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# EFFECT OF HYPERBARIC OXYGENATION ON LONGITUDINAL GROWTH OF BONES

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The increased rate of growth in length of bones after fractures and ostemyelites in children gave rise to a surge of interest in the induction of such growth stimulation in experimental animals in the middle of the 19th century (*Stanley* 1849, *Ollier* 1867, *von Langenbeck* 1869). As early as 1869 *von Langenbeck* implanted ivory plugs in the medullary cavity of the femur and the tibia of a young dog for growth stimulation; after 11 weeks the leg was 10 mm longer than the contralateral one. In his classical monograph on bone regeneration *Ollier* in 1867 reported that removal of the periosteum of the tibia of rabbits resulted in a relative increase of the growth in length of the bone by 2–5 mm within 3 months. He also reported a less regular effect of the inplantation of nails in long bones and of the division of nerves in rabbits and dogs. Vasomotor paralysis with increased flow was considered as a possible cause of the stimulation of the length growth in these experiments. In 1856 *Broca* described a case with an arteriovenous aneurysm of a lower limb which was 3 cm longer than the contralateral leg. *Krause* (1861) published a similar case with an arteriovenous aneurysm of the one forearm which was 4 cm longer than the other.

On the basis of the above observations it could be assumed that epiphyseal hyperaemia was a common growth stimulant, and in 1887 *Helferich* reported the use of induced venous stasis as a clinical therapy: in a boy and in a girl with leg length disparity the difference was said to be reduced by 1.5–2 cm by prolonged stasis of the shorter limb.

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In more recent years stimulation of epiphyseal growth in length has received extensive attention. Thus, *Pearse & Morton* (1930) confirmed the stimulating effect of *venous stasis* on the healing of fractures in dogs and *Hutchinson & Burdeaux* on the growth in length (1954). Artificial *arteriovenous fistulae* have also been found to increase the rate of growth in the length of bones in the dog (*Janes & Musgrove* 1950, and *Keek & Kelly* 1964). *Hiertonn* (1961) and others have reported important growth stimulation after femoral arteriovenous shunting in children. *Intraosseous foreign bodies e.g.* solutions, metals, textiles, skin, ivory and bacteria have been used with varying success (*Wu & Miltner* 1930, *Kishikawa* 1936, *Chapchal & Zeldenrust* 1948, *Wilson* 1951, *Pease* 1952, *Herndon & Spencer* 1953, *Trueta* 1953, *Wilson & Percy* 1956, *Elo* 1960). *Medullary curettage* by *Ferguson* (1930) and *Hansson & Wiberg* (1963), *medullary plugging* by *Trueta* (1953) have been tried but with only fair success (*Carpenter, Dalton* 1956, *Jansen* 1957, *Ståhl* 1957). The effect of *division of nerves* and nerve roots has been reviewed and studied by *Troupp* (1961) and *Ring* (1961). *Buchtala* (1948) found *ultrasound* treatment of the epiphysis to produce some acceleration of growth in length, an effect which could not be confirmed by *DeForest et al.* (1953) or by *Vaughan & Bender* (1959). The effect of *roentgen irradiation* has been studied by *Baunach* (1935), *Reidy et al.* (1947) and *Barnhard* (1963), who found small doses to have no clear effect and large doses to retard growth of the epiphysis in birds and dogs. *Short wave diathermy* stimulates bone growth in rats according to *Doyle & Smart* (1963), but not in dogs (*Granberry & Janes* 1963). *Electric stimulation* has been found to have no effect (*Granberry & Janes* 1963) or an irregular and varying effect (*Richards & Stofer* 1959 and *Haas* 1963). *Local heating* has given no stimulation (*Ring & Lee* 1958). The influence of *fractures* on bone growth in children has recently been reviewed by *Guldhammer* (1963)—on the leg, and *Emnéus & Hedström*—on the arm (1964).

Though it may be regarded as established that growth in length of a bone is often accelerated by osteomyelites, fractures, foreign bodies, arteriovenous fistulae and venous stasis, no unequivocal evidence has been produced in support of the assumption of *Hutchinson & Burdeaux* (1954) that such acceleration is due to venous stasis hyperaemia as a primary or secondary effect in all cases cited. In this connection it may be convenient to mention an interesting observation by *Servelle* (1948). He reported venographically verified unilateral congenital venous abnormalities of the leg in 14 human beings, whose affected leg was

0.8–9.6 cm longer than the contralateral one, while no such difference could be found in any of 25 patients with chronic lymphoedema elephantiasis. The difference was so regular that he thought that it might be used in the distinction between the two diseases. In other words, growth in length was promoted by the congestion of the blood but not by the lymph stasis.

