# Heart mitochondria in rats submitted to chronic hypoxia

**J. Cervós Navarro[1], R.Ch. Kunas[2], S. Sampaolo[2] and U. Mansmann[2]**

[1]Instituto de Ciencias Neurológicas y Gerontológicas, Universitat Internacional de Catalunya, Barcelona, Spain, [2]Department of Neuropathology and [3]Department of Medical Statistic and Epidemiology, Freie Universität Berlin, Hindenburgdamm, Berlin, Germany

**Summary.** The effect of prolonged exposure to normobaric hypoxia on the mitochondria of myocard of rats exposed for several weeks to 8 and 7% $O_2$ has been morphometrically evaluated. Twelve male Wistar rats housed in Nalgene cages (2 per cage) with a batch of six cages placed in plexiglass chambers were maintained in air/$N_2$ mixtures containing different concentrations of $O_2$. Six animals kept in similar cages under normoxia served as controls. When at day 60 the $FIO_2$ was reduced to 8%, the weight increase stagnated and after the 81st test day, on which the hypoxic animals were subdivided into 8% and 7% groups the weight curve showed a decrease in the mean body weight for both groups. The arrest and the following loss of weight beyond the 85th day may be interpreted as the expression of a limit reached in the compensation capacity. In the 8%-group the shape of the mitochondria varied more markedly often with budding and furrowing of the surface. In the 7%-group bizarre shapes and wide variations in size with a decided shift towards larger mitochondria were noteworthy. While rats kept under 8% oxygen exhibited a numerical increase in myocardial mitochondria compared to controls, the mitochondria of the 7%-group were numerically reduced. The results suggest that hypoxia of 8% oxygen is compensatable, if only to some extent, by an increasing surface of mitochondrial membranes, and that further reduction of oxygen causes compensation mechanisms to fail as seen by the severe alterations of the mitochondrial population of the cardiomyocyte in the 7%-group.

**Key words:** Heart, Mitochondria, Chronic hypoxia, Ultrastructural morphometry

## Introduction

Human tolerance to severe chronic hypoxia has been examined in two high altitude studies: one was the 1981 American Medical Research Expedition to Mount Everest and the other the Operation Everest II (OEII)

undertaken in 1985. OE II was a chamber study, in which subjects breathed air decompressed to the equivalent of 8,848 m for six weeks. But though numerous papers have been published from OE II and a great amount of data has been obtained on cardio-vascular and pulmonary acclimatization of man to extreme altitudes, only few experimental studies have been performed.

It has been repeated that morphological changes in acute ischemia first manifest in the mitochondria which can be regarded as sensitive indicators of hypoxia. The importance of mitochondrial $Ca^{2+}$ regulation (Miyata et al., 1992; Silverman, 1993) and the protective effect of phosphocreatine in acute hypoxia were discussed. However, no recent studies on mitochondrial changes in chronic hypoxia are available.

In a model of prolonged exposure to normobaric hypoxia (Cervós-Navarro et al., 1991; Patt et al., 1997) we examined the effect on the myocard of rats exposed for several weeks to 8 and 7% $O_2$ with special regard to changes in mitochondrial morphology.

## Materials and methods

Eighteen male Wistar rats (*Rattus norwegicus*) with at an initial age of 6 months and a body weight of 299±17.7 g were used. Twelve animals were kept under hypoxic conditions, while six animals served as controls.

### Exposure to chronic hypoxia

The rats were housed in Nalgene cages (2 per cage) with a batch of six cages placed in plexiglass chambers with a volume of 0.36 m$^3$. Air or gas mixtures containing different concentrations of oxygen were pumped into the chambers at a rate of 1800 l/hour. The whole gas volume of the chambers was replaced 5 times per hour. The flow of the gas mixture including $N_2$ was regulated by flowmeters. The gas concentrations (including $CO_2$), temperature and relative humidity were monitored and kept constant with the aid of an oxymeter and a $CO_2$-meter (Oxytest, "Uras M"; Hartmann and Braun AG, Frankfurt). Chamber concentrations of $CO_2$ were never higher than 0.5%. Rats of the experimental group were exposed to successively higher degrees of

normobaric hypoxia. The duration of each step and the constitution of the gas mixture were chosen on the basis of preliminary experiments: adaptation to a given level of hypoxia, which allowed passage to lower oxygen concentrations, was established when haematocrit values and blood gases revealed a steady state. The final schedule (Table 1) was: 7 days normoxia, 15 days at 15% $O_2$ and 22 days for each of the further levels of hypoxia: 12, 10 and 8% $O_2$. After this time 6 animals were maintained at the 8% level for a further 15 days (8% group). The other 6 were exposed to 7% $O_2$ for 17 days (7% group). Every 3 days, the chambers were opened in order to be cleaned and to change food and water, which produced normoxic conditions for a few minutes. At these timepoints the rats were weighed.

