

2. With whole casein a curve showing a lag period before a linear portion is obtained and with  $\beta$ -casein a very flat curve is given. A steep straight line was obtained with  $\alpha_2$ -casein and a sigmoid curve with  $\alpha_1$ -casein.

3. With mixtures of  $\alpha_2$ - and  $\beta$ -casein or  $\alpha_1$ -casein the slope of the curves decreased linearly as the proportion of  $\beta$ - or  $\alpha_1$ -casein increased, and a lag period was observed with some mixtures of  $\alpha_2$ - and  $\beta$ -casein only.

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## The Effect of Development on the Composition of a Long Bone of the Pig, Rat and Fowl

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The earlier investigators of the chemistry of bone growth (Weiske, 1889; Hammett, 1925*a-c*; Kramer & Shear, 1928; Swanson & Job, 1937) worked largely on whole bones and were therefore concerned with bones as organs. More recently, those interested in the chemistry of bone growth have directed their attention to the cortex. The matrix of this tissue consists of the substances that constitute the connective tissue of skin and muscle. Bone differs from the connective tissue of these organs, however, in its characteristic 'hardness'. This property is attributed to the deposition of calcium salts within the matrix. Whereas earlier workers paid little attention to the organic matrix, the more recent investigators have studied its composition and its relationship to the bone minerals, and particularly the calcium.

Burns & Henderson (1936*a*) studied the influence of age from birth to adult life on the concentrations of calcium and carbonate in the cortical

bone of kittens and puppies. The work of Baker, Butterworth & Langley (1946) on the calcium:nitrogen ratio of carefully cleaned bone tissue was extended by Weidmann & Rogers (1950) in a comparative study of the degree of calcification of cortical bone from adult rats, rabbits and cats. These investigators subsequently showed (Rogers, Weidmann & Parkinson, 1952) that 89-97% of the nitrogen in the bone tissue of the adult rabbit, ox and man is present as collagen. The value for ox bone was later confirmed by Eastoe & Eastoe (1954). In later work Weidmann & Rogers (1958) found an increase in the calcium:nitrogen ratio in the cortical bone of the rabbit from about the fifth day of life and in that of the cat from about the ninth day. No similar work appears to have been done on the cortical bone of the pig, rat or fowl.

One of the characteristic signs of maturation in mammalian long bones is the appearance and growth of the secondary centres of ossification in the

cartilaginous epiphyses. Burns & Henderson (1936b), in their work on puppies and kittens, made one of the few studies that there have been on the changes in composition of the epiphyses during growth. Their study was limited to the changes in the percentages of water and calcium.

The work described here on the rat was originally undertaken as a contribution to a study of the effects of accelerated growth (Widdowson & McCance, 1960), and certain aspects of the composition of rat bone have been reported elsewhere (Dickerson & Widdowson, 1960a). Repetition will be avoided as far as possible except where it is necessary for comparison with the other species. The rat is a very convenient animal in which to study the epiphyses because, over most of its life span, the epiphyses of the femur can readily be separated from the remainder of the bone.

The study of the pig and fowl bones provides the normal background against which to interpret the composition of the bones of undernourished animals and of animals that were undernourished and then rehabilitated (Dickerson & McCance, 1961).

Careful dissection of the bones and analysis of the different parts has made it possible to calculate the composition of the whole bone in the different species at the various ages.

Similar work on the effect of pre- and post-natal growth on the composition of the human femur is reported separately (Dickerson, 1962).

## EXPERIMENTAL

*Material.* The pigs were of a predominantly Large White breed. Foetal bones were analysed at 46, 65 and 90 days' gestation. Two litters were used at 46 days, four litters at 65 days and three litters at 90 days. At each stage the bones of several foetuses from the same litter were pooled for analysis. Bones were also investigated from seven newborn piglets, six piglets aged 20–45 days, five pigs aged 65–143 days, and three aged over 300 days, which are subsequently referred to as '1-year-old' animals.

The rats used were those described by Dickerson & Widdowson (1960a). The values obtained for the composition of the bones of rats reared in large and small litters have been averaged to obtain the values for the various constituents given in this paper, on the assumption that they represent the limits of normal variation at any age.

The work on the fowl was carried out on the same or similar animals to those described by Dickerson (1960).

In the rat and the fowl the femur was used. In the pig the post-natal study was confined to the humerus, but at birth and at the prenatal stages both the humerus and the femur were analysed.

*Methods.* The method of cleaning and dissecting the bones has been described elsewhere (Dickerson & Widdowson, 1960a). The bone fragments were dried at 105° until constant in weight, extracted three or more times with light petroleum at room temperature, reheated and weighed. The

difference in weight before and after treatment with light petroleum was assumed to be fat. The fragments of small bones were then powdered with a pestle and mortar, and the larger pig and fowl bones in a Culatti hammer mill.

