phosphate supplementation and that instead of the continuous measurement of oxaloacetate production we have incubated the enzyme plus substrate for a finite period of 10 minutes.

The reaction was carried out in 10 ml centrifuge tubes at 37° C. Buffered homogenate (0.05 to 0.15 ml of a 1:10 in 0.01 *M* phosphate buffer, pH 7.4) was pre-incubated for 20 minutes and aspartate then added. After a further 10 minutes α-ketoglutarate was added and the enzyme reaction allowed to proceed for exactly 10 minutes. The reaction was stopped by the addition of 0.5 ml 30% (w/v) trichloracetic acid and the tubes cooled. After centrifugation the supernatant was decanted into tubes graduated at 10 ml and previously charged with 1.0 ml of *N* NaOH. 2.0 ml of 0.5 *M* borate buffer, pH 9.2, were added and the volume made up to 10 ml. The optical density of this solution was then measured at 280 mμ. Further tubes, at each enzyme level, containing all the reactants were submitted to the same procedure except that the trichloracetic acid was added immediately prior to the α-ketoglutaric acid, and these served as blanks. The oxaloacetate concentration was determined by reference to a standard curve constructed by measuring the optical density of different amounts of oxaloacetate treated in the same way.

Using the method described here the enzyme assay is linear over a range of 5–15 mg of tissue (fresh weight). The sensitivity of the estimation may be seen from Fig. 2 in which the optical density of oxaloacetic acid solutions treated as above is expressed as a function of the concentration. For comparison, a similar curve, calculated from the data of NISONOFF, HENRY AND BARNES[2] is included.

Errors due to the decarboxylation, both spontaneous and catalysed, of the oxaloacetic acid produced enzymically are negligible, since we have calculated from the data of NISONOFF, HENRY AND BARNES[2] that this could not exceed 2.5% of the total oxaloacetate produced.

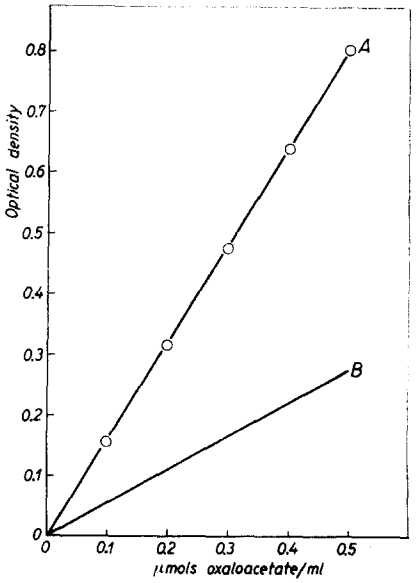

Fig. 2. Relation between oxaloacetic acid concentration and optical density. Curve A represents the relationship obtained using the method described above. Curve B is a similar curve calculated from the data of NISONOFF, HENRY AND BARNES[2].

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DISSIMILATION OF DL-ALANINE-1-^{14}C BY RAT BRAIN HOMOGENATES*

by

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We would like to report data which indicate that the brain can dissimilate amino acids other than glutamic acid to an appreciable extent. Adult rats of Spray Doli Strain weighing around 150–200 grams were used. After sacrificing these animals by decapitation, the entire brain (1.5 g) was im-

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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.