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Changes in skeletal proportions of the rat in response to hypoxic stress

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INTRODUCTION

It was noted many years ago that exposure of humans and animals to new environments for the first time is associated with structural and physiological changes which produce characteristics approaching those of native populations (Barcroft, 1925) and without which toleration of the new environment is not possible (Hingston, 1924). This process is now termed 'acclimatization'.

In 1925 Barcroft reviewed work concerned with acclimatization to the hypoxic stress of high altitude. Morphological and physiological differences were recognized in the *Cholos* of Peru (Indians native to high altitude), including reduced stature and the possession of wider and deeper chests in comparison with their sea-level counterparts. Such changes in shape were thought to be of adaptive significance.

The present experiments were performed to study changes in shape and growth rates of rats during and after exposure to hypoxic stress produced by decompression. Changes in body proportions may be effected by changes in the amount and distribution of the soft tissues as well as the growth rate and state of maturity of the skeleton, but the greater reliability of skeletal measurements makes them more satisfactory as indicators of change in shape. The present investigation is based on measurements, from radiographs, of the dimensions and states of maturity of certain regions of the skeleton of hypoxic rats and their correlation with changes in body weight.

MATERIALS AND METHODS

Four litters of four male Wistar rats aged 21 days were divided into equal groups of experimental and control animals in such a way that each experimental animal had a litter mate control. The experimental animals were subsequently exposed at 28 days of age to a pressure of 390 mmHg for 4 weeks (for an account of methods of maintenance see Hunter & Clegg, 1973). Whole-body radiographs were made of all the animals at the ages of 35, 45 and 56 days (i.e. after 7, 17 and 28 days of exposure). Following removal of the experimental animals from the hypoxic environment, further radiographs were taken 4 weeks later (84 days of age) and 12 weeks later (140 days of age).

Radiographs were taken with a Picker portable X-ray machine. The anode-film distance was 76 cm and the film was Agfa-Gevaert Osray M unscreened film (automatic development). Exposure time was $2\frac{1}{4}$ sec at 9 mA and 60 kV.

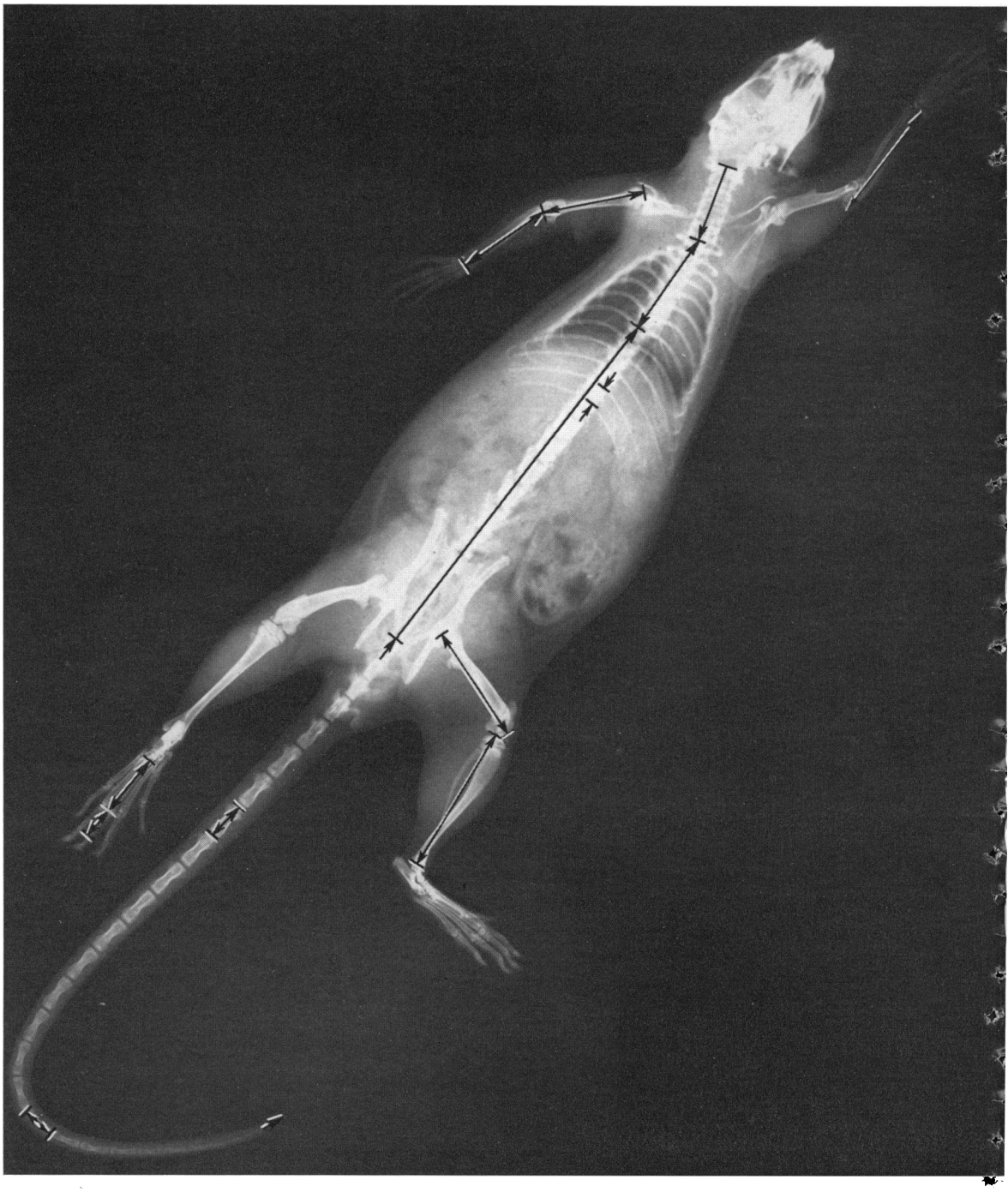

Fig. 1. Whole-body radiograph of a 56 day old control rat. ×0·8.
The skeletal dimensions indicated are described in the text.

To avoid movement during radiography, animals were anaesthetized with ether, laid prone on the X-ray film and positioned as recommended by Hughes & Tanner (1970). The limbs were fixed in position with Sellotape. Fig. 1 shows a radiograph of a control rat in the correct position. Unfortunately this positioning made it difficult to measure head length and width, but it allowed consistent measurement of the following skeletal dimensions, which are also indicated in Fig. 1:

Body length (*cranio-sacral distance*): from the cranial edge of the first cervical vertebra to the junction of the sacrum with the first caudal vertebra.

Tail length: from the cranial edge of the first caudal vertebra to the fleshy tip of the tail.

13th *thoracic vertebra* 8*th caudal vertebra* 18*th caudal vertebra*	Craniocaudal lengths.
Humerus and radius of the right forelimb *Ulna of the left forelimb*	From the proximal to the distal ends, including epiphyses.
Tibia of the left hind limb *Third metatarsal and third proximal phalanx of the right hind limb*	From the proximal to the distal ends, including epiphyses.

