Soluble and Mitochondrial Forms of Tyrosine Aminotransferase. Relationship to Human Tyrosinemia*

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ABSTRACT: The paradoxical finding of tyrosinemia and increased urinary *p*-hydroxyphenylpyruvate in a patient whose liver biopsy contained normal *p*-hydroxyphenylpyruvate hydroxylase and virtual absence of soluble tyrosine aminotransferase led to studies of the latter enzyme.

Two new methods for the assay of tyrosine aminotransferase are described together with the results of studies of tissue and subcellular distribution of this enzyme in human and rat tissues. Only a mitochondrial form of tyrosine aminotransferase was present in the patient's liver. Both soluble and mitochondrial forms were found in normal liver, heart, skeletal muscle, and brain. Neither form was demonstrable in skin and erythrocytes and only small amounts were demonstrable in leukocytes. Hydrocortisone induced increased amounts of enzyme in both mitochondrial and soluble fractions of rat liver. The lack of mitochondrial *p*-hydroxyphenylpyruvate and the presence of glutamic dehydrogenase was shown to influence the ratio of tyrosine to glutamate produced by mitochondrial tyrosine aminotransferase. The implications of these findings to the normal metabolism of tyrosine and to tyrosine metabolism in this patient are discussed.

Lin *et al.* (1958) described the occurrence of tyrosine aminotransferase[1,2] in the supernatant of rat liver homogenates after centrifugation at 13,000–14,000*g*. This form of the mammalian enzyme has been studied extensively and has been shown to be under diurnal (Wurtman and Axelrod, 1967) and hormonal control (Lin and Knox, 1958; Kenny, 1962; Litwack *et al.*, 1963; Holten and Kenny, 1967; Gelehrter and Tompkins, 1967; Peterkofsky and Tompkins, 1967). In a pioneering study of this enzyme, appreciable amounts of activity were reported to be present in the "precipitate" fraction, but the authors did not identify the organelle with which this activity was associated (Canellakis and Cohen, 1956). More recently a bound form of the enzyme has been reported in the nuclear fraction (Litwack *et al.*, 1963) and in the mitochondrial fraction (Rowsell *et al.*, 1963) of rat liver homogenates.

A decreased activity of tyrosine aminotransferase and of *p*-hydroxyphenylpyruvate hydroxylase was found by Kretchmer *et al.* (1956, 1957) in liver samples from premature as compared with full-term infants, and may account in part for the transitory neonatal elevation of tyrosine levels observed in a significant proportion of premature infants (Levine *et al.*, 1941; Shear *et al.*, 1967). Persistent tyrosinemia and tyrosyluria have been described in patients who manifest a common set of clinical and biochemical aberrations, and in whom genetic studies are consistent with an autosomal recessive pattern of inheritance of a defect in *p*-hydroxyphenylpyruvate hydroxylase (Shear *et al.*, 1967; Gentz *et al.*, 1965; Scriver *et al.*, 1967; La Du, 1966). Medes (1932) described the first patient with tyrosinuria and interpreted her findings as consistent with a defect in the oxidation of *p*-hydroxyphenylpyruvate, *i.e.*, *p*-hydroxyphenylpyruvate hydroxylase activity. This interpretation has been questioned by La Du (1966, 1967); however, it is impossible to verify the site of the metabolic defect since enzyme assays were not then available.

In pursuing an investigation of a 2-year-old patient with markedly elevated tyrosine blood levels and an increase in urinary *p*-hydroxyphenylpyruvate and *p*-hydroxyphenyllactate (Campbell *et al.*, 1967; R. A. Campbell, N. R. M. Buist, and E. Jacinto, in preparation), we studied the appropriate enzymes of the tyrosine-oxidizing system in a liver biopsy. The clinical and

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[1] The following abbreviations and trivial names are used: tyrosine aminotransferase, L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5); glutamate dehydrogenase, L-glutamate:NAD(P) oxidoreductase (EC 1.4.1.3); hydrocortisone, hydrocortisone sodium succinate (Solu-Cortef).

[2] The following reagents were obtained from commercial sources: L-tyrosine, L-phenylalanine, pyridoxal 5′-phosphate, α-ketoglutaric acid (monopotassium salt), *p*-hydroxyphenylpyruvate, keto–enol tatuomerase (porcine kidney type II) were supplied by Sigma Chemical Co.; diethyldithiocarbamic acid (diethylammonium salt) from K & K Laboratories; 4-chloro-*o*-phenylenediamine from Eastman Organic Chemicals; hydrazine, mercaptoethanol, and activated charcoal (Nuchar C-190N) from Matheson Coleman and Bell; hydrocortisone sodium succinate from Upjohn; L-tyrosine (side chain-2,3-^{3}H) batch 1, 3570 mCi/mM, from Nuclear-Chicago; Celite Filter Cel from John Manville; 2,5-diphenyloxazole and 1,4-[bis-2-(5-phenyloxazolyl)]-benzene from Packard Instrument Co.; Special Clinical Chemistry Control Serum, Hyland; and ninhydrin from Pierce Chemical Co.

