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TISSUE OXYGEN AND CARBON DIOXIDE TENSIONS IN HEALING RABBIT TIBIAS

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Recent studies have demonstrated that tissue gas tensions can be measured by implanting a Silastic tube into the target organ (Niinikoski & Hunt 1972, Kivisaari & Niinikoski 1973). Silastic is highly permeable to respiratory gases and, therefore, the fluid filling the tube quickly equilibrates to the average PO_2 and PCO_2 of the surrounding tissue.

This paper reports determinations of tissue oxygen and carbon dioxide tensions in healing rabbit tibias with the above method. Baseline tissue gas tensions and the response to systemic hyperoxia and hypercarbia were recorded. Additionally, response of bone PO_2 and PCO_2 to occlusion of local circulation was determined, and bone PCO_2 was recorded after intravenous administration of acetazolamide, an inhibitor of carbonic anhydrase.

MATERIAL AND METHODS

Oxygen and carbon dioxide tensions of healing bones were measured by the method originally developed for the determination of PO_2 and PCO_2 in soft tissue wounds (Kivisaari & Niinikoski 1973). The tonometer was made from a gas permeable, barium-impregnated, x-ray positive Silastic tube, 17 cm long, with an outside diameter of 1.5 mm and an inside diameter of 1.1 mm. The tubing is commercially available in sterile packings and is commonly used in ventriculoatriostomies for the treatment of hydrocephalus.*

Twelve white male rabbits weighing 2.5–3.5 kg served as experimental animals. The rabbits were anesthetized with intravenously administered nembutal. The left hind leg was shaved and wiped thoroughly with iodine-alcohol solution. Through

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* Atrial Catheter J, B 190, Extracorporeal Medical Specialties Inc., King of Prussia, Pennsylvania, U.S.A.

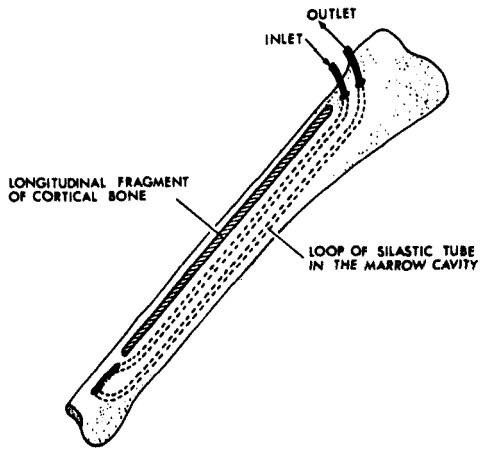

Figure 1. The experimental design for measuring tissue gas tensions in the rabbit tibia injured by implantation of the Silastic tube.

a medial incision, periosteum was split and the surface of the cortex was exposed. A longitudinal fragment of cortex was cut out with a dental drill with a fissured taper. The medulla was removed as completely as possible and stored under sterile conditions. The inner surface of the cortex was then abraded with the drill to ensure a circumferential injury of the cortical bone. Two pairs of holes, 7.5 cm apart, were made anteriorly in both ends of the tibia, and the Silastic tube was fed into its position in the marrow cavity through these holes (Figure 1). The medullary material was replaced and covered by the fragment of cortex. The periosteal incision was sutured, and the tube ends were fixed with a silk ligature. The skin was closed over the tube ends with a continuous polypropylene suture and the wound was sealed with Nobecutan® spray.*

For the measurements of tissue gas tensions the rabbits were lightly anesthetized with intramuscularly injected fentanyl citrate** and fluanison*** as described by Silver (1969). The ends of the tonometer were exposed through a small incision, and oxygen and carbon dioxide tensions in the healing bone were determined by the method of Kivisaari & Niinikoski (1973).

For continuous monitoring of tissue gases the gas permeable Silastic tube was repeatedly filled with hypoxic saline solution (PO_2 between 3 and 8 mmHg) for two minutes. During this period PO_2 and PCO_2 equilibrations of 95 and 85 per cent were achieved between saline and the tissue in contact with the tube. After the equilibration period the fluid was sampled into an Astrup-type glass capillary tube

* Bofors, Nobel-Pharma, Sweden.

