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0014-4754/86/020126-06$1.50 + 0.20/0

Short Communications

Adaptive heart and breathing frequencies in 4 ecologically differentiating chromosomal species of mole rats in Israel

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Summary. Breathing (f_R) and heart (f_H) frequencies decreases as aridity increases in 4 chromosomal species of the *Spalax ehrenbergi* that inhabits humid to arid habitats in Israel in the order 2n = 52, 58, 54, 60. Breathing frequencies were 50.0, 46.9, 45.9, and 43.4% of the expected values, and f_H were 37.6, 32.7, 27.8, and 25.8% for 2n = 52, 58, 54, and 60, respectively. The decrease of f_R and f_H has a genetic basis and correlates with the metabolism of the mole rat.
Key words. Physiological adaptation; speciation; subterranean mole rat.

The actively speciating superspecies *Spalax ehrenbergi* has recently radiated into 4 chromosomal species while colonizing increasingly arid zones. The species 2n = 52 inhabits the humid northern region of Israel, and the 2n = 58 is situated in a less humid region south of the 2n = 52. To the northeast lies the range of the 2n = 54 in the drier Golan Heights[1,2], and the species 2n = 60 lives in the southern, most arid part of its distribution range. Radiation into new ecological niches should be concomitant with physiologic adjustments[3,4]. Adaptation to fossorial life in the underground atmosphere (hypoxia and hypercapnia) greatly affects almost every mechanism along the gas transport system of the mole rat[5–8]; therefore, one would expect to find differences between the 4 chromosomal species in their gas exchange physiology, which is under selective environmental pressure[9]. In the present study we compared resting breathing frequency (f_R) and resting heart frequency (f_H) of the 4 chromosomal species. Frequency of both heart and respiration in the mole rat is very low compared to that found in similar-sized mammals, which is typical of adaptation to an underground atmosphere[6,7]. Therefore, 2 opposing trends may be suggested for the mole rat: (1) If a hypoxic-hypercapnic environment selects for low f_H and f_R, then in the north, where precipitation clogs the air pores in the heavy soil (2n = 52), low f_R and f_H are expected compared to the aerated lighter, dry soil of 2n = 60. (2) The metabolic rate of mole rats decreases as aridity increases[3,4]. A reduced 0_2 demand from 2n = 52 to 2n = 60 is expected to correlate with reduced convection of both air and blood, and therefore f_H and f_R are expected to decrease from the humid (2n = 52) to the arid (2n = 60) habitats.

Materials and methods. Thirty-seven mole rats were used in the experiment. The f_H and f_R were not measured in every animal; thus, f_R was measured in 30 mole rats, and f_H was monitored in

Experientia 42 (1986), Birkhäuser Verlag, CH-4010 Basel/Switzerland

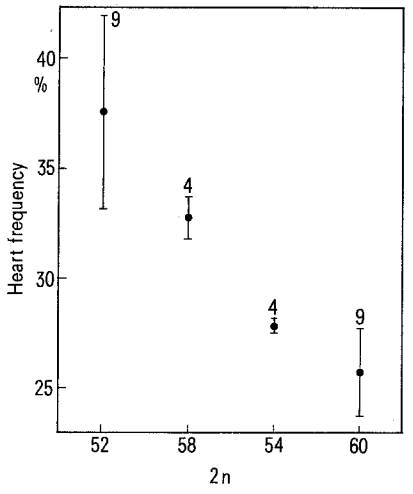

Figure 1. Heart frequency (mean ± SE) as percent of value expected for the animal's body mass[11], plotted for the 4 chromosomal species of mole rat (abscissa) in order of increasing aridity. Sample size appears above each group.

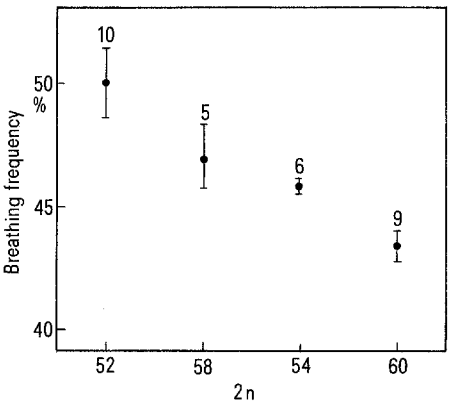

Figure 2. Breathing frequency (mean ± SE) as percent of value expected for the animal's body mass[11], plotted for the 4 chromosomal species of mole rat (abscissa) in order of increasing aridity. Sample size appears above each group.

