Histol Histopath (1993) 8: 247-255

Histology and Histopathology

Pathological changes in organs of rats chronically exposed to hypoxia. Development of pulmonary lipidosis

M. Wolman[1], J. Cervós-Navarro[2], S. Sampaolo[2] and A. Cardesa[3]

[1]Department of Pathology, Tel Aviv University Sackler Faculty of Medicine, Tel Aviv, Israel, [2]Department of Neuropathology, Free University of Berlin, Berlin, Germany, and [3]Department of Pathology, University of Barcelona, Barcelona, Spain

Summary. Rats were exposed to chronic normobaric hypoxia of progressively increasing severity; down to 8% or 7% oxygen concentrations. In addition to loss of weight, pathology revealed congestion, haemorrhages, hypertrophy of the heart involving mainly the right ventricle, thickening of arteries, ischaemic changes in the myocardium and extramedullary haematopoiesis in the spleen. Changes not described up until now were: 1) sheets of foam cells in the pulmonary alveoli; 2) foamy and solid storing cells in the spleen; 3) mucoid changes in the atrioventricular valve leaflets; 4) hyperplasia of the juxtaglomerular apparatus; 5) atrophy of the adrenal glomerulosa and hyperplasia of medulla; 6) atrophy of the perifollicular B-cell zone in the spleen; and 7) lipid pigment deposition in various organs. The findings indicate that severe chronic hypoxia induces a significant pulmonary lipidosis similar to that caused by amphiphilic cationic drugs, presumably by inhibiting hydrolytic enzyme activities. The observations are of importance in human hypoxic conditions and open the possibility of their rational treatment.

Key words: Chronic hypoxia, Pathology, Lung lipidosis, Splenic lipidosis, Foam cells

Introduction

Chronic hypoxia occurs in humans and animals either under normal atmospheric pressure (normobaric) or under hypobaric conditions. Hypobaric hypoxia occurs during climbs to high altitudes and, occasionally, in high-altitude flights. Normobaric hypoxia may occur in patients with chronic lungs or cardiac diseases. An advantage of normobaric hypoxia studies over experiments using hypobaric conditions is that, in the former, the effects of hypoxia are not mixed with the effects of low pressure. Normobaric hypoxia, however, is not quite comparable to chronic heart and lung diseases, as in the latter, defective oxygenation is complicated by deranged CO_2 elimination.

Rats were found by Olson and Dempsey(1987) to represent a good model for the study of human ventilatory adaptation to chronic hypoxia. The present report is part of an extensive study of normobaric hypoxia on rats and cats. Changes in the nervous system yielded results (Cervós-Navarro et al., 1991) outside the scope of the present paper. The present study describes the effect of chronic exposure at hypoxia on various organs. We are not aware of any previous study on the pathological changes of organs in comparable experimental conditions of hypoxia.

Offprint requests to: Prof. Dr. med. J. Cervós-Navarro, Institut für Neuropathologie, Klinikum Steglitz, Hindenburgdamm 30, D 1000 Berlin 45, Germany

Materials and methods

Ninety-three male Wistar rats, weighing 180-230 grams at the beginning of the experiment, were used in the present study. Of these, 25 were exposed to 7% oxygen before being killed, 12 to 8% oxygen, while 8 served as controls for the 7% O_2 group, and another 8 for the 8% group. These figures do not include rats which were found dead in their cages in the course of the exposure to hypoxia (2 in the 8% group and 4 in the 7% group).

An additional group of 40 rats (12 at 7% and 12 at 8% oxygen, each with a normoxic control group of 8 rats) served for haematological and biochemical estimations performed prior to their killing.

Procedure for inducing hypoxia

The rats were housed in Nalgene cages (2 per cage) with a batch of six cages placed in plexiglass chambers 0.36 m^3 in volume. 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. Flowmeters regulated the flow of N_2 and of air in the mixture and the gas concentrations (including CO_2) as well as temperature and relative humidity were

monitored and kept constant with the aid of an oxymeter «Oxytest» and a CO_2-meter «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 (given in Table 1) 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 used in the presently-described experiments was: 7 days normoxia, 15 at 15% O_2 and 22 days for each of the further levels of hypoxia: 12, 10 and 8% of oxygen. Length of exposure to 7% O_2 varied, as rats were often killed when they presented haematuria, gait disturbance, or apathy and somnolence. Every 3 days, the chambers were opened to clean them and to change food and water, which produced normoxic conditions for a few minutes. During this procedure the rats were weighed. Seven rats of the 7% oxygen group underwent repeated electroencephalography and were therefore exposed to normoxic conditions during and after implantation of screw electrodes and during the tracing, for about 32 hours altogether. These EEG rats differed from the others in their responses and will be discussed as a separate group.

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

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

Table 1. Environmental conditions in the hypoxic chambers

Atmospheric Pressure	mmHg	795±4
Temperature	ºC	27
Humidity	%	60

Inspiratory O_2 (vol%)	Gas supply to the chambers (l/min) Air	N_2	Duration of each step of hypoxia (days
21	30	0	7
15	22.5	7.5	15
12	18	12	22
10	15	15	22
8	12	18	22
7	10.5	19.5	Group A: 4-11
			Group B: 12-20
			Group C: 12-20*
			Group D: 21-55*

*: rats of groups C and D underwent intracranial insertion of electrodes

Physiological parameters

Body weights were measured every three days. Haematological and physiological parameters, which were determined in another batch of similarly-treated rats, were measured after 20 days of th 8% hypoxia in this group, and between the 12th and 20th day of exposure to the lowest O_2 level (the 7% group). All parameters of Table 2 were determined on arterial blood samples; the rats were taken out of their hypoxic or normoxic chambers, anaesthesized with ketamine-

Table 2. Phisiological parameters.