Samples of the bone powders weighing 50–300 mg., or the whole bone fragments in the case of those not large enough for powdering, were heated with 6N-HCl (1 ml./50 mg. of bone) in sealed tubes in a boiling-water bath for 48 hr. After hydrolysis, the mixture was transferred to a graduated flask and neutralized with NaOH solution. Just sufficient HCl was then added to dissolve the precipitated calcium phosphate. After making up to volume with distilled water, samples of the solution were taken for the estimation of Ca, P and N by the methods described by Dickerson & Widdowson (1960b) and for collagen by the Neuman & Logan (1950) procedure with the stronger solution of CuSO<sub>4</sub> as suggested by Baker, Lampitt & Brown (1953). Rat collagen was assumed to contain 13.4% (Neuman & Logan, 1950) and pig collagen 13.5% of hydroxyproline (Eastoe, 1955). Fowl collagen contains 14% of hydroxyproline (Leach, 1957). The collagen of all species was assumed to contain 18% of N.

## RESULTS

### *Cortical bone*

Tables 1–3 show the composition of dry fat-free cortical bone from the pig, rat and fowl respectively. Ratios of the amounts of the constituents are also shown.

In each species the percentage of total N fell from the youngest to the oldest bone, and the percentages of Ca and P rose. These changes are reflected in the rise in the Ca/N ratio. In the mammals the percentage of collagen increased initially, and then decreased to a lower level during subsequent growth. The values for the contribution of collagen N to the total N show that when the percentage of collagen in the bone was increasing it was replacing other nitrogenous constituents in the bone. The fall in the percentage of collagen during later development was due to the rise in the percentage of minerals. In general terms the pattern of the overall changes in the composition of fowl bone was similar to that in the mammals.

The above changes were not regular ones in either of the three species. In the pig (Table 1) the percentage of total N fell between 46 and 65 days' gestation, rose between 65 days and term, and then fell steadily during postnatal growth. The fall in the percentage of total N between 46 and 65 days' gestation was accompanied by a big rise in the percentages of Ca and P and in the Ca/N ratio. The Ca/collagen ratio also increased. After 65 days the Ca/N ratio fell to a minimum value in the cortical bone of the newborn piglets. After birth the ratio rose gradually and reached its highest value in the tissue from the animals of a year old. There was no regular change in the Ca/P ratio in pig bone with age.

Table 1. *Composition of cortical bone from pig's humerus*  
 Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age and (no. of analyses)	Composition of dry fat-free solids (g./100 g.)				Ca/N	Ca/collagen	Ca/P	Collagen N $\times$ 100
	Total N	Collagen	Ca	P				
46 days' gestation (2)	5.81 (5.27, 6.35)	15.7 (15.5, 15.8)	18.9 (18.6, 19.1)	9.0 (8.6, 9.4)	3.28 (2.94, 3.62)	1.21 (1.18, 1.23)	2.12 (2.04, 2.20)	49.0 (45.0, 53.0)
65 days' gestation (4)	4.02 ( $\pm 0.38$ )	18.4 ( $\pm 1.14$ )	26.0 ( $\pm 0.72$ )	11.6 ( $\pm 0.29$ )	6.51 ( $\pm 0.76$ )	1.42 ( $\pm 0.08$ )	2.25 ( $\pm 0.08$ )	82.6 ( $\pm 9.8$ )
90 days' gestation (3)	4.38 ( $\pm 0.25$ )	21.5 ( $\pm 2.0$ )	25.5 ( $\pm 0.39$ )	11.1 ( $\pm 0.30$ )	5.77 ( $\pm 0.35$ )	1.18 ( $\pm 0.15$ )	2.27 ( $\pm 0.11$ )	88.6 ( $\pm 8.2$ )
Newborn (7)	4.57 ( $\pm 0.28$ )	22.2 ( $\pm 0.53$ )	25.1 ( $\pm 0.77$ )	10.9 ( $\pm 0.16$ )	5.51 ( $\pm 0.29$ )	1.13 ( $\pm 0.03$ )	2.28 ( $\pm 0.09$ )	87.9 ( $\pm 5.6$ )
20-45 days (6)	4.24 ( $\pm 0.11$ )	21.8 ( $\pm 0.74$ )	25.0 ( $\pm 0.47$ )	11.1 ( $\pm 0.44$ )	5.90 ( $\pm 0.32$ )	1.15 ( $\pm 0.04$ )	2.25 ( $\pm 0.08$ )	92.5 ( $\pm 4.0$ )
65-143 days (5)	4.00 ( $\pm 0.30$ )	19.6 ( $\pm 0.69$ )	26.1 ( $\pm 0.73$ )	11.9 ( $\pm 0.20$ )	6.58 ( $\pm 0.63$ )	1.23 ( $\pm 0.17$ )	2.19 ( $\pm 0.09$ )	88.5 ( $\pm 5.5$ )
1 year (3)	3.70 ( $\pm 0.17$ )	18.6 ( $\pm 0.10$ )	27.1 ( $\pm 0.42$ )	12.5 ( $\pm 0.20$ )	7.32 ( $\pm 0.35$ )	1.46 ( $\pm 0.07$ )	2.17 ( $\pm 0.06$ )	90.5 ( $\pm 3.6$ )

