OXYGEN TRANSPORT AND BLOOD BUFFERING CAPACITY IN THE MEDITERRANEAN PINE VOLE

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Oxygen transport and blood buffering capacity in the Mediterranean pine vole.– Blood samples of the Mediterranean pine vole (*Microtus duodecimcostatus* de Sélys-Longchamps) were analysed to determine blood buffering capabilities and some parameters of the blood respiratory system. Specimens were live-trapped in an orange orchard in Monte Gordo (Algarve, Portugal). The low values of the oxygen pressure and oxygen saturation of the blood, obtained in anesthetized voles, combined with the high levels of the carbon dioxide pressure, bicarbonate and 2,3-diphosphoglycerate found, appear to reflect no difficulties in the delivery of oxygen to the tissues and efficient blood buffering capacity under hypoxic-hypercapnic stress. This mechanism is expected to be useful for the living in a subterranean environment where these voles spend almost their entire life.

Key words: *Microtus*, Eco-physiology, Blood oxygen affinity, Western Mediterranean.

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INTRODUCTION

The hypoxia and high levels of carbon dioxide (CO_2) occurring within burrow systems may create respiratory difficulties to burrowing mammals. In fact, the concentrations of CO_2 found in rodent burrows (e.g. STUDIER & BACA, 1968; CHAPMAN & BENNETT, 1975; ARIELI et al., 1977) are within the chronic toxicity range that has been defined for humans (CAIN, 1981). DARDEN (1972) has suggested that the great insensitivity of fossorial mammals to CO_2 may be due to an efficient buffering capacity of their blood when compared with air-breathing mammals.

The Mediterranean pine vole, a rodent which occurs in southeastern France and southern parts of Spain and Portugal, has proven to be morphologically adapted to a fossorial life style (MADUREIRA, 1984). It is a strictly herbivorous vole which excavated extensive tunnels up to 40 cm below the surface of the soil, where it spends almost its entire life and obtains much of its food. Its habitat is restricted to areas of grassland including crops and open woodland (CORBET & OVENDEN, 1980; NIETHAMMER & KRAPP, 1982; MADUREIRA, 1984). However, in spite of the natural habits and structural characteristics of these voles being rather well-known, practically no quantitative data on their physiological attributes are yet available.

In this study, some respiratory properties and acid-base parameters of the blood of Mediterranean voles are examined in an attempt to evaluate the types of responses which enable them to remain underground for long periods. Moreover, the results are compared with literary data concerning fossorial and non-fossorial mammals.

MATERIAL AND METHODS

1. Experimental animals

Twenty-four adult specimens (11♀♀ and 13♂♂) ranging in body mass from 21.50 to 33.29 g were used. All of the specimens were live-trapped at the entrances of their burrows, in an orange orchard in Monte Gordo (Algarve, Portugal).

The voles were housed in small-sized terraria partly filled with soil. Air temperature was kept close to that of the outside, with natural photoperiod. Voles were fed apples, carrots, potatoes and water ad lib. No body mass loss was observed during this period. The experiments were conducted from September to December 1985. All measurements were made within two days of capture.

2. Blood analysis

All the analyses were made during daytime, almost without exception between 14:00 and 16:00 hours. The voles were anesthetized with ether and within the first minute of anesthesia mixed venous blood was drawn, by cardiac puncture, into heparinized syringes. Approximately 0.35 ml of blood was taken from each animal. The blood samples for pH and gas analyses were kept anaerobically on ice, into a closed syringe, until determination.

Analyses were initiated immediately after sampling and completed within 2 hours of blood withdrawal. Hemoglobin concentration (Hb) and oxygen saturation of the blood (SO_2) were measured in duplicate by photometry of a capillary fraction hemolysed directly by ultrasound, using an oximeter OSM 2 (Radiometer, Copenhagen), according to the method of SIGGARD-ANDERSEN (1977). Erythrocyte concentrations of 2,3-diphosphoglycerate (2,3-DPG) were assayed from extracts, deproteinized with trichloroacetic acid immediately after the blood sampling, with a commercial enzymatic test kit (Boehringer-Mannhein) (HELLERSTEIN & BUNTHRARUNGROJ, 1976; SAMAJA et al., 1983). Oxygen pressure (PO_2), carbon dioxide pressure (PCO_2), pH, base excess (B.E.) and bicarbonate concentration (HCO_3^-) were measured in duplicate, at 37 °C, with standard electrodes for blood gas and pH, in a IL 1302 pH/Blood Gas Analyser.

Due to the small amount of blood sampled from each vole not all of these analyses were conducted in each specimen.

RESULTS

Physiological characteristics of the blood of *M. duodecimcostatus* are summarized in tables 1 and 2.

Table 1. Hemoglobin (Hb) and 2,3-diphosphoglycerate (2,3-DPG) values of ten Mediterranean pine voles. X. Mean; S.D. Standard deviation.
Valores de hemoglobina (Hb) y 2,3-difosfoglicerato (2,3-DPG) de diez topillos comunes. X. Media; S.D. Desviación estándar.

