OTHER work in this laboratory has shown that the percentage of total body calcium increases during normal growth and development and to a somewhat larger extent in females than in males; also, that the calcium content of the body may be influenced by that of the food (1, 2). The rat, a presumably representative mammal in this respect, has been used in experiments which involved the analysis of the body for its calcium content, while the validity of the same principles in human nutrition is indicated by the results of calcium balance experiments with growing children upon different levels of calcium intake (3). The present paper deals with the distribution of the body calcium as between the skeletal system including the teeth, on the one hand, and, on the other hand, the soft tissues including the blood and lymph. Determinations in separate organs were discontinued because the smallness of the amounts involved made comparisons, in our judgment, of doubtful value. The extent to which the body calcium is concentrated in the skeletal system is, however, determinable with satisfactory accuracy in the rat; and the present findings upon this species are probably fairly representative of the higher vertebrates generally.

EXPERIMENTAL

The animals were killed by chloroform. After careful removal of any dust which may have adhered to the fur, the skin, muscles, and all other soft parts were dissected away from the skeleton with special care that no trace of bone should be removed with the soft tissues. (On the other hand, traces of muscle left with the skeleton would not measurably affect the determinations of cal-
calcium. Previous work in this laboratory has shown that boiling to loosen the muscle is not advisable where calcium is to be determined, as it dissolves too much of the calcium from the bone.)

The alimentary tract was removed and the weight of its contents subtracted from the live weight to obtain the true (net) body weight of the animal. In ashing the soft parts for analysis, the alimentary tract was omitted to avoid any possibility of contamination of the sample with food residues, previous work in this laboratory having shown that the calcium content of the walls of the alimentary canal is negligible.

In several cases the brain was analyzed separately. It contained only about 1 mg. of calcium, even in fully adult rats. As this is too small an amount to have any significant influence upon the total amount of calcium found in the skeleton, it has been considered sufficient in other cases to dissect away the other soft parts leaving the brain and cord with the skeletal system. This greatly reduced the largest danger of experimental error; namely, the possibility of accidental transfer of scrapings or small splinters of bone to the soft tissues during the dissection.

Each part or collection of parts which was to constitute a separate sample was ashed in a silica dish in a muffle, and the calcium of the ash determined by the modified McCrudden method (1).

Because of indications of appreciable calcification of the trachea in some cases, considerable numbers of individual tracheae were ashed and analyzed separately. Those of animals up to 2 months of age were found to contain less than 0.5 mg. of calcium. Tracheae of rats from 3 months to 15 months old showed about 1 to 4 mg. of calcium, the average amount increasing with the age.

**Skeletal Calcium in Percentage of Total Body Calcium**

*In Adults from Fully Normal Diets*—In thirty-four male rats of 6 months and older averaging about 1 year of age, the skeletal system showed $99.33 \pm 0.018$ per cent of the total body calcium found. In thirty-one female rats of similar age and nutritional history, the skeletal system showed an average of $99.32 \pm 0.019$ per cent of the total body calcium.

*In Younger Rats from Fully Normal Diets*—The well known fact that the skeleton is less calcified at birth than at maturity

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1 The precision measure here used is the classical probable error.
suggests the question whether the younger animals have a lesser proportion of their total body calcium in their skeletal systems. At 2 months, eight males showed skeletal calcium of 99.06 per cent, and eight females, 99.08 per cent, of the total calcium of their bodies; at 3 months, five males 99.1, two females 99.2 per cent; at 4 to 5 months, ten males 99.4 per cent, four females 99.3 per cent. Here it appears that at the age of 2 months the skeletal tissues have an appreciably lesser share of the total body calcium than in the normal adult; but that the adult status in this respect is reached at between 3 and 5 months in the rat, or between the time of puberty and that of attaining full adult size.

*In Adult Rats from a Low Calcium Dietary*—In a recent study of a rather low calcium diet in this laboratory (4) it was found that adults of the second generation thus fed were of less than average adult calcium content. Here the paucity of intake of calcium had apparently kept the adults in the condition of uncompleted ossification ordinarily found only in the young. In these adults of low calcium content the percentage of total body calcium which was found in the skeletal system at 1 year of age was: for three males 99.0, 99.0, and 99.0; for five females 99.0, 98.9, 99.0, 99.0, and 98.9 per cent, respectively. While these cases are probably too few to justify statistical treatment, their uniformity and the fact that the normal average has above been established with a high degree of precision, together seem to justify the conclusion that in the adults with low calcium content as well as in the young animals in which the normal process of calcification (ossification) is still incomplete, there is a slightly smaller percentage of the total body calcium in the skeletal system than is the case in the normally calcified adult.

In all cases we find higher percentages of the total calcium in the skeletal system than was reported by McCann and Barnett (5) but this is explained, first by the fact that they were not interested in as small differences as those with which we are here concerned; and, second, that they boiled their rats and included the water with the soft parts, which technique increases the calcium attributed to the soft parts and decreases that of the bones.

**SUMMARY**

Of the total body calcium of normally developed adult rats on fully adequate diets, the percentage contained in the skeletal
system (bones and teeth) was found to be: in thirty-four males an average of 99.33 per cent with a probable error of 0.018; and in thirty-one females an average of 99.32 with a probable error of 0.019. These averages are thus established with a relatively high degree of precision. The reasons for some lower estimates in earlier work are explained.

In rapidly growing immature rats, while the normal process of calcification is still unfinished, and also in adult rats which were still of low calcium content because of calcium-poor food, a somewhat smaller percentage of the total body calcium was found in the skeletal system; but even in the cases of this kind (within the range of the present investigation) the skeletal system contained something of the order of 99 per cent of the total amount of calcium in the body.

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THE NORMAL DISTRIBUTION OF CALCIUM BETWEEN THE SKELETON AND SOFT TISSUES
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