The chromosomal species tested

Chromosomal species (2n)	Sample size (N)	Sex (M/F)	Weight (g) mean ± SD
52	11	2/9	131 ± 21
54	8	1/7	132 ± 10
58	7	1/6	112 ± 13
60	11	4/7	117 ± 20

26 animals. Bigger samples were taken from the populations of 2n = 60 and 52 because they inhabit the 2 extreme ecological niches and represent the more chromosomally distant genetic types[10]. The number and weight of the animals are given in the table. The mole rats of 2n = 60 were captured in Lahav and Anza, the 2n = 58 population from Zipori and Afiq, the 2n = 54 mole rats from El-Al, and the 2n = 52 population were trapped in Ma'alot, Kiriat Shmona, and Kerem Ben-Zimra (see localities and geographic distributions of the 4 species in refs. 2 and 9). The mole rats spent more than 10 months in captivity; thus, any acclimation to different environmental conditions should have faded away during captivity, in which the animals stayed at normoxia, 21 °C, and 70% relative humidity. Heavier animals were too big for the experimental chamber; therefore, the number of females in the sample was greater than the number of males.

The experimental system consisted of a cylindrical glass chamber (18 cm long and 6.3 cm in diameter) equipped with an 8-copper-plate floor. Each copper plate was welded to a wire leading to an external plug, and at any time 3 of the 8 plugs could be connected to an EKG preamplifier of Dinograph chart recorder (Beckman). An ultrasensitive Validyne pressure transducer (DP-103-40) was used to record respiratory frequency.

An animal was weighed and then placed in the chamber after coating the copper plates with electrolyte gel. The chamber was then placed in a thermoregulated bath (31 °C) while humidified air was pumped through the chamber (700 ml/min). The experimental system was confined in a Faraday cage to reduce the electrical noise. The mole rat was allowed to adjust to the chamber for at least 1 h before measurements were started. Collecting data was done for at least 1 h (at 10-min intervals) only when the animal was at rest. The 5 lowest measurements of f_H and f_R were taken for the final analysis.

Because of the expected effect of the size of an animal on its f_H and f_R, we standardized these 2 variables by dividing the measured value by the expected value for the body mass of the animal[11]. We compared the 4 chromosomal species using Spearman's rank correlation, correlating either f_R or f_H with the aridity order of the 4 species.

Results. Respiratory frequency as a function of order of aridity is plotted in figure 1. Breathing frequency decreased as the aridity increased: the correlation coefficient was significant both for the measured f_R and for the percent of the expected f_R ($r = 0.63$, $p < 0.01$ and $r = 0.66$, $p < 0.01$, respectively). The distribution of f_H among the 4 chromosomal groups was similar to that of f_R (fig. 2): the correlation coefficient of measured f_H and measured/expected f_H versus order of aridity was significant ($r = 0.51$, $p < 0.01$ and $r = 0.55$, $p < 0.01$, respectively).

Discussion. A similarity between the f_H and f_R of the 4 chromosomal species of the mole rat was found (figs. 1 and 2). Relatively high frequencies were shown for 2n = 52. Intermediate frequencies were found for the 2 species which border 2n = 52, namely, from the southern, more humid climate, 2n = 58, and from the eastern, drier climate, 2n = 54. The lowest frequencies were recorded for the southern 2n = 60, of the driest climate. The high variability of 2n = 52 could be due to the 3 different sources of these mole rats, and the low variability of 2n = 54 could be related to the single trapping location. The trend in heart and breathing frequencies agrees with the reduction of basal metabolic rate as a function of aridity[3,4]. Thus, besides the low f_R and f_H which are typical of fossorial mammals and reflect the improved extraction of 0_2 and the potential to elevate f_R and f_H at hypoxia[6-8], the reduced metabolic rate as aridity increases causes further reduction of both f_R and f_H, as has been shown for a desert canid[13]. The differences between the chromosomal species are genetically determined, because the animals spent a long time in captivity to eliminate any previous acclimatization and to correlate with other genetically determined traits such as swimming ability[12], tissue gas tension[9], and nonshivering thermogenesis[4]. The adaptive pattern found provides one of the many morphological, physiological, and behavioral factors associated with the active speciation and adaptation of subterranean mole rats in Israel.

Acknowledgments. We thank A. Beiles for his help in the analysis. E. Nevo thanks S. Simson for field work and the Israel Discount Bank Chair of Evolutionary Biology and the 'Ancell-Teicher Research Foundation for Genetics and Molecular Evolution' (established by Florence and Theodore Boumritter of New York) for financial support.

