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Craniofacial morphology and growth in the rat. Cephalometric analysis of the effects of a low calcium and vitamin D-deficient diet

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INTRODUCTION

In earlier studies, a disordered calcification pattern of dentine and an increased metabolic activity in the odontoblast region have been shown in young rats fed a low calcium and vitamin D-deficient diet (Engström, Linde & Magnusson, 1977*a*; Engström, Granström & Linde, 1977*b*; Engström, Jontell & Linde, 1978; Engström, 1980). These results from dentine are consistent with studies on bone in hypocalcaemic and vitamin D-deficient young rats (Ferguson & Hartles, 1963; Baylink, Stauffer, Wergedal & Rich, 1970; Yoshiki & Yanagisawa, 1974). Furthermore, increased enzymatic activities in the metaphysis region of the tibia have been reported by Wergedal (1969) in low calcium and vitamin D-deficient rats.

Vitamin D deficiency is known to change the growth and form of the skull in growing humans (Weinmann & Sicher, 1955). Thus, in view of the previous findings of changes in metabolism of mineralized tissues induced by a low calcium and vitamin D-deficient diet, it may be expected that osteogenesis in different growth sites of the skull should be affected.

The aim of the present investigation was therefore to study the influence of low calcium and vitamin D-deficient diet on the growth and form of the skull of the growing rat, using a cephalometric radiographic technique. It was shown that this diet caused changes in the craniofacial growth pattern. This technique could also be used to obtain an intra-individual cephalometric description of normal growth and form at different ages during postnatal growth in the rat.

MATERIAL AND METHODS

Animals

Pregnant rats of the Sprague–Dawley strain were obtained from Anticimex, Sollentuna, Sweden, and were fed a diet adequate with regard to nutritional needs. A total number of 30 pups, five male pups in each litter, were weaned normally, and were then fed a diet adequate with regard to the nutritional needs until they reached a weight of 75 g. The animals were then divided into two groups with 15 rats in each. One group continued on a control diet (R47) and the second group was fed a diet low in calcium and vitamin D-deficient (R25). The two groups were fed their respective diet for two weeks. The diets were from Astra Ewos AB, Södertälje, Sweden and were previously described (Engström *et al.* 1977*a*).

Table 1. *Radiographic registration at different weights in the normal (R47) group and deficient (R25) group*

($\bar{x}$, Mean value; S.D., standard deviation)

Day after parturition	10	15	20	25	30	42	44
	Normal group						
Body weight (g)							
$\bar{x}$	15·3	30·2	45·5	60·1	75·3	125·4	175·2
S.D.	3·0	4·4	4·9	6·8	3·2	6·5	11·8
Radiographic registration longitudinal	×	×	×	×	×	×	×
Radiographic registration methodologic study	×	.	.	.	×	.	×
Day after parturition	10	20	25	25	30	42	44
	Deficient group						
Body weight (g)							
$\bar{x}$	.	.	.	.	75·4	.	125·4
S.D.	.	.	.	.	4·4	.	6·5
Radiographic registration longitudinal	.	.	.	.	×	.	×

Table 2. *Definition of reference points and lines used in the cephalometric analysis*

Points

Po	The most posterior point on the cranial vault.
N	A point on the nasofrontal suture.
A	The most anterior point on the nasal bone.
E	The intersection between the frontal bone and the most superior-anterior point of the posterior limit of the ethmoid bone.
So	The intersection between the posterior border of the basisphenoid and the tympanic bulla.
Pr	The most inferior and anterior point on the alveolar process of the premaxilla.
Bu	A point on the premaxilla between jaw bone and the lingual surface of the upper lingual incisors.
Iu	The most prominent point between the incisal edges of the upper incisors.
Mu	A point on the intersection between the maxillary bone and the mesial surface of the upper first premolar.
Ii	The most prominent point between the incisal edges of the lower incisors.
Id	The most inferior and anterior point on the alveolar process of the mandible.
Mn	A point in the deepest part of the antegonial notch curvature.
M1	A point on the intersection between the mandibular alveolar bone and the mesial surface on the first premolar.
B1	A point on the intersection between the lingual surface of the lower incisors and the most anterior part of the lingual alveolar bone.

Lines

PoEL through the points Po and E
SoEL through the points So and E
ANL through the points A and E
PrEL through the points Pr and E
BuEL through the points Bu and E
IuEL through the points Iu and E
MuBuL through the points Mu and Bu
MnIdL through the points Mn and Id
M1B1L through the points M1 and B1
MuPrL through the points Mu and Pr
M1IiL through the points M1 and Ii
PrIuL through the points Pr and Iu
IdIiL through the points Id and Ii

Registration

The animals were weighed at different times after parturition. When the normal animals weighed 15, 30, 45, 60, 75 and 125 g, and at the end of the dietary experiment (Table 1), lateral radiographs were taken with the skull of the animal fixed in a specially constructed craniostat. The animal was subjected to light ether anaesthesia. The points of fixation were the external auditory meati with the mid-sagittal plane of the skull horizontally oriented. A standard dental X-ray machine (Type D9, Ritter A-G, Karlsruhe, BRD) was used at 50 kV and 10 mA throughout the whole study, the length of exposure being varied from 0·2 to 0·4 seconds. The focus to film distance was constant at 50 cm. The film was Kodak occlusal ultraspeed film DF-45 (Eastman Kodak Co, Rochester, N.Y., U.S.A.).

The films were developed, fixed and rinsed in a standard X-ray developer at 20–24 °C for a total time of four minutes. The image of the radiograph was copied in Ilford photographic paper (Ilfospeed, 3·24M) enlarged four times. The amount of enlargement of the skull in the radiograph was on average 7·5%. The following cephalometric analysis was made directly on the photographic prints.

