

A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia¹

N. VÖLKEL, U. WIEGERS, V. SILL, AND J. TRAUTMANN

From I Medizinische Universitätsklinik Hamburg, Martinistrasse 52, 2000 Hamburg 20, Germany

Vökel, N., Wieggers, U., Sill, V., and Trautmann, J. (1977). *Thorax*, 32, 578-581. **A kinetic study of lung DNA-synthesis during simulated chronic high-altitude hypoxia.** Rats were exposed to chronic hypobaric hypoxia at a simulated altitude of 4250 m for 3, 6, 9, 12, 20, and 35 days. The *in-vitro* incorporation of ³H-thymidine into the DNA of lung tissue was measured and compared with that of normoxic controls: the obtained time course study showed a maximum increase of 345% on the ninth day of hypoxia, indicating a marked stimulation of cellular proliferation. Between the 12th and 20th day of hypoxia, the lung DNA-synthesis reached control values. No significant change in the DNA-concentration of the lungs was registered. The response to hypoxia was less impressive in rat livers used as controls.

Pulmonary hypertension is a common manifestation of altitude exposure. This has been described in different species by many authors (Alexander *et al.*, 1960; Arias-Stella and Saldaña, 1962; Grover *et al.*, 1963; Burton *et al.*, 1967; Barer *et al.*, 1970; Abrahams *et al.*, 1971; McMurtry *et al.*, 1973). The pulmonary hypertension is the result of a fixed increase in pulmonary vascular resistance due to structural changes in the pulmonary blood vessels that occur in the course of long-term hypoxia.

Although the histological changes are well documented by Arias-Stella and Saldaña (1962) and by Heath (1959), it is not clear whether they are hypertrophic or hyperplastic, and we are also ignorant of the time course of these changes. In order to investigate the mechanism involved and to monitor the onset and the kinetics of the expected cellular proliferation the present cyto-kinetic study of pulmonary response to chronic hypoxia was designed.

Methods

Thirty female Wistar rats weighing 220-250 g were used for the experiments. They were randomly divided into groups of three: six groups were kept in a decompression chamber for 3, 6, 9, 12, 20, and 35 days respectively and were exposed to a simulated altitude of 4250 m. Three groups were taken as controls at the beginning of the

study and on day 3, 12, and 35 respectively. The animals were exposed to hypoxia for 22 hours daily and they were allowed access to food and water. After anaesthesia with 2 mg/kg pentobarbital sodium (Nembutal[®]) a medium thoracotomy was performed, and the middle lobe of the right lung was excised and cut into slices. Two hundred milligram portions of each lung were immediately incubated in 3 ml Krebs-Ringer medium containing 20 μ Ci of ³H-thymidine (H³-thymidine, 24.6 Ci/mmol, Amersham-Buchler, Braunschweig) at 35°C for 60 minutes. The incubation was stopped by the addition of 5 ml ice-cold 3N TCA. The slices were homogenised in 10 ml 5% TCA (w/w) and centrifuged at 3000 g for 5 minutes. The DNA of the sediment was hydrolysed in 5 ml 5% TCA at 95°C for 30 minutes. The insoluble material was removed by centrifugation. One hundred microlitre samples of the supernatant were measured in duplicate for both radioactivity and DNA (Burton, 1968). The DNA synthesis rate was expressed as specific radioactivity (cpm/ μ g DNA). Liver slices in portions of approximately 300 mg were run as controls.

Results

Rats that were exposed to chronic hypobaric hypoxia (440 Torr) for a variable period of time (3-35 days) increased their lung DNA synthesis considerably. Figure 1 shows a rapid and continu-