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0014-4754/86/020131-03$1.50 + 0.20/0

Muscle proteinase activities during compensatory growth and atrophy

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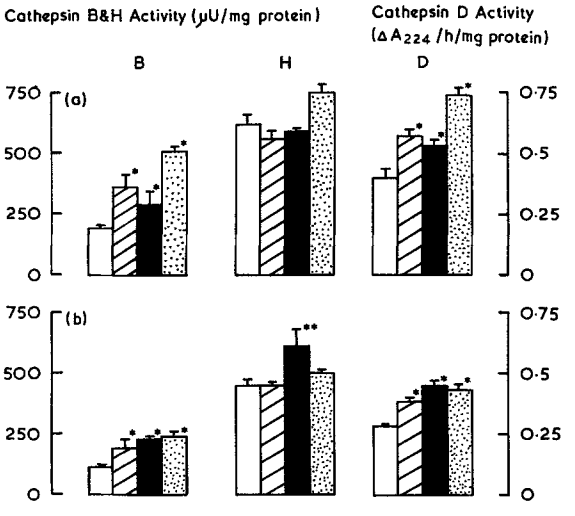
Summary. Specific activities of cathepsins B and D, but not H, increased in both the tenotomized gastrocnemius and functionally overloaded soleus muscles, thus correlating with previously reported increases in protein degradation. Subsequent denervation of the overloaded soleus caused an additive increase in proteolysis, suggesting a possible greater lability of proteins in this muscle.
Key words. Cathepsin B, D and H; skeletal muscle; tenotomy; denervation; protein degradation; compensatory growth; muscle atrophy.

Alterations in work demands are known to induce appreciable changes in the size of skeletal muscle(s); reduced activity generally leads to atrophy while increased functional demands evoke compensatory growth[1]. This plasticity of muscle clearly offers selective advantages to the organism, within a framework of biological efficiency and economy. Tenotomy of a lower hind limb muscle (e.g. gastrocnemius) is an experimental model which has been widely used, because it simultaneously induces muscle wasting and adaptive growth; the former is induced in the tenotomized muscle itself, while the latter arises in functional synergists (e.g. soleus[2]) which are left to perform proportionately more work if flexing the ankle. This form of compensatory growth has been extensively studied, with changes in fiber type proportions, metabolic enzymes and polymorphic forms of contractile proteins having been described[2-4]. In attempting to explain how the additional growth occurs good agreement exists, from a variety of approaches (both in vivo[2,5] and in vitro[4]), that protein synthesis is increased in such functionally overloaded muscles. The role played by protein degradation is however much less clear. Few studies have been undertaken in this area and the available information is contradictory; one study[2] has suggested a decrease in the degradative rates while another[5] has indicated an increase. These discrepencies clearly need to be resolved.

Surprisingly, much less overall information is available concerning the time related changes in the tenotomized muscle. Although it has only been possible to obtain indirect (i.e. calculated) rates of protein breakdown in this muscle[5], it has been suggested that increased proteolysis, rather than any appreciable change in protein synthesis, is primarily responsible for the observed muscle atrophy[5]. It is therefore important to establish precisely what role protein degradation plays in influencing muscle size in both experimental situations, for as yet this remains uncertain.

Results and discussion. The aim of this study is to resolve these uncertainties by measuring the activities of three proteinases believed to be involved in the catabolism of intracellular proteins. Two of these enzyme activities, i.e. cathepsin B and D, have recently been shown to change in accord with induced alterations in the rate of muscle protein degradation[6]. Such a correlation is further supported in the present study. That is, higher proteinase activities were found in the control soleus (fig. a), compared with the gastrocnemius (fig. b), which is in keeping with the higher rate of protein turnover in the former muscle[7,9]. In addition, the specific activities of both cathepsins B and D, were found to increase significantly in both the tenotomized gastrocnemius (fig. b) and the overloaded soleus (fig. a). In contrast, cathepsin H remained remarkably resistant to change.



Changes in cathepsin B, D and H activities in the soleus and gastrocnemius muscles after various experimental procedures. Total activities of cathepsin B, H and D were measured in homogenates (1:50 H_2O, w/v) of soleus (a) or gastrocnemius (b) muscles isolated from 55 g Charles River (CD strain) male rats, either 3 or 6 days after various operative procedures had been performed. Cathepsin D was assayed against denatured hemoglobin, at pH 3.5; activity is expressed (on right axis) as the change in absorbance of perchloric acid-soluble materials when measured at 224 nm[7]. Cathepsins B and H were measured against the synthetic substrates Z-Phe-Arg-NMec and Arg-NMe, at pH 6.0 and 6.8 respectively[8]; in both cases one unit of activity (left axis) represents the amount of enzyme capable of releasing 1 μmol of aminomethylcoumarin/min. Each value is the mean ± SEM of at least 5 muscles. Student's t-test was used to determine the level of statistical significance (* $p < 0.01$; ** $p < 0.025$), in all cases being compared to control muscle values of contralateral, sham-operated limbs. Soleus muscles (a) are denoted as control (□), 3-day functionally overloaded (▨), 3-day denervated (■), or 3-day overload followed by 3 further days of denervation (▩). Gastrocnemius muscles (b) are denoted as control (□), 3-day tenotomized (▨), 3-day denervated (■) or 3-day tenotomized followed by 3 additional days denervation (▩).