Control rats were housed in similar cages and chambers under normoxic conditions with pure air being pumped in all the time.

All procedures adhered to the German laws for protection of animals.

### Tissue preparation

The animals were killed in a specially constructed chamber containing a guilloutine which enabled the maintenance of the hypoxic conditions before and during the decapitation.

The heart was removed after sternotomy and resection of the lateral thoracic areas. The pericardial vessels were resected prior to weighing of the heart. Blocks were obtained by removing an approximately 1 mm-thick tissue disc parallel to the diaphragma cordis at the mid-ventricular level. The tissue disc thus corresponded to a cross section of the heart on the ventricular plane. From the right ventricle (R), the interventricular septum (S) and the left ventricle (L), three blocks each were removed from the tissue disc, i.e. altogether 9 blocks per heart. Depending on the thickness of the ventricular wall, the tissue blocks varied from less than 1 mm to 3 mm in length and from 0.5 mm to 1 mm in width. They were fixed in glutaraldehyde and embedded in epoxid prior to electronmicroscopy.

### Quantitative methods

Morphometric evaluation was performed based on a mean of 10 micrographs of each myocardial region, i.e. altogether 30 per heart in an 8,000-fold initial magnification. Detailed enlargements in a 15,700-fold magnification were prepared from two images each on the average. The images were taken following the principle of chance developed according to Weibel and Gomez (1962) and based on two requirements: 1. The images must be taken according to a system of constant distances. 2. The images evaluated must not include parts of nuclei, vessels or extensive intercellular substance. This reduces the number of assessable images to an average of 6 per myocardial region, i.e. 18 per heart.

**Table 1.** Hypoxia model.

| DESIRED $FIO_2$ (%) | GAS MIXTURE AIR: | CHAMBERS N2 (l/min) | DURATION (days) | SIMULATED ALTITUDE (m over sea level) |
|---|---|---|---|---|
| 15 | 22.5 | 7.5 | 15 | 2,600 |
| 12 | 18 | 12 | 22 | 4,450 |
| 10 | 15 | 15 | 22 | 5,800 |
| 8 | 12 | 18 | 25/40* | 7,400 |
| 7 | 10.5 | 19.5 | 17 | 8.350 |

air pressure: 759±4 mmHg: T: 23 °C: relative air humidity: 60%: $FIO_2$: inspiratory $O_2$-partial pressure; *: divided groups of animals in 8% and 7%

The planimetric measurement of the micrographs was performed using an IBAS 2 computer. The computer received the image data directly from the negatives (6x6 cm) via a video camera. The standard measuring surface was marked by a square frame (a = 3.444 $\mu$m) that was projected onto the screen with constant coordinates. Its size was determined in such a way that the distance from the edge of the screen to the measuring frame corresponded approximately to the maximal extent of the mitochondria. This enabled mitochondria that were cut by the frame still to be measured in their full extent on the screen. All mitochondria were measured which were situated completely inside the measuring frame or were cut by two adjacent and predefined sides of the measuring square.

No consideration was given to any mitochondria situated outside the measuring square or cut by the two other sides of the square. The mitochondria to be measured by the computer were encircled with a pointing device (mouse). A program based on the principle of contrast enhancement enabled the thus encircled mitochondria to be demonstrated as white areas on a black background, which were then counted and individually calculated. The data of the average total of 6 micrographs for each myocardial region were combined and stored for further processing.

### Stereology

The three indices V ($\mu$m$^3$ volumen), Nv ($\mu$m$^{-3}$ number) and $V_v$ (%) were calculated according to the formulas developed by Weibel and Gomez (20).
V: The mean volume per mitochondrion is obtained by simple division of the volume density ($V_v$) by the numerical density ($N_v$):

$$V = \frac{V_v}{N_v}$$

$N_v$: According to the authors, it is inadmissible to interpret the mitochondrion sections (NA) counted per standard measuring surface in terms of a numerical density. This is dependent on the size distribution (K)

and shape of the mitochondria:

$$N_v = \frac{K \times NA^{3/2}}{\beta V_v^{1/2}}$$

The form coefficient ß for ellipsoidal structures is a function of the axial ratio 23λ = L/D (longitudinal to transverse diameter). From about 250 measurements, a mean value of λ = 3.1 was determined for the mitochondria of the micrographies applied here. This enabled ß = 2 to be determined with the nomograph developed by Weibel and Gomez (1962).

