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From the Department of Orthopedic Surgery, University Hospital, Lund, Sweden
(Head: Professor Gunnar Wiberg), and the Institute of Physiology,
Laboratory of Aviation and Naval Medicine, University of Lund, Sweden.

GROWTH IN LENGTH OF BONES
IN CHANGE OF OXYGEN
AND CARBON DIOXIDE TENSIONS

BY

BJÖRN MAURITZ PERSSON

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Statistical adviser: Leif Pedersen, B.A.

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CHAPTER 1.

INTRODUCTION

This work is part of a research into the longitudinal growth of bones against the clinical background of leg length disparity in children. Hundreds of publications have appeared during the last century, describing different conditions resulting in stimulated or retarded growth in length. The clinical aspect of this problem was reviewed by *Goff* in 1960, citing 638 references on the topic. The acceleration of growth in length, reported during the last century, after fractures, osteomyelites or arterio-venous fistulae in limbs, has led to series of experimental efforts to induce increased growth rate for therapeutic equalizing of leg length disparity caused by one-sided stimulation, as mentioned, or by retardation after poliomyelitis, epiphyseal growth plate injuries or congenital defects. These experiments were recently reviewed and analysed by *Sundén* (1967) in a table listing about 120 papers. Periosteal stripping, curettage of the medullary cavity, implantation of a great number of different materials into the medullary cavity, plugging of the cavity, fracturing of bones, application of heat, electricity, roentgen radiation, ultrasound, shortwave diathermy, venous stasis, creation of femoral arterio-venous fistulae, immobilisation, nerve sectioning, nerve root sectioning, sympathectomy and mechanical stress and strain have all been tried. Summarizing these observations there is no doubt, that *direct* trauma to the growing long bones, whether caused by fracture, periosteal stripping or medullary plugging (*Hansson 1967*), does cause a certain longitudinal growth acceleration in a considerable percentage of cases, except when the epiphyseal growth plate itself is injured or the trauma is minimal. The same applies to chronic inflammation, whether caused by infection, rheumatoid arthritis (*Brattström 1963*), implantation of foreign materials or other necrotizing influences. Finally, *indirect* measures, such as arterio-venous fistulae and peripheral nerve divisions, have shown an enhancement of growth in length (*Sundén 1967*), while induced venous stasis has produced more diverging results.

Mechanism of overgrowth

Arterial hyperaemia

The underlying cause of longitudinal over-growth is mostly considered to be increased arterial blood flow in the growth plate regions (*Langenskiöld* 1957, *Hansson & Wiberg* 1963, *Vanderhoeft et al.* 1963). This should apply during fracture healing (*Levander* 1929) and after periosteal loosening of a diaphysis (*Ollier* 1867, *Brodin* 1955, *Elo* 1960) as well as after experimental interruption of the intramedullary vessels (*Ferguson* 1933). By blocking the medullary canal (*Cavadias & Trueta* 1965) this interruption is made more lasting and will cause a compensatory increase in blood flow to the metaphysis through vessels near the growth plate, thereby inducing an increased growth rate. Also when the femoral arterio-venous fistula was introduced as a measure for growth stimulation the effect was believed to be mediated through a hyperaemia distal to the fistula (*Janes & Musgrove* 1950). Later studies on the actual blood flow in these cases have, however, shown a decreased blood flow distally (*Holman* 1955). Registration of growth in length and of the blood flow in bone under the same circumstances was made by *Sundén* (1967). He demonstrated an increased growth after peripheral nerve division, measured by oxytetracycline labelling, and a simultaneous increase in bone blood flow, measured as heat clearance. This is in support of the hyperaemia theory, further strengthened by the fact that the same growth acceleration was achieved by immobilisation through Achilles tendon division in rabbits as well. Immobilisation in plaster of rabbit legs has been shown to cause an increase in pO₂ and pH of the intramedullary blood as well as an increased Sr⁸⁵-clearance (*Semb* 1966) which also favours the hyperaemia theory. The important rôle, played by the skeletal blood flow, has also led to successive attempts to stimulate growth in length by direct implantation of a patent artery into a long bone (*Dickerson* 1966).

Venous stasis

The major opposition to the theory of arterial hyperaemia has come from authors believing a venous stasis to be the causative agent in cases of one-sided over-growth in length. This should be the mechanism with trauma to a long bone, as well as in femoral arterio-venous fistulae and in venous stasis induced by tourniquet (*Hutchison & Burdeaux* 1954). Arterio-venous fistula is the most reliant stimulant of growth experimentally and clinically, generally leading to a more reliable increase in length than trauma and inflammation (*Broca* 1856, *Janes & Mugrove* 1950, *Cooley et al.* 1960, *Hierton* 1961, *Janes &*

Jennings 1961). *Birnstingl* (1962) reported 2.3 cm increase in the femur and 4.3 cm increase in the tibia in a case of a femoral fistula demonstrated by serial femoral angiography. He considered this dominating tibial growth acceleration to be supportive evidence for the hypothesis, that venous stasis, caused by the fistula, was the causative mechanism. This opinion seems to be shared by *Colt & Iger* (1963) and by *Keck & Kelly* (1964) who angiographically, demonstrated an arterial back-flow in the distal vein; they called this an "active venous stasis". The haemodynamics of arterio-venous fistulae have been subjected to several investigations (*Holman* 1955, *Henrie et al.* 1959, *Hillman et al.* 1959, *Stein et al.* 1959, *Ingebrigtsen et al.* 1963, *Weinman et al.* 1964). Venous stasis distally is a common finding although the collateral circulation, which is very pronounced, and the back-flow in the veins, both complicate the interpretation regarding effects on the growth plates. *Hillman et al.* (1959) conclude about induced stasis and arterio-venous fistulae: "venous stasis is the common denominator in these two conditions, which produce limb overgrowth and would seem to be related etiologically. The exact mechanism by which venous stasis produces limb over-growth is still unexplained". Venous stasis induced for therapeutic purposes, was first reported by *Helferich* (1887), who claimed to have reduced leg inequality in two children by prolonged tourniquet-stasis of the shorter legs. The method was repeated later in humans (*Bier* 1903) and in animal experiments (*Borel* 1922, *Kishikawa* 1936, *Peck* 1957, *Colt & Iger* 1963, *Keck & Kelly* 1964) with diverging results. In 1948 *Servelle* published a series of venographically verified unilateral, congenital, venous abnormalities with stasis in 14 humans, whose affected legs were 0.8—9.6 cm longer than the contralateral ones, while no differences could be found in any of 25 patients with chronic lymphoedema elephantiasis. *Hutchison & Burdeaux* (1954) are among those, who found induction of venous stasis to stimulate the growth rate. They express the opinion that venous stasis, is responsible for the over-growth, not only in arterio-venous fistulae, but also in fractures and osteomyelites. Attempts to measure the actual blood-flow in bone during venous stasis have been contradictory (*Shaw* 1964, *Walderrama & Trueta* 1965, *White & Stein* 1965).

Growth stimulating substances

No successful efforts seem to have been made to demonstrate the mechanism, by which a venous stasis is supposed to accelerate the growth. *Foster & Kirtley* (1959) believe that possibly the stasis supplies an increase of growth stimulating substances. This has also been proposed as a mechanism in other conditions with over-growth of bones (*Lacroix* 1947, *Wray & Goodman* 1961). Even when dealing with leg length inequality as here, one must not forget the possibility of

local liberation of stimulating substances of the same type as in cases of total body growth aberrations, that is hormonal and nutritional factors, but acting locally.

Pressure and tension

For completeness it is necessary to mention also mechanical loading and unloading as measures to influence growth of bones although this has very little application in this investigation. The temporary epiphyseodesis with staples according to Blount (*Blount & Clarke* 1949) is proof of the growth retarding possibilities using pressure, demonstrated previously by *Wegner* (1878) also using staples and wire cerclage. The pressure necessary to stop growth has been determined (*Strobino et al* 1952, *Blount & Zeiter* 1952). The possibility that growth can be accelerated by tension has not been demonstrated convincingly (*Smith & Cunningham* 1957).

Increase in temperature

The living cell and its enzymatic, oxidative systems, must have some optimal temperature and oxygen tension for the physiological processes to proceed normally. As over-growth can be induced, there might be a zone left on the side of the normal temperature or oxygen tension, where an increased turnover could take place. The temperature factor has been paid attention to in the literature. Supposing increase in temperature to be a common denominator for all conditions of over-growth mentioned above, *Granberry & Janes* (1963) tried to heat a growth plate in dogs by microwave diathermy but with no positive result. On the other hand *Doyle & Smart* (1963) observed increased growth in rats after short-wave diathermy, and so did *Ashoub* (1958) by housing mice for 4 weeks at two extreme temperatures, recording an increase in tail-length of 15 per cent in the warmer environment.

After creating arterio-venous fistulae *Janes & Musgroove* (1950) observed increased longitudinal bone growth in dogs and they recorded an increased medullary temperature in the femur of the operated legs compared to the unoperated. *Brodin* (1955) measured the intramedullary temperature in the tibia after one-sided periosteal loosening but observed a temperature variation within the cavity of such a magnitude that side comparisons were impossible. *Richards & Stofer* (1959) also tried to study the temperature factor. They say: "temperature and oxygen tension are probably the two most important physical variables

affecting living cells. Physical variation of either or both of these might influence the rate of growth of bone". They observed an increased growth in length in both dogs and rats using an electrically heated sling around the femora, but unfortunately the method produced such a necrotizing effect that even fractures resulted. Heating was attempted also by *Ring & Lee* (1958) using carbon resistors implanted into the bony epiphysis of the ulna in rabbits but they induced only increase in width.

The importance of the temperature factor has thus been observed but no conclusive results have been presented as to the possibilities of using it for growth rate alterations.

The oxygen factor

Concerning the possibilities of inducing changes in the growth of long bones by alteration of the oxygen tension no reports can be found in the literature. In spite of this many authors mention increase in oxygen availability as a probable factor in observed accelerations in bone growth. Since the discovery of oxygen by *Priestley* in 1774 much has been written regarding oxygen in medicine. This literature has been reviewed by *Bean* (1945, 1964) and with regard to the use of oxygen at increased barometric pressure also by *Linehan* (1964) and by *Jacobson et al.* (1965). The fundamental importance of oxygen in general has even led to trials of injection of gaseous oxygen in arteries in conditions of ischaemia (*Fey & Boxberg* 1956) as well as injection of diluted hydrogen peroxide but not in connection with growth studies.

Tissue culturing technics have been employed to study oxygen effects on growth in general; *Cooper et al.* (1958) studied the growth of animal cells and virus, *Ollodart & Blair* (1965) demonstrated increased growth of bacterias in oxygen tensions up to 1 atm.abs., (atmosphere absolute=760 mm Hg) and bacteriostasis on further increase of the oxygen tension. *Nelsen* (1958) studied the early development of chick and frog embryos and recorded some stimulation in certain stages. *Goldhaber* (1958) observed increased osteoclastic activity of cultured bone at 95 per cent oxygen in the gaseous phase at 1 atm.abs., and pointed out the similarity to the effects caused by parathyreoid hormone. *Vaes & Nicols* (1962) found increased metabolism in rat bone cultures at increased oxygen tension. *Shaw & Basset* (1967) cultured chick embryo tibiae under different oxygen concentrations in the gaseous phase and found maximal osteogenesis at 35 per cent, but at 5 per cent and at 95 per cent of oxygen there was a pronounced decrease in osteogenesis and chondrogenesis.

The effects of increased oxygen tension on healing of standardized surgical wounds have been studied by *Lundgren & Sandberg* (1965) reporting decreased healing in normal rats and acceleration in anemic rats during hyperbaric

oxygen therapy at intervals, but in dogs augmented healing has been reported (Beckham & Hitchcock 1965). Studying the survival of split skin grafts in 48 humans Perrins (1967) recorded an increase in the taken area of 29 per cent and an increase in complete graft taking from 17 to 64 per cent after hyperbaric oxygenation at 2 atm.abs. of pure oxygen breathing for two hours twice daily during three days after operation.

Also in the case of fracture healing oxygen has lately aroused interest. Coulson et al. (1966) report accelerated healing in femoral fractures of the rat at hyperbaric oxygenation in intervals for 1—3 weeks, tested by mechanical resistance and radioactive calcium activity. This finding is complemented by Makley et al. (1967) observing impaired healing of fractures in rats at reduced barometric pressure corresponding to 18 000 feet of altitude. Periosteal proliferation in mice has been studied by Manspeizer & Tonna (1967) who found decreased proliferation at 5 per cent oxygen and increased at 100 per cent oxygen in the environment measured by tritiated thymidine and autoradiography. A search of the literature has actually revealed one work mentioning also growth in length and the influence of increased oxygen tension and that is Wright & Howard-Flanders (1957) investigating the growth in length of mice-tails after roentgen radiation combined with 100 per cent of oxygen in an environment of atmospheric pressure varying from 0.1 to 3.0 atm.abs.. They found radiosensitivity decreased during hypoxia and increased during hyperoxia as measured by the length of the tails. In a control group they varied the oxygen tension without radiation and did not observe any effects on the length of the tails. The data given and the short period of treatment do not allow any conclusions to be drawn concerning the unplanned observations on the relationship between longitudinal growth of bones and the environmental partial pressure of oxygen.

Purpose of investigation

The purpose of this investigation can now be advanced in the light of what is said above, concerning factors influencing longitudinal bone growth, especially the oxygen factor. In the literature on over-growth of bones increase in the oxygen tension is almost regularly mentioned as a hypothetic cause of the growth stimulation, but no experimental efforts to prove this have appeared. Preliminary observations on change in the longitudinal growth of the proximal tibial metaphysis of rabbits during hyperbaric oxygenation for repeated short intervals during a single day have been published earlier (Persson 1967) and the aim has been to extend the study for more exact documentation of the effects and for evaluation of such secondary consequences, as oxygen toxicity

and carbon dioxide changes, which have to be considered during change in the environmental oxygen tension. Regarding the controversial opinions about hyperaemia or venous stasis as the responsible factor for accelerated growth in length it has been considered to be of great interest to see, whether a direct alteration in the respiratory oxygen tension can induce a change in the growth rate, especially as the oxygen factor itself, on the cellular plane, must be of a key importance, not earlier studied in this respect. Except for the oedema promoting tendency a major character of venous stasis compared to arterial hyperaemia is probably the lower tissue tension of oxygen and higher tension of carbon dioxide and consequently a study of the influence of these gases on growth could shed some light on the problem of overgrowth of bones, increasing our knowledge about oxygen therapy in general and about the physiology of the growing epiphyseal plate in particular.

MATERIAL AND METHODS

Choice of animal

Because of the toxicity of pure oxygen inhaled for an extended period it was evident that the method chosen, should allow determination of growth rates with a high accuracy over very short periods of time, that is about one day. Therefore the intravital tetracycline labelling method seemed to be the best for quantitative measurements (see later). Concerning the choice of animal it was clear that it had to be some relatively fast growing type to allow observations on changes during the short times made possible by the oxygen toxicity and secondly, it had to be easily available for the comparatively long series necessary, in a study dealing with comparisons between different animals and not as hitherto for the above mentioned method, between the right and left sides. These conditions allow for the use of rabbits, rats, mice and guinea-pigs, but as the method hitherto had been used for longitudinal growth estimations mainly in rabbits, (*Hulth & Olerud 1962, Hansson 1964 and 1967, Sundén 1967*) this favoured the choice of rabbits, especially as they had often been used in bone growth investigations in general, as well as in the literature on oxygen toxicity and breathing physiology (*Dittmer & Grebe 1958*). The rabbits used were always white to make the intravenous injections easier in the young animals used, aged between 27 and 42 days at the day of treatment. The age was chosen because of the easier preparation and the greater growth rate during this time (*Hansson 1967*). The sex of the animal was not taken into account (*Hansson 1967*).

Care of animals

The general care of animals was the same in test and control as follows; Rabbits were bought from three breeders, who had noted the day of birth, and were transported to the hutches with their mothers. They lived together for a

couple of days before the experiments. The hutches were in-door with daylight, with electric light added during winter between about 07.00 and 17.00. The temperature in the hutches was about 18—23°C. Humidity was not specially controlled. The rabbits were fed on pellets and water ad libitum and by milk from their mothers as long as they suckled them. During the 8 hrs. of treatment (see later) both test and control rabbits were taken away from the mothers, but were given pellets and water in the treatment boxes. They were weighed on the first and on the last day of the experiment.

After the choice of rabbits to be used for the study, one had to consider which aberrant animals to exclude during the experiments.

Exclusion of animals

Among young rabbits at the time of weaning a considerable percentage will die or be temporarily diseased. Further more, some of them will be regaining health during the time of growth measurements, and this will give an increased deviation in the figures of growth, making necessary very long series, unless these factors could be reduced in a way, not influencing the reliability of the effects to be studied. This problem is of special importance when growth rate investigations are performed for studying influences on the whole rabbit, comparing different individuals, in comparison to right and left side studies in the same animal. This is consequently an added difficulty but the method used in this way allows for increased possibilities. The preliminary studies (Persson 1967) had shown that some principles had to be introduced for exclusion of unhealthy animals, because even grossly undetectable diseases in the rabbits could severely affect the growth rates recorded. It was thought unsatisfactory in this investigation to exclude animals below a certain weight (Hansson 1967), as different breeders could have different standard weights at a certain age because of genetic or nutritional factors. It was also thought insufficient to exclude animals because of clinical impression of disease (Hansson 1967, Sundén 1967), and after the preliminary experiences the following conditions for excluding animals were adopted:

1. Spontaneous death during the experiment (only complete observation series were used).
2. Loss of weight between the start and the end of the experiment (precision of the instrument ± 5 grams).
3. Diarroe observed during the experiment.
4. Unacceptable quality of preparations for microscopy (allowing for less than 5 readings).

5. Disease made evident by abnormal growth rate, defined as being more than 100 microns below the arithmetic mean of litter-mates exposed to the same treatment (observed during any of the three 24-hrs. periods measured).
6. Animal left single in a litter after exclusions above.

(For definitions of preparations, readings and growth periods see later in this chapter).

These six principles have been followed throughout the investigation with the following comments. Regarding point 1, there are no exceptions; complete readings were made impossible by the death of the animal. Point 2 has proved to be well founded on the fact that decreased growth rate was practically always observed in animals with decrease in weight and after some time these animals were not prepared for microscopy at all. Point 3 was used in the way that animals being soiled by their diarrhoea, were excluded. Regarding, point 4, 30 animals were accepted in the beginning of the investigation with less than five readings, but only in cases where no difference between consecutive time periods were recorded. With regard to point 5 finally, 2 animals were accepted, also in the beginning and in cases where the difference between consecutive periods was not more than 10 microns, thereby negligibly affecting the calculations. A full account of excluded animals is given in Table 1. Each animal is given with its identification number, the last letter plus the following roman figures designating the number within its litter and the first one or two letters designating the litter. This allows one in reading the tables, given in the results, to identify the origin of the excluded animals. The total number of excluded animals is 148, but this should be compared to the total number of animals before this exclusion, that is 1079, giving a reasonable percentage of exclusion for rabbits at this age. In all 13 animals died spontaneously after registration at the institution but before any treatment, and are therefore not listed in Table 1. The distribution of animals given in Table 1 further shows, that spontaneous deaths were more common among test animals, of which 4 died during administration of the maximal doses of oxygen and 4 while given the minimum doses. This is further discussed later. In the group of unacceptable preparations there are also more test animals but the distribution is even over the whole material comprising 532 tests and 399 controls. In the other groups there is no remarkable difference between the number of test and control animals excluded.

From the studies of *Hansson* (1967) it was known that there was a successive decrease in growth rate after the age of 30 days (Fig. 15) but no correlation between weight and growth within each litter. It was therefore considered to be of interest to see how the selection principles, used in this study, influenced on a possible correlation between these factors. There was a considerable number of control animals with notes on age, weight and growth in length of the proximal tibial metaphysis measured on the same day as they were weighed. In all 237 animals were grouped according to age and analysed regarding a correlation between growth and weight at the given age. It turned out to be a

Table 1.
Specification of animals excluded during the investigation. The groups are in accordance with the list of principles for exclusions given in text.

1. Spontaneous death		2. Loss of weight		3. Diarrhoea		4. Unacceptable preparations		5. Abnormal growth		6. Single animal	
Test	Contr	Test	Contr	Test	Contr	Test	Contr	Test	Contr	Test	Contr
QH III	FGH III	EVV III	FRH III	CIH II	CIV II	FRH II	VV IV	EEV III	OV I	NH V	CZH III
QH IV	CAV III	GXV I	FRV III	CIV I	CLH III	EUH I	GUH I	FGH I	NH IV	DBH III	BVV I
VH I	DDH II	EMV V	DYV III	DOV I	CTV I	GTV III	EEV I	GCV V	EKH I	BRV I	
VH III		CSH I	EEV II	DRH IV	DOV III	GUH IV	CKH IV	DEH III	DOH IV		
CSV I		EBV I	CIH IV	FDH V	DPV II	EEH III	CKV I	DFH I	DBH I		
CSV II		CZV III	CIV IV	CZH IV	DRH I	CQH III	ENV IV	HFH II	DBV II		
CTH III		DKV V	EAV I	CZH II	DRH III	CRV II	EPH II	EDV IV	DIV V		
CTV III		DIV II	ECV I	CZV I	CZV II	CKH I	FZH I	EEV IV	GCH V		
DPH I		GDH II	CZH I	DBH II	DKV IV	CKV II	ERH IV	EMV III	FZV I		
DBV I		BRV II	CZV IV	GAH II	GEH II	DIH III	BRH IV	FVH I	HQH V		
DKV III		CXH IV	DKV I	BRH II	FVV IV	GEH I		FZV III	HQV IV		
GDV II		DGV III	ENV III	BVH II	FUH VI	ENH III			BTV III		
ECH II		BVH II	FVH V	BVH I	ENV V	ENV V					
		CAV I	FVV III	DEV I	GDV III	GDV III					
			ESV I		FVV V	FVV V					
			BRH III		FVH IV	FVH IV					
			DGH I		FXH I	FXH I					
			DGH III		FXH II	FXH II					
			DCV I		ERH I	ERH I					
					ESV III	ESV III					
					BRV III	BRV III					
					DDH III	DDH III					

positive correlation, which was highly significant in three age-groups and insignificant in eight age-groups. When the groups were put together as listed below in 5-day groups it was significant in all the groups, implying a greater daily growth in length at a higher weight.