** Fentanyl®, Orion, Helsinki, Finland.

*** Sedalande®, Orion, Helsinki, Finland.

by filling the tonometer with another dose of hypoxic saline from 1 ml syringe. The capillary was mounted into a micro-sample injector*, and the sample was emptied into a thermostated cuvette containing either an O_2 or CO_2 electrode. The electrodes were connected with a gas monitor PHM 71.*

Zero adjustment of the O_2 electrode was obtained with gaseous nitrogen and calibration took place with aerated saline, PO_2 150 mmHg, using the capillary sampling technique. The CO_2 electrode was also calibrated by the capillary sampling technique using saline solutions of two known CO_2 tensions (26 and 63 mmHg).

After induction of the anesthesia, baseline tissue gas tensions in the healing tibias were determined. The responses of tissue PO_2 and PCO_2 to breathing of a gas mixture containing 95 per cent O_2 and 5 per cent CO_2 were then recorded. The gas mixture was supplied by means of a head tent and the O_2 and CO_2 concentrations inside the tent were checked with a gas analyzer. In some animals the blood flow to the limb was hindered by a tight tourniquet to see the effect of vascular occlusion on tissue gas tensions. Response of bone tissue gases to the release of circulation was also recorded. In six animals bone PCO_2 was recorded after a single intravenous injection of acetazolamide**, an inhibitor of carbonic anhydrase (Minkin & Jennings 1972). In each animal acid-base equilibrium and oxygen and carbon dioxide tensions of the arterial blood were determined at short intervals in samples taken from the ear artery.

After the measurements were completed the rabbits were sacrificed and the bones with the implanted Silastic tubes were removed for histologic study. The samples were fixed, decalcified, and sections stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

A rapid decline in the baseline bone PO_2 from 32 to 25 mmHg occurred within the first three days postimplantation (Figure 2). During the following 52 days the PO_2 rose gradually to 42 mmHg. The maximum PO_2 in the healing tibia during breathing of 95 per cent O_2 and 5 per cent CO_2 rose progressively from the first day value of 35 mmHg to a level of 140 mmHg by the 30th day. Thereafter, no further increase was noted in the response to systemic hyperoxia.

The baseline PCO_2 in the healing tibia rose from 61 mmHg on the first day to 85 mmHg on day 3 (Figure 3). Thereafter, the PCO_2 gradually declined to 57 mmHg by the 30th day, a level which was maintained until the end of the observation period. The maximum PCO_2 during breathing of 95 per cent O_2 and 5 per cent CO_2 increased from 65 to 101 mmHg during the first three days after implantation and then decreased to a level of 65 mmHg by the 30th day.

Occlusion of the circulation to the leg by means of a tourniquet

* Radiometer, Copenhagen, Denmark.

** Diamox®, Lederle, U.S.A.

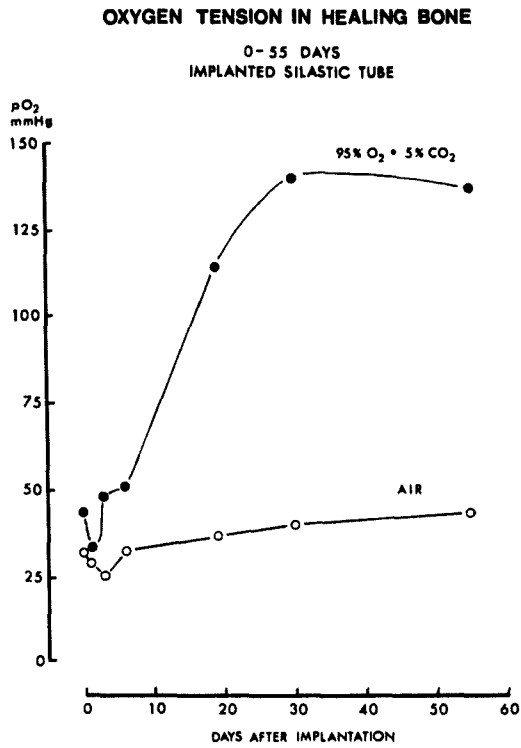

Figure 2. Oxygen tensions in healing rabbit tibias. Maximum tissue oxygen tensions during 1 h exposure to 95 per cent O_2 and 5 per cent CO_2 are also shown. The figures represent single determinations.

decreased the bone PO_2 from baseline values to a minimum of 2–5 mmHg within a few minutes (Figure 4). At the same time the bone PCO_2 was elevated to values well above 100 mmHg. After the release of circulation the gas tensions returned to normal levels in ten minutes.

