THE GROWTH IN LENGTH OF THE LONG BONES IN THE MADDER-FED PIG[1]

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INTRODUCTION

It has long been known that the bones of the limbs grow in length by additions to the ends of the diaphyses, that the increments are unequal at the two ends, and that the greater increment is constant in position for a given bone (Keith (12), Sharpey-Schafer (16)).

These facts have been established (1) experimentally, (2) by observation of the bones of madder-fed animals, and (3) lately by radiographic observations of the ends of human bones

Experimental. That the limb bones grow in length by additions to their ends was first demonstrated by Stephen Hales (8) in 1727. He pierced two small holes half an inch apart in the tibia of a growing chicken with a sharp-pointed iron. Two months later he found that the distance between the two marks was still the same. He concluded that the bone between the marks had not distended lengthwise over the period of two months, and thus experimentally threw doubt on the common belief in the interstitial growth of bone. Further, he noted that the bone as a whole had grown an inch in length and that the growth was mostly at the upper end of the bone. These fundamental experiments of Hales were repeated with minor modifications of technique and on different animals by Duhamel (4) (silver stylets, pigeon and dog), John Hunter (11) (lead shot, pig), Flourens (6) (silver balls, rabbit) and Ollier (15) (lead shot, rabbit). Gatewood and Mullen (7) have recently again confirmed the conclusions of these observers by placing shot at measured intervals in the bones of young rabbits.

Madder Feeding. The colouring of bone by madder, first described in England by Belchier (1) in 1736, was employed by Duhamel (5), John Hunter (11), Humphry (10) and Kölliker (13) to investigate the mode of growth of the long bones. By madder feeding Duhamel certainly demonstrated that the shaft grows in width by sub-periosteal deposit; but there appears to be some doubt whether he thus confirmed his conclusions from the silver stylet experiments concerning growth in length. It is generally presumed that John Hunter did confirm his conclusions from the shot experiments by observations on the long bones of his madder-fed pigs, thus noting that length is obtained by additions to the ends of the shaft, and that there is a greater increment at one end than the

[1] Based on part of a thesis (June, 1929) accepted by the University of Birmingham for the degree of M.D. ("Observations on the Growth of the Limb Bones in the Pig").

other; but these observations do not appear to be recorded by him. Humphry certainly noted these facts and explains "that extension of the shaft takes place most quickly and is most prolonged, at the end where the bone can best bear the weakening consequent upon the more rapid changes in the growing matrix; and that is usually the larger end."

Radiography. Harris (9) has recently demonstrated radiographically that transverse striations near the diaphysial ends of the long bones of a non-rachitic child could be accurately dated over a period of two years. These transverse striations are held by Harris to represent periods of relative cessation of growth, corresponding to illnesses. The fact that the intervals between them remain constant as the bones grow in length strikingly confirms the other evidence that growth in length takes place only at the ends of the diaphyses and provides a means of measuring the relative amounts of growth at the two ends of human long bones.

The situation and direction of the nutrient canal has also been used by Digby (3) to show the unequal amount of growth at the diaphysial extremities. Human long bones were sectioned longitudinally through the plane of the nutrient canal and the plane of the canal projected into the medullary cavity until a point was reached midway between the walls of the section. This point Digby supposed to be the site of primary ossification[1].

The present contribution to the subject is based on the measurement of growth increments, during given periods, to the ends of the diaphyses of the limb bones of madder-fed pigs. These measurements are sufficiently accurate to permit statements of the relative amounts and calculation of the relative rates of bone growth at the two ends of the diaphyses. A method of graphic representation has been employed to demonstrate these relative amounts and rates.

MATERIAL AND METHODS

I am indebted to Prof. Brash for placing at my disposal the limb bones of a dozen madder-fed pigs varying in age between 80 and 587 days. These pigs came from three separate litters, were of mixed breed (sow, middle-large white; boar, Berkshire), and had all been castrated or spayed according to sex. They had all been fed from weaning with barley meal and "sharps" to which madder had been added; and in the case of one of the litters the sow had also been madder-fed for a fortnight before she farrowed and during lactation.