Age in days	Number	r-value	t-value
26—30	116	0.39	4.53 × × ×
31—35	93	0.32	3.21 × ×
36—40	28	0.78	6.32 × × ×
total	237	0.17	2.64 × ×

It is notable that the significance of correlation in the total material is smaller than in any of the age-groups, which means that a negative factor increases by the fact that an older animal grows less than a younger, after the age of 30 days (Fig. 15). It was assumed that the day of birth was not noted by the breeder with a higher accuracy than one or two days. This made it reasonable to use the 5-day groups above. The demonstrated correlation between weight and growth in this material was not of consequence in the present study, because this was based on differences in growth between consecutive days in the same animal, and with littermates in test and control groups. These were made with an approximately even distribution of weights.

Method for measuring of growth

The reasons for selecting the intravital oxytetracycline labelling method for this investigation are mentioned in the beginning of this chapter. Since the discovery by *André* (1956) that tetracyclines are deposited in bone tissue this has been further studied by *Milch et al.* (1958). The method was used for lamellar bone growth measurements only (see *Hansson* 1967) until it was shown that tetracyclines were deposited in the border between the metaphysis and the growth plate, marking the endochondral longitudinal bone growth as well (*Hulth & Olerud* 1962). These possibilities were developed and used by *Hansson* (1964 and 1967) also reviewing the earlier literature. In this investigation the oxytetracycline technic has been used as described below, according to *Hansson*.

The rabbits were given oxytetracycline (Terramycin®, Pfitzer) intravenously in a marginal ear vein 4 times each, at intervals of 24 hrs. ± 5 minutes. In a smaller part of the investigation the injections were given with intervals of 12 hrs., ± 2.5 minutes. These four injections allowed the determination of

longitudinal growth during three consecutive time periods. Further injections had shown an increasing indefiniteness of line identification in the microscopical sections because of reabsorption and remodelling of the bone trabeculae of the metaphysis (Fig. 1). The doses of oxytetracycline was 1.0—1.5 mg/kg body-weight, being without toxic effect on growth in length (Hansson 1967). The gas exposures were made in defined time relation to the oxytetracycline injections (Fig. 2 and 3). Within half an hour after the fourth injection the animals were killed by intravenous injection of Evipan® (Bayer), about 100 mg/kg body-weight. The left, proximal tibia was removed; chosen because of its high growth rate in combination with the comparatively flat shape of its epiphyseal growth plate (Hansson 1967). For this investigation of changes in growth during exposure

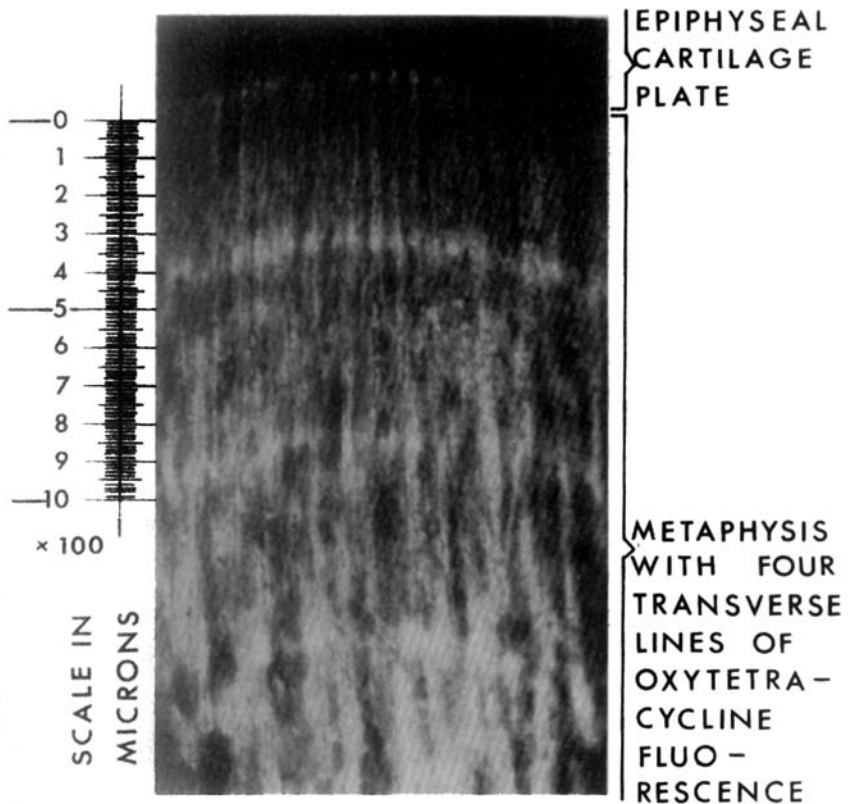


Fig. 1.
Proximal tibial metaphysis of growing rabbit. Microphotograph in ultraviolet fluorescence. Oxytetracycline labelling of growth at intervals of 24 hrs. Measuring scale showing the magnification.

of the whole animal to alteration of respiratory gas tensions no important advantage could be expected by utilizing more than one epiphyseal growth region. The piece of bone was fixed in concentrated ethanolum (99.5 per cent) for 12—72 hrs.. The bony epiphysis was then horizontally divided near the growth plate and the diaphysis was sawed off as well about 5 mm distal to the plate. The resulting piece was sagittally cut in one lateral and one medial half, of which the lateral was used for sectioning. Parallel to the metaphyseal trabeculae seen in the sagittal cut surface the anterior quarter was cut off with a razor-blade knife and the posterior quarter was used. In the new surface, the cut was again controlled or adjusted parallel to the planes of the trabeculae of the metaphysis on both the surfaces exposed. Further cutting of bone sections with the razor-blade knife were made parallel to the trabecular system. The cutting was then made in the sagittal plane and about 10 sections were taken and put into Xylol for 5—10 minutes. The thickness was occasionally checked and ranged between 60—120 microns as measured with a micrometer screw-gauge. Between 6 and 10 sections were then mounted in liquid DePeX (Gurr) on glass and covered with glass. After drying in darkness the sections were examined in an ultraviolet (mercury lamp OSRAM HBO 200 W) fluorescence microscope (Zeiss) with a dark-field condenser (NA 0.65/0.85) and measured with a calibrated ocular (12.5 \times), the objective being 10 \times . The primary filters used were BG 12/4+BG 38/2.5 and the secondary filter 53. With this equipment the longitudinal growth rate was measured as three distances between the metaphyseal borders of the four lines, in such a way that the readings were always made with the measuring scale projected along unbroken bone trabeculae in positions, where they were nearly at right angles to the growth cartilage plate (Fig. 1). The error of this method has been determined earlier by side comparisons (Hansson 1967, Persson 1967, Sundén 1967). One or two readings were made on each section, and all together about 5—10 readings were made. The arithmetic mean number of readings in each animal was 7.3 in the entire material. To be accepted, readings should be possible along the same trabecula for all the three consecutive time periods used. For the further calculations the arithmetic means of these readings were used, giving in microns the longitudinal growth rates of the proximal tibial metaphysis during three consecutive periods of time. The first period, before the gas treatment, gave one control value, to which the following growth changes could be referred. A second type of growth control value was achieved by using about half of each litter as a reference group, simultaneously treated with air only. This gave a measure of possible growth effects caused by stress factors from change in housing and handling during the day of gas treatment, and secondly, it allowed for possibilities of "double blind" readings during microscopy, with decodification of the identification numbers on the glasses made after the measuring.

Statistical methods

The statistical analysis was based on differences in growth between each of the three consecutive time periods measured in the same animal. In the group of animals, where pure oxygen was tested, it was specially analysed whether the statistical result was influenced by the fact that rounded figures were used in this investigation. The mean value of the readings in each animal at each time period was rounded off, 466 was put to 470 for instance. This was done because the measuring stab of the microscope did give only two figures (Fig. 1). The analysis demonstrated that the statistical significances were uninfluenced by this measure. For each step of the investigation about 10—20 animals were exposed identically with a certain test gas and about the same number of animals to air as a control. In this way it was possible to follow the changes in growth within each animal after a certain exposure and after the air exposure and then to compare the differences of effects in the two samples. In the bottom of each table, given in the chapters on results, the arithmetic mean of growth in each time period is given, as well as the mean difference between two consecutive time periods, the standard deviation of these differences, the t-value with degrees of significance (Student's test), and the n-value.

$$\text{standard deviation} = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (X_i - m)^2}$$

$$t\text{-value} = \frac{m_d - \mu_d}{\frac{S_d}{\sqrt{n}}} \quad \text{with } n-1 \text{ degrees of freedom.}$$

The two sided alternative hypothesis was used in the Student's test as the direction of a possible change in growth could not be predicted. The test within the the material of each table is given in its bottom, but the tests between control groups and the test-gas groups, that is between two different tables, are given in the corresponding text in each chapter. For this comparison the following test function was used, again with a two-sided alternative hypothesis.

$$t = \frac{m_x - m_y - (\mu_x - \mu_y)}{S \sqrt{\frac{1}{n_x} + \frac{1}{n_y}}} \quad \text{with } n_x + n_y - 2 \text{ degrees of freedom}$$

It was tested that the variables of the samples were normally or approximately normally distributed, which is required for the test. It is also necessary that

the standard deviations of the populations are the same, which was tested by the function $F = \frac{S_x^2}{S_y^2}$ with $n_x - 1$ respectively $n_y - 1$ degrees of freedom. In some parts of the material this was not the case and then a non parametric test was used, namely Wilcoxon's rank-sum test (T), or Mann-Whitney's test (U). In these cases T or U is written in the corresponding text.

In the interpretation of the results conventional probability levels have been used with the following symbols written:

—	$p > 0.05$	(not significant)
×	$0.01 < p < 0.05$	(almost significant)
××	$0.001 < p < 0.01$	(significant)
×××	$p < 0.001$	(highly significant)

Abbreviations:

$\sum_{i=1}^n X_i$	=the sum of the values of the observations (from 1 to n)
n	=the number of observations
x	=the individual observation in one time period
y	=the observation in the following time period of the same individual
x_i	=the series of individual observations from 1 to n
m	=the arithmetic mean of the observations
m_d	=the arithmetic mean of differences $x - y$ in the sample
μ_d	=the arithmetic mean in the population of $x - y$
S_d	=standard deviation of the sample of the difference $x - y$
m_x	=the arithmetic mean of the sample for the variable x
m_y	=the arithmetic mean of the sample for the variable y
μ_x	=the arithmetic mean of the population for the variable x
μ_y	=the arithmetic mean of the population for the variable y
n_x	=the number of observations in the sample for the variable x
n_y	=the number of observations in the sample for the variable y
S_x	=standard deviation of the sample for the variable x
S_y	=standard deviation of the sample for the variable y
S	=the weighed standard deviation of the samples for the variables x and y
t	=t-distribution according to Student
F	=F-distribution (Dixon & Massey, 1957)
T	=the rank sum according to Wilcoxon's test (—, —)
U	=value in the U-test according to Mann-Whitney (—, —)
p	=probability level

Method for change of gas tensions

With the aim of analysing the effects on growth in length during change in oxygen and carbon dioxide tensions, a method was preferred, which was not immobilizing and not traumatic to the bone and simple enough for the long series necessary. The most convenient seemed to be to house the animals for a defined period in chambers, where they could breathe the gases to be tested.

When the animals were to be exposed to the different breathing gases they were placed in either of two boxes, one for the gas to be tested and one for the air as control. Five to ten animals could be housed in each of the boxes, measuring $60 \times 35 \times 35$ cm. The litters were divided between the boxes as listed in the tables of the following chapters. The gases were supplied to the boxes through rubber tubes from standard gas cylinders. The gas cylinders were delivered by Syrgas AB Alfax, Malmö, Sweden, with the gases purified for medical use. The gas mixtures were made with an accuracy of ± 0.3 per cent and tested by the manufacturer (Orsat — analysis). On three occasions, as an additional control, it was also checked at the Laboratory of Aviation and Naval Medicine, Institute of Physiology. In a Scholander apparatus the deviations from the ordered concentrations were not more than 0.1—0.2 per cent. This department also delivered the cylinders with compressed air for the controls. At the Pressure Chamber Laboratory of this institute the gas exposures were also carried out. The boxes, housing the animals, were placed in the hyper- or hypobaric chamber, where the desired pressure was reached in a couple of minutes. Thus there were two methods of changing the gas tensions, that is by change in gas concentrations of the cylinders and by variation in the barometric pressure of the chambers. The gas cylinders had a volume of 40 or 50 liters and a starting pressure between 150 and 200 kp/cm². By calculating the volume and the change in pressure it was possible to estimate the flow from the cylinders to the boxes. The flow was controlled by means of an orifice, through which gas was supplied at adjustable feeding pressure from the reducing valve (Aga, Sweden) on the cylinder. This flow had been calibrated with a Douglas bag in the pressure chamber at the actual working pressures (manometers: NAF, Sweden), and the volume was later measured in a standard wet gas meter outside the chamber. These precautions about the flow rate of gases were taken because it was necessary to use a flow rate big enough to wash out the carbon dioxide produced by the animals during the 8 hrs. of treatment. The flow was let out of the boxes after passage, through holes in the lid. The ventilation needed was calculated to be 400 liters/kg bodyweight of animals per hour as follows. The maximal CO₂-concentration, which could be tolerated as harmless was supposed to be equivalent to 0.5 per cent at 1 atm.abs. i.e. 0.25 per cent at 2 atm.abs.. The production of CO₂ is not more than equal to the consumption of oxygen, that is about 0.8 liters/kg/hr. (*Dittmer & Grebe 1958*). This was rounded to

1.0 liter/kg/hr. at 1 atm.abs.. If the CO₂ production was 1.0 liter/kg/hr. and 0.25 per cent could be tolerated the flow must be 400 liters/kg/hr., i.e. 6.7 liters/min. per kg of animal. The actual flow used was between 400 and 800 liters/kg/hr. checked continuously as mentioned above. On two occasions it was also checked by Scholander analysis of the gas, leaving the boxes. The calculations were verified. This flow through the boxes also gave a homogenisation of gases within the boxes, further made certain by an extra increase in flow at the beginning of each exposure. Finally the flow prevented an increase of the temperature of the boxes because of the gas temperature decrease during the gas expansion. The temperature was occasionally recorded and found to be between 20 and 26°C within the boxes. The humidity of the air from the cylinders was close to 0 but the animals were given water freely in an open cup. Temperature, humidity, light, draught and noise were equal in the test and control hutches. The lids of the hutches were transparent allowing free inspection through the windows of the pressure chamber. Further details on concentrations and pressures of the tested gases are given in the chapters on results.

Periods of time for change in gas tensions and for measuring of growth

The oxygen exposures should be short enough to avoid the oxygen toxicity, which is proportional both to the time and the partial pressure (*Bean* 1945). At the same time the exposures had to be long enough to allow an accurate calculation of the growth in length. The method chosen allows labelling with 12 hrs. intervals (*Persson* 1968). Shorter intervals seem to lead to increased difficulties in separation of the line frontiers in the microscopic sections. Thus the periods of growth labelling should not be shorter than 12 hrs., but to allow sufficient time for transport of the animals to and from the place of treatment, and time for the intravenous injections as well, 8 hrs. of treatment was considered to be convenient. A longer time would have limited the possible pressure of oxygen used, because of toxicity. This toxicity is of two major types. One acute form of toxicity affects the central nervous system (*Bert* 1878) and is seen at higher tensions of oxygen, that is about 3 atm.abs. and more. The second type is the pulmonary toxicity (*Smith* 1899), which can be elicited at oxygen pressures over 0.6 atm.abs. during extended treatments (*Barach* 1926). With increasing partial pressures of oxygen the time for this lung damage to evolve is progressively decreased. These problems are well known in humans and experimental animals (*Bean* 1945, 1964, *Gerschman* et al. 1964) and hold for the rabbit as well (*Binger* et al 1927). This reduces the possible advantage of using longer periods of exposure and consequently it was decided in this investigation

to use a time of treatment of 8 hrs.. Treatment given in intervals for instance of 2+2+2 hrs. as in clinical praxis, reduces toxicity but has the disadvantage of masking effects in case of diphasic responses (Persson 1967) and was therefore not used in this investigation. The treatment was used only once in each animal, because a repetition for several days would have led to another two important draw-backs, that is acclimatisation effects on haematopoiesis (Altland & Highman 1952, Brooksby et al. 1966), and secondly it would have reduced the possibilities of analysing the time lags between the treatments and the changes in growth rate.

The relation between the time periods and the 8 hrs. of treatment used in this investigation is illustrated in Fig. 2 and Fig. 3 for the 24 and the 12 hrs. systems used respectively. As mentioned earlier only three consecutive periods of growth were measured in each animal. The maturing time of the cartilage cells, from the layer of the reserve cells to the layer of degenerating and calcifying cells, has been determined to be 2—4 days (Messier & Leblond 1960, Kember 1960) and it was evidently necessary to observe the possible effects on growth for 4—5 days after the treatment. This problem was solved by using three consecutive series of animals, overlapping each other with one day for the coupling together, making possible analysis of changes in growth for an extended time. This is also illustrated in Fig. 2. Figures 2 and 3 also illustrate that the gas treatment was given almost until the end of the period 0. The time lag between the end of treatment and the following injection for tetracycline labelling of growth was less than 1 hr. in series with 24 hrs. periods, and half an hour in series with 12 hrs. periods. The reason for this was, that there could be expected to be some withdrawal effects after the treatments. In this way it was possible to localize immediate effects and immediate withdrawal effects on different sides of the labelling lines, and they were not allowed to compensate

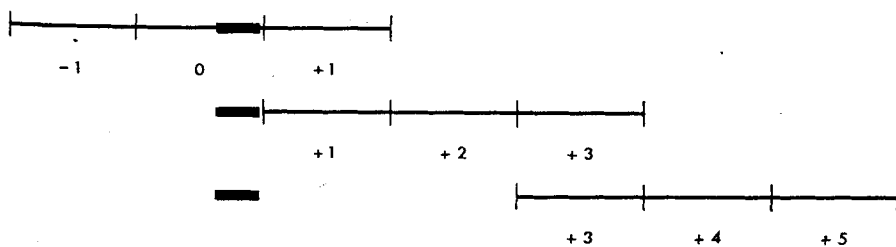


Figure 2.

The time relation between the three periods of 24 hrs. used for the longitudinal growth measurements in each animal, and the 8 hrs. of treatment given during the period named 0, and the overlapping in time between each series, allowing 7 consecutive periods of 24 hrs. to be compared.

| = time for injection of oxytetracycline
 ■ = time of exposure to test gases

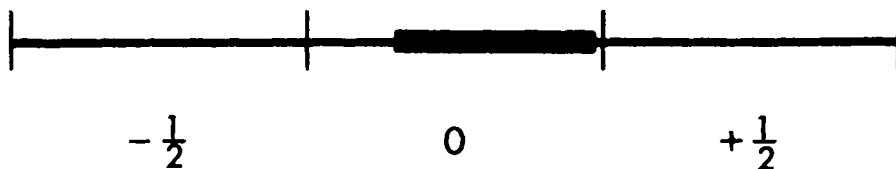


Figure 3.

The time relation between the three periods of 12 hrs. used for the longitudinal growth measurements in each animal, and the 8 hrs. of treatment given during the period named 0. The other periods are named $-\frac{1}{2}$ and $+\frac{1}{2}$ respectively to distinguish from other series, where periods of 24 hrs. were used.

| = time for injection of oxytetracycline
 ■ = time of exposure to test gases

each other within the same period in case of diphasic effects on the growth rate (Persson 1967). The reasons given in this text for not using more than 8 hrs. of treatment do not apply to later testing of decreased oxygen and increased carbon dioxide tensions, as the toxicity factors are not at all the same. Anyhow it was decided to use the same system of periods to allow for better possibilities for comparisons of the sensitivity of growth to the different test gases tried.

It was considered to be unimportant at which time in the day the injections were given as long as it was at the same time each day. For practical reasons they were given between 16.30 and 17.00 with the numbered animals in a constant order. The ordinary time for injection was less than one minute for each animal and each litter was begun with at a constant time each day, making the time factor equal in tests and controls. This holds for the entire material where periods of 24 hrs. were used. When periods of 12 hrs. were used the treatment was started at 11.00 and ended at 19.00 and the injections were given before 19.30, thereby marking the end of period 0. On the next morning the last injection of tetracycline was given before 07.30, that is after another 12 hrs. exactly for each animal, again with the animals in a constant order. In this case the time chosen for injection could be of some importance, if there were a diurnal variation in growth rate, as suggested by Simmons & Nichols (1966). This possibility has been separately studied (Persson 1968) but no significant variation was found. However, as long as the investigation is carried out as a comparison between tests and controls, this possible factor is equalized.

