2721

Effect of the Supply of Oxygen on the Tensile Strength of Healing Skin Wound and Granulation Tissue

By

E. Kulonen, J. Niinikoski and R. Penttinen

Abstract

KULONEN, E., J. NIINIKOSKI and R. PENTTINEN. *Effect of the supply of oxygen on the tensile strength of healing skin wound and granulation tissue.* Acta physiol. scand. 1967. *70.* 112—115.

Experimental granulomas were induced in rats with subcutaneously implanted viscose cellulose-sponges. The rats were kept for 10 days in normobaric atmospheres containing 21, 35 or 70 % (v/v) oxygen. After killing, the tensile strengths of the granulomas and skin wounds were measured. In granulomas the tensile strengths were: in 21 % O_2 107.2±9.7 g (23 determinations), in 35 % O_2 190.2±18.5 g (41), in 70 % O_2 221.0±24.6 g (26) and in skin wounds 431.3±32.8 g (29), 534.0±22.8 g (40) and 574.1±32.9 g (24), respectively. The analysis of variances confirmed that the variation between the groups is larger than within the groups (for the granulomas $P<0.001$ and for the skin wounds $P<0.005$).

This work was inspired by the finding that oxygen is necessary for the synthesis of collagen in incubated slices of sponge-induced granulation tissue (Kao *et al.* 1963). We wished to learn whether this dependence could be extended to whole animals living in normobaric atmosphere where the concentration of oxygen could be varied. A preliminary report has appeared (Niinikoski, Penttinen and Kulonen 1966).

Relevant observations in the literature are few. In rats which had been kept intermittently in hyperbaric (2 atm) oxygen the wound healing was retarded (Lundgren and Sandberg 1965). On the other hand, the regeneration of wound epithelium was more rapid at sea level than in the mountains of 3 000 m (Utkina 1958). Also, Beckham and Hitchcock (1965) reported that hyperbaric oxygenation of tissues may influence beneficially the healing of wounds. Intravascular injection of hydrogen peroxide improved the healing of leg sores (Balla *et al.* 1964). They observed that no systematic study has been performed on the effect of oxygen on the wound healing with reference to the tensile strength.

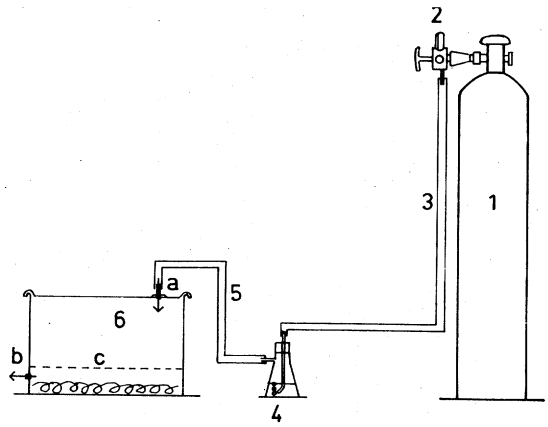

Fig. 1. Experimental arrangement. From a gas cylinder (*1*) the oxygen-nitrogen mixture flows (usually at the rate of 5 l/hr) through the reducing valve (*2*) and a 5-mm tubing (*3*) to a washing bottle (*4*) which serves as both a flow-meter and a humidifier and then through further tubing (*5*) to a box (*6*), where the rats are kept. The air-tight box (20×30×38 cm) is made of Perspex-sheets. There are small holes for the entry (*a*) and the exit (*b*) of the gas. The rats live on a removable wire floor (*c*) of stainless steel and the bottom of the box is covered with saw dust, which is changed every 3rd day.

Experimental

Induction of granulomas. Two or four pieces of viscose cellulose-sponge (20×10×10 mm each) were implanted subcutaneously in the backs of male rats (Wistar strain, weight 200±30 g). The pieces had been cut into two equal halves, fixed to the original position with stitches, and sterilized by boiling in 0.9 % (w/v) NaCl solution for 30 min. The length of the skin wound was 3 cm and it was closed with five sutures of cotton yarn.

Arrangement of the variations in the supply of oxygen. The set-up is shown schematically in Fig. 1. Three rats were kept simultaneously in each box (*6*), fed with the standard laboratory diet and water *ad libitum*. For the feeding and cleaning the boxes were opened once every day. The cylinders (*1*) contained mixtures of oxygen and nitrogen (O_2 21 %, 50 % and 100 %, v/v, respectively). In the boxes the final concentrations of oxygen were 18 %, 35 % and 70 %, respectively, as ascertained with Scholander's apparatus (kindly placed to our disposal by the Department of Physiology, University of Turku). The CO_2-concentration inside the boxes reached during the 24-hr period a level of about 5—6 % which caused some hyperventilation. The relative humidity in the boxes was about 90 % and the temperature 2—3° C above the ambient temperature (*cf.* Hulpieu and Cole 1944). No signs of O_2-intoxication, *e. g.* convulsions and pulmonary edema, were observed, probably because even the highest actual concentration of oxygen was no more than 70 %.

Measurements. Ten days after the implantation the rats were killed and the tensile strengths of the granulomas and of the skin wounds were measured as described by Viljanto (1964). The granulomas were stored frozen for chemical analyses.

