The Effect of Age upon the Lipids of the Long Bones of the Rat[1] (34158)

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Recent work has shown that the bones of animals contain a significant amount of lipid (1), and there has been speculation that these lipids are involved in the process of bone formation or calcification (2). As the rate of bone formation varies with age, it would be logical to expect variations in bone lipids also to occur and therefore the present study was carried out to determine the effect of age upon the lipid content of the organic matrix of rat bone.

Materials and Methods. Ten RVH hooded male and female rats of 10, 20, 30, and 50 days of age were used. The 30- and 50-day animals were litter mates while the 10- and 20-day-old rats came from each of two different litters with the same male parent. Lactating mothers and experimental animals were maintained on Purina Labina and tap water *ad libitum*. The animals were decapitated and the femorae and tibiae were removed and cleaned of all soft tissues. The proximal 10% of the femur and the distal 10% of the tibiae were discarded. The epiphyses were removed and the metaphyses were separated from the diaphyses by transection with a saw under a cold saline drip. The marrow was removed with a high pressure jet of cold distilled water. This has been shown previously to remove almost all of the marrow (1, 2). The epiphyses, the metaphyses, and the diaphyses were individually lyophilized at —40° and then material from five animals of the same age and sex was pooled and ground for 1.5 min in a Spex Mixer Mill.[2] The lipids were extracted and washed according to the method of Folch *et al.* (3). The total lipids were determined gravimetrically and the phospholipids were determined according to the method of Marinetti (4). The triglycerides were analyzed according to the method of Van Handel *et al.* (5) and the total fatty acid and total cholesterol were determined according to the method of Albrink (6).

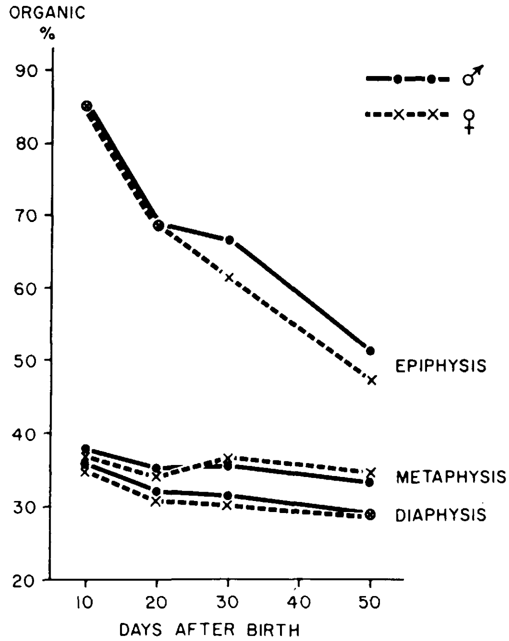

FIG. 1. Percentage of organic content.

The ash content of an aliquot of dried defatted bone residue was determined by ashing in a muffle furnace at 680° for 48 hr. The percentage of organic matter present was calculated by subtracting the ash content from 100%. All values were expressed as milligrams of lipid per gram of organic material. The total calcium and total inorganic phosphorus of the ash were determined by the methods of Yanigasawa (7) and Gomori (8).

Results. The values for organic content are shown in Fig. 1. As shown, there is a gradual fall in the percentage of the organic

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[2] Spex Industries, Scotch Plains, N.J.

fraction in the epiphysis. There is also a slight fall in the values in both the metaphysis and diaphysis although the curves are not as steep. Statistical analysis of the results was not possible due to an insufficient amount of material.

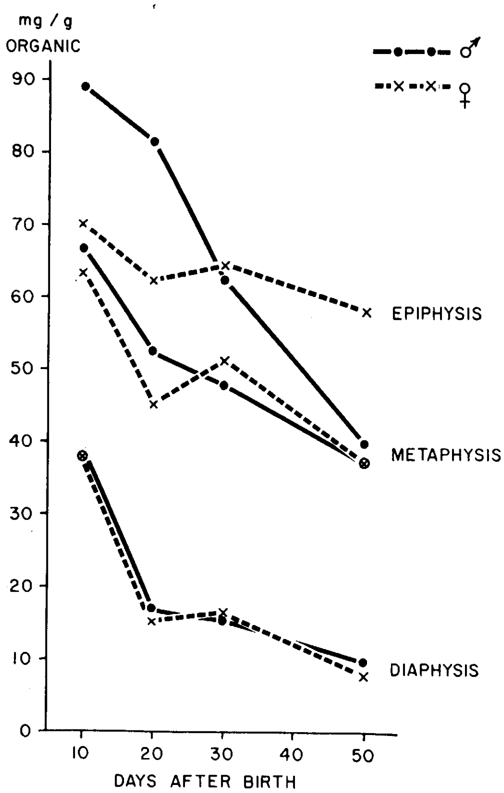

FIG. 2. Total lipid content.