Physical and Physiological data

As a background to the description of the results obtained, some basic data from respiratory physiology will be recapitulated. Air contains 20.95 vol. per cent of oxygen, 79.02 vol. per cent of nitrogen and 0.03 vol. per cent of carbon dioxide

(Dittmer & Grebe 1958). The percentage of nitrogen given includes 0.95 vol. per cent of inert gases. At a barometric pressure of 760 mm Hg in dry air this means an oxygen partial pressure of 158 mm Hg. Inspired into the alveoli the gas tension of oxygen has been reduced to just a little above 100 mm Hg because of the occurrence of water vapor and carbon dioxide, being about 47 and 40 mm Hg respectively. From the alveolar air, oxygen diffuses readily over to blood. In the capillary blood of the lungs, pO_2 is close to the alveolar gas tension, but it will be reduced by venous admixture corresponding to the ventilation/perfusion ratio and the resulting arterial oxygen tension is about 94 mm Hg. In the peripheral capillary beds this oxygen tension is reduced to 40 mm Hg in the venous blood returning to the lungs. In blood, oxygen is carried in two ways, i.e. combined with haemoglobin, and in physical solution. The special affinity of oxygen for haemoglobin makes the arterial saturation to be about 97 per cent at the normal pO_2 of 94 mm Hg. Each gram of haemoglobin can combine with 1.34 ml of oxygen and if the normal haemoglobin content is 15 g per cent this means that the haemoglobin in blood carries about 20 ml of oxygen per 100 ml blood under normal circumstances. Meanwhile the amount of oxygen in physical solution is about 0.3 ml/100 ml. As this physical solution has a linear relation to the gas tension in the gaseous phase, it means that giving 100 per cent of oxygen in the respiratory air the pO_2 in the arterial blood will be about 673 mm Hg, increasing the oxygen content by 2.2 vol. per cent, of which 1.7 vol. per cent is in physical solution (Linehan 1964). This means that an increase in tension of oxygen by 6.7 times increases the carrying capacity about 11 per cent. This moderate increase in capacity is, however, combined with a considerable increase in the diffusion pressure. The cited figures hold approximately for the rabbit as well (Chapter 7).

When the environmental barometric pressure is changed by means of a pressure chamber (Chapter 3) the gas tension of oxygen will be altered simultaneously. If the pressure is raised to 2 atm.abs., (atm.abs.=atmospheres absolute=760 mm Hg), that is 1520 mm Hg, the oxygen tension in air will be about 304 mm Hg. If the animals are breathing pure and dry oxygen at the same pressure, the oxygen partial pressure will be 1520 mm Hg and the oxygen content in the blood will be increased from about 20 ml/100 ml to about 25.2 ml/100 ml.

In Chapter 4 the results will be given of decreased oxygen tensions by the means of a hypobaric chamber, that is a pressure tank, where the barometric pressure can be reduced by vacuum pumps. The same principles applies to this, that is for instance, if the pressure is reduced to half, that is, from 760 to 380 mm Hg, the oxygen partial pressure will be reduced from 158 to 79 mm Hg if there is no water vapor. At 6000 meters altitude the barometric pressure is about 354 mm Hg, the oxygen partial pressure about 76 mm Hg, and the alveolar oxygen tension about 35 mm Hg, the alveolar carbon dioxide tension finally

is about 30 mm Hg, compared to 40 at sea level (*Luft* 1961). This oxygen tension corresponds to an oxygen saturation of the haemoglobin of about 68 per cent, which means that the oxygen carrying capacity per 100 ml of blood has been reduced from 20 to about 13.6 vol. per cent. The oxygen lack causes a hyperventilation, which reduces the alveolar carbon dioxide tension. At 5000 meters the alveolar oxygen tension, still according to *Luft* (1961), is about 42 mm Hg, at 4000 meters it is about 51 mm Hg, at 3000 meters it is about 62 mm Hg and the alveolar carbon dioxide tension is then about 36 mm Hg.

In Chapter 6 the investigation of increased carbon dioxide tension in air will be described. When the normal carbon dioxide tension is raised from about 0.04 vol. per cent to 3 vol. per cent, the alveolar carbon dioxide tension increases from about 45.5 to 47.5 mm Hg. At 5 vol. per cent of carbon dioxide in air the alveolar tension is about 49.5 mm Hg, and at 7 vol. per cent finally, the alveolar carbon dioxide tension is about 58 mm Hg in humans (*Barcroft & Margaria* 1931, *Patterson et al.* 1955). At this carbon dioxide tension the ventilation rate had increased from about 12 to about 80 liters/min. and it was about the highest carbon dioxide tension that could be tolerated by the healthy test subjects. After 18 hrs. in about 4 per cent carbon dioxide in air the ventilation is still about 200 per cent of the normal (*Dittmer & Grebe* 1958). After 15—20 min. of 3.5 per cent carbon dioxide breathing in 16 humans the jugular venous pH in blood had decreased from a mean of 7.35 to 7.32 and the arterial pH had decreased from 7.39 to 7.35 (*Patterson et al.* 1955).

After this short presentation of some basic figures, illustrating important principles from the respiratory physiology, the results, obtained by testing the effects on longitudinal bone growth from short time alterations of respiratory gases as specified later, will be described.

CHAPTER 3.

RESULTS OF INCREASED OXYGEN TENSIONS

The preliminary studies of the effects on growth during increased respiratory oxygen tension for 2+2+2 hrs. with 100 per cent oxygen at 2 atm.abs. during one day, earlier referred to, had indicated an increased growth in length during the exposure, followed by a depression the day after. On the basis of these results the following questions were considered for further studies:

Does omitting of the pauses in the intermittent oxygen exposure disclose a growth acceleration of significant magnitude, earlier hidden by a diphasic effect between the periods of hyperoxygenation and the pauses between?

Does an observation, extended in time after the oxygen exposure reveal later effects, which can be related to younger maturing stages of the cartilage cells, which may not reach the calcification zone until after a couple of days?

Does there exist a dose-response relationship between effects on growth and the oxygen tension used during the 8 hrs. of treatment?

This part of the investigation comprises a total of 193 rabbits, 102 treated with increased tensions of oxygen and 91 litter-mates treated simultaneously with air for control. The oxygen, when said to be 100 per cent pure, contained less than 0.5 per cent inert gases, which can be ignored. They are a part of the air also and they have no proved biological function. The results are given under four headings below, named A—D. The percentage of oxygen in the gas mixtures, conducted through the boxes, is specified, and the ambient pressure is given in atm.abs.. The pressure specified means the normal ambient pressure for the day of the experiment, and under A another 1 atm.abs. achieved by raising the pressure this amount in the hyperbaric chamber, where the boxes were stationed. The barometric pressure on the days of treatment varied only between 745—770 mm Hg, which was not compensated for. During the 8 hrs. of hyperbaric oxygenation under A, the pressure in the chamber was allowed to vary ± 0.1 atm.abs. at most, which means ± 5 per cent.

A. 80 and 100 per cent oxygen at 2 atm.abs. with growth recording periods of 12 hrs.

This experimental series was intended to answer the first question, if omitting of the treatment pauses earlier tried, would disclose a significant change in growth, hitherto covered as being diphasic within the growth period measured. Pure oxygen at 2 atm.abs. was first tried, but turned out to be over the limit of toxicity for the treatment of 8 hrs. used. Therefore 80 per cent oxygen was tried for the same time and the same pressure, that is an oxygen pressure of 1.6 atm.abs.. In the first group 2 animals out of 7 died of respiratory failure soon after the end of exposure, and in the second group 2 of 13 died with the same symptoms. In both cases it was animals in the same litter that reacted in this way, and another 5 animals (not excluded) in these two litters showed symptoms of respiratory distress, but survived the measuring period. The post mortem examination of the lungs disclosed macroscopic "hepatisation", i.e. alveolar exudation, blood engorgement and haemorrhages as described by *Lorrain Smith* (1899), in 11 out of 18 rabbits treated with 80 or 100 per cent oxygen for 8 hrs.. In three of the cases the changes were slight as also in one of the controls. Microscopic examination was not made as these changes have been thoroughly studied (*Smith* 1899). As toxicity reactions occurred in both groups, it was considered to be justified to add them together for the statistical evaluation. The arithmetic mean of the changes seemed to be the same as well, and the figures are given in Table 2 and 3. The effects on growth is illustrated in Fig. 4 as alteration of mean differences between consecutive time periods. The mean values given in Tables 2—3 show an increase in growth during period 0 of 4 per cent in the oxygen group, compared to 2 per cent in the control, and a decrease during period $+1/2$ of 12 per cent in the oxygen group compared to 3 per cent in the control. The statistical comparison between changes in test and control eliminates the significance of the stimulation observed during hyperoxygenation ($-1/2 \rightarrow 0 : t=1.35^-$), but shows a significant decrease in growth for the oxygen group during the 12 hrs. after the treatment ($0 \rightarrow +1/2 : U=2.60^{xx}$). Because of the toxicity reactions observed, these exposures were not continued. The changes observed are illustrated in Fig. 4.

As the sensitivity of the measuring method was not expected to allow shorter observations, it was necessary to lower the partial pressure of oxygen used, and 100 per cent at 1 atm.abs. was therefore chosen, being simpler to handle than 50 per cent oxygen at 2 atm.abs..

MEAN GROWTH CHANGE IN MICRONS

PERIODS OF 12 HRS.

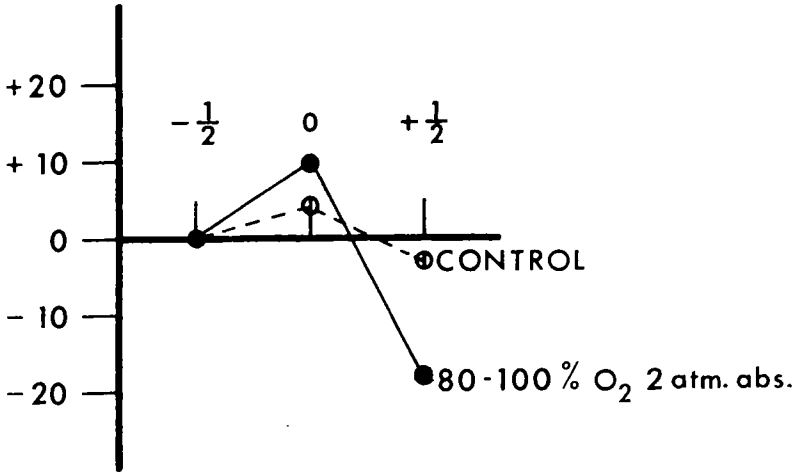


Figure 4.

Mean growth changes in microns between three consecutive time periods of 12 hrs.. The oxygen group was treated in 80 or 100 % of O₂ at 2 atm.abs. for 8 hrs. during period 0, the control group simultaneously treated with air at the same pressure.

MEAN GROWTH CHANGE IN MICRONS

PERIODS OF 12 HRS.

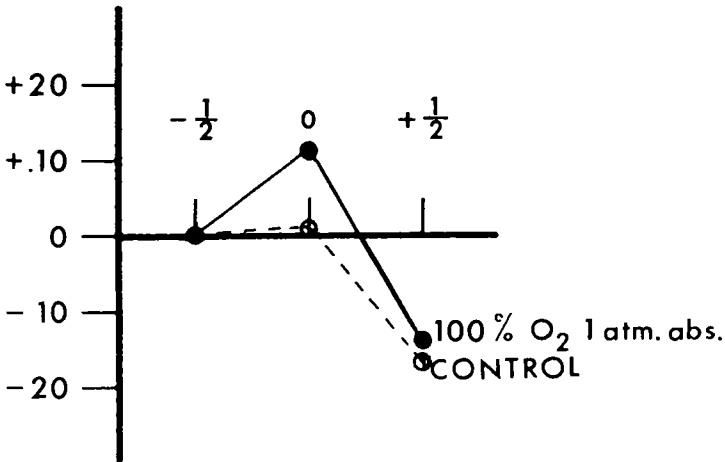


Figure 5.

Mean growth changes in microns between three consecutive time periods of 12 hrs.. The oxygen group was treated in 100 % O₂ at 1 atm.abs. for 8 hrs. during period 0, the control group simultaneously treated with air at the same pressure.

Table 2.

Increased oxygen tension by exposure to 80 and 100 per cent oxygen at 2 atm.abs. in hyperbaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$).

Animal	$-1/2$	0	$+1/2$
TH I	250	280	240
TH II	240	280	270
TH III	260	250	280
TH IV	230	240	220
UH I	270	270	250
UH II	260	280	260
UH III	270	280	280
UH IV	250	250	240
UV II	260	270	270
UV III	250	250	240
VH II	250	250	170
VH IV	220	240	190
VV I	230	230	170
OH I	280	290	260
OH II	260	290	220
OH III	260	280	240
QH I	240	230	180
QH II	230	230	200
Mean growth	251	261	232

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	+10	-28
Stand. dev.	14.14	27.49
t-value	$+3.0 \times \times$	$-4.38 \times \times \times$
n-value	18	18

Table 3.

Control animals for Table 2. Air breathing at 2 atm.abs. for 8 hrs. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$).

Animal	$-1/2$	0	$+1/2$
OV II	260	260	230
QV I	220	200	210
QV II	250	240	240
QV III	230	230	230
QV IV	280	290	260
RV I	280	280	260
RV II	260	250	240
RV III	250	260	250
RV IV	300	290	280
TV I	270	280	270
TV II	240	250	270
TV III	230	260	260
UV I	250	270	280
UV IV	260	260	250
VV II	230	230	230
VV III	210	230	210
Mean growth	251	255	248

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	+4	-7
Stand. dev.	13.10	14.01
t-value	+1.15 ⁻	-1.96 ⁻
n-value	16	16

B. 100 per cent oxygen at 1 atm.abs. with growth recording periods of 12 hrs.

This part describes the immediate effects, up to 12 hrs. after the end of the exposure to 100 per cent oxygen at 1 atm.abs.. No animals died or developed respiratory distress. Only one animal showed traces of the pulmonary "hepatisation" observed in A. Consequently this partial pressure of oxygen is about the highest tolerable for 8 hrs. in young rabbits. Individual values and mean values of growth rates are given in Tables 4 and 5. The changes are further illustrated in Fig. 5 as mean differences in test and control. The mean values given in Tables 4 and 5 show an increase in growth during period 0 of 5 per cent in test, compared to 0 per cent in control, and a decrease in growth during period $+1/2$ of 11 per cent in test compared to 7 per cent in control. Statistically the first change is significant ($t=2.86^{**}$), the second is insignificant ($t=1.56^{-}$).

This part B had given a determination of the highest tolerable doses of oxygen for 8 hrs. and shown that the increase in growth, earlier not significant, was statistically reliable. It was now necessary to illuminate on the second question, whether an observation of growth, extended in time after the therapy, would disclose further changes, referable to different stages of the cartilage cell development. This demanded an observation time of 5 days (*Messier and Leblond* 1960) after the exposure with a hyperoxygenation of 100 per cent at 1 atm.abs., as determined to be the highest dose, which could be tolerated.

C. 100 per cent oxygen at 1 atm.abs. with growth recording periods of 24 hrs.

With the partial pressure of oxygen and time of exposure identical with that in part B it was considered to be justified for this prolonged observation to change the growth recording periods from 12 to 24 hrs. as the earlier effects were already determined. This would allow an easier handling in respect of time consumption, and give a reduced relative error of measurements, which was supposed to outweigh the possible advantage of using 12 hrs. as the period for the study. For changes in growth occurring later in time than 12 hrs. after the gas exposure it could be presumed to be a reaction, flattened out in time, thereby making it sufficient to extend the periods in use to 24 hrs.. In the preliminary investigation (*Persson* 1967) the 24 hrs. period had also been used.

The test groups were given 100 per cent of oxygen at 1 atm. abs. for 8 hrs. during period 0, the controls simultaneously exposed to air from gas cylinders.

Table 4.

Increased oxygen tension by exposure to 100 per cent oxygen at 1 atm. abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$).

Animal	$-1/2$	0	$+1/2$
SH I	270	290	270
SH II	280	290	280
SH III	280	290	250
XH I	240	230	220
XH II	210	220	200
XH III	210	200	200
XV I	170	190	180
ADH I	270	290	250
ADH II	280	300	260
ADH III	280	300	250
ADV I	300	300	260
EVH I	230	250	210
EVH IV	240	270	210
EVV I	250	250	200
EXH I	260	280	240
EXH II	240	250	210
EXV II	260	280	240
EYH I	290	290	280
EYH III	280	270	250
EYV I	270	290	240
FRV I	150	180	140
FRV II	160	180	150
FSH II	190	210	200
FSH III	190	190	180
FSV I	160	170	170
FSV II	180	190	180
FTH I	260	280	240
FTH II	220	250	230
FTH IV	250	250	230
FTV II	210	200	200
FU I	210	210	210
FU III	170	190	170
Mean growth	233	245	219

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	+12	-26
Stand. dev.	13.77	17.57
t-value	+4.75 × × ×	-8.36 × × ×
n-value	32	32

Table 5.

Control animals for Table 4. Air breathing at 1 atm.abs. for 8 hrs. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$).

Animal	$-1/2$	0	$+1/2$
SV I	300	300	280
SV II	280	270	250
XV II	230	240	240
XV III	220	220	210
ADV II	310	310	270
ADV III	290	300	250
EVH II	250	250	220
EVH III	250	250	220
EVH V	250	280	230
EVV IV	260	260	250
EXH III	260	260	230
EXV I	240	280	220
EYH II	280	280	270
EYV II	280	290	260
EYV III	300	290	260
FRH I	200	210	200
FSH I	210	210	210
FSH IV	210	200	200
FSV III	190	200	190
FSV IV	170	170	190
FTH III	260	250	250
FTV I	240	240	240
FTV III	270	250	220
FU II	250	240	240
FU IV	220	210	200
FU V	220	220	200
Mean growth	248	249	231

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	+2	-18
Stand. dev.	12.55	18.91
t-value	+0.63 ⁻	-4.98 ^{x x x}
n-value	26	26

The results are given in Tables 6 and 7 showing in comparison a significant increase of 3 per cent between period -1 and period 0 ($t=3.42^{**}$). The continued observation for 5 periods of 24 hrs. after period 0 did not reveal any statistically significant alteration in the continued growth rate of the metaphysis with comparison between test and control. ($0 \rightarrow +1: t=0.23^-$, $+1 \rightarrow +2: t=0.19^-$, $+2 \rightarrow +3: t=0.04^-$, $+3 \rightarrow +4: t=1.24^-$, $+4 \rightarrow +5: t=0.89^-$). This is illustrated in Fig. 6. This does not, however, indicate that the growth in test and control was the same after the end of the oxygen treatment. From the statistical point of view, on the contrary, it means that the acceleration of growth, observed during the treatment with 100 per cent of oxygen, continued as a growth rate on a little higher level than in the control group. It might be that the growth stimulation subsided slowly and extended over several periods after the treatment, making each step so small that the method did not allow measuring of the changes. The recorded stimulation was 3 per cent, and the error of the method is 1—2 per cent (Hansson 1967, Persson 1967), which makes this possibility probable. To evaluate this and to answer the question as to when the stimulation ends, the absolute values of growth were compared. This is made in Fig. 7 as a quotation of the mean value of growth in the test with that in the control group for the same day. The growth in the test group is thereby expressed as a percentage of the control for each day. In this way the overlapping groups in period +1 and +3 respectively were utilized by summing, which makes the samples larger on these two days. This comparison indicated that the stimulation subsided from +4 per cent to 1 per cent from period +2 to +4 (Fig. 7).

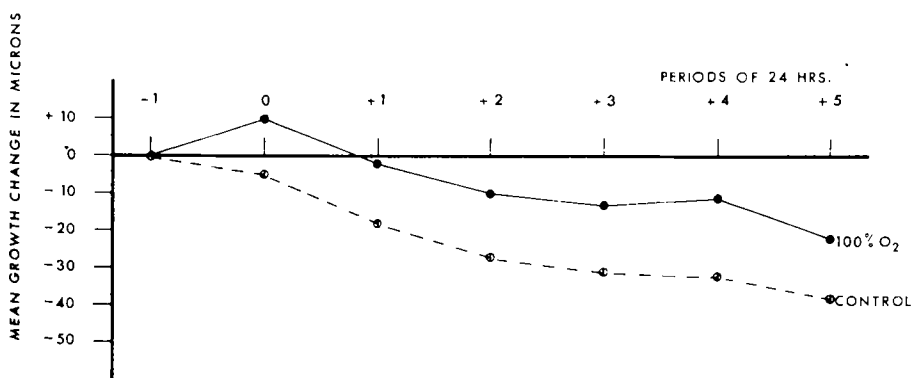


Figure 6.

Longitudinal growth of the proximal tibial metaphysis in rabbits at increased oxygen tension by exposure to 100 % of oxygen at 1 atm.abs. for 8 hrs. during period 0. Growth recordings by oxytetracycline labelling for 7 consecutive time periods of 24 hrs. Mean growth changes in microns form day to day, measured within the same animal. Figures made continous by overlapping observations in period +1 and +3.

Table 6.

Increased oxygen tension by exposure to 100 per cent oxygen at 1 atm. abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals.

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DYH I	550	550	540	EDH III	400	400	410	EFH I	470	460	450
DYH III	540	550	530	EDH IV	470	470	480	EFH III	440	450	420
DYH IV	540	540	540	EDH V	450	450	430	EFH IV	500	500	470
DYH V	500	510	480	EDV III	420	410	410	EFV III	510	510	510
DYV IV	530	520	520	EEH I	390	400	410	EGH I	490	490	490
DZH II	510	520	510	EEH IV	420	420	420	EGH III	510	510	500
DZH III	480	500	470	EEH VI	430	430	440	EGH IV	520	520	510
DZH IV	540	530	510	EJH I	500	480	480	EGV II	510	530	510
DZH V	520	510	500	EJH II	510	490	470	EHH II	500	510	500
DZV II	510	500	510	EKH II	520	500	510	EHH V	550	550	540
DZV IV	510	510	490	EKV II	540	530	500	EHV I	500	500	490
GTH I	500	500	500	EKV III	520	500	460	EHV II	540	550	550
GTH II	450	470	480	EL I	450	430	400	EHV IV	470	470	460
GTV I	410	440	440	EMH I	530	500	490				
GUV I	500	520	490	EMH III	500	490	500				
GUV II	520	540	530	EMH VI	450	490	510				
GV I	500	510	500	EMV I	570	540	540				
GV IV	490	510	480	EMV II	520	520	520				
GXH II	540	560	540								
GXH III	510	530	520								
Mean growth	508	518	505		477	469	466		501	504	492

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	+10	-12	-8	-4	+3	-11
Stand. dev.	14.14	12.61	16.65	17.20	7.51	10.77
t-value	+3.24 ^{xx}	-4.50 ^{xxx}	-1.98 ⁻	-0.96 ⁻	+1.48 ⁻	-3.61 ^{xx}
n-value	21	21	18	18	13	13

Table 7.