K is determined by the size distribution of the objects. The values for K usually vary between 1.01 and 1.1. Different values had to be determined for K in conformity with the different size distribution of the mitochondria in Groups C, 8%, and 7% (appendix, histograms 1-3). For the three groups of the present study, the range between 1.01 and 1.1. was subdivided in conformity with their variation coefficients according to the formula:

$$K = 0.09 \times \frac{V_{kn}^{-1}}{V_{k1}} \times \frac{V_{Ki}}{V_{k1}} + 1.01$$

$V_{kn}$ is the variation coefficient with the highest value, $V_{k1}$ that with the lowest, and $V_{ki}$ are all variation coefficients $< V_{k1}$. Determinations were done for K(C) = 1.096, for K(8H) = 1.09 and for K(7H) = 1.1.

$V_v$: According to the principle of Delesse, the relative volume fraction of the mitochondria is: $V_v \approx A_A$ where $A_A$ is equal to the relative surface fraction of the unit area comprised by all mitochondrion slices.

### Statistics

Assuming a normal distribution of numerical and volumetric density, the t-test for independent random samples was performed for these parameters. Since a normal distribution of the mitochondrion size V cannot be assumed, the Mann-Whitney test was used for V. Only for the group of 8% hypoxia was no statistical test applied because the loss of two animals reduced their number to n=4. A relation of the indices V, $N_v$, and $V_v$ to each other and to other parameters (body and heart weight) was tested by calculating the correlation coefficient r.

## Results

Figure 1 depicts the development of body weight in the course of the experiment. In the first three stages of hypoxia (FIO$_2$ 15%, 12% and 10%), the animals showed a continuous increase in body weight, though the weight curve took a somewhat flatter course than in the controls. When at day 60 the FIO$_2$ was reduced to 8%, the weight increase stagnated, and a plateau was reached at a value of about 365g compared to a weight of about

405g for the control animals (p < 0.04). After the 81st test day, on which the hypoxic animals were subdivided into 2 groups of a further reduced FIO$_2$ of 7% and a continued FIO$_2$ of 8%, the weight curve showed a decrease in the mean body weight for both groups down to 318.5 g for the 8%-group (p<0.001) and 306.7 g for the 7%-group (p<0.001). In contrast, the control animals reached a mean weight of 423.0 g.

The mean absolute heart weight of the hypoxic animals was 2.1±0.38g in the 8%-group and 2.08±0.54g in the 7%-group and thus differed markedly (p<0.001) from that of the controls (1.45±0.08 g). Calculation of the relative heart weight with reference to 100 g body weight disclosed nearly a doubling from 0.345±0.025 g (controls) to 0.665±0.095 g (8%) (p<0.001) and 0.68±0.162 g (7%) (p<0.001) respectively.

Hearts taken from the hypoxic animals were not only larger than those from the normoxic ones, but their considerably darker aspect clearly distinguished them from the control hearts. Intramural arteries were thickened and often partly hyalinized. Myocardial infarcts were seen in 2 animals of the 7%-group and in one of the 8%-group.

The cross section through the center of the ventricles parallel to the diaphragma cordis revealed a markedly enlarged right ventricle with a slightly thickened wall. In contrast, the left hearts of the hypoxic and normoxic animals were approximately the same size, though the lumina of the left ventricles were smaller in the hypoxic animals than in the controls. The findings corresponded to a ventricular hypertrophy that was eccentric on the right and concentric on the left.

In both the 7% and 8% O$_2$ groups about 60% of the animals showed various degrees of mucoid change and oedema in the mitral and tricuspid leaflets. Endocardial
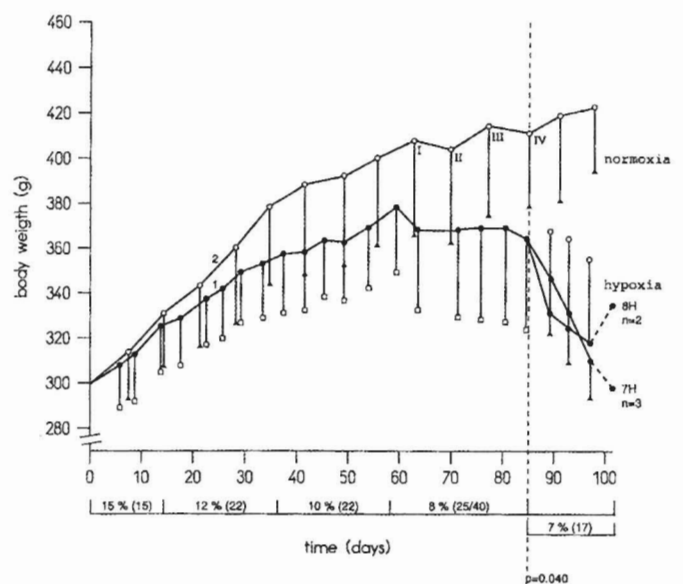