Table 2. *Composition of cortical bone from the rat's femur*  
 Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age in days and (no. of analyses)	Composition of dry fat-free solids (g./100 g.)				Ca/N	Ca/collagen	Ca/P	Collagen N $\times$ 100
	Total N	Collagen	Ca	P				
Newborn (1)	5.86	11.73	18.8	10.25	3.21	1.60	1.84	36.0
7 (2)	6.13 (6.12, 6.15)	14.8 (14.6, 15.1)	18.4 (17.6, 19.2)	9.78 (9.32, 10.25)	3.00 (2.88, 3.12)	1.23 (1.20, 1.27)	1.88 (1.87, 1.89)	43.6 (43.0, 44.2)
14 (4)	5.44 ( $\pm 0.27$ )	15.4 ( $\pm 0.56$ )	19.9 ( $\pm 1.05$ )	10.09 ( $\pm 0.78$ )	3.74 ( $\pm 0.38$ )	1.30 ( $\pm 0.06$ )	1.92 ( $\pm 0.05$ )	51.0 ( $\pm 2.96$ )
21 (6)	5.23 ( $\pm 0.63$ )	12.08 ( $\pm 1.35$ )	19.9 ( $\pm 3.08$ )	10.0 ( $\pm 1.46$ )	3.87 ( $\pm 0.77$ )	1.65 ( $\pm 0.19$ )	1.99 ( $\pm 0.07$ )	42.0 ( $\pm 7.4$ )
33 (6)	4.38 ( $\pm 0.35$ )	15.2 ( $\pm 0.63$ )	25.4 ( $\pm 1.15$ )	12.2 ( $\pm 0.41$ )	5.84 ( $\pm 0.43$ )	1.67 ( $\pm 0.09$ )	2.08 ( $\pm 0.04$ )	62.8 ( $\pm 3.8$ )
46 (6)	3.53 ( $\pm 0.37$ )	12.7 ( $\pm 3.12$ )	23.4 ( $\pm 4.87$ )	10.8 ( $\pm 2.34$ )	6.61 ( $\pm 0.80$ )	1.86 ( $\pm 0.06$ )	2.16 ( $\pm 0.01$ )	64.1 ( $\pm 9.1$ )
56 (6)	3.72 ( $\pm 0.37$ )	14.7 ( $\pm 2.44$ )	25.4 ( $\pm 2.91$ )	12.1 ( $\pm 1.55$ )	6.84 ( $\pm 0.73$ )	1.73 ( $\pm 0.097$ )	2.10 ( $\pm 0.043$ )	71.2 ( $\pm 5.74$ )
77 (6)	3.60 ( $\pm 0.22$ )	17.5 ( $\pm 0.95$ )	28.8 ( $\pm 1.54$ )	13.3 ( $\pm 0.94$ )	7.78 ( $\pm 0.21$ )	1.59 ( $\pm 0.09$ )	2.11 ( $\pm 0.117$ )	88.1 ( $\pm 4.42$ )
108 (6)	3.36 ( $\pm 0.10$ )	15.9 ( $\pm 1.34$ )	28.8 ( $\pm 0.45$ )	13.6 ( $\pm 0.42$ )	8.58 ( $\pm 0.14$ )	1.81 ( $\pm 0.33$ )	2.12 ( $\pm 0.03$ )	85.1 ( $\pm 4.98$ )
158 (6)	3.03 ( $\pm 0.088$ )	14.8 ( $\pm 1.01$ )	29.0 ( $\pm 0.85$ )	13.3 ( $\pm 0.69$ )	9.57 ( $\pm 0.19$ )	1.97 ( $\pm 0.09$ )	2.18 ( $\pm 0.08$ )	86.9 ( $\pm 4.74$ )

Table 3. *Composition of cortical bone from the fowl's femur*

Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age in weeks and (no. of analyses)	Composition of dry fat-free solids (g./100 g.)				Ca/N	Ca/ collagen	Ca/P	Collagen N $\times$ 100 Total N
	Total N	Collagen	Ca	P				
Newly hatched (3)	5.20 ( $\pm 0.10$ )	19.2 ( $\pm 0.14$ )	18.9 ( $\pm 0.77$ )	8.8 ( $\pm 0.57$ )	3.59 ( $\pm 0.10$ )	0.97 ( $\pm 0.02$ )	2.17 ( $\pm 0.06$ )	66.7 ( $\pm 1.14$ )
2½ (3)	4.9 ( $\pm 0.19$ )	17.4 ( $\pm 0.39$ )	22.6 ( $\pm 0.5$ )	10.6 ( $\pm 0.33$ )	4.62 ( $\pm 0.27$ )	1.30 ( $\pm 0.02$ )	2.13 ( $\pm 0.05$ )	63.8 ( $\pm 3.16$ )
4 (4)	4.36 ( $\pm 0.38$ )	17.4 ( $\pm 1.61$ )	22.8 ( $\pm 0.63$ )	10.9 ( $\pm 0.20$ )	5.27 ( $\pm 1.34$ )	1.32 ( $\pm 0.11$ )	2.09 ( $\pm 0.09$ )	71.9 ( $\pm 1.34$ )
15 (3)	4.42 ( $\pm 0.04$ )	21.4 ( $\pm 1.06$ )	26.5 ( $\pm 0.26$ )	11.3 ( $\pm 0.37$ )	5.98 ( $\pm 0.05$ )	1.24 ( $\pm 0.06$ )	2.33 ( $\pm 0.05$ )	86.9 ( $\pm 4.44$ )
27-42 (12)	3.72 ( $\pm 0.15$ )	19.3 ( $\pm 0.70$ )	26.0 ( $\pm 0.74$ )	11.5 ( $\pm 0.29$ )	7.01 ( $\pm 0.34$ )	1.35 ( $\pm 0.07$ )	2.26 ( $\pm 0.06$ )	93.8 ( $\pm 4.5$ )