Results and discussion

The averages of the tensile strengths are shown in Fig. 2. The favorable effect of the increased oxygen concentration is clear especially in the range of 21—35 % O_2 At higher concentrations the curves level off. On the hyperbaric conditions we do not have any experience. At lower concentrations of oxygen (about 12 %) the tensile strengths are decisively decreased in comparison with the results in air (336.2 ± 25.6 g for skin wounds and 43.8 ± 3.9 g for granulomas, $n = 23$ for both).

The most marked effect of oxygen in the tensile strength of the healing wounds is thus observed when its supply is varied starting from the composition of air. This is not necessarily true for the epithelial regeneration where hyperbaric oxygen may be more efficient.

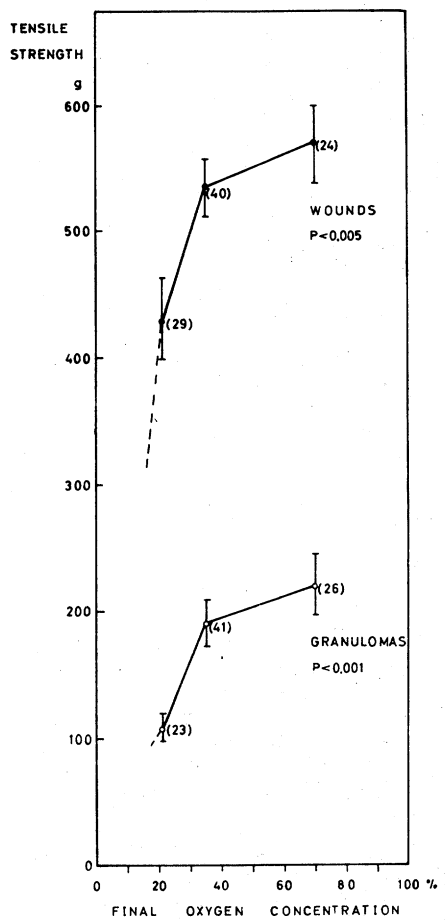

Fig. 2. The average tensile strengths of the skin wounds and the granulomas (ten days after implantation) plotted against the actual oxygen concentrations in the breathing gas. The tensile strength of the skin wounds is given in g/cm of healing wound and of the granulomas in g/cm² of the cross-sectional area. The number of experiments is indicated in the parentheses and the variation by the vertical bars which express the standard error of the mean. The statistical significance of the effect of the oxygen concentration is calculated by the analysis of the variances.

Chemical analyses on the granulomas have confirmed that the effect on the tensile strength depends on corresponding changes in the amounts of collagen hydroxyproline (unpublished work). The increased supply of oxygen may cause either an increased hydroxylation of proline, bound in collagen precursor, or a less specific stimulation of the growth of the granuloma. Our data are not yet conclusive enough to decide between these two alternatives. A general effect of oxygen may be demonstrated, for example, in the higher content of nucleic acids in the granulomas from those rats which have been kept at the O_2-concentration of 35 or 70 %. How far the synthesis of collagen is regulated by the availability of oxygen for the hydroxylation of proline remains to be investigated.

The financial support by institutional grants from Sigrid Jusélius Foundation and U.S. Department of Agriculture, Foreign Research and Technical Programs Division, is gratefully acknowledged.

We are indebted to Dr. P.-O. Barr, Karolinska Institutet, Stockholm, Sweden, for advice and criticism.

References

BALLA, G. A., J. W. FINNEY, B. L. ARONOFF, D. L. BYRD, G. J. RACE and J. T. WILLIAMS, Use of intra-arterial hydrogen peroxide to promote wound healing. *Amer. J. Surg.* 1964. *108.* 621—629.

BECKHAM, P. H. and C. R. HITCHCOCK, Effect of hyperbaric oxygenation on wound strength in dogs: a preliminary report. *Hyperbaric Oxygenation. Proceedings of the Second Congress* (Ed. I. M. Ledingham). E. & S. Livingstone Ltd., Edinburgh and London, 1965. 397—408.

HULPIEU, H. R. and V. V. COLE, The effect of humidity and temperature on oxygen toxicity. *J. Lab. clin. Med.* 1944. *29.* 1134—1138.

KAO, K.-Y.-T., W. E. HITT, R. L. DAWSON and T. H. MCGAVACK, Connective tissue VIII. Factors affecting collagen synthesis by sponge biopsy connective tissue. *Proc. Soc. exp. Biol.* (N.Y.). 1963. *113.* 762—766.

LUNDGREN, C. and N. SANDBERG, Influence of hyperbaric oxygen on the tensile strength of healing skin wounds in rats. *Hyperbaric Oxygenation. Proceedings of the Second International Congress* (Ed. I. M. Ledingham). E. & S. Livingstone Ltd., Edinburgh and London, 1965. 393—396.

NIINIKOSKI, J., R. PENTTINEN and E. KULONEN, Effects of oxygen supply on the tensile strength of healing wound and of granulation tissue. Abstracts of XII Scandinavian Congress of Physiology. *Acta physiol. scand.* 1966. *68.* Suppl. 277. 146.

UTKINA, O. T., Regeneration of skin epithelium in healing wounds, under normal conditions and at reduced barometric pressure. *Byul nauchn tr ryazansk otd vses nauchn obstrch anat. gistol. i embriol.* 1958. *4.* 26—29; *Biol. Abstr.* 1964. *45.* 6289—90.

VILJANTO, J., Biochemical basis of tensile strength in wound healing. *Acta chir. scand.* 1964. Suppl. *333.* 25—29.