As is shown in Fig. 2, there is also a precipitous drop in the total lipid of all regions. The male epiphyseal value falls to below that of the female whereas the curves are approximately equal for the metaphysis and diaphysis. The phospholipid values are shown in Fig. 3 with no differences found between the male and female levels. The cholesterol values are shown in Fig. 4 with a slight increase shown between the ages of 20 and 30 days. Figure 5 demonstrates the fall in total fatty acid and Fig. 6 the fall in triglycerides. It is to be noted that there is a sex difference at 50 days in the epiphyseal fatty acid, cholesterol, and triglyceride.

Table I demonstrates the change in the Ca:P ratio of the two sexes at various ages. A

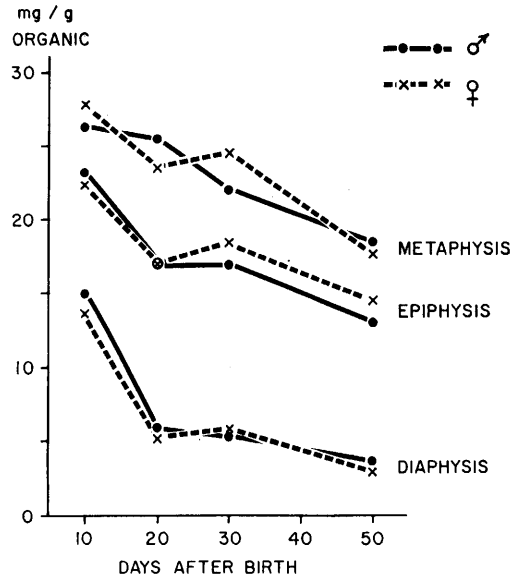

FIG. 3. Phospholipid values.

gradual increase in this value was seen in all regions with age and it was noted that the diaphyseal values were always higher than the metaphyseal ones and that these in turn were higher than the epiphyesal levels. As shown the calcium content of both the epiphysis and diaphysis tended to increase as the age of the animal advanced but there was very little change in the metaphysis. On

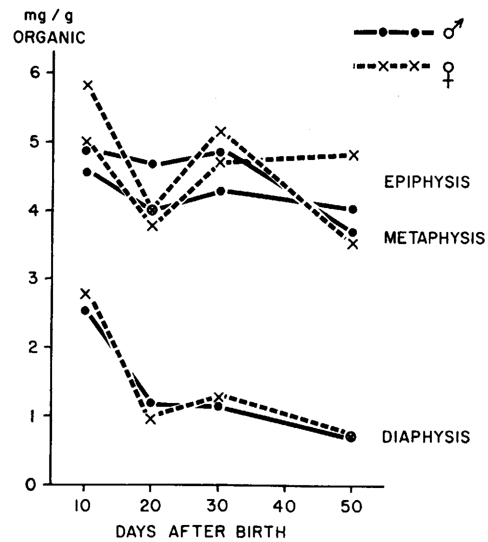

FIG. 4. Cholesterol values.

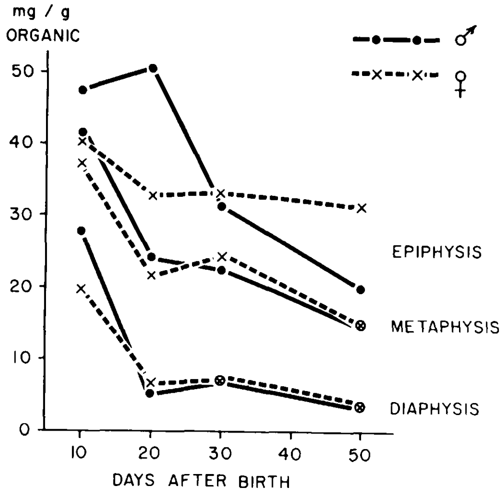

FIG. 5. Total fatty acid values.

the other hand, the phosphorus content was almost unchanged in the metaphysis and diaphysis, with a slight increase shown in the epiphyseal value. There was a gradual increase in the calcium:phosphorus ratio from epiphysis to metaphysis to diaphysis.

Correlation coefficients were calculated for all lipid values in both epiphysis, metaphysis, and diaphysis. No correlation was found for the metaphyseal values but clear inverse correlations were found between the calcium content and phospholipid ($\gamma = 0.99$), cholesterol ($\gamma = 0.99$), triglyceride ($\gamma = 0.92$), and fatty acid ($\gamma = 0.96$).

Discussion. The absolute values recorded in this study are similar to those found previously for 50-day-old rats (1). It is known that there are metabolic differences between the epiphysis, metaphysis, and diaphysis, with the epiphysis containing a large amount of cartilaginous tissue as well as newly formed bone which undoubtedly accounts for the high organic content of approximately 85% at 10 days, falling to 50% at 50 days. At 10 days the epiphysis is largely cartilaginous. The metaphysis, which contains a larger proportion of newly formed and less heavy mineralized bone, had an organic content of about 35% and dropped only slightly from the values of the younger animal. The diaphysis, in which the rate of new bone formation is much less, contained less organic material: 30% in the 50-day animal. All lipid values were higher in the epiphysis than in the metaphysis with the exception of phospholipid, and the diaphyseal values were consistently lower than those of the other two regions. It is of interest that the phospholipid value was higher in the metaphysis than it was in the epiphysis as new bone formation occurs primarily in the metaphyseal region. It is tempting to correlate the higher phospholipid value with the production of large amounts of new bone. If phospholipids were involved with bone formation, this would be expected although, of course, it does not prove the connection.