Femur of the left hind limb: from the most proximal border of the head of the femur to the distal border of the distal epiphysis.

Dial indicator calipers measuring to 0·1 mm (John Bull; British Indicators) were used for measuring bone lengths on radiographs. The distances between the bones measured and the film were small and relatively constant, so no corrections for parallax were made.

Skeletal maturity was determined from the radiographs by the method of Hughes & Tanner (1970). It was found difficult to score the humerus and femur, so these bones were not utilized.

(*a*) Tail scores – vertebrae 8 to 18.

(*b*) Forelimb scores – radius, ulna, 2nd, 3rd and 4th metacarpals, and 2nd, 3rd and 4th proximal phalanges.

(*c*) Hind limb scores – tibia, calcaneus, 2nd, 3rd and 4th metatarsals, and 2nd, 3rd and 4th proximal phalanges.

RESULTS

Fig. 2 is a radiograph of an experimental animal at 56 days of age, after 28 days of exposure to hypoxia. The reduced size of its skeleton is obvious when it is compared with Fig. 1 (a radiograph of a control animal of the same age).

Body weight, body length and tail length

Fig. 3 indicates the effect of hypoxia on body length (*a*), body weight (*b*) and tail length (*c*). The small standard errors indicate the accuracy of the methods of measurement. Prolonged hypoxia produced a decrease in the rate of gain of body weight,

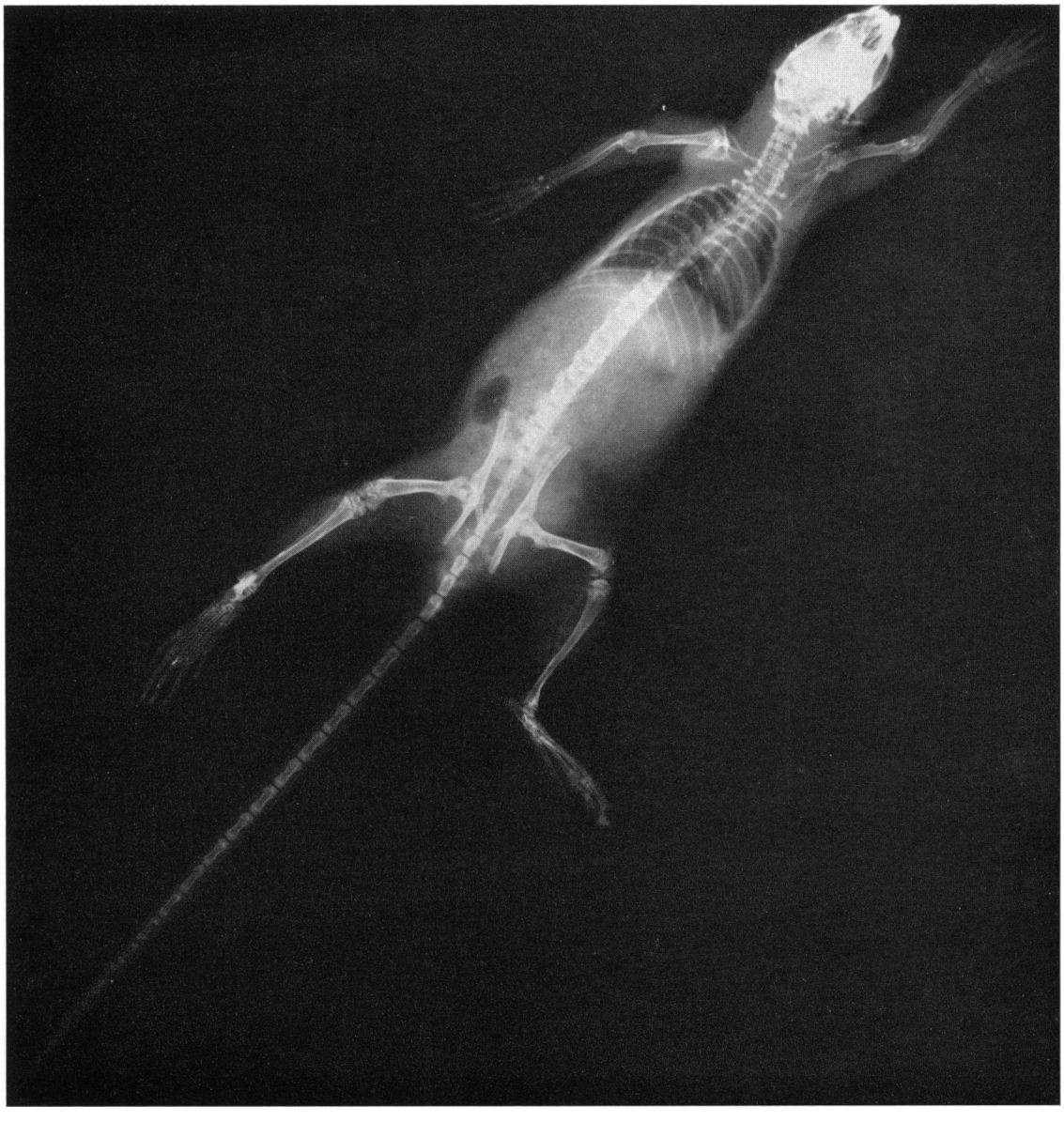

Fig. 2. Experimental litter mate of the animal shown in Fig. 1. ×0·8. Exposure to hypoxia from 28th to 56th day of life. The markedly smaller size is evident and the greater immaturity of the skeleton is seen when the appearances of the epiphyses are compared.

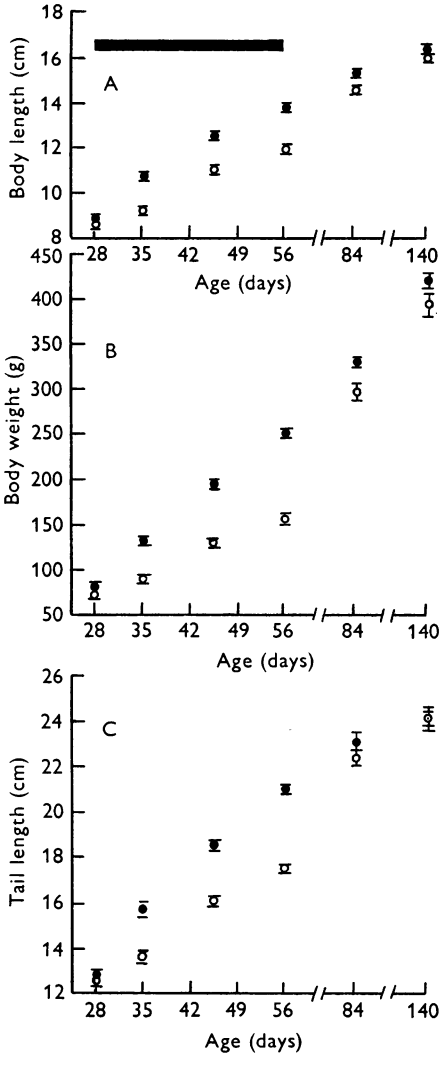

Fig. 3. The effect of hypoxia on (A) body length, (B) body weight, and (C) tail length. ○, experimental animals; ●, control animals. (Means ± s.e.)

followed, after removal of the stress, by an increase in the rate of gain above control values ('catch-up'). There were similar changes in the growth of the axial skeleton. After an exposure of as little as one week, comparison with controls showed a highly significant retardation in both body length and tail length, which increased with duration of exposure (Table 1). After 12 weeks' recovery, although body length and tail length had almost completely caught up, the mean of experimental body weights was still almost significantly less than that of the control animals.