A low dose of acetazolamide (5–35 mg/kg) had no influence on the bone PCO_2, whereas a dose of 100 mg/kg given to three rabbits increased the mean PCO_2 of the healing bone from 68 to 101 mmHg within three hours on day 19.

During air breathing the arterial blood PO_2 varied from 80 to 95 mmHg and the arterial blood PCO_2 was between 40 and 50 mmHg. While the rabbit was breathing 95 per cent O_2 and 5 per cent CO_2 the PaO_2 was between 400 and 500 mmHg, and the $PaCO_2$ was approximately 70 mmHg. Three hours after administration of acetazolamide there

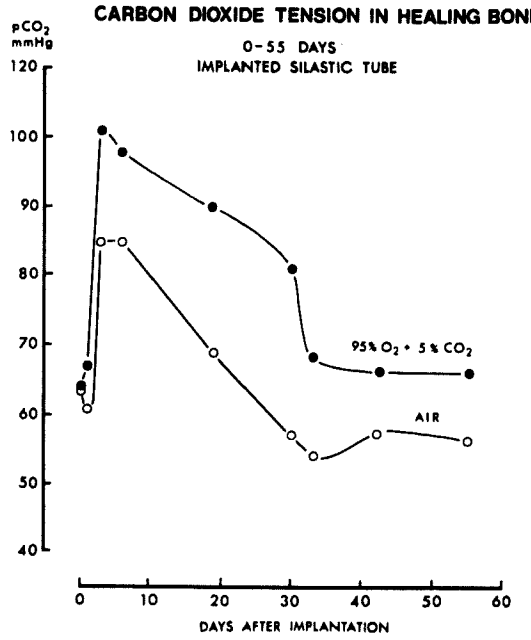

Figure 3. Carbon dioxide tensions in healing rabbit tibias. Maximum tissue carbon dioxide tensions during 1 h exposure to 95 per cent O_2 and 5 per cent CO_2 are also shown.

was a slight metabolic acidosis but the $PaCO_2$ remained essentially unchanged.

On histologic examination, the Silastic tube was surrounded with a trabeculated structure of rapidly calcifying bone by the 19th day (Figure 5 a). On the 30th day, the trabeculation had greatly diminished. By the 55th day further remodeling had taken place (Figure 5 b). The tonometer itself produced very little tissue reaction. No dead space was noted around the tube and no tonometer became infected.

DISCUSSION

The significance of adequate oxygen supply in healing bone has been demonstrated both experimentally (Coulson et al. 1966, Makley et al. 1967, Prasad & Reynolds 1968, Wray & Rogers 1968), and with clinical proof (Slack et al. 1965). Repair of fractures responds to changes in arterial oxygen tensions, and the main targets of oxygen in healing bone appear to be the synthesis of collagen and mineralization (Basset

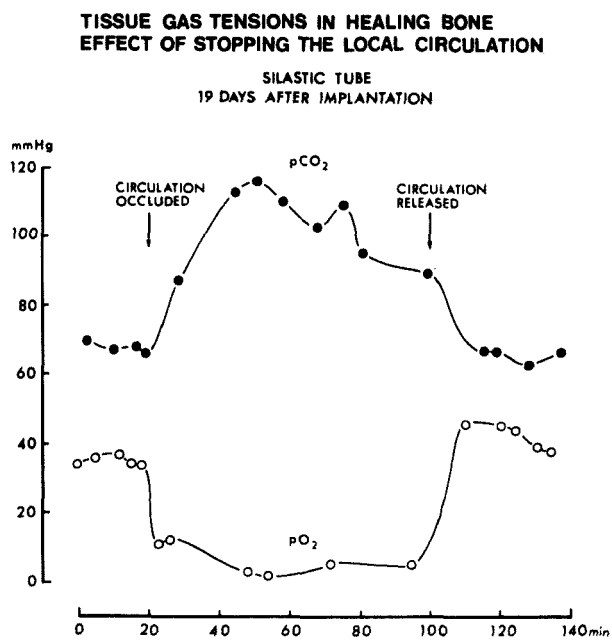

Figure 4. Effect of occlusion of local circulation on tissue gas tensions in the healing rabbit tibia. The measurement was performed 19 days after implantation of the Silastic tube.

& Herrmann 1961, Yablon & Cruess 1968, Niinikoski et al. 1970, Penttinen 1972).

