CLXIX. CHANGES IN GROWTH AND WATER CONTENT OF THE BONES OF NEWLY BORN PUPS AND KITTENS.

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HAMMETT [1925] concluded that "The percentage of water in bones of both sexes decreases with age. This is largely due to ash deposition. The increase in percentage of organic matter is a minor factor." He also pointed out, "If the percentage values be charted it is seen that the curves for ash and water simulate that of a monomolecular autocatalyzed reaction." Bone growth and ossification are however the results of numerous changes taking place simultaneously. A study of these individual changes in normal bone growth was undertaken before the study of certain pathological conditions. The methods used have been described [Burns & Henderson, 1935]. Cats and dogs were used, as they were available and of suitable size. Some work was also done on the herbivores, goats, sheep and rabbits, but as they showed the presence of certain species differences they will be dealt with in a subsequent paper.

The cats and dogs used were bred domestically and the mothers were given ample mixed domestic diets. The mother cats received ¼ pint of milk per head per day normally; this was increased to ½ pint during lactation. The bitch received ½ pint usually and 1 pint during lactation. The animals were free to play in the sunshine. Ample calcium and phosphate were added to the diet in the form of bones and fish heads. The young weaned themselves gradually, and were put on to the same diet as their mothers with the necessary addition to the quantity of milk. Weaning was complete at 21–25 days. Figures were also secured from two adult cats and one adult dog, of unknown breed and diet, and from pooled samples from the long bones of the hind-legs of four embryos from a large mongrel bitch. The age of the embryos was unknown, but they were hairless and the fresh weight of the sixteen femora and humeri was 0·819 g. which may be compared with 1·463 g., the weight of the two femora of a newly born pup from a much smaller terrier bitch.

The first factor studied in these animals was the relation between age and the relative proportion of epiphysis to whole bone both fresh and dry (Table I).

Table I. *Epiphysis as % of total femur.*

	Kittens											Puppies				
	Litter 1			Litter 2				Litter 3*	Litter 7*							
Age (days) ...	0	3	16	7	11	17	45	160	0	3	10	1	24	60	160	Embryos
% fresh weight	40·0	49·8	43·3	47·9	46·8	38·0	34·6	29·9	39·8	39·1	45·4	44·8	41·3	44·0	—	68·1
% dry weight	23·1	30·4	28·0	30·2	30·0	22·2	27·7	27·9	22·1	17·2	27·2	22·4	24·2	37·4	29·0	43·6
Ca % dry epiphysis	—	—	—	1·22	1·11	1·68	9·5	15·6	—	—	—	0·88	3·99	9·6	—	0·43

* Same mother as litter 2.

In litter 1 which was studied from birth, there was an increase in the weight of both fresh and dry epiphysis relative to the whole bone in the first 3 days of extra-uterine life. In litter 7, these increases occurred between the 3rd and 10th days. Thereafter there was a steady decrease in the percentage of the fresh bone attributable to epiphysis. At an age of about 20 days, however, the proportion of the weight of the dry bone due to epiphysis began to increase as the epiphysis ossified. (This ossification is indicated in Table I by the calcium content of the dry epiphysis.) On the other hand, in the puppies, no significant change took place in the first 60 days in the proportion of the epiphysis in the fresh bone. In the dry bone, the proportion of the weight due to epiphysis increased with ossification and then fell with the continued growth of the shaft. In the embryos, the epiphysis formed a much larger proportion of either wet or dry bone than at any stage after birth. In the case of the kittens, two animals from another litter from the same mother as litters 2 and 3 gave figures at 23 days which agreed satisfactorily with those shown in the table. Two kittens 50 days old, however, from a cat of larger breed had relatively much heavier and larger epiphyses than those shown in the table, indicating that breed influences relative rates of growth.

Femur, humerus and tibia were all examined from each animal and all changed in the same way. The figures for the humerus and femur in the kittens only differed significantly in the 160-day animal in which the epiphysis formed 35·2 and 32·2 % of the wet and dry humerus respectively as against 29·9 and 27·9 % in the femur. The epiphysis of the tibia at all stages after the first few days of life was smaller in proportion to the bone than were those of the other two. In the puppies, the same general relations were found.