Cephalometric analysis

The cephalometric analysis contained both linear and angular measurements and was adapted for rats from analyses described for guinea-pigs by Stenström & Thilander (1967, 1970, 1972) and rabbits by Hellqvist (1972). The distances were measured with calipers and the angles with a protractor graded in halves of a degree. The

Table 3. *Cranial anatomical characteristics were represented by the following cephalometric characteristics given*

Cranial anatomical characteristics	Cephalometric characteristics
Distances	
Total skull length	Po–A
Neurocranial length	Po–E
Cranial base length	So–E
Nasal bone length	N–A
Viscerocranial height	E–Mu
Viscerocranial length	E–Pr, E–Bu
Palatal length	Mu–Bu, Mu–Pr
Mandibular corpus length	Mn–Id
Mandibular lingual alveolar bone	M1–B1
Erupted upper incisor length	Pr–Iu, E–Iu
Erupted lower incisor length	Ii–Id, M1–Ii
Angles	
Cranial vault to cranial base	PoEL/SoEL
Nasal bone to cranial base	ANL/SoEL
Nasal bone to cranial vault	ANL/PoEL
Premaxilla to cranial base	PrEL/SoEL, BuEL/SoEL
Maxilla–premaxilla to cranial base	MuBuL/SoEL, MuPrL/SoEL
Upper incisors to cranial base	IuEL/SoEL
Premaxilla to cranial vault	PrEL/PoEL, BuEL/PoEL
Maxilla–premaxilla to cranial vault	MuBuL/PoEL, MuPrL/PoEL
Upper incisors to cranial vault	IuEL/PoEL
Upper incisor inclination	MuPrL/PrIuL, MuBuL/PrIuL
Lower incisor inclination	M1IiL/IdIiL, M1B1L/IdIiL
Mandibular corpus to cranial vault	MnIdL/PoEL, M1B1L/PoEL
Mandibular corpus to cranial base	MnIdL/SoEL, M1B1L/SoEL

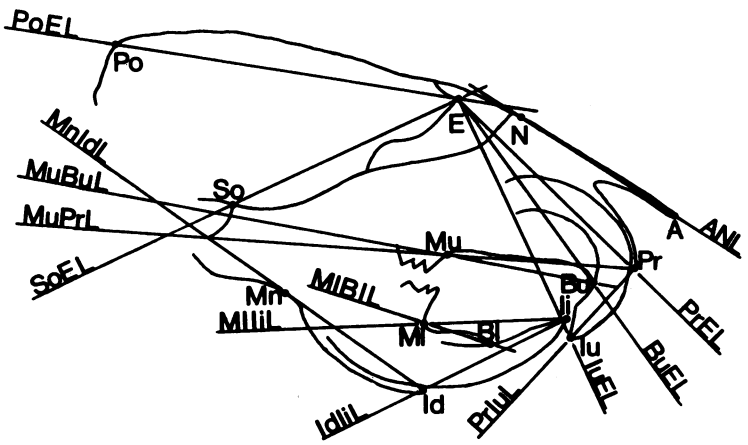


Fig. 1. (*a*) Reference points and lines on the lateral skull radiographs used in the cephalometric analysis. (*b*) A lateral radiograph of the rat skull is seen opposite for comparison.

distances were read by estimation of the nearest half millimetre. The points and lines of reference are shown in Table 2 and Figure 1.

The cranial anatomical characteristics in the cephalometric analysis were represented by the distances and angles as shown in Table 3.

Methodological error

In order to evaluate the methodological error in the measurements, repeated radiographic registrations and cephalometric analyses were performed on normal rats when weighing 15, 75 and 175 g (Table 1). Five animals of each weight were used. A radiographic registration was made on the 15 animals on four different occasions, the interval between registrations being 6 hours, allowing the animals to recover from anaesthesia and minimizing the influence of growth. Following termination of the radiographic registrations, photographic prints (as described above) were made and cephalometric analysis performed. The magnitude of the different components of variation in the technique was estimated by variance analysis.

Statistical analysis

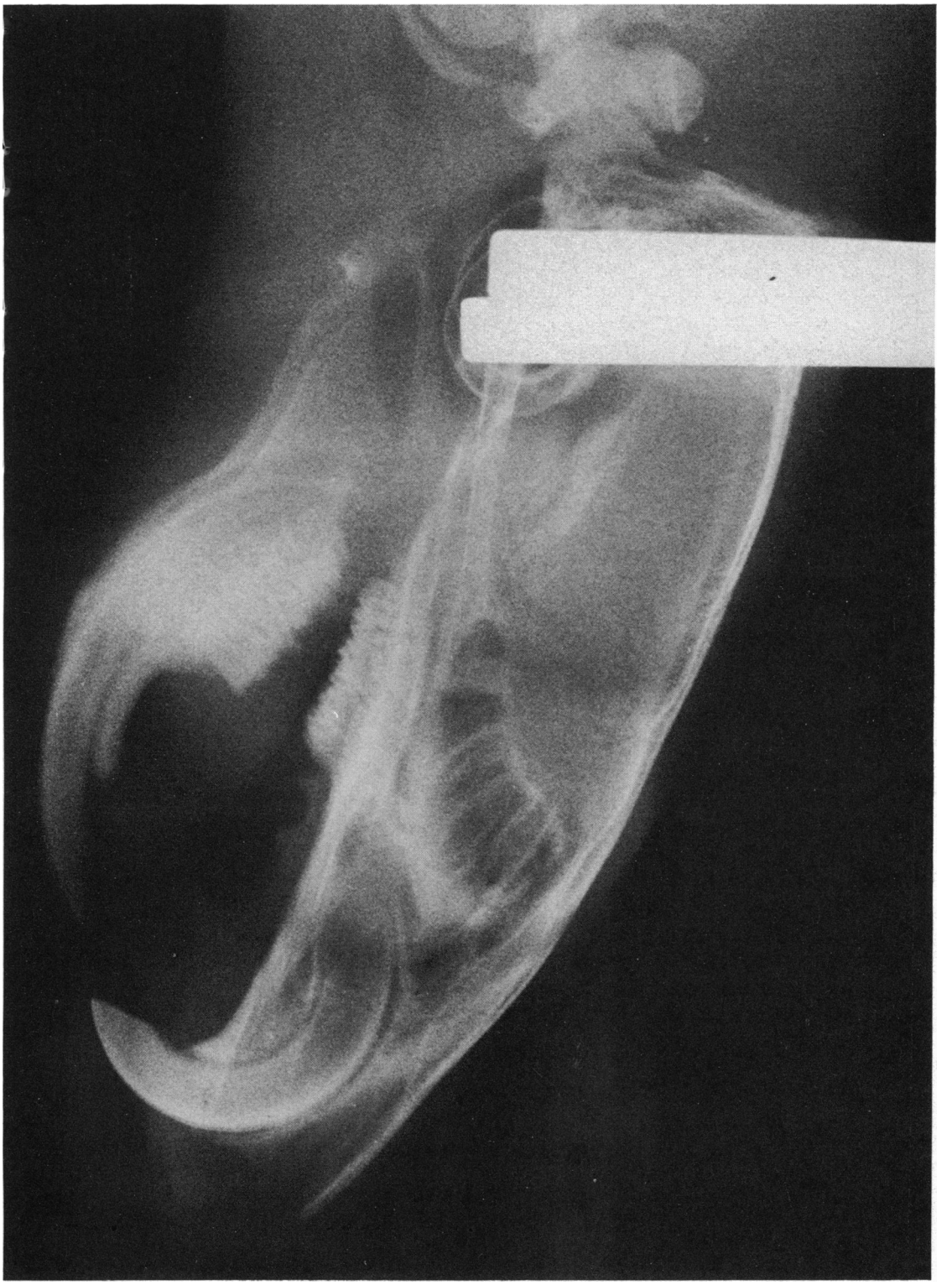
Analysis of variance (Bailey, 1973) was performed in order to evaluate the total methodological error and the ability of the method used to describe morphological changes during postnatal development. The Student's *t* test (Bailey, 1973) was used in order to evaluate possible morphological differences between the normal group (R47 diet) and the deficient group (R25 diet).