The supply of oxygen to the repair area is diffusion-limited. Measurements with ultramicro oxygen electrodes have shown that oxygen tension gradients are steep between the capillary and the healing tissue a few microns away (Silver 1969, Niinikoski et al. 1972). A substantial portion of any injured tissue exists in conditions of low oxygen tension which may be far from optimal.

In a study with semimicro needle electrodes Brighton & Krebs (1972) measured oxygen tensions below 10 mmHg in fracture hematoma whereas newly-formed cartilage and fiber bone showed oxygen tensions between 20 and 40 mmHg. To the best of our knowledge, no study has been reported of the measurement of tissue carbon dioxide tension in healing bone. According to Cuervo et al. (1971), the PCO_2 in the extra-cellular fluid at calcifying sites of cartilage in rats varies between 38 and 43 mmHg. Laurnen & Kelly (1969) observed no changes in the arterial-venous differences in oxygen content, PO_2, PCO_2 or pH

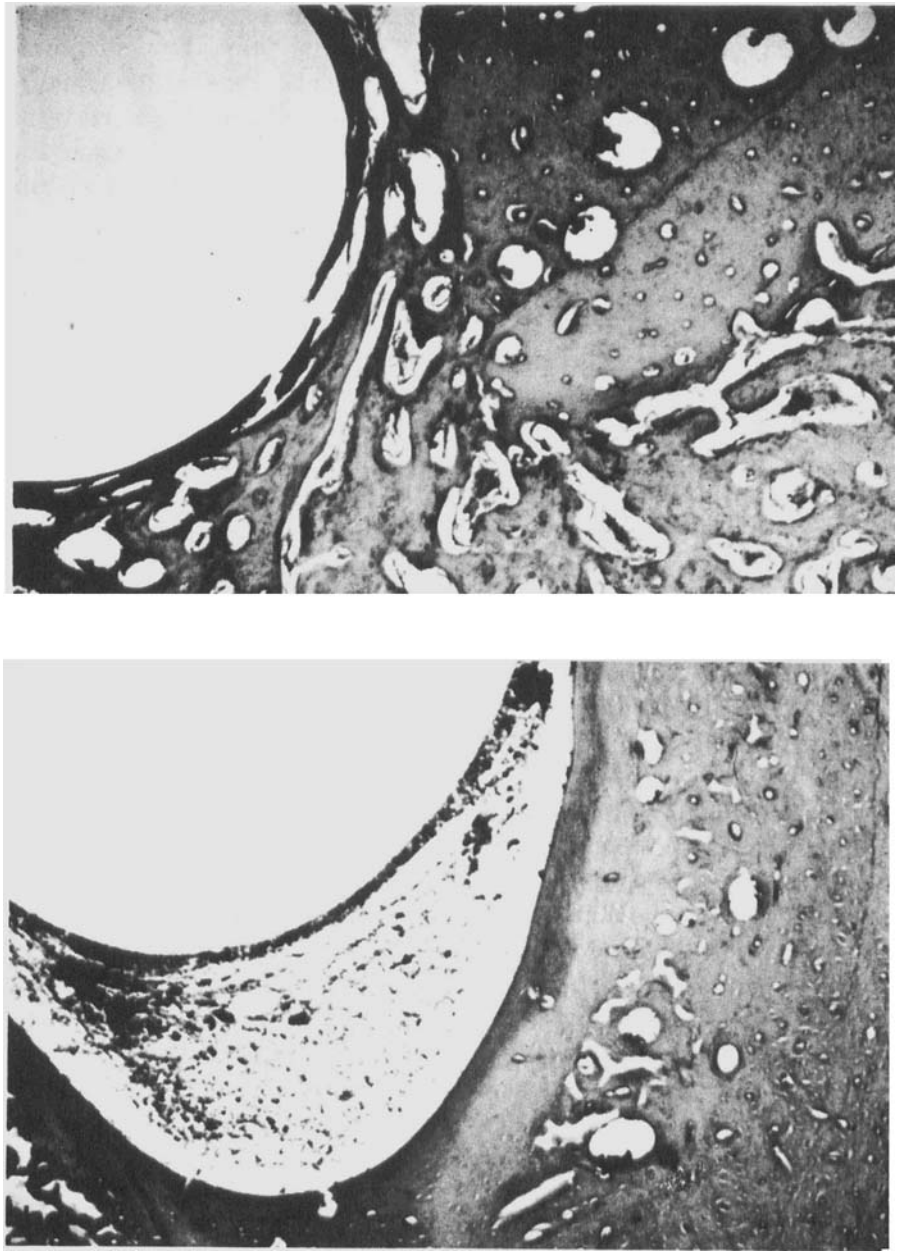

Figure 5. (a) Photomicrograph of the Silastic tube surrounded by trabeculated bone 19 days after implantation. Hematoxylin and eosin stain. (b) Silastic tube in the marrow cavity of the healing rabbit tibia 55 days after implantation. The inner cortex looks virtually normal.