[1] The ulna in the pig is an exception to the general rule that the shaft grows in thickness by sub-periosteal addition on all sides. Instead of deposit there is some absorption on the ventral surface of the shaft which is contiguous to the dorsal surface of the radius. There is deposit on the medullary side of the ventral wall of the shaft of the ulna, and thus a growth movement of the shaft as a whole in a backward direction away from the radius. It is clear that Digby's proposition that the point in the centre of the medullary cavity in the line of the prolonged nutrient canal indicates the site of primary ossification can only be true of bones whose shafts can be shown to increase uniformly in thickness in all directions (this point is referred to more fully in my thesis).

All the pigs grew well as evidenced by steadily increasing weights. Their ages and details of madder and non-madder periods are noted in the following table. The horizontal lines separate pigs of different litters. (Pigs Nos. 12, 9 and 8 belong to the same litter.)

Table I

Pig No.	Age days	Madder period days	Growth without madder days
14	80	72	8
15	91	77	14
16	108	84	24
17	126	105	21
12	140	61	28
1	169	68	29
2	197	97	28
3	225	125	28
4	279	153	54
6	362	207	84
9	475	271	126
8	587	394	116

The madder was withdrawn from the food for varying periods, increasing according to the age and consequent diminution in rapidity of growth, before the animals were slaughtered. They were thus treated by the "indirect madder method" (employed by Duhamel, John Hunter and Humphry) whereby the new bone of the non-madder periods shows up white against the background of the thoroughly maddered skeleton. A short discussion of the advantages and disadvantages of the "direct" and "indirect" madder methods will be found in the paper by Prof. Brash (2) on "The growth of the alveolar bone."

The skeletons were macerated in a weak solution of KOH (0·1 per cent.) at 50° C., a method which appears to emphasise the contrast between the "old red" and "new white" bone.

The epiphyses of the limb bones separated readily except in the case of the oldest animals in which the bony union of the distal humeral and proximal radial epiphyses was first seen in No. 6 (362 days). No additional epiphyses had united in No. 9 (475 days). In the oldest pig, No. 8 (587 days), in addition to the above two, the distal tibial epiphysis had also united. It has been possible to ascertain from bones of older pigs that at 647 days in addition to the foregoing, the proximal ulnar, femoral, and the distal fibular epiphyses have united. The remaining epiphyses, distal radial, ulnar, femoral, proximal humeral, tibial and fibular, have united in a pig aged 729 days.

Before sectioning the diaphyses in order to measure additions of new bone at the ends, observations were made on the surface distribution of new bone, on the position of "stationary" areas and areas showing active absorption. Full details of these observations were recorded in the original thesis, and an account given for each bone of the "modelling process" (first described by John Hunter for the neck of the femur and in greater detail for other bones by

Kölliker) by which bones retain their shape as they grow in length and thickness. The only point to be noted here is that the addition of new bone to the ends of the diaphyses is obvious at a glance, since the white ends are invariably separated from the white bone surrounding the middle of the shaft by areas of older red bone (varying in extent according to the length of the non-madder period) with or without evidences of absorption according to the contour of the surface. These areas of absorption may and frequently do extend from the older red over the new white bone almost to the edge of the expanded growing ends of the diaphyses.

THE GROWTH IN LENGTH OF THE DIAPHYSES

In order to obtain reasonably accurate measurements of the amount of new bone added to the ends of the diaphyses longitudinal sections were cut. The bones of the left side were used for this purpose, and before the sections were made the maximum length of each diaphysis was taken on the osteometric board. The maximum lengths of the diaphyses are recorded in Table II. The lengths of a few of the older bones were measured in section as one of their epiphyses had united. These measurements are indicated in italics.

Table II. *Measurements of length in cm.*

Pig No.	14	15	16	17	12	1	2	3	4	6	9	8
Age in days	80	91	108	126	140	169	197	225	279	362	475	587
Humerus	9·5	9·6	10·0	10·7	10·8	10·5	11·4	12·2	11·9	*12·0*	*12·5*	*15·0*
Radius	7·1	7·0	7·6	7·7	8·4	7·7	8·8	9·3	9·2	*10·3*	*10·0*	*12·5*
Ulna	10·0	9·9	10·6	11·0	11·9	11·1	12·6	13·4	13·1	14·6	14·3	17·9
Femur	11·5	11·5	12·2	13·2	13·6	12·9	13·8	15·8	15·5	16·4	15·7	19·4
Tibia	10·6	10·2	11·1	11·5	12·0	11·6	13·1	13·9	13·5	14·7	14·4	*17·0*
Fibula	9·1	8·6	9·6	10·0	10·8	10·1	11·4	12·1	11·7	13·0	12·9	16·1

The sections of the diaphyses were made through long axes corresponding to their maximum lengths, and in order to obtain the best display of the new bone at each end, the plane of the section was approximately sagittal for the humerus, radius and ulna, and approximately coronal for the femur, tibia and fibula.