		CONTROL	8%O_2	7%O_2
RBC	10^{12}/l	6.700±0.500	10.500±1.100	11.800±1.500
PCV	%	40.500±1.700	70.000±1.500	74.000±1.300
Hb	g/dl	15.000±1.000	24.800±1.100	30.200±1.200
PLTS	10^{10}/l	68.430±17.960	40.500±17.030	23.680±8.970
Glucose	mMol/l	5.500±0.800	3.800±0.800	3.100±0.600
Lactate	mMol/l	1.500±0.500	4.500±1.200	2.200±0.800
pH		7.433±0.013	7.359±0.017	7.276±0.053
PaO_2	mmHg	93.000±2.200	41.000±2.000	34.000±0.700
$PaCO_2$	mmHg	39.250±1.300	22.350±2.100	18.100±1.000

Table 3. Weights of organs expressed as organ/body weight ratio.

	Body weight (g)	Heart	Lung	Liver $1x10^{-3}$	Spleen	Kidney	Adrenal
	± SD						
CONTROLS (6)	448.833±52.094	4±1	5±1	3.5±0.3	2±0.5	4±1	0.04±0.02
8% RATS (7)	345.857±30.174 p<0.001	5±0.9	5±0.2	3.5±0.5	4±0.1 p<0.014	4±0.1	0.04±0.003
7% RATS							
Group A (5)	338.000±26.449 p<0.002	8±2 p<0.003	8±1 p<0.02	4±0.6	6±2 p<0.006	4±0.9	0.06±0.01
Group B (8)	251.125±42.202 p<0.001	6±0.8 p<0.001	9±2 p<0.01	3±0.4	7±1	4±1	0.03±0.01
Group C* (2)	333.000±53.814	6±1	6±0.5	4±0.5	7±0.1	3±0.3	0.01±0.005
Group D (5)	282.200±39.638 p<0.001	5±1	5±1	4±1	7±3 p<0.01	3±0.5 p<0.05	0.02±0.003

Student's t-test for independent samples. Number of rats given in brackets. *: because of the low number of animals statistical analysis was not performed. Groups C and D underwent electroencephalography with consequent repeated exposure to normoxia.

xylazine and their tail artery was cannulated. After recovery from anaesthesia, the animals were returned to the chambers and 3 hours later arterial blood samples were withdrawn with the animals situated in the chambers. The findings of these tests are summarized in Table 2. Body weights are given in Table 3.

Pathology

Most animals were killed by decapitation. Four rats of the 8% group and 8 of the 7% O_2 were perfused with 0.9% NaCl and killed with cold 4% formaldehyde during mechanical ventilation after having been anaesthesized with ketamine. Autopsy was performed and the organs were weighed, fixed in cold buffered 4% formaldehyde, embedded in paraffin wax and sectioned at 5 µm. Sections were regularly stained by the H.E., van Gieson, and P.A.S. and Perls' iron procedures. Whenever necessary, other special stains were used and will be described in Results.

Results

Weights

Body and organ weights measured in 33 animals are shown in Table 3. In order to obtain comparable data, the organs were weighed after 3 days of submersion in formaldehyde and included the left of paired organs. As the various animals differed in their body weights, Table 3 shows the ratios of organ to whole body weight.

Macroscopical findings

Haemorrhages and haemorrhagic effusions and severe congestion of organs were observed in most rats. In 5 of the 12 rats of the 7% group gastric ulcers were found.

Microscopical findings

Heart

Ischaemic changes were found in the hearts of all rats, with occasional foci of fresh necrosis and perivascular infiltrates and fibrosis of different intensities. Large infarcts were seen in only 2 animals of the 7% group and in one of the 8% hypoxia group out of the 37 exposed to hypoxia, and in one of them and in 2 of the same group of 7% hypoxia other mural thrombi occurred in the left ventricles. Intramural arteries were

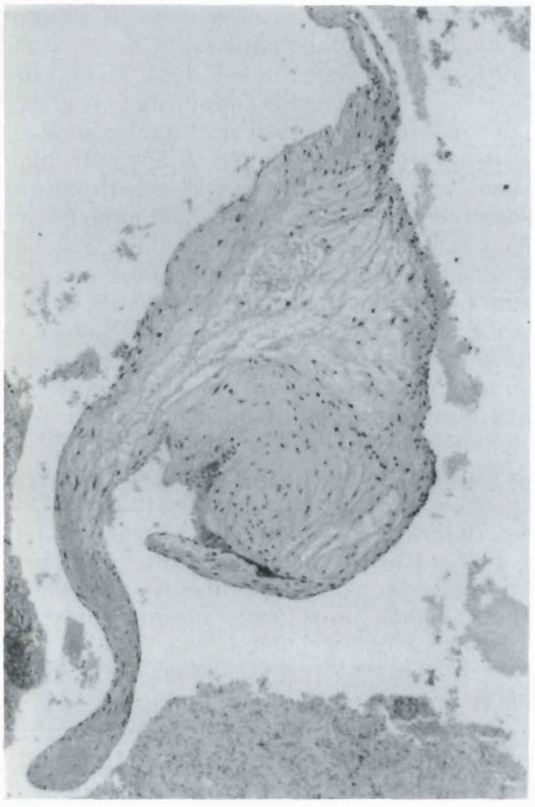

Fig. 1. *Mucoid change and oedema in mitral leaflet in a 7% hypoxia rat.* H&E. x 90