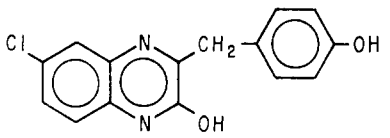


FIGURE 1: Proposed structure of the chromophoric quinoxyline derivative formed in the reaction of 4-chloro-*o*-phenylenediamine with *p*-hydroxyphenylpyruvate.

biochemical findings in this patient differ from those described in the genetically determined defect in *p*-hydroxyphenylpyruvate hydroxylase and from the patient described by Medes. A seemingly paradoxically normal amount of *p*-hydroxyphenylpyruvate hydroxylase and virtual absence of soluble tyrosine aminotransferase activity were found at the time of liver biopsy. A bound form of tyrosine aminotransferase was demonstrated in the insoluble fraction from the liver biopsy of this patient.

This observation led us to investigate the tissue and subcellular distribution of tyrosine aminotransferase in human and rat tissue homogenates. For these studies two new methods of assay were developed and are described: a colorimetric method and a radiochemical procedure which depends upon release of tritium. A possible rationale to account for the findings in this patient is presented.

Experimental Procedures and Results

Preparation of Tissue Homogenates. Male, Sprague–Dawley rats weighing 150–300 g were used in all animal studies reported. Homogenates of tissues from decapitated animals or from biopsies of human tissues were prepared using a Potter-Elvehjem homogenizer in a medium of either 0.25 M sucrose or 0.14 M KCl solutions containing 0.001 M EDTA. Early studies demonstrated that in addition to a soluble form, tyrosine aminotransferase activity was also present in the 20,000*g* precipitate and could not be eluted by repeated washing with the homogenizing medium. Subcellular fractionation according to the procedure of Hogeboom (1955) was used in all subsequent studies to separate supernatant, mitochondrial, microsomal, and nuclear fractions. These were assayed for succinoxidase (King, 1965) and lactic dehydrogenase (White, 1956) and examined by electron microscopy to establish the relative purity. Mitochondrial fractions were washed in six to ten volumes of 0.14 M KCl to remove adsorbed proteins. Following fractionation, samples were dialyzed against 0.2 M phosphate buffer (pH 7.4) containing 0.001 M mercaptoethanol and 0.001 M EDTA as described by Kenny (1962).

Protein estimation was done by alkaline hydrolysis modified after Crestfield *et al.* (1963), followed by ninhydrin color development (Moore and Stein, 1954) as adapted to the autoanalyzer (Johnson *et al.*, 1966) and compared with a protein standard (Hyland) which was carried through the same procedure. A set of leucine standards was also run to check the reagents.

Tyrosine Aminotransferase Assay. A. THE ENOL BORATE TAUTOMERASE METHOD of Lin *et al.* (1958) was employed in preliminary studies on supernatants prepared from fresh biopsies (liver) from controls and from the patient with tyrosinemia.

B. COLORIMETRIC. A procedure based upon the reaction of 4-chloro-*o*-phenylenediamine with keto acids to produce a chromophoric quinoxyline derivative (Fellman *et al.*, 1962) was adapted to the assay of tyrosine aminotransferase. Crude 4-chloro-*o*-phenylenediamine was purified by crystallization; 10 g dissolved in a minimum volume of 1 N HCl was treated with 8 g of activated charcoal and filtered. The slightly pink filtrate was poured into 200 ml of concentrated HCl and the white precipitate of 4-chloro-*o*-phenylenediamine was immediately filtered on a sintered-glass funnel and dried over P_2O_5 *in vacuo*. This recrystallized material has been stable for 6 years. Solutions of 50 mg of 4-chloro-*o*-phenylenediamine in 50 ml of 85% phosphoric acid were prepared fresh each day.

Advantage was taken of the fact that the amino group acceptor, α-ketoglutaric acid, combines with hydrazine to form a stable pyridazine which does not react whereas the product, *p*-hydroxyphenylpyruvate, does react with 4-chloro-*o*-phenylenediamine. Stoichiometric amounts of *p*-hydroxyphenylpyruvate and 4-chloro-*o*-phenylenediamine were reacted and the product exhibited an infrared spectrum consistent with 2-hydroxy-3-(*p*-hydroxyphenyl)-6-chloroquinoxyline (Figure 1): ν_{CH} 2940, 2850 cm^{-1}, $\nu_{Quin\ C=N}$ 1602 cm^{-1}, ν_{Cl} 820 cm^{-1}. The ultraviolet absorption spectrum shows a peak at 382 mμ (ϵ 4860).