Control animals for Table 6. Air breathing at 1 atm.abs. for 8 hrs. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals.

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DYH II	520	520	510	EDH I	410	410	410	EFH II	420	420	440
DYV I	550	550	540	EDH II	370	370	380	EFV I	500	500	480
DYV II	530	530	510	EDV I	430	440	450	EFV II	520	520	520
DZH I	500	500	500	EDV III	460	460	450	EFV IV	490	480	470
DZH IV	540	530	510	EDV V	390	390	380	EGH II	490	490	470
DZV I	510	530	480	EEH II	410	410	440	EGV I	520	510	500
DZV III	430	430	430	EEH V	320	280	310	EGV III	520	530	500
DZV V	490	510	500	EEV V	390	360	350	EHH I	520	530	530
DZV VI	460	490	450	EJH III	490	440	390	EHH III	530	540	540
GTH III	450	420	440	EKH III	470	470	460	EHH IV	500	490	490
GTH IV	490	490	490	EKV I	470	470	480	EHV III	510	500	500
GTV II	430	410	400	EL II	510	510	500				
GTV IV	450	440	430	EMH II	530	530	510				
GUH III	530	510	490	EMH IV	540	520	510				
GUV III	500	490	480	EMH V	590	580	580				
GV II	520	510	500	EMV IV	540	530	500				
GV III	510	500	490	EMV IV	490	490	490				
GV V	530	510	490								
GXH I	510	490	470								
GXV II	540	530	520								
GXV III	530	520	500								
Mean growth	501	496	482		459	451	446		502	501	495

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-5	-13	-9	-4	-1	-6
Stand. dev.	14.70	14.26	16.54	19.70	8.31	13.65
t-value	-1.63	-4.28 × × ×	-2.20 ×	-0.86	-0.36	-1.55
n-value	21	21	17	17	11	11

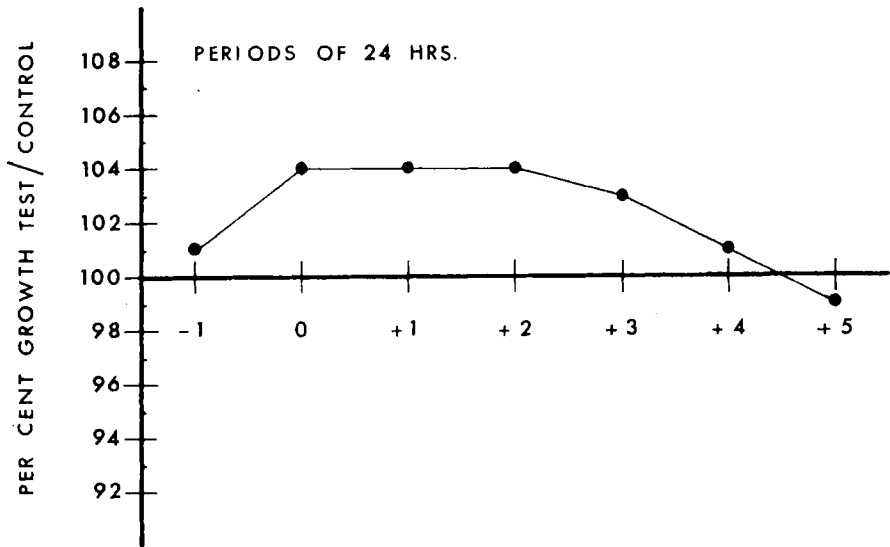


Figure 7.

Per cent growth in length from the proximal tibial growth plate of rabbits treated with 100 % O₂ at 1 atm.abs. for 8 hrs. during period 0, compared to control groups of littermates simultaneously treated with air from gas cylinders.

As the gases tried might influence the respiration and because of the fact that rabbits regulate their heat by panting instead of sweating some attention had to be paid to the *temperature factor*. In testing 100 per cent of oxygen at 1 atm.abs. for 8 hrs. the rectal temperatures were measured with a mercury thermometer in 14 animals before and within a minute after the exposure. They ranged between 38.0 and 39.5°C, but there was no difference between tests and controls. This is in accordance with *Valenzuela (1877)*. The temperature of the two boxes was also the same at the end of the experiment.

D. 100 to 21 per cent oxygen at 1 atm.abs. with growth recording periods of 12 hrs.

A preliminary test of 80 per cent and 40 per cent of oxygen in nitrogen for 8 hrs. at 1 atm.abs. was made in 25 animals but the effects were insignificant. It was therefore considered not worth carrying on with, to avoid a further splitting up of the material. These values are therefore not reported in detail and

an analysis of these pressures has not been carried out, because a determination of the limits of the effects was not essential for this investigation.

Regarding the questions in the beginning of this chapter the following statements can be made. The omitting of the intervals of the hyperbaric oxygenation earlier reported did show an influence by increased oxygen on the longitudinal growth of the metaphysis, which was statistically significant. An acceleration of growth starting during exposure to 100 per cent of oxygen at 1 atm.abs. was observed. Further, an extended observation for 5 days after the exposure did not reveal statistically significant changes, but probably a gradual decrease in growth back to the level of the control groups during periods +3 and +4 occurred (Fig. 7). A dose-response relationship existed so that toxicity reactions occurred when the partial pressure of oxygen was increased above 1 atm.abs. for 8 hrs., eliminating the stimulation.

After the examination of effects on growth caused by increased oxygen tensions of the respiratory air it seemed to be valuable for the interpretation, to study as well the effects of reduced oxygen tensions.

CHAPTER 4.

RESULTS OF DECREASED OXYGEN TENSIONS

The simplest way to reduce the oxygen tension for groups of animals during 8 hrs. seemed to be by using a hypobaric chamber. It was considered indicated to begin with this method, although a lowering of the environmental pressure would reduce the partial pressure of other gases in air as well. These other gases were not expected to influence the growth rates. The control animals were housed outside the chamber and the test animals inside the chamber, kept in an open box. Pellets and water were given freely to both as always. The pressure in the chamber was lowered to pressures corresponding to 3000, 4000, 5000 and 6000 meters respectively in four series. Exposure and measuring times were as described in Chapter 2 (Fig. 2 and 3). According to the transformation list of the station 3000 meters was equivalent to 526 mm Hg, 4000 meters was 462 mm Hg, 5000 meters was 405 mm Hg and 6000 meters was equivalent to 354 mm Hg. An adjustable spring-loaded valve kept the pressure constant by letting in a small flow of air, which increased if the pumps were about to lower the pressure too much. The hypobaric chamber was cylindrical, length 225 cm, diameter 254 cm, and this magnitude made special efforts for temperature regulation and carbon dioxide control unnecessary. The results will be described in the order the experiments were performed, beginning with the moderate hypoxia, accomplished by reducing the pressure to the equivalent of 3000 meters with growth recording in half-days. Stepwise in different series the oxygen lack was increased in simulated altitudes from 3000 to 6000 meters, and after the 12 hrs. series of growth recordings the same degrees of hypoxia were analysed with growth recording periods of 24 hrs. for a more extended observation time. This part of the investigation comprises 461 animals, distributed as specified in the tables.

A. Air at 3000 meters altitude with growth recording periods of 12 hrs.

The experimental results are given in Table 8 to be compared with the control result in Table 9. There was no significant effect on growth at 3000 meters (0.70 atm.abs.) for the 8 hrs. of exposure used. The comparison values statistically were $-1/2 \rightarrow 0$: $t=0.90^-$ and $0 \rightarrow +1/2$: $t=0.04^-$.

Table 8.

Reduced oxygen tension by exposure to 3000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rate in microns during successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
AYH I	220	220	200
AYH II	180	160	170
AYH III	170	160	150
AYH IV	190	180	150
AYH V	180	170	160
AZV I	150	130	140
AZV II	150	150	150
AZV III	180	190	180
AZV IV	180	180	150
AZV V	180	180	160
BBH I	310	300	300
BBH II	300	310	300
BBH III	310	310	300
BBV I	300	290	300
BBV II	300	300	290
BBV III	300	290	300
BDV I	130	130	130
BDV II	140	170	130
BDV III	120	130	130
Mean growth	210	208	199

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow 1/2$
Mean diff.	-2	-8
Stand. dev.	11.82	14.63
t-value	-0.78 ⁻	-2.51 ^x
n-value	19	19

Table 9.

Control animals for Table 8, 10 and 11 (3000—6000 meters in hypobaric chamber), resting out-side the chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
YV I	220	220	210
YV II	240	250	250
YV III	250	250	230
ZV II	250	250	250
ZV III	250	250	250
AAV II	260	260	250
ACV II	280	280	260
ACV III	260	290	250
AKV II	310	300	300
AKV III	300	310	320
AKV IV	290	290	290
AKV V	280	270	260
AUV I	260	260	250
AVV I	250	260	250
BBH IV	310	310	300
BBV IV	300	290	280
BDV IV	120	110	110
Mean growth	261	262	254

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow 1/2$
Mean diff.	+1	-8
Stand. dev.	9.93	11.31
t-value	+0.39-	-3.00 $\times \times$
n-value	17	17

B. Air at 4000 meters altitude with growth recording periode of 12 hrs.

The influences on growth at 4000 meters (0.61 atm.abs.) are given in Table 10 to be compared with the control values in Table 9. There is an almost significant decrease in growth in comparison between the period before the treatment and the period of treatment and this significance is unaltered in comparison with the control group ($-1/2 \rightarrow 0$: $t=2.57^{\times}$, $0 \rightarrow +1/2$: $t=0.51^{-}$).

Table 10.

Reduced oxygen tension by exposure to 4000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
AUH I	290	280	270
AUH II	280	280	240
AUH III	290	270	260
AUH IV	290	290	260
AUV II	240	230	240
AUV III	290	270	250
AVH I	230	210	220
AVH II	240	250	250
AVH III	280	270	250
AVV II	260	250	250
Mean growth	269	260	249

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	-9	-11
Stand. dev.	9.94	16.63
t-value	-2.86^{\times}	-2.09^{-}
n-value	10	10

C. Air at 5000 meters altitude with growth recording periods of 12 hrs.

The results of testing growth at 5000 meters (0.54 atm.abs.) are given in Table 11 to be compared with the control animals in Table 9. The differences are statistically insignificant ($-1/2 \rightarrow 0$: $t=1.13^-$, $0 \rightarrow +1/2$: $t=1.72^-$) in comparison between test and control animals, but significant within the test group (Table 11). The direction of the changes in growth is the same as at 4000 meters.

Table 11.

Reduced oxygen tension by exposure to 5000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
YH I	230	230	230
YH II	270	270	240
YH III	240	240	240
YH IV	240	250	200
YH V	220	220	230
YV IV	240	240	230
AKH I	290	290	280
AKH II	280	280	260
AKH III	310	320	300
AKH IV	310	300	260
AKH V	310	290	260
AKV V	300	270	260
Mean growth	270	267	249

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow 1/2$
Mean diff.	-3	-18
Stand. dev.	11.55	17.65
n-value	-1.00 ⁻	-3.43 ^{××}
t-value	12	12

Table 12.

Reduced oxygen tension by exposure to 6000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
ZH I	240	220	220
ZH II	240	200	200
ZH III	270	230	230
ZH IV	240	230	220
ZVI	230	220	220
AAH I	260	260	210
AAH II	250	230	220
AAV I	260	250	220
ACH I	270	250	240
ACH II	270	270	230
ACH III	280	250	240
ACV I	260	240	240
Mean growth	256	238	224

Statistical analysis of differences in growth between the consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	-18	-13
Stand. dev.	13.37	17.25
t-value	-4.74^{***}	-2.68^*
n-value	12	12

D. Air at 6000 meters altitude with growth recording periods of 12 hrs.

The results of testing growth at a simulated altitude of 6000 meters (0.47 atm.abs.) are given in Table 12 to be compared with the control values in Table 9. There is a decrease in mean growth of 7 per cent in comparison between the day before and the day of treatment and a further decrease of another 5 per cent to the day after. The first decrease is highly significant also compared to the controls ($-1/2 \rightarrow 0$: $t=4.52^{***}$, $0 \rightarrow +1/2$: $t=0.96^-$).

This part of the investigation (A—D) had shown that there was a decreased growth in length during air-breathing at reduced barometric pressure, persisting during the first 12 hrs. after the exposure. The changes are highly significant only at 6000 meters altitude. The next step was to follow these effects for an extended period after the treatment. On the same grounds as given in Chapter 6 measuring periods of 24 hrs. were used for the continued study. This is given below (E—H).

E. Air at 3000 meters altitude with growth recording periods of 24 hrs.

The results of these exposures to air at 3000 meters (0.70 atm.abs.) for 8 hrs. are given in Table 13 to be compared to the controls in Table 14. There are no significant effect at this comparison even during this extended observation for 5 days after the end of treatment.

Table 13.

Reduced oxygen tension by exposure to 3000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CEH I	540	540	500	AXH I	390	380	370	BAH I	490	450	420
CEH II	530	530	510	AXH II	390	390	380	BAH II	450	450	440
CEH IV	540	540	510	AXH III	310	310	310	BAH III	490	490	450
CFH II	520	510	500	AXH IV	220	220	220	BAH IV	530	520	500
CFH IV	530	530	500	AXH V	260	260	270	BAV I	440	440	430
CFV II	510	530	510	AXV I	370	400	350	BAV II	500	490	480
CFV III	520	520	510	AXV II	320	330	370	BAV III	570	560	560
CGH II	540	540	540	AXV III	340	340	340	BAV IV	480	480	480
CGH IV	530	520	520	AXV IV	370	380	370	BC I	520	520	520
CGH V	500	500	490					BC II	550	550	550
CGV I	510	510	530					BC III	540	530	520
CGV V	530	540	500					BC IV	540	540	510
Mean growth	525	526	510		330	334	331		512	507	492

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	+1	-16	+4	-3	-5	-15
Stand. dev.	7.93	17.82	11.30	23.45	11.98	13.91
t-value	+0.36 ⁻	-3.07 [×]	+1.18 ⁻	-0.43 ⁻	-1.62 ⁻	-3.79 [×]
n-value	12	12	9	9	12	12

Table 14.

Control animals for Tables 13, 15, 16 and 17 (3000—6000 meters in hypobaric chamber), resting out-side the chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CEH III	530	530	490	ALV II	540	550	550	BAH V	480	470	440
CEV I	530	530	520	ALV III	510	510	510	BCV I	550	550	540
CFH I	510	520	520	AIV IV	580	590	560	BEH III	460	450	450
CFH III	540	540	540	AMV II	560	550	550	BEH IV	510	500	480
CFV I	540	550	560	AMV III	620	610	590	BGV II	380	380	370
CFV IV	520	520	510	AMV IV	590	590	580	BGV III	340	340	330
CGH I	510	510	500	ANV III	530	540	530	BGV IV	350	350	330
CGH III	580	580	570	ANV IV	560	550	550	BHV III	450	450	450
CGV II	550	550	550	AOV II	490	490	490	BJV IV	370	400	380
CGV III	550	550	530	AOV III	490	480	460	BJV V	250	240	240
CGV IV	560	560	560	APH V	490	470	500	BKH III	530	530	510
CPH IV	470	470	450	ARV V	550	550	550	BKV I	600	600	590
CPV II	520	520	500	ASH III	340	350	350	BKV II	580	560	530
CPV III	470	470	470	ASH IV	370	380	380	BLH III	590	600	590
CPV IV	460	460	460	ATH III	340	340	330	BLV I	570	560	560
CQH II	550	550	550	ATH IV	350	360	350	BLV II	490	490	490
CQV I	520	520	520	AXH VI	400	380	390	BNV II	560	550	550
CQV III	560	560	540					BNV III	580	570	560
CRH I	490	490	490					BOV III	610	600	560
CRH III	510	500	490					BOV IV	550	560	550
CRV I	520	520	520								
CRV III	500	500	470								
CHH II	540	540	500								
CHH IV	550	540	530								
CHV I	520	500	500								
CJH I	520	520	500								
CKV III	550	540	500								
CLH IV	550	540	540								
CLV I	550	550	540								
CLV IV	570	570	500								
CLV VI	490	480	470								
CMH I	600	600	600								
CNH I	480	490	490								
CNV II	580	590	550								
COH I	560	560	550								
COH III	600	600	590								
CSH II	550	550	500								
CSH III	550	520	500								
CVS III	450	430	420								
CTH II	570	550	550								
CTH IV	560	540	510								
CUH I	550	520	450								
CUH III	510	510	510								
CUV II	550	530	520								
CUV III	570	550	520								
Mean											
growth	534	529	514		489	488	484		490	488	475

Table 14 continued

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-4	-15	-1	-4	-3	-13
Stand. dev.	10.35	18.42	10.54	13.26	10.70	11.64
t-value	-2.88 ^{xx}	-5.50 ^{xxx}	-0.46 ⁻	-1.28 ⁻	-1.04 ⁻	-4.80 ^{xxx}
n-value	45	45	17	17	20	20

F. Air at 4000 meters altitude with growth recording periods of 24 hrs.

The results of studying growth during air-breathing at 4000 meters (0.61 atm.abs.) for 8 hrs. are given in Table 15 to be compared to the control values in

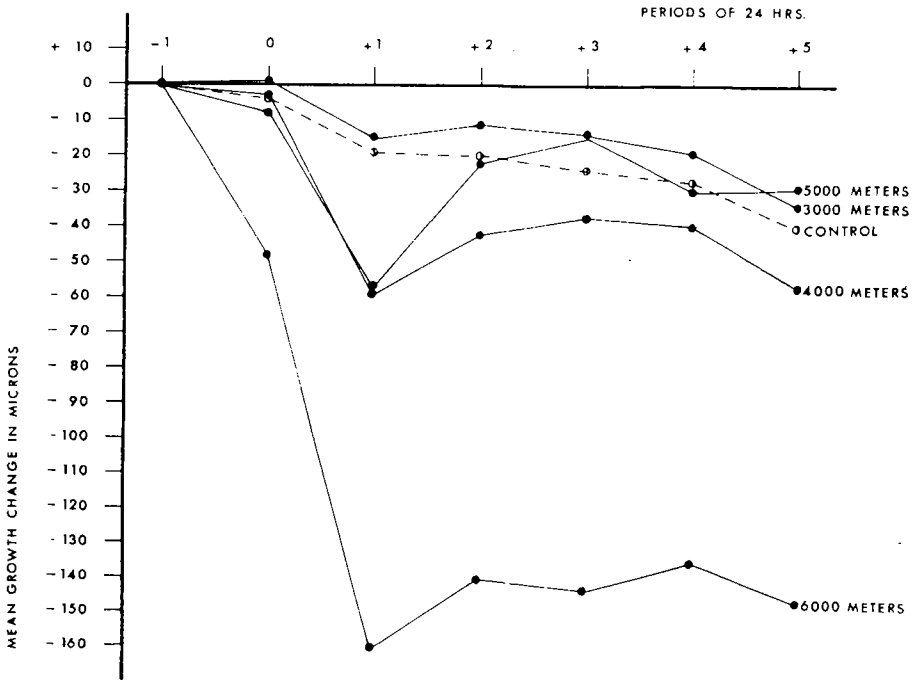


Figure 8.

Longitudinal growth of the proximal tibial metaphysis in rabbits at reduced oxygen tension by exposure to simulated altitudes of 3000, 4000, 5000 and 6000 meters for 8 hrs. during period 0. Growth recordings by oxytetracycline labelling for 7 consecutive time periods of 24 hrs. Mean growth changes in microns from day to day, measured within the same animal. Figures made continuous by overlapping observations in period +1 and +3.

Table 14. This comparison shows a highly significant decrease in growth from the day of exposure to the day after ($t=5.97^{***}$) and further by a return towards normal as a highly significant increase between period +1 and +2 ($t=4.61^{***}$). The first decrease is about 11 per cent, the following increase about 5 per cent between periods +1 and +2. There is a further increase of 2 per cent between periods +2 and +3 ($t=1.92^-$). The effects are illustrated in Fig. 8. After period +3 there are no significant changes (+3 \rightarrow +4: $t=0.13^-$, +4 \rightarrow +5: $t=1.01^-$).

Table 15.

Reduced oxygen tension by exposure to 4000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CPH I	500	500	460	ASH I	300	320	310	BEH I	470	470	460
CPH II	470	470	450	ASH II	320	330	330	BEH II	490	480	480
CPH III	490	490	440	ASV I	310	340	350	BGH I	380	370	370
CPV I	490	500	450	ASV II	350	370	360	BGH II	380	370	330
CQH I	520	510	430	ASV III	270	290	310	BGH III	400	390	360
CQH IV	570	560	510	ASV IV	310	340	340	BGH IV	410	420	370
CQV II	540	540	460	ATH I	360	370	380	BGV I	370	370	360
CRH II	500	500	430	ATH II	300	310	310	BJV I	350	350	340
CRH IV	490	470	410	ATV I	290	290	320	BJV II	350	360	330
				ATV II	320	350	350	BJV III	320	320	320
				ATV III	350	360	360				
				ATV IV	300	310	320				
Mean growth	508	504	449		315	332	337		392	390	372

Statistical analysis of differences in growth between the consecutive periods above:

	-1 \rightarrow 0	0 \rightarrow 1	1 \rightarrow 2	2 \rightarrow 3	3 \rightarrow 4	4 \rightarrow 5
Mean diff.	-3	-56	+17	+5	-2	-18
Stand. dev.	8.66	19.44	9.85	11.68	7.89	18.14
t-value	-1.15 ⁻	-8.57 ^{***}	+5.86 ^{***}	+1.48 ⁻	-0.80 ⁻	-3.14 ^x
n-value	9	9	12	12	10	10

G. Air at 5000 meters altitude with growth recording periods of 24 hrs.

This study of the effects on growth at exposure to 5000 meters (0.54 atm.abs.) for 8 hrs. is described in Table 16, with the control animals listed in Table 14. A comparison between the two materials showed no immediate effect at exposure ($-1 \rightarrow 0$; $t=1.50^-$) but with comparison of changes between period 0

Table 16.