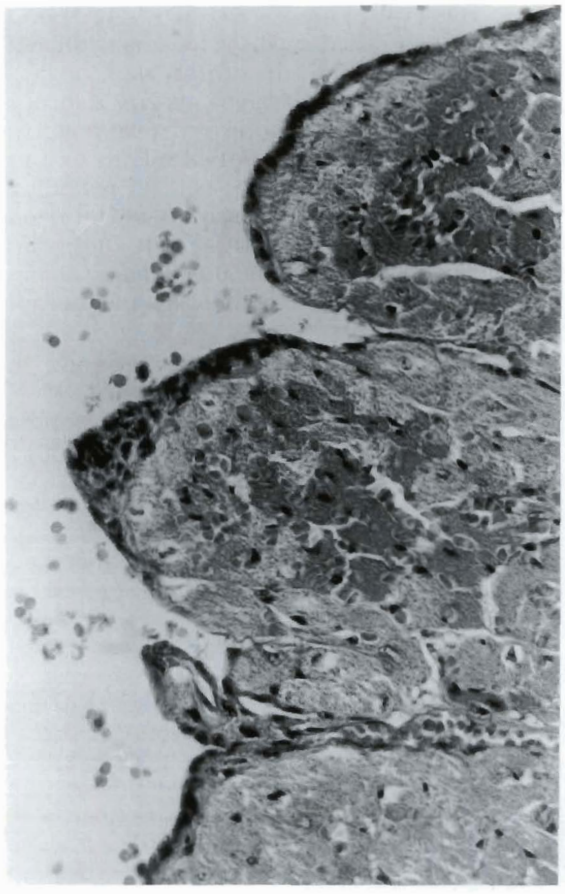

Fig. 2. *Endothelial swelling on endocardial surface with stratification and* bulging in the same rat. H&E. x 360

thickened and often partly hyalinized.

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 (Fig. 1). Endocardial surface endothelium was mostly swollen and in 3 cases belonging to the 7% and 2 to the 8% groups was stratified and bulged into the lumen (Fig. 2).

Lungs

The most striking finding in the lungs of rats exposed to hypoxia was the presence of masses of intraalveolar foam cells. The tiny vacuoles responsible for the foamy structure appeared mostly empty and occasionally granular in paraffin sections (Figs. 3, 4). The foam cells were primarily situated in subpleural alveoli, but with increased infiltration sheets of foam cells appeared peribronchially and occasionally also deep in the parenchyma. The extent of this infiltration varied in accordance with the degree of hypoxia and with its duration. Thus, maximal foam cell infiltration involving numerous alveoli occurred in 7/25 rats of the 7% O_2 and 1/12 of the 8% group. The only severely hypoxic rat which did not exhibit foam cells was an animal which underwent EEG and was on the 7% O_2 regimen for 7 days. Also in other EEG rats, the extent of pulmonary foam cell infiltration was very much lower than in rats which were not exposed for long periods to normoxia in the course of the experiment.

In some cases, foam cells appear to have burst and their contents spilled into the alveolar lumen (Fig. 5). Occasionally, lipid pigment granules, which emitted yellow autofluorescence under UV light and were stained by Sudan black B and PAS in paraffin sections, were observed in the foam cells. No iron-containing pigment was observed in the lungs of the hypoxic rats. In 5 cases from both groups, pulmonary edema was noted. Occasionally, slight or moderate interstitial infiltration occurred. In all cases, the arterial walls were thickened and often hyalinized.

Liver

The findings in the liver were surprisingly unremarkable. In only two instances, both belonging to the 8% O_2 group, were necrotic foci detected. No fatty change and no extramedullary haematopoiesis was found in any hypoxic animal. Swelling of Kupffer cells and presence of lipid pigment in them was noted in about half the rats exposed to 7% hypoxia and less frequently

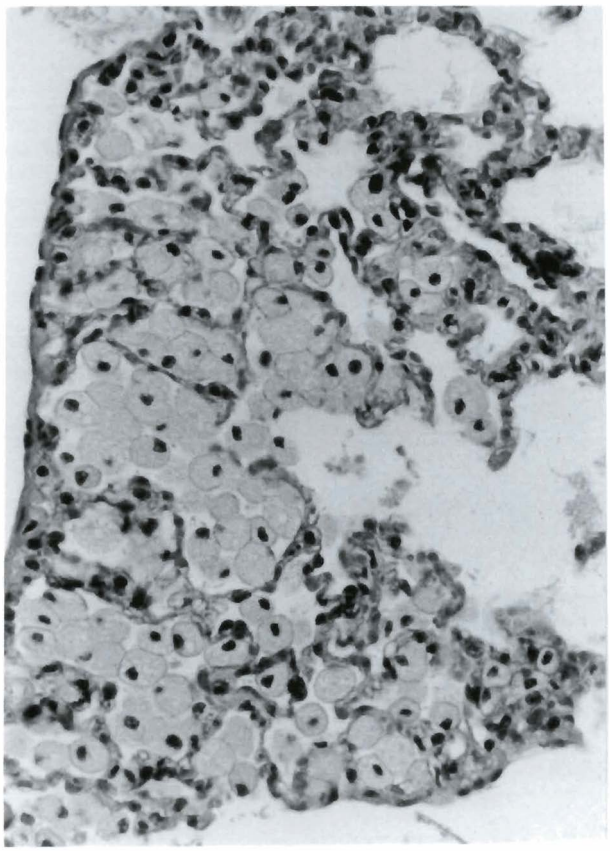

Fig. 3. Subpleural accumulation of foam cells in a 7% hypoxia rat. H&E. x 360

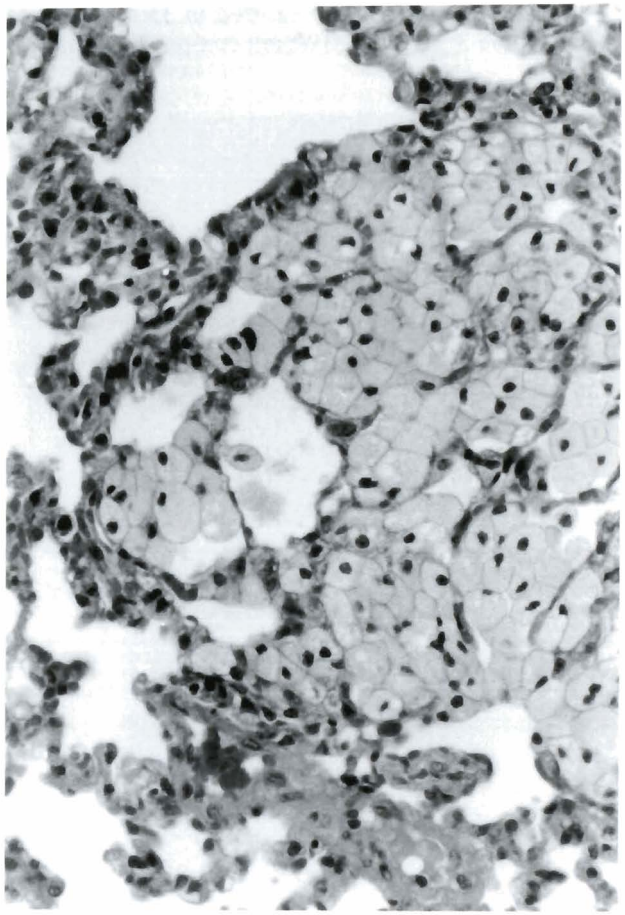

Fig. 4. Granular appearance of intralveolar foam cells in a 7% hypoxia rat. Sudan black B staining of a paraffin-embedded section. x 360

