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DISTRIBUTION PER VOLUME BONE TISSUE
OF CALCIUM, PHOSPHORUS AND NITROGEN FROM
INDIVIDUALS OF VARYING AGES AS COMPARED
WITH DISTRIBUTION PER UNIT WEIGHT

By

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Variations with age in the composition of bone tissue have been demonstrated by means of chemical analyses in investigations of both human material and material from various species of animals.

For this purpose, the degree of mineralization has been expressed either as a ratio Ca/N or P/N, or as the weight per cent of calcium or phosphorus. Variations in the composition of the mineral salts have been measured in terms of the Ca/P ratios.

Thus, *Baker, Butterworth & Langley* (1), *Rogers, Weidman & Parkinson* (2) and *Dickerson* (3) have investigated human compact bone tissue, dissected free of soft tissues, and have found that the Ca/N ratio increases with age. When the concentrations of calcium and nitrogen was determined as percentages by weight, it was found that the amount of calcium increased with age while that of nitrogen decreased. *Dickerson* also determined the phosphorus content, and found a somewhat lower P/N ratio during the foetal stage and the first years of life; no variations in the Ca/P ratio could be detected.

Vogt (4) has made similar determinations of calcium, phosphorus and nitrogen in spongy bone tissue from crista ilica; he found no definite variation with age for the Ca/N ratio, but the percentages of calcium and nitrogen both increased with age. *Vogt* also found that the concentration of phosphorus and the P/N ratio both reached a maximum at about 40-50 years of age, while the Ca/P ratio was at a minimum at this age.

Strobino & Farr (5), *Cartier* (6), *Weidman & Rogers* (7) and others

have investigated different animal species, and have demonstrated a corresponding increase in the Ca/N ratio with age. *Weidman & Rogers* investigated the rabbit and the rat, and found in addition a certain variation with age of the Ca/P ratio, which sank for a short period after birth and subsequently increased. The highest Ca/P ratios were found in full-grown animals.

Strandh (8) has studied the variations with age in the composition of Haversian systems. These were dissected from ground sections of compact bone tissue from human subjects aged 9 months, 6 years, 17 years, 52 years and 76 years. *Strandh* found that the Ca/N ratio increased with age, in agreement with results of the macroscopic investigations of bone tissue cited above. The P/N ratio was found to be lower for the nine-months subject than for the older subjects, but the latter showed no definite variations with age. The Ca/P ratio was relatively high for the nine-months infant, lower for the six-year-old child, and increased with age for the other individuals.

The methods of studying the mineralization of bone tissue which have hitherto been employed, whether by way of the Ca/N, P/N and Ca/P ratios or by determining the percentages by weight of the three elements, give no information regarding any variations in density. Moreover, the presence of water and organic material in the bone tissue often makes it difficult to define the weight exactly. It was therefore considered of interest to supplement current knowledge on this topic, as outlined above, with determinations of the weight by volume of Ca, P and N in bone tissue at various ages.

MATERIAL AND METHODS

The investigation was carried out on compact bone tissue from human femur diaphyses taken from autopsies comprising 49 cases aged between 22 days and 90 years, all free of any known skeletal diseases. Previous to investigation, the bone material was stored at -20°C .

Cross-sections of the femur diaphyses were ground down to a thickness of $350\ \mu$. They were microradiographed with a Philips X-ray PW 1010/20 at 30 kV and 20 mA. Under guidance of the microradiograms, parallel-epipeds of lamellar bone (ca. $500\ \mu$) of periosteal or endosteal type were dissected from each femur diaphysis. Portions containing canal systems were avoided, and bone tissue was selected, which had an X-ray absorption closely to that of interstitial lamellar bone which always shows the highest X-ray absorption.

The material was divided into the following age-groups: under 1 year, 1-10 years, 11-20 years, 21-30 years, 31-50 years, 51-70 years and 71-90 years. In the material from the 71-90 age-group there was almost complete absence of both periosteal and endosteal lamellar bone. In this group, highly mineralized Haversian systems were

TABLE 1

The Occurrence in Different Age-Groups of Calcium, Phosphorus and Nitrogen in Lamellar Bone and Harversian Systems Expressed as Percentages by Weight and as Weights per unit Volume. Density of the Bone Tissue and the Weight Ratios Ca/N, P/N and Ca/P. Mean Values and the Standard Deviation of the Mean.

