# Effect of Changes in Inspired Oxygen and Carbon Dioxide Tensions on Wound Tensile Strength:

## An Experimental Study

FREDERICK O. STEPHENS, M.D., F.R.C.S., F.A.C.S., F.R.A.C.S., THOMAS K. HUNT, M.D., F.A.C.S.

*From the Department of Surgery, The University of California, San Francisco, San Francisco, California 94122*

CLINICIANS have long been searching for ways to obtain "supernormal" healing in wounds. However, in a recent review Prudden *et al.*[8] concluded that, with the single exception of cartilage powder, nothing has yet been found to promote better than normal healing.

We have, however, abundant evidence that healing processes are oxygen-dependent and that the supply of available oxygen at the healing edge is extremely precarious.[3] Indeed, some healing processes are apparently limited by the available oxygen supply.[4-7] In theory, an improvement in the supply of available oxygen to the healing wound should provide an environment in which more rapid healing could result. Conversely, reduction in oxygen tension in the healing wound might be expected to impede wound healing.

Reduced atmospheric oxygen lowers the $P_{O_2}$ in the healing wound. A moderate increase in atmospheric oxygen raises the $P_{O_2}$ in the healing wound only slightly, although $P_{CO_2}$ rises somewhat more. This suggests that when extra oxygen is supplied to the wound, it will be consumed.

In the first part of this study the tensile strength of healing wounds in rats was determined in relation to decreased, normal, and increased partial pressures of oxygen in inspired air.

Increased atmospheric oxygen tension may not be fully reflected in oxygen available to the healing wound because of physical and physiological limitations of supplying blood vessels. Although the physical damage to the local microcirculation cannot be immediately repaired, it may be possible in increase blood flow in undamaged vessels by physiological means. Tissue blood flow is partly regulated by $P_{CO_2}$; increased $P_{CO_2}$ results in small-vessel dilation and increased blood flow. The apparent effect of increased amounts of oxygen in enhancing wound healing, as suggested in previous studies,[5-7] could have been due to the associated rise in $P_{CO_2}$ in the wound. Therefore, in the second part of this study, the tensile strength of healing wounds in rats was determined in relation to combinations of normal and increased atmospheric oxygen with normal and increased atmospheric carbon dioxide.

Finally, determinations were made of the effect of increased atmospheric carbon dioxide on $P_{O_2}$, $P_{CO_2}$, and pH of tissue fluid in healing wounds.

Submitted for publication August 7, 1970.

This work was financed by United States Public Health Service Grant GM 12829. This work was carried out during Dr. Stephens's sabbatical year as Visiting Professor and Fulbright Fellow in the Department of Surgery, University of California, San Francisco.

Request reprints: Dr. Thomas K. Hunt, Department of Surgery, University of California, San Francisco, San Francisco, California 94122.

TABLE 1. *Experiment I. Atmospheric Oxygen and Wound Tensile Strength*

| | No. of Animals | Average Wound Tensile Strength (Gm.) | Standard Error | Statistical Significance of Difference with Control Group | Average Change in Weight (Gm.) |
|---|---|---|---|---|---|
| Group 1<br>10% oxygen | 12 | 445.6 | 27.4 | $p < 0.05$ | +2 |
| Group 2 (control)<br>20% oxygen | 12 | 517.3 | 22.5 | | +30 |
| Group 3<br>40% oxygen | 12 | 563.1 | 31.3 | $p < 0.025$ | +31 |

Table 1 shows the average tensile strength of wounds and standard error of the mean in the three groups of animals kept in oxygen concentrations. Average changes in body weight at the end of one week are also shown.

## Methods

Seventy-two young male Sprague-Dawley rats were anesthetized with ether and hair was removed from the back with clippers. Operations were performed under clean but not strictly sterile conditions.

All animals were fed a standard pellet diet and were given water ad libitum. They were housed in individual compartments of "Perspex" containers, the floors of which were covered with wood shavings. The individual compartments measured 20 cm. × 8 cm. and were 18 cm. deep.

Gases were supplied to the containers at atmospheric pressure; the temperature was maintained at 25° C. to 27° C. and humidity was between 70 and 90%.