The changes in tail length were truly representative of bone growth retardation because exposure to hypoxia did not result in any alteration in the number of ossified

Table 1. *Mean value* (± S.E.) *of the different skeletal dimensions of hypoxic and control rats*

(Significance of mean experimental-control differences estimated using Fisher's paired *t*-test.)

			Period of exposure						Period of recovery			
	Before exposure		7 days		17 days		28 days		28 days		84 days	
Measurement	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
Body length (cm)	8·60*** ±0·06	8·88 ±0·10	9·23**** ±0·08	10·58 ±0·07	10·96**** ±0·08	12·46 ±0·06	11·86**** ±0·10	13·72 ±0·08	14·46*** ±0·13	15·15 ±0·10	15·93 ±0·17	16·20 ±0·16
Tail length (cm)	12·55 ±0·22	12·82 ±0·14	13·53*** ±0·19	15·60 ±0·29	15·92**** ±0·18	18·42 ±0·17	17·37**** ±0·19	20·85 ±0·14	22·33* ±0·23	22·95 ±0·33	24·05 ±0·34	23·98 ±0·48
Body weight (g)	73·50 ±2·57	79·00 ±2·49	87·10**** ±2·57	128·60 ±3·90	128·70**** ±2·26	190·50 ±4·23	156·80**** ±4·39	246·10 ±2·82	293·70*** ±6·76	325·30 ±3·66	386·33* ±12·37	412·20 ±8·61
13th thoracic vertebra (cm)	0·29 ±0·01	0·28 ±0·01	0·30 ±0·01	0·35 ±0·01	0·36 ±0·01	0·43 ±0·02	0·42*** ±0·01	0·49 ±0·01	0·52** ±0·01	0·57 ±0·01	0·58 ±0·01	0·60 ±0·01
8th caudal vertebra (cm)	0·53* ±0·01	0·55 ±0·01	0·60*** ±0·01	0·67 ±0·01	0·68**** ±0·01	0·83 ±0·01	0·76*** ±0·02	0·91 ±0·01	1·03 ±0·01	1·04 ±0·01	1·13 ±0·02	1·13 ±0·01
18th caudal vertebra (cm)	0·42 ±0·01	0·44 ±0·01	0·47*** ±0·01	0·52 ±0·01	0·55**** ±0·01	0·66 ±0·01	0·61**** ±0·02	0·71 ±0·01	0·79 ±0·01	0·82 ±0·02	0·88 ±0·01	0·86 ±0·02
(R) humerus (cm)	1·69 ±0·01	1·70 ±0·01	1·82*** ±0·01	1·91 ±0·02	2·02**** ±0·01	2·18 ±0·01	2·18*** ±0·02	2·37 ±0·02	2·54*** ±0·01	2·67 ±0·02	2·88 ±0·05	2·92 ±0·03
(R) radius (cm)	1·54 ±0·02	1·55 ±0·02	1·62**** ±0·01	1·73 ±0·01	1·79**** ±0·01	1·96 ±0·01	1·93**** ±0·01	2·13 ±0·02	2·27** ±0·01	2·36 ±0·02	2·54 ±0·02	2·53 ±0·03
(L) ulna (cm)	2·11 ±0·03	2·14 ±0·02	2·21** ±0·02	2·35 ±0·03	2·41*** ±0·02	2·63 ±0·03	2·60*** ±0·03	2·83 ±0·02	3·03 ±0·01	3·12 ±0·03	3·37 ±0·03	3·42 ±0·03
(L) femur (cm)	2·01 ±0·01	2·01 ±0·01	2·12** ±0·02	2·23 ±0·02	2·33*** ±0·03	2·51 ±0·02	2·40**** ±0·04	2·71 ±0·04	2·85* ±0·05	2·96 ±0·03	3·15 ±0·05	3·21 ±0·07
(L) tibia (cm)	2·46 ±0·02	2·51 ±0·03	2·63**** ±0·02	2·84 ±0·02	2·94**** ±0·02	3·21 ±0·03	3·16**** ±0·02	3·49 ±0·02	3·70*** ±0·02	3·88 ±0·04	4·08 ±0·03	4·16 ±0·04
(R) 3rd metatarsal (cm)	1·18 ±0·01	1·18 ±0·02	1·23 ±0·01	1·29 ±0·02	1·34*** ±0·02	1·44 ±0·02	1·42*** ±0·03	1·50 ±0·02	1·50 ±0·02	1·53 ±0·02	1·51 ±0·02	1·53 ±0·01
(R) 3rd phalanx (cm)	0·52 ±0·02	0·50 ±0·01	0·56 ±0·01	0·57 ±0·01	0·58**** ±0·01	0·65 ±0·01	0·60*** ±0·01	0·67 ±0·01	0·68 ±0·01	0·69 ±0·01	0·68 ±0·01	0·70 ±0·01

* $0{\cdot}02 < P < 0{\cdot}05$. ** $0{\cdot}01 < P < 0{\cdot}02$. *** $0{\cdot}001 < P < 0{\cdot}01$. **** $P < 0{\cdot}001$.

Table 2. *Regression coefficients of body and tail lengths on logarithms of body weight*

Regression	Age range: 28–56 days (hypoxic) Experimentals	28–56 days (hypoxic) Controls	84–140 days (recovery) Experimentals	84–140 days (recovery) Controls
Body length (cm) on log body weight (g)	9·738 ± 0·289	9·756 ± 0·262	12·149 ± 0·754	10·798 ± 0·931
Tail length (cm) on log body weight (g)	14·177 ± 0·711	15·971 ± 0·557	11·691 ± 3·714	11·894 ± 4·960

caudal vertebrae which were added as the tail developed. The mean number of ossified caudal vertebrae in the experimental animals after one month of hypoxia was 28·0 ± 0·3, while that of litter mate controls of the same age was 27·8 ± 0·2.

Table 2 shows the relationships between body and tail lengths and the logarithm of body weight. Differences between experimental and control groups in the slopes of the regression lines were not statistically significant.