In the rat (Table 2) the percentage of total N increased a little during the first 7 days of life. There was little change in the percentage of Ca. The Ca/N ratio fell and the cortex may have been a little less calcified at 7 days of age than it was at birth. The Ca/collagen ratio was also lower at 7 days and did not increase appreciably until after 14 days. The changes in the percentage of collagen were very erratic during the first 46 days of life and only after 46 days did the changes follow a pattern similar to that in pig bone. There was a steady rise in the Ca/P ratio during the first 46 days of life.

In the fowl (Table 3) the percentage of collagen fell and the percentages of Ca and of P rose during the first 2½ weeks after hatching. The increase in the amount of collagen after 4 weeks of age was accompanied by an increase in the percentages of Ca and P. The Ca/collagen ratio did not change between 2½ and 27-42 weeks of age.

The cortical bone of newborn piglets was more mature in its composition than that of the newborn rat or newly hatched chicken. With the exception of the percentage of collagen, the cortical bone of the newborn rat was similar in its composition to that of pig fetuses at 46 days' gestation. Bone from pigs 1 year old was less highly calcified than rat bone at 158 days but was a little more calcified than that of the fowl at 27 weeks. The replacement of non-collagen N by collagen N was almost complete in the pig by 65 days of gestation, whereas in the rat it was not complete until 56-77 days after birth, and in the fowl until between 4 and 27 weeks of age.

#### *Epiphyses*

Table 4 shows that on a wet-weight basis the proportion of the pig's femur and humerus accounted for by the epiphyses fell steadily between 46 days' gestation and term. On a dry-weight basis the proportion fell between 46 and 65 days

and appeared to change little during the remainder of foetal development. There was, however, a change in the composition of the dry fat-free solids over the whole period. The percentage of collagen increased steadily both on an absolute basis and relative to the amount of total N. The rise in the amount of Ca was small until between 90 days and term, and a change in the composition of the epiphysal cartilage with respect to its nitrogenous constituents preceded the development of the ossification centres. The amount of P increased more slowly than that of Ca and there was an increase, particularly between 90 days and term, in the Ca/P ratio. At each age the mean percentage of Ca was higher in the epiphyses of the humerus than it was in those of the femur.

The contribution of the epiphyses to the weight of the rat's femur also falls during growth (Dickerson & Widdowson, 1960a) and Fig. 1 shows the changes in the composition of their dry fat-free solids with respect to collagen N, non-collagen N (calculated as the difference between the total N and the collagen N) and Ca. The amount of non-collagen N fell rapidly during the first 21 days of life, more slowly between 21 and 46 days and then more rapidly up to 77 days, reaching the mature level at 108 days. The first part of the fall in the amount of non-collagen N was accompanied by a rise in the amounts of both collagen and Ca. After 56 days the amount of collagen also fell, whereas that of Ca continued to increase. There was a similar rise in the Ca/P ratio of the rat's epiphyses to that described above in those of the pig. In the rat the ratio rose from 1.0 at birth to 1.9 at 21 days and continued to rise slowly to a value of 2.2 at 158 days. The epiphyses of the newborn rat's femur were considerably less mature, as judged by their lower Ca content, than those of the pig's femur.

Table 4. *Composition of the epiphyses of the pig femur and humerus*

Values are expressed as g./100 g. of dry fat-free solids. Standard deviations are shown ( $\pm$ ).