In the first two experiments (60 rats) one standard skin wound 6 cm. long was made in the back of each animal. The wound was begun 2 cm. caudal to the scapula and ran parallel to the midline and 1.5 cm. to the left. All wounds were closed in an identical fashion with continuous 4–0 silk atraumatic sutures. No dressings were applied.

**Experiment I.** Thirty-six rats were studied in three groups of 12. The rats weighed between 252 and 350 Gm. (average 295 Gm).

*Group 1.* Group 1 rats were kept in an atmosphere of 10% oxygen, less than 0.5 $CO_2$, and approximately 89.5% nitrogen.

*Group 2.* Group 2 rats were kept in an atmosphere of 20% oxygen, less than 0.5% $CO_2$, and approximately 79.5% nitrogen.

*Group 3.* Group 3 rats were kept in an atmosphere of 40% oxygen, less than 0.5% $CO_2$, and approximately 59.5% nitrogen.

**Experiment II.** Twenty-four rats were studied in four groups of six each. These rats were a little younger than the rats used in Experiment I and weighed between 242 and 280 Gm. (average 262 Gm.).

*Group 1.* Group 1 rats were kept in an atmosphere of 20% oxygen, less than 0.5% $CO_2$, and approximately 79.5% nitrogen.

*Group 2.* Group 2 rats were kept in an atmosphere of 20% oxygen, 8% $CO_2$, and 72% nitrogen.

*Group 3.* Group 3 rats were kept in an atmosphere of 40% oxygen, less than 0.5% $CO_2$, and approximately 59.5% nitrogen.

*Group 4.* Group 4 rats were kept in an atmosphere of 40% oxygen, 8% $CO_2$, and 52% nitrogen.

## Measurements of Wound Tensile Strength

After 7 days the 60 animals in Experiments I and II were anesthetized with ether, sutures were removed, weights were recorded, and the tensile strength of the wounds was measured by a tensiometer, as described by Sandblom, Peterson and Muren.[9] Appropriate precautions were taken as outlined by Douglas.[1] Each wound was tested three times, once each in its anterior, central, and posterior positions, as described elsewhere.[2]

**Experiment III.** Twelve rats were anesthetized with ether and one steel wire cylinder, 4 cm. long and 1 cm. in diameter was inserted subcutaneously into each flank. The technic used was similar to that previously described.[3] These rats were then divided into two groups of six each.

*Group 1.* Group 1 rats were kept in an atmosphere of 20% oxygen, less than 0.5% $CO_2$, and approximately 79.5% nitrogen.

*Group 2.* Group 2 rats were kept in an atmosphere of 20% oxygen, 8% $CO_2$, and 72% nitrogen. At the end of one week, wound fluid was aspirated from the cylinders and $P_{O_2}$, $P_{CO_2}$, and pH of the wound fluid were determined as previously described.

## Results

**Experiment I.** Animals that breathed 10% oxygen had a significant reduction in wound tensile strength after one week when compared with control animals breathing 20% oxygen (Table 1). Animals breathing 40% oxygen had a significant increase in wound tensile strength. The group given 10% oxygen gained significantly less weight but no significant difference was found in the weight gain of animals in the 20% and 40% oxygen groups.

**Experiment II.** The differences in tensile strength between Groups 1 and 2, Groups 1 and 3, Groups 2 and 4, and Groups 3 and 4, were statistically significant (Table 2).

As in Experiment I, the animals breathing 40% oxygen and less than 0.5% $CO_2$ had significantly stronger wounds than animals breathing 20% oxygen and less than 0.5% $CO_2$. The difference in these younger animals was in fact apparently greater than in equivalent groups of animals in Experiment I.

The increase of inspired carbon dioxide from less than 0.5% to 8% not only failed to increase wound tensile strength, but actually resulted in significantly reduced tensile strength in animals breathing both 20% and 40% oxygen.