*Brodin* (1955) found the increased growth of the rabbit tibia after periosteal loosening to be associated with an increase in the *blood supply* as judged by photometric determination of the fluorescence of the bone after injection of sodium oxypyrene–sulphonate. Using i.v. $P^{32}$ injection *Watanabe* (1962) reported increased blood supply to the epiphysis after medullary curettage, as measured by the radioactivity of bone ash from standardized pieces of the tibia. This increase was proportional to the increase in growth recorded. It was not determined whether these observations depended on tissue affinities altered by the trauma or on change in the arterial blood flow or the blood volume content only.

Summing up there is thus ample evidence that hyperaemia is a cause of accelerated longitudinal growth of bones. This gives rise to the question whether there is an *increased arterial flow* in the affected region as indicated by the experiments of *Trueta* or a mere passive *venous congestion,* as suggested by *Hutchinson & Burdeaux.* One main difference between the two theories is the amount of oxygen offered to the growth plate and, in other words, is the growth accelerated by an increased oxygen supply *per se* to the extent that it follows the increased oxygen content of the arterial blood during hyperbaric oxygen breathing. A search of the literature failed to reveal any reports on this problem. It was therefore considered legitimate to study the effect of hyperbaric oxygenation on he rate of bone growth and the preliminary results are presented below.

## MATERIAL AND METHODS

The *material* consisted of 64 white rabbits of the same breed. The animals were 5–7 weeks old, they weighed 600–1800 g and represented 12 litters. Pure oxygen at 2 atm. absolute pressure was given to 20 rabbits, air at 2 atm. abs. to 18 litters mates, pure oxygen at 1 atm. abs. to 6 rabbits and air at 1 atm. abs. to 12 rabbits. The animals were weighed at the beginning and the end of the experiments. Eight animals (4 that received pure oxygen and 4 that received air) were excluded because of loss of bodyweight.

The equipment for *oxygenation* by pure oxygen breathing consisted of 2 glass hutches in a pressure chamber, pure oxygen and air in standard cylinders (200 kp),

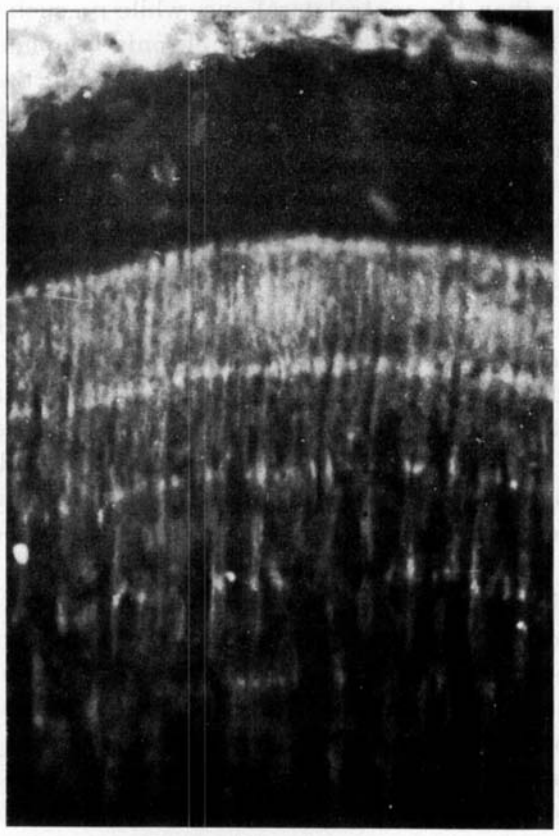


*Figure 1. Oxytetracycline fluorescence lines on the metaphyseal side of the proximal epiphyseal growth cartilage of the tibia of rabbits. Interval between the lines 24 hours, distance about 300 microns.*

and flowmeters to check the flow through the hutches. Pure air or oxygen was conducted through the hutches in the pressure chamber at the rate of 800 litres/hr/kg bodyweight to prevent accumulation of carbon dioxide. Two to four animals were placed in each hutch. Litter mates were placed in the 2 hutches and treated simultaneously. As in clinical practice, the animals were treated with oxygen for three 2-hour periods on a single day, with an interval of 1–2 hours between consecutive periods of treatment. During these intervals the rabbits were resting together outside the hutches. Before and after the day of treatment the animals were kept in the same cage as the mother. The animals were fed on rabbit pellets and carrots which were given at the same hours every day.

