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Note on the Chloride Content of the Mineral Constituents of the Skeleton

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By adding ^{36}Cl to the diet of pregnant mice and to their offspring until they are outgrown uniformly labelled animals are obtained. Prior to administering non-labelled diet to the outgrown labelled mice the total activity of the body of one member of the litter is determined. All ^{36}Cl with the exception of that sequestered in the skeleton is then removed by keeping the mice for 6 months on non-radioactive diet. After that date the skeleton is found to contain 0.73 % of the total activity which the sister mouse had prior to removal of the radioactive diet. From this figure and the chloride content of the mouse the chloride sequestered in the skeleton of a 35 g mouse is calculated to amount to 0.35 mg. While almost 90 % of the bone sodium is excess sodium, the corresponding figure for chloride is about 10 only.

In view of the only slightly differing size of the hydroxyl and fluoride ion, the hydroxyl ions of the bone apatite can be replaced by fluoride ions. Furthermore some of the fluoride may be present in the bone mineral as calcium fluoride.

The fluoride content of the bone apatite is determined by that of the plasma, which in turn depends on the fluoride content of the food. The fluoride content of the earth's crust is much lower than that of the seawater, the mineral constituents of the skeleton of mammals living in the sea is, correspondingly, very much, about eleven times, larger than that of mammals living on land, which contain 0.05 % fluoride only. The skeleton of fish living in the Baltic, which has a low fluoride content, have a much lower fluoride percentage (0,06 %) than the skeleton of fish living in the Atlantic (0.43 %). Incorporation of fluoride into the mineral constituents of the bone was in recent years much investigated, mainly in connection with the observation that the presence of fluoride in the mineral constituents of teeth increases their resistance to caries.

The radius of the chloride ion is much larger (1.81 Å) than that of the fluoride ion (1.33 Å) and calcium chloride being very soluble we can expect to find slight amounts of chloride in the mineral constituents of the bone. While the

X-ray diagram of fluoroapatite is almost identical with that of hydroxyapatite that is far from being the case for chloroapatite[9]. The fluids circulating in the bone tissue having a high chloride content (about 300 mg/100 ml); this has to be quantitatively removed prior to the determination of the amount of chloride incorporated into the mineral constituents. Such removal by chemical treatment of the bone encounters great difficulties. However, when labelling the skeleton all through with radiochloride and then placing the animal, *e. g.* the mouse, on diet containing non-radioactive chloride for several months, all exchangeable radiochloride will be removed and excreted, the radiochloride fixed in the mineral constituents alone remaining in the skeleton.

In the human half of the exchangeable chloride is removed in the course of 14 days [1] and replaced by chloride of the food and after 6 months, *i. e.* after 12 periods, all exchangeable chloride initially present will be practically absent. In the mouse, with its high metabolic rate, the removal rate of chloride can be expected to be still higher than in man. The removal can be accelerated by increased chloride feeding. Besides the chloride present in the standard biscuits fed we added 0.2 % NaCl to the water the mice were drinking after they were put on non-radioactive diet. To the water administered to the mice in the first phase of the experiment ^{36}Cl of 0.67 μ C activity per liter was added as sodium chloride weighing 9.3 mg. The labelled chloride was administered to pregnant mice about 2 weeks prior to gestation. After gestation the administration of labelled chloride was continued for 4 months when the animals were fully grown. One member of each litter was then killed and its total ^{36}Cl content and that of its skeleton determined. The remaining members of the litters were investigated 6 months later.

The bone was ashed in the presence of sodium carbonate. The activity of 100 mg, thus of an infinite thick layer of the samples obtained was determined, the counts registered being multiplied by the total weight of the ash-sodium carbonate mixture.

The total ash of the first member of the litter of the mice investigated had a total activity of 2 780 counts per min, mean value 2 910 $\pm$ 452 counts, that of the mineral constituents of the skeleton of the first investigated offspring 56.6 counts. This was prior to biological removal of all exchangeable chloride from the skeleton but after the removal of some of the latter in the course of the isolation of the mineral constituents.

By keeping the mice on an activity-free diet for 6 months the activity of the mineral constituents declined in the average to 21.2 counts (*cf.* Table 1).

The total chloride content of a 35 g mouse taken to be 48 mg, the activity of 1 mg of the body chloride prior to removal of the active food was 60.6 counts per min. As the total skeleton after biological removal of all exchangeable chloride had an activity of 21.2 counts per min the sequestered chloride content of the bone mineral amounted to 0.35 mg or 0.73 % of the total body chloride.

The sequestered fraction of the bone calcium of the mouse, that non-replaceable by circulating calcium, was found [2] to be 67.2 $\pm$ 7.9 %. The corresponding figure for bone sodium is stated to be 60—70 [3], 65 [4], 60 [5] and 69 [6]

Table 1. Counts per min. ^{36}Cl activity of the skeleton of 33—36 g mice after keeping on non-active diet, thus after biological removal of exchangeable radiochloride, for 6 months

18.2
24.6
25.1
28.6
25.1
26.6
25.5
16.4
19.4
12.1
211.6 : 10 = 21.2 ± 2.08
m.e.v. = ± 0.208

by different authors. Thus about a similar percentage of excess sodium and of excess calcium is prevented from interchanging with their circulating atoms. The sequestration of bone constituents is presumably due to the fact that a contact between these constituents and the circulating body fluids is obstructed. To arrive at the total excess chloride content of the bone of the mouse we correspondingly have to multiply the figure of 0.35 mg found for the non-exchangeable bone chloride by about 1.5, thus arriving at the result that the total excess bone chloride content of our 35 g mice amounts to about 0.53 mg.

In contrast to the bone sodium which is to a large extent present as excess sodium in the skeleton it can be shown that the chloride present as excess chloride makes out 1/10 only of the total bone chloride.

From 234 meqiv sodium present in 1 kg of dry human bone 84.9 %, thus 46 g, was found by Edelman *et al.*[5] to be excess sodium in a 70 kg man. For the dog Edelman and associates found 89.5 % of the bone sodium to be excess sodium and a similar figure is stated by Miller and associates [7]. 1 kg of rat bone was found to contain 125 meqiv excess sodium [8]. As to the total chloride content of 1 kg fat free bone this was found to amount to 19 meqiv only [5], thus to less than the extracellular bone sodium which makes out 25 meqiv. While excess bone sodium is to a marked extent responsible for the difference between total and extracellular body sodium, for chloride this difference is almost entirely due to the presence of intracellular chloride in the soft tissues.

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