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SHORT COMMUNICATIONS

A sensitive and specific isotopic assay for the estimation of tyrosine transaminase

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WHEN RATS are kept under standard lighting conditions and are given access *ad libitum* to a standard laboratory diet (Purina chow), the activity of tyrosine transaminase (1-tyrosine, 2-oxoglutarate amino transferase, EC 2.6.1.5) in rat liver shows marked diurnal variations.[1-3] This biological rhythm is independent of adrenocortical function[1,2] and appears to be generated by an amino acid constituent of the diet, possibly tryptophan.[4] When the animal eats, the amino acid is preferentially delivered to the liver via the portal vein; it can then act as a signal to initiate the rise in enzyme activity..

It became of interest to determine whether tyrosine transaminase (TT) activity also varies diurnally in organs which are not perfused with portal venous blood, such as the brain and the adrenals. Low levels of enzyme activity could be demonstrated in these and other nonhepatic organs by using the standard spectrophotimetric assay procedures.[5,6] However these methods were not entirely satisfactory for several reasons. They lacked chemical specificity, since any reaction product that absorbed light energy at 310 mμ (ref. 5) or 331 mμ (ref. 6) might be taken as an index of TT activity. Moreover, measurements of enzyme activity were often only slightly higher than blank readings and were variable because of the presence of nonspecific light-absorbing compounds in the large amounts of the enzyme preparations used. Hence, we have developed an isotopic method for estimating TT activity. This assay is sensitive, simple and chemically specific; it can be used to study the enzyme in any tissue. Sixty or more determinations can be performed by a single technician in 3 hr, each using as little as 250 μg liver.

The assay is based upon the change in the polarity of tyrosine which follows the action of TT. The enzyme causes the amino acid to lose its only amine group and thereby to form *p*-hydroxyphenyl puruvic acid (PHPPA). This compound is largely un-ionized at an acid pH and can thus be quantitatively separated from the substrate by extraction into an organic solvent.

Tissues are routinely homogenized in 9 vol. of chilled isotonic potassium chloride, and 10 μl (1 mg) of the 100,000 *g* supernatant fluid is used for the TT assay. The remainder of the incubation mixture is prepared as follows: 500 μl 1-tyrosine (7×10^{-3}M in pH 7·6 phosphate buffer, 10^{-1}M), 10 μl pyridoxal phosphate (10^{-2} M in water), 20 μl alpha-keto glutaric acid ($4{\cdot}5 \times 10^{-2}$ M in water) and 1 μl L-3,5-^{3}H-ditritiotyrosine (0·1 μc; New England Nuclear Corp., 5 c/m-mole) per assay tube are combined; 0·5-ml portions of the mixture are then pipetted into 15-ml glass-stoppered centrifuge tubes. The enzyme preparation is added, and the mixture is incubated at 37° for 20 min. The reaction is stopped by the addition of 0·5 ml of 2 N hydrochloric acid, and the deaminated radioactive material is extracted into 6 ml of a mixture of toluene and isoamyl alcohol (9:1). The organic phase is washed with 1 ml of 1 N HCl; a 4-ml portion is transferred to a vial and counted in a liquid scintillation spectrophotometer after the addition of 2 ml ethanol and 10 ml phosphor [0·4% 2,5-diphenyloxazole and 0·005% 1,4-di (2,5-phenyloxazole) benzene in toluene]. About 80 per cent of the product (^{3}H-PHPPA) is recovered by this procedure. Less than 1 per cent of the ^{3}H-tyrosine remains in the organic solvent. A correction is made for this amount by incubating the assay mixture with denatured enzyme or by running zero-time blanks. The reaction is linear with time for at least 20 min and with enzyme concentration over a range of 0·1 to 10·0 mg liver. Duplicate determinations of the TT activity in 1 mg liver generally differ by less than 2 per cent.

The radioactive product obtained when ^{3}H-tyrosine was incubated with rat liver as described above was identified as ^{3}H-PHPPA by ascending paper chromatography. ^{3}H-PHPPA was separated from ^{3}H-tyrosine by a butanol:acetic acid:water (8:2:2) system; the R_f of the PHPPA was 0·81, while that of the amino acid was 0·35. Essentially all of the radioactivity present in the reaction mixture could be accounted for as either tyrosine or PHPPA.

The concentrations of the reactants are critical in assays for TT activity. The enzyme has a high K_m for tyrosine (estimated at $1{\cdot}19 > 10^{-3}$ M). Since this concentration is almost as great as the amount of tyrosine which can be dissolved in water at 37°, it is not possible to assay the enzyme at maximum velocity; small changes in the tyrosine concentration of the medium (i.e. during the course of the reaction) can produce large changes in apparent enzyme activity. In our procedure, sufficient tyrosine to make a 7×10^{-3} M solution is dissolved in water by heating. The resulting solution is kept warm until it is used. A small amount of the tyrosine crystallizes out of the medium during the course of the reaction; this use of a supersaturated solution ensures that the concentration of substrate remains relatively stable. Radioactive tyrosine of any specific activity can be used with this assay, since the final specific activity will depend solely upon the unlabeled material, which is supersaturated.

High concentrations of alpha-keto glutaric acid (i.e. 5×10^{-2} M) markedly inhibit TT. When this cofactor is omitted from the incubation medium, the activity of the unpurified enzyme in hepatic supernatant fluid is only 15 per cent that obtained when the complete assay mixture is used. The addition of pyridoxal phosphate in concentrations ranging from 10^{-4} M to 10^{-1} M stimulates the reaction about 2-fold. TT activity is not enhanced by adding diethyldithiocarbamate to the medium. A more complete separation of tyrosine and PHPPA can be obtained by extracting the radioactive product into pure toluene or into chloroform; however, the net recovery of PHPPA is appreciably lower under these conditions.

TABLE 1. TISSUE DISTRIBUTION OF TYROSINE TRANSAMINASE ACTIVITY IN THE RAT*

Tissue	Spectrophotometric method	Isotopic method
	% Liver activity	
Liver	100	100
Heart	31	22
Adrenal	12	5
Kidney	8	8
Brain	3	1

* The spectrophotometric assay used was the method of Diamondstone.[6] The hepatic TT activity was estimated to be 66 ± 4 μmole/g/hr by the spectrophotometric method and $71 \pm 7\mu$ mole/g/hr by the isotopic method. The livers were taken from 6 rats at 10:00 a.m.

The tissue distribution of tyrosine transaminase was studied by spectrophotometric and isotopic methods. The results, illustrated in Table 1, indicate that the spectrophotometric method tends to overestimate TT activity in heart and adrenal.

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Department of Nutrition and Food Science,
Massachusetts Institute of Technology,
Cambridge, Mass., U.S.A.

RICHARD J. WURTMAN
FRANCES LARIN

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