Table 1 also indicates changes in the depths of the three vertebrae measured. The two caudal vertebrae reacted in a similar manner, having caught up after four weeks' recovery, but the response of the 13th thoracic vertebrae was slow, both in retardation and in recovery.

Limb bone lengths

Fig. 4 indicates the effect of hypoxia on some limb bone lengths. Growth of the larger limb bones (humerus, radius, femur, tibia) was significantly retarded in all experimental animals after only one week of hypoxia and the degree of retardation increased with duration of exposure. Apart from those for the ulna (not shown), values remained significantly different up to 4 weeks' recovery, but after 12 weeks were back to normal, although differences in tibial lengths verged on statistical significance. The responses of the metatarsal and phalanx were less marked than in the more proximal limb bones. No significant differences were recorded after 7 days' exposure and normal lengths were achieved before 4 weeks' recovery.

Table 3 shows the regression coefficients for the sum of the major limb bone lengths (femur, tibia, humerus and radius) plotted against body weight, a relationship based on Schreider's (1964) limb length/body weight ratio for man. The regression was altered significantly by hypoxic stress, producing a line with a steeper gradient, but there was a return to normal after 12 weeks' recovery. Thus, it is apparent that although the limb bones of hypoxic animals were significantly shorter than those of the controls, they were growing longer in relation to body weight, and began to do so soon after the onset of the stress.

To examine whether any one bone was responsible for the increase in the limb length/body weight ratio, the percentage retardation in each of the separate bones involved in the ratio was determined. Table 4 indicates the results. There appears to have been very little difference in the percentage retardation in the separate bones by the end of the 28 day period of hypoxia, although the femur was most retarded, but

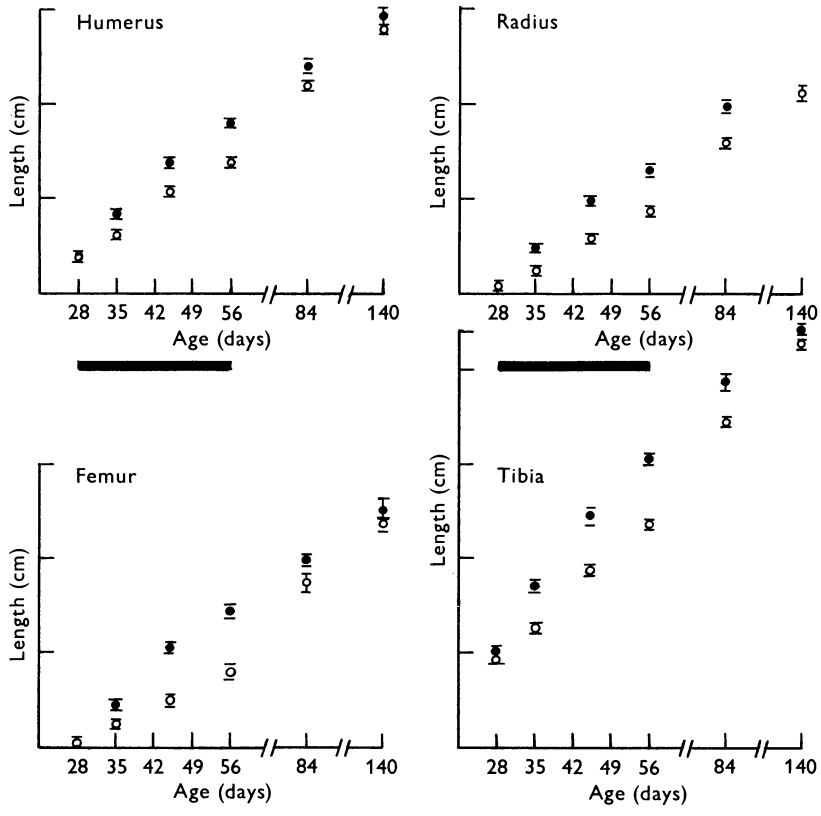

Fig. 4. The effect of hypoxia on growth of some of the long bones. ○, experimental animals; ●, control animals. (Means ±s.e.)

Table 3. *Regression coefficients of total limb bone lengths (humerus + radius + femur + tibia) on body weight*

Age range			
28–56 days (hypoxic)		84–140 days (recovery)	
Experimentals	Controls	Experimentals	Controls
0·2300**** ± 0·0010	0·0170 ± 0·0004	0·0120 ± 0·0014	0·0110 ± 0·0015

**** Highly significant experimental − control difference ($P < 0·001$).

the more distal long bones (radius and tibia) were affected most strongly after one week's hypoxia. All bones caught up to the same extent following recovery.

This initially greater retardation of the distal as opposed to the proximal major limb bones cannot be extrapolated to the limb as a whole, in the sense of a general disto-proximal gradient of retardation. This is evidenced by Fig. 5, in which the percentage retardations of all the measured bones of the hind limb are plotted against

Table 4. *Percentage retardation in the lengths of limb bones of the rat during and after exposure to hypoxia (means ± S.E.)*

Period	Age (days)	Humerus	Radius	Femur	Tibia
Hypoxic	35	4·964 ± 0·409	6·522 ± 0·849	4·855 ± 1·232	7·259 ± 1·018
	45	7·445 ± 0·504	8·556 ± 0·896	7·315 ± 1·413	8·466 ± 1·052
	56	8·187 ± 0·990	9·472 ± 1·133	11·454 ± 1·154	9·550 ± 0·798
Recovery	84	3·585 ± 0·575	3·667 ± 1·032	3·695 ± 1·352	4·410 ± 0·932
	140	1·431 ± 1·324	−0·222 ± 1·756	1·673 ± 2·608	1·729 ± 0·833