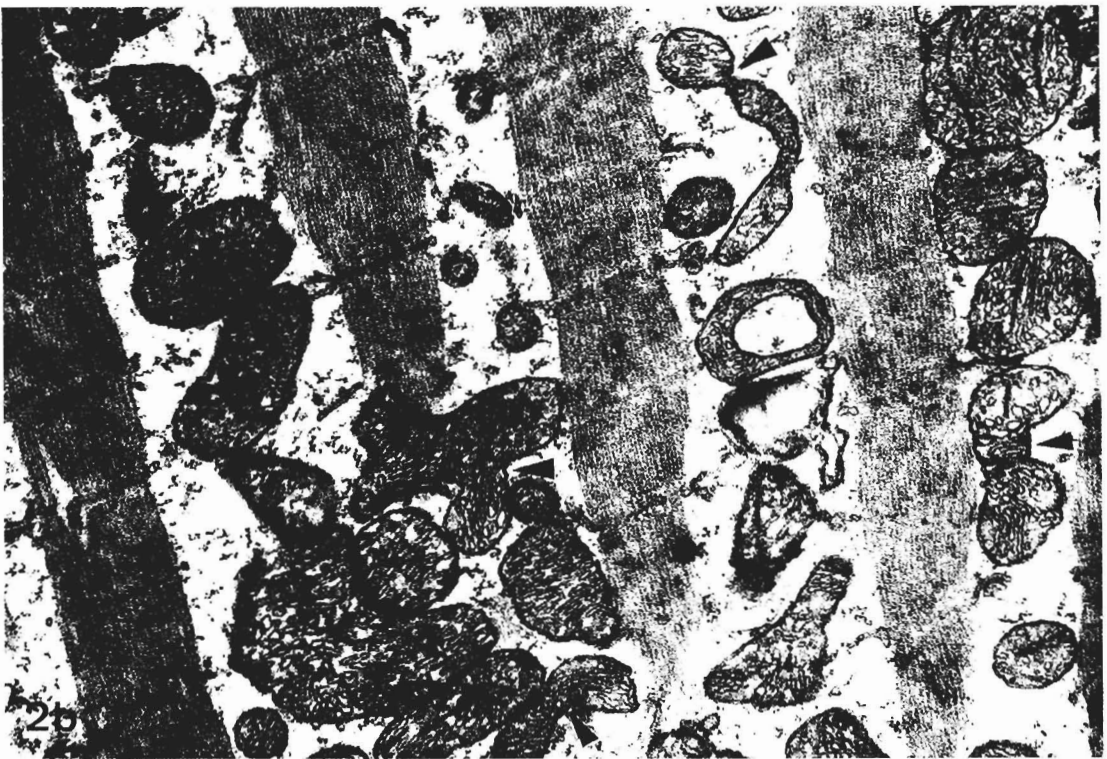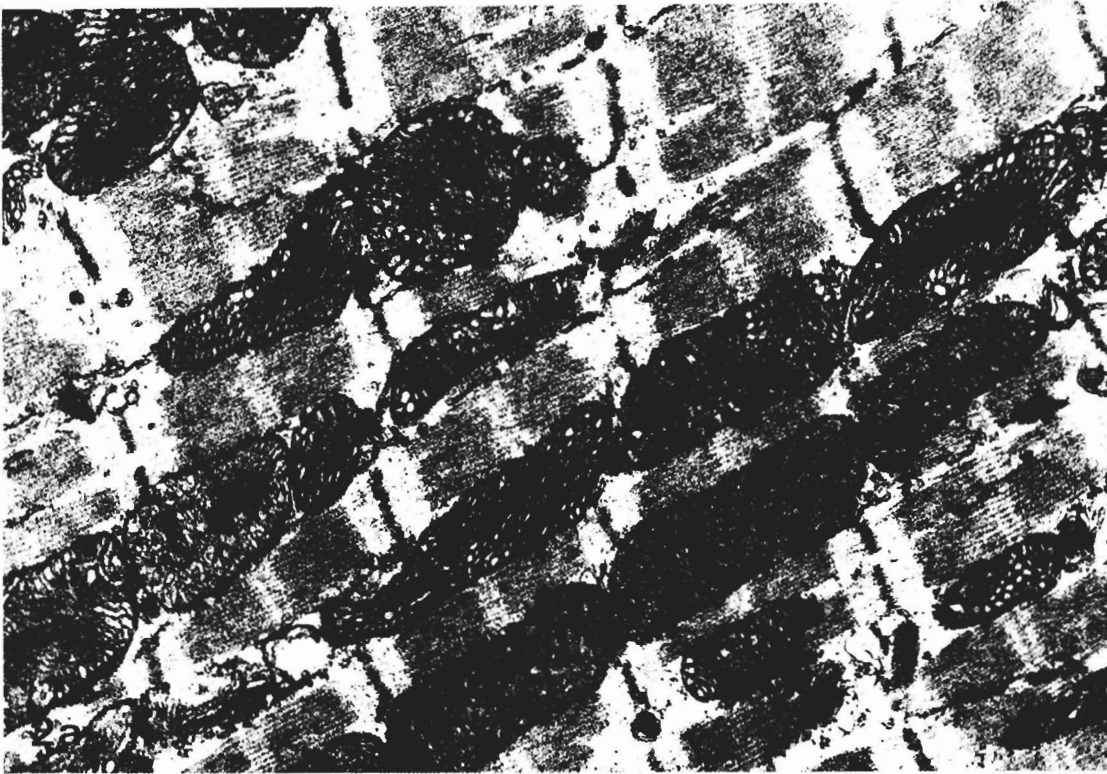**Fig. 1.** Development of body weight in normoxia and hypoxia.