Water and fat contents of epiphysis, diaphysis and whole bone at different ages were next studied (Table II). The fat content was negligible in the first few weeks of life. In the three litters of kittens shown in Table II, and one other (litter 4) in which only the whole bone was examined, there was a definite rise in water content of the whole bone in the neonatal period. In litter 7, this rise occurred between the 3rd and 10th days. After this the water content fell steadily. The initial increase was mainly due to an increase in the water of the diaphysis during this period, whilst the subsequent fall was due to the decrease in the proportion of fresh epiphysis to whole bone and to the decreasing water content of the epiphysis. After the first rise the water of the diaphysis remained roughly constant up to 50 days of age, whilst the water of the epiphysis fell from 79·5 to 68·5 %. Estimations on animals from other litters all accorded with those shown.

No consistent and significant difference in the water contents of femur and humerus was observed as reported by Hammett [1925] for rats. Out of 11 animals in which femur and humerus were compared, the femur contained more water than the humerus in 10, but differences averaged 2 % and never exceeded 3 %. When femur and tibia were compared, the femur again contained more water in 10 cases, and the differences ranged from 1 to 5 % and averaged 2 %. When diaphyses were compared instead of bone, the femur again generally contained more water than either humerus or tibia (average difference 3 %), and in two cases differences as high as 10 % were noted.

In the puppies the diaphyseal water content rose steadily from 1 to 60 days of age, whilst the epiphyseal water content decreased. For the whole bone, the water content was roughly constant over this period. It is possible that the maximum point found in the kittens was missed in the puppies because of the few samples available. In the embryos, the water contents of epiphysis and

Table II.

		Kittens													Pups			
		Litter 1			Litter 7*			Litter 2*				Litter 3*	Cat					
Age (days) ...	...	<1	3	16	<1	3	10	7	11	17	45	160	Adult	Embryos	<1‡	24	60	160‡
Bone	Water %	59·3	70·0	69·4	66·0	58·7	67·8	64·8	68·1	63·6	60·5	41·4	36·4†	72·0	63·6	63·0	62·6	45·5
Bone	Fat %	—	—	0·4	—	—	—	1·0	0·8	1·7	0·8	12·1	—	—	0·5	1·4	2·8	4·1
Epiphysis	Water %	79·4	81·6	80·2	81·1	81·8	80·8	77·9	79·5	78·8	68·5	45·4	—	82·1	81·9	78·4	68·8	—
Epiphysis	Fat %	—	—	0·7	—	—	—	0·7	0·5	0·3	0·7	10·0	—	—	0·0	0·0	1·7	—
Diaphysis	Water %	45·9	58·4	61·2	56·0	43·9	57·2	52·7	58·0	54·4	56·3	39·7	—	50·4	48·8	52·2	57·2	—
Diaphysis	Fat %	0·0	0·2	0·7	—	—	—	1·3	0·7	2·5	0·9	13·0	—	—	1·9	2·0	4·2	—

* Litters from same cat. † Fat and water. ‡ Litters from same dog.

Table III.

	Kittens										Pups		
	Litter 1			Litter 7			Litter 4						
Age (days) ...	<1	3	7	<1	3	10	1	8	25	33	<1	24	60
Water femur %	59·3	70·0	69·4	66·0*	58·7*	67·8*	61·0	64·9	65·7	61·5	63·6	63·0	63·2
Width of femur mm.	—	—	—	—	—	—	3	3	4	5	2·5	5·0	6·0
Width of cavity mm.	Pin point	Cortex paper thickness	—	—	Pin point	Much wider	1	1·5	3	3·5	Pin point	2·5	4·5
Texture of cortex	Very hard	Very brittle	Brittle	Tibia hard Femur brittle	Femur hard Tibia less hard	Mod. hard	Very hard	Hard	Brittle	Brittle	Very hard	Hard	Brittle

* Femur and tibia.

diaphysis did not differ much from those of the bones of the newly born pups, but owing to the large proportion of epiphysis to whole bone, the water content of the whole bone was relatively higher.

Differences between femur, tibia and humerus in the pups were of the same order as in the kittens, but the femur in these animals did not so consistently contain more water, the differences being fairly evenly distributed in the two directions.