**Experiment III.** The $P_{O_2}$ of wound fluid taken from animals exposed to 20% oxygen and 8% atmospheric $CO_2$ was slightly altered from that of animals kept in 20% oxygen and less than 0.5% $CO_2$, but this difference was not statistically significant (Table 3). The $P_{CO_2}$, however, was significantly elevated. At the same time the pH

TABLE 2. *Experiment II. Effect of Different Atmospheric Oxygen and $CO_2$ Tensions on Wound Tensile Strength*

| | No. of Animals | Average Wound Tensile Strength | Standard Error | Average Change In Weight (Gm.) |
|---|---|---|---|---|
| Group 1<br>20% oxygen<br>< 0.5 $CO_2$ | 6 | 518.1 | 27.4 | +15 |
| Group 2<br>20% oxygen<br>8% $CO_2$ | 6 | 388.8 | 18.4 | −9 |
| Group 3<br>40% oxygen<br>< 0.5% $CO_2$ | 6 | 571.3 | 31.5 | +25 |
| Group 4<br>40% oxygen<br>8% $CO_2$ | 6 | 460.1 | 32.4 | +19 |

Table 2 shows the average tensile strength of wounds and standard errors of the mean in the four groups of animals in different oxygen and carbon dioxide atmospheres. Average changes in body weight at the end of one week are also shown.

TABLE 3. *Experiment III. Effect of Different Atmospheric $CO_2$ Tensions Gas Tension in Wound Fluid*

| | $P_{O_2}$ | Standard Deviation | $P_{CO_2}$ | Standard Deviation | pH | Standard Deviation |
|---|---|---|---|---|---|---|
| Group 1<br>20% oxygen<br>< 0.5% $CO_2$ | 15.6 | 4.6 | 69.8 | 6.5 | 7.25 | 0.03 |
| Group 2<br>20% oxygen<br>8% $CO_2$ | 13.9 | 3.9 | 84.6 | 11.3 | 7.35 | 0.05 |
| Significance of difference between groups 1 and 2 | not significant | | $p < 0.001$ | | $p < 0.001$ | |

Table 3 shows average measurements of $P_{O_2}$, $P_{CO_2}$, and pH of wound fluid from rats kept in normal and raised atmospheric $CO_2$.

of the wound fluid was found, in fact, to be slightly but significantly raised.

## Discussion

Wound tensile strength is largely dependent upon normal collagen formation and accumulation.[12] Recent studies have clarified the role played by oxygen and protocollagen hydroxylase in collagen formation.[11] Probably even more important is the need for oxygen to provide energy for protein synthesis. Available oxygen for these processes is dependent upon partial pressure of oxygen in blood, capillary blood flow, and diffusion of oxygen from the nearest functioning blood vessels to the healing wound edge.

Oxygen tension at the actual healing wound edge and at the surface of granulation tissue has been found to be precariously low and it has been suggested that even in "normal" wounds the rate of healing may be governed by oxygen supply.[3, 5] Experimental evidence by two independent laboratories indicates that increased atmospheric oxygen will promote wound healing,[6, 7, 13] although other authorities have remained unconvinced.[8]

The present studies provide evidence that an increase in $P_{O_2}$ in inspired air of normal rats results in an increase in wound tensile strength at one week. This evidence is consistent with the theory that the consumption of oxygen in the wound is largely dependent upon the available supply of oxygen to the healing wound edge.

The addition of excess $CO_2$ to the inspired air, however, not only failed to promote wound healing, but in fact, was associated with reduced wound tensile strength. It seems likely that in the healing wound, normal $CO_2$ accumulation is sufficient to provide conditions for maximal small vessel dilatation and that a further large supply of $CO_2$ produces metabolic changes which impair healing. Although we found that exposure to 8% $CO_2$ for one week increased the $P_{CO_2}$ in wound fluid, pH also rose. Presumably, this reflects the generalized metabolic alkalosis which compensates for the prolonged respiratory acidosis produced in these animals.

Previous experiments showing increased accumulation of wound collagen in animals kept in high-oxygen environments have been open to possible criticism that the accumulated $P_{CO_2}$ in the wound and not the increased oxygen, accelerated the collagen accumulation. Niinikoski *et al.*, in their experiments, allowed $CO_2$ to accumulate in the atmosphere to about 5%.[7] Their experiments, therefore, are open to the same criticism. We have shown, however, that increased $P_{CO_2}$ in the atmosphere and in the wound is detrimental to healing and militates against the accelerating effect of increased oxygen. The $p_{CO_2}$ of 84 mm. Hg reached in the wound fluid is comparable to the wound $P_{CO_2}$ of 85 mm. Hg at 5 days in rabbits treated with 40% oxygen in a previous unpublished study. It is now almost certain that the rate of collagen accumulation in wound healing is directly proportional to the $P_{O_2}$ of blood delivered to the wound.