On sectioning long bones prepared by the indirect madder method a beautiful demonstration is obtained of the mode of growth in length by addition to the ends of the diaphyses (figs. 1–4).

In each of these figures (1–4) the areas of bone unstained by madder are represented white. Those at the ends and surrounding the shafts of the diaphyses represent new bone laid down during the second non-madder period (29 days). The white area of bone on the inside of the shaft was laid down during the pre-madder growth. Bone stained by madder is stippled in the case of cancellous and black in the case of compact bone.

At each end of the diaphysis the new white bone already noted on the surface stands out in contrast to the old red bone of which the greater part of the shaft is composed. The whole end of the bone is white, and this new white

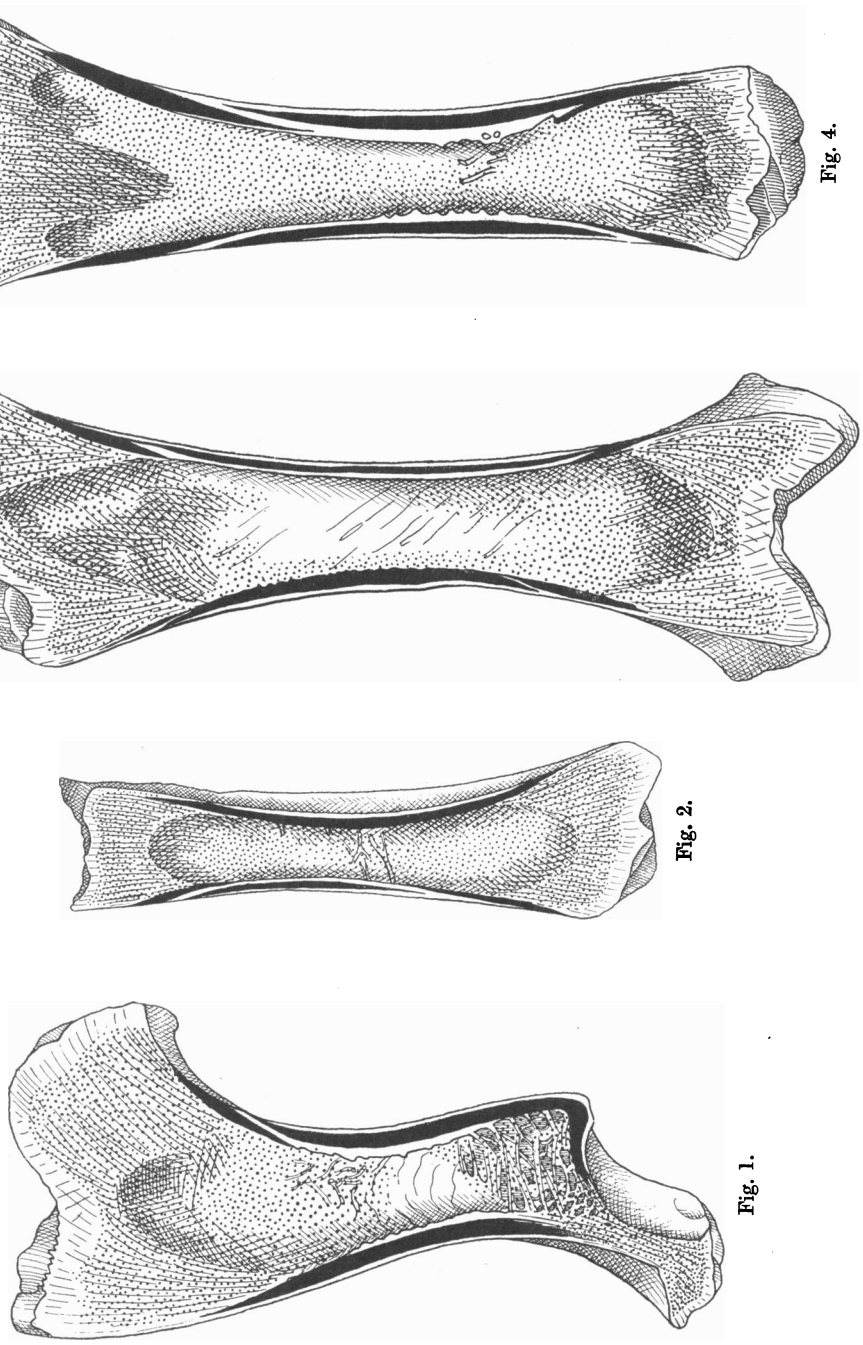

Fig. 1. Sagittal section of diaphysis of right humerus of pig 169 days old (No. 1).
Fig. 2. Sagittal section of diaphysis of right radius of same pig as fig. 1.
Fig. 3. Coronal section of diaphysis of right femur of same pig as fig. 1.
Fig. 4. Coronal section of diaphysis of right tibia of same pig as fig. 1.
Scale nat.

cancellous bone is usually separated from the medullary cavity by a layer of red cancellous bone. The thickness of the new white bone and of the older red cancellous bone which is absorbed as the medullary cavity extends, varies according to the length of the non-madder period. The transition from old to new bone is sharp and allows of fairly accurate measurements of the thickness of the new bone and certainly of accurate comparisons of the amount laid down at the two ends. In every long bone the increments at the two ends are found to be unequal and the greater increment constant in position for a given bone.