in the 8% O_2 animals. Haemosiderin-containing macrophages were rarely found.

Spleen

Storing macrophages were often found. They were easily detected in about half of the animals of both groups and were numerous and prominent whenever exposure to 7% hypoxia was of 20 days or more. Most storing cells were different from pulmonary foam cells. Some appeared foamy with numerous tiny vacuoles, but mostly they had a large, clear cytoplasm and occasionally looked similar to Gaucher cells with a «cloudy» cytoplasm (Fig. 6). Some of these cells stained with different intensity with PAS.

In 21 rats of 25 of the 7% O_2 group, the Malpighian follicles were compressed and small. This was found to occur in only 4 from 12 in the 8% O_2 rats. In 3/4 of the rats of the 7% hypoxia group the perifollicular B-cell zone was absent, or markedly thinned, occasionally with nuclear debris and other evidence of cell destruction (Fig. 7). In the 8% O_2 rats, this occurred in half of the animals. Extramedullary haematopoiesis was prominent in all rats. Abundant lipid pigment was found in the splenic macrophages of all animals. Haemosiderin deposition, although obvious, was of a lesser degree in the hypoxic compared to the control rats. In two cases of the 7% hypoxia rats, splenic infarcts were observed.

Kidneys

All animals exhibited thickened arteries. In about 1/3 of the rats exposed to 7% hypoxia, the glomeruli were almost bloodless and apparently hypercellular. This rarely occurred in the 8% O_2 group. In most instances, however, glomeruli were severely congested. The juxtaglomerular apparatus (JGA) was prominent and appeared hyperplastic in about half of the animals of the 7% O_2 group (Fig. 8). This only occurred in one rat of the 8% group and was also rare in the EEG-examined rats. The appearance of the JGA cells and the granularity varied in different cases. Hyaline casts were found in the tubuli of 9 hypoxic rats. In one rat, tubular necrosis was observed and in another, a renal infarct.

Adrenals

In about half of the rats of the 7% O_2 group, the glomerulosa layer was thinned, consisting of compact, lipid-depleted cells and occasionally it was almost completely obliterated. (Fig. 9). The appearance of the fasciculata varied between lipid-rich and exhausted cells,

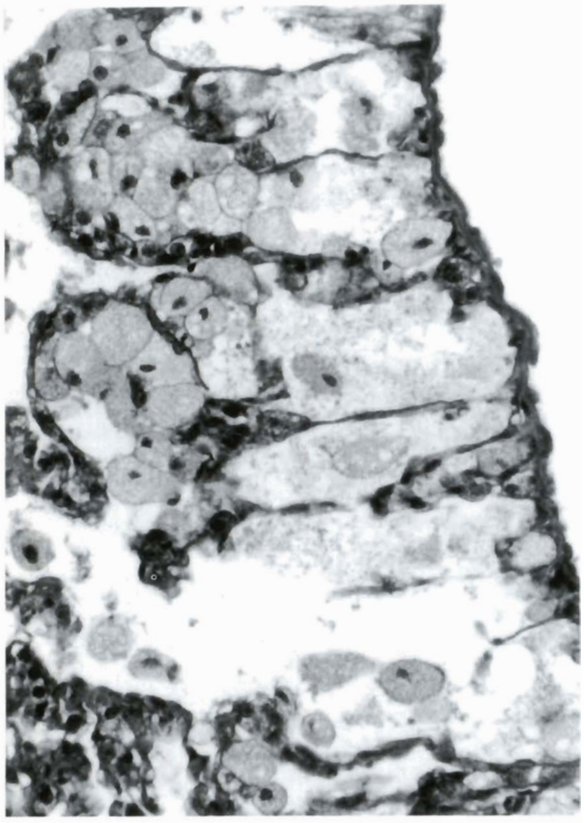

Fig. 5. Ruptured foam cells with spilling of contents into alveolar lumina. Sudan black B staining of a paraffin-embedded section. x 360

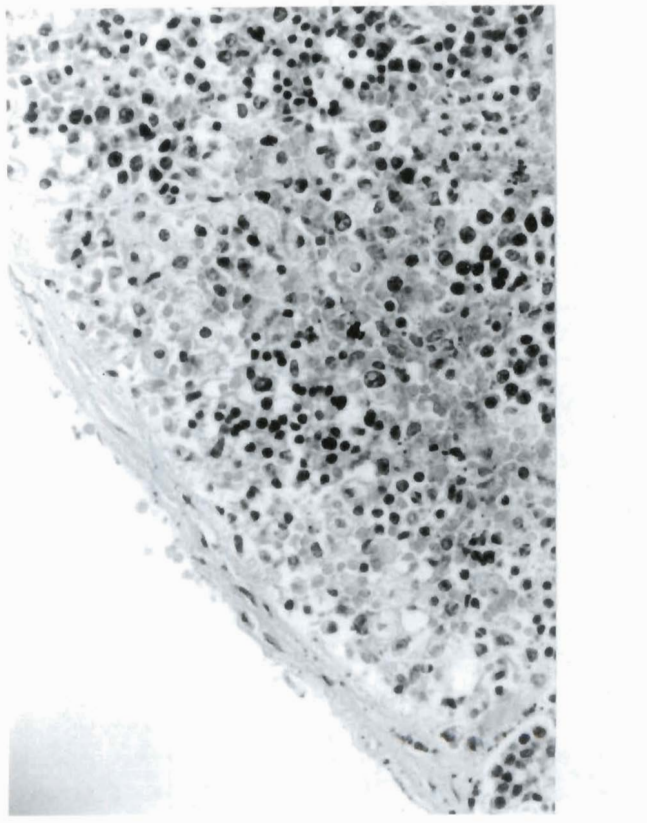

Fig. 6. Storing cells, mostly foamy, in the spleen of a rat exposed to 7% hypoxia. H&E. x 400

without obvious relation to the intensity of length of hypoxia. The reticularis was mostly severely congested, occasionally with blood extravasations.