Table III shows that these changes in the water content of the bone are associated with changes in the growth processes and in the texture of the bone. The newly born kitten from litter 1, three 24-hour old kittens from litters 4 and 5, and the newly born pup had bones which were almost solid in the middle of the shaft and were extremely hard and difficult to cut but did not splinter. In two 3-day kittens (litter 6), and the 8-day animal from litter 4 the bone cortex was fairly thick and hard but the marrow cavity was definitely wider. On the other hand, in the 3-day-old animal from litter 1, although the bone was wider than that of the newly born litter-mate, the cortex was of paper thickness and so brittle that it fell to pieces when cut. All the bones of litter 2 from 7 days upwards showed well-marked marrow cavities, and their cortices were quite brittle. From Table III it may be seen that the widths of bone and marrow cavity were such that the 8-day bone (litter 4) would lie inside the marrow cavity of its 25-day litter-mate. The latter again would almost lie inside the cavity of its 33-day old litter-mate. Similarly the bone of the newly born pup would lie inside the 24-day bone, and this again almost inside the bone of the 60-day pup. The increase in the water of the diaphysis, and the consequent increase in or maintenance of the water content of the bone was thus mainly associated with the widening of the marrow cavity, which was accompanied by rapid destruction of bone. Between 0 and 3 days in litter 1, 8 and 25 days in litter 4, and between 1 and 24 days and again between 24 and 60 days in the pups, the entire cortex was eaten away and a new cortex built up. During this period of rapid growth the texture of the cortex underwent a marked change and became very brittle and splintered readily. The changes in the texture of the cortex and in the water content and width of marrow cavity did not exactly coincide, e.g. in the 8-day animal of litter 4, a fairly wide cavity and high water content were found in bones with fairly hard cortex. In both pups and kittens the cortex remained brittle and splintered readily when cut, until its increasing thickness made cutting with an ordinary knife impossible. This stage was reached between the ages of 60 and 160 days. The nature of the changes determining the alteration in the cortex of the bone is being studied histologically.

The question of whether the solidity of the bones at about the time of birth represents a store of calcium laid up during the stages of slow growth at the end of the intra-uterine period, or whether it merely marks a stage of bone development, is being examined by the study of those animals in which the bones of the new-born are at different stages of ossification. The results of these studies will be communicated shortly. The fact however that one 3-day-old tibia was more solid than that of its new-born litter-mate suggests that the beginning of rapid bone breakdown does not necessarily coincide with birth.

The slight increase in the water content of the epiphysis between 0 and 3 days of age (litter 1, Table II), was accompanied by an increase of 25 % in the weight of the epiphysis, whilst the diaphysis increased by 10 % in length, but lost by about 10 % in weight. In litter 2, both epiphysis and diaphysis increased by 100 % in weight between 7 and 11 days, but the epiphysis made no further gain between 11 and 17 days, whilst the diaphysis increased by a further 50 %. In litter 7 on the other hand, the bones of the new-born and 3-day animals were equal in weight and length, and the fresh epiphysis formed the same proportion of the fresh bones in each, but the diaphysis of the 3-day-old bone contained much more solid (organic and inorganic) than that of the new-born. Bone growth is thus represented by a large number of simultaneous reactions whereby organic tissue is laid down and ossified in certain places, while both organic and inorganic materials are removed from others.

Hammett [1925] found consistent differences in the rates of growth in the femur and humerus of rats.

There was no consistent difference in the rates of growth of femur, humerus and tibia in puppies and kittens, although the tibia at all ages was slightly smaller than either of the other two (Table IV).

Table IV. *Weight in g.*

	Kittens					Pups			
Age (days) ...	7	11	17	45	160	1	24	60	160
F.	0·714	1·496	1·628	4·886	18·70	1·463	9·164	20·10	48·9
H.	0·890	1·506	1·831	4·674	16·84	1·901	9·696	19·03	47·8
T.	0·510	1·013	1·282	3·839	16·71	0·906	5·855	14·74	41·4

It seems possible that the differences which he noted in water content were associated with the differences in rates of growth.

Summary.

1. The water content of very young bone is determined mainly by the size and water content of the epiphysis and of the marrow cavity.

2. Shortly after birth in pups and kittens there is an increase in the water content of the long bones, due mainly to an increase in the water content of the diaphysis.

3. During this period, there is a rapid increase in the size of the marrow cavity, and a change in the texture of the bone cortex.

4. These changes are found in femora, humeri and tibiae. There is no consistent difference in water content between these bones.

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References.

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