It should be noted here also that the contiguous surfaces of the diaphyses and epiphyses present a marked contrast which is best observed in section; while the ends of the diaphyses are entirely composed of new white bone, the diaphysial surfaces of the epiphyses, provided that the bones have been previously thoroughly maddered, remain red at the end of even the longest non-madder period. It is evident on section that epiphyses grow in size by peripheral additions except on their diaphysial surfaces, and that the contribution made by the growth of the epiphyses to the total length of a bone depends entirely upon additions to their joint surfaces beneath the articular cartilage. Macewen's (14) comment on the term "epiphysial cartilage" may in this connection be noted and emphasised. "This cartilage," he says, "might more appropriately be called diaphysial, as its more active portion pertains to the diaphysis."

MEASUREMENTS OF NEW BONE AT THE DIAPHYSIAL ENDS

The growth increments at the ends of all the diaphyses, as represented by the layer of new white bone laid down during the respective non-madder periods, have been measured in the axes of maximum length, and are recorded in Table III. Measurements in italics are for part only of the non-madder period, since the respective epiphyses have united during this period.

Table III. *Diaphysial growth. Measurements in mm.*

Pig No.		14	15	16	17	12	1	2	3	4	6	9	8
Age in days ...		80	91	108	126	140	169	197	225	279	362	475	587
Age of new bone ...		8	14	24	21	28	29	28	28	54	84	126	116
Humerus:	Proximal	3·0	2·8	3·4	3·5	4·4	4·2	3·0	2·3	3·5	4·2	6·0	5·0
	Distal	1·4	1·4	1·4	1·5	1·7	1·3	1·5	1·0	1·2	*0·5*	*0·5*	Nil
Radius:	Proximal	1·4	1·0	1·4	1·2	1·8	1·3	1·0	0·7	1·2	*0·5*	*0·5*	Nil
	Distal	2·8	2·2	2·6	2·8	3·2	2·8	2·3	2·0	3·7	4·0	8·0	5·2
Ulna:	Proximal	1·5	1·1	1·5	1·4	2·0	1·5	1·2	0·9	1·4	1·2	3·0	2·7
	Distal	3·0	2·3	2·5	3·0	3·3	3·0	2·8	2·2	3·2	3·8	7·8	5·0
Femur:	Proximal	1·8	1·7	2·5	2·5	4·8	3·0	1·3	1·5	2·5	3·0	4·0	3·0
	Distal	3·2	3·2	4·5	4·2	6·8	5·0	3·0	3·0	4·5	5·0	6·0	4·5
Tibia:	Proximal	3·8	3·2	3·3	4·0	6·4	3·5	3·5	3·4	4·5	5·0	7·8	5·5
	Distal	1·8	1·5	2·0	1·6	3·5	2·3	1·6	1·2	1·3	1·2	2·4	*1·5*
Fibula:	Proximal	3·5	3·0	3·2	3·7	6·2	3·2	3·3	3·0	4·1	4·6	7·6	5·0
	Distal	1·8	1·5	2·0	1·8	3·1	2·0	1·4	1·2	1·4	1·5	3·0	2·0

