mediately removed, homogenized in phosphate buffer, and incubated in a Warburg flask into which the radioactive amino acid had been introduced. A square of filter paper was placed in the center well containing 0.2 ml of 5% KOH to collect the $^{14}CO_2$. The flask was swept with oxygen and placed in the water bath at 37° C. After incubation, the filter paper was removed immediately, dried and counted.

The Tables (I and II) indicate that the $^{14}CO_2$ production from the DL-alanine by brain is about one third that produced by kidney and about one half that produced by liver.

When the supernatant obtained by centrifugation of the homogenate is incubated similarly, it also displays appreciable capacity for DL-alanine dissimilation. Acetone powder may be used instead of fresh brain tissue.

TABLE I

$^{14}CO_2$ PRODUCTION FROM LABELED DL-ALANINE-1-^{14}C BY VARIOUS RAT TISSUE HOMOGENATES

Tissue	*Counts of $^{14}CO_2$ produced* × 100 / *Counts of administered dose*
Brain	0.37
Kidney	0.99
Liver	0.64
Spleen	0.02
Blood	0.008

TABLE II

$^{14}CO_2$ PRODUCTION FROM LABELED DL-ALANINE-1-^{14}C BY RAT BRAIN HOMOGENATE

Time of incubation	*Counts of $^{14}CO_2$ produced* × 100 / *Counts of administered dose*
15 minutes	0.11
40 minutes	0.43
65 minutes	1.11
118 minutes	1.60

Table 1:

Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) (except the flask which contained 2.0 ml of fresh blood, drawn from the heart, in presence of substrate only) and 0.1 mg ($1.2 \cdot 10^{-3}$ mc) of DL-alanine. The flasks were incubated at 37° C for 45 minutes.

Table 2:

Each incubation flask contained 1.5 g of tissue suspended in 2.0 ml of 0.1 *M* phosphate buffer (pH 7.4) and 0.2 mg ($2.4 \cdot 10^{-3}$ mc) of DL-alanine. The flasks were incubated for indicated times.

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THE UPTAKE OF ^{32}P IN THE FEMUR OF GROWING RACHITIC AND NORMAL RATS AS COMPARED WITH THE UPTAKE IN THE TOTAL SKELETON

by

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In a series of experiments, recently performed in this laboratory, we determined the uptake of inorganic ^{32}P in the femur of growing rachitic and normal rats. We were also interested in the amount of ^{32}P taken up by the total skeleton. As it is known that the uptake of ^{32}P is different for the various parts of the skeleton[1,2], it seems hazardous to calculate the total uptake of ^{32}P from the uptake in the femur on the basis of ^{31}P content or weight, as some authors do[3,4]. We, therefore, determined in a number of animals the uptake in both femur and total skeleton one hour after intraperitoneal injection. These data gave us an impression of the amount of ^{32}P to be expected in the total skeleton once the tracer content of the femur was known.

The animals, young white rats of the Wistar strain, had subsisted after weaning on a somewhat modified Steenbock Black rachitogenic diet containing 1.20% Ca and 0.30% P. The animals indicated as normal received a protecting amount of calciferol (1.05 microgram per week). One hour after injection of about 15 μC of ^{32}P as $Na_2H^{32}PO_4$ the animals were killed with ether.

After the skin had been removed and the internal viscerae taken out, the carcasses were brought into a 2% solution of a mixture consisting of 45% Na_2CO_3, 30% soap powder and 25% water (Gold-dust Washing Powder method[5]). The solution was subsequently heated till 96° C. After remaining on that temperature for about 5 minutes the rats were taken out and it proved comparatively easy to remove the soft tissue from the skeletons. The skeletons were dried at 100° C for three days. One femur was removed from each skeleton and both this femur and the rest were ashed at 600° C. The ashes were dissolved in HCl and analyzed for ^{31}P and ^{32}P. The results are given in Table I.

TABLE I

UPTAKE OF ^{32}P IN THE FEMUR AND IN THE TOTAL SKELETON OF NORMAL AND RACHITIC RATS ONE HOUR AFTER INTRAPERITONEAL INJECTION

Rat No.	*Weight g*	*Femur ^{31}P in percentage of total ^{31}P*	*Femur ^{32}P in percentage of total ^{32}P*	*$\frac{\text{Percentage } ^{32}P}{\text{Percentage } ^{31}P}$ in femur*
1857 N	116	3.8	5.2	1.4
1846 N	123	3.7	5.0	1.3
1847 N	119	4.2	5.4	1.3
888 R*	92	2.8	4.4	1.6
904 R	97	2.8	—	—
1858 R	118	3.2	5.2	1.6
1863 R	122	3.2	5.0	1.6
1855 R	120	3.6	6.2	1.7

* sacrified 30 min after injection

Our data show that the uptake of ^{32}P by the femur in short time experiments is higher than that of the average skeleton, if calculated on the basis of ^{31}P content. This is seen both in the rachitic and in the control group. The percentage of ^{31}P found in the femur seems lower in the rachitic group. This difference does not show itself when comparing the ^{32}P percentages. If we combine all the values found for the ^{32}P percentage in the femur (normal and rachitic) we obtain 5.2 ± 0.2 (standard error). As this figure might be influenced by the somewhat higher values found for 1855 R we generally take a value of 5.0%. This agrees nicely with the value of 4.8% calculated from data obtained by NORRIS AND KISIELESKI[6] with ^{45}Ca, using 250 gram Sprague Dawley rats.

Data obtained by NEUMAN *et al.*[7,8] show that, after an initial rapid uptake of ^{32}P, the isotope level of the femur increases only very slowly. The same was shown by DOLS *et al.*[4] to be true for the uptake in the total skeleton. Experiments in this laboratory proved that the initial rapid uptake is completed within the first 5–15 minutes after the injection of the tracer*. So we think it is reasonable to assume that the value of 5%, found for the ^{32}P content of the femur as compared to the total content of the skeleton one hour after injection of the radiophosphate, may be applied in the whole range of "short time experiments" from about 15 minutes on. This enables us to calculate the isotope content of the total skeleton in these experiments by simply multiplying the amount found in one femur by a factor 20.

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