**Fig. 2. a.** Control animals. The shape of the mitochondria is mainly regular. **b.** Hypoxia 8%. The shape of the mitochondria varied markedly. Buccing of the surface (arrowheads). x 45,000

surface endothelium was mostly swollen.

In the group of control animals, the shape of the mitochondria was mainly ellipsoidal regardless of the section plane. In the 8%-group the shape of the mitochondria varied more markedly often with budding and furrowing of the surface (Fig. 2). In the 7%-group the bizarre shapes and wide variations in size with a decided shift towards larger mitochondria were noteworthy (Fig. 3).

While rats kept under 8% oxygen exhibited a numerical increase in myocardial mitochondria ($N_v$) compared to controls (Table 2) with a concomitant reduction in their size (V) in all three heart regions (R, S, L), though without major changes in the relative mitochondrial fraction of the sarcoplasm ($V_v$), the mitochondria of the 7% group were markedly enlarged and the numerical density reduced. This increase in the volumetric density of the mitochondria took place in all three heart regions, the interventricular septum evincing the greatest changes.

**Table 2.** Morphometric results.

| | C | | mit C: 8%, n=4 | | | 7% | | | |
|---|---|---|---|---|---|---|---|---|---|
| | x | SD | x | SD | $d_1$ | x | SD | $d_1$ | $d_2$ |
| V | 0.51 | 0.11 | 0.43 | 0.13 | -15.7 | 0.70 | 0.21 | +37.3 (4) | +62.8[8] |
| R Nv | 62.74 | 8.90 | 87.51 | 27.88 | +39.5 (1) | 56.85 | 18.93 | -9.4 | -35.0[9] |
| Vv | 31.10 | 3.31 | 34.03 | 5.10 | +9.4 | 36.06 | 4.61 | +15.9 (5) | +6.0 |
| V | 0.61 | 0.19 | 0.58 | 0.23 | -4.9 | 1.03 | 0.28 | 68.9 (6) | +77.6[10] |
| S Nv | 54.71 | 14.09 | 62.70 | 22.07 | +14.6 | 43.03 | 7.46 | -21.3 | -31.4[11] |
| Vv | 31.84 | 6.13 | 31.75 | 2.45 | -0.3 | 42.49 | 7.16 | 33.4 (7) | 33.8[12] |
| V | 0.67 | 0.17 | 0.44 | 0.06 | -34.3 (2) | 0.70 | 0.34 | +4.5 | +59.1[13] |
| L Nv | 53.05 | 13.36 | 75.52 | 8.49 | +42.4 (3) | 59.59 | 17.48 | +12.3 | -21.1[14] |
| Vv | 33.09 | 1.28 | 32.77 | 2.09 | -1.0 | 36.72 | 5.80 | +11.0 | -12.1 |

[1]: $p<0.036$; [2]: $p<0.017$; [3]: $p<0.009$; [4]: $p<0.039$; [5]: $p<0.029$; [6]: $p<0.006$; [7]: $p<0.010$; [8]: $p<0.012$; [9]: $p<0.025$; [10]: $p<0.025$; [11]: $p<0.006$; [12]: $p<0.003$; (13): $p<0.047$; (14): $p<0.036$. C: control group; 8% and 7%: test groups with 8% and 7% $O_2$ at the end of the experiment respectively; R, S, L: right ventricle, septum interventriculare, left ventricle; V: mean volume of mitochondrion ($\mu m^3$); $N_v$: numerical density (x $10^{-2}/\mu m^3$); $V_v$: volume density (%). Mean values (x) with standard deviation (SD); $d_1$: relative difference (%) to controls; $d_2$ relative difference between groups 7H and 8H.