The delivery of oxygen to the wound is a sensitive function of many physiologic determinants such as local blood flow, cardiopulmonary function, blood volume, and temperature. Local blood flow is in turn dependent upon other factors such as the number, tightness, and time of removal of sutures and the activity of the patient. Our studies in this field are reported elsewhere.[10] In brief, we believe that the absence of restrictive and unnecessary dressings, early mobilization of patients, and early removal of sutures consistent with maintenance of wound edge apposition are of fundamental importance in the healing of "normal" clean wounds. These factors are probably in part operative through the delivery of oxygen to the wound.

The degree of "acceleration" shown here is relatively slight. The authors do not suggest that oxygen therapy should be given to patients with cleanly incised and closed wounds. Of more interest therapeutically are those patients with decreased oxygen delivery, extensive wounds, respiratory failure, and poor local perfusion. Oxygen therapy may be of benefit in some of these conditions. Further studies seem warranted.

## Summary

Evidence is given to show that the rate of healing in "normal" wounds is dependent upon available oxygen supply.

Wounds in rats kept in an atmosphere of 10% oxygen had reduced tensile strength and wounds in animals kept in 40% oxygen had increased tensile strength when compared at the end of one week with wounds in animals kept in 20% oxygen.

The imposition of excessive $P_{CO_2}$ (8%) to inspired air not only failed to increase but actually reduced wound tensile strength of animals kept in 20% and 40% oxygen atmospheres. This confirms that increased wound healing associated with oxygen is directly due to the increased available oxygen and is not a secondary result of increased $P_{CO_2}$.

Excessive atmospheric $P_{CO_2}$ (8%) given to animals breathing 20% oxygen increased the $P_{CO_2}$ and also the pH of wound tissue fluid.

## Acknowledgments

We thank Mrs. Gunta Grisliss and Julie Shewvan for assistance in the studies.

## References

1. Douglas, D. M.: Wound Healing and Management. London, E. & S. Livingstone, 1963.
2. Ehrlich, P. and Hunt, T. K.: Effects of Cortisone and Vitamin A on Wound Healing. Ann. Surg., **167**:324, 1968.
3. Hunt, T. K., Twomey, P., Zederfeldt, B. and Dunphy, J. E.: Respiratory Gas Tensions and pH in Healing Wounds. Amer. J. Surg., **114**: 302, 1967.
4. Hunt, T. K. and Zederfeldt, B.: Nutritional and Environmental Aspects of Wound Healing. In: J. E. Dunphy and W. Van Winkle, Jr., eds., Repair and Regeneration. New York, McGraw-Hill, 1969, p. 217.
5. Hunt, T. K., Zederfeldt, B. and Goldstick, T. K.: Oxygen and Healing. Amer. J. Surg., **118**:521, 1969.
6. Hunt, T. K. and Dunphy, J. E.: Effects on Increasing Oxygen Supply to Healing Wounds. Brit. J. Surg., **56**:705, 1969.
7. Niinikoski, J.: Effect of Oxygen Supply on Wound Healing and Formation of Experimental Granulation Tissue. Acta Physiol. Scand., suppl. 334, 1969.
8. Prudden, J. F., Wolarsky, E. R. and Balassa, L.: The Acceleration of Healing. Surg. Gynec. Obstet., **128**:1321, 1969.
9. Sandblom, P. H., Peterson, P. and Murren, A.: Determination of the Tensile Strength of the Healing Wound as a Clinical Test. Acta Chir. Scand., **105**:252, 1953.
10. Stephens, F. O., Hunt, T. K. and Dunphy, J. E.: A Study of Traditional Clinical Therapy on the Tensile Strength of Skin Wounds in Rats (in preparation).
11. Udenfriend, S.: Formation of Hydroxyproline in Collagen. Science, **152**:1335, 1966.
12. Viljanto, J.: Biochemical Basis of Tensile Strength in Wound Healing. Acta Chir. Scand., suppl. 333, 1964.
13. Yablon, I. G. and Cruess, R. L.: The Effect of Hyperbaric Oxygen on Fracture Healing in Rats. J. Trauma, **8**:186, 1968.