Enzyme assays were carried out as follows: a standard 3.5-ml reaction mixture contained 5.1 mM L-tyrosine, 4.3 mM α-ketoglutarate, 0.034 mM pyridoxal 5′-phosphate, 3.41 mM *N,N′*-diethyldithiocarbamate, 200 mM potassium phosphate buffer (pH 7.4) which was used to dissolve all reagents except *N,N′*-diethyldithiocarbamate, and 0.4 ml of sample to be tested for enzyme activity. The dialyzed tissue samples were preincubated for 5 min with pyridoxal 5′-phosphate at 37°. L-Tyrosine and *N,N′*-diethyldithiocarbamate were added and the reaction was started by the addition of α-ketoglutarate. Controls consisting of boiled enzyme samples and the identical reaction mixtures were run in parallel. After 30-min incubation at 37°, 1 ml of 10% sulfosalicylic acid was added to both reaction and control tubes. All tubes were then centrifuged, 0.5 ml of supernatant was transferred to a 2 × 15 cm tube, and 0.5 ml of 2 M hydrazine was added. After 15 min, 3 ml of 4-chloro-*o*-phenylenediamine solution was added to each tube and mixed well. The tubes were placed in a boiling-water bath, shaken several times, and cooled quickly after 10 min. Optical densities were read at 380 mμ against a 4-chloro-*o*-phenylenediamine reagent blank which had been carried through the same procedure. The amount of *p*-hydroxyphenylpyruvate formed was estimated from the difference between optical densities of the boiled enzyme controls and the reaction tubes and was calculated directly from a standard curve (Figure 2). Enzyme activity, expressed as mmoles of *p*-hydroxyphenylpyruvate produced per 30 min, was related to the protein content of the sample to obtain specific activities.

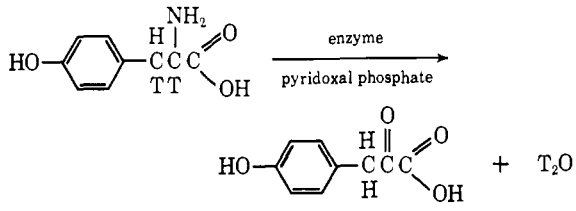
TABLE I: Tissue Distribution of Soluble and Mitochondrial Tyrosine Aminotransferase Activity.[a]

Cell Fraction	n	Tissue				
		Liver	Heart	Muscle	Kidney	Brain
Rat	3					
Soluble		0.029	0.028	0.007	0.017	0.007
Mitochondrial		0.042	0.025	0.040	0.037	0.027
Human	2					
Soluble		0.036	0.009	0.002	0.002	0.003
Mitochondrial		0.028	0.027	0.002	0.011	0.014

[a] Activity is expressed as mmoles of *p*-hydroxyphenylpyruvate produced in 30 min per mg of protein assayed by the radiochemical technique.

These were expressed as mmoles of *p*-hydroxyphenylpyruvate produced in 30 min per mg of protein.

C. RADIOCHEMICAL ASSAY. This assay is based upon the release of tritium from the α- and β-carbons of L-tyrosine-2,3-^{3}H when tyrosine is converted into *p*-phenylpyruvate. The tritium at the α position is directly released; that at the β position exchanges as a result of an α-carbonyl activation. Because pyridoxal 5'-phosphate



can catalyze nonenzymatic activation and thus carry on "model" reactions with α-amino acids (Snell, 1955), one necessarily encounters a consistent nonenzymatic tritium release which is treated as a reagent blank.

Enzyme assays were carried out under conditions identical with those described for the colorimetric procedure, except that 1.2 μCi/ml of L-tyrosine-2,3-^{3}H was added to the reaction mixture. Supernatants from reaction mixtures and from boiled enzyme controls were poured over charcoal–Celite columns[3] which were placed in 1.5 × 10 cm polyethylene tubes and centrifuged at 1000 rpm for 5 min to obtain a clear filtrate of T_2O, free of tyrosine. Evidence that the filtrate contained no tyrosine was obtained by drying such filtrates in a rotary evaporator following which no detectable radioactive residue was found. Assay for tritium was carried out by placing 0.5 ml of the filtrate and 19 ml of a solution containing 2 g of 2,5-diphenyloxazole and 50 mg of 1,4-[bis-2-(5-phenyloxazolyl)]benzene in 250 ml of absolute ethanol plus 250 ml of toluene (Buhler, 1962) in a scintillation vial. The samples were counted with a Beckman CPM 100 liquid scintillation counter.