Reduced oxygen tension by exposure to 5000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CHH I	490	490	420	ALH I	480	500	510	BHH I	350	350	360
CHH III	540	530	510	ALH II	510	530	540	BHH II	390	360	360
CHV II	530	500	440	ALH III	520	530	550	BHH III	380	350	350
CHV III	520	510	500	ALV I	450	500	510	BHH IV	310	260	260
CJH III	510	510	450	AMH I	490	520	520	BHV I	420	420	420
CKH II	560	570	520	AMH II	530	570	570	BHV II	350	350	350
CKH III	550	540	510	AMH III	530	550	540	BKH I	550	530	540
CLH I	540	530	480	AMH IV	530	580	580	BKH II	480	470	460
CLH II	550	520	510	AMV I	530	540	540	BLH I	610	610	610
CLH V	530	510	460	ANH I	490	540	590	BLH II	560	550	550
CLV II	540	540	460	ANH II	500	540	540				
CLV III	570	560	530	ANH III	540	590	590				
CLV V	550	540	540	ANH IV	470	550	550				
CMH II	600	590	490	ANV I	510	550	550				
CMH III	580	570	480	ANV II	540	550	560				
CNH II	560	560	460								
CNV I	570	560	510								
COH II	560	560	490								
COH IV	570	570	560								
Mean growth	548	540	491		508	543	549		440	425	426

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	8	-49	+35	+7	-15	+1
Stand. dev.	10.15	31.18	19.95	13.97	17.16	4.83
t-value	-3.62 ^{x x}	-6.92 ^{x x x}	+6.73 ^{x x x}	+1.85 ⁻	-2.76 ^x	+0.65 ⁻
n-value	19	19	15	15	10	10

and period +1 there was a highly significant decrease ($U=4.08^{***}$) measuring 11 per cent in mean value. After this there was a highly significant increase in growth ($U=4.48^{***}$) between period +1 and period +2, measuring 6 per cent. The following days showed no significant changes between consecutive days in the test group but in the control group there was a significant decrease in growth of unknown cause during the last day (Table 14).

H. Air at 6000 meters altitude with growth recording periods of 24 hrs.

The results of studying growth at 6000 meters (0.47 atm.abs.) for 8 hrs. are given in Table 17, with controls in Table 14. At this altitude a comparison of the groups showed a highly significant decrease in growth already between period -1 and the period of exposure (0), measuring 9 per cent ($T=33.5^{***}$). The earlier observed decrease given in part D is verified and followed by a further decrease the day after treatment of another 20 per cent now highly significant ($T=23.5^{***}$). The increase between period +1 and +2 observed at 4000 and 5000 meters is still significant at 6000 meters ($U=2.92^{**}$) in spite of the pronounced reduction observed during the first two periods at this altitude. During period +2 and until +5 there is still no significant delayed change in growth in comparison between tests and controls (+2 → +3: $U=0.43^-$, +3 → +4: $t=2.02^-$, +4 → +5: $t=0.05^-$). Many animals seemed to regain a normal growth during these days (Table 17) but the mean change does not fully compensate for the decreased growth in the first days (Fig. 8).

As pronounced effects had been observed at the altitudes studied it was considered to be of no value to proceed further, as the hypoxia, if further increased, would severely influence on the general condition of the animals, which was not the intention. The growth reactions described in A—H were caused by reduced barometric pressure, and it was considered necessary to investigate, if the same changes could be induced by a reduction of the oxygen concentration alone at a normal barometric pressure. This was necessary, not only to exclude other factors of the hypobaric condition used, which might influence on growth, but also to prepare the way for the investigation of the importance of the carbon dioxide pressure because reduction of the oxygen tension induces a hyperventilation, which gives a hypocapnia. The first step was to try 12 per cent of oxygen at normal pressure, corresponding to about 4500 meters of altitude in air-breathing.

Table 17.

Reduced oxygen tension by exposure to 6000 meters altitude in hypobaric chamber for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CSH IV	520	500	350	AOH I	420	480	480	BNH I	560	600	590
CTH I	530	450	310	AOH II	510	540	490	BNH II	600	610	600
CTV II	570	480	430	AOH III	460	480	450	BNH III	590	610	600
CUH II	600	550	420	AOV I	440	450	420	BOH I	560	540	560
CUH IV	530	500	410	APH I	470	480	480	BOH II	560	570	540
CUV I	570	550	440	APH II	520	510	490	BOH III	530	530	490
				APH III	400	400	440	BOH IV	550	550	530
				APH IV	440	440	440	BOV I	510	520	510
				ARV I	460	500	520	BOV II	560	560	560
				ARV II	430	450	470				
				ARV III	420	450	460				
				ARV IV	430	460	460				
Mean growth	553	505	393		450	470	467		558	566	553

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-48	-112	+20	-3	+8	-12
Stand. dev.	30.61	37.10	19.54	25.35	16.42	17.16
t-value	-3.87 ^x	-7.36 ^{xxx}	+3.54 ^{xx}	-0.45 ⁻	+1.42 ⁻	-2.14 ⁻
n-value	6	6	12	12	9	9

I. Twelve per cent oxygen in nitrogen at 1 atm.abs. with growth recording periods of 24 hrs.

Twelve per cent oxygen corresponds to about 4500 meters altitude, that is a barometric pressure of 434 mm Hg. The exposure was for a duration of 8 hrs. during period 0. The results are given in Table 18 with the control animals in Table 19. The statistical comparison between changes in tests and controls shows a significant decrease in growth (-6 per cent) between the period of exposure and the day after ($U=2.93^{xx}$). A subsequent increase, reported in part F-H, is observed (Table 18) but not statistically significant ($U=1.03^{-}$). The other comparisons between changes in tests and controls showed no differences either ($+1 \rightarrow +2 : U=1.03^{-}$, $+2 \rightarrow +3 : t=1.02^{-}$, $+3 \rightarrow +4 : U=0.25^{-}$, $+4 \rightarrow +5 : t=1.87^{-}$). It was considered to be indicated to further lower the oxygen tension.

Table 18.

Reduced oxygen tension by exposure to 12 per cent oxygen in nitrogen for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DLH I	470	490	420	DKH II	440	430	440	ENH I	430	420	420
DLV I	500	480	430	DKH III	420	430	430	ENH IV	380	360	360
DLV II	400	380	350	DKH V	440	440	440	ENV I	440	420	390
DLV IV	480	480	460	DJH I	450	470	430	EOH I	440	420	380
DMH I	530	520	490	DJH IV	450	420	390	EOH IV	470	450	440
DMH II	510	490	450	DJV IV	460	460	410	EOV III	450	450	430
DMH III	500	500	500	GEV II	530	520	510	EPH I	460	440	460
DMH IV	480	470	460	GEV III	550	540	540	EPH III	470	470	470
DMV I	520	490	430	GFH I	460	510	470	EPH V	490	480	490
DNH I	570	560	490	GFH III	410	440	440	EPV II	440	420	400
DNH II	540	550	490	GFV I	450	530	510	EPV III	500	500	500
DNV II	530	510	460	GFV II	400	450	440	FGH IV	370	410	410
								FHH II	520	520	510
								FHV I	450	380	370
								FHV II	460	450	470
								FHV IV	500	500	490
								FJH I	540	540	530
								FJH II	490	460	470
								FJV I	510	510	500
								FJV II	450	450	440
								FJV IV	510	500	510
Mean growth	503	489	450		455	470	454		465	455	450

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-14	-39	+15	-16	-10	-5
Stand. dev.	17.46	21.56	32.05	19.75	19.87	15.04
t-value	-3.15 ^{xx}	-7.19 ^{xxx}	+1.62 ⁻	-2.78 ^x	-2.42 ^x	-1.60 ⁻
n-value	12	12	12	12	21	21

Table 19.

Control animals for Table 18 (12 per cent oxygen in nitrogen) exposed to air for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DLH II	490	490	480	DKH I	410	450	430	ENH II	480	470	440
DLH III	520	500	500	DKH IV	460	450	460	ENH V	450	430	420
DLH IV	490	480	470	DKV II	490	490	490	ENV II	430	400	360
DLV II	500	490	480	DIH II	470	470	460	EOH II	410	410	400
DMH V	530	530	500	DIH V	460	460	440	EOH III	430	410	400
DMV II	510	520	500	DIV I	460	460	470	EOV I	490	480	430
DMV III	550	550	520	DIV III	450	450	440	EOV II	460	450	450
DMV IV	550	550	540	GEH III	470	450	430	EPH IV	510	500	490
DMV V	520	520	510	GEV I	500	500	500	EPV I	490	480	470
DNH III	550	530	520	GFH II	500	500	500	EPV IV	530	520	510
DNV I	560	560	550	GFH IV	440	430	380	FGH II	390	390	400
				GFV III	460	450	450	FGH V	400	410	410
				GFV IV	390	400	400	FHH I	500	510	490
								FHH III	490	480	450
								FHH IV	490	480	450
								FHV III	530	520	510
								FJH III	520	510	510
								FJH IV	500	490	490
								FJH V	510	510	500
								FJV III	530	520	510
Mean growth	526	523	507		458	458	450		477	469	456

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-4	-16	0	-8	-9	-14
Stand. dev.	8.42	9.38	14.14	16.25	9.33	15.01
t-value	-1.59	-6.26 ^{***}	0	-1.88	-4.07 ^{***}	-4.17 ^{***}
n-value	11	11	13	13	20	20

K. Ten per cent oxygen in nitrogen at 1 atm.abs. with growth recording periods of 24 hrs.

Ten per cent oxygen corresponds to an altitude of about 5900 meters or a barometric pressure of 365 mm Hg. The exposure was for a duration of 8 hrs. as always. Rabbits can survive even 8 or 7 per cent oxygen (Binger 1927), but

they can not keep a normal weight beyond 5500 meters (*Champbell 1935*). The results are given in Table 20, with the control animals in Table 21. The tendencies earlier described were again observed. The relative changes in growth were -7 per cent during the day of exposure compared to the day before, again -7 per cent between period 0 and period +1, there after $+8$ per cent to period +2. A comparison of the absolute values of growth during the following periods to +5 shows that the control animals in average have a little better growth rate than the test groups, which illustrates that the changes at this degree of hypoxia probably are affecting the growth for an extended time after return to normal oxygen tension. This was also the case at 6000 meters earlier described. The statistical comparison between changes in tests and in controls demonstrates an almost significant decrease in growth from the day before to the day of treatment ($U=2.02^{\times}$) and a further decrease to the day after treatment which was significant ($U=2.93^{\times\times}$). After this time there was no statistical significance in the differences between changes in test and in control up to five days after the end of the exposure to 10 per cent of oxygen compared to air ($+1 \rightarrow +2: t=1.21^{-}$, $+2 \rightarrow +3: t=0.09^{-}$, $+3 \rightarrow +4: t=0.69^{-}$, $+4 \rightarrow +5: t=0.27^{-}$).

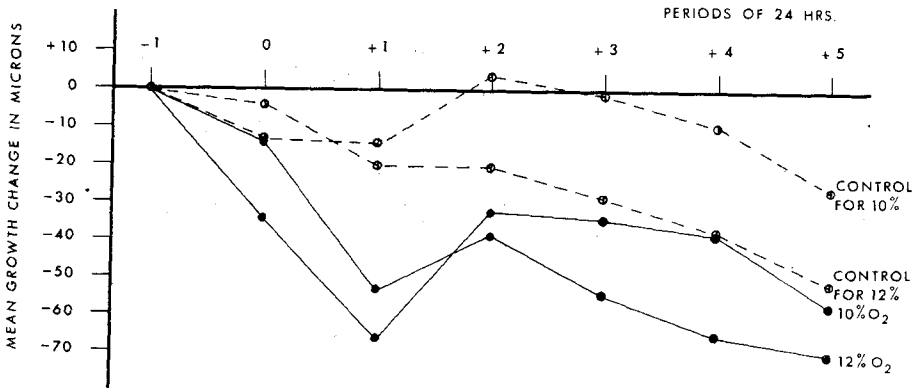


Figure 9.

Longitudinal growth of the proximal tibial metaphysis in rabbits at reduced oxygen tension by exposure to a gas mixture with 10 and 12 % of oxygen respectively in nitrogen at 1 atm.abs.. Exposure time 8 hrs. during period 0. Growth recordings by oxytetracycline labelling for 7 consecutive time periods of 24 hrs. Mean growth changes in microns from day to day, measured within the same animal. Figures made continous by overlapping observations in period +1 and +3.

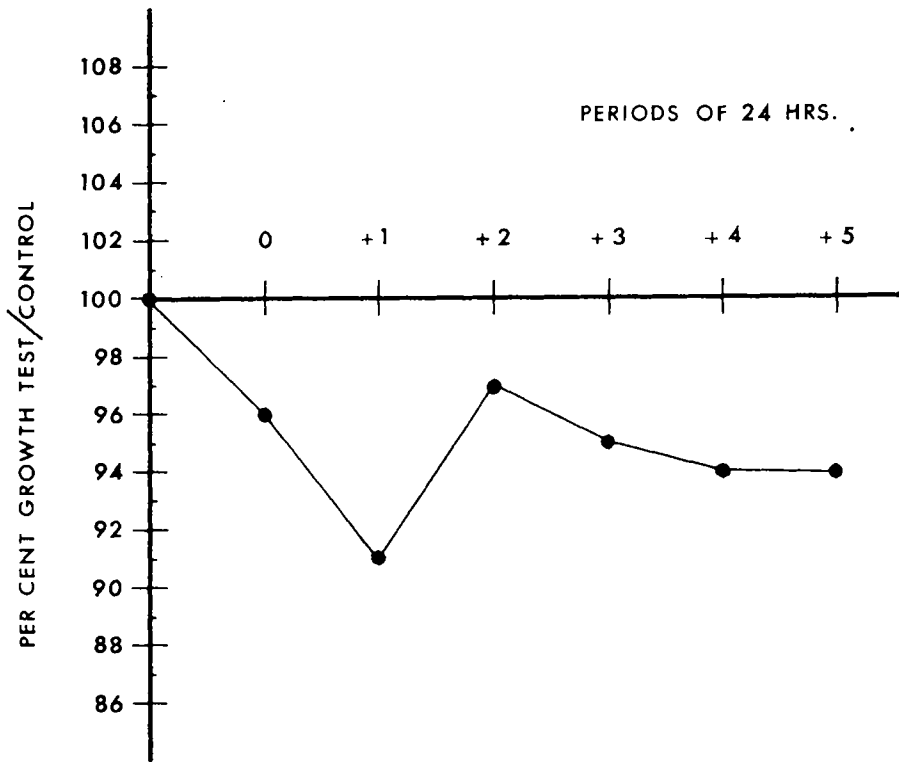


Figure 10.

Per cent growth in length from the proximal tibial growth plate of rabbits treated with 10 % O₂ in N₂ at 1 atm.abs. for 8 hrs. during period 0, compared to control groups of litter mates simultaneously treated with air from gas cylinders.

The decrease in growth during hypoxia is again observed for 24 hrs. after the end of treatment (Fig. 9). The increase that followed between period +1 and +2 is not statistically significant in the test of 12 and 10 per cent of oxygen reported above, which it was at the test of growth during hypobaric conditions corresponding to 4000, 5000 and 6000 meters altitude. Looking at the absolute values of growth in the 10 per cent oxygen series, however, the same increase is observed during that time. This is illustrated in Fig. 10, where test values have been divided by the control of the same day, as described in Chapter 3 for 100 per cent oxygen (Fig. 7).

Table 20.

Reduced oxygen tension by exposure to 10 per cent oxygen in nitrogen for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DOH I	500	440	390	DSV I	440	440	440	FDH I	450	450	440
DOH II	450	430	410	DSV III	480	480	460	FDH II	460	450	450
DOV IV	500	480	430	DT II	510	520	530	FEH II	500	500	500
DPH III	470	350	260	DUV II	490	540	530	FEH III	500	500	490
DPH IV	460	420	430	DUV VI	500	550	550	FEV I	530	540	500
DPV III	430	370	280	DVH I	370	390	410	FFH I	470	470	440
DRH II	400	350	350	DVH II	320	360	420	FFH III	520	540	480
DRV I	400	380	350	DXV II	460	540	510	FFV II	520	550	530
DRV IV	410	390	380	DXV V	480	490	480	FFV IV	510	510	500
FAH I	480	440	390	DXV VI	440	520	480	EAH I	390	380	360
FAH III	490	480	460					EAH II	420	440	400
FAH V	440	400	360					EAH III	430	410	360
FBH I	420	400	390					EAV III	450	430	410
FBH IV	420	370	390					EAV III	450	430	410
FCH I	450	430	420					EBH I	350	340	340
FCH IV	470	470	460					EBH II	250	250	280
FCV I	520	500	470					ECV II	300	270	240
FCV II	530	510	510					ECV III	300	260	250
Mean growth	457	423	391		449	483	481		432	429	410

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-34	-32	+34	-2	-4	-19
Stand. dev.	27.06	32.64	30.62	28.21	18.01	21.76
t-value	-5.40 ^{xxx}	-4.19 ^{xxx}	+3.51 ^{xx}	-0.22	-0.81	-3.56 ^{xx}
n-value	18	18	10	10	17	17

Table 21.

Control animals for Table 20 (10 % oxygen in nitrogen) exposed to air for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
DOH III	470	460	450	DSH I	570	560	540	FDH III	430	430	430
DOV II	490	490	480	DT III	500	500	500	FDH IV	440	440	420
DPH II	450	420	410	DUV IV	500	510	490	FEH I	550	560	550
DPV I	420	360	350	DVH III	460	490	480	FEH IV	520	500	500
DRV II	400	390	400	DVH IV	400	400	490	FEV II	570	570	560
DRV III	420	390	360	DVH V	500	520	490	FEV III	510	500	490
FAH II	430	410	410	DXV II	460	540	510	FFH II	550	550	530
FAH IV	450	450	450	DXV III	470	500	490	FFH IV	570	570	520
FBH II	440	420	450	DXV IV	480	480	480	FFV I	540	530	540
FBH III	410	400	400					FFV III	500	520	480
FBH V	400	400	400					EAH IV	380	340	320
FCH II	430	430	430					EAV II	360	360	370
FCH III	520	530	530					EBH IV	370	360	350
FCV III	540	540	540					EBV III	330	310	260
FCV IV	550	540	550					ECH I	410	400	390
								ECH III	370	340	300
Mean growth	455	442	441		482	500	497		463	455	438

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-13	-1	+18	-3	-8	-17
Stand. dev.	17.51	13.02	27.28	36.74	14.83	19.22
t-value	-2.80 ^x	-0.40 ⁻	+1.96 ⁻	-0.27 ⁻	-2.02 ⁻	-3.51 ^{x x}
n-value	15	15	9	9	16	16

Height of the cartilage plate after 10 per cent of oxygen for 8 hrs.

It was clear that a lowering of the partial pressure of oxygen in the respiratory air did affect the growth in length of the metaphysis. This influence was in a certain proportion to the degree of hypoxia induced with no changes at 3000 meter, and severe changes at 6000 meters altitude for 8 hrs. in unacclimatized animals. The question was now whether this effect on the growth rate of the metaphysis represented a temporary shift of the calcification border or affected the total epiphyseal cartilage plate. Theoretically three different possibilities existed:

1. The hypoxia acted mainly in the zone of degeneration and calcification, where the cartilage cells meet the blood vessels again.
2. The hypoxia acted more evenly over all the maturing stages of the cartilage cells from the reserve layer to the degenerating layer.
3. The hypoxia acted more in the top layers of proliferating cells than in the metaphyseal end.

This question could possibly be answered by measuring of the thickness of the whole cartilage plate simultaneously with a recording of the metaphyseal growth rate by tetracycline labelling as above. The same three possibilities regarding the position of the most sensitive cells presumably existed also at increased doses of oxygen earlier described, but as the effects on growth observed during hyperoxia were quantitatively smaller it was considered to be advisable to study this first at a reduced oxygen pressure.

If conditions agreed mostly to point one above, there could be expected to be an increased height of the plate, as the younger cells then should have continued the growth, while the calcification border had slowed down during decreased oxygen tension as recorded at 10 per cent of oxygen in nitrogen described above in this chapter. This slow-down was maximal in period +1, up to 24 hrs. after the end of the exposure to 10 per cent of oxygen. This moment was therefore chosen for sacrificing 32 rabbits at the age generally used. Half of the series was treated in low oxygen, half in air. The proximal tibia of one side was taken for fixation in formaline, decalcified, sectioned with a microtome and mounted. Standard staining with haematoxylin-eosin was used and the height of the cartilage plate was measured with the same optic equipment as elsewhere in this investigation. The readings were made "blind", with decodification of tests and controls after the reading. Efforts were made to measure on sections where the columns of the cells were longitudinally cut. The other tibia in each animal was taken for ultraviolet microscopy of the oxytetracycline labelling made simultaneously. The growth rates showed the decreases described. The statistical comparison of the height of the plate in tests and controls showed an almost significant difference, the height being less in animals exposed to 10 per cent of oxygen ($t=2.17^x$, 31 degrees of freedom). The differences were

quantitatively small. This result implies that the effect was not solely localised to the degenerating layer of the cartilage plate but to a major part. Point three was excluded by the fact that decreases in growth were observed already during the exposure (Fig. 9 and 10). If the height of the plate had been found to be unchanged by the hypoxia it had then been necessary to measure it immediately at the end of the exposure to rule out a possibility, that it had regained its thickness during the 24 hrs. after exposure, which now were allowed to pass. There is a parallelity between the metaphyseal growth and the height of the cartilage plate in less acute experiments (*Hansson 1967*) but to limit the present work it was not carried on further, and the other experiments performed were not supplemented by this method.