| Age group | 1 year | | 1-10 years | | 11-20 years | | 21-30 years | | 31-50 years | | 51-70 years | | 71-90 years | |
|--------------------------------|--------|--------|------------|--------|-------------|--------|-------------|--------|-------------|--------|-------------|--------|-------------|----------|
| | 6 | | 7 | | 7 | | 4 | | 10 | | 5 | | 10 | |
| Lamellar bone | | | | | | | | | | | | | | |
| Density | 1.79 | ± 0.02 | 1.87 | ± 0.02 | 1.90 | ± 0.01 | 1.90 | ± 0.03 | 1.91 | ± 0.02 | 1.94 | ± 0.01 | 1.91 | ± 0.02 |
| Percentage by weight | 23.8 | ± 0.2 | 24.9 | ± 0.3 | 25.0 | ± 0.3 | 25.9 | ± 0.5 | 26.4 | ± 0.1 | 26.5 | ± 0.2 | 26.2 | ± 0.2 |
| | 10.5 | ± 0.2 | 10.9 | ± 0.1 | 11.2 | ± 0.1 | 11.1 | ± 0.2 | 11.1 | ± 0.1 | 11.2 | ± 0.1 | 10.9 | ± 0.1 |
| | 4.64 | ± 0.02 | 4.55 | ± 0.07 | 4.48 | ± 0.07 | 4.45 | ± 0.10 | 4.43 | ± 0.04 | 4.29 | ± 0.04 | 4.47 | ± 0.05 |
| | | | | | | | | | | | | | (27.0) | (11.2) |
| | | | | | | | | | | | | | | (4.34) |
| Haversian systems | | | | | | | | | | | | | | |
| Weight per unit volume (µg/µl) | 427 | ± 6 | 449 | ± 5 | 470 | ± 7 | 492 | ± 11 | 507 | ± 5 | 514 | ± 5 | 505 | ± 5 |
| | 188 | ± 3 | 205 | ± 2 | 213 | ± 3 | 211 | ± 5 | 214 | ± 3 | 216 | ± 3 | 211 | ± 3 |
| | 83 | ± 1 | 85 | ± 2 | 85 | ± 1 | 84 | ± 1 | 84 | ± 1 | 83 | ± 1 | 86 | ± 1 |
| | | | | | | | | | | | | | | (520) |
| | | | | | | | | | | | | | | (217) |
| Ratios | Ca/N | 5.13 | ± 0.07 | 5.28 | ± 0.09 | 5.57 | ± 0.11 | 5.86 | ± 0.20 | 6.01 | ± 0.05 | 6.17 | ± 0.09 | 5.88 |
| | P/N | 2.24 | ± 0.02 | 2.40 | ± 0.04 | 2.52 | ± 0.04 | 2.52 | ± 0.09 | 2.55 | ± 0.04 | 2.61 | ± 0.03 | 2.46 |
| | Ca/P | 2.28 | ± 0.03 | 2.20 | ± 0.02 | 2.22 | ± 0.02 | 2.33 | ± 0.01 | 2.37 | ± 0.02 | 2.38 | ± 0.03 | 2.40 |
| | | | | | | | | | | | | | | (6.06) |
| | | | | | | | | | | | | | | (2.53) |
| | | | | | | | | | | | | | | ± 0.03 |
| | | | | | | | | | | | | | | 2.40 |
| | | | | | | | | | | | | | | ± 0.01 |

The figures in brackets under the age-group 71-90 years are values calculated to be applicable for a three per cent higher degree of mineralization than that in highly mineralized Harversian systems.

dissected out instead, and were subsequently freed from the contents of the Haversian canals. The degree of mineralization of highly mineralized Haversian systems has previously been found to be about 3-4 per cent lower than that of interstitial lamellar bone (8, 9). It is therefore necessary to make a corresponding correction when comparing these specimens with lamellar bone from the younger age-groups.

The volume of the dissected structures (the parallel-epipeds) could be calculated by applying planimetric methods to microradiograms taken from above and from the side.

The weighing and analysis of the structures was carried out in the manner described in a previous paper (8).

RESULTS

Table 1 gives the occurrence of the elements Ca, P and N in lamellar bone and Haversian systems expressed as percentages by weight and as weights per unit volume ($\mu\text{g}/\mu\text{l}$). The table also gives the density of the bone tissue and the ratios Ca/N, P/N and Ca/P. Each value corresponds to the mean of 4-10 individuals, and the spread is given as the standard deviation of the mean. For the highest age-group (71-90 years) the values given are those obtained for highly mineralized Haversian systems. The figures in brackets are the values which are calculated to be applicable for a three per cent higher degree of mineralization, *i.e.* equivalent to highly mineralized lamellar bone.