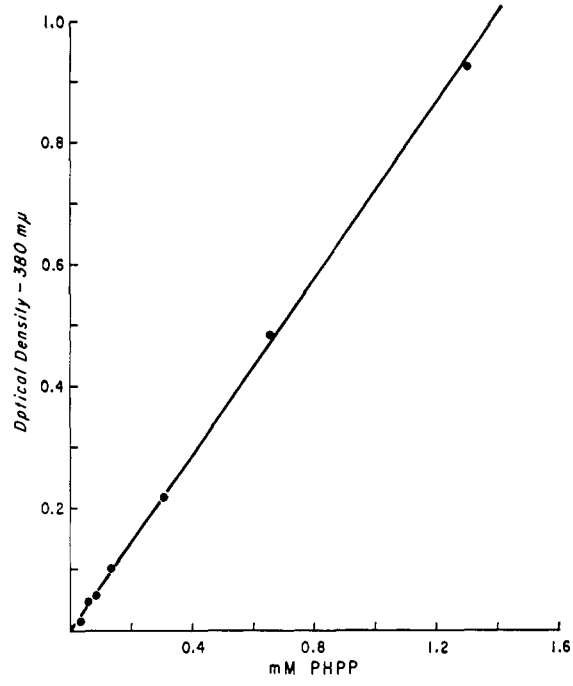


FIGURE 2: Standard curve for colorimetric procedure. Absorbance at 380 mμ (ordinate) of product formed by reacting known amounts of *p*-hydroxylphenylpyruvate (abscissa) with 4-chloro-*o*-phenylenediamine.

Results of the colorimetric assay are presented in Figures 2–4. They demonstrated that the chromophoric quinoxyline product formed by reaction of 4-chloro-*o*-phenylenediamine with *p*-hydroxyphenylpyruvate obeys Beer's law through a useful optical density range and can be used to calculate the amount of product formed. Data from the progress curve (Figure 3) indicate that the appearance of product falls off gradually after 30 min requiring that rates be based on incubation times of 30 min or less. A linear relationship of amount of enzyme to appearance of product is shown in Figure 4. Similar results were obtained with the radiochemical assay and are presented in Figures 5 and 6.

[3] Charcoal–Celite columns were made by layering 100 mg of charcoal followed by 500 mg of a 50:10 Celite–charcoal mixture in a 0.5 × 12 cm glass column fitted at the bottom with a sintered glass disk. The columns were packed dry by centrifugating at 1000 rpm for 5 min.

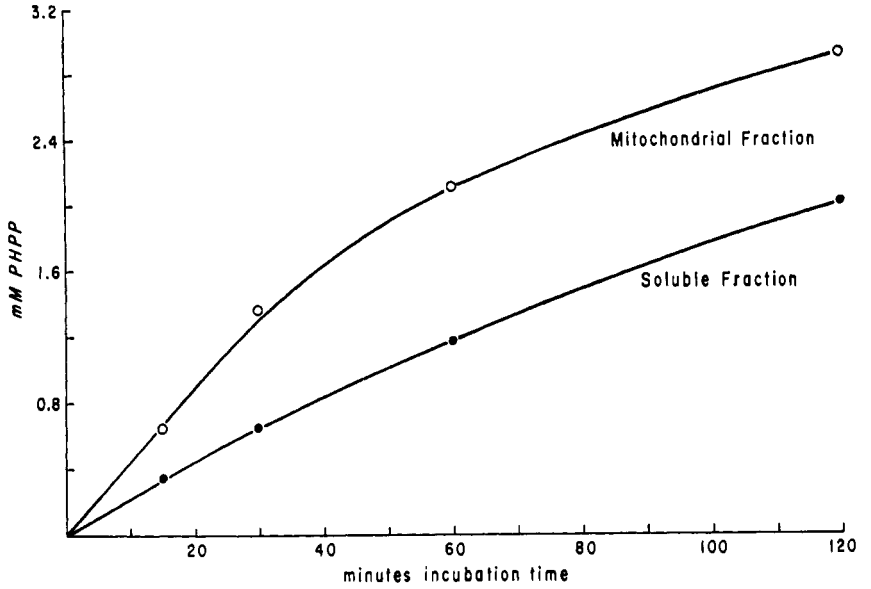


FIGURE 3: Progress curve for colorimetric procedure. Increase in product expressed as millimoles of *p*-hydroxyphenylpyruvate (ordinate) with time in minutes (abscissa) per milliliter of tissue fraction.

