A BIOCHEMICAL STUDY OF BONE GROWTH.

III. CHANGES IN THE COMPOSITION OF THE ASH DURING GROWTH.

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Earlier reports, dealing with another line of investigation, have indicated that the percentage composition of bone ash shifts with age with respect to calcium, magnesium, and phosphorus (1, 2). The figures given in this paper establish the shift as a fact and permit an evaluation of its direction and significance.

Table I gives the means, and their probable errors, of the calcium, magnesium, and phosphorus of the ash of the bones at the stated ages. The values were derived from the data which served as the basis of the first two papers of this series (3, 4). They thus constitute a third phase in the study of the chemistry of bone growth, in that they extend the picture to include the differentiation which takes place in the ash of the bones during growth.

Before going on to the discussion of the changes incident to growth, the trends of systemic and sex differences may be noted.

There are no systemic or sex differences in the magnesium percentage of the ash.

There is a general tendency for the calcium percentage of the ash of the femur to be slightly less than that of the humerus. There is also a general tendency for the calcium percentage of the ash of the bones of the male to be less than that of the female. The relation is reversed at 30 and 75 days of age. This reversal coincides with periods of disturbance in differential development of bone, as already discussed in detail (3). It indicates that these disturbances are reflected in ash composition in as far as calcium is concerned.

The phosphorus percentage of the ash tends to be slightly greater in the femur than in the humerus, and in the bones of the male than
in those of the female. It is noteworthy that, contrary to a priori expectation, the direction of systemic and sex differences in phosphorus percentage is the opposite of that of calcium. This indicates that the phase of metabolism in which calcium is utilized in bone growth, is not completely representative of the phosphorus metabolism.

The tendency towards the presence of a higher percentage of phosphorus in the bones of the male and in the femur is correlatable with the fact that the bones of the male contain more organic matter than do those of the female, and the fact that the femur has more organic matter than the humerus. It may be, therefore, that these systemic and sex differences in phosphorus percentage of the ash are to be attributed to the differences in organic matter.

Although the differences just noted are small in degree, the consistency of their occurrence makes them suggestive.

Turning now to the changes in ash composition with age, it is
seen from the table that the calcium percentage of both bones of both sexes increases with age to a statistically valid degree. The greater part of the increase in the bones of the male occurs during the growth period from 23 to 65 days of age. Thereafter but little change is seen, though a drift upwards is indicated. In the female, while the greater part of the increase also occurs during the period from 23 to 65 days, stabilization is not evident until 100 days of age. These sex differences are concordant with those exhibited by the ash as a whole, as has been discussed in an earlier paper (3). They are similarly interpretable. The significant point is that the differentiation of ash with respect to its calcium content is directed by the same sex differential factors of influence as regards time of action during growth as is the differentiation of the bone as a whole with respect to its growth by increments of ash.

The percentage of magnesium in the ash of both bones of both sexes decreases to a statistically valid degree during the growth period from 23 to 150 days of age. The greater part of the decrease in the bones of the male takes place between 50 and 65 days of age, and in the female between the ages of 65 and 75 days. Thereafter the changes are inconsiderable. This sex difference in time of decrease and stabilization is comparable with that which is exhibited by the water of the bones. For if the magnesium percentage values be expressed as a rate of change in percentage on unit basis, it is found that the time relations of the dip and recovery in the curve are quite the same as those for the water percentage calculated on the same basis (3). This being so, it is possible that there is an association of some sort between the changes in magnesium percentage of the ash and the water percentage of the bones, and the rôle of the sex-specific factors therein.

The percentage of phosphorus in the ash of both bones of both sexes decreases to a statistically valid degree during the growth period from 23 to 150 days of age. The greater part of the decrease is completed by the time the rats are 75 days of age. Thereafter the changes are inconsiderable.

Phosphorus and calcium are thus seen to change in opposite directions, contrary to a priori expectation. This may be interpreted as indicating a change in the type of Ca-P complex laid down with age, or an intramolecular change with age, or as an expression of a difference in type metabolism in which an associa-
tion of phosphorus with organic matter increments is present as distinct from the association of phosphorus with calcium in the process of calcification. The ultimate interpretation must rest on further data.

The significant point of these findings is that the greater part of the shift in ash composition during growth, with respect to calcium, magnesium, and phosphorus, takes place during the period when the chemical differentiation of the bone as a whole leading to the structure which is sex- and systemically characteristic of the adult from the chemical point of view, is taking place, and that the incidence of stabilization at the end of puberty is coterminous with that of the stabilization of chemical differentiation of the bone as a whole.

SUMMARY.

The percentage composition of the ash of the humerus and femur of male and female albino rats with respect to calcium, magnesium, and phosphorus changes during the growth period from 23 to 150 days of age.

The changes are practically complete at 75 days of age. That is to say by the end of the puberal period.

The changes consist of an increase in the calcium and a decrease in the magnesium and phosphorus percentage of the ash.

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BIBLIOGRAPHY.