Proportional increment

The rate of changes in weight gain and skull characteristics, such as distances and angles, was expressed as percentage proportional increment. The calculation of proportional increment (P.I.) was made as follows:

$$\frac{\text{value at } t_2 \times \text{value at } t_1}{\text{value at } t_1} \times 100.$$

At the time t_1 the animal was of lesser age than at the time t_2.



RESULTS

Methodological error

The measured values of skull characteristics have a variability which is composed of methodological error, growth changes and biological variations. The results of variance analysis on the cephalometric values of skull characteristics *at different ages* showed that the methodological error (residual in Table 4) was significantly lower than the registered growth changes (between registrations in Table 4). The biological variation (between animals in Table 4) was low compared to the registered growth changes (between registrations in Table 4).

The cephalometric characteristics shown in Table 4 were selected from a total of 42 in order to represent the linear dimensions (total skull length, viscerocranial length, palatal length, cranial base length and neurocranial length) as well as the relations of maxilla, nasal bone, mandible and maxillary incisors to the cranial base, and of the nasal bone to the cranial vault.

Table 4. *Growth characteristics in the normal rat. Variance analysis performed on values from longitudinal cephalometric registrations on* 15 *normal rats at the following weights:* 15, 30, 45, 60, 75, 175 g ($xx = P < 0{\cdot}01$)

Cephalometric characteristic	Source of variation	Degrees of freedom	Mean square	Variance ratio	Variability
ANL/PoEL	Between animals	14	2·44	1·17	—
	Between registrations	5	439·89	211·48^{xx}	—
	Residual	71	2·08	—	1·44
IuEL/SoEL	Between animals	14	8·89	1·08	—
	Between registrations	5	55·51	6·76^{xx}	—
	Residual	71	8·21	—	2·86
MuBuL/SoEL	Between animals	14	113·59	4·70^{xx}	—
	Between registrations	5	36·82	1·54	—
	Residual	71	23·93	—	4·89
MnIdL/SoEL	Between animals	14	2·02	0·19	—
	Between registrations	5	53·22	5·06^{xx}	—
	Residual	71	10·50	—	3·24
ANL/SoEL	Between animals	14	129·77	1·13	—
	Between registrations	5	1278·60	11·22^{xx}	—
	Residual	71	113·91	—	10·67
M1B1L/SoEL	Between animals	14	23·60	1·73	—
	Between registrations	5	96·69	7·12^{xx}	—
	Residual	71	13·57	—	3·68
MuPrL/SoEL	Between animals	14	3·18	1·42	—
	Between registrations	5	25·85	11·59^{xx}	—
	Residual	71	2·24	—	1·49
Po–A	Between animals	14	47·02	1·08	—
	Between registrations	5	6242·26	143·43^{xx}	—
	Residual	71	43·52	—	6·59
Mu–Bu	Between animals	14	5·58	0·12	—
	Between registrations	5	587·51	12·98^{xx}	—
	Residual	71	45·24	—	6·72
So–E	Between animals	14	2·78	1·14	—
	Between registrations	5	899·08	368·47^{xx}	—
	Residual	71	2·44	—	1·56
E–pr	Between animals	14	2·62	1·22	—
	Between registrations	5	232·56	108·67^{xx}	—
	Residual	71	2·14	—	1·46
Po–E	Between animals	14	2·11	1·25	—
	Between registrations	5	1314·36	782·36^{xx}	—
	Residual	71	1·68	—	1·30

In the methodological study it was found that repeated registrations (between registrations in Table 5) did not significantly increase the methodological error. As in the cephalometric study on the growth and form of the skull at different ages, the total methodological error (residual in Table 5) was smaller than the differences in skull characteristics between animals in different developmental stages (between animals in Table 5). The cephalometric characteristics presented in Table 5 were selected in order to evaluate the error when analysing linear dimensions (Po–A), angular measurements (PoEL/ANL) and the possibility of relating the position of the mandible to the cranial base (MnIdL/SoEL).

In the evaluation of the cephalometric method employed in this study for investigating growth changes in the craniofacial regions, the method thus seemed to be sufficient with respect to describing morphological changes in the linear dimensions and the relations between anatomical parts of the skull, the exception being the relation between the mandible and other parts of the skull (Tables 4 and 5).

Growth changes in the skull during development in normal rats

The measurements describing the findings in this section are given in Tables 6 and 7.

The angle between the cranial vault and the nasal bone (PoEL/ANL) increased during the period studied, meaning a flattening of the upper aspect of the skull in lateral view. The angle between the cranial vault and the cranial base (PoEL/SoEL) increased slightly.

The premaxilla and maxilla were found to rotate in a dorsal direction. This was indicated by the relationship between the cranial base and the different planes studied, since the angles PrEL/SoEL and BuEL/SoEL were increased. A similar finding was made when the relationship between the maxilla and the cranial vault was studied (angles PrEL/PoEL and BuEL/PoEL). The palate moved in the same anterior direction as the maxilla since the palatal plane became increasingly more parallel with the cranial vault (angle MuBuL/PoEL). This was also indicated when the palatal plane was related to the cranial base, as this angle (MuBuL/SoEL) decreased. The relationship between the palatal plane through the alveolar anterior

Table 5. *Variance in cephalometric radiographic registration*

(The error of method was tested by repeated radiographic registrations, 4 occasions on 15 normal animals. The animal body weights and respective number were 15 g, $n = 5$, 75 g, $n = 5$ and 175 g, $n = 6$. The result of the variance analysis performed on the cephalometric measurements made on the radiographs are shown. ($xx = P < 0{\cdot}01$).)