At this point it was necessary to consider the possibility that the changes observed were not directly due to the reduced oxygen tension, but to a respiratory alkalosis with hypocapnia caused by the hyperventilation, which was induced by the oxygen lack. This hyperventilation did not influence on the body temperature, as measured immediately after the exposure to a simulated altitude of 5900 meters for 8 hrs. The rectal temperatures before and after treatment showed no differences between 7 treated animals and 5 controls. To prevent the out-washing of carbon dioxide during the hypoxemic hyperventilation, thereby keeping $p\text{CO}_2$ and the pH of the blood near the normal values (*Honda & Krenzer 1966*) it was decided to add a certain dose of carbon dioxide to the gas with reduced oxygen content. The aim was to see whether this CO_2 addition would change the effects of the hypoxia described above.

RESULTS OF DECREASED OXYGEN TENSION COMBINED WITH INCREASED CARBON DIOXIDE TENSION.

A. Ten per cent oxygen plus five per cent carbon dioxide in nitrogen at 1 atm.abs. with growth recording periods of 24 hrs.

The reason for addition of carbon dioxide to the gas with reduced oxygen tension is given in the end of Chapter 4. It was natural to continue with 10 per cent of oxygen in nitrogen because of the pronounced effects on growth observed at that concentration of oxygen. The problem was to select the concentration of carbon dioxide to be added for preventing the out-washing of carbon dioxide during the hyperventilation, which was induced by the oxygen lack in the experiments described in Chapter 4. It was decided to use 5 per cent carbon dioxide admixture to reach an approximately normal alveolar CO_2 tension. If this concentration would give a slight accumulation of carbon dioxide instead, this factor could be separately studied later. In this part of the investigation the same technic as described in Chapter 4 was used, only with the difference that the gas cylinders contained 10 per cent oxygen plus 5 per cent carbon dioxide all in nitrogen. The treatment was for 8 hrs. at 1 atm.abs. with growth recording periods of 24 hrs. as earlier described.

The results are given in Table 23, with the control values in Table 24. The effects are further recorded in Fig. 11, as the mean differences between consecutive time periods in tests and controls separately. The mean growth in the test group showed a decrease of 4 per cent from the period before treatment to the period of treatment, and a further decrease of 8 per cent to the period after exposure followed by an increase in growth of 8 per cent between period +1 and +2. After this time there was no statistical difference between the changes in test and control groups. The statistical comparison values were: $-1 \rightarrow 0$: $U=3.68^{xxx}$, $0 \rightarrow +1$ $U=4.40^{xxx}$, $+1 \rightarrow +2$: $t=5.52^{xxx}$, $+2 \rightarrow +3$: $t=0.41^-$, $+3 \rightarrow +4$: $t=0^-$, $+4 \rightarrow +5$: $t=0.95^-$. The degrees of freedom can be seen in Table 23 and 24.

As in chapter 3 the *temperature* was measured to see whether the hyperventilation induced by this hypoxia had influenced on the body heat regulation. In all 15 rabbits, with 7 controls and 8 test animals were used for this. In opposit to the increased and decreased oxygen exposures an effect was now measurable. At the end of the exposure to 5 per cent carbon dioxide plus 10 per

Table 23.

Reduced oxygen tension with addition of carbon dioxide to prevent hyperventilatory hypocapnia. Exposure to 10 per cent oxygen plus 5 per cent carbon dioxide in nitrogen at 1 atm.abs. for 8 hrs. during period 0, with growth recording periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5). Longitudinal growth rates in microns in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
GBH I	490	470	460	FVH II	420	410	420	FYH I	490	490	460
GBH II	480	460	460	FV V I	380	410	430	FYH II	480	480	420
GBH V	410	410	410	FV V II	350	370	400	FYH V	460	460	460
GCH I	530	510	500	FXH III	550	550	550	FYV I	480	480	460
GCH II	480	460	450	FXH IV	530	530	520	FYV II	470	470	460
GCH III	500	470	450	FXVI	520	530	520	FZH IV	380	370	360
GCV II	450	430	420	GGH II	470	490	480	FZV II	400	390	400
GDH III	390	300	230	GGH III	480	510	500	GAH III	510	500	490
HDH II	460	460	400	GGH IV	520	570	540	GAV II	400	400	400
HDH III	490	460	420	GGV II	530	550	540				
HDH IV	450	440	350	GHH II	360	390	350				
HDV I	480	460	410	GHH III	400	460	440				
HEH II	460	390	370	GHH V	360	420	380				
HEH III	520	510	470	GHV I	460	500	480				
HEH IV	520	510	480	GJ II	430	480	460				
HEV IV	470	440	380	GJ IV	410	440	390				
HFH I	490	470	400	GJ V	420	450	450				
HFH IV	540	510	450	GKH II	510	550	530				
HFV IV	450	450	370	GKV I	500	510	520				
				GKV II	480	500	540				
Mean growth	477	453	415		454	481	472		452	449	434

Statistical analysis of differences in growth between consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-24	-38	+27	-9	-3	-14
Stand. dev.	22.41	28.73	19.49	23.15	5.00	20.68
t-value	-4.61 ^{xxx}	-5.83 ^{xxx}	+6.19 ^{xxx}	-1.74 ⁻	-2.00 ⁻	-2.09 ⁻
n-value	19	19	20	20	9	9

Table 24.

Control animals for Table 23 (10 per cent oxygen plus 5 per cent carbon dioxide in nitrogen). Exposed to air for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
GBH III	450	420	440	FVH III	450	450	450	FYH III	450	450	460
GBH IV	440	440	440	FVV IV	370	350	350	FYH IV	500	490	460
GCH IV	410	420	430	FXH V	540	530	530	FYV III	470	490	470
GCV I	410	410	410	FXV II	550	550	550	FYV IV	490	480	480
GCV III	440	430	450	FXV III	530	520	520	FZH II	440	430	420
GCV IV	480	480	480	FXV IV	550	540	540	FZH III	390	380	390
GDH IV	450	450	440	GGH I	490	490	450	GAH I	440	440	430
GDV I	420	420	420	GGV I	490	510	460	GAV I	470	460	450
HDH I	500	490	490	GGV III	480	500	490	GAV III	440	440	440
HDI II	420	400	420	GGH I	450	450	440				
HDI III	510	500	470	GHH IV	400	390	380				
HEH I	500	500	500	GHV II	490	480	440				
HEH V	440	450	460	GHV III	390	390	380				
HEV I	430	440	430	GHV IV	340	340	330				
HEV II	520	510	510	GJ I	480	480	470				
HEV III	510	510	500	GJ III	420	420	410				
HFH III	470	470	460	GKH I	490	470	470				
HFV I	470	480	490	GKH III	500	500	500				
HFV II	450	440	440	GKV III	510	530	510				
HFV III	450	460	460								
Mean growth	459	456	457		469	468	456		454	451	444

Statistical analysis of differences in growth between the consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-3	+1	-2	-12	-3	-7
Stand. dev.	10.70	12.10	11.67	15.37	10.00	13.23
t-value	-1.04-	+0.37-	-0.59-	-3.28 ^x	-1.00-	-1.51-
n-value	20	20	19	19	9	9

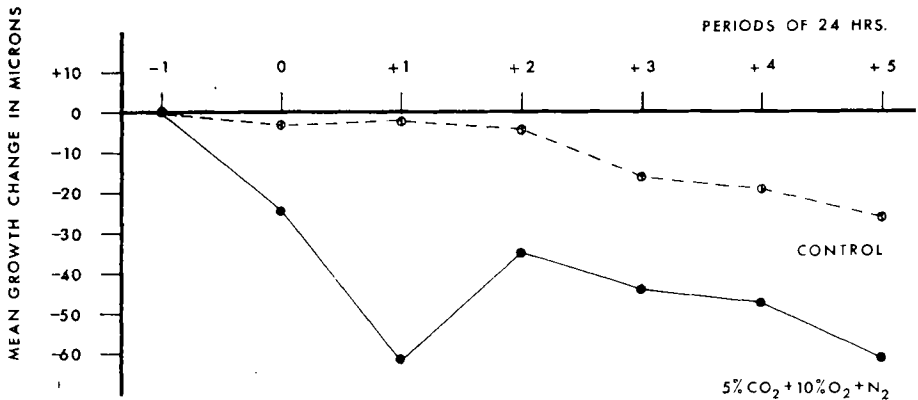


Figure 11.

Longitudinal growth of the proximal tibial metaphysis in rabbits at exposure to a gas mixture with 10 % of oxygen and 5 % of carbon dioxide in nitrogen at normal barometric pressure. Exposure time 8 hrs. during period 0. Growth recordings by oxytetracycline labelling for seven consecutive days. Mean growth changes in microns from day to day, measured within the same animal. Figures made continuous by overlapping observations in period +1 and +3.

cent oxygen all in nitrogen, the rectal temperature of the control animals was in mean 38,6 °C and in the test group 37,9°C. The difference was significant, which means that the temperature factor had to be taken into consideration later (Chapter 7). Further more, this hyperventilation, when influencing on the temperature, could be expected to influence also on the *water balance of the animals*. To evaluate this possibility 6 animals were exposed as above and the haematocrite and the haemoglobin values of venous blood was determined before and immediately after. The haematocrite values ranged between 33 and 44 per cent but without differences between tests and controls. The haemoglobin was between 9.6 and 13.6 g per cent, also with no difference between tests and controls.

It was considered indicated also to study the possible rôle played by carbon dioxide alone when given in increased dose at an unreduced oxygen partial pressure.

RESULTS OF INCREASED CARBON DIOXIDE TENSION IN AIR

The results hitherto presented have shown a variation in growth on alteration of the inspired oxygen tension. It has also been demonstrated that the decrease in growth at reduced oxygen is not due to the concomitant reduction of the carbon dioxide tension during the hypoxaemic hyperventilation. These observations on hypoxaemia with and without hypocapnia also represent observations on decreased (Chapter 4) and undecreased (Chapter 5) carbon dioxide tension. As the effects on growth were not changed by the difference in carbon dioxide tension, the conclusion can be drawn that a reduction of carbon dioxide does not influence on the growth as measured here, with the limitation of being guilty only in hypoxaemia. As mentioned in the introduction, venous stasis has been held responsible by various authors for stimulated growth in general, and this makes the study of the carbon dioxide factor interesting. Does an increased carbon dioxide tension accelerate the growth rate, for instance, by a vasodilation in bone? To begin with this question can be raised irrespective of the mechanism involved.

It was considered advisable to start with a fairly small dose to avoid carbon dioxide toxicity, which might be present for instance as a depression on the cartilage cell metabolism. The percentages chosen were 3 per cent, 5 per cent and 7 per cent. Higher doses are not tolerated by human volunteers (Fig. 12). Much higher doses can be given to animals before death occurs, but it was considered to be of little interest in this investigation to further increase the carbon dioxide tension because of the pronounced effects on the general condition of animals under such circumstances.

A. Three per cent carbon dioxide in air at 1 atm.abs. with growth recording periods of 24 hrs.

The results are given in Table 25, with control animals in Table 26. The statistical comparison between changes in test and control showed no differences. There were no particular changes in the mean values between the seven consecutive days studied.

Table 25.

Increased carbon dioxide tension at normal oxygen tension by exposure to 3 per cent carbon dioxide in air at 1 atm.abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0,+1, +2, +3) in two overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3
BPH I	530	530	520	BQH I	610	600	600
BPH II	570	570	560	BQH II	540	540	540
BPH III	560	560	560	BQH III	590	590	590
BPV I	570	570	570	BQH IV	590	580	570
BPV II	570	570	560	BQV III	580	570	570
BPV III	550	550	550	BQV IV	570	570	570
BPV IV	580	570	560	BSH I	460	460	450
BPV V	430	430	430	BSH II	500	500	450
BMH II	550	540	520	BSH III	470	450	470
BMH III	550	530	500	BSH IV	480	450	440
Mean growth	546	542	533		539	531	525

Statistical analysis of differences in growth between consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3
Mean diff.	-4	-9	-8	-6
Stand. dev.	6.99	9.94	10.33	17.76
t-value	-1.80 ⁻	-2.84 ^x	-2.45 ^x	-1.07 ⁻
n-value	10	10	10	10

Table 26.

Control animals for Tables 25, 27 and 28 (3, 5, 7 per cent CO₂). Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
BHM I	530	550	520	BQV I	590	590	590	BXV I	540	530	500
BPH IV	540	540	540	BQV II	600	600	600	BXV II	540	540	530
BPH V	560	550	550	BSV I	530	520	500	BVV I	410	400	350
BTH III	590	590	600	BSV II	470	440	410	CAH II	450	400	350
BTH IV	550	550	550	BUH III	450	450	390	CAH III	370	380	400
BTV IV	530	520	520	BUV II	480	480	480	CAH IV	480	480	480
CBH I	560	570	590	BUV III	480	480	480	DCH II	390	390	390
CBV I	470	470	410	BYH II	420	440	420	DCV III	430	410	400
CBV III	520	510	500	BYH III	420	430	420	DDV I	460	460	460
CBV IV	550	540	540	BYV I	410	400	400	DDV II	460	440	480
CCV I	520	520	510	BYV II	370	350	340	DEH II	520	520	510
CCV II	520	520	510	BZV I	610	600	590	DEV III	500	500	490
CCV III	520	520	530	BZH II	610	610	600	DEV IV	510	510	510
CCH IV	500	500	500	BZV II	550	560	560				
CDV I	500	500	500	DFH III	520	530	510				
CDV II	510	510	490	DFH IV	460	470	460				
CVH III	550	550	510	DFV III	470	470	470				
CVV II	520	510	500	DHH II	550	550	550				
CVV III	550	550	530	DHH IV	540	540	530				
CXH I	500	480	480	DHV II	560	550	530				
CXH II	450	430	410	HQH III	490	490	480				
CYH IV	530	530	510	HQH IV	470	480	490				
CYH V	450	470	470	HRH III	510	500	500				
CYV III	510	530	530	HRH IV	550	540	550				
CYV IV	520	530	530	HRH V	520	520	520				
Mean											
growth	522	522	513		505	504	495		466	457	450

Statistical analysis of differences in growth between consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	0	-8	-2	-9	-9	-7
Stand. dev.	10.60	17.00	10.68	14.53	15.53	25.94
t-value	-0.19	-2.47 ^x	-0.75	-3.03 ^x	-2.15	-0.96
n-value	25	25	25	25	13	13

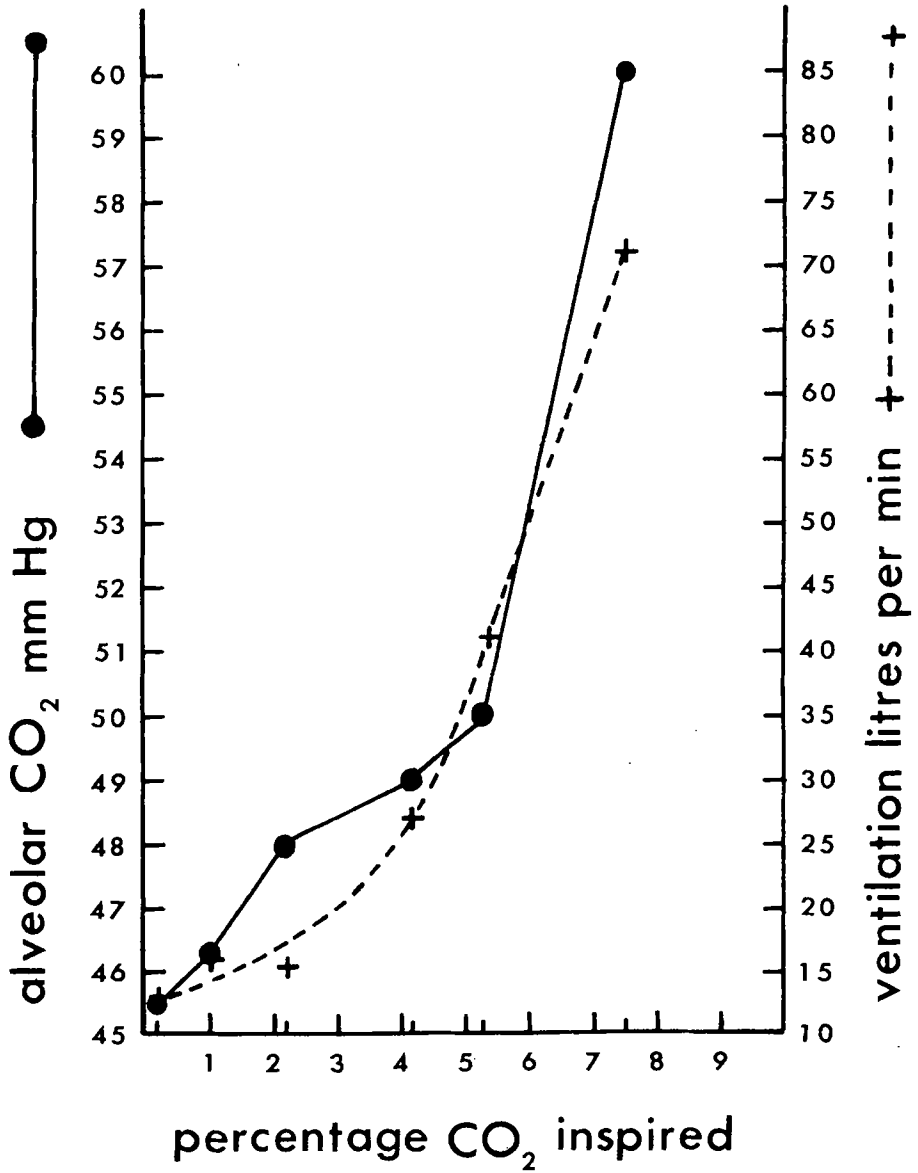


Figure 12.

Alveolar CO₂ pressure and ventilation volume at different percentage of CO₂ breathing in man. (After: Barcroft J., Margaria R., *J. Physiol.* [London] 72: 175, 1931).

B. Five per cent carbon dioxide in air at 1 atm.abs. with growth recording periods of 24 hrs.

The results are given in Table 27, with the control animals in Table 26. The statistical comparison between changes in test and control still demonstrated no significant differences.

Table 27.

Increased carbon dioxide tension at normal oxygen tension by exposure to 5 per cent carbon dioxide in air at 1 atm.abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
BTH I	570	560	550	BUH I	450	480	470	BXH I	510	500	500
BTH II	550	540	540	BUH II	440	440	440	BXH II	550	540	530
BTH V	560	550	530	BUH IV	490	490	450	BXH III	590	580	540
BTV I	560	570	560	BUV I	500	500	480	CAH I	530	530	510
BTV II	590	590	580	BYH I	370	390	350	CAH V	490	490	450
BTH V	510	500	500	BYH IV	400	410	380	CAV II	470	460	480
CBH II	540	550	530	BYV III	360	360	350	CAV IV	490	500	490
CBH III	540	520	530	BZH I	530	520	500				
CBH IV	560	550	520	BZH III	550	540	540				
CBH V	550	550	550	BZV III	570	550	550				
CBV II	530	530	510								
CCH I	500	500	490								
CCH II	520	520	520								
CCH III	500	500	500								
CDH I	400	440	480								
CDH II	500	500	500								
Mean											
growth	530	529	524		466	468	451		519	514	500

Statistical analysis of differences in growth between consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	-1	-5	+2	-17	-4	-14
Stand. dev.	13.26	15.92	14.76	15.67	7.86	21.49
t-value	-0.19-	-1.26-	+0.43-	-3.42 ^x	-1.45-	-1.76-
n-value	16	16	10	10	7	7

Table 28.

Increased carbon dioxide tension at normal oxygen tension by exposure to 7 per cent carbon dioxide in air at 1 atm.abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during successive periods of 24 hrs. (-1, 0, +1, +2, +3, +4, +5) in three overlapping series of animals:

Animal	-1	0	+1	Animal	+1	+2	+3	Animal	+3	+4	+5
CVH I	550	550	560	DFH II	530	530	530	DCH I	410	370	330
CVH II	550	550	530	DFV II	550	540	540	DCH III	400	400	410
CVH IV	530	520	510	DGH II	560	550	550	DCV II	360	360	380
CVV I	540	540	530	DGV I	550	540	540	DDH I	510	510	510
CXH III	450	430	410	DGV II	540	570	520	DDV III	490	480	480
CXH V	370	400	400	DHH I	530	530	530	DEH I	500	510	500
CYH I	540	540	540	DHH III	550	550	540	DEH IV	530	530	500
CYH II	510	500	480	DHV I	540	540	520	DEV II	490	480	490
CYV I	500	510	510	DHV III	540	520	500				
CYV II	500	510	490	HQH I	450	450	460				
				HQH II	380	400	430				
				HQV I	480	460	420				
				HQV II	460	460	460				
				HQV III	460	460	440				
				HRH I	490	500	500				
				HRH II	540	530	530				
Mean growth	504	505	496		509	508	501		461	455	450

Statistical analysis of differences in growth between consecutive periods above:

	-1 → 0	0 → 1	1 → 2	2 → 3	3 → 4	4 → 5
Mean diff.	+1	-9	-1	-8	-6	-5
Stand. dev.	13.70	11.00	13.10	19.15	15.06	20.70
t-value	+0.23 ⁻	-2.59 [×]	-0.38 ⁻	-1.57 ⁻	-1.18 ⁻	-0.69 ⁻
n-value	10	10	16	16	8	8

C. Seven per cent carbon dioxide in air at 1 atm.abs. with growth recording periods of 24 hrs.

The results are given in Table 28, with the control animals in Table 26. The statistical comparison gave the following values: -1 → 0: $t=0.14^-$, 0 → +1: $t=0.10^-$, +1 → +2: $t=0.09^-$, +2 → +3: $t=0.25^-$, +3 → +4: $t=0.43^-$, +4 → +5: $t=0.18^-$. The insignificant changes in growth recorded are illustrated in Fig. 13 for 3, 5 and 7 per cent carbon dioxide in air and the control

animals simultaneously treated with air alone. The controls are added together in one table here, as also in Chapter 4 (A—H) for the studies utilizing the hypobaric chamber.