¹Supported by the Deutsche Forschungsgemeinschaft grant No. Si 207/2

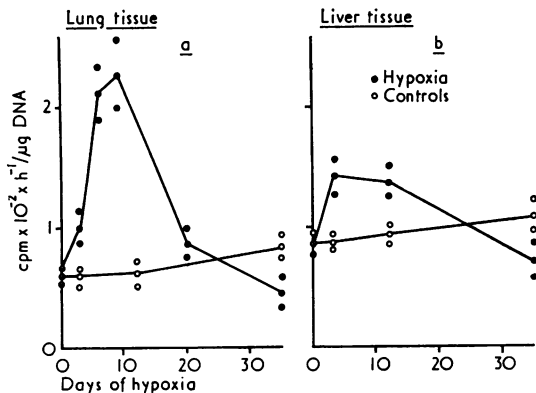


Fig. 1 Study of ^3H -thymidine incorporation by lung and liver parenchyma during normoxia and hypoxia: (a) Mean values of the *in vitro* ^3H -thymidine incorporation of rat lungs reach a maximum on the ninth day of hypobaric hypoxia (440 Torr. $n=3$). In spite of persisting hypoxia the cell proliferation decreases after the ninth day to control values ($n=3$); (b) stimulation of liver cell proliferation during hypoxia is less pronounced ($n=3$).

ous rise of the *in-vitro* incorporation of ^3H -thymidine into the lung tissue and a subsequent decrease of the incorporation rate to the level of normoxic control animals in spite of prolonged hypoxia. Preliminary studies showed that the initial increase of DNA synthesis is almost linear during the first three days. Figure 1 shows a typical experiment; earlier experiments gave similar results: the maximum stimulatory response to chronic hypoxia is seen on the 9th day of the experiment and makes up 220–345% of the controls. Between the 15th and the 20th day of hypoxia the lung DNA synthesis has reached control values and tends to fall even below control values if hypoxia is maintained further (day 35). The mean DNA synthesis rates of the normoxic control rats show a small increase during the 35 days of the study which is not significant.

The DNA content of the hypoxic lungs ($n=6$), expressed as μg DNA/mg dry tissue weight, shows no significant changes during a hypoxic period lasting for 12 days (Fig. 2). The DNA-synthesis rate of rat liver tissue responded in a similar manner, although the response was less pronounced.

Discussion

The morphological adaptation to altitude hypoxia is seen in right ventricular hypertrophy (Alexander *et al.*, 1960; Grover *et al.*, 1963; Barer *et al.*,

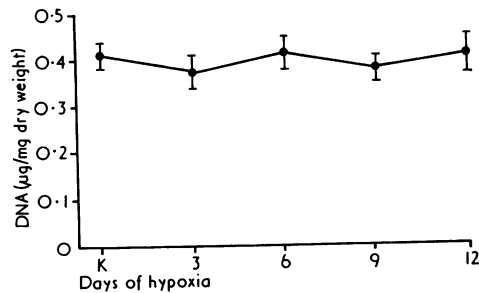


Fig. 2 Means and SEM of lung tissue DNA content. Time course during a hypoxic period of 12 days ($n=6$). The animals were sacrificed on day 3, 6, 9, and 12: the controls (K) were sacrificed on day 6 of the experiment. The changes in the DNA concentration are not significant.

1970; Abraham *et al.*, 1971; Heath, 1975; Pfeiffer *et al.*, 1976; Suggett and Mungall, 1976), medial hypertrophy of the pulmonary trunk (Heath, 1975), and medial muscularisation of the small pulmonary arterioles (Heath, 1959; Naeye and Bickerman, 1959; Meessen, 1960; Arias-Stella and Saldaña, 1962; Barer *et al.*, 1970). These changes are encountered in rats of all age groups after a period of chronic hypoxia due to simulated high altitude (McGrath *et al.*, 1973). Similar changes are found during long-term hypercapnia (Lodi and Viswanathan, 1974).

Exposure to low oxygen in a decompression chamber serves as an effective model to produce the reversible histological vascular changes resulting from chronic hypoxia (Kay *et al.*, 1970; Abraham *et al.*, 1971; Heath 1975; Urbanová *et al.*, 1975) in the course of chronic bronchitis or long lasting emphysema (Naeye and Bickerman, 1959; Heath, 1970). We used this model to design a pilot study in search of a simple parameter to measure cell proliferation in the lung under hypoxic conditions.