Cephalometric characteristic	Source of variation	Degrees of freedom	Mean square	Variation ratio
Po–A	Between animals	14	1214·85	2169·37^{xx}
	Between registrations	3	0·15	0·26
	Residual	47	0·56	—
	Variability: 0·74			
MnIdL/SoEL	Between animals	14	4·75	0·53
	Between registrations	3	2·41	0·27
	Residual	47	8·65	—
	Variability: 2·94			
ANL/PoEL	Between animals	14	22·51	72·61^{xx}
	Between registrations	3	0·31	0·23
	Residual	47	1·32	—
	Variability: 1·14			

Table 6. *Craniofacial growth changes in normal rats*

(Values ($\bar{x}$: mean ± S.D.: standard deviation) found for the cephalometric characteristics at given body weights.)

Cephalometric characteristic	15 g		30 g		45 g		60 g		75 g	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
ANL/PoEL	144·8	2·2	115·5	1·3	158·2	1·3	155·7	1·7	156·5	1·1
PrEL/PoEL	131·5	1·9	135·6	1·3	142·7	1·7	142·5	2·4	145·1	1·6
BuEL/PoEL	119·3	2·0	125·1	1·7	133·5	1·7	132·2	2·4	135·1	1·4
IuEL/PoEL	115·0	2·0	119·0	1·5	124·9	1·5	124·3	2·1	126·1	1·6
PoEL/SoEL	28·2	1·0	29·1	1·2	31·3	1·3	32·4	1·0	32·3	0·9
MuBuEL/PoEL	17·1	1·9	11·3	2·3	3·6	1·5	7·7	1·8	6·3	2·2
MnIdL/PoEL	16·1	2·4	13·6	2·0	11·7	1·8	10·2	2·0	10·1	2·4
M1B1L/PoEL	9·1	2·7	13·2	1·9	11·5	2·4	7·5	3·2	7·9	4·0
MuPrL/PoEL	2·0	0·9	1·8	1·5	3·0	1·0	1·6	1·2	1·5	1·0
PrEL/SoEL	102·9	1·4	107·3	3·8	111·0	1·3	110·4	1·5	112·7	1·9
BuEL/SoEL	91·0	1·6	95·8	2·1	102·1	1·3	100·5	1·4	102·6	2·0
IuEL/SoEL	89·0	6·4	90·2	1·1	93·5	1·2	92·3	1·2	93·6	1·9
MuBuL/SoEL	45·1	2·0	40·9	2·2	34·7	1·5	40·2	1·5	38·3	1·5
MnIdL/SoEL	44·3	2·8	41·7	2·4	43·5	1·4	42·0	4·9	42·3	2·4
ANL/SoEL	115·3	4·0	122·5	1·5	126·4	1·3	122·6	1·5	124·0	1·5
M1B1L/SoEL	37·3	2·3	38·3	3·7	42·8	2·8	39·0	3·9	40·1	4·0
MuPrL/SoEL	30·1	1·9	31·5	2·1	27·9	1·0	31·6	1·6	30·4	1·0
MuPrL/PrIuL	61·7	2·1	60·3	1·9	61·7	2·4	61·0	1·7	59·7	2·5
M1IiL/IdIiL	28·4	1·8	30·5	1·8	31·9	2·1	28·8	1·5	29·1	3·5
MuBuL/PrIuL	78·3	1·8	69·8	1·8	69·0	2·0	70·3	2·0	68·1	2·4
M1B1L/IdIiL	30·5	2·4	39·2	1·8	45·4	8·1	40·7	2·9	43·2	3·5
Po–A	103·5	1·6	124·7	1·2	136·5	1·6	143·7	1·5	143·3	1·9
E–Mu	24·7	0·7	29·3	1·2	32·2	1·2	34·5	1·3	34·0	1·5
Po–E	74·7	1·1	86·5	0·9	92·1	1·4	92·1	1·5	91·5	0·8
N–A	25·1	1·0	31·6	1·6	38·7	1·7	43·3	1·8	44·0	1·5
E–Pr	33·9	1·2	41·9	1·7	48·6	1·8	54·8	1·6	55·9	1·1
E–Bu	35·3	1·0	40·4	2·2	46·7	1·3	52·5	1·4	53·6	1·5
E–Iu	38·9	1·0	45·1	1·5	54·3	1·2	60·7	1·5	61·1	1·4
E–So	48·4	1·4	51·9	1·6	61·0	1·1	62·3	1·8	61·9	1·4
Mu–Pr	25·8	1·1	35·4	1·0	41·1	1·0	44·0	1·1	44·9	2·1
Mu–Bu	25·8	1·1	29·9	2·3	34·6	1·1	36·4	1·5	36·3	1·5
Pr–Iu	12·1	0·8	13·4	1·1	16·0	1·9	19·3	0·8	19·9	0·6
M1–Ii	23·3	1·2	30·1	2·5	33·0	1·3	37·3	1·6	36·7	2·1
M1–B1	14·3	2·3	20·3	1·5	21·5	0·7	21·7	1·2	21·3	1·4
Ii–Id	16·0	1·4	21·4	1·1	26·6	1·7	32·3	1·1	32·2	1·5
Mn–Id	33·7	2·3	40·1	2·6	41·7	2·2	43·4	1·8	43·1	2·1

margin and the cranial base or cranial vault was only slightly changed during the period studied (angles MuPrL/SoEL and MuPrL/PoEL). The incisal edges became increasingly more anteriorly situated (angles IuEL/SoEL and IuEL/PoEL). It was also noted that the curvature of the incisors increased during the period, as indicated by the angles describing the relationship between the mandible or maxillary alveolar and basal bone and the incisal edges (MuPrL/PrIuL, MuBuL/PrIuL and MiIiL/IdIiL).

The change in relations between different parts of the skull was not consistent but varied during the weight period 15–45 g. Above 45 g of body weight the growth pattern was relatively stable according to the description given above (Tables 6 and 7).

The linear dimensions of the normal rat cranium increased up to a body weight of 60 g. Between 60 and 75 g no change was noted. Above this body weight a continuous increase in linear dimensions up to 175 g was noted. The rate of changes in linear dimensions, given as P.I. of distances, was on the whole highest at body weights between 30 and 45 g (Tables 6 and 7).

Table 7. *Craniofacial growth changes in normal rats, given as proportional increment (P.I.)*

(Proportional increment (%) indicates the amount of change in cephalometric characteristics which occurred between registrations at given body weights. Percentage error (coefficient of variation) was less than 13·2.)