As this part of the investigation had not disclosed any changes in growth at a moderate increase in the carbon dioxide tension in air, it was considered to be advisable to increase the sensitivity of the test by reducing the observation time to 12 hrs., thereby covering $\frac{2}{3}$ of period 0 with the carbon dioxide exposure instead of $\frac{1}{3}$ as in A—C above. Growth measuring periods of 12 hrs. were used also in Chapters 3—4. It was decided convenient for the comparisons to keep the same time periods although the toxicity character of carbon dioxide at the doses applied did not necessitate this. As no effects had been observed with the carbon dioxide exposures so far, 7 per cent carbon dioxide in air was chosen for the 12 hrs. study.

D. Seven per cent carbon dioxide in air at 1 atm.abs. with growth recording periods of 12 hrs.

The results are given in Tables 29 and 30. The statistical comparison between test and control showed no significant influence on the growth rate from this

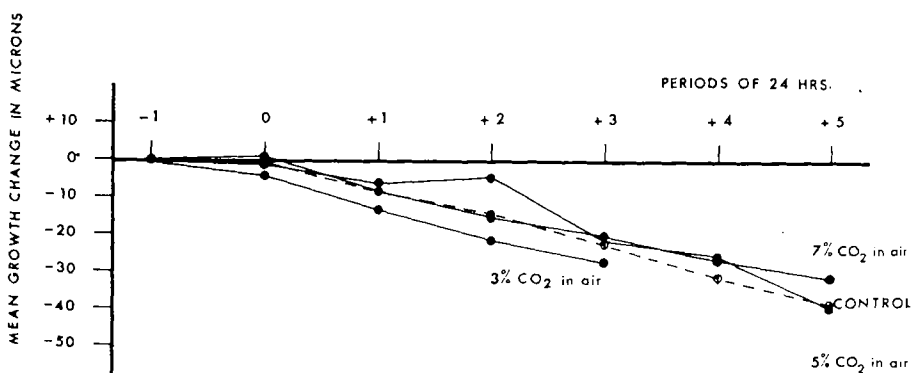


Figure 13.

Longitudinal growth of the proximal tibial metaphysis in rabbits at exposure to increased concentrations of carbon dioxide (3, 5, 7%) in air at 1 atm.abs. for 8 hrs. during period 0. Growth recordings by oxytetracycline labelling for seven consecutive days. Mean growth changes in microns from day to day, measured within the same animal. Figures made continuous by overlapping observations in period +1 and +3.

Table 29.

Increased carbon dioxide tension at normal oxygen tension by exposure to 7 per cent carbon dioxide in air at 1 atm.abs. for 8 hrs. during period 0. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
ERH II	220	220	200
ERH III	240	230	210
ERV II	250	250	240
ERV III	250	250	240
ESH III	170	160	160
ESH IV	240	230	210
ESV II	250	250	240
ETH I	270	260	260
ETH III	280	270	270
ETV II	270	260	260
HOH I	210	210	210
HOH III	230	240	230
HOH IV	240	230	230
HOH V	230	230	240
HOV I	230	230	230
EPH I	270	270	260
HPH II	250	250	250
Mean growth	241	238	232

Statistical analysis of differences in growth between consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	-4	-6
Stand. dev.	6.06	8.70
t-value	-2.40 ^x	-2.79 ^x
n-avalue	17	17

partial pressure of carbon dioxide in air. ($-1/2 \rightarrow 0$: $U=0.64^-$, $0 \rightarrow +1/2$: $U=0.98^-$).

Carbon dioxide at a concentration of 7 per cent in air is a breathing stimulant and this hyperventilation could be expected to lower the body temperature. (Chapter 6) In 18 animals, divided into 9 in test and 9 in control the rectal

Table 30.

Control animals for Table 29 (7 % CO₂ in air). Exposed to air for 8 hrs. during period 0. Longitudinal growth rates in microns during three successive periods of 12 hrs. ($-1/2$, 0, $+1/2$):

Animal	$-1/2$	0	$+1/2$
ERH V	250	240	240
ERV I	190	200	200
ERV IV	250	250	240
ESH I	190	170	180
ESH II	170	170	160
ESV IV	260	250	260
ETH II	240	250	240
ETV I	280	270	260
ETV III	250	290	220
HOH II	230	230	240
HOV II	210	210	210
HOV III	220	220	220
HOV IV	230	220	230
HPH III	240	240	240
HPH IV	240	240	230
Mean growth	230	230	225

Statistical analysis of differences in growth between consecutive periods above:

	$-1/2 \rightarrow 0$	$0 \rightarrow +1/2$
Mean diff.	0	-5
Stand. dev.	13.63	19.59
t-value	0	-1.05
n-value	15	15

temperatures were measured immediately at the end of the 8 hrs. but no significant difference was observed in this case in contrast to the study of 10 per cent O₂+5 per cent CO₂ in nitrogen. Therefore no determination was performed on haemoglobin and haematocrite values because the hyperventilation, even when inducing a reduced temperature, did not alter these values (Chapter 6).

This part of the investigation had not demonstrated any effects on the longitudinal growth rates of the proximal tibial metaphysis during or after increase in the respiratory carbon dioxide concentrations to 3—7 per cent for 8 hrs. and with an observation time of 6 consecutive periods of 24 hrs., thus covering the maturing time of the epiphyseal cartilage cells in the growth plate (*Messier & Leblond* 1960). The partial pressure of carbon dioxide in venous blood is in man about 50 mm Hg (*Patterson et al.* 1955) which would correspond to about 6.5 per cent CO₂ in air at a pressure of 760 mm Hg. The use of 7 per cent here has consequently been well above the normal venous blood tension. The partial pressures used in this investigation must have been sufficiently high for the purpose of the study. A further elevation of the carbon dioxide tension up to growth depressing concentrations was beyond the scope of this study.

CHAPTER 7.

DISCUSSION

The activity of investigation into the bone growth and related problems is considerable. A search of the literature through the years 1964—67 by the MEDLARS system of automatic data analysis at the Karolinska Institutet, Stockholm, Sweden, yielded 612 references on *Bone Growth* and 377 on *Blood Flow in Bone and Extremities*. The aspect from which the author has investigated this problem concerning endochondral growth of bones seems not to be reported, but it has been used in tissue cultures and in studies on wound healing, fracture healing and periosteal proliferation (Chapter 1). Changes in the blood circulation, arterial as well as venous, is closely interrelated to changes in the tensions of the respiratory gases of the tissues, besides the concomitant alteration in the transport of other components of the blood. It was decided that the growth in length of bones should be analysed at a primary change in the gaseous pressures, to see whether a knowledge in this uninvestigated sector of bone physiology would shed some light on the clinical problem of leg length inequality.

A sensitive **method** for recording of growth in length was necessary, because the exposures to the test gases had to be short if acclimatization reactions (*Altland & Highman 1952*) and the toxicity of oxygen (*Bean 1945*) were to be avoided (Chapter 2). This means that a daily recording of the growth had to be possible, because the oxygen toxicity is induced within hours during pure oxygen breathing at increased barometric pressure both in rabbits and humans (*Binger et al. 1927, Bean 1964*). The intravital oxytetracycline labelling method was well known at the institution and was the most sensitive for quantitative measurements of daily growth in length in rabbits (*Hansson 1967*). Therefore, the choice of this method needs no further discussion.

With the aim of inducing alteration first in the oxygen tension and secondly also in the carbon dioxide tension of the blood it seemed convenient to perform this by respiratory gas changes. In this way it was possible to utilize data from

the literature of respiratory physiology and at the same time to perform the experiments without direct influences on the extremities, as for instance in perfusion technics. This would introduce other factors influencing on growth such as trauma, immobilisation, venous stasis or denervation (*Sundén 1967*). It was decided as a compromise between reduction of errors of the method and avoiding of the oxygen toxicity to use 8 hrs. of treatment with increased oxygen tensions; The same times were used for the whole investigation to make comparisons easier, although the toxicity factors of the different exposures were not the same. The treatment was given only once and continuously during the end of the day named period 0 (Fig. 2 and 3). This localisation was intended to prevent coverings of eventually disphasic responses (*Persson 1967*).

Experimental works with growing rabbits always result in a considerable mortality during the period when they are no longer feeding from the mother. To reduce errors from this, defined conditions were set out for exclusion of aberrant animals in a way which was intended not to influence on the reliability of the results obtained (Chapter 2). By the 6 principles for exclusion, the total number of animals in this investigation was reduced from 1079 to 948, which seems reasonable in rabbits of this age (Table 1). Of this material 237 rabbits were controls with notes on age, weight and longitudinal growth of the proximal tibial metaphysis all measured on the same day. With the rabbits arranged in groups of 5 days difference in age, there was found to be a significant or highly significant positive correlation between the weight and the growth in length (Chapter 2). This is not in accordance with *Hansson (1967)*, but his analysis was performed within each litter and with animals excluded below defined weight limits.

The **statistical treatment** of the material is described in Chapter 2. It was decided that comparisons of changes in growth should be based on changes within each animal, thereby reducing the variation in the samples regarding their growth in absolute values. Only three consecutive time periods of growth were measured in each animal, and to cover the total maturing time of the cartilage cells (*Kember 1960, Messier & Leblond 1960*) three overlapping series of animals were used in each study thereby giving a series of 7 consecutive days of growth (Fig. 2 and 3). The mean differences of growth from day to day within the same animal are illustrated in the figures. Some figures are in addition made up as vertical comparisons of the growth in absolute mean values between a test group and a control group, both made up of litter-mates.

Some basic principles and data from the respiratory physiology are mentioned in the end of chapter 2, concerning mostly alveolar and blood gas tensions at the partial pressures of gases used in this investigation. Many of these values are from human experiments but as the rabbit has about the same oxygen dissociation curve (Fig. 14) of haemoglobin and the same pH, the same principles apply to them, although the absolute values can be a bit different because of differences in body temperature, temperature regulation, respiratory dead space

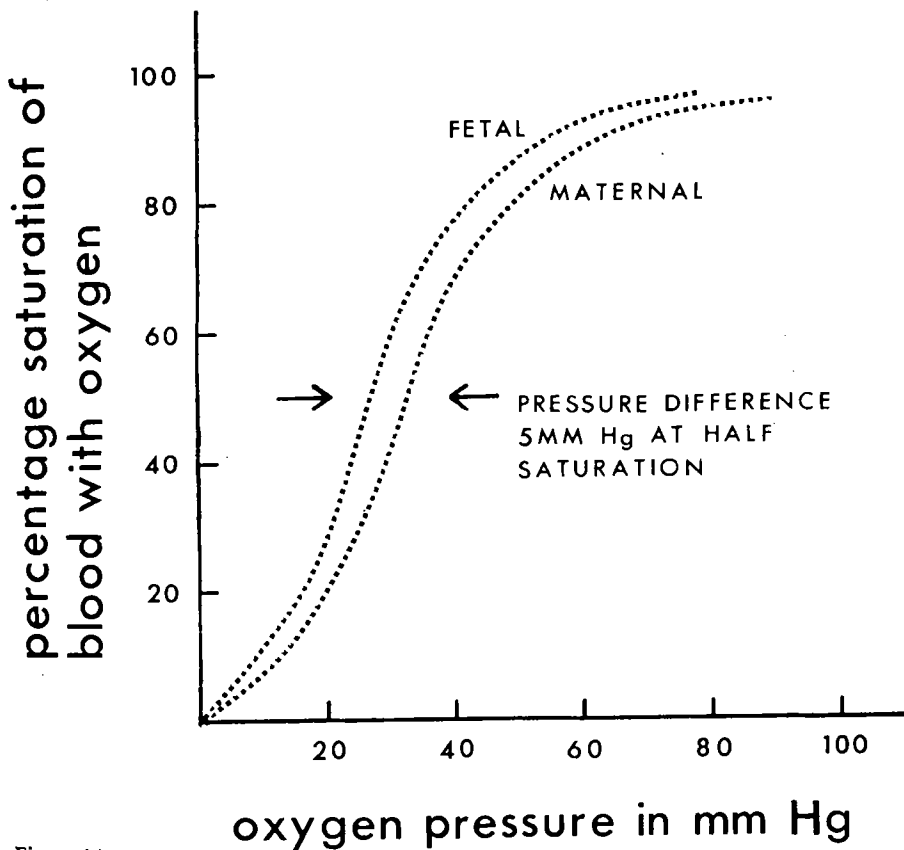


Figure 14.

Oxygen dissociation curves of maternal and fetal bloods from rabbits near term. (After: Barron, D. H., Meschia, G. Sympos. quant. Biol. 19: 93, 1954).

and so on. These differences are not unknown (*Dittmer & Grebe 1958*), but in this investigation the absolute quantitative changes were of less importance than the principal alterations induced. For this reason it was considered to be unnecessary to make special analyses of the blood gas tensions, pH and so on, in the rabbits used for the study. For the ages used in this investigation, it can be deduced from the curves of haemoglobin dissociation at birth and at adulthood (*Fig. 14*), that the actual values have been somewhere between the two curves and very near the human dissociation curve (*Dittmer & Grebe 1958*).

The investigation begins with testing of **increased oxygen tensions**(Chapter 3) with omittance of the intervals earlier tried (*Persson 1967*). It proved to be above the limit of oxygen toxicity to use 100 per cent oxygen for 8 hrs. at 2 atm.abs. as judged by autopsy of the lungs of the animals. There was, however,

a small but statistically insignificant increase in growth during the oxygen treatment at 2 atm.abs., followed by a significant decrease in growth during the 12 hrs. after withdrawal of oxygen. This might be the result of the oxygen toxicity to the lungs (*Smith 1899*) as it was not observed when the oxygen pressure was reduced to 100 per cent at 1 atm.abs.. At this tension there was no mortality and no lung changes either and the increase in growth during the treatment was now statistically significant, measuring 5 per cent in absolute mean value compared to the controls. The results mentioned above were achieved with growth recording periods of 12 hrs.. The decrease in growth during the time period immediately after the hyperoxygenation, observed at 2 atm.abs., was not significant after a continuous exposure for 8 hrs. at 1 atm.abs. of pure oxygen. When the observation time was extended for 5 days after the gas exposure (Chapter 3, C) the same changes were observed, with a significant increase in growth (+3 per cent compared to the control) during the period of hyperoxygenation, but with no further changes with comparison between test and control for the following 5 days. This should imply that the stimulation of growth, which was observed to start during the hyperoxygenation, continued for the following time periods. If it had ceased suddenly, there should have been a decrease in growth again with comparison between the changes in test and control from some period to the next, and this was not the case. Only during period +5 in the test group, there was a significant decrease in growth, but in comparison with the changes in the control group this significance was lost. There was also a significant decrease in growth during period +1 compared to period 0 but this is of the same magnitude both in test and control. The reason for this decrease in both groups might be that the animals had been taken away from their mother for the first time during the day before, and also that intravenous injections had been started the day before that. Such stress factors could have some temporary influence on the growth rate. There is also another possible factor in this decrease. From the earlier investigations of hyperoxygenation at 1 and 2 atm.abs. but with growth recording periods of 12 hrs. reported above, there had been significant signs of such a decrease. When the measuring microscopy is performed as a blind test, it is possible, that expectancies of changes, influence on the readings, within the limits of error of the labelling line identification. But the blind handling in this case will affect the control and test equally, thereby eliminating some significances when the comparison is performed between the changes in test and in the control. Returning to the observations made at 1 atm.abs. during 100 per cent of oxygen breathing for 8 hrs. and with growth recording periods of 24 hrs. the question remains to be answered, if the stimulation during the exposure proceeds also on the following days, which is suggested by the fact that no later decrease is statistically verified. Looking at the absolute values of growth rates, (Tables 6 and 7) it seems as if the increase in growth during period 0 in the test group is lost during periods +1, but in the control group the values of growth decrease

as much. During period -1 the growth differs only 7 microns in mean value, during period 0 it differs 22 microns and during period +1 it differs 23 microns between test and control. The same difference in absolute growth rates between test and control remains during period +1 to +3 (Tables 6 and 7) but in the last series of animals, covering periods +3 to +5 there seems to be no difference in the mean values. This could be a coincidence but it implies that the stimulation had ended after about 72 hrs. that is during period +3 about. This is illustrated in Fig. 7. The type of calculation used for Fig. 7, has not been used for the statistics because it introduces another error, which was considered to be principally larger, i.e. comparison of changes in growth rates, which are not within the same animal. Unless the samples are very large it can not be expected that the mean growth in the control group should be the same as in the test group, as measured in absolute values of growth. It is probable, that the stimulation recorded in period 0, measured to about 3 per cent above the control, is so near the error of the method (*Hansson 1967, Persson 1967*) that it was impossible to get a significant measure of the return to a normal growth rate, if this return occurs over a couple of days around period +3, thus measuring only some per cent from one day to the other. It should also be noted that, at the age of rabbits used, there is a normal decrease in growth rates of about 1 per cent daily (Fig. 15). Variations in this could influence on occasional samples.

To conclude on the analysis of increased oxygen tensions, there was found to be a small stimulation of the longitudinal growth of the tibial, proximal metaphysis during breathing of 100 per cent of oxygen at 1 atm.abs. for 8 hrs.. In the series with 24 hrs. as the labelling period, the increase was 3 per cent. When the labelling period was 12 hrs. instead, the increase should have been about 6 per cent theoretically as will be explained below. It was noted to be 5 per cent (Chapter 3). This means that the actual increase in growth during the oxygen treatment in period 0, (that is 8 hrs. of the 12 or 24 hrs. used as the labelling interval) should have been about 7.5 to 9 per cent above the growth rate before treatment. This conclusion can be made because of the positioning of treatment in relation to the oxytetracycline injections (Fig. 2—3) in such a way, that period 0 ends very close to the end of the oxygen treatment. This makes the hrs. before the start of treatment, still included in period 0, to have a growth rate like that of the control day named period -1, as long as there is no diurnal variation (*Persson 1968*). It is not possible to say if the increase in growth rate mentioned was the same during all the 8 hrs. of hyperoxygenation. It is of interest to compare this degree of stimulation, with the values reported after peripheral nerve division (*Sundén 1967*), also in rabbits and with the oxytetracycline method. In comparison between the operated and the non operated side, there was a difference of 23 per cent on the sixth day after the nerve section, with the higher growth rate on the paralyzed side. This seems to be a greater stimulation, but it should be remembered, that there might have

been a certain retardation also on the control side, due to the catabolic phase after the operation. This factor has been shown to influence on the growth rate, measured with the same technic by *Hansson* (1967). He recorded a relative stimulation of growth after medullary plugging of tibia, measuring up to 10 per cent above the control side. This is more of the magnitude measured in the present study. It is not possible, with the available information, to say whether the calculated growth acceleration (+7.5 to 9 per cent) observed during hyperoxygenation was near or far from the maximal growth rate of the cartilage, that is, if oxygen, at least for a short time, can speed up the growth rate fully, or if other factors are of greater importance in addition.

The effects on growth seen during oxygen breathing may theoretically be due to some secondary factor and not to the changes in oxygen availability itself. However, breathing 100 per cent of oxygen does not seem to influence on arterial pH and $p\text{CO}_2$ (*Dittmer & Grebe* 1958) although smaller changes occasionally are reported. Heart rate, blood pressure and vascular resistance are also nearly unchanged (*Egger et al.* 1962). Ventilation volumes have been reported to be slightly decreased (*Hejneman* 1943) but also to be increased (*Lambertsen et al.* 1953). It has been advocated to be an accumulation of carbon dioxide in tissues during hyperbaric oxygenation, due to blocking of the haemoglobin by the oxygen (*Bean* 1945). *Lambertsen et al.* (1953) have shown that this is not the case in rabbits, breathing 3.5 atmospheres of pure oxygen. This was investigated by analysis of subcutaneous carbon dioxide gas depots and there was no increase above the controls until after 4 hrs. of oxygen treatment, that is when the pulmonary toxicity influences on the gas exchanges. Thus, there is no unequivocal support for the possibility that the growth effects of oxygen is mediated by the abovementioned factors. Changes in the respiratory oxygen tensions influence also on the blood circulation, which will be discussed below in connection with decreased oxygen tensions and changes in the carbon dioxide tension, which are also vasoactive.

Results of decreased oxygen tensions are given in Chapter 4. This part of the investigation comprises 461 accepted animals, distributed on hypoxic conditions corresponding to air-breathing at 3000, 4000, 5000 and 6000 meters altitude in a hypobaric chamber, and on exposures to 12 and 10 per cent of oxygen in nitrogen at normobaric environmental pressure. The experiments showed that 3000 meters (corresponding to a $p\text{O}_2$ of about 108 mm Hg) did not statistically affect the growth rates of the proximal tibial metaphysis for the time of exposure used. At 4000 meters there was a highly significant decrease in growth from the day of exposure to the day after, and another highly significant increase in growth from the first day after to the second day after exposure (Chapter 4, F). The arithmetic mean of the decrease was 11 per cent, and of the following increase it was 5 per cent in comparison between changes in the test and in

the control group. The result at 5000 meters were about the same. At 6000 meters the effects were still more pronounced, now with a highly significant decrease in growth on the day of treatment compared to the day before, measuring 9 per cent in mean difference. This was followed by a further decrease of 20 per cent to the day after treatment, still highly significant. From period +1 to period +2 after treatment there was again a significant increase in growth, now measuring a little more than 4 per cent only.