Age and (no. of analyses)	Proportion of bone (%)				Collagen		Ca		P		Collagen N $\times$ 100		Ca/P			
	Femur		Humerus		Femur	Humerus	Femur	Humerus	Femur	Humerus	Femur	Humerus	Femur	Humerus		
	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Total N	Total N	Total N	Total N	Total N	Total N	Total N	Total N	Total N	Total N		
46 days' gestation (2)	74.6	53.8	74.6	51.2	8.31 (7.67, 8.94)	7.37 (6.67, 8.07)	12.5 (11.2, 13.7)	13.1 (12.4, 13.8)	0.82 (0.68, 0.96)	0.87 (0.60, 1.13)	1.03 (0.99, 1.06)	0.90 (0.87, 0.93)	27.4 (22.6, 32.2)	32.5 (27.7, 37.2)	0.80 (0.69, 0.90)	0.95 (0.69, 1.21)
65 days' gestation (4)	59.8	29.6	59.7	27.0	8.83 ( $\pm 0.42$ )	8.74 ( $\pm 0.53$ )	20.0 ( $\pm 2.23$ )	21.3 ( $\pm 1.42$ )	1.11 ( $\pm 0.12$ )	1.30 ( $\pm 0.30$ )	1.03 ( $\pm 0.09$ )	1.41 ( $\pm 0.48$ )	41.0 ( $\pm 5.73$ )	44.0 ( $\pm 4.05$ )	1.07 ( $\pm 0.02$ )	0.95 ( $\pm 0.17$ )
90 days' gestation (2)	53.6	28.2	51.5	23.2	9.95 (9.90, 9.99)	10.58 (7.76, 13.4)	29.9 (29.3, 30.4)	32.4 (24.9, 34.9)	1.38 (1.00, 1.76)	1.79 (1.77, 1.80)	0.91 (0.77, 1.05)	1.12 (1.06, 1.17)	54.1 (53.3, 54.8)	55.8 (53.6, 57.9)	1.49 (1.30, 1.68)	1.61 (1.51, 1.70)
Newborn (6)	46.3	29.0	39.5	25.2	9.83 ( $\pm 0.25$ )	9.67 ( $\pm 0.50$ )	34.4 ( $\pm 1.85$ )	33.8 ( $\pm 2.62$ )	5.08 ( $\pm 0.74$ )	6.34 ( $\pm 0.79$ )	2.88 ( $\pm 0.87$ )	2.85 ( $\pm 0.27$ )	63.2 ( $\pm 1.95$ )	63.2 ( $\pm 2.05$ )	2.19 ( $\pm 0.27$ )	2.23 ( $\pm 0.29$ )

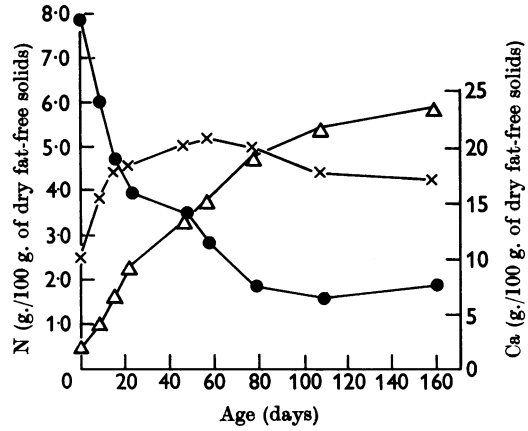


Fig. 1. Effect of development on the percentage of collagen N, non-collagen N (calculated as the difference between the total N and the collagen N) and Ca in the dry fat-free epiphyses of the rat femur. x, Collagen N; ●, non-collagen N; Δ, Ca.

Whole bone

Tables 5-7 show the composition of the whole humerus of the pig, and the whole femur of the rat and fowl respectively at the different ages. After 20-45 days of age in the pig and 4 weeks of age in the fowl there was a large rise in the percentage of fat in the fresh bones. In the rat's femur there was no increase in the percentage of fat between birth and 158 days. In each species the amount of fat in the bones during the early stages of growth was, on the whole, small, and throughout development the major part of the fall in the water content of the fat-free bones was due to the deposition of mineral. The rise in the percentage of total N played a smaller role. The proportion of the total N accounted for by collagen increased in all the species. In the pig humerus, for instance, the increase was from about 40% at 46 days' gestation to 71% at birth, and to 81% at maturity.

The percentages of Ca and P in the pig's humerus fell slightly between 90 days' gestation and term. Similar changes took place in the composition of the rat's femur during the first 7 days of postnatal life. The humerus of the newborn piglet was more highly mineralized than the femur of the newborn rat. The latter was, in fact, only a little more mature with respect to its mineral content than the pig's humerus at 46 days' gestation. With the exception of the higher concentration of collagen, the femur of the fowl at hatching was similar in composition to that of the newborn rat. These changes in the composition of the whole bone are the result of the changes in the composition of the different parts of the bone and in the contribution

of the different parts to the whole. In the early stages of growth the relatively large epiphyses with their high water and low Ca contents exert a considerable 'diluting' effect on the constituents in the remainder of the bone. Table 4 shows that they did not share in the fall in the degree of calcification of the pig femur between 65 days' gestation and term. If they are excluded from the results for the whole bone the Ca/N ratio of the remainder fell from  $5.88 \pm 0.62$  at 65 days to  $3.72 \pm 0.39$  at term. This fall is highly significant ( $P < 0.01$ ) and, since it is greater than that which takes place in the cortical bone, the major part of it must be attributed to a fall in the degree of calcification of the cancellous bone of the metaphyses.

### DISCUSSION

The use of three species for this investigation has helped to bring out general principles. At the same time it has emphasized that species vary in the time of life at which the major changes occur and also in the magnitude of these changes. Further, the detailed study of the pig and rat has shown that the changes which take place during growth are apparently subject to some fluctuation.