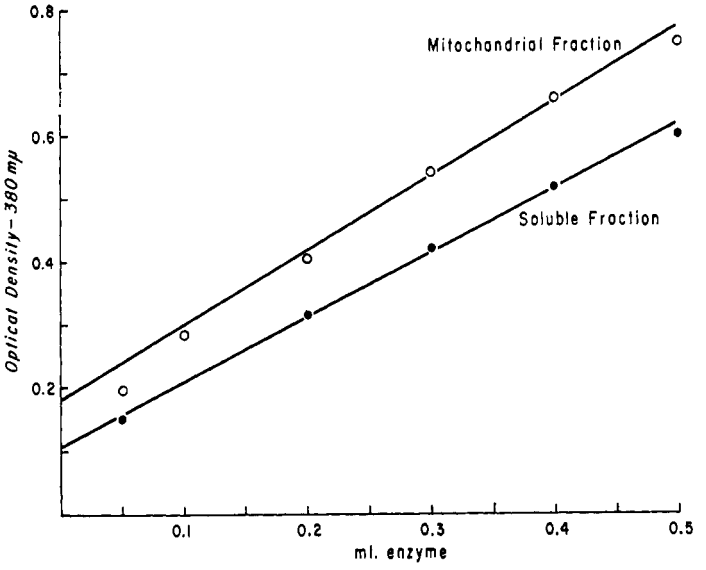


FIGURE 4: Linear relationship between amount of enzyme and amount of product for colorimetric procedure. Increase in *p*-hydroxyphenylpyruvate as measured by absorbance at 380 mμ (ordinate) is related to volume of tissue fraction from rat liver (abscissa).

Phenylalanine has been shown to serve as substrate for the soluble form of tyrosine aminotransferase (Lin *et al.*, 1958). This was confirmed for both soluble and mitochondrial forms of rat and human liver tyrosine aminotransferase with the colorimetric method.

The substrate and cofactor requirement of the tissue fractions made it clear that no detectable L-amino acid oxidases were operating in the conversion of tyrosine into *p*-hydroxyphenylpyruvate. Thus, the absolute requirement for α-ketoglutaric acid, the stimulation of activity by small amounts of pyrodoxal 5'-phosphate, the fact that the reaction proceeded as well under anaerobic conditions, and the formation of glutamate support the conclusion that the assay specifically determined the tyrosine aminotransferase activity in the tissue fractions examined. It is unlikely that other tyrosine aminotransferases, such as the pyruvate transaminase studied by Rowsell *et al.* (1963), were being measured, since all reported assays were done on dialyzed samples to which only tyrosine and α-ketoglutarate were added as substrate.

Tissue Distribution. The specific activity of soluble and mitochondrial tyrosine aminotransferase as determined by the radiochemical method in tissues from three rats and from two humans are presented in Table I. Human leukocyte soluble and mitochondrial fractions have small but detectable amounts.

Introduction by Hydrocortisone. The observation that soluble tyrosine aminotransferase increased rapidly after hydrocortisone injection (Lin and Knox, 1958) led us to investigate the effect of this hormone on both soluble and mitochondrial forms of the enzyme. Male, Sprague–Dawley rats weighing 180–230 g were fed standard Purina laboratory chow and maintained under controlled

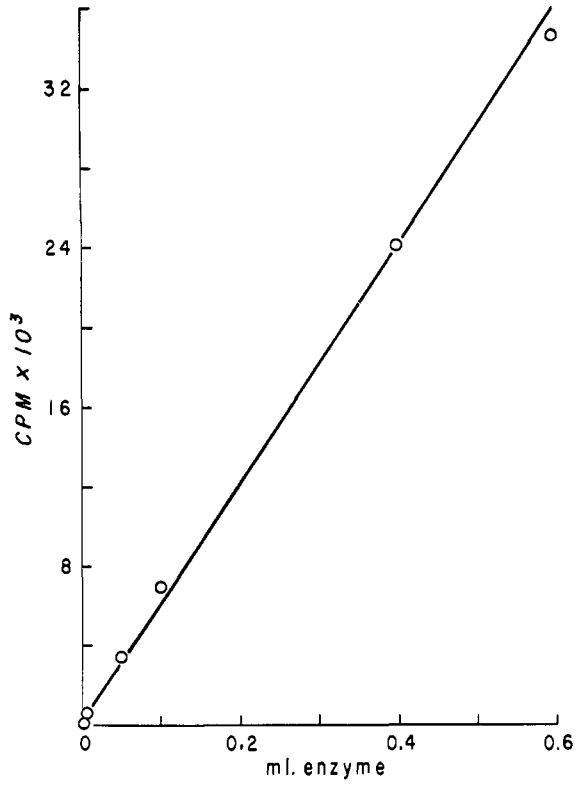


FIGURE 5: Standard curve for radiochemical procedure. Velocity expressed as counts per minute of tritium released per 30 min against varying amounts of mitochondrial suspensions. The enzyme solution contained 28 mg/ml of protein.