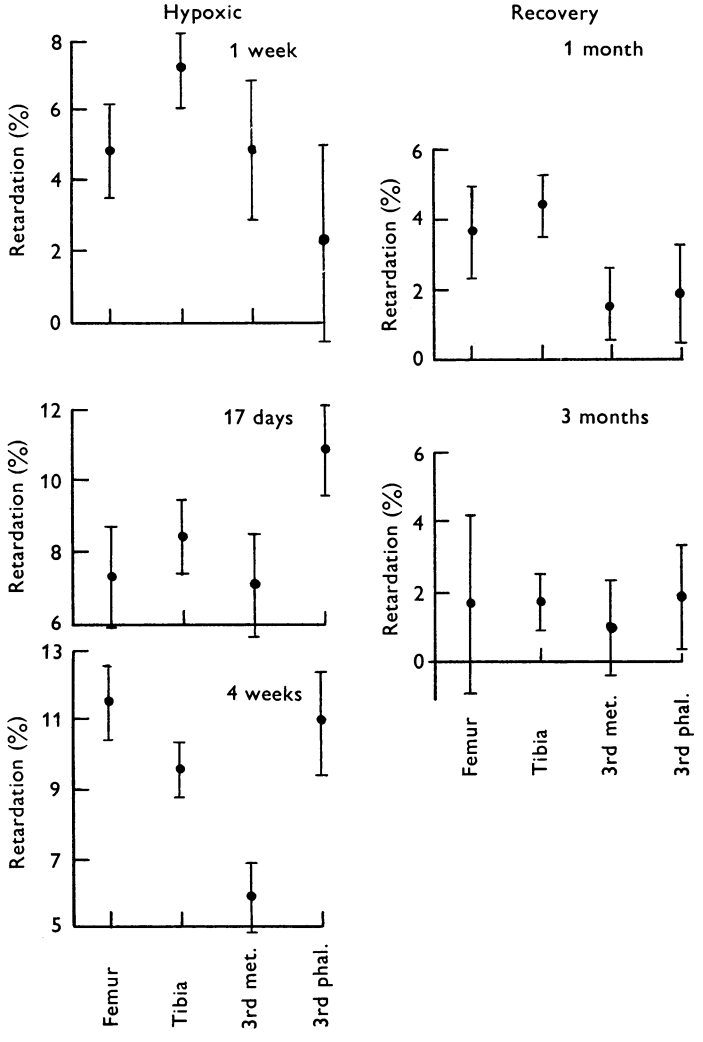

Fig. 5. The percentage retardations (means ± S.E.) in some bone lengths after different periods of hypoxia and of recovery.

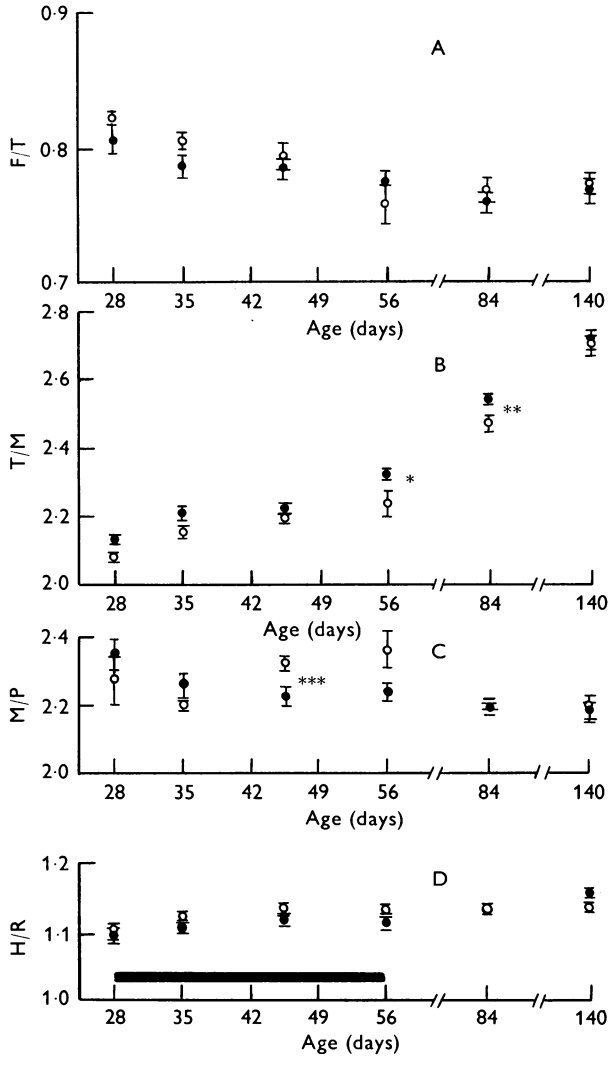

Fig. 6. Ratios of bone lengths (means ± S.E.) in experimental animals (○) and controls (●). F/T, femur/tibia; T/M, tibia/3rd metatarsal; M/P, 3rd metatarsal/3rd proximal phalanx; H/R, humerus/radius. * $0{\cdot}02 < P < 0{\cdot}05$. ** $0{\cdot}01 < P < 0{\cdot}02$. *** $0{\cdot}001 < P < 0{\cdot}01$.

the duration of exposure or of recovery. After 28 days' exposure, the metatarsal was least retarded, and it also recovered most quickly. Thus no consistent gradient could be established.

Fig. 6 indicates the development of various limb-length ratios with age, and the effect of exposure to, and recovery from, hypoxia. Changes in the relationships with age are determined by the relative growth rates of the limb bones. In control animals the femur/tibia ratio decreased with age, indicating that the tibia was growing faster than the femur. Similarly, the tibia was growing faster than the metatarsal, which was

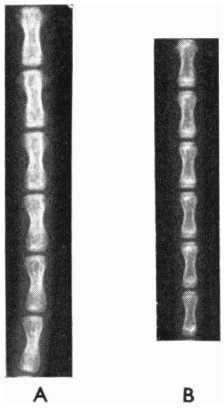

Fig. 7. Radiograph (× 1·6) of the eighth to thirteenth caudal vertebrae of (A) a control animal aged 56 days and (B) its experimental litter mate at the same age (natural size). Note the greater size and maturity of the vertebrae in the control animal.

in turn growing slower than the phalanx. The humerus was growing faster than the radius.

These relationships were not altered significantly by exposure to hypoxia, except in the case of the tibia/metatarsal ratio after 28 days' exposure and 28 days' recovery, when the metatarsal grew faster in relation to the tibia than in the controls; and in the case of the metatarsal/phalanx ratio at 17 days' and possibly 28 days' exposure, when the metatarsal grew faster in relation to the phalanx. It therefore appears that the metatarsal grew abnormally fast in relation to its neighbouring bones during exposure to hypoxia. This result is also indicated in Fig. 5.

Skeletal maturity

Fig. 7 is an enlargement of a radiograph of the tails of the two animals compared in Figs. 1 and 2. Equivalent caudal vertebrae in the experimental animals had persistent epiphysial plates, while those of the controls were passing through various stages of fusion: consequently, lower maturity ratings were awarded to the experimental animals than to the controls.

Table 5 indicates the average maturity scores for the separate bones of both control and experimental rats from the ages of 28 to 140 days. In the experimental group there was a general retardation of skeletal maturity throughout the body, which was most noticeable in the tail (Table 5*c*). Some individual limb bones had maturity scores greater than those for the controls during the early stages of exposure (Table 5*a*, *b*) but when the scores were added for each region, significant retardation was observable.

Fig. 8 indicates the discrepancy between control and experimental scores for each region after 7, 17 and 28 days' hypoxia and 4 and 12 weeks' recovery. It can be seen that the retardation of the whole skeleton scores (Fig. 8*a*) increased with exposure up to about 17 days, when values became constant; after 12 weeks' recovery all values had returned to normal.

Table 5*a*. *Effect of hypoxia on forelimb maturity scores in the rat* (*means* ± S.E.)

(Maximum possible scores are: radius, 14; ulna, 15; metacarpals, 13; phalanges, 11.)