A raised *in-vitro* incorporation of ^3H -thymidine into lungs submitted to increasingly longer periods of hypoxia suggests a stimulation of cell proliferation. As in postnatal life, this rise of lung DNA synthesis may occur to match a new functional requirement (Crocker *et al.*, 1970). Rats exposed to high altitude for three days show a 50% increase in pulmonary artery pressure (Moret and Duchosal, 1976), and after 10 days of simulated high altitude the mean percentage wall thickness of small pulmonary vessels is significantly raised (Reid, 1975). Our time course study provides data for increased cellular DNA synthesis activity that is upheld for several days. DNA synthesis may be

the terminal event in a chain of metabolic processes which are preceded by changes of the DNA precursor pool resulting in a lag between the application of the stimulus and the onset of DNA synthesis (Novi, 1976). The delay varies in duration from hours to a day (Baserga, 1968). Thus a 345% increase in the DNA synthesis rate after nine days of hypoxia due to a reduction of the capacity of the DNA precursor pool seems improbable.

It is surprising that the proliferative system is turned on vigorously during an early induction phase but seems insensitive to further hypoxia. This finding may explain the results of Naeye (1962) who was unable to demonstrate a change in the mitosis index in mice lungs after chronic hypoxia. In contrast to the proliferation rate, which returns to control values and tends to reach even lower levels, the lung tissue DNA concentration remains raised, reflecting the postmitotic increase in the total cell number.

Our data suggest a transient stimulation of mitotic activity, but we cannot define the type of cell that accounts for this proliferation. Whether we deal with arteriolar wall muscle cells, intima cells, alveolar cells, fibroblasts or the mast cell hyperplasia that has been reported to occur around small vessels of hypoxic lungs (Kay and Grover, 1975; Mungall and Barer, 1975; Mungall, 1976) can only be shown by morphological studies.

A preliminary qualitative statement can be made on the basis of our first autoradiographic findings. A significantly higher index of radioactive label in the hypoxic lungs compared with the controls is found in the mesenchymal cells, in the walls of small pulmonary arteries, and in the bronchial tissue. A detailed quantitative evaluation of these results is in preparation.

References

- Abraham, A. S., Kay, J. M., Cole, R. B., and Pincock, A. C. (1971). Haemodynamic and pathological study of the effect of chronic hypoxia and subsequent recovery of the heart and pulmonary vasculature of the rat. *Cardiovascular Research*, **5**, 95-102.
- Alexander, A. F., Will, D. H., Grover, R. F., and Reeves, J. T. (1960). Pulmonary hypertension and right ventricular hypertrophy in cattle at high altitude. *American Journal of Veterinary Research*, **21**, 199-204.
- Arias-Stella, J., and Saldaña, M. (1962). The muscular pulmonary arteries in people native to high altitude. *Medicina Thoracalis*, **19**, 484-493.
- Barer, G. R., Cook, C., Clegg, E. J., and Shaw, J. W. (1970). Pulmonary vascular and cardiac changes in rats and mice exposed to chronic hypoxia (Abstract). *Journal of Pathology*, **101**, P i-ii.