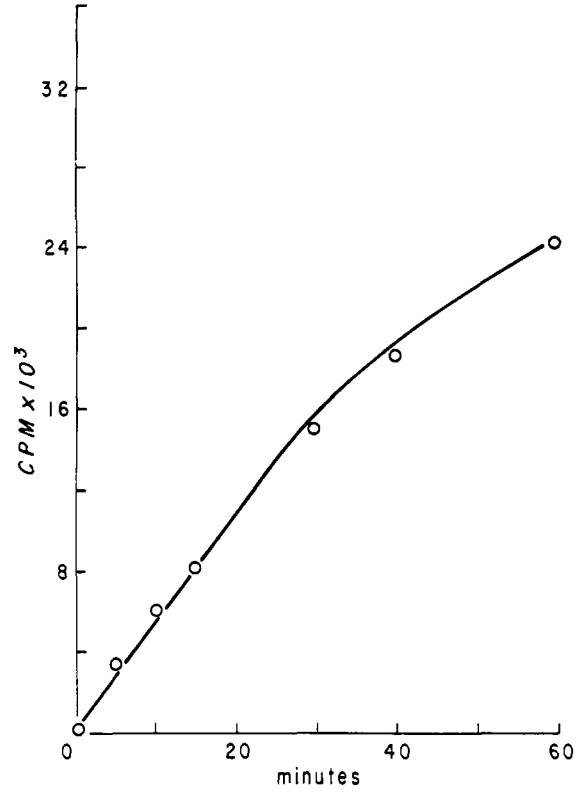


FIGURE 6: Progress curve for radiochemical procedure. Linear relationship between tritium release and tyrosine aminotransferase activity in rat liver mitochondrial fraction. Activity is expressed as cpm × 10^3/sample incubated up to 1 hr in the standard assay procedure using the radiochemical technique described in the text. Washed mitochondrial suspensions contained 28 mg of protein/ml.

lighting conditions (14-hr darkness from 5 PM to 8 AM). Two experiments were done. In the first, three rats were given 30 mg/kg of hydrocortisone intraperitoneally and sacrificed 5 hr later. In the second, six rats were given 1 g/kg of hydrocortisone following a 12-hr fast and were sacrificed 8 hr later after continued fasting. Control animals in both experiments differed from the treated group only in that they received saline instead of hydrocortisone. Soluble and mitochondrial fractions were prepared from the liver and assayed by the colorimetric method in the first study. The same fractions were prepared, assayed by both the colorimetric and radiochemical method and for lactic dehydrogenase in the second study. Two of the treated animals in the second study died before 8 hr. The results of both studies (Table II) show a fourfold increase of soluble and a twofold increase of mitochondrial tyrosine aminotransferase specific activity in liver fractions from the treated group. The specific activities of lactic dehydrogenase, which is restricted to the soluble fraction (Dixon and Webb, 1964), indicate less than 1% contamination of mitochondrial fractions with soluble protein. Regression analysis of the tyrosine aminotransferase results for the two methods in the second study show a correlation of 0.98 with a value of $b = 1.29$. The lower results with the colorimetric method have been observed consistently, and are believe to reflect instability of *p*-hydroxyphenylpyruvate.

Studies of Liver Biopsy from Patient with Tyrosinemia. We found normal levels of *p*-hydroxyphenylpyruvate hydroxylase in the liver biopsy from the patient reported by Campbell *et al.* (1967; R. A. Campbell, N. R. M. Buist, and E. Jacinto, in preparation). Tyrosine aminotransferase as assayed by the enol borate–tautomerase method of Lin *et al.* (1958) and by the two methods described in this paper all showed negligible activity in the soluble fraction, but normal mitochondrial tyrosine aminotransferase activity (Figure 7).

Glutamate Production by Tyrosine Aminotransferase. The possibility that glutamate is cycled back to α-ketoglutarate by mitochondrial enzymes was tested. One such enzyme, glutamate dehydrogenase, was shown using the method of Strecker (1955) to be active in mitochondrial preparations but not in the soluble fraction. The disappearance of tyrosine and recovery of glutamate in assay mixtures containing dialyzed soluble and mitochondrial fractions from rat liver is presented in Table III. The reactions were stopped by boiling and the supernatants were diluted tenfold with 0.2 M acetate buffer (pH 2.2). The quantitative analysis of the amino acids were carried out using a Spinco 120 analyzer (Spinco-Beckman, Palo Alto, Calif.). The procedure of Moore *et al.* (1958) was used with modifications as described in the June 1965, instruction manual, A-1M-3, for the Model 120-C amino acid analyzer.