*Growth was measured* by the oxytetracycline (OTC) labelling method described by *Hansson* 1964. The OTC was injected intravenously in a dose of 1 mg/kg bodyweight each 24 hours. Incorporation of the OTC in the zone of calcification on the metaphyseal side of the growth cartilage produces distinct bands of fluorescence in an

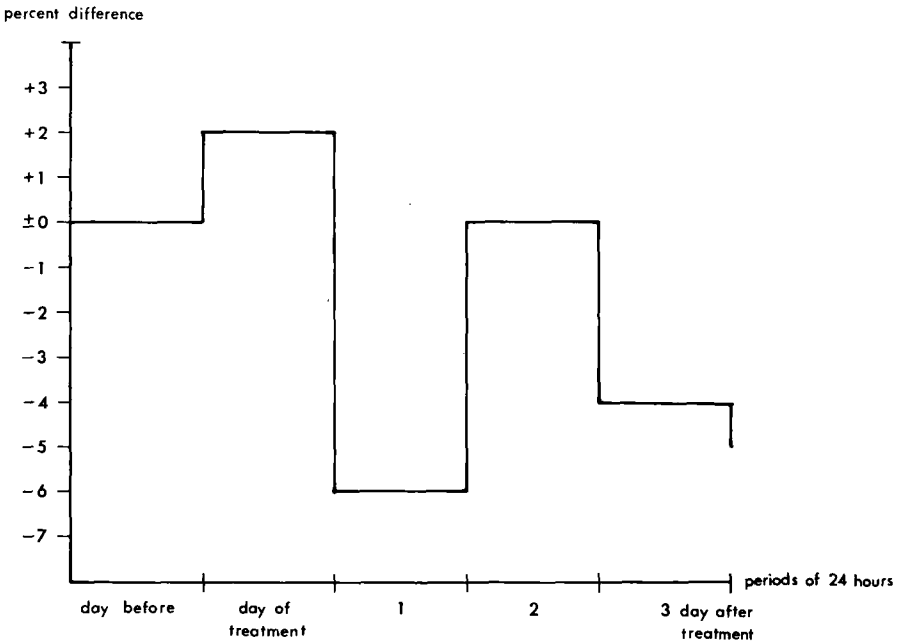


*Figure 2. Percent difference of epiphyseal growth distance in periods of 24 hours between oxygen and air treated rabbits with one single day of treatment for 2 + 2 + 2 hours at 2 atm. abs. of pure oxygen compared to pure air breathing.*

ultraviolet light microscope with a dark field condenser. The distance between consecutive bands of OTC is a measure of the growth per 24 hours (Figure 1). Each animal received 4 to 6 injections which thus allowed measurement of growth during three to five 24-hour periods. The first interval was used as a normal value, the second as the day of treatment and the subsequent days for studying the continued effect on growth. The animals were treated in the pressure chamber between the second and the third injections, the third injection being given within one hour of the end of the last period of oxygenation, so that the growth rate during oxygenation fell on one side and the growth rate after withdrawal of oxygenation on the other side of the third injection line. The proximal part of the left tibia was used for the microscopical sections. The rabbits were killed by intraperitoneal injection of Evipan® about 1 hour after administration of the last dose of OTC. The proximal tibia was excised and fixed in pure ethanol for 1–3 days. Slices 60–120 $\mu$ in thickness were cut with a razor blade placed parallel to the metaphyseal trabeculae, as judged in one sagittal and one frontal cut surface. Still according to *Hansson*, the slices were then treated in xylol for 5–10 minutes and mounted in DePeX on glass slides. Microscopic measurements of the line intervals were made with a standardized measuring ocular. The mean of 5–10 slices measured was used.

To test the reliability of the method in the hands of the writer, 15 animals were given 4 injections each and the growth was measured on the 3 consecutive periods of 12 hours used. For comparison both proximal tibiae were excised, treated and