Period	Age (days)	(L) radius Exp.	(L) radius Cont.	(L) ulna Exp.	(L) ulna Cont.	(R) metacarpals 2nd Exp.	(R) metacarpals 2nd Cont.	(R) metacarpals 3rd Exp.	(R) metacarpals 3rd Cont.	(R) metacarpals 4th Exp.	(R) metacarpals 4th Cont.	(R) phalanges 2nd Exp.	(R) phalanges 2nd Cont.	(R) phalanges 3rd Exp.	(R) phalanges 3rd Cont.	(R) phalanges 4th Exp.	(R) phalanges 4th Cont.	Whole forelimb. Mean difference (control − experimental)
Normal	28	12·00 ±0	12·00 ±0	10·83 ±0·17	10·67 ×0·21	6·67 ±0·21	6·83 ±0·67	8·33 ±0·33	7·50 ±0·50	7·33 ±0·33	7·67 ±0·33	5·83 ±0·17	6·33 ±0·33	7·67 ±0·21	8·00 ±0·37	7·50 ±0·22	7·50 ±0·34	−0·67 ±1·31
Hypoxic	35	12·00 ±0	12·17 ±0·17	11·33 ±0·21	11·17 ±0·17	7·00 ±0	7·00 ±0	8·67 ±0·33	9·17 ±0·31	8·83 ±0·40	8·17 ±0·40	7·00 ±0·52	8·50 ±0·50	8·33 ±0·21	8·50 ±0·22	8·67 ±0·21	8·50 ±0·34	1·33 ±1·20
	45	12·50 ±0·34	12·17 ±0·17	11·17 ±0·17	12·17 ±0·17	7·33 ±0·33	8·33 ±1·05	9·00 ±0	9·67 ±0·21	8·67 ±0·21	9·50 ±0·62	8·83 ±0·17	9·00 ±0·26	8·33 ±0·21	9·00 ±0	8·50 ±0·34	9·33 ±0·21	4·67 ±1·94
	56	12·33 ±0·21	12·17 ±0·17	12·17 ±0·31	12·33 ±0·21	11·00 ±0·37	12·00 ±0·26	10·33 ±0·21	11·00 ±0·26	10·00 ±0	0·133 ±0·21	9·83 ±0·31	10·83 ±0·17	9·67 ±0·21	10·17 ±0·31	9·67 ±0·33	10·50 ±0·22	4·33** ±1·33
Recovery	84	13·17 ±0·17	13·17 ±0·17	13·83 ±0·40	14·00 ±0·37	12·17 ±0·17	12·67 ±0·21	11·83 ±0·17	12·17 ±0·17	11·83 ±0·17	12·33 ±0·21	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	1·50 ±0·72
	140	13·50 ±0·22	13·50 ±0·22	14·50 ±0·22	14·67 ±0·21	12·33 ±0·21	12·17 ±0·17	12·17 ±0·17	12·17 ±0·17	12·50 ±0·22	12·17 ±0·17	11·00 ±0	11·00 ±0	10·83 ±0	11·00 ±0	10·83 ±0·17	11·00 ±0	0 ±0·78

** $0{\cdot}01 < P < 0{\cdot}02$.

Table 5*b*. *Effect of hypoxia on hind limb maturity scores* (*means* ± S.E.)

(Maximum possible scores are: tibia, 15; calcaneum, 9; metatarsals, 13; phalanges, 11.)

Bone ... Period	Age (days)	(R) tibia Exp.	(R) tibia Cont.	(L) calcaneum Exp.	(L) calcaneum Cont.	(R) metatarsals 2nd Exp.	(R) metatarsals 2nd Cont.	(R) metatarsals 3rd Exp.	(R) metatarsals 3rd Cont.	(R) metatarsals 4th Exp.	(R) metatarsals 4th Cont.	(R) phalanges 2nd Exp.	(R) phalanges 2nd Cont.	(R) phalanges 3rd Exp.	(R) phalanges 3rd Cont.	(R) phalanges 4th Exp.	(R) phalanges 4th Cont.	Whole hind limb. Mean difference (control − experimental)
Normal	28	9·17 ±0·31	9·17 ±0·17	6·00 ±0	6·00 ±0	8·33 ±0·40	8·00 ±0·26	8·67 ±0·21	8·00 ±0·37	8·50 ±0·22	8·67 ±0·42	6·33 ±0·21	7·55 ±0·62	6·00 ±0	6·17 ±0·17	6·00 ±0	6·00 ±0	−0·17 ±1·62
Hypoxic	35	10·00 ±0	9·83 ±0·17	6·00 ±0	61·7 ±0·17	9·33 ±0·49	9·83 ±0·17	9·33 ±0·21	9·00 ±0·63	9·00 ±0·37	0·90 ±0·63	7·67 ±0·62	8·83 ±0·17	7·17 ±0·60	8·67 ±0·21	7·17 ±0·60	8·17 ±0·48	3·83* ±1·49
	45	11·17 ±0·31	12·17 ±0·17	6·50 ±0·22	7·00 ±0·26	9·33 ±0·49	10·00 ±0	9·83 ±0·67	9·00 ±0·63	9·00 ±0·51	8·83 ±0·60	8·67 ±0·21	9·50 ±0·22	8·50 ±0·22	9·17 ±0·17	8·17 ±0·48	9·17 ±0·17	4·33*** ±1·15
	56	12·00 ±0	12·17 ±0·31	7·00 ±0·26	7·50 ±0·22	9·83 ±0·17	10·00 ±0	9·50 ±0·50	10·00 ±0	9·50 ±0·22	10·00 ±0	9·67 ±0·21	10·17 ±0·17	9·17 ±0·17	10·00 ±0·26	9·33 ±0·21	9·83 ±0·31	4·33*** ±0·96
Recovery	84	13·00 ±0·26	13·50 ±0·17	9·00 ±0	9·00 ±0	11·00 ±0·26	11·67 ±0·21	11·17 ±0·31	11·83 ±0·17	11·67 ±0·21	11·83 ±0·17	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	2·83** ±0·79
	140	13·67 ±0·33	13·50 ±0·34	9·00 ±0	9·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	11·00 ±0	0·50 ±0·43

* $0·02 < P < 0·05$. ** $0·01 < P < 0·02$. *** $0·001 < P < 0·01$.

Table 5*c*. *Effect of hypoxia on maturity scores of caudal vertebra* (*means* ± S.E.)

(Maximum possible score for each vertebra is 13.)