The adrenal medulla of the rats of the 7% O_2 group was hyperplastic in 2/3 of the animals. Such medullary hyperplasia was expressed in increased size of the medulla, formation of nodules and, occasionally, penetration of medullary nests into the cortex (Fig. 10). Many cells in the hyperplastic medulla appeared clear and agranular (Fig. 11).

Discussion

The present study describes the effect of chronic exposure to hypoxia, the severity of which was gradually increased, on various organs. We are not aware of any previous study on the pathological changes of organs other than the cardiovascular and nervous system in comparable experimental conditions of hypoxia. One of the most obvious changes which occurs in chronic hypoxia is the extreme loss of body weight (Büchner, 1957), which was also observed in our experiments. Our findings show, however, that weight of the parenchymatous organs was not markedly affected, and in relation to total body weight it was even increased. The loss in weight was clearly due to atrophy of muscles and disappearance of the majority of body fat, which could be seen in our animals. While in acute hypoxia, when body fat is not lost, severe fatty change in the liver, heart and brain are invariably found (Büchner, 1957), in our rats no fat deposition was noted in the liver or heart, as no lipid deposit could be mobilized.

The hypoxic rats of this experiment exhibited haemorrhages in various organs. Haemorrhages in hypoxic rats in relation to defective clotting were also observed by previous authors (Haymaker and Strughold, 1957), and might be related to the thrombocytopaenia observed by us. As haemosiderin deposition around the haemorrhages was absent in almost all cases, it is likely that the haemorrhages contributed to the run-down condition of the rats, which were therefore killed soon after the haemorrhages occurred.

The reasons underlying the minimal occurrence of haemosiderin in our rats are not quite clear. The phenomenon might possibly be related to increased uptake of ferritin needed to maintain the polycythaemic state. Polycythaemia *per se* with presence of mainly young erythrocytes in the blood stream might also be a

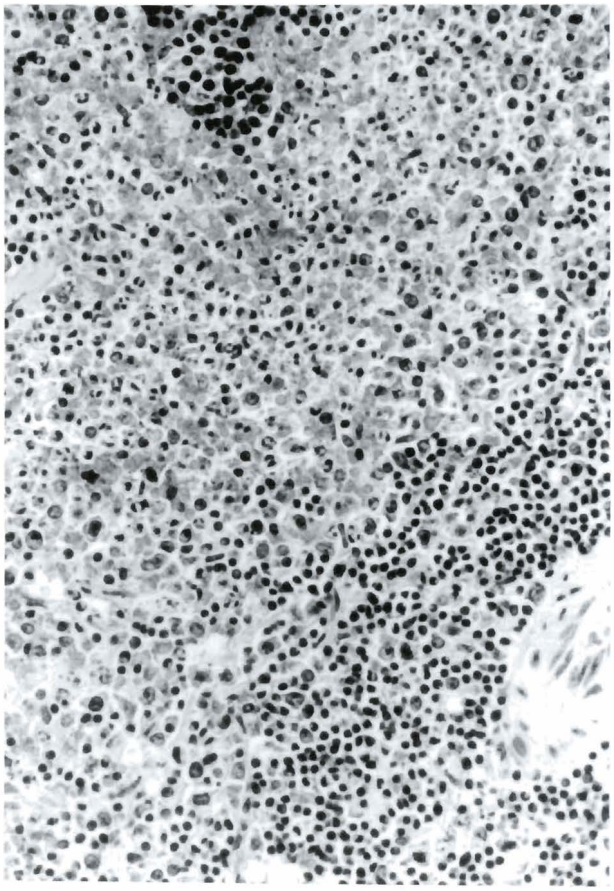

Fig. 7. Cell necrosis with nuclear debris in the perifollicular zone of the spleen in 7% hypoxic rat. H&E. x 360

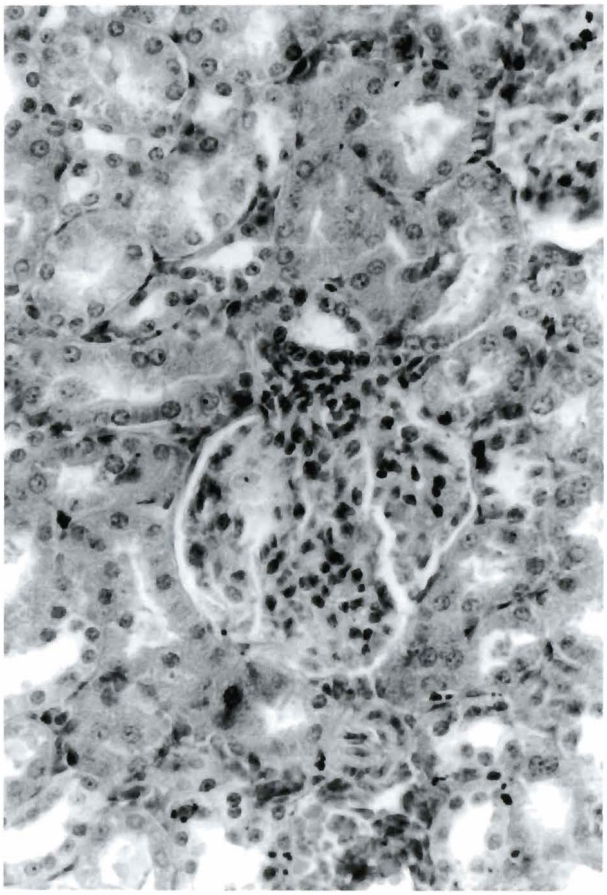

Fig. 8. Hyperplastic juxtaglomerular apparatus in a 7% hypoxic rat. H&E. x 360

contributory factor.