when blood from a fractured canine tibia was compared with that from the control side. O_2 consumption and CO_2 production, however, must have increased since blood flow was invariably elevated at the fracture site. Dulce et al. (1960) suggested the presence of a functional carbonic anhydrase system in bone which could aid in the mineralization and demineralization of bone by controlling the local hydrogen ion secretion and pH.

The present results are comparable to those of an earlier study in which tissue oxygen tensions in healing rabbit tibias were measured by continuous perfusion of the implanted Silastic tube (Niinikoski & Hunt 1972). The normal sequences of change in PO_2 (Figure 2) are identical and the responses to systemic hyperoxia are generally similar to the findings of the earlier method. The advantages of the capillary sampling technique over continuous perfusion of the tonometer have been discussed elsewhere (Kivisaari & Niinikoski 1973).

The foreign body reaction that normally occurs around the Silastic tube consists normally of only two to four cell layers. Blood vessels frequently lie within a distance of 40–50 μ from the tube. The Silastic tubing perfused or filled with hypoxic saline solution measures and extracts oxygen from a distance of at least 1.0 mm (Kivisaari & Niinikoski 1973). Therefore, oxygen extracted into the sample probably derives not only from the marrow cavity but also from the inner cortex.

The bone-healing model used in this study differs markedly from a normal fracture of the tibial shaft. In the present model a small external callus is formed along the whole length of the tibia but the main process occurs in the marrow cavity. Here the trauma site is filled with fibrous tissue which quickly calcifies (Figure 5 a). Possibly because of a good blood supply, cartilage formation does not occur, and fibrous or spongy bone is formed directly. Remodeling is evident by the 30th day, and by the 55th day the bone looks almost normal (Figure 5 b).

During the first three days after implantation the bone PO_2 was rather low and its response to systemic hyperoxia was small (Figure 2). This was probably partly due to a transient reduction of local blood flow in the marrow cavity caused by clotting after the fresh trauma. Between the third and 30th days, the baseline PO_2 and the response to systemic hyperoxia increased gradually, probably due to the developing vascularity and the decreasing oxygen consumption in the repair tissue. During the remodeling phase the bone oxygen tensions remained virtually unchanged.

The high tissue carbon dioxide tensions between days 3 and 6 were probably due to accumulation of carbon dioxide because of impaired circulation and/or to increased production of carbon dioxide by the rapidly regenerating tissue (Figure 3). When the healing progressed the bone PCO_2 gradually decreased. Between days 30 and 55 the bone PCO_2 was about 55 mmHg. The increase of the bone PCO_2 during breathing of 95 per cent O_2 and 5 per cent CO_2 was proportional to the concomitant respiratory acidosis from the third day onwards.

When the circulation in a limb was interrupted by a tourniquet, the bone PO_2 fell abruptly so that most of the decrease occurred within two minutes (Figure 4). After the occlusion of circulation the bone PCO_2 gained its maximum within the first 30 minutes and then declined slightly. These results suggest marked oxygen consumption in the healing bone tissue. When all available oxygen has been consumed no more carbon dioxide is accumulated. After the tourniquet was removed the bone PO_2 increased momentarily above the normal level, probably due to reactive hyperemia or transient decline in oxygen consumption.

The local carbon dioxide tension may play a physiological role in the process of calcification. Bone mineralization appears to occur in conditions of rather high tissue PCO_2 (cf. Figure 3). High concentrations of carbonic anhydrase have been detected at calcifying sites of cartilage (Cuervo et al. 1971). On the other hand, Minkin & Jennings (1972) showed that inhibitors of carbonic anhydrase inhibited parathyroid hormone-induced resorption of bone in organ culture and suggested the presence of a functional carbonic anhydrase system in bone linked to the mechanism of bone resorption.