RATES OF GROWTH

Since the non-madder growth periods of the individual bones vary, having been increased with the age of the animal in order to obtain about the same amounts of new bone as the general rate of growth diminished, the growth increments cannot be directly compared. The table of measurements of new bone (Table III) shows the increments during periods varying from 8 to 126 days. In order to compare rates of growth in young and old bones the amount of new bone laid down at the proximal and distal diaphysial ends has been calculated per week (Table IV). Figures in italics are calculated in the same manner as the remaining ones, but from measurements of new bone over an unknown and shorter growth period than in the previous table, since growth must have ceased with the union of epiphyses in the situations indicated; consequently the rates shown are comparatively small.

Table IV. *Rates of diaphysial growth per week. Measurements in mm.*

Pig No.		14	15	16	17	12	1	2	3	4	6	9	8
Humerus:	Proximal	2·63	1·4	1·0	1·16	1·1	1·05	0·75	0·58	0·44	0·35	0·33	0·30
	Distal	1·22	0·7	0·4	0·5	0·42	0·32	0·38	0·25	0·15	*0·04*	*0·02*	Nil
Radius:	Proximal	1·22	0·5	0·4	0·4	0·45	0·32	0·25	0·18	0·15	*0·04*	*0·02*	Nil
	Distal	2·44	1·1	0·74	0·7	0·80	0·7	0·58	0·5	0·47	0·33	0·44	0·31
Ulna:	Proximal	1·31	0·55	0·43	0·46	0·5	0·37	0·3	0·22	0·17	0·1	0·16	0·16
	Distal	2·62	1·15	0·71	1·0	0·80	0·7	0·7	0·55	0·4	0·31	0·43	0·30
Femur:	Proximal	1·57	0·85	0·71	0·8	1·2	0·75	0·32	0·37	0·31	0·25	0·22	0·18
	Distal	2·80	1·6	1·3	1·4	1·7	1·25	0·75	0·75	0·55	0·41	0·33	0·27
Tibia:	Proximal	3·33	1·6	0·94	1·3	1·6	0·87	0·87	0·85	0·56	0·41	0·43	0·31
	Distal	1·57	0·75	0·57	0·5	0·87	0·58	0·4	0·3	0·16	0·1	0·13	*0·09*
Fibula:	Proximal	3·07	1·5	0·91	1·2	1·5	0·8	0·82	0·75	0·51	0·38	0·42	0·28
	Distal	1·57	0·75	0·57	0·9	0·77	0·50	0·35	0·3	0·17	0·12	0·16	0·11

From this table it is clear that there is a gradual diminution in the rate of growth in every bone as one passes from the younger to the older bones. In the youngest bones (80 days) the ends with the greatest increments have an average growth of 2·82 mm. per week, while the ends with the lesser increments have an average growth of 1·41 mm. per week. Therefore the ends with the greatest increments are making approximately twice the growth of the ends with the lesser increments at this age. At nearly a year old (362 days) the average growth of the ends with the greater increments is 0·37 mm. per week, while that of the ends with the lesser increments is 0·11 mm. per week, the ends with the greater increments making approximately three times the growth of the ends with the lesser increments.

About a year old the distal epiphysis of the humerus and the proximal epiphysis of the radius begin to unite, so that the increment shown for the diaphysial ends adjacent to these epiphyses in No. 9 (475 days) was laid down early in the non-madder period (126 days) and not throughout this period as in the younger bones. In No. 8 (587 days) union of the above-mentioned epiphyses took place before the non-madder period (116 days), and no new

bone is laid down at the diaphysial ends adjacent to these united epiphyses. The distal epiphysis of the tibia had united in No. 8 (587 days), so that the increment at this end of the diaphysis is that for the early part of the non-madder period (116 days).