Cephalometric characteristic	15–30 g	30–45 g	45–60 g	60–75 g	75–175 g
ANL/PoEL	4	4	−1	0	2
PrEL/PoEL	3	5	0	1	1
BuEL/PoEL	4	6	−1	2	1
IuEL/PoEL	3	4	0	1	0
PoEL/SoEL	3	7	3	0	0
MuBuEL/PoEL	−33	−68	114	−19	36
MnIdL/PoEL	−15	−14	−12	1	30
M1B1L/PoEL	45	−1	−6	5	37
MuPrL/PoEL	−10	64	−45	−8	59
PrEL/SoEL	4	3	1	2	1
BuEL/SoEL	5	6	−1	2	1
IuEL/SoEL	1	3	−1	1	0
MuBuL/SoEL	−9	−15	−15	−4	5
MnIdL/SoEL	−5	4	−3	1	10
ANL/SoEL	6	3	−3	−1	−1
M1B1L/SoEL	2	11	−9	3	8
MuPrL/SoEL	2	−11	−15	−3	1
MuPrL/PrIuL	−2	2	−1	−1	−7
M1IiL/IdIiL	7	4	−9	1	0
MuBuL/PrIuL	−10	−1	1	−3	−5
M1B1/IdIiL	28	15	−10	6	12
Po–E	15	6	0	0	13
N–A	25	22	11	1	23
E–Pr	23	16	12	1	23
E–Bu	14	15	12	2	23
E–Iu	16	20	11	0	21
E–So	7	17	2	0	12
Mu–Pr	37	16	6	−1	20
Mu–Bu	15	15	5	−0	21
Pr–Iu	11	19	20	2	28
M1–Ii	26	7	12	−2	18
M1–B1	42	5	1	1	12
Ii–Id	34	24	21	0	21
Mn–Id	18	3	4	1	11
Po–A	20	9	5	0	17
E–Mu	15	10	7	−1	16

Body weight development in normal (R47 group) rats compared to the deficient (R25 group) rats

The total weight was increased continuously in the normal rat during the period studied (Fig. 2). On the whole, the proportional increment of body weight (Fig. 3) increased during the early growing stage (age 0–20 days post-parturition) and decreased during the late growing stage (20–44 days post-parturition). Incidental peaks of proportional increment of normal body weight were noted, first during the late growing stage, at a time when the pups begin to supplement their diet with non-milk food and second around day 40 (post-parturition), which indicated a pre-pubertal growth spurt.

The proportional increment of weight was lower in the deficient group (R25) than in the normal group (R47) (Fig. 3). Accordingly, the total body weight was lower in

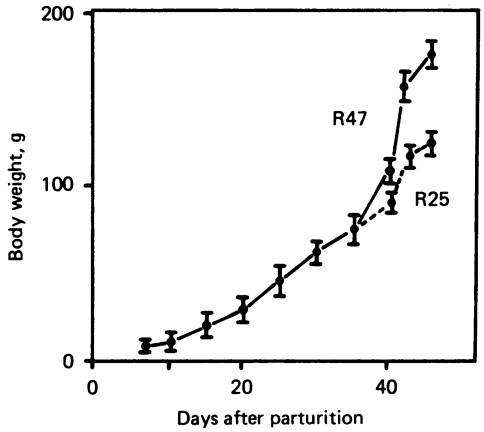


Fig. 2. Body weight (mean value ± S.D.: g) at different days after parturition of normal rats (——) and of deficient rats (---).

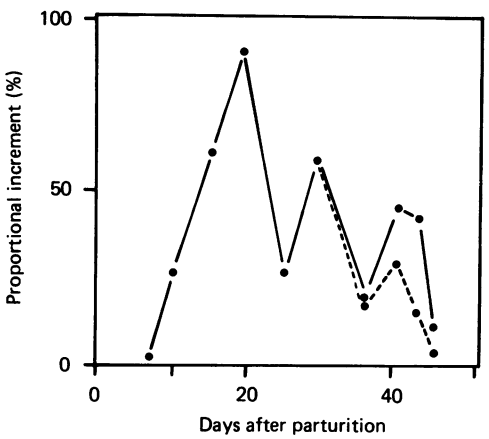


Fig. 3. Proportional increment of body weight in normal rats (——) and in deficient rats (---). (Cv was less than 10·2 %.)

the deficient group when compared to the normal group at the end of the experimental period (Fig. 2). However, the prepubertal growth spurt indicated by a peak of P.I. in the late growing stage appeared at the same time in the two groups (Fig. 3).

Differences in the growth and form of the skull between deficient and normal rats of the same age

The main findings on the growth and form of the skull in the animals fed the deficient diet when compared to the animals on the normal diet were a decreased total anterior–posterior length (distance Po–A), a decreased vertical height (distance E–Mu) and a decreased cranial base length (distance So–E) (Tables 8 and 9). The main effect in length was the decreased length of the neurocranium (distance Po–E), since the nasal bone seemed to be unchanged (distance N–A). The difference in length was also reflected in that the length of the viscerocranium (distances E–Pr and E–Bu) was decreased in the deficient R25 group. No change was found in the

Table 8. *Differences between deficient and normal rats in craniofacial morphology*

(Values of cephalometric characteristics ($\bar{x}$ (mean) ± S.E.M. (standard error of the mean); n = 0·5) found for the normal group (R47 diet) and the deficient fed group (R25 diet). $P <$ indicates statistical significant differences found with Student's t test when the two groups are compared.)