The presence of extramedullary haematopoiesis in the spleen is easily explained by the effect of hypoxia on the increased production of red blood cells. Splenic congestion and the haematopoiesis might have been responsible for the frequent atrophy of the follicles. We have, however, no explanation for the atrophy of the perifollicular zone which occurred in many rats.

The most remarkable finding in the rats after chronic exposure to hypoxia was the appearance of pulmonary and also, to a lesser extent, splenic lipidosis, with accumulation of foam cells in both organs. The material stored in the foam cells in our rats was mainly lipid because: a) it occurred in globular, well circumscribed vacuoles, indicating its hydrophobic nature; b) it was mostly dissolved during paraffin embedding; c) it was occasionally admixed with chromolipid (lipid pigment) granules; and d) in a histochemical-ultrastructural study (following article) is displayed the characteristics of lipid inclusions (Cervós-Navarro et al., 1993).

Lipid deposition has been known for years to complicate interstitial pneumonitis (Waddel et al., 1954) and post-obstructive lipid pneumonia (Cohen and Cline, 1972; Verbeken et al., 1989). Furthermore, there are evidences that chronic hypoxia affects phospholipid metabolism in the lung (Kumar et al., 1980).

Influx of alveolar macrophages in human under conditions of hypoxia has been observed in high-altitude pulmonary edema (Schoene, 1987). No evidence has been published till now that this occurs in hypoxic states

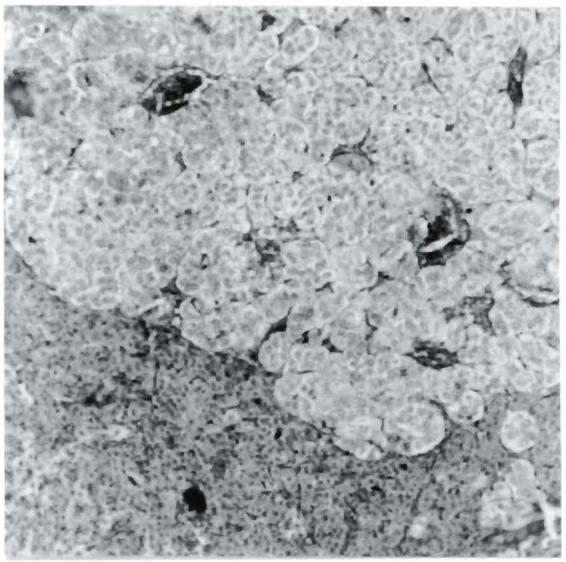

Fig. 10. Hyperplastic adrenal medulla in a 7% hypoxic rat. Nodular appearance and penetration into the cortex. H&E. x 80

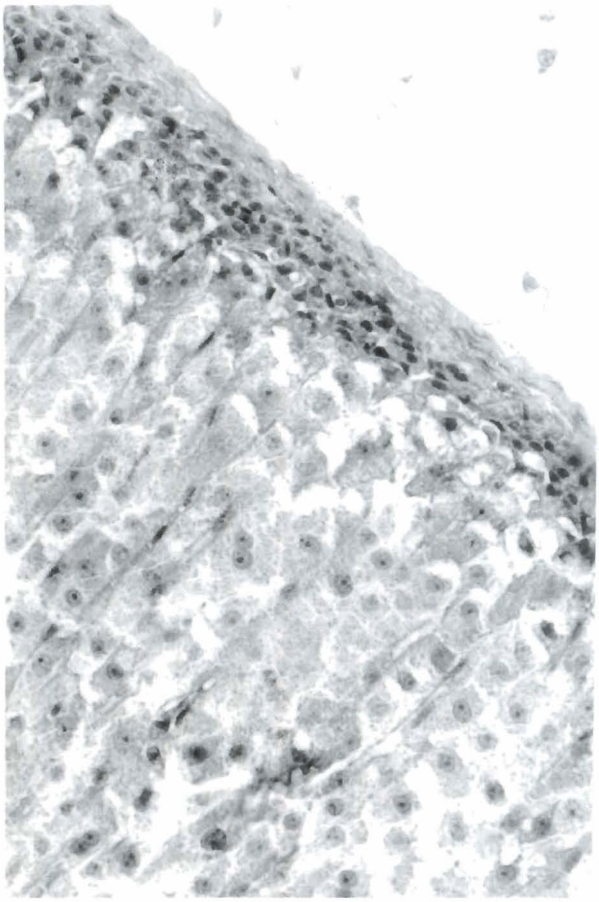

Fig. 9. Atrophic glomerulosa with cells devoid of lipid adjoining a lipid droplet-rich fasciculata in an adrenal of a 7% hypoxia rat. H&E. x 350

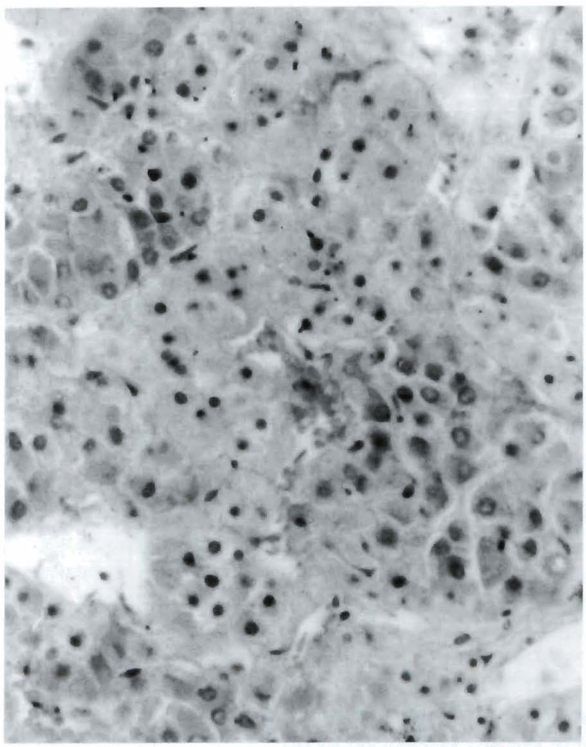

Fig. 11. Hyperplastic adrenal medulla in a rat killed after 24 days of 8% hypoxia. Numerous clear agranular cells. H&E. x 360

different from hypobaria. However, our findings may have been influenced by the length and severity of hypoxic conditions in our model.