In the present study intravenous administration of carbonic anhydrase inhibitor caused a marked rise in bone PCO_2 at the phase of rapid regeneration and calcification. Simultaneously, the arterial blood PCO_2 remained unchanged suggesting that the rise in bone PCO_2 is due to a local effect of the carbonic anhydrase inhibitor.

SUMMARY

Tissue gas tensions were measured in healing rabbit tibias by means of an implanted Silastic tonometer. During the course of the healing, tissue oxygen tensions increased progressively and carbon dioxide tensions underwent a gradual decline. In all phases of repair, bone tissue gases responded to systemic hyperoxia and hypercarbia. Occlusion of local circulation resulted in tissue anoxia and accumulation

of carbon dioxide. Acetazolamide, an inhibitor of carbonic anhydrase, elevated the carbon dioxide tension in the bone but not in the blood which supports earlier data indicating the presence of a functional carbonic anhydrase system in actively metabolizing bone tissue.

REFERENCES

Basset, C. A. L. & Herrmann, I. (1961) Influence of oxygen concentration and mechanical factors on differentiation of connective tissue in vitro. *Nature (Lond.)* **190**, 460–461.

Brighton, C. T. & Krebs, A. G. (1972) Oxygen tension of healing fractures in the rabbit. *J. Bone Jt Surg.* **54**-A, 323–332.

Coulson, D. B., Ferguson, A. B. Jr. & Diehl, R. C. Jr. (1966) Effect of hyperbaric oxygen on the healing femur of the rat. *Surg. Forum* **17**, 449–450.

Cuervo, L. A., Pita, J. C. & Howell, D. S. (1971) Ultramicro-analysis of pH, PCO_2 and carbonic anhydrase activity at calcifying sites in cartilage. *Calcif. Tiss. Res.* **7**, 220–231.

Dulce, H. J., Siegmund, P., Korber, F. & Schutte, E. (1960) Zur Biochemie der Knochenauflösung. III Über das Vorkommen von Carboanhydratase im Knochen. *Hoppe-Seylers Z. physiol. Chem.* **320**, 163–167.

Kivisaari, J. & Niinikoski, J. (1973) Use of Silastic tube and capillary sampling technic in the measurement of tissue PO_2 and PCO_2. *Amer. J. Surg.* **125**, 623–627.

Laurnen, E. L. & Kelly, P. J. (1969) Blood flow, oxygen consumption, carbon-dioxide production, and blood-calcium and pH changes in tibial fractures in dogs. *J. Bone Jt Surg.* **51**-A, 298–308.

Makley, J. T., Heiple, K. G., Chase, S. W. & Herndon, C. H. (1967) The effect of reduced barometric pressure on fracture healing in rats. *J. Bone Jt Surg.* **49**-A, 903–914.

Minkin, C. & Jennings, J. M. (1972) Carbonic anhydrase and bone remodelling: sulfonamide inhibition of bone resorption in organ culture. *Science* **176**, 1031–1033.

Niinikoski, J. & Hunt, T. K. (1972) Oxygen tensions in healing bone. *Surg. Gynec. Obstet.* **134**, 746–750.

Niinikoski, J., Hunt, T. K. & Dunphy, J. E. (1972) Oxygen supply in healing tissue. *Amer. J. Surg.* **123**, 247–252.

Niinikoski, J., Penttinen, R. & Kulonen, E. (1970) Effect of hyperbaric oxygenation on fracture healing in the rat. *Calcif. Tiss. Res.*, Suppl. 4, 115.

Penttinen, R. (1972) Biochemical studies on fracture healing in the rat with special reference to the oxygen supply. *Acta chir. scand.*, Suppl. 432, 1–32.

Prasad, G. C. & Reynolds, J. C. (1968) Effect of environmental factors on the repair of bone in vitro. *J. Bone Jt Surg.* **50**-B, 401–408.

Silver, I. A. (1969) The measurement of oxygen tension in healing tissue. *Progr. Resp. Res.* **3**, 124–135.

Slack, W. K., Thomas, D. A. & Perrins, D. (1965) Hyperbaric oxygenation in chronic osteomyelitis. *Lancet* **i**, 1093–1094.

Wray, J. B. & Rogers, L. S. (1968) Effect of hyperbaric oxygenation upon fracture healing in the rat. *J. surg. Res.* **8**, 373–378.

Yablon, I. G. & Cruess, R. L. (1968) The effect of hyperbaric oxygen on fracture healing in rats. *J. Trauma* **8**, 186–202.

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