Cephalometric characteristic	R47		R25		
	$\bar{x}$	S.E.M.	$\bar{x}$	S.E.M.	$P<$
Po–E	103·4	0·5	96·9	0·6	0·001
N–A	54·2	0·4	53·1	0·6	n.s.
E–Pr	69·0	0·4	64·5	0·6	0·001
E–Bu	66·0	0·5	62·3	0·3	0·001
E–Iu	74·1	0·5	72·0	0·3	0·001
E–So	69·8	0·5	66·1	0·9	0·001
Mu–Pr	54·1	0·5	53·7	0·4	n.s.
Mu–Bu	44·1	0·7	44·8	0·3	n.s.
Pr–Iu	25·6	0·5	24·3	0·2	n.s.
M1–Ii	43·6	0·5	43·7	0·5	n.s.
M1–B1	23·9	0·5	24·5	0·4	n.s.
Ii–Id	39·4	0·8	39·4	0·8	n.s.
Mn–Id	48·1	0·6	43·9	0·5	0·001
Po–A	168·1	0·7	158·7	0·7	0·001
E–Mu	40·5	0·3	37·2	0·3	0·001
ANL/PoEL	159·6	0·3	155·5	0·3	0·001
PrEL/PoEL	146·5	0·2	148·2	0·3	0·001
BuEL/PoEL	136·3	0·2	138·3	0·3	0·001
IuEL/PoEL	125·9	0·3	128·4	0·3	0·001
PoEL/SoEL	32·4	0·3	33·2	0·3	n.s.
MuBuL/PoEL	8·5	0·9	6·1	0·5	0·009
MnIdL/PoEL	13·2	0·7	12·7	0·5	n.s.
M1B1L/PoEL	10·9	1·0	11·9	1·3	n.s.
MuPrL/PoEL	2·3	0·3	2·5	0·2	n.s.
PrEL/SoEL	113·7	0·4	115·0	0·3	0·011
BuEL/SoEL	103·5	0·4	105·0	0·3	0·001
IuEL/SoEL	93·2	0·4	95·2	0·3	0·001
MuBuL/SoEL	40·3	0·8	39·1	0·3	n.s.
MnIdL/SoEL	46·7	0·8	46·2	0·7	n.s.
ANL/SoEL	122·7	0·4	126·3	0·3	0·001
M1B1L/SoEL	43·7	1·5	45·8	1·5	n.s.
MuPrL/SoEL	30·8	0·4	30·5	0·4	n.s.
MuPrL/PrIuL	55·5	0·6	61·6	0·4	0·001
M1IiL/IdIiL	29·0	0·7	29·2	0·5	n.s.
MuBuL/PrIuL	64·6	0·7	70·1	0·5	0·001
M1B1L/IdIiL	48·5	1·0	48·7	0·8	n.s.

incisors (distances Pr–Iu and Ii–Id), in the parts of the mandible studied (distance M1–B1) or in the length of the palate (distance Mu–Bu).

No change in relationship between the cranial base and the cranial vault (angle PoEL/SoEL) could be found when the R25 group and the R47 group were compared (Tables 8 and 9). In lateral view, the upper aspect of the skull was more curved in the deficient groups when compared to the normal group; this was reflected in a decreased angle between the cranial vault and the nasal bone (angle PoEL/ANL). The relationship between the palate and the cranial base (angle MuBuL/SoEL) did not show any significant difference between the two groups. However, the relation between the palate and the cranial vault (angle MuBuL/PoEL) was significantly more parallel in the deficient group. On the whole, the viscerocranium was in a more anterior and upward position in the deficient group when compared to the normal group (angles

Table 9. *A summary of the difference found in the radiographic cephalometric study of skull morphology between the deficient (R25) group and the normal groups, (a) with the same age and (b) with the same body weight*

Anatomical characteristic	R25 group compared to a group with the same age	R25 group compared to a group with the same body weight
Skull length	Decreased	Increased
Neurocranial length	Decreased	No difference
Viscerocranial length	Decreased	Increased
Height	Decreased	Increased
Cranial base length	Decreased	No difference
Cranial base/cranial vault	No difference	No difference
Viscerocranium/cranial vault	Changed relation	Changed relation
Viscerocranium/cranial base	Changed relation	Changed relation
Palate/cranial base	No difference	No difference
Nasal bone/cranial vault	Changed relation	Changed relation
Palatal length	No difference	No difference
Incisal edge, upper incisor	Changed position	Changed position
Incisal edge, lower incisor	No difference	Changed position
Curvature, upper incisor	Greater	Greater
Curvature, lower incisor	No difference	Greater

PrEL/PoEL, BuEL/PoEL, PrEL/SoEL, BuEL/SoEL) as indicated by the positions of the palate. Accordingly, the upper incisors showed the same change in the deficient group (angles IuEL/PoEL and IuEL/SoEL). A greater curvature was found for the upper incisors in the deficient group (angles MuBuL/PrUiL and MuPrL/PrIuL), whereas no difference could be demonstrated for the lower incisors (angles M1Ii/IiIdL and M1B1L/IdIiL).

Difference in the growth and form of the skull between deficient and normal rats with the same body weight

The length of the skull (distance Po–A) was significantly greater in the deficient R25 group but the length of the nasal bone (distance N–A) and the length of the neurocranium (distance Po–E) did not differ between the two groups (Tables 9 and 10). The height of the skull (distance E–Mu) was significantly greater in the R25 group when compared to the normal group. No difference with respect to cranial base (distance So–E) was noted. The palate was longer in the deficient (R25) group (distance Mu–Bu) as was the length of the viscerocranium (distance E–Pr and E–Bu). No significant difference was found in the corpus part of the mandible (distance Mn–Id) between the two groups. The distances between the incisal edges (upper and lower) and the anterior marginal part of the alveolar bones were significantly greater in the deficient group when compared to the normal group (distances Id–Ii and Pr–Iu).

The relationship between the cranial base and the cranial vault (angle PoEL/SoEL) did not differ between the groups (Tables 9 and 10). The angle between the nasal bone and the cranial vault (PoEL/ANL) was significantly smaller in the deficient group. The viscerocranium was more anteriorly positioned in the deficient group when compared to the normal group, both when related to the cranial base (angles PrEL/SoEL and BuEL/SoEL) and to the cranial vault (angles PrEL/PoEL and BuEL/PoEL). The relationships between the palatal plane and the cranial vault (angle MuBUL/PoEL) or the cranial base (angle MuBuL/SoEL) did not show any significant differences when the two groups were compared. The upper incisors were

Table 10. *Differences between deficient and normal rats in craniofacial morphology*

(The values of cephalometric characteristics in 125 g (b.w.) normal rats are compared to values of the deficient fed group (R25 diet), Table 8. $P <$ indicates statistical significant difference found with Student's t test. ($\bar{x}$ (mean) $\pm$ S.E.M.; $n = 15$.)