The density shows a highly significant increase with age on comparison of the youngest age-groups, and is also significantly higher in the 51-70 age-group than in the 1-10 age-group. The calcium content also increases with age. This increase exhibits a larger percentage if the content is expressed per unit volume or as a Ca/N ratio rather than as a percentage by weight. The occurrence of phosphorus, calculated as a percentage by weight, increases with age up to the 11-20 age-group, and appears to be unaltered thereafter. When expressed in terms of unit weight per unit volume the phosphorus increase exhibits a larger percentage than if the content is expressed as a percentage by weight. The nitrogen content decreases with age up to the 51-70 age-group when it is measured as a percentage by weight; when expressed in terms of unit weight per unit volume, however, it shows no variation with age.

DISCUSSION

The results obtained show good agreement with those from previous investigations. However, the introduction of volume determinations has yielded additional information on the nature of the variations with age. The nitrogen content is found to be unaltered with age when

measured per unit volume, while it decreases with age if measured per unit weight. This and the increase of density with age show that the changes with age do not consist of a replacement of organic material by mineral salt, but rather a deposition of this salt in cavities such as osteocyte lacunes and central canals. This conclusion is in agreement with the results obtained by *Sisson* and co-workers (11) and others from microradiographic investigations of normal bone tissue at various ages.

The increase with age of the calcium content but not the phosphorus content in the higher age-groups, together with the increase in the Ca/P ratio, suggests that a further calcium salt is deposited as well as apatite. *Bergström & Bell* (12) and *Burns & Henderson* (13) have shown that the carbonate content of bone tissue increases with age, and it is therefore reasonable to interpret the above-mentioned variations in terms of a filling-up of the cavities with mineral salt, which in the higher age-groups consists partly of calcium carbonate.

SUMMARY

Determinations of the weight by volume of calcium, phosphorus and nitrogen in human compact bone was performed on 49 autopsy cases aged between 22 days and 90 years. The calcium content was found to increase with age. The phosphorus content increases with age up to the 11–20 age-group and appears thereafter to be unaltered. The increase of these elements exhibits a larger percentage if the content of them is expressed in terms of unit weight per unit volume rather than as a percentage by weight. The nitrogen content decreases with age up to the 51–70 age-group when measured as a percentage by weight; when expressed in terms of unit weight per unit volume, it shows no variation with age. The density of the bone tissue was found to increase significantly with age up to the 1–10 group and it is significantly higher in the 51–70 age-group than in the 1–10 age-group.

RESUME

Des déterminations du poids par le volume du calcium, du phosphore et de l'azote dans le tissu compact des os humains ont été pratiquées dans 49 cas d'autopsie de sujets âgés de 22 jours à 90 ans.

On a constaté que la teneur en calcium augmente avec l'âge. La teneur en phosphore augmente avec l'âge jusqu'au groupe entre 11 et

20 ans et semble rester inchangée par la suite. L'augmentation de ces éléments représente un plus large pourcentage de la teneur si celle-ci est exprimée en unité de poids par unité de volume plutôt qu'en un pourcentage du poids. La teneur en azote décroît avec l'âge jusqu'au groupe entre 51 et 70 ans lorsqu'elle est mesurée comme un pourcentage du poids; lorsqu'elle est exprimée en unité de poids par unité de volume, il n'y a aucune variation avec l'âge. On a constaté que la densité du tissu osseux augmente d'une manière notoire avec l'âge dans le groupe de 1 à 10 ans et elle est sensiblement plus élevée dans le groupe d'âge de 51 à 70 ans que dans celui de 1 à 10 ans.

ZUSAMMENFASSUNG

Bestimmungen des Volumengewichtes von Calcium, Phosphor und Stickstoff im menschlichen Kortikalknochen wurde an 49 Autopsiefällen, die 22 Tage bis 90 Jahre alt waren, vorgenommen. Der Calciumgehalt nimmt mit dem Alter zu. Der Phosphorgehalt nimmt mit dem Alter bis zur 11-20 jährigen Gruppe zu um hernach unverändert bestehen zu bleiben. Die Zunahme dieser Elemente weist einen höheren Prozent auf, wenn ihr Inhalt im Verhältnis von Gewichtseinheit per Volumeneinheit anstatt als Gewichtsprozent ausgedrückt wird. Der Stickstoffgehalt nimmt mit dem Alter bis zur 51-70 jährigen Altersgruppe ab, wenn er als Gewichtsprozent gemessen wird. Wenn er jedoch im Verhältnis von Gewichtseinheit zu Volumeneinheit ausgedrückt wird, zeigt er keine Veränderung mit dem Alter. Es wurde gefunden, dass die Dichtigkeit des Knochengewebes mit dem Alter bis zu der 1-10 Gruppe bedeutend zunimmt und dass sie in der 51-70 jährigen bedeutend grösser ist als in der 1-10 jährigen Gruppe.

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