The calcium:phosphorus ratios found are similar to those found by Dickerson (9) in rats of similar ages. The alterations are probably explained in two ways. In the first place, a large proportion of the calcium and phosphorus precipitated in the epiphysis in the younger animal is undoubtedly in the zone of provisional calcification with a smaller part being derived from the bony epiphysis itself. As the animal ages, the relative contributions are altered as there is more bone in relation to cartilage. The epiphyseal ratios are thus a mixture of different forms of calcium and phosphorus. The alterations found in the metaphyseal and diaphyseal ratios parallel those reported by Termine and Posner (10) who described amorphous calci-

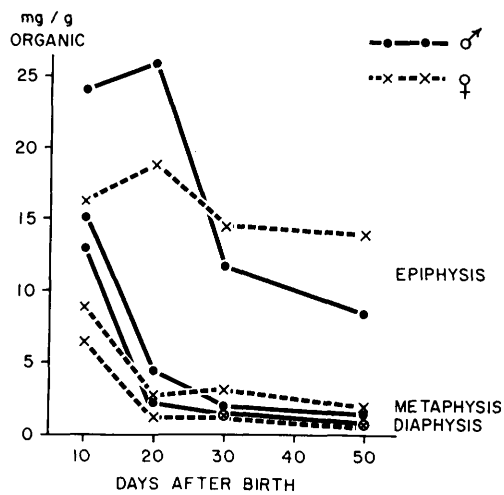

FIG. 6. Triglyceride values.

TABLE I. Calcium and Phosphorus Content of 100 g of Dry Fat-Free Bone.

		Calcium (g)		Phosphorus (g)		CA:P molar ratio	
Part	Age of rat	Male	Female	Male	Female	Male	Female
Epiphysis	10	3.7	3.1	2.2	2.0	1.30	1.20
	20	11.8	10.9	5.4	5.5	1.69	1.54
	30	10.4	14.1	5.9	6.9	1.37	1.58
	50	18.3	20.0	9.1	9.6	1.56	1.61
Metaphysis	10	26.4	26.2	12.0	12.1	1.71	1.68
	20	23.1	27.5	12.2	12.4	1.47	1.72
	30	23.7	24.4	12.2	11.7	1.51	1.61
	50	25.0	27.9	12.4	12.1	1.56	1.79
Diaphysis	10	25.3	25.9	12.1	12.2	1.62	1.64
	20	26.4	34.3	12.6	12.8	1.62	2.08
	30	34.3	35.7	12.8	12.9	2.08	2.14
	50	35.3	36.5	13.1	13.2	2.10	2.14

um phosphate which is present in large amounts in the young animal or in newly formed bone. As the animal ages, the relative amount of amorphous calcium phosphate decreases and the calcium:phosphorus ratio increases.

The inverse correlation observed between the calcium content and the various lipid values is also of interest. It is noteworthy that the metaphysis failed to adhere to the patterns noted in other regions of the bones. The possibility of these lipids being used or bound during calcification suggests itself and fits in with the theories advanced by Wuthier (12).

There have been several suggestions in the literature that phospholipids are involved in bone formation. Irving (11) found a material at the epiphyseal line and in other regions where calcium was being precipitated which would stain with Sudan black B and he suggested that this was a phospholipid. Wuthier (12) found increasing amounts of lipid during proliferation and calcification of the epiphyseal cartilage and suggested a binding of phospholipids during calcification with a protein–polysaccharide–lipid–calcium complex being put forward as a possibility. The patterns reported here are compatible with Wuthier's observations and strongly suggest a link between rates of bone formation and lipid content. Cruess and Clark (1, 13) found a large increase in the phospholipids derived from bones of animals with hypervitaminosis D, and suggested that the increased rate of bone formation was responsible for this rise. Despite these suggestions, the exact role played by phospholipids in bone formation or calcification remains obscure. The well-known affinity of phospholipids for calcium or their ability to form membrane-like structures as suggested by Johnson (2) are the most plausible possibilities.

It is of more than passing interest that the metaphysis, where the majority of new bone formation occurrs, is the only region where there is a greater amount of phospholipid than in the epiphysis and it is also of note that the curves are somewhat flatter for phospholipid than they are for all other lipid values except cholesterol. This tends to support but not prove an association between phospholipids and bone formation.

Summary. An analysis of the lipids of all regions of rat bones at varying ages has been carried out. There was a precipitous drop in the total lipid of the epiphysis, metaphysis, and diaphysis with this fall being paralleled by a drop in the phospholipid, cholesterol, triglyceride, and fatty acid. The phospholipid value was higher in the metaphysis than the other two regions of the bone during the

active growth period, and it is felt that this is associated with the extremely active bone formation being seen in the metaphysis.

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