Cephalometric characteristic	125 g $\bar{x}$	125 g S.E.M.	125 g compared to R25, $P <$
ANL/PoEL	156·6	0·3	0·001
PrEL/PoEL	145·0	0·6	0·001
BuEL/PoEL	135·4	0·4	0·001
IuEL/PoEL	126·4	0·2	0·001
PoEL/SoEL	31·8	0·5	n.s.
MuBuEL/PoEL	7·8	0·4	n.s.
MnIdL/PoEL	10·2	1·2	0·020
M1B1L/PoEL	5·0	1·6	0·001
MuPrL/PoEL	0·5	0·3	0·001
PrEL/SoEL	113·2	0·6	0·009
BuEL/SoEL	103·4	0·5	0·001
IuEL/SoEL	94·0	0·5	0·05
MuBuL/SoEL	40·0	0·4	n.s.
MnIdL/SoEL	44·4	1·2	n.s.
ANL/SoEL	124·2	0·4	0·001
M1B1L/SoEL	35·0	0·8	0·001
MuPrL/SoEL	31·0	0·3	n.s.
MuPrL/PrIuL	58·8	0·9	0·007
M1IiL/IdIiL	25·4	0·5	0·001
MuBuL/PrIuL	66·8	0·7	0·001
M1B1L/IdIiL	40·8	1·0	0·001
Po–A	154·4	1·1	0·001
E–Mu	34·8	0·3	0·001
Po–E	95·0	0·6	n.s.
E–Pr	61·2	0·5	0·005
E–Bu	57·6	0·8	0·001
E–Iu	64·6	0·8	0·001
E–So	64·0	0·6	n.s.
Mu–Pr	47·8	0·7	0·001
Mu–Bu	43·4	0·7	n.s.
Pr–Iu	21·8	0·5	0·001
M1–Ii	39·2	0·4	0·001
M1–B1	21·0	0·3	0·001
Ii–Id	33·0	0·7	0·001
Mn–Id	44·2	0·6	n.s.
N–A	52·9	0·5	n.s.

more anteriorly positioned in the deficient group when compared to the normal group (angles IuEL/PoEL and IuEL/SoEL). The curvature of the incisors was greater in the deficient group than in the normal group (angles MuPrL/PrIuL, MuBuL/PrIuL, M1Ii/IiIdL and M1M1L/IiIdL).

DISCUSSION

In studying changes in the growth and form of the skull during postnatal development, a cephalometric technique is well suited, mainly because of the possibility of following the development of each animal. The radiographic technique has been used in cross sectional studies to investigate growth aberrations due to experimentally induced disturbances in different species (Asling & Frank, 1963; Stenström &

Thilander, 1967, 1970, 1972; Hellqvist, 1972) and in studies to investigate the intra-individual postnatal development of the skull in the rat (Spence, 1940; Asling & Frank, 1963; Hughes & Tanner, 1970; Hughes, Tanner & Williams, 1978). On the other hand, the cephalometric radiographic techique has been suggested as unreliable due to the great methodological error (Cleall, Wilson & Garnett, 1969).

In the methodological part of the present study, the combined components of variation of the methodological error, such as the influence of individual and biological variation and repeated registrations, were found to be significantly smaller than the recorded growth changes or differences found between normal and low calcium and vitamin D-deficient rats. The cephalometric radiographic technique therefore seems to be of value in studies on experimentally induced growth aberrations of the rat skull. This is in contrast to the findings of Cleall *et al.* (1969).

Characteristics of the Sprague–Dawley strain are a continuously increased body weight (with the absence of a pre-pubertal growth spurt) and the fact that the normal growth of the skull is ahead of the body weight gain (Farris & Griffiths, 1967; Hughes & Tanner, 1970; Falkmer & Waller, 1972; Acheson, Macintyre & Oldham, 1959). The latter characteristic was also found in the present study. However, the proportional increment of body weight indicated, at the end of the period studied (30–44 days post-parturition), a growth spurt which coincided with an increase in skull dimensional proportional increment.

The highest growth rate of the normal skull occurred in the early growing stage (0–20 days post-parturition), with a predominance of neurocranial growth. A shift of the predominance of growth to the viscerocranium was noted in the period of the late growing stage (20–44 days parturition). This was also observed by Moss & Baer (1956) and by Hughes *et al.* (1978). The normal growth of the skull in the late growing stage seemed to continue in a constant direction. The same result was obtained by Spence (1940), using radiographic technique, and by Cleall *et al.* (1969), using vital staining technique. Together with the shift to a predominance of viscerocranial growth in the late growing stage, this indicated an alteration of the form of the skull from klinorhynchal to orthocranial, as suggested by Moss (1958) and further shown by Baer (1954) and Pucciarelli (1978).

In the present study the low calcium and vitamin D-deficient diet was found to decrease the rat body weight gain. The same was found by Steenbock & Herting (1955). The growth and form of the skull were also changed in the late growing stage, the main effect being on the viscerocranium.

The fact that the growth in the viscerocranium seemed to dominate in comparison to that in the neurocranium in the late growing stage of the normal rat might explain the finding that the main effect of the deficient diet was on the viscerocranial part of the skull and in growth sites in the border areas between the viscerocranial and the neurocranial parts of the skull.

A disturbed osteogenesis in various growth sites of the skull might be expected since indications for a disturbed osteogenesis in rats low in calcium and vitamin D-deficient have been found in previous studies on bone (Ferguson & Hartles, 1963; Wergedal, 1969; Baylink *et al.* 1970; Yoshiki & Yanagisawa, 1974) and dentine (Yoshiki & Yanagisawa, 1974; Engström *et al.* 1977*a*, *b*). Furthermore, skull growth in human rickets is known to be changed (Weinmann & Sicher, 1955), which is of interest, because human rickets is a metabolic disturbance similar to the dietary disturbance experimentally induced in this study (Rasmussen & DeLuca, 1963).

Because osteogenic activity has been documented in sutures, synchondroses and

periosteal surfaces of normal rats even older than those used in the present study (Cleall *et al.* 1969), it seems probable that the altered growth and form of the skull in the low calcium and vitamin D-deficient rats were primarily caused by a disturbed osteogenesis in different craniofacial growth sites. As the linear dimensions as a whole were found to be decreased in the deficient animals, this indicated a failure of adequate bone formation in sutures, synchondroses and periosteal areas. The change in relations between different parts of the skull found in the deficient rats indicates a disturbed capacity for accomplishing normal remodelling growth in response to influences of the functional matrix during the experimental period. In accordance with this suggestion, an effect on the remodelling capacity has been previously noted in tibial bone of low calcium and vitamin D-deficient rats by Stauffer, Baylink, Wergedal & Rich (1972). The change in the form of the skull in the deficient rats could not be explained by a simple arrest of development, since differences in form were also found when the deficient animals were compared to normal rats of the same body weight, the main differences being in the viscerocranium.

To conclude, in the low calcium and vitamin D-deficient rats impaired increase in body weight as well as impaired growth of the skull were found. The cephalometric radiographic technique, used to study the growth and form of the skull, was found to be well suited for the purpose of detailed description. Since the growth and form of the skull in the low calcium and vitamin D-deficient rats were found to be changed, both when compared to rats of the same age and to rats of the same body weight, these indicated a disturbed osteogenesis in growth sites determining the growth and form of the viscerocranium and its relation to the neurocranium. To elucidate the basic mechanisms behind the disturbed osteogenesis in these deficient rats, further studies on possible metabolic changes in craniofacial growth sites seem to be of interest.