Vertebra no. ...		8		9		10		11		12		13	
Period	Age (days)	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
Normal	28	9·50 ±0·34	9·83 ±0·17	9·17 ±0·31	9·67 ±0·21	9·17 ±0·31	9·67 ±0·21	8·83 ±0·17	9·00 ±0	8·67 ±0·33	9·00 ±0	8·50 ±0·34	8·83 ±0·17
Hypoxic	35	10·83 ±0·40	11·00 ±0·37	10·33 ±0·42	10·50 ±0·22	9·67 ±0·21	10·33 ±0·21	9·50 ±0·22	10·33 ±0·21	9·50 ±0·22	9·83 ±0·67	9·33 ±0·21	9·83 ±0·17
	45	10·67 ±0·42	12·33 ±0·21	10·00 ±0	12·00 ±0	10·00 ±0	12·00 ±0	10·00 ±0	11·33 ±0·21	10·00 ±0	11·33 ±0·21	10·00 ±0	11·17 ±0·17
	56	11·50 ±0·50	12·66 ±0·21	11·17 ±0·54	12·17 ±0·17	10·33 ±0·33	12·00 ±0	10·00 ±0	12·00 ±0	10·00 ±0	12·00 ±0	10·00 ±0	12·00 ±0
Recovery	84	12·83 ±0·17	13·00 ±0	12·67 ±0·21	12·67 ±0·21	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0
	140	13·00 ±0	12·83 ±0·17	13·00 ±0	12·50 ±0·22	12·33 ±0·21	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0

Vertebra no. ...		14		15		16		17		18		All vertebrae. Mean difference (control − experimental)
Period	Age (days)	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	
Normal	28	8·50 ±0·34	8·67 ±0·33	8·50 ±0·34	8·50 ±0·34	8·33 ±0·42	8·50 ±0·34	8·17 ±0·40	8·33 ±0·42	7·67 ±0·49	8·33 ±0·42	0 ±1·24
Hypoxic	35	9·17 ±0·17	9·83 ±0·17	9·17 ±0·17	9·83 ±0·17	8·83 ±0·17	9·83 ±0·17	8·83 ±0·17	9·67 ±0·17	8·50 ±0·22	9·50 ±0·22	6·83*** ±1·33
	45	10·00 ±0	11·33 ±0·21	10·00 ±0	11·17 ±0·17	9·83 ±0·17	11·00 ±0	9·83 ±0·17	11·00 ±0	9·67 ±0·21	11·00 ±0	15·83**** ±1·20
	56	10·00 ±0	11·83 ±0·17	10·00 ±0	11·67 ±0·21	10·00 ±0	11·33 ±0·21	10·00 ±0	11·17 ±0·17	10·00 ±0	11·00 ±0	15·17**** ±1·11
Recovery	84	11·83 ±0·17	12·00 ±0	11·83 ±0·17	11·83 ±1·67	11·67 ±0·21	11·83 ±1·17	11·67 ±0·21	11·67 ±0·21	11·67 ±0·21	11·50 ±0·22	0·50 ±1·34
	140	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	12·00 ±0	11·83 ±0·17	11·83 ±0·17	11·83 ±0·17	11·67 ±0·21	−1·17 ±0·65

*** $0{\cdot}001 < P < 0{\cdot}01$. **** $P < 0{\cdot}001$.

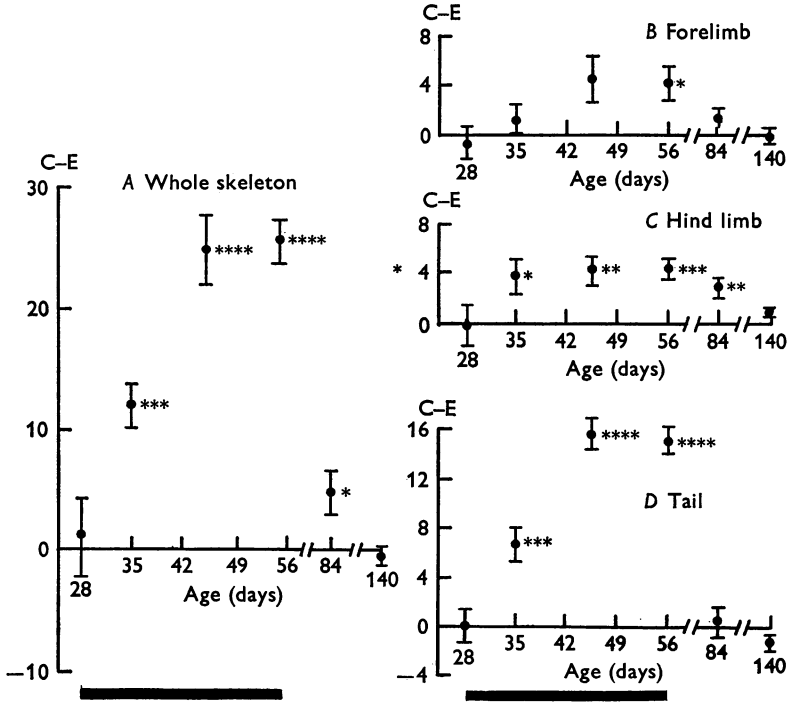

Fig. 8. Skeletal maturity in different regions of the rat after varying periods of hypoxia. C–E, mean (±S.E.) control maturity score–experimental maturity score. * $0{\cdot}02 < P < 0{\cdot}05$. ** $0{\cdot}01 < P < 0{\cdot}02$. *** $0{\cdot}001 < P < 0{\cdot}01$. **** $P < 0{\cdot}001$.

It appears that differences in tail maturity (Fig. 8*d*) contributed most to the differences in whole skeleton scores, but this may have been an artefact produced by the method of measurement; one cannot be sure that a single unit of retardation in one bone is the same as in another. The curves for the three regions differed in shape. The hind limb (Fig. 8*c*) appeared to react more quickly than the forelimb (Fig. 8*b*) and differences remained greater after 4 weeks' recovery. Tail maturity (Fig. 8*d*) returned to normal after 4 weeks' recovery, so that the deficit observed in the whole skeleton at this time (Fig. 8*a*) was due mainly to lower scores in the hind limb. The discrepancy between control and experimental scores reached a maximum (sooner or later) in all regions after prolonged hypoxia. There may possibly have been a craniocaudal gradient in delayed maturity, the forelimb being less likely to be 'knocked' from its curve than the hind limb, and the tail being most retarded. However, it appears that, despite this greater susceptibility, the tail also possessed a greater ability to recover once the stress had been removed.

DISCUSSION

It is apparent from these results that growth in size of the rat skeleton was generally retarded during hypoxic stress of 390 mmHg and that during the recovery period the increased weight gain of the animal was accompanied by an acceleration

of growth in bone length. These findings are consistent with those of Acheson & MacIntyre (1958). The stress did not, however, affect the body uniformly, so that although tail and body length grew in proportion to body weight, the limbs grew longer in relation to this parameter than in litter mate control animals. Unlike exposure to cold (Lee, Chu & Chang, 1969) hypoxia did not lead to any reduction in the numbers of caudal vertebra.

Hypoxic rats were lighter than litter mate controls, a finding similar to that in mice (Hunter & Clegg, 1973) but they had body lengths and tail lengths in proportion to their weights. The finding that the normal body length/body weight ratio was maintained during hypoxia is consistent with the results of other workers studying the effects of other forms of stress on body proportions (Outhouse & Mendel, 1933; Acheson & MacIntyre, 1958; Tanner, 1962; Lee *et al.* 1969; Mosier, 1969).