As the epiphysial cartilage begins to disappear and the epiphysis unites with the diaphysis, growth in length at that end of the bone must cease, except in so far as the articular surface of the epiphysis itself may continue to grow.

It is also quite obvious from these tables that the greater growth in length at one end of a long bone is due not so much to the fact that the epiphysial centre of ossification appears earlier at one end and remains longer ununited there, but to the greater rate of diaphysial growth throughout the growth period, as originally pointed out by Humphry.

The rates of diaphysial growth of individual bones now being known between 80 and 587 days, it is possible, taking the length of the youngest bone as a starting-point, to calculate the growth increments that might be expected to be added to it at the stage of each successive older bone. The differences in age of the successive older bones do not always correspond to the non-madder growth periods, so that it is necessary to calculate from the rates of growth the amount of growth for these periods of differences in age. The results of these calculations are set out in Table V. Figures in italics, being calculated from those of the previous table which are for a shorter growth period than stated, are comparatively small.

Table V. *Expected growth increments of diaphyses. Measurements in mm.*

Pig No.		14	15	16	17	12	1	2	3	4	6	9	8
Age in days ...		80	91	108	126	140	169	197	225	279	362	475	587
Difference in age ...		—	11	17	18	14	29	28	28	54	83	113	112
Humerus:	Proximal	—	2·19	2·42	2·98	2·2	4·20	3·00	2·32	3·38	4·20	5·28	4·8
	Distal	—	1·09	0·97	1·28	0·84	1·28	1·52	1·00	1·15	*0·48*	*0·32*	Nil
Radius:	Proximal	—	0·78	0·97	1·02	0·9	1·28	1·00	0·72	1·15	*0·48*	*0·32*	Nil
	Distal	—	1·73	1·8	1·8	1·6	2·8	2·32	2·0	3·61	3·96	7·04	4·96
Ulna:	Proximal	—	0·86	1·04	1·18	1·0	1·48	1·2	0·88	1·30	1·2	2·56	2·56
	Distal	—	1·8	1·72	2·57	1·6	2·8	2·8	2·20	3·08	3·72	6·88	4·8
Femur:	Proximal	—	1·33	1·71	2·05	2·4	3·00	1·28	1·48	2·38	3·00	3·52	2·88
	Distal	—	2·66	3·15	3·59	3·4	5·00	3·00	3·00	4·23	4·92	5·28	4·32
Tibia:	Proximal	—	2·51	2·28	3·34	3·2	3·48	3·48	3·40	4·31	4·92	6·88	4·96
	Distal	—	1·18	1·39	1·29	1·74	2·32	1·6	1·2	1·23	1·2	2·08	*1·44*
Fibula:	Proximal	—	2·4	2·21	3·08	3·0	3·2	3·28	3·00	3·92	4·56	6·72	4·48
	Distal	—	1·18	1·39	2·31	1·54	2·0	1·40	1·2	1·31	1·44	2·56	1·76

The data provided by this table have been used to illustrate the relative rates and amounts of growth at the two ends of the humeral, radial, femoral and tibial diaphyses. The expected length of the diaphysis at each stage has been contrasted with the length of the actual specimen (figs. 5 and 6).

The horizontal base line represents 80 to 600 days on a scale of one day per 0·33 mm. The vertical scale represents length (scale $\times \frac{1}{3}$). The stippled areas represent the amount of expected increment at each end of the youngest