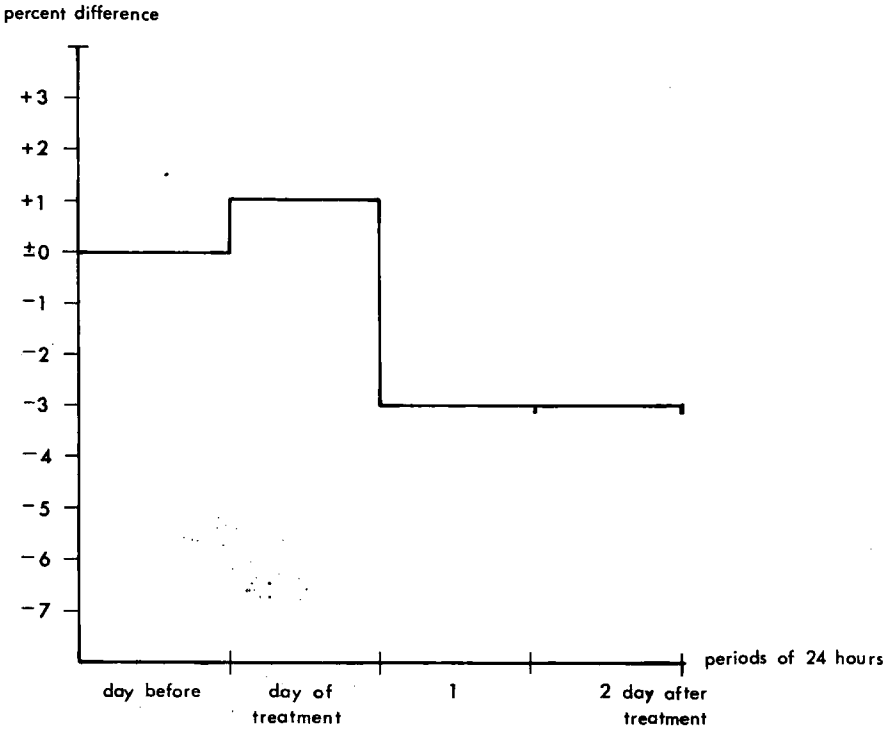


*Figure 3. Percent difference of epiphyseal growth distances in periods of 24 hours between oxygen and air treated rabbits with one single day of treatment for 2 + 2 + 2 hours at 1 atm. abs. of pure oxygen compared to pure air breathing.*

measured in the way described. The differences between the right and left sides of the 45 observations were added and compared with the mean of each interval and thereby gave the mean error of the method, which was found to be 9 microns or 3.7 per cent, including possible differences between the sides, error of obliqueness of the plane of sections relative to the plane of the trabeculae, mounting angulation, reading error and calculation.

## RESULTS

The results are summarized in Figures 2 and 3, which give the mean growth ratios on the control day, the day of treatment and the subsequent days of observation. In each of the 4 groups the mean growth noted was divided by the mean of the first day (before treatment—normal value) which was said to represent 100 units. The values thus obtained for growth in the oxygen group were divided by the corresponding figures recorded in the corresponding air group. In this way factors other than oxygen were excluded *i.e.* temperature,

humidity, pressure and other possible stressing factors as far as they were the same in the group treated with oxygen and in the one treated with air.

Figure 2 gives the results obtained with oxygen breathing at 2 atm. abs.; Figure 3, for oxygen at 1 atm. abs. No experiments were made at 3 atm. abs. because of the risk of toxic effects of the oxygen. The lungs of the animals were regularly examined post mortem. The findings indicated a slight stimulation (about 2 per cent) during oxygen breathing followed by a pronounced depression of growth rate (about 7 per cent) the day after exposure (see Figures 2 and 3). Statistical significance was found only for the depression of growth just mentioned. The material was not large enough to allow final conclusions but continued studies since performed lend support to all the tendencies observed in the present series.

## DISCUSSION

The 2 per cent increase of the rate of growth in length of the rabbit tibia during pure oxygen breathing for 2 + 2 + 2 hours in a single 24 hour period implies that the increase in rate of growth during the actual 6 hours of treatment exceeded 2 per cent assuming that growth proceeded at a normal rate during the intervals between the consecutive exposures. But judging from the deceleration of growth on the day after treatment, the rate of growth between and after the 3 exposures to oxygen might have been suppressed and thereby masked part of an increase in growth during the actual treatment. This possibility is being checked in investigations now in progress.

The inhalation of oxygen at an increased partial pressure suppresses the circulation in the limbs as measured by venous occlusion plethysmography and by Xenon clearance (*Bird & Telfer* 1965). At 2 atm. abs. of pure oxygen the reduction was about 28 per cent. If these figures hold for the rabbit and for the exposure periods used in the present investigation as well as for the blood flow in the epiphyseal bone per se it would decrease the supply of blood-borne growth substances including oxygen. According to Handbook of Respiration (*Dittmer & Grebe* 1958) the oxygen capacity of 100 ml of blood is 20 ml during air brathing at 1 atm. abs. and this figure is raised to 20.9 ml of haemoglobin bound oxygen plus another 4.3 ml in physical solution during oxygen breathing at 2 atm. abs. This means that a rise in inspired partial pressure of oxygen from about 150 mm Hg to about 1500 mm Hg increases the oxy-