**Fig. 3.** Hypoxia 7%. Bizarre shapes of larger mitochondria. x 45,000

## Discussion

"High" altitude is considered to begin at about 3,000 m (FiO$_2$ 14.5%) where the first signs of adaptation are observed (La Manna, 1992). The oxygen pressure at this altitude is the boundary between mild and moderate hypoxia. Successful adaptation may be possible up to, at most, 6,000 m (FiO$_2$ 10%) and this defines the upper limit for moderate hypoxia. Severe hypoxia is considered when FiO$_2$ is below 10%.

The arrest of weight gain and the following loss of weight, if severe hypoxia of an FIO$_2$ below 10% is prolonged beyond the 85th day may be interpreted as a limit reached in compensation capacity. Complete failure in the ability to adapt can be assumed in the fifth stage of hypoxia, corresponding to an FIO$_2$ of 7%. This is in accordance with the results of different authors (Harik et al., 1995) who reported slower growth rates or a decrease in body weight during chronic hypobaric hypoxia in rats. Other experiments showed that rats continuously exposed to a simulated height of 5,486 m (corresponding to an FIO$_2$ of 10.5%) did not reach the initial values for the body weight even after three months. Our results show that a partial increase in the body weight can take place at a moderately reduced FIO$_2$, suggesting that gradual lowering of the FIO$_2$ creates more favorable conditions for weight development of the animals.

Decreased food consumption has been reported by mountain expeditions at high altitude. Rose et al. (1988) found that prolonged exposure to increasing hypobaric hypoxia was associated with a reduction in carbohydrate preference and body weight despite access to ample varieties and quantities of food. In studies in high altitude or in hypobaric chambers other factors such as changes in the food intake and fluid metabolism can overlap the effect of pure hypoxia. In our model of normobaric hypoxia changes in food and water intake and liquid metabolism do not overlap the effects of pure hypoxia, and our results show that hypoxia can be a sufficient cause for weight loss.

In contrast to the findings of Costa et al. (1988) in chronic hypobaric hypoxia and Martorana et al. (1986) in emphysemic dogs, a macroscopically visible cardiac hypertrophy could be demonstrated for the hypoxic animals in the present study. The oxygen supply was lower than in the study of Costa (FiO$_2$ 12%), and thus the hearts were exposed to greater stress. The present study confirms the results of Kaaja and Are (1982), who produced cardiac hypertrophy of 100% and more in rats exposed to hypobaric hypoxia of 9.5% O$_2$ for 40 days. Kononova (1979) was able to demonstrate a weight loss for the septum under chronic hypoxia but found an increase for the right and left ventricle. This is similar to the right heart hypertrophy known to occur in pulmonary hypertension and, in hypoxia, the right heart is subject to exceptional strain due to the enhanced pulmonary artery pressure (Jin et al., 1990). Pulmonary hypertension and right ventricular hypertrophy were developed in about 70% of animals after 7 days of exposure in a normobaric chamber at an FiO$_2$ of 10% (Zhao et al., 1994).

In chronic obstructive lung disease accompanied by hypoxia and hypercapnia right heart failure often occurs when pulmonary arterial pressure is only modestly elevated. In contrast, when hypoxia and hypercapnia are absent, as in primary pulmonary hypertension, heart failure does not usually occur until pulmonary pressure is more significantly elevated (Reeves et al., 1994). Since in our model normocapnia was maintained during the whole experiment, hypoxia should be the triggering mechanism leading to myocardial hypertrophy.

In the controls, the values of the mitochondrial indices V, N$_v$ and V$_v$ are in good agreement with several studies in which the material was perfused. The application of immersion fixation thus appears to exert no major influence on the morphometric parameters of mitochondria.

However, there is a considerable range of normal values for mitochondria reported for the rat heart independent of the fixation procedure varying between 22.4% and 40.2%.

The different values of the indices determined for the groups of 8% and 7% oxygen content have been reported by others. In different studies of experimental hypoxia results vary depending on the type of chronicity and the degree of hypoxia, though the diversity of myocardial regions (left, right ventricle, inner and outer part of the ventricular wall, papillary muscle) have to be taken into account. As for the average size of the mitochondria, most authors demonstrated a decrease, but an increase was also found. It was not possible to find in the literature a stereological evaluation of all three indices examined here (V, N$_v$, V$_v$) in various myocardial regions and under different intensities of hypoxia.