SUMMARY

The influence of a low calcium and vitamin D-deficient diet on the growth and form of the skull of the growing rat was studied using a cephalometric radiographic technique. First, this technique was used to obtain an intra-individual cephalometric description of the normal growth and form of the skull at different ages during postnatal growth in the rat. It was shown that the methodological error and the biological variation between animals were significantly lower than the registered growth changes, thus demonstrating the suitability of the technique. In the animals fed the low calcium and vitamin D-deficient diet an impaired increase in body weight was found. This diet also caused changes in cranial dimensions, both when the deficient animals were compared with control animals of the same age and with control animals with the same weight. It was concluded that the vitamin D-free and low calcium diet caused a disturbed osteogenesis in growth sites determining the growth and form of the viscerocranium and its relation to the neurocranium.

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REFERENCES

ACHESON, R. M., MACINTYRE, M. N. & OLDHAM, E. (1959). Technique in longitudinal studies of the skeletal development of the rat. *British Journal of Nutrition* **13**, 282–292.

ASLING, A. C. & FRANK, H. R. (1963). Roentgen cephalometric studies on skull development in rats. Normal and hypophysectomized females. *American Journal of Physiology* **21**, 527–537.

BAER, M. J. (1954). Patterns of growth of the skull as revealed by vital staining. *Human Biology* **26**, 80–126.

BAILEY, N. T. J. (1973). *Statistical Methods in Biology*. London: English University Press Ltd.

BAYLINK, D., STAUFFER, M., WERGEDAL, J. & RICH, D. (1970). Formation, mineralization and resorption of bone in vitamin D-deficient rats. *The Journal of Clinical Investigation* **49**, 1122–1134.

CLEALL, J. F., WILSON, G. W. & GARNETT, D. S. (1969). Normal craniofacial skeletal growth of the rat. *American Journal of Physical Anthropology* **29**, 225–242.

ENGSTRÖM, C., LINDE, A. & MAGNUSSON, B. C. (1977*a*). Odontoblast metabolism in rats deficient in vitamin D and calcium. I. A histochemical survey. *Journal of Oral Pathology* **6**, 359–366.

ENGSTRÖM, C., GRANSTRÖM, G. & LINDE, A. (1977*b*). Odontoblast metabolism in rats deficient in vitamin D and calcium. II. Changes in activities of alkaline phosphatases. *Journal of Oral Pathology* **6**, 367–372.

ENGSTRÖM, C., JONTELL, M. & LINDE, A. (1978). Odontoblast metabolism in rats deficient in vitamin D and calcium. III. Protein synthesis *in vitro*. *Journal of Oral Pathology* **7**, 227–253.

ENGSTRÖM, C. (1980). Odontoblast metabolism in rats deficient in vitamin D and calcium. IV. Lysosomal and energy metabolic enzymes. *Journal of Oral Pathology* **9**, 246–254.

FALKMER, S. & WALLER, T. (1972). *Försöksdjurskunskap*. Stockholm: Allmänna Förlaget.

FARRIS, E. J. & GRIFFITHS, J. Q. H. (1967). *The Rat in Laboratory Investigation*. New York: Hafner Publishing Co.

FERGUSON, H. W. & HARTLES, R. L. (1963). The effect of vitamin D on the bones of young rats receiving diets low in calcium or phosphorus. *Archives of Oral Biology* **8**, 407–420.

HELLQVIST, R. (1972). Facial skeleton growth after periosteal resection. *Scandinavian Journal of Plastic and Reconstructive Surgery, Suppl.* **10**.

HUGHES, P. C. R. & TANNER, J. M. (1970). A longitudinal study of the growth of the black-hooded rat: methods of measurement and rates of growth for skull, limbs, pelvis, nose–rump and tail-length. *Journal of Anatomy* **106**, 349–370.

HUGHES, P. C. R., TANNER, J. M. & WILLIAMS, J. P. C. (1978). A longitudinal radiographic study of the rat skull. *Journal of Anatomy* **127**, 83–91.

MOSS, M. L. & BAER, M. J. (1956). Differential growth of the rat skull. *Growth* **20**, 107–120.

MOSS, M. L. (1958). Rotation of the cranial components in the growing rat and their experimental alteration. *Acta anatomica* **32**, 65–86.

PUCCIARELLI, H. M. (1978). Craniofacial development of the rat with respect to vestibular orientation. *Acta anatomica* **100**, 101–110.

RASMUSSEN, H. & DELUCA, H. F. (1963). Calcium homeostasis. *Ergebnisse der Physiologie* **53**, 108–173.

SPENCE, J. M. (1940). Method of studying the skull development of the living rat by serial cephalometric roentgenograms. *The Angle Orthodontist* **10**, 127–139.

STAUFFER, M., BAYLINK, D., WERGEDAL, J. & RICH, C. (1972). Bone repletion in calcium deficient rats fed a high calcium diet. *Calcified Tissue Research* **9**, 163–172.

STEENBOCK, H. & HERTING, D. C. (1955). Vitamin D and growth. *Journal of Nutrition* **57**, 449–468.

STENSTRÖM, S. J. & THILANDER, B. L. (1967). Facial skeleton growth after bone grafting to surgically created premaxillo-maxillary suture defects: An experimental study on the guinea pig. *Plastic and Reconstructive Surgery* **40**, 1–12.

STENSTRÖM, S. J. & THILANDER, B. L. (1970). Effects of nasal septal cartilage resections on young guinea pigs. *Journal of Plastic and Reconstructive Surgery* **45**, 161–170.

STENSTRÖM, S. J. & THILANDER, B. L. (1972). Healing of surgically created defects in the septal cartilages of young guinea pigs. *Journal of Plastic and Reconstructive Surgery* **49**, 194–199.

WEINMANN, J. P. & SICHER, H. (1955). *Bone and Bones. Fundamentals of Bone Biology*. St. Louis: C. V. Mosby Co.

WERGEDAL, J. E. (1969). Enzymes of protein and phosphate catabolism in rat bone. *Calcified Tissue Research* **3**, 67–73.

YOSHIKI, S. & YANAGISAWA, T. (1974). The role of Vitamin D in the mineralization of dentin in rats made rachitic by a diet low in calcium and deficient in vitamin D. *Calcified Tissue Research* **15**, 295–302.