The fundamental change that takes place in the composition of a bone during growth is an increase in its degree of mineralization, and Hammett (1925*a*) concluded that this was largely responsible for the fall in the percentage of water, and that the increase in the percentage of fat-free organic material played a minor role. The same is probably true in the bone tissue of the cortex (Robinson & Elliott, 1957). The present work has added to this picture by showing that there is also a change in the nature of the nitrogenous constituents in the bone as a whole and in the two parts of the bone, the epiphyses and the cortex, which have been analysed separately.

The total nitrogen in a bone may be divided crudely into collagen nitrogen and non-collagen nitrogen. The former has been estimated and the latter obtained by difference from the total nitrogen. The non-collagen nitrogen consists principally of the cell proteins, blood and the nitrogen of the mucopolysaccharides. The fall in the proportion of non-collagen nitrogen in the bone could be due to a fall in one, or all, of these constituents. The heterogeneity of a bone makes it difficult to interpret these findings very specifically, but Sobel, Marmorston & Moore (1954) have shown that there is a fall in the percentage of mucopolysaccharide relative to collagen in the rat femur during growth. Some of this fall must be due to the replacement of cartilage by bone in the epiphyses and the consequent decrease in the percentage of chondroitin sulphates in them. Precise information

Table 5. *Composition of the pig humerus*

Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age and (no. of analyses)	No. of bones analysed	Mean humerus weight (g.)	Composition of fresh bone (g./100 g.)		Composition of fat-free bone (g./100 g.)						Ca/N
			Fat	Water	Water	Total N	Collagen	Ca	P		
46 days' gestation (2)	20	0.08	Nil	80.5	80.5 (80.3, 80.7)	1.29 (1.16, 1.42)	2.80 (2.78, 2.82)	1.88 (1.86, 1.91)	0.94 (0.94, 0.95)	1.39 (1.34, 1.44)	
65 days' gestation (4)	32	0.47	Nil	67.0	67.0 ( $\pm 2.15$ )	1.81 ( $\pm 0.10$ )	6.01 ( $\pm 0.35$ )	6.18 ( $\pm 0.68$ )	2.80 ( $\pm 0.38$ )	3.40 ( $\pm 0.40$ )	
90 days' gestation (3)	14	2.48	Nil	65.5	65.5 ( $\pm 4.6$ )	2.24 ( $\pm 0.04$ )	8.21 ( $\pm 0.37$ )	6.89 ( $\pm 0.80$ )	3.04 ( $\pm 0.27$ )	3.06 ( $\pm 0.33$ )	
Newborn (6)	6	5.44	Nil	63.7	63.7 ( $\pm 2.5$ )	2.33 ( $\pm 0.27$ )	8.44 ( $\pm 0.49$ )	5.99 ( $\pm 0.49$ )	2.60 ( $\pm 0.21$ )	2.57 ( $\pm 0.26$ )	
20-45 days (6)	6	24.9	1.73	59.4	60.4 ( $\pm 3.0$ )	2.69 ( $\pm 0.56$ )	9.57 ( $\pm 1.29$ )	7.18 ( $\pm 1.07$ )	3.33 ( $\pm 0.42$ )	2.75 ( $\pm 0.62$ )	
65-143 days (5)	5	132	15.2	45.6	52.4 ( $\pm 2.2$ )	2.04 ( $\pm 0.25$ )	10.9 ( $\pm 1.03$ )	10.1 ( $\pm 0.91$ )	4.60 ( $\pm 0.36$ )	3.83 ( $\pm 0.20$ )	
1 year (3)	3	404	39.7	22.0	36.2 ( $\pm 1.4$ )	3.53 ( $\pm 0.72$ )	14.9 ( $\pm 1.01$ )	14.8 ( $\pm 0.26$ )	6.75 ( $\pm 0.20$ )	4.32 ( $\pm 0.84$ )	