- N. Völkel, U. Wiegers, V. Sill, and J. Trautmann
- Baserga, R. (1968). Biochemistry of the cell cycle: a review. *Cell and Tissue Kinetics*, **1**, 167-191.
- Burton, K. (1968). Determination of DNA concentration with diphenylamine. *Methods in Enzymology*, **12 B**, 163.
- Burton, R. R., Besch, E. L., and Smith, A. H. (1967). Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. *American Journal of Physiology*, **214**, 1438-1442.
- Crocker, T. T., Teeter, A., and Nielsen, B. (1970). Postnatal cellular proliferation in mouse and hamster lung. *Cancer Research*, **30**, 357-361.
- Grover, R. F., Reeves, J. T., Will, D. H., and Blount, S. G. Jr. (1963). Pulmonary vasoconstriction in steers at high altitude. *Journal of Applied Physiology*, **18**, 567-574.
- Heath, D. (1959). Structural alterations of pulmonary vessels in response to pulmonary hypertension. In *Pulmonary Circulation, International Symposium 1958*, edited by W. R. Adams and I. Veith, pp. 122-125. Grune and Stratton, New York.
- Heath, D. (1970). Hypoxic hypertensive pulmonary vascular disease. *Progress in Respiration Research*, **5**, 13-16.
- Heath, D. (1975). Reversibility of cardiopulmonary changes in rats induced by simulated high altitude. *Progress in Respiration Research*, **9**, 118-120.
- Kay, J. M., Abraham, A. S., Cole, R. B., and Pincock, A. C. (1970). The effect of chronic hypoxia and subsequent recovery on the heart and pulmonary circulation of the rat (Abstract). *Journal of Pathology*, **101**, P ii.
- Kay, J. M., and Grover, R. F. (1975). Lung mast cells and hypoxic pulmonary hypertension. *Progress in Respiration Research*, **9**, 157-164.
- Lodi, S. T. K., and Viswanathan, R. (1974). Effect of 7% CO₂ breathing on muscular pulmonary arteries and on the right ventricle in rats. *Respiration*, **31**, 201-207.
- McGrath, J. J., Procházka, J., Pelouch, V., and Ostadal, B. (1973). Physiological responses of rats to intermittent high-altitude stress: effects of age. *Journal of Applied Physiology*, **34**, 289-293.
- McMurtry, I. F., Frith, C. H., and Will, D. H. (1973). Cardiopulmonary responses of male and female swine to simulated high altitude. *Journal of Applied Physiology*, **35**, 459-462.
- Meessen, H. (1960). Die Pathomorphologie der Diffusion und Perfusion. *Verhandlungen der Deutschen Gesellschaft für Pathologie*, **44**, 98-127.
- Moret, P. R., and Duchosal, F. (1976). Effets du propranolol sur l'hypertension pulmonaire, l'hypertrophie ventriculaire droite et les altérations métaboliques myocardiques dues à l'hypoxémie aiguë de haute altitude. *Schweizerische medizinische Wochenschrift*, **106**, 1564-1566.
- Mungall, I. P. F. (1976). Hypoxia and lung mast cells: influence of disodium cromoglycate. *Thorax*, **31**, 94-100.
- Mungall, I. P. F., and Barer, G. R. (1975). Lung vessels and mast cells in chronically hypoxic rats. *Progress in Respiration Research*, **9**, 144-150.

- Naeye, R. L. (1962). Hypoxemia, effects on the pulmonary vascular bed. *Medicina Thoracalis*, **19**, 494–501.
- Naeye, R. L., and Bickerman, H. A. (1959). The effects of hypoxemia on the pulmonary arterial bed of humans and rats (Abstract). *Federation Proceedings*, **18**, 497.
- Novi, A. M. (1976). Molecular basis of a control mechanism of DNA synthesis in mammalian cells. *Klinische Wochenschrift*, **54**, 961–968.
- Pfeiffer, H. G., Daum, S., Goerg, R., Blümel, G. (1976). Kontraktilität des rechten Ventrikels bei *Cor pulmonale chronicum* (Abstract no. 20). *Cor pulmonale chronicum. Symposium, Munich, 1976*.
- Reid, L. (1975). Morphology of pulmonary circulation in health and disease. *Kongressberichte wissenschaftliche Tagung der Norddeutschen Gesellschaft für Lungen- und Bronchialheilkunde*, **14**, 333–347.
- Suggett, A. J., and Mungall, J. P. F. (1976). The effects of drugs on the right ventricular hypertrophy and pulmonary vascular changes of chronic hypoxia: an experimental study (Abstract no. 64). *Cor pulmonale chronicum, Symposium, Munich, 1976*.
- Urbanová, D., Ressler, J., Widimský, J., Ošťádal, B., Pelouch, V., Procházka, J. (1975). Pulmonary vascular changes and their reversibility induced by intermittent high altitude exposure. *Progress in Respiration Research*, **9**, 126–129.

Requests for reprints to: Dr. N. Völkel, I Medical University Clinic, 2, Hamburg 20, Martinstrasse 52, Germany.