Hypoxic stress produced animals with limbs which were relatively long compared with body weight, body length and tail length. It is tempting to suggest that there may be some adaptive significance attached to this change in relative proportions, for example, to provide an equally mobile animal with a reduced central mass and associated oxygen utilization. However, it may also simply represent a difference in the type of growth in the long bones compared with the axial skeleton.

The metatarsals of the hind limb appear to have grown at an abnormally fast rate, compared with neighbouring bones, during hypoxic stress. This area of the hind limb is particularly associated with heat dissipation and the relatively increased growth rate may have been related to this. Certainly, the tail and paws of hypoxic rodents are very pink in colour. This may be caused by increased blood flow to the extremities (Korner & Uther, 1969; Aorki & Robinson, 1969), which would be associated with increased heat dissipation. Possibly an increase in blood flow to the paws may have had an effect on bone growth similar to that produced during heat exposure (Harrison, Morton & Weiner, 1959; Grant, 1963). Harrison *et al.* (1959) suggested that there is a causal relationship between increased tail length and increased vascularization, although this has not been proved. In the present experiments, however, proportionality between tail length and body weight was maintained during hypoxic stress, so the explanation above cannot be applied to growth of this appendage.

It is known that stress may or may not permanently alter certain body proportions, depending on the timing and intensity of the period of its application, and this was discussed by Tanner (1962) in relation to the effects of malnutrition. Acheson & MacIntyre (1958) demonstrated in rats that, under the stress of starvation, retardation of linear growth was greater than retardation of skeletal maturation, so that bones whose epiphyses were near fusion remained permanently stunted. However, Tanner (1962) proposed that, more often than not, catch-up of linear growth would be proportionately greater than for maturation, so that a normally proportioned adult would result. Despite this, it would seem likely that importance should be attached to the suggestion of Acheson & MacIntyre (1958) that the effect of stress on bone growth depends on the amount of growth achieved before the stress is introduced, and on the amount of growing time left when the stress is removed.

All bones studied in hypoxic rats of the present experiments were reduced in length when compared with their controls. After removal from the stress, even though they were maturing at different rates, they all eventually caught up to

control values, irrespective of the initial stage of maturation or the amount of retardation. These findings would at first seem to be inconsistent with those of Acheson & MacIntyre (1958), but it is known that none of the bones had reached maturity at the time of removal from the hypoxic stress, so that all were capable of resuming growth before epiphysial fusion was completed. It is impossible to say whether prolongation of the duration of the stress beyond the time of completion of maturation would have produced permanent stunting in the bones concerned. Presumably stress would be most likely to produce permanent stunting if it were applied just prior to epiphyseal closure, because the results indicate that maturity was retarded in a similar way to growth in length. The phalanges of both the forelimbs and the hind limbs were the bones whose epiphysial cartilage plates were nearest to fusion when the stress was applied, but the retardation of their maturity was similar to that of other bones in the body in which epiphysial closure was not so imminent. Fig. 5*d* shows that the lengths of the 3rd metatarsal and phalanx returned more quickly to normal than those of the tibia or femur, suggesting that growth was accelerated to a greater extent in those bones nearer to fusion, so that normal proportions could be regained.

Similar results were observed in the tail. Table 5*a* indicates that there was a maturity gradient in the tail, the more proximal vertebrae being the most mature, but that after return to normal pressure, catch-up occurred in a differential manner, so that all bones regained control values. The 8th and 18th caudal vertebrae were retarded in length to similar degrees and subsequently regained control lengths, irrespective of their stages of maturity. The 13th thoracic vertebra, however, recovered more slowly than the caudal vertebrae (Table 1). The stage of maturity of this bone when the stress was applied is not known, but it is reasonable to assume that it was more mature than the caudal vertebrae studied because of the craniocaudal maturity gradient in the tail. Perhaps, therefore, it had passed a point when 'catch-up' can proceed more quickly in bones reaching epiphysial fusion. It seems that although bone growth and maturity may be dissociated during prolonged periods of severe stress, catch-up can occur differentially, so that adults of proportions as near normal as possible can result.

There seems to be some evidence (Fig. 5) to support Tanner's suggestion (1962) that there is a greater stunting effect on faster growing limb bones. For example, the faster growing tibia was more quickly stunted than the neighbouring femur or metatarsal (Fig. 5*a*). After 28 days' hypoxia, however, it was less stunted than the femur but more stunted than the metatarsal (Fig. 5*c*). This latter bone, in turn, was less stunted than the faster-growing phalanx (Fig. 5*c*).

Associated with findings such as those above, Tanner (1963) proposed that there is a 'time-tally' mechanism present in the body which monitors any changes in size and regulates growth to ensure that normal size is maintained whenever possible. Mosier (1969) extended this proposal to a mechanism controlling body proportions. He demonstrated that there was a greater effect on growth in body weight than in skeletal length under stress produced by fasting, corticosterone injection, or propylthiouracil feeding, so that animals of abnormal body proportions were produced. However, he also showed that the relationship returned to normal after the stress was lifted, and to explain this he proposed that the body is capable of registering

relative growth, so that changes in shape can be monitored and body proportions kept fairly constant. Tanner (1963) suggested that if a system, such as his 'time-tally' for size, was to monitor body proportions it would have to be regulated peripherally, because, if a central mechanism were responsible, one would expect accelerated growth of the whole body to occur when, for example, a limb was amputated. This 'peripheral regulator' is consistent with the suggestion of MacArthur & Chiasson (1945) that growth of each appendage depends on a basic genetic rate determinant acted upon by factors determining body size.

In the present experiments tail length and body length were depressed in proportion to body weight, while the limbs remained relatively long. However, all had returned to normal by 84 days after return to normal pressure. One would therefore expect there to be at least two 'regulators', one for the tail and another for the limbs, because, for differential catch-up to occur, one would expect regulator mechanisms for at least each catch-up variable. In the hind limb alone, however, stunting and catch-up occurred at different rates in the individual bones. This would be inconsistent with the idea of a single 'regulator' for each limb, and suggests that each bone has its own 'regulator'. However, it seems likely that there must also be some 'central' system, perhaps of a different nature altogether from that proposed by Tanner (1963), to monitor general body shape. A mechanism similar to that proposed by MacArthur & Chiasson (1945) seems most likely.

SUMMARY

Growing rats were subjected to hypoxic stress produced by decompression. Changes in skeletal length and maturity and in body weight were observed in exposed animals and compared with the changes in litter mate controls. Exposure to hypoxia was associated with non-uniform retardation of skeletal growth, so that changes in body shape occurred. Dissociation of skeletal growth and maturation was also observed during exposure, but differential catch-up growth during recovery resulted in normally proportioned adults.

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