Table 6. *Composition of the rat femur*

Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age in days and (no. of analyses)	No. of bones analysed	Mean femur weight (g.)	Composition of fresh bone (g./100 g.)			Composition of fat-free bone (g./100 g.)					Ca/N
			Fat	Water	Water	Total N	Collagen	Ca	P	Ca/N	
Newborn (1)	26	0.01	Nil	71.0	71.0	2.56	3.72	2.24	1.39	0.88	
7 (2)	40	0.03	1.6	73.2	74.5 (74.2, 74.9)	2.25 (2.16, 2.34)	4.28 (4.21, 4.35)	2.02 (1.80, 2.25)	1.26 (1.22, 1.31)	0.90 (0.77, 1.04)	
14 (2)	36	0.06	2.2	65.5	67.0 (64.4, 69.6)	2.64 (2.56, 2.72)	6.23 (6.12, 6.35)	3.60 (2.82, 4.38)	2.01 (1.61, 2.42)	1.37 (1.04, 1.71)	
21 (6)	24	0.12	1.9	59.6	60.7 ( $\pm 0.93$ )	2.93 ( $\pm 0.10$ )	7.47 ( $\pm 0.4$ )	5.00 ( $\pm 0.63$ )	2.65 ( $\pm 0.22$ )	1.71 ( $\pm 0.22$ )	
33 (6)	12	0.23	0.32	58.6	58.7 ( $\pm 2.28$ )	3.06 ( $\pm 0.39$ )	8.23 ( $\pm 0.51$ )	6.88 ( $\pm 0.69$ )	3.31 ( $\pm 0.33$ )	2.26 ( $\pm 0.22$ )	
46 (6)	12	0.34	1.02	49.1	49.7 ( $\pm 2.14$ )	3.28 ( $\pm 0.21$ )	10.12 ( $\pm 1.23$ )	8.82 ( $\pm 1.04$ )	4.72 ( $\pm 1.0$ )	2.70 ( $\pm 0.39$ )	
56 (6)	12	0.46	1.2	42.1	42.6 ( $\pm 2.39$ )	3.50 ( $\pm 0.29$ )	11.6 ( $\pm 0.56$ )	11.6 ( $\pm 1.37$ )	6.10 ( $\pm 1.11$ )	3.36 ( $\pm 0.63$ )	
108 (6)	12	0.69	1.1	27.5	27.8 ( $\pm 2.24$ )	3.50 ( $\pm 0.26$ )	13.8 ( $\pm 1.69$ )	18.2 ( $\pm 0.84$ )	7.94 ( $\pm 0.36$ )	5.23 ( $\pm 0.21$ )	
158 (6)	12	0.80	2.1	25.8	26.4 ( $\pm 2.54$ )	3.32 ( $\pm 0.16$ )	12.9 ( $\pm 0.75$ )	19.1 ( $\pm 0.77$ )	8.60 ( $\pm 0.49$ )	5.72 ( $\pm 0.30$ )	

Table 7. *Composition of the fowl femur*

Values are means of the number of analyses shown. Standard deviations are shown ( $\pm$ ).

Age in weeks and (no. of analyses)	No. of bones analysed	Mean femur weight (g.)	Composition of fresh bone (g./100 g.)			Composition of fat-free bone (g./100 g.)					Ca/N
			Fat	Water	Water	Total N	Collagen	Ca	P	Ca/N	
Newly hatched (3)	23	0.16	2.39 ( $\pm 0.99$ )	70.3 ( $\pm 0.39$ )	72.3 ( $\pm 1.1$ )	2.20 ( $\pm 0.09$ )	7.08 ( $\pm 0.38$ )	2.90 ( $\pm 0.04$ )	1.41 ( $\pm 0.09$ )	1.37 ( $\pm 0.10$ )	
2½ (3)	12	0.5	6.20 ( $\pm 1.98$ )	52.4 ( $\pm 3.6$ )	55.8 ( $\pm 2.7$ )	2.88 ( $\pm 0.21$ )	9.37 ( $\pm 1.30$ )	6.04 ( $\pm 0.51$ )	2.92 ( $\pm 0.11$ )	2.10 ( $\pm 0.12$ )	
4 (3)	9	1.17	1.5 ( $\pm 0.33$ )	53.7 ( $\pm 0.5$ )	54.6 ( $\pm 0.30$ )	3.04 ( $\pm 0.07$ )	9.91 ( $\pm 0.73$ )	6.90 ( $\pm 0.33$ )	3.33 ( $\pm 0.60$ )	2.26 ( $\pm 0.07$ )	
15 (3)	3	15.7	16.6 ( $\pm 2.46$ )	40.6 ( $\pm 2.69$ )	48.7 ( $\pm 1.73$ )	3.55 ( $\pm 0.92$ )	12.9 ( $\pm 0.21$ )	9.00 ( $\pm 0.43$ )	4.17 ( $\pm 0.43$ )	2.53 ( $\pm 0.06$ )	
27-42 (10)	10	22.17	15.0 ( $\pm 5.4$ )	28.9 ( $\pm 5.2$ )	34.6 ( $\pm 7.0$ )	3.52 ( $\pm 0.42$ )	13.7 ( $\pm 2.0$ )	12.8 ( $\pm 2.1$ )	5.56 ( $\pm 0.84$ )	3.78 ( $\pm 0.44$ )	

is lacking about the proportion of mucopolysaccharide in the cortex of any of the species employed in the present study. Rogers (1949) has shown, however, that the percentage of hexosamine, and therefore presumably of mucopolysaccharide, decreases in human cortical bone during postnatal growth. Since there is only a small fall in the proportion of non-collagen nitrogen after birth in human bone (Dickerson, 1962) it might be inferred that similar but larger falls in the hexosamine content of the cortical bone of other species would take place during growth and particularly at the times when the contribution of collagen to the total nitrogen is increasing.

Although cortical bone may be referred to as 'bone tissue' when compared with the more complex organ of which it is only a part, it is far from homogeneous. Pratt (1959) has shown that the shaft of the rat femur consists at different ages of different proportions of 'metaphysis', 'splinting diaphysis' and 'primitive diaphysis'. The cortical bone obtained from the femora of the newborn, 7-day-old and possibly the 14-day-old rats probably consisted entirely of 'primitive diaphysis'. This 'primitive diaphysis' has a higher cell density than the 'splinting diaphysis', which at later stages would have contributed an increasing proportion of the sample. This anatomical study indicates that a fall in the proportion of cell proteins probably accounted for some of the fall in the proportion of non-collagen nitrogen.