	Hb (g%)	DPG (μmoles/ml)	DPG (μmoles/g Hb)
X	13.58	3.50	25.43
S.D.	1.10	0.95	5.55

Table 2. PO_2, SO_2 and acid-base status of the blood of *Microtus duodecimcostatus*. X. Mean; S.D. Standard deviation; n. Number of voles.
PO_2, SO_2 y estatus ácido-base de la sangre de M. duodecimcostatus. *X. Media; S.D. Desviación estándar; n. Número de especímenes.*

	SO_2 (%)	PO_2 (mm Hg)	PH	PCO_2 (mm Hg)	HCO_3^- (mM/l)	B.E. (mM/l)
X	25.97	27.61	7.19	86.57	32.98	2.28
S.D.	19.14	13.30	0.09	16.35	5.32	5.62
n	10	15	15	15	15	15

DISCUSSION

1. Respiratory properties of blood

The average hemoglobin value in the Mediterranean pine voles blood (13.58 ± 1.10 g%) is similar to that in normoxic rat or other air-breathing small mammals blood (e.g. MITRUKA & RAWNSLEY, 1981).

STUDIER & BACA (1968) have suggested that a high PCO_2 in the blood of subterranean rodents, as a consequence of high carbon dioxide levels in burrows, may lead to a respiratory acidosis or a Bohr-shift of the blood-oxygen dissociation curve sufficient to compensate the hypoxia which might result from the lower oxygen tensions. The average value of blood CO_2 pressure found (86.57 ± 16.35 mmHg) is very high when compared, for instance, with values for mice (40.0 ± 5.4 mmHg) or rats (42.0 ± 5.7 mmHg) (MITRUKA & RAWNSLEY, 1981). However, the occurrence of a short period of hypoventilation, less than one minute, between anesthesia and blood sampling can not be excluded. On the other hand, the results show a low average SO_2 (25.97 ± 19.14 %), even for venous blood, compared with blood PO_2 (27.61 ± 13.30 mmHg). Assuming an average $n = 2.5$ for Hill coefficient we obtain a P50 *in vivo* of approximately 41.7 torr. However, when corrected for the low pH observed, assuming a Bohr effect of –0.5 P50, at pH of 7.4 a value of P50 standard near 33.4 torr (SEVERINGHAUS, 1966; ABERMAN et al., 1975) was found.

The low average SO_2 which was obtained suggests that the operating range for the oxygen equilibrium curve, in this particular experiment, corresponds to less than 50 % saturation.

Moreover, the 2,3-DPG levels found in the blood of *M. duodecimcostatus* are very high (3.50 ± 0.95 µmol/ml), when compared to those found for other small mammals (e.g. AR et al., 1977). This suggests a low oxygen affinity *in vivo* which may facilitate the delivery of oxygen to the tissues during the permanence within burrows. This adaptation and the corresponding right-shifted oxygen dissociation curve seems to be similar to the one reported in low-altitude animals kept for long periods at high altitude (e.g. JOHANSEN et al., 1976; AR et al., 1977).

2. Blood buffering capacity

The average PCO_2 in the blood of the Mediterranean pine voles as well as the high HCO_3^- concentration values found, suggests an elevated buffering capacity of the blood in these animals. This is probably a response of the blood buffer system which protects the individuals from CO_2 retention and from a decresing in the pH of venous blood (table 2).

The average bicarbonate concentration found in *M. duodecimcostatus* (32.98 ± 5.32 mM/l) is higher than in humans (27.0 mM/l), rabbits (18.0; 22.8 mM/l) or rats (19.8 mM/l) (CHAPMAN & BENNETT, 1975).

The Mediterranean pine voles, in spite of spending the major part of the day underground, also spend each day a certain amount of time on the surface. They seem to be able to support and adjust their blood respiratory capability to marked differences in the gaseous atmosphere of their surrounding environment.

Data obtained through the blood analyses performed in *M. duodecimcostatus* as the low PO_2 and SO_2 values, suggest the adaptability of these rodents to hypoxia. Moreover, the high PCO_2 agree with the idea of a certain insensitivity of these small mammals to CO_2 which can be likely explained by the high blood buffering capacity of their blood as assayed by the bicarbonate content found (32.98 ± 5.32 mM/l). On the other hand as the pH of mixed venous blood is rather low (7.19 ± 0.09), the possible existence of a diminished sensitivity of the respiratory control receptors, which react to low pH, could also be important.

Their adaptive mechanism to hypoxia and rathter low PO_2 is also interesting. The high levels of 2,3-DPG (3.50 ± 0.95 µmol/ml) together with the low pH and high PCO_2 values found, will cause a shift to the right of the blood-oxyhemoglobin dissociation curve, in

situations of hypoxia or hypercapnia, increasing the oxygen supply to tissues.

Results suggest that the respiratory strategy used by the Mediterranean pine voles to maintain an adequate oxygenation can be based in the high concentration of red cell 2,3-DPG which seems to be the main parameter controlling the supply of oxygen to the tissues.

RESUMEN

Transporte de oxígeno y capacidad tampón en la sangre del topillo común.

En este trabajo se analizan algunos parámetros de la sangre de *Microtus duodecimcostatus* para determinar la capacidad tampón y el sistema de transporte de oxígeno.

Este topillo que vive en galerías puede soportar grandes variaciones de la composición gaseosa circundante.

La estrategia respiratoria que permite a este topillo vivir en ambientes con oxígeno insuficiente, puede basarse en la alteración de la curva de disociación del oxígeno, y a la regulación del equilibrio ácido-base (tabla 2). La concentración de 2,3-DPG de los glóbulos rojos será el principal parámetro en el control del desvío directo de su curva de disociación (tabla 1).

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