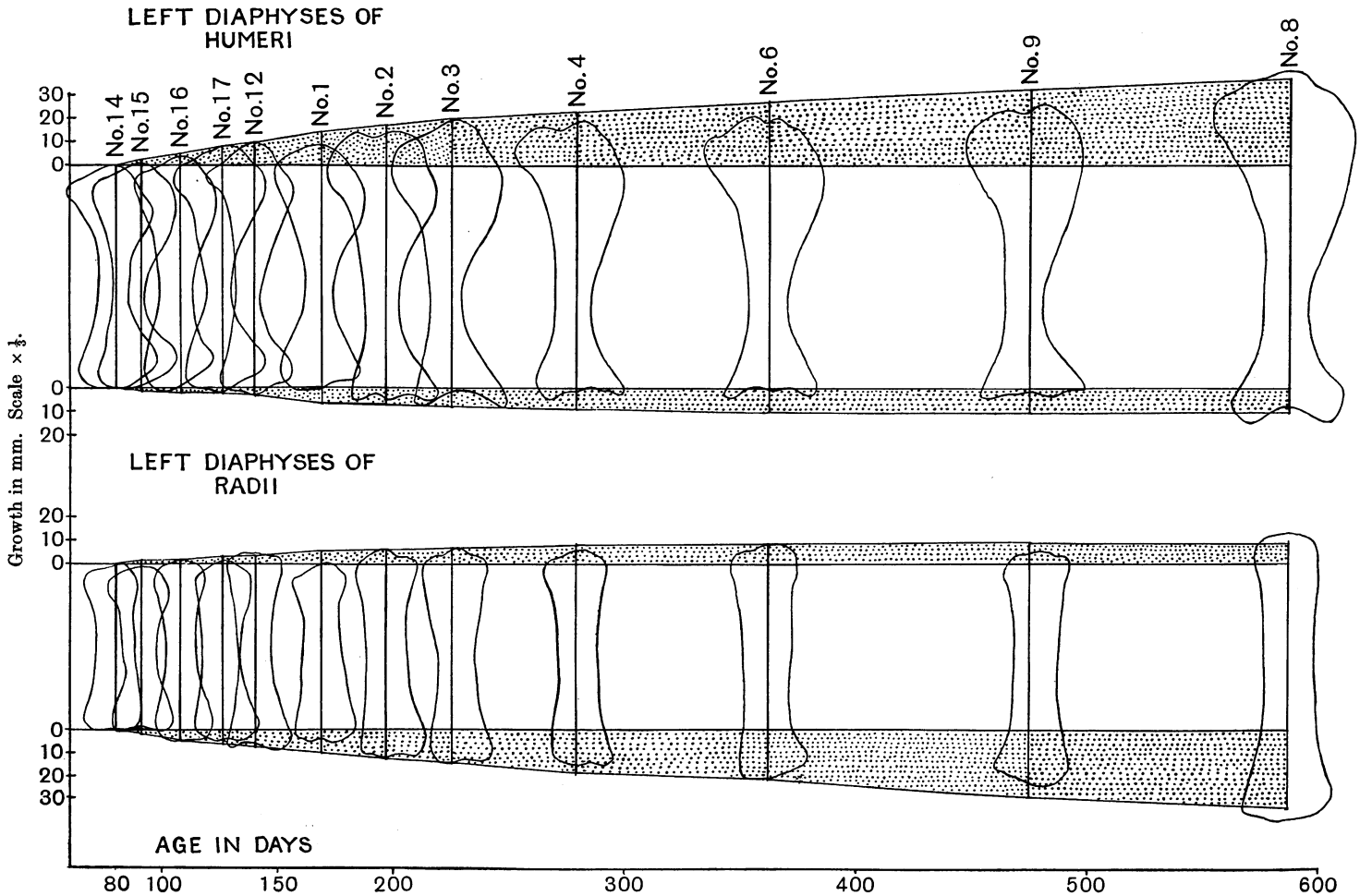

Fig. 5. Graphic representation of relative rates and amounts of growth at ends of humeral and radial diaphyses of madder-fed pig as explained in text.