gen capacity from 20 to 25.2 ml/100 ml of blood, an increase of about 25 per cent. The vasoconstriction in question would tend to reduce the attempted tissue hyperoxygenation as well as the supply of other bloodborne growth factors. Another point of interest is the possible retention of carbon dioxide caused by the reduced transport capacity of the haemoglobin when blocked by the hyperoxygenation. The maturation time for the cells of the growth plate has been determined to be 2–4 days from the zone of proliferation to the zone of calcification (*Messier & Leblond* 1960) and this causes difficulties in the interpretation of the diphasic stimulation and retardation recorded. The stimulation during the day of treatment was achieved without any venous stasis but may be with a local carbon dioxide accumulation. It was probably achieved without any increase in local blood flow but with a rise in arterial oxygen pressure. These problems will receive further attention.

Apart from the information that may be obtained on the physiological role played by oxygen in bone growth it may be of interest for a future development of therapeutic hyperbaric oxygenation in orthopaedics to know the effect of oxygen treatment in the longitudinal growth. Because of its quantitative measurability it forms another parameter of the biological effects of hyperbaric oxygenation.

## SUMMARY

From clinical experience and experimental work on longitudinal growth of bones it is known that the growth rate can be accelerated locally by certain inflammatory conditions as well as by venous stasis and arteriovenous shunts. In an attempt to investigate whether this stimulation is caused by *arterial hyperaemia* or by passive *venous stasis* young rabbits were exposed to pure oxygen breathing at one and two atm. abs. pressure for 2 + 2 + 2 hours on one single day and the growth rate of the proximal tibial growth plate was studied by oxytetracycline-labelling according to *Hansson*. The subsequent daily growth was compared with that in litter mates exposed to air at the same pressures for the same periods. Oxygen breathing seemed to accelerate growth by 2 per cent during the day of treatment, followed by a deceleration of 7 per cent the day after treatment. The results are discussed, mechanisms involved are evaluated and the continued work is outlined.

RESUME

L'expérience clinique et les travaux d'expérimentation ont montré que dans la croissance longitudinale des os, le taux de croissance peut être accéléré localement par certaines conditions inflammatoires, ainsi que par une stase veineuse ou des dérivations artério-veineuses. Dans le but de rechercher si cette stimulation est causée par *hypérémie artérielle* ou par *stase veineuse* passive, de jeunes lapins ont été exposés à aspirer de l'oxygène pur à une pression d'une ou 2 atm. abs. pendant 2 + 2 + 2 heures dans le même jour. Le taux de la croissance de la plaque de croissance tibiale proximale a été étudié par la méthode de l'oxytétracycline selon *Hansson*. La croissance journalière subséquente a été comparée avec celle des animaux de contrôle exposés à l'air aux mêmes pressions pendant les mêmes périodes. L'aspiration d'oxygène semble accélérer la croissance de 2 pour cent pendant la journée du traitement. Elle est suivie ensuite par une décélération de 7 pour cent le jour qui suit le traitement. Les résultats sont discutés, il est porté une appréciation sur le mécanisme en jeu et il est donné un aperçu sur la poursuite des travaux.

ZUSAMMENFASSUNG

Aus klinischen Erfahrungen und experimentellen Untersuchungen ist es gut bekannt, dass das Längenwachstum der Knochen durch gewisse endzündliche Zustände sowie durch venöse Stauung und arterio-venösen Shunt beschleunigt werden kann.

Um ausfindig zu machen, ob diese Anregung durch arterielle Hyperämie oder durch venöse Stauung verursacht sei wurden junge Kaninchen einer reinen Sauerstoffatmung unter dem Druck von einer und von zwei Atmosphären während 2 + 2 + 2 Stunden eines einzigen Tages exponiert. Die Wachstumsschnelligkeit der proximalen tibialen Epiphysenplatte wurde durch "Oxytetracyklin Labelling" ad mod. *Hansson* studiert.

Der beobachtete tägliche Knochenwuchs wurde mit dem Knochenwuchs von Vergleichstieren, die Luft des gleichen Druckes und während der gleichen Zeit exponiert worden waren, verglichen.

Die reine Sauerstoffatmung gab während des Tages der Behandlung eine Beschleunigung des Wachstums von 2 Prozent und eine Vezögerung des Wachstums von 7 Prozent am folgenden Tage.

Die Ergebnisse wurden diskutiert, der Mechanismus beurteilt und der Plan der zukünftigen Arbeit besprochen.

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