The increase in numerical density observed in the 8% group together with a decrease in the average size and an unaltered mitochondrial mass was also found by Costa et al. (1988) in rats exposed to hypobaric hypoxia (440 mmHg corresponding to an FIO$_2$ of 12%) for 9-11 months. The morphometric values were also similarly altered in the right ventricle of dogs 6 months after emphysema induction by papain ventilation. Changes in the mitochondrial indices similar to our findings in the 8% group were reported in rats exposed to isolated physical strain (running wheel).

Similar to our 7% group a decrease in number with a concomitant increase in mean size of mitochondria was demonstrated by Guski et al. (1981) and Kononova (1982) under severe hypoxic conditions, in which rats were exposed for 5/6 hours a day to a hypobaric hypoxia corresponding to a level of 6,000 m (FiO$_2$ 9.7%) and 9,000 m (FiO$_2$ 6.5%). The duration of the experiment was 90 days. This increase in the size of the mitochondria has also been observed after ligature of a coronary artery.

None of the animals in our experimental model evidenced a decrease in mitochondrial volume such as that which is described for rat hearts submitted to strain

by hypertension by Rakusan and Tomanek (1986). Lund and Tomanek (1980) exposed both spontaneously hypertensive rats (Okamoto-Aoki breed) and rats with artificial aortic constriction to a hypobaric hypoxia corresponding to a height of 6,100 m for 6 weeks. They came to the conclusion that chronic hypoxia induces a number of structural changes which differ from those generated by a pressure load and which are also prevalent in the presence of concomitant hypertension. Our results corroborate this assumption.

Above 7,000 m maximal oxygen consumption falls precipitously. Tolerance to extreme altitude is critically dependent on barometric pressure, and even seasonal changes in pressure probably affect climbing performance near the summit of Mt. Everest. However, cardiac function is astonishingly well maintained up to extreme altitudes. The relationship between cardiac output and work rate is the same as at sea level, and there is evidence that myocardial contractility is well preserved in spite of the extreme hypoxemia (West, 1990). In contrast to acute hypoxia, the cardiac ejection is not increased in chronic hypoxia. An increased heart rate has only been observed above 6,000 m. Due to an elevated pulmonary arterial pressure and the increased hematocrit, however, the heart is under an additional strain even at medium heights. Exposure to an extreme altitude results in electrocardiographic changes compatible with pulmonary hypertension, but maximal exercise at 8,848 m is not accompanied by any electrocardiographic evidence of ischemia despite severe hypoxemia and alkalosis (Malconian et al., 1990).

The question arises as to the way in which particularly the right heart copes with this increased strain in the presence of a reduced oxygen supply. It has been repeatedly confirmed that the heart cannot significantly increase the $AVDO_2$. A decisive adaptation mechanism for physical exertion, namely dilatation of the coronary arteries with a consecutive multiple increase of the blood flow (coronary reserve), appears to have no significance in chronic hypoxia. Therefore, the changes in mitochondria of the cardiac muscle seem to play a major role in the adaptation to chronic hypoxia. They reflect the border line between compensation and decompensation.

The differences between the two experimental groups with regard to the three indices V, $N_v$, and $V_v$ pointed to a change in the compensatory reactions of the mitochondria to the two different levels of chronic hypoxia. In the 8% group, the incipient decompensation concluded from the final loss of body weight was not yet manifest in the mitochondria morphometry. Notwithstanding the decrease in the average size of the mitochondria, the increase in the surface-to-volume ratio by budding and fourrowing enlarges the surface for the oxygen-transporting system. The significant increase in mitochondria surface is consistent with the observation made by Terrados et al. (1990) on changes of oxidative enzyme activities in skeletal muscle with training in hypoxia. The results appear to justify the assumption that the morphometric changes in the mitochondria of the 8% oxygen group represent a compensated state.

Assuming that the values applicable for the 7% group were similar to those for the 8% group when the animals were at this hypoxia level, a qualitative and quantitative change is apparent in the mitochondria by the increased standard deviations of all three indices, particularly of the volume. Together with the steep drop in the body-weight curve, a definite decompensation must be assumed for the 7% group similar to an ischemic situation.

In conclusion, while hypoxia of 8% oxygen can be compensated to some extent by increasing the surface of mitochondrial membranes, further reduction of oxygen causes compensatory mechanisms to fail as seen by the severe alterations of the mitochondrial population of the cardiomyocyte. 7% hypoxia corresponds to an altitude of little less than 9,000 m which is certainly rarely reached by humans. Nevertheless, as chronic obstructive lung disease with severe disturbance of oxygen exchange leads to hypoxia and hypercapnia and arteriosclerosis to a deficient supply of oxygen to the cell, our model may depict the changes on the ultrastructural level to be expected in these very common diseases.

