely that oxygen may at times be lower in concentration than in the open air. It seems plausible from studies on the oxygen dissociation curve of animal blood that the position of the curve is not fixed by any single characteristic of the animal but rather is related to the composite physiological requirements of the animal in the environment in which it lives successfully. It would seem implausible that the curve is not a pattern of overall adaptation.

Summary. The respiratory function of the blood of prairie dogs (*Cynomys ludovicianus ludovicianus* (Ord)) has been studied. The position of the oxygen dissociation curve is to the left of other rodents closely related to this species and of similar size. The prairie dog blood resembles in some respects small mammals found at high altitudes and in other respects animals living near sea level. This is considered an adaptation to the habits and habitat of the species.

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Estrogens and Transport of Ova in the Rat.* (29441)

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The effectiveness of estrogens in interrupting pregnancy in the rat and mouse was first demonstrated by Parkes and Bellerby(1) and Smith(2). Later Burdick and his associates (3,4,5,6) reported that large amounts of estrogens accelerated ova transport while small amounts caused retention of ova in the Fallopian tubes where they ultimately degenerated. Dreisbach(7) has been successful in interrupting pregnancy in rats with a single subcutaneous injection of 0.4 mg/kg estrone up to the eleventh day post coitum (p.c.) He has further shown that as little as 20 μg/kg was effective during the period of tubal transport, while 100 μg/kg was necessary to prevent implantation after the ovum was free in the uterus. Edgren and Shipley(8) found that by single injection estrone was most effective in interrupting pregnancy in badger rats on day 3 of gestation (ED50 = 4.2 μg, compared with 13 μg on day 1) whereas from day 7 on, doses up to 160 μg were ineffective. Greenwald(9) reported that the effect of 10 μg estradiol cyclopentyl propionate (ECP) administered to rat on day 1 was to increase tubal and uterine motility so that the fertilized ova were expelled from the uterus by 48 hours p.c. The effectiveness of 5 μg of ECP in accelerating ovum transport varied from animal to animal.

In the rabbit, Pincus and Kirsch(10) found ova retained in the Fallopian tubes after administering estrone and related compounds during preimplantation stage. Noyes *et al*(11) lost many transferred rabbit eggs in estradiol-treated hosts, 23% passing into the vagina 10 to 78 hours after transfer. Black and Asdell(12) gave 2.5 mg estradiol to mated rabbits, but do not record, in contrast, any retention of eggs in the Fallopian tubes. Greenwald(13) reported that ovum transport in the rabbit is accelerated with 25 μg ECP whereas with 250 μg ECP retains ova at the ampullary-isthmic junction for as long as 5 days. These results were confirmed in a subsequent study(14) which indicated that the mechanism of interruption of pregnancy in rabbits by ECP depended on the dose level of the hormone.

Deanesly(15) observed the expulsion of guinea pig eggs from the Fallopian tubes when a very small quantity of estradiol benzoate is given immediately after mating. She has further shown that once the fertilized eggs have passed normally into the uterus, implantation is not prevented by as much as 30 or 50 μg of estradiol benzoate in the guinea pig, but post implantation development may be affected.

In this paper we describe the effects upon implantation and ovum transport in the rat (Sprague-Dawley) of estrone administered before implantation. The methods of pregnancy timing, of hormone administered and implant determination are those described previously(16).

Estrone and implantation. Various dosages of estrone (E_1) in 0.1 ml sesame oil were injected subcutaneously during the preimplantation stage (day 1 through 3) as indi-

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