Supernatant fractions exhibited a ratio of tyrosine lost to glutamate formed of unity, in the presence or ab-

TABLE II: Induction of Liver Tyrosine Aminotransferase by Hydrocortisone.[a]

		Colorimetric		Radiochemical	
	n	Soluble	Mitochondrial	Soluble	Mitochondrial
Control	3	0.038 ± 0.007	0.029 ± 0.004		
Hydrocortisone	3	0.153 ± 0.062	0.064 ± 0.004		
Control	6	0.032 ± 0.008	0.037 ± 0.005	0.046 ± 0.015	0.041 ± 0.008
Hydrocoristone	4	0.151 ± 0.028	0.076 ± 0.011	0.191 ± 0.057	0.088 ± 0.011
Lactic dehydrogenase[b]	10	4.80 ± 0.60	0.003 ± 0.010		

[a] Liver tyrosine aminotransferase activity is expresses as mean specific activity, plus and minus standard deviation, mmoles of *p*-hydroxyphenylpyruvate produced in 30 min per mg of protein, as measured by the 4-chloro-*o*-phenylenediamine colorimetric and radiochemical techniques. [b] Liver lactic dehydrogenase activity assayed to demonstrate relative purity of fractions is expressed as the mean specific activity, International Enzyme Units per milligram.

TABLE III: Recovery of Glutamate from Soluble and Mitochondrial Tyrosine Aminotransferase System.

		Assay Mixture		Recovery		Tyrosine Lost
Enzyme Source	Incubation Time (min)	α-Ketoglutarate[a]	Tyr[a]	Glu[a]	Tyr[a]	Glu Recovered
Supernatant	30		5.0		4.94	
	30	4.3	5.0	0.60	4.23	1.1
	60	4.3	5.0	0.92	4.10	0.9
	60[b]	4.3	5.0	0.93	3.78	1.2
Mitochondrial	30		5.0		4.98	
	30	4.3	5.0	0.24	4.68	1.3
	60	4.3	5.0	0.28	3.15	6.4
	60[b]	4.3	5.0	0.40	4.43	1.2

[a] Micromoles per milliliter. [b] Without NADP.

sence of NADP. However, in 60 min the mitochondrial enzyme system consumed greater than six times more tyrosine than glutamate recovered. When the NADP was withheld from this system the ratio observed was unity.

Discussion

Reported methods for assaying tyrosine aminotransferase include the enol borate tautomerase method of Lin *et al.* (1958) and the benzaldehyde method of Diamondstone (1966). Both of these have the minor disadvantage of a high reagent blank and the first depends upon a ready source of pure tautomerase. The two methods described here are at least as sensitive. The colorimetric method can be used for measuring other reactions which produce α-keto acids; the results showing transamination of phenylalanine as an alternate substrate represent one such example.

The radiochemical method, in principle, should provide a very sensitive method for assaying any enzyme reaction which involves tritium exchange at the α-carbon position. In addition to tyrosine aminotransferase, other transamination reactions and L-amino acid oxidase, amino oxidase, β-dehydrase, and desulfhydrase reactions are prime candidates for this kind of assay and require only suitably labeled substrates and properly selected adsorbants. Dowex 50-X8 (200–400 mesh) in the sodium form has been found to substitute for the charcoal–Celite columns in adsorbing a number of possible substrates. Deuterium exchange at the α-carbon of tyrosine in the presence of pyridoxal, which in principle presages this approach, was first described by Konikova *et al.* (1947).

We have not had opportunity to compare the two methods described here with the radiochemical methods using ^{14}C-labeled substrates recently reported by Weinstein *et al.* (1967) and by Gabay and George (1967).

The initial stimulus for developing these methods and for the studies reported is the apparently unique error in tyrosine metabolism in the patient reported by Campbell *et al.* (1967; R. A. Campbell, N. R. M. Buist, and E. Jacinto, in preparation). The hepatic cell defect