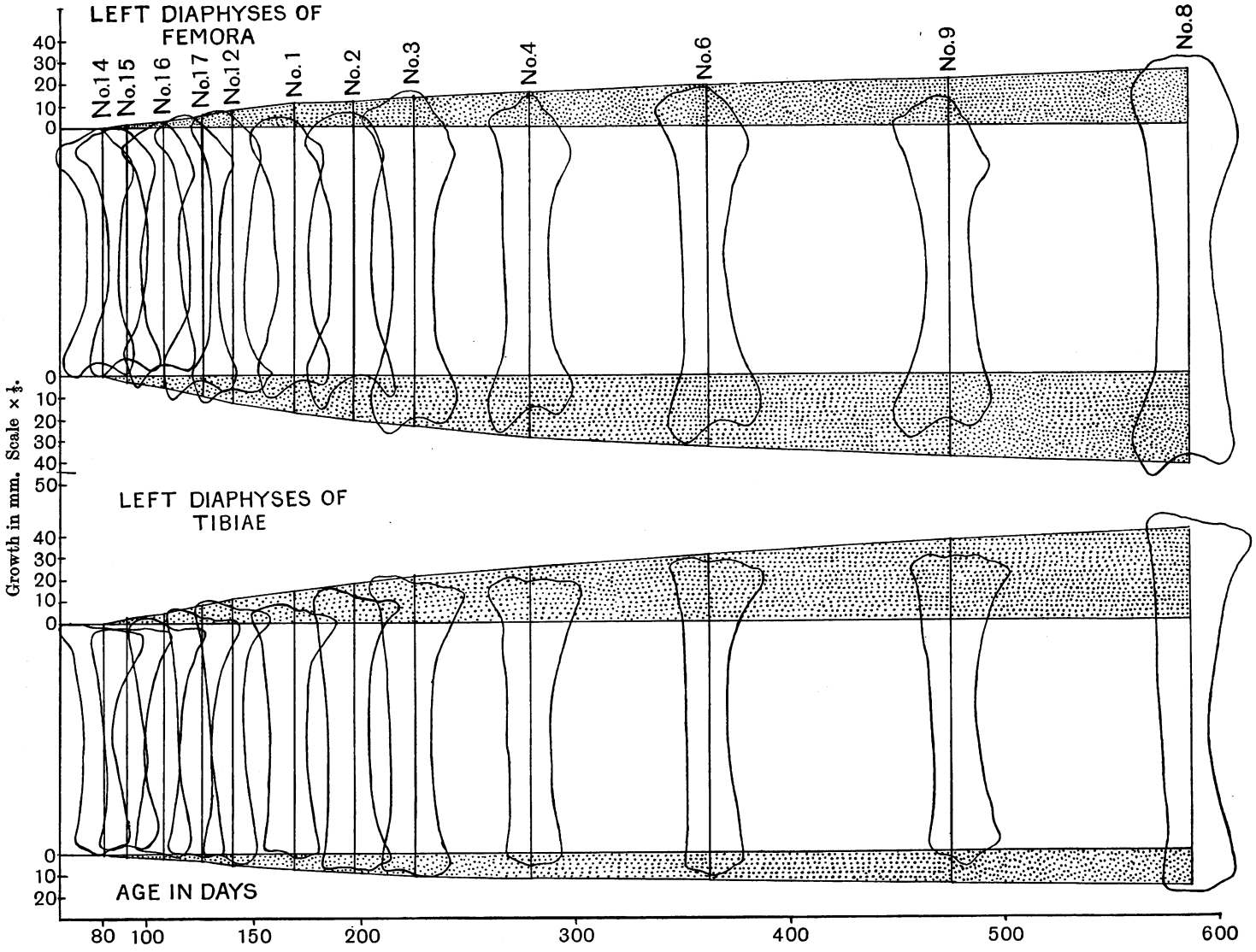

Fig. 6. Relative rates and amounts of growth at ends of femoral and tibial diaphyses of madder-fed pig. Scale as in fig. 5.

diaphysis and at the ages of the older bones a vertical line is drawn to represent the expected length which the youngest diaphysis might have reached. Over the expected length has been drawn the outline of the individual bone for comparison.

On this basis the diaphyses of the long bones of various ages may be superimposed in proportion to their growth in length to demonstrate the relative amounts and rates of growth at the two ends.

The fact that some of the diaphyses are shorter while others are longer than the expected length may be accounted for by the circumstance that we are dealing with the bones of individual pigs with mixed inheritance, though indeed it is notorious that individual pigs of pure breed also vary considerably in their rates of growth.

SUMMARY AND CONCLUSIONS

The amounts and quantitative ratios of new bone added to the two ends of the limb bones are demonstrated for the bones of madder-fed pigs over given periods.

The rate of growth diminishes with age, the diminution being more rapid at the end which has the lesser increment (the proportion of growth being 2 : 1 at 80 days as compared with 3 : 1 at 362 days).

From measurements of growth increments in madder-fed pigs it is possible to superimpose a series of long bones of increasing ages to show the relative amounts of growth at the ends of their long axes.

In conclusion I would like to express my thanks to Prof. Brash for his advice and help in the preparation of this paper.

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