Studies of undecalcified bone with the electron microscope have demonstrated the intimate relationship between the crystallites of bone mineral and the collagen fibres (Robinson & Watson, 1952, 1955; Jackson & Randall, 1956). The fact that in the pig and fowl cortex there is only a small rise in the calcium/collagen ratio with age is in agreement with the view that the collagen fibres are rapidly calcified to about 80% saturation and only slowly attain full saturation (Neuman & Neuman, 1958). The finding of a larger rise in the calcium/collagen ratio in the rat cortex with age is puzzling on the basis of this concept. It is possible, however, that the collagen fibres in this species are of smaller diameter and a given mass of them is able to accommodate more mineral. Differences in fibre diameter may also be one factor to be considered in trying to account for the different degrees of calcification of adult bone of different species found by Weidmann & Rogers (1950) and brought out again in the present study. A single sample of cortex may contain bone with a range of degrees of calcification (Amprino & Engström, 1952; Davies & Engström, 1954). Any measure of the degree of calcification of cortical bone therefore gives only an average value for the different microscopical structures within the sample.

The rat was the only species investigated in which there was a steady increase during growth in the calcium/phosphorus ratio in the cortex. The change from 1.84 to 2.16 in the first 46 days of life was greater than that reported by Kramer & Shear (1928). Kramer & Shear attributed the rise in calcium/phosphorus ratio to a rise in the proportion of calcium carbonate in the bone crystals. The deposition of calcium carbonate could have been one factor contributing to the present results, but the major part of the rise in the calcium/phosphorus ratio during the first 46 days of life in the rat is probably due to a fall in the proportion of ester phosphates from the cells.

There is some evidence that the pig's humerus develops at a slightly faster rate than its femur. Hammett (1925*a, b*) found a similar difference in the rate of development of these two bones in the rat. The relative degree of maturity of homologous bones of different species at comparable stages of development is also different. The percentage of calcium in the epiphyses of the pig's femur shows that this bone is much more mature in the newborn animal than the femur of the newborn rat, or that of the newborn human baby (Dickerson, 1962). The chemical development of the skeletal muscle of pigs also takes place at a faster rate than that of human muscle, and it has been suggested that this is to be associated with the greater activity of young piglets (Dickerson & Widdowson, 1960*b*).

Nutrition affects the rate of growth of bones and also their maturation (Appleton, 1929). Both may be affected, and fast-growing bones may also mature chemically at a faster rate than more slowly growing ones (Dickerson & Widdowson, 1960*a*). The rate at which a bone increases in size is related to the activity of the epiphysial plate and to the production of new matrix. In certain circumstances the latter may proceed at a faster rate than the calcification of the matrix and result in the new bone's being less highly calcified than that previously laid down. Burns & Henderson (1936*b*) found that there was a fall in the percentage of calcium in the cortex of kitten bones during the first 7–13 days of life. Weidmann & Rogers (1958) have confirmed this and suggested that it might be due to nutrition. Slater & Widdowson (1961) have since shown that the fall in the degree of calcification of kitten bones can to some extent be prevented by giving calcium phosphate supplements. The changes in the rat femur during the first 7 days of life are probably similar to those in young kittens.

The hold-up in the chemical maturation of the pig's humerus during the latter part of gestation may be due to the growth of the bones outstripping the supply of calcium crossing the placenta. On the other hand, there may not be enough ATP or some



other substance in the foetal bones to make use of all the calcium reaching the foetus. This is not likely, however, for the urine of newborn piglets contains only 1.3–3.6 m-equiv./l. (E. M. Widdowson, personal communication).

### SUMMARY

1. The humerus of the pig has been analysed at various stages of pre- and post-natal development, and the femur of the rat and fowl at various stages of postnatal development.

2. In the cortical bone, species differences were found in the time of life at which the major changes in composition took place and also in the magnitude of these changes. Rat bone at 158 days was more highly calcified than pig bone at one year or fowl bone at 27–42 weeks, but newborn-rat bone was in many ways comparable with that of pig foetuses at 46 days' gestation.

3. The prenatal epiphyseal development of the pig's humerus was a little more advanced than that of the femur at the same age. At birth the epiphyseal development of the pig's femur was considerably more advanced than that of the rat's femur.

4. In the dry fat-free epiphyses of the rat femur the fall in the percentage of non-collagen nitrogen during the first 56 days of postnatal life was accompanied by a rise in percentages of both collagen and calcium. After 56 days the percentage of collagen also fell, but that of calcium continued to rise.

5. In the whole fresh bones of the pig and fowl the fall in the percentage of water during later postnatal growth was largely due to an increase in the percentage of fat. In the rat there was no increase in the percentage of fat with age. In all three species the fall in the percentage of water in the fat-free bones resulted mainly from a rise in the percentage of minerals.

6. There was a significant fall in the degree of calcification of the pig's humerus between 65 days' gestation and term when the epiphyses were excluded. The major part of this fall was probably due to a fall in the degree of calcification of the metaphyses.

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