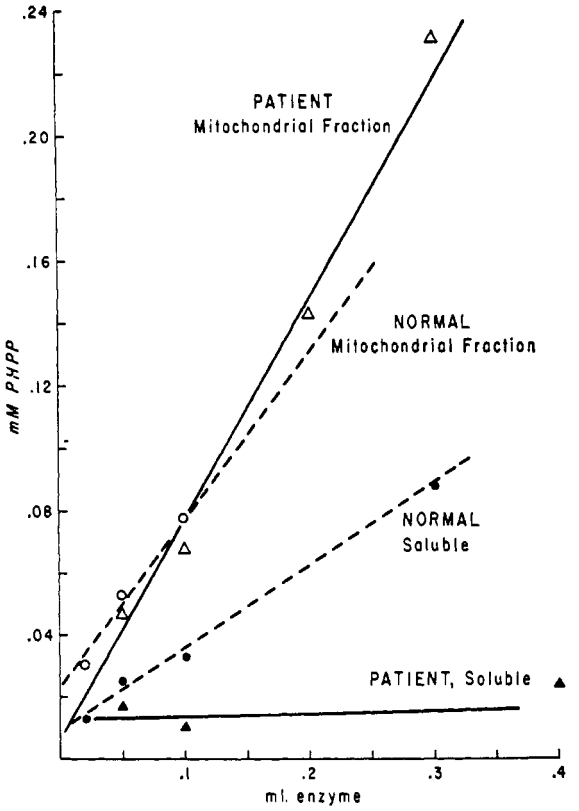


FIGURE 7: Comparison of tyrosine aminotransferase activity determined by radiochemical assay of liver fractions from patient (△—△) and from normal control (○——○). Volumes of soluble and mitochondrial fractions, designated by solid and open symbols, respectively, were varied between 0.025 and 0.4 ml, and results are expressed as mmoles of *p*-hydroxyphenylpyruvate formed in 30 min determined by the radiochemical technique.

in soluble tyrosine aminotransferase would be expected to block the metabolism of tyrosine to *p*-hydroxyphenylpyruvate in the soluble multienzyme system (Knox and LeMay-Knox, 1951) which normally converts tyrosine into CO_2 and water. Blood and urine levels of tyrosine should increase. The observation (N. R. M. Buist, 1968, personal communication) that the patient excretes large amounts of *p*-hydroxyphenylpyruvate in his urine (150–350 mg/24 hr) indicates that this step is not completely blocked. The question then becomes one of why intact soluble *p*-hydroxyphenylpyruvate hydroxylase fails to establish normal steady-state levels of *p*-hydroxyphenylpyruvate?

Although there are only minor differences between the specific activities of the two forms of tyrosine aminotransferase, the greater amount of protein in the cytosol would account for the major fraction of total activity. In the absence of soluble enzyme activity in the patient, the mitochondrial enzyme is expected to operate at an increased rate for several reasons. Firstly, it is exposed to higher concentrations of tyrosine. Of perhaps greater influence is the mitochondrial capacity to regenerate α-ketoglutarate from glutamic acid by oxidation or *via* the tricarboxylic acid cycle. This effect is reflected in an increase in the ratio of net tyrosine lost to net glutamate formed (Table III).

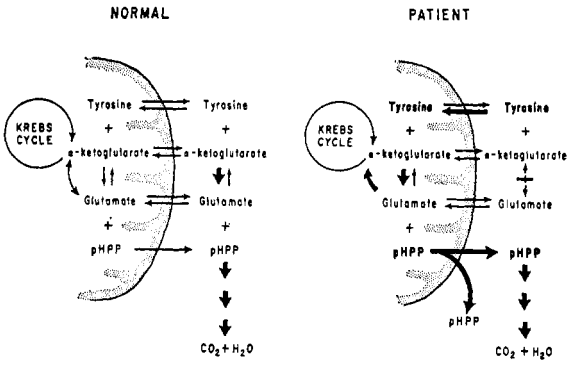


FIGURE 8: Schematic diagram of mitochondrial and soluble tyrosine oxidizing system in normal cells and of changes in this system from absence of soluble tyrosine aminotransferase in the patient.

Mitochondria do not contain *p*-hydroxyphenylpyruvate hydroxylase. Williams and Sreenivasan (1953) using manometric methods to measure the complete tyrosine oxidizing system found activity only in the 25,000*g* supernatant and none in the precipitate. In support of this indirect evidence, we assayed rat liver mitochondria and found no *p*-hydroxyphenylpyruvate hydroxylase activity (J. H. Fellman and P. Vanbellinghen, 1968, unpublished observations). It may therefore be presumed that the intact soluble tyrosine oxidizing system is required for catabolizing the *p*-hydroxyphenylpyruvate produced in the mitochondria under normal steady-state conditions (Figure 8). The increased urinary excretion of *p*-hydroxyphenylpyruvate associated with defective soluble tyrosine aminotransferase activity may result from a relative failure of soluble *p*-hydroxyphenylpyruvate hydroxylase to deal with the new steady-state concentration of *p*-hydroxyphenylpyruvate or to spilling of some of this metabolite from renal tubular cells before it can be handled by the oxidizing system in the cytosol.

Acknowledgments

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