## References

Cervós-Navarro J., Sampaolo S. and Hamdorf G. (1991). Brain changes in experimental chronic hypoxia. Exp. Pathol. 42, 205-212.

Costa L.E., Boveris A., Koch O.R. and Taquini A.C. (1988). Liver and heart mitochondria in rats submitted to chronic hypobaric hypoxia. Am. J. Physiol. 255, C123-129.

Guski H., Meerson F.Z. and Wassilew G. (1981). Comparative study of ultrastructure and function of the rat heart hypertrophied by exercise or hypoxia. Exp. Pathol. 20, 108-120.

Harik S.I., Hritz M.A. and La Manna J.C. (1995). Hypoxia-induced brain angiogenesis in the adult rat. J. Physiol. 485, 525-530.

Jin H., Yang R.H., Chen Y.F., Jackson R.M. and Oparil S. (1990). Atrial natriuretic peptide attenuateds the development of pulmonary hypertension in rats adapted to chronic hypoxia. J. Clin. Invest. 85, 115-120.

Kaaja R. and Are K. (1982). Myocardial LDH isoenzyme patterns in rats exposed to cold and/or hypobaric hypoxia. Acta Med. Scand. (Suppl) 668, 136-142.

Kononova V.A. (1979). Structural changes in the rat myocardium in adaptation to altitude hypoxia. Biull. Eksp. Biol. Med. 88, 497-500.

Kononova V.A. (1982). Quantitative analysis of ultrastructure cardiomyocyte mitochondria during adaptation to altitude hypoxia. Biull. Eksp. Biol. Med. 94, 116-118.

La Manna J.C. (1992). Rat brain adaptation to chronic hypobaric hypoxia. In: Oxygen transport to tissue. XIV. Advances in experimental medicine and biology. Erdman W. and Bruley D.F. (eds). Plenum Press. New York. Vol 317. pp 107-114.

Lund D.D. and Tomanek R.J. (1980) The effects of chronic hypoxia on the myocardial cell of normotensive and hypertensive rats. Anat. Rec. 196, 421-430

Malconian M., Rock P., Hulgren H., Donner H., Cymerman A., Groves B., Reeves J., Alexander J., Sutton J., Nitta M. and Houston C. (1990). The electrocardiogram at rest and exercise during a

simulated ascent of Mt. Everst (Operation Everest II). Am. J. Cardiol. 65, 1475-1480.

Martorana P.A., Even van P., Wüsten B. and Schaper J. (1986). Increase in number and decrease in size of mitochondria profiles in myocytes of the right ventricle of dogs with experimental emphysema. Am. Rev. Respir. Dis. 134, 580-584.

Miyata H., Lakatta E.G., Stern M.D. and Silverman H.S. (1992). Relation of mitochondrial and cytosolic free calcium to cardiac myocyte recovery after exposure to anoxia. Circ. Res. 71, 605-613.

Patt S., Sampaolo S., Théallier-Jankó A., Tschairkin I. and Cervós-Navarro J. (1997). Cerebral angiogenesis triggered by severe chronic hypoxia displays regional differences. J. Cereb. Blood Flow Metabol. 17, 801-806.

Rakusan K. and Tomanek R.J. (1986). Distribution of mitochondria in normal and hypertrophic myocytes from the rat heart. J. Moll. Cell Cardiol. 18, 299-305

Reeves J.T., Welsh C.H. and Wagner P.D. (1994). The heart and lungs at extreme altitude. Thorax 49, 631-633.

Rose M.S., Houston C.S., Fulco C.S., Coates G., Sutton J.R. and Cymerman A. (1988). Operation Everest II: Nutrition and body composition. J. Appl. Physiol. 65, 2545-2551.

Silverman H.S. (1993). Mitochondrial free calcium regulation in hypoxia and reoxygenation: relation to cellular injury. Basic Res. Cardiol. 88, 483-494.

Terrados N., Jansson E., Sylven C. and Kaijser L. (1990). Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? J. Appl. Physiol. 68, 2369-2372.

Weibel E.R. and Gomez D.M. (1962). A principle for counting tissue structures in random sections. J. Appl. Physiol. 17, 343-348.

West J.B. (1990). Tolerance to severe hypoxia: Lessons from Mt. Everest. Acta Anaesthesiol. Scand. (Suppl.) 34, 18-23.

Zhao L., Hughes J.M.B. and Winter R.J.D. (1994). Reversal of pulmonary vascular remodelling following hypoxic exposure: no effect of infusion of atrial natriuretic factor and neutral endo-peptidase inhibitor. Cardiovasc. Res. 28, 519-523.