These results were obtained in a hypobaric chamber. To rule out the possibility, that the effects on growth were caused by other factors than the reduction in the oxygen pressure, the analysis was continued by testing 12 per cent and 10 per cent of oxygen in nitrogen at a normal barometric pressure. The same tendencies were again observed, with a decrease in growth from the day before to the day of treatment, measuring 7 per cent at 10 per cent of oxygen. From the day of treatment to the day after there was another significant decrease of 7 per cent. In this connection it should be remembered that the relation between exposure and measuring periods was the same as in the pure oxygen studies. This means that the reduction of 7 per cent recorded during the hypoxic exposure implied that the actual reduction were three times greater, that is about 21 per cent during the 8 hrs. of treatment within the 24 hrs. of oxygen labelling in period 0. If the same considerations are applied to the studies on simulated altitudes we can deduce that the decrease in growth during hypoxic conditions (4000—6000 meters) were between 10.5 per cent and 33 per cent. The growth rate during the day after treatment was in general a little less depressed than these figures, contrary to the impression given by the illustrations (Fig. 8 and 9). Returning to the study of 10 per cent of oxygen in nitrogen there was no statistical significance in the increase of growth from period +1 to +2 contrary to the altitude exposures. Looking at the absolute values, however, there was such an increase, which was significant in the 10 per cent oxygen test group and not significant in the corresponding control group. With comparison between the two groups this significance was lost. The mean difference between periods +1 and +2 was +6 per cent, which is the same magnitude as earlier observed in the hypobaric chamber. The samples used at the 10 per cent of oxygen test were, however, fairly small at these time periods (10 and 9 animals in test and control). It was therefore concluded, on the basis of the total material, that the effects on the longitudinal growth were the same, whether the hypoxia was caused by means of a hypobaric chamber or by exposing the animals to a gas mixture with oxygen of the corresponding partial pressure. For the utilized time of exposure, it can further be concluded that 3000 meters does not influence on growth, that is a barometric pressure of about 520 mm Hg and an oxygen partial pressure of about 110 mm Hg, compared to the normal pressure 159 mm Hg. At 4000 and 5000 meters there were pronounced but temporary effects on growth. At 6000 meters or at 10 per cent of oxygen (pO_2 about 75 mm Hg) the reduction was more pronounced.

ced, but now not fully reversible. This is in accordance with Campbell's findings (1935), that rabbits were able to acclimatize to 12 per cent but not to 10 per cent of oxygen, at which they did not keep their normal weight. If the tested degrees of hypoxia are transformed to alveolar oxygen partial pressure (*Luft* 1961) and from that to haemoglobin oxygen saturation (*Roughton* 1965) it is found that there was no influence on the growth rate at an arterial haemoglobin oxygen saturation above 90 per cent and that the changes were evident but lasting only for 24 hrs. after exposure, if the haemoglobin oxygen saturation was somewhere between 70 and 90 per cent. If the oxygen saturation was reduced to about 65 per cent the mean growth rates did not fully return to the normal levels for the observation time used, that is up to 5 days after the hypoxia.

Reduced oxygen tension induces a hyperventilation, which in turn lowers the carbon dioxide tension (Chapter 2). On the other hand pure oxygen breathing could have a slight tendency to increase the tissue carbon dioxide content by reducing the ventilation and by blocking parts of the carbon dioxide transport (*Bean* 1945). For these two reasons it seemed necessary to study effects from alteration in the carbon dioxide tensions also. Actually the study, reported above on decrease in oxygen, represents a study on decrease in carbon dioxide as-well. Taking the 10 per cent oxygen in nitrogen for example, this represents a condition with the alveolar pO_2 lowered from about 100 to about 34 mm Hg, and at the same time a lowering of the alveolar pCO_2 from 40 to about 30 mm Hg in humans (*Luft* 1961). By adding 5 per cent carbon dioxide to the 10 per cent of oxygen in nitrogen, it was considered possible to counteract the out-washing of the carbon dioxide during the hyperventilation. In this way, it should be possible to compare the effects on growth from low oxygen + low carbon dioxide with a condition of low oxygen alone.

In Chapter 5 results are presented from testing of **ten per cent of oxygen in combination with five per cent of carbon dioxide in nitrogen**. A comparison of the changes in growth from day to day between test and control showed, that there was a decrease in growth between the day before and the day of exposure, measuring 4 per cent in mean, a further decrease of 8 per cent during the 24 hrs. after exposure (+1), and finally an increase during the next day (+2) measuring 8 per cent. All these differences between the test group and the control group were highly significant. This means that the effects on growth in length of the metaphysis were about the same in the hypoxic experiments, whether the carbon dioxide tension was allowed to be reduced by the hyperventilation or not. This in turn means that the change in growth was induced by the changes in oxygen tension.

At this point it is of interest to discuss the differences between hypocapnic and normocapnic hypoxia, which differ with regard to the haemoglobin dissociation curves leading to differences in the amount of oxygen available

at a given oxygen tension. *Lennox and Gibbs* (1932) have determined the oxygen and carbon dioxide contents of arterial and venous femoral blood in humans inhaling various mixtures containing these gases and nitrogen. At an oxygen concentration of 6—12 per cent in nitrogen the arterial oxygen content decreased from 17.5 to 16.5 vol. per cent, and the venous oxygen content from 13.5 to 12.3 vol. per cent. At the same time the arterial carbon dioxide content decreased from 51.4 to 49.5 vol. per cent and the venous carbon dioxide content changed from 54.1 to 54.3 vol. per cent as a mean of 4 test subjects. When 4—6 per cent of carbon dioxide was added to the same oxygen concentration the arterial oxygen content decreased from 18.8 to 16.5 vol. per cent, the venous oxygen content from 15.6 to 13.3 vol. per cent and the carbon dioxide content increased in arterial blood from 47.7 to 49.1 vol. per cent and in venous blood from 50.2 to 51.0 vol. per cent. The changes recorded means changes from normal air breathing to the test gas specified after 10—15 min.. The referred values imply that the addition of carbon dioxide was enough to counteract the reduction in carbon dioxide tension caused by hypoxic hyperventilation at an oxygen concentration of 8—12 per cent in nitrogen. About the same inspiratory gas concentrations were used in the present investigation. It also means that the addition of carbon dioxide did not reduce the magnitude of decrease in the arterial oxygen content during 8—12 per cent of oxygen breathing. This could have been suspected for the reason that the added carbon dioxide further increases the hyperventilation caused by the hypoxia. On the other hand it could also have been expected to be a reduced oxygen content in the blood after the addition of carbon dioxide, due to the increased acidity and the resulting "Bohreffect" on the haemoglobin dissociation curve, which gives a reduction in the oxygen binding capacity at increased carbon dioxide tensions (*Steen* 1968). The reported oxygen contents above (*Lennox & Gibbs* 1932) showed this tendency. It is possible that these two counteracting effects (hyperventilation and Bohr-effect) of the added carbon dioxide explain why no difference was demonstrated in the growth rate studies. Effects on the blood flow could also be expected from the added carbon dioxide (see later).

For a further understanding of the possible effects caused by carbon dioxide on growth, it was studied during normoxic conditions also. **Addition of carbon dioxide to air** could influence on growth rate by influence on the oxygen tensions, due to hyperventilatory increase of the alveolar ventilation, or to Bohr-effects on the haemoglobin dissociation curve as discussed above for the hypoxaemia experiments. It could also influence on the bone blood flow (see later). Growth recordings were made at 3, 5 and 7 per cent of carbon dioxide in air at 1 atm. abs. for 8 hrs.. No significant effects on the growth rates were observed (Chapter 6). Observations were made for five days after the end of the exposures. An increased growth could have been expected by the fact that the hyper-

ventilation, that is induced, increases the alveolar oxygen tension. Furthermore, an increased oxygen availability in the bone could have been caused by the Bohr-effect. In contrast to its consequences during hypoxic conditions, it has theoretically a promoting effect on the oxygen transport during normoxia. This is due to the fact that the dissociation curves at normocapnia and at hypercapnia coincide at a normal arterial oxygen tension, but divide at a reduced oxygen tension, liberating more oxygen in the capillary system. The gain in oxygen availability caused by this mechanism is probably too small to give measurable effects on growth, and the same is probably the case with the gain due to hyperventilation. The moderate increase in growth caused by a five-folded oxygen tension (Chapter 3) makes this conclusion reasonable. On the other hand it could have been a slight depressing effect on the cell metabolism from the increased carbon dioxide tension, counteracting the increased oxygen availability. Finally, it is also possible that carbon dioxide influences on the bone blood flow (see later).

In addition to the discussion of the separate results above, some common factors will be considered below, that is the possible effects caused by changes in temperature and in the bone blood flow.

As mentioned **temperature** has been considered to be a possible regulant of growth rate in bones, although no unequivocal results have been presented as to the possibilities of using it as a stimulant of growth (Chapter 1). Rabbits are known to regulate temperature by breathing, and this fact introduces the possibility that effects on growth at changes in the gaseous environment are concomitant with alterations in the body temperature. To evaluate this possibility rectal temperatures were recorded in some of the series. Exposure to 100 per cent of oxygen at 1 atm.abs. did not influence on the temperatures (Chapter 3). In this case the conclusion is that the increase in growth simultaneously recorded was not due to change in temperature. It was expected to be a maximal hyperventilation at exposure to a combination of decreased oxygen and increased carbon dioxide tensions. This test was therefore chosen for the next determination of temperatures, that is the exposure to 10 per cent of oxygen and 5 per cent of carbon dioxide in nitrogen (Chapter 5). There was found to be a significant decrease in body temperature at the end of the exposure, measuring in mean 0.7°C . In this case there were concomitant decreases in temperature and in growth. To see whether this decrease was due to the hypoxia or to the increased carbon dioxide tension in the inspired gas, temperature recordings were also performed at the same oxygen tension, but without addition of carbon dioxide. It was performed on 7 animals after 8 hrs. at 5900 meters, corresponding to an oxygen concentration of 10 per cent at 1 atm.abs.. No changes were observed compared to the temperatures before exposure and to 5 control animals. Addition of 7 per cent carbon dioxide to

air for 8 hrs. did not change the temperature either (Chapter 6). The conclusion is that the changes in growth were not concomitant with changes in the rectal temperature except in one experiment, which does not warrant any conclusion as to the possibility that the growth can be altered by local change in temperature. Determination of haemoglobin content and haematocrit value (Chapter 5) did not show any change during the exposure, indicating that the decrease in temperature was coincident with any significant loss of fluid.

The next special factor is the **influences on the blood flow** from the alteration in gas tensions studied. It is well documented that both oxygen and carbon dioxide may be vasoactive, altering the blood flow with changes in tension. This has been studied regarding the femoral blood flow (*Lennox & Gibbs 1932*). Increased oxygen tensions gave slightly reduced flow rates (*Bird & Telfer 1966*), decreased oxygen tensions gave considerable increases (*Boon & Marotta 1964*), and finally increased carbon dioxide tensions gave slight increases in sympathectomized limbs but slight decreases in normal limbs (*Steck & Gellhorn 1939*). There are however no reports on the changes in the intraosseous blood flow under the circumstances mentioned. Therefore this was specially studied using the heat clearance method described by *Sundén (1967)* and the gas tensions used in the present investigation. There turned out to be no consistent effects measurable on the blood flow in the epiphysis. These experiments will be separately published (*Persson & Sundén 1968*). The indication at present is, that the effects on growth described in this investigation, were achieved without measurable changes in the bone blood flow.

The next part of the discussion will be devoted to the **time lags between the exposures and the recorded effects on growth**, with the intention of evaluating which cells of the cartilage plate, that have highest sensitivity to the treatments. This problem is presented in Chapter 4 where determinations of the height of the cartilage plate are reported. For the following discussion a summarizing figure (Fig. 16) is given concerning some principal growth effects obtained with increased and decreased oxygen tensions and increased carbon dioxide tensions. The figure depicts the growth ratios between test and control for each day with litter-mates in both groups. It is made up from the absolute values of growth in microns. The overlapping samples in the time periods +1 and +3 have been summarized. From this figure (Fig. 16) it is clear that the effects on growth were recorded already during the exposure to the artificial gas mixtures, rich or poor in oxygen. This means that the effect was elicited in the zone of degenerating cells where the tetracycline was built in, labelling the growth process. The curves give the impression that the hypoxic depression

(10 per cent $O_2 + N_2$) is greater during the day after exposure, than during the exposure. This is due to the fact, earlier mentioned, that the day of exposure includes about 16 hrs. of normal growth before the 8 hrs. of treatment and decrease in growth. This means that the actual growth rate under the hypoxic condition was somewhat three times greater than recorded in the figure. In other words, the decrease in growth rate is about the same during the hypoxic exposure as during the following 24 hrs. (period +1). If the same reasoning is applied to the pure oxygen breathing, it would imply, that the growth in period 0, recorded in Fig. 16 as a stimulation of 4 per cent in reality comprised 16 hrs. of normal growth rate and 8 hrs. of about 12 per cent increase in growth. This means that the stimulation is greater during the oxygen exposure than during the following days. The conclusion must be, that the hyperoxygenation accelerated the growth during the treatment and that this acceleration slowly subsided during 1—4 days after. In conclusion it may be suggested that *degeneration and calcification of the cartilage cells is an oxygen depending process*, easily disturbed by changes in the oxygen tension of blood. This could also be expected by the fact that this is the zone where the cartilage cells meet the blood vessels of the metaphysis (Trueta & Amato 1960).

The question can now be raised, whether the changes in metaphyseal growth rate represent only alterations in the position of the calcifying frontier of a temporary duration, or if they represent true changes in the growth in length of the bone. In other words, are the effects limited to the degenerating zone, or are they affecting a more extended series of cells in different stages of maturation? This problem was studied by determinations of the height of the cartilage plate in combination with the growth recordings (Chapter 4). After exposure to decreased oxygen partial pressure there was an almost significant decrease in the height of the plate, indicating effects also on cartilage cells at other maturing stages than those undergoing degeneration. Measurement of the cartilage plate has not yet been made at increased oxygen tensions, although it seems interesting to know, whether the recorded growth acceleration represents a temporary advancement of the calcifying frontier with a decrease in the height of the plate, or if it is combined with an unchanged or increased height of the cartilage plate, signifying that the stimulation also affected the younger cells of the cartilage.

The demonstrated sensitivity to changes in the oxygen tension and the lack of sensitivity to changes in the carbon dioxide tension does not mean that it should be possible to increase the height of a person by increasing the environmental oxygen tension. The oxygen toxicity, especially to the lungs, would very soon eliminate the stimulation, and might even result in a total decrease in growth. On the other hand, the demonstrated importance of the oxygen tension might have some application in conditions of decreased oxygen availa-

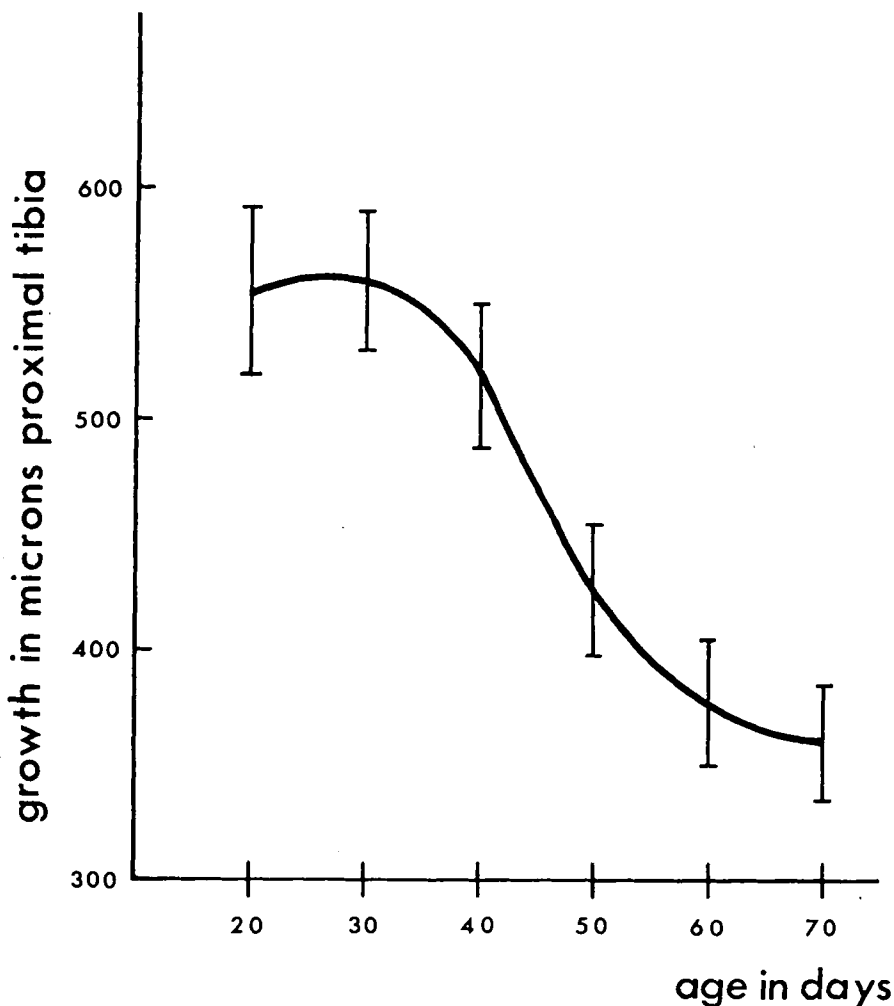


Figure 15.

Growth rate from the proximal growth plate of the tibia of rabbits according to Hansson (1967).

bility, due to circulatory disturbances (Ackerman & Brinkley 1966), as for instance in pathological fracture healing (Coulson et al. 1966, Goulon et al. 1968). Regarding the introductory question of venous stasis or arterial hyperaemia as the possible mechanism behind unilateral stimulation of bone growth, this investigation does not positively support the assumption that venous stasis should stimulate the growth. Arterial hyperaemia seems more in line with the

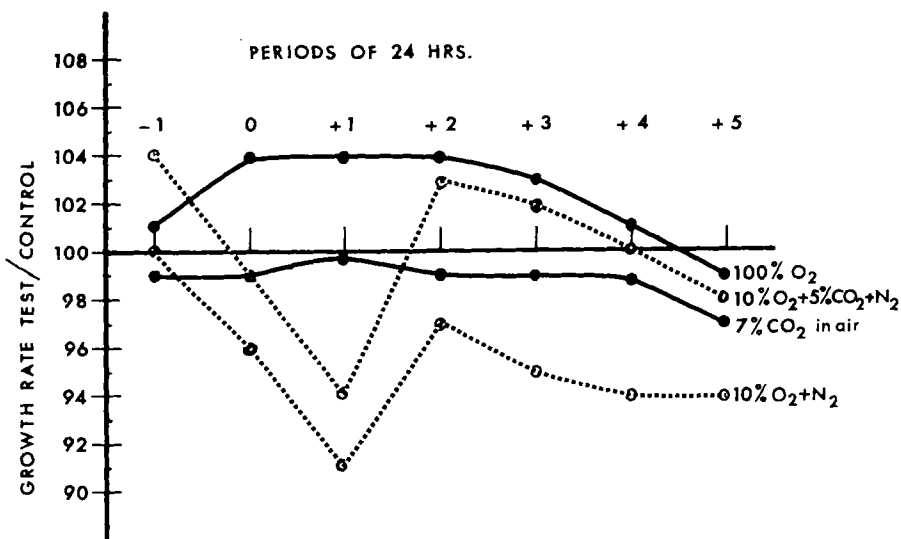


Figure 16.

Daily growth in length of the proximal tibial metaphysis in rabbits, measured by oxytetracycline labelling, given as growth rates of tests in per cent of growth in control groups. Respiratory gas altered for 8 hrs. during period 0. Gas compositions given in figure. Ambient pressure 1 atm.abs.

findings, but on the other hand, it can not be assumed that venous stasis is always concomitant with a decrease in the local blood flow. It is theoretically possible that a venous stasis with an increased venous pressure can be compensated by a local arteriolar dilatation, keeping the pressure difference and the blood flow on a normal level. Attempts to measure the bone flow during venous stasis are so diverging in the results that this might be the case (Herzig & Root 1959, McPherson et al. 1961, Valderrama & Trueta 1965, White & Stein 1965). If venous stasis stimulates growth it must not, anyhow, be combined with any significant reduction of the local oxygen tension, unless it comprises other factors compensating for the hypoxic growth retardation.

CHAPTER 8

SUMMARY

Leg length inequality in children is sometimes caused by one-sided growth stimulation after fractures, osteomyelites or arterio-venous fistulae. This knowledge has resulted in experimental and clinical attempts to induce such a stimulation in a short leg. This literature is shortly reviewed with special reference to the suggested causative factors, particularly arterial hyperaemia, venous stasis, local temperature and the local oxygenation.

For studying the oxygen factor in this respect, and secondly also the carbon dioxide factor, young rabbits were exposed to changes in the partial pressures of the respiratory gases, utilizing pressure chambers and artificial gas mixtures. The exposures were for 8 hrs. and given only once. Defined principles were set up for exclusion of abnormal animals. The longitudinal growth of the proximal tibial metaphysis was recorded by intravital oxytetracycline labelling and ultraviolet microscopy, performed as a blind test.

It was found that oxygen tensions above 100 per cent at 2 atm.abs. was above the tolerance, due to the pulmonary oxygen toxicity. At 100 per cent of oxygen at 1 atm.abs. there was a stimulation of growth during the exposure, subsiding for about 1—4 days. When the oxygen tension was reduced below normal, there was a decrease in growth during the exposure and during the following 24 hrs. of about the same magnitude, but thereafter the growth approached the normal. The oxygen tension limits of these effects were determined. The height of the cartilage plate seemed to be reduced one day after the hypoxia implying effects on an extended series of maturing stages of the cartilage cells. For elimination of the hyperventilatory hypocapnia under the hypoxic condition, carbon dioxide was added to the low oxygen mixture, without changing the growth effects. Addition of carbon dioxide to air did not influence on the growth within the doses tested. Rectal temperatures were checked without showing co-variation in more than one case.

It was concluded from the immediate effects on growth at changes in the oxygen tension, that the cartilage cells in the zone of degeneration and calcification were very sensitive, implying an oxygen depending nature of this process. The lack of sensitivity to changes in the carbon dioxide tension was discussed with reference to venous stasis and arterial hyperaemia as mechanisms behind over-growth of a long bone.

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Björn Mauritz Persson

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