The appearance of the foam cell aggregates and their distribution in the lungs were practically identical to those observed in chlorcyclizine lipidosis (Gaton and Wolman, 1979) and in other lipidoses caused by amphibilic cationic drugs (Lüllmann et al., 1978; Hruban, 1984). The distribution of the nests of foam cells in the lungs of our rats, as well as in animals treated with various cationic drugs, suggests a relationship to pulmonary lymph flow. Their concentration near the pleural surface and around bronchial and arterial trunks corresponds to the flow of lymph and suggests that the lipid-laden cells might have migrated out of lymphatic spaces.

The chromolipid granules found in various organs represent products of free radical attacks on lipids and are mostly associated with increased oxidative activity (Wolman, 1981). Mild normobaric hypoxia (10% O_2) decreases lung O_2 radical production *in vitro,* which promptly return to basal levels with restoration of normoxia (Archer et al., 1989). Therefore, it may be very possible in our experiment, that repeated short exposure to normoxia activated O_2 radical production. Otherwise, haemosiderin, one of the oxidation catalysts with the highest concentration in damaged tissues, was not increased in any organ of our rats. It is known, however, that chronic hypoxic states can also induce free radical formation with consequent lipid peroxidation (Fridovich, 1979), possibly through exhaustion of cellular reserves of antioxidants.

The severe atrophy of the glomerulosa layer of the adrenals and the apparent hypertrophy of the juxtaglomerular apparatus (JGA) probably indicate exhaustion of mineralocorticoid secretion by the adrenal, and compensatory hypertrophy of the JGA. These findings are in accord with changes in plasma renin and aldosterone found in acute exposure of humans to hypoxia (Sutton et al., 1977; Ou and Tenney, 1979); also in rats chronic hypoxia impaired angiotensin metabolism (Jackson et al., 1986). The variable lipid droplet content of the fasciculata cells observed by us corresponds to changes which commonly occur in various stages of stress. The hyperplasia of the adrenal medulla is presumed to correspond to increased adrenergic-noradrenergic activity. This and the hyperlipaemic effect of hypoxia (Louhija, 1969), might have played a role in the thickening of arteries observed by us in various organs. In the lungs, thickening of arteries is known to be mainly due to pulmonary hypertension (Hislop and Reid, 1967; Emery et al., 1981).

Most of our findings in the heart correspond to previous observations. The dilatation and hypertrophy, mainly of the right ventricle, the focal necroses, perivascular infiltrates, fibrosis and occasional infarcts are well known effects of hypoxia. In addition to these changes, however, we observed mucoid change, often accompanied by oedema in leaflets of the atrio-ventricular valves in the experimental rats. This change did not occur in the majority of EEGed rats. It is tempting to suggest that chronic and almost uninterrupted hypoxia might also have affected some enzymes active in the metabolism of glycosamino-glycans. Intermittent hypoxia, with periods of normoxia alternating with periods of hypoxia, has been shown by others not to affect pulmonary hypertension and polycythemia which developed in rats continuously exposed to hypoxia (Kay, 1980). In the present experiments, however, even a relatively short exposure to normoxia markedly reduced the loss of weight and the changes in the lungs, cardiac valve leaflets and JGA.

The present observations have a bearing on clinical problems. Presence of sheets of foam cells in the lungs observed in amphibilic cationic drugs-induced pulmonary lipidoses are known to cause dyspnea in humans and animals (Lüllmann et al., 1978). These drugs are endocytosed in lysosomes and are believed to inhibit some lysosomal hydrolases by binding to their substrates (Lüllmann et al., 1978), by directly inhibiting the enzymes, often by complexing with them (Kubo and Hostetler, 1985; Joshi et al., 1989), and/or by locally raising the pH and interfering with the fusion of endocytic vacuoles with lysosomes (Kielian and Cohn, 1982). In fact, inhibition of acid phospholipases was demonstrated in such cases and was believed to be responsible for the pulmonary lipidosis (Stern et al., 1983; Heath et al., 1985; Gonmori et al., 1986; Kacev, 1988). Chronic hypoxic changes in lung and spleen in our rats may have a similar genesis.

Although massive lipid deposition in pulmonary macrophages, as observed in our rats, has not yet been described in patients suffering from chronic lung disease and cardiac anomalies as well as persons exposed to noxious agents in the atmosphere, its occurrence in a less severe degree is probably common, as some phospholipases were shown to be inhibited by hypoxia (Grynberg et al., 1988). If such deposition also occurs in humans exposed to chronic hypoxia it would add a considerable further burden on the pulmonary gas exchange.

Should this notion prove to be correct, phospholipase-containing aerosols might help such patients as well as climbers of high mountains. It is furthermore, important that pathologists and cytologists who examine chronic pulmonary and cardiac patients should pay special attention to the presence of eventual lipid vacuoles in the debris- and haemosiderin-containing macrophages.

References

Archer S.L., Nelson D.P. and Weir E.K. (1989). Simultaneous measurement of O_2 radicals and pulmonary vascular reactivity in rat lung. J. Appl. Physiol. 67, 1903-1911.

Büchner F. (1957). Die Pathologie der cellulären und geweblichen Oxydationen. Die Hypoxidosen. In: Handbuch der allgemeinen Pathologie. Vol IV. Der Stoffwechsel II. Büchner F., Letter E. and

Roulet F. (eds). Springer-Verlag. Berlin. pp 569-650

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

Cervós-Navarro J., Sampaolo S., Heye N. and Wolman M. (1993). Histochemical and electron microscopical observations of the lipidosis in chronic hypoxia. (in preparation).

Cohen A.B. and Cline M.J. (1972). *In vitro* studies of the foamy macrophage of postobstructive endogenous lipoid pneumonia in man. Am. Rev. Resp. Dis. 106, 69-78.

Emery C.Y., Bee D. and Barer G.R. (1981). Mechanical properties and reactivity of vessels in isolated perfused lungs of chronically hypoxic rats. Clin. Sci. 61, 569-580.

Fridovich I. (1979). Hypoxia and oxygen toxicity. In: Advances in Neurology. Vol. 26. Fahn S., Davis J.N. and Rowland L.P. (eds). Raven Press. New York. pp 255-259.

Gaton E. and Wolman M. (1979). Histochemical study of the pathogenesis of chlorcyclizine-induced pulmonary lipidosis. Histochemistry 63, 203-207.

Gonmori K., Morita T. and Mehendale H.M. (1986). Effects of chlorphentermine on incorporation of (^{14}C) choline in the rat lung phospholipids. Lipids 21, 230-234.

Grynberg A., Nalbone G., Degois M., Leonardi J., Athias P. and Lafont H. (1988). Activities of some enzymes of phospholipid metabolism in cultured rat ventricular myocytes in normoxic and hypoxic conditions. Biochem. Biophys. Acta 958, 24-30.

Haymaker W. and Strughold H. (1957). Atmospheric hypoxidosis. In: Handbuch der speziellen pathologischen Anatomie und Histologie 13/1 B. Lubarsch O., Henke F. and Rössle. R. (eds). Springer-Verlag. Berlin. pp 1673-1711.

Heath M.F., Costa-Jussa F.R., Jacobs J.M. and Jacobson W. (1985). The induction of pulmonary phospholipidoses and the inhibition of lysosomal phospholipases by amiodarone. Br. J. Exp. Pathol. 66, 391-397.

Hislop A. and Reid L. (1967). New findings in pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. Br. J. Exp. Pathol. 57, 542-554.

Hruban Z. (1984). Pulmonary and generalized lysosomal storage induced by amphibilic drugs. Environ. Health Perspect. 55, 53-76.

Jackson R.M., Narkantes A.J. and Oapril S. (1986). Impaired pulmonary conversion of angiotensin I to angiotensin II in rats exposed to chronic hypoxia. J. Appl. Physiol. 60, 1121-1127.

Joshi U.M., Rao P., Kodavantis S., Lockard V.G. and Mehendale H.M. (1989). Fluorescence studies on binding of amphibilic drugs to isolated lamellar bodies: relevance to phospholipidodis. Biochem. Biophys. Acta 1004, 309-320.

Kacev S. (1988). Role of phospholipase C in chlorphentemine-induced pulmonary phospholipidosis. Proc. Soc. Exp. Biol. Med. 188, 35-39.

Kay J.M. (1980). Effect of intermittent normoxia on chronic hypoxic pulmonary hypertension, right ventricular hypertrophy and polycythemia in rats. Am. Rev. Resp. Dis. 121, 993-1001.

Kielian M.C. and Cohn Z.A. (1982). Intralysosomal accumulation of polyanions II. Polyanion internalization and its influence on lysosomal pH and membrane fluidity. J. Cell Biol. 93, 875-882.

Kubo M. and Hostetler K.Y. (1985). Mechanisms of cationic amphibilic drug inhibition of purified lysosomal phospholipase A1. Biochemistry 24, 6515-6520.

Kumar R., Hedge K.S., Krishna B. and Sharma R.S. (1980). Combined effect of hypoxia and cold on the phospholipid composition of lung surfactant in rats. Aviat. Space Environ. Med. 51, 459-462.

Louhija A. (1969). Hypertriglyceridemia in rats at stimulated high altitudes. Experientia 25, 248-249.

Lüllmann H., Lüllmann-Rauch R. and Wassermann A. (1978). Lipidosis induced by amphibilic cationic drugs. Biochem. Pharmacol. 27, 1103-1108.

Olson E.B. and Dempsey J.A. (1987). Rat as a model for human like ventilatory adaptation to chronic hypoxia. J. Appl. Physiol. 44, 763-769.

Ou L.C. and Tenney S.M. (1979). Adrenocortical function in rats chronically exposed to high altitude. J. Appl. Physiol. 47, 1185-1187.

Schoene R.B. (1987). High-altitude pulmonary edema: patho-physiological and clinical review. Ann. Emerg. Med. 16, 987-992.

Stern N., Tietz A., Gaton E. and Wolman M. (1983). Effects of chlorcyclizine on pulmonary lipid metabolism in rats. Biochem. Biophys. Acta 754, 166-173.

Sutton R.J., Viol G.W., Gray G.W., McFadden M. and Keane P.M. (1977). Renin, aldosterone, electrolyte, and cortisol responses to hypoxic decompression. J. Appl. Physiol. 43, 421-424.

Verbeken E.K., Demedtes M., Vanwing J., Denmeffe G. and Lauweryns J.M. (1989). Pulmonary phospholipid accumulation distal to an obstructed bronchus. A moprhologic study. Arch. Pathol. Lab. Med. 113, 886-890.

Waddel W.R., Sniffen R.C. and Whytehead L.L. (1954). The etiology of chronic interstitial pneumonitis associated with lipid deposition. J. Thorac. Surg. 28, 134-144.

Wolman M. (1981). Factors affecting lipid pigment formation. In: Age Pigments. Sohal R.S. (ed). Elsevier/North-Holland Biochemical Press. Amsterdam. pp 265-281.

Accepted November 6, 1992