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Decarboxylation to Tyramine: A Major Route of Tyrosine Metabolism in Mammals

(tyrosine aminotransferase pathway/decarboxylase inhibitor)

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ABSTRACT **Metabolism of tyrosine was examined in mice, some of which had been treated with an inhibitor of aromatic-L-amino-acid decarboxylase. The results of the study indicate that as the plasma and tissue levels of tyrosine are elevated, decarboxylation to tyramine becomes the predominant route of metabolism. At the highest dose of tyrosine used (1.5 g/kg), it was found that 42% of the administered dose was decarboxylated within 6 hr and only 11.5% was metabolized by the tyrosine aminotransferase pathway.**

It has been known for more than 60 years that in animals tyrosine is metabolized to homogentisic acid (1, 2). The initial step in the pathway leading to homogentisic acid was shown to be catalyzed by tyrosine aminotransferase (3–5). Since the early 1950's it has been accepted that the major route of tyrosine metabolism involves transamination to *p*-hydroxyphenylpyruvic acid followed by oxidation to homogentisic acid and the subsequent formation of fumaric and acetoacetic acid.

The decarboxylation of aromatic amino acids, although known to occur (6, 7), has been considered to be a minor route of metabolism for these compounds. Recently, we have reported that following the elevation of the plasma levels of aromatic amino acids, decarboxylation of tyrosine, phenylalanine, and tryptophan by aromatic-L-amino-acid decarboxylase (decarboxylase, EC 4.1.1.26) can constitute a major metabolic pathway for these amino acids (8). This conclusion was based on the observation that upon oral administration of any of these amino acids their plasma levels were further elevated when inhibitors of decarboxylase were also administered. We have now analyzed the metabolism of tyrosine in detail and have established that decarboxylation is quantitatively an important pathway. With increased tyrosine intake decarboxylation can predominate over transamination in the overall metabolism of this amino acid *in vivo*.

MATERIALS AND METHODS

Male MF_1 mice (25–30 g) were obtained from Marland Farms, Wayne, N.J. Uniformly labeled L-[^{14}C]tyrosine (380 Ci/mole) was obtained from New England Nuclear Corp., L-[^{14}C]-tyramine (50 Ci/mole) from Schwarz Mann, and uniformly labeled [^{14}C]*p*-hydroxyphenylpyruvic acid (25 Ci/mole) from Amersham Searle. *p*-Hydroxyphenylpyruvic acid, L-tyrosine, and β-glucuronidase Type-H2 (130,000 units/ml) were purchased from Sigma. IRC 50 was furnished by Mallinkrodt and the decarboxylase inhibitor Ro 4-4602 [N^1-*dl*-seryl-N^2-(2,3,4)-trihydroxybenzylhydrazine] was supplied by Hoffmann-La Roche Inc., Nutley, N.J.

The mice were pretreated with the monoamine oxidase inhibitor, pargyline (50 mg/kg), 24 hr before and just prior to the oral administration of [^{14}C]tyrosine. In order to minimize the effects of bacteria on the metabolism of tyrosine, neomycin (2 g/liter) was placed in the drinking water 24 hr prior to the initiation of the experiment and during its course. In addition, the animals were fasted for 24 hr prior to the administration of tyrosine and received an aqueous suspension of sulfasuxidine (2 mg/kg) 24 hr before and concomitant with the administration of tyrosine. In addition to the tracer dose of [^{14}C]tyrosine, some mice were also given an oral dose of tyrosine ranging from 0.5 to 1.5 g/kg and/or the decarboxylase inhibitor Ro 4-4602 (50 mg/kg intraperitoneally). The mice were then placed in individual 600-ml glass beakers for periods of time ranging from 2 to 8 hr, after which they were killed.

The stomach, intestinal tract, and feces were removed and homogenized in 3 volumes of distilled water, using a Polytron homogenizer (Brinkman). This homogenate was then centrifuged at 30,000 × *g* for 15 min and 1 ml of β-glucuronidase was added to a 10-ml aliquot of the supernatant followed by a 150-min incubation at 37°. The reaction was stopped by the addition of trichloroacetic acid to a final concentration of 5% followed by centrifugation at 30,000 × *g* for 15 min.

The remainder of each carcass plus any voided urine was homogenized with a Waring blendor in 3 volumes of water. A 10-ml aliquot was removed and saved for determination of [^{14}C]tyrosine incorporation into protein (see below). Another 10-ml aliquot of the homogenate was incubated with β-glucuronidase, and proteins were precipitated with trichloroacetic acid as described for the gastrointestinal tract above. The supernatant was applied to a 0.5 × 4-cm IRC 50 (H^+ form) column previously adjusted to pH 6.5. The effluent, plus an additional 5 ml of water was collected (*acidic fraction*). The adsorbed material (neutral and basic compounds) was eluted with 5 ml of 4 M ammonia. After concentration to 2 ml by evaporation under nitrogen, this fraction was adjusted to pH 6.5 and added to a fresh IRC 50 column which had been adjusted previously to pH 6.5. The effluent was collected and the column was then washed with 5 ml of 0.5 M sodium phosphate buffer, pH 6.5, followed by 14 ml of water. This fraction was combined with the initial effluent to constitute the *neutral fraction*. The *basic fraction* was obtained by elution with 5 ml of 5 M ammonia. Each

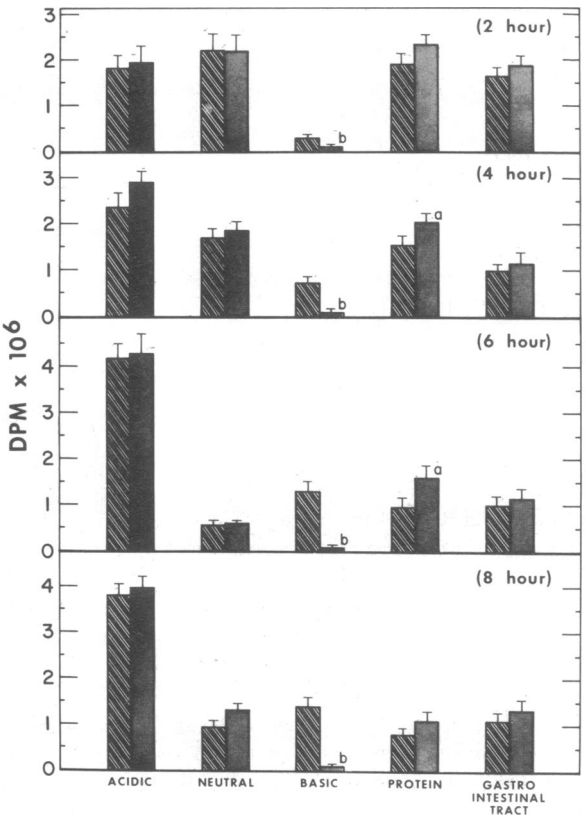

FIG. 1. Metabolic disposition of a tracer dose of orally administered tyrosine. One group of mice was given 9.1 × 10^6 dpm of uniformly labeled [^{14}C]tyrosine (*stippled bars*) in 0.3 ml of distilled water by mouth and a second group received in addition 50 mg/kg of the decarboxylase inhibitor Ro 4-4602 intraperitoneally (*hatched bars*). All animals were pretreated with the monoamino oxidase inhibitor, pargyline, and an antibacterial agent, as described under *Methods*. Four animals from each group were killed at each time point and the various fractions were separated as described under *Methods*. The results are expressed as mean dpm ± SEM; the numbers on the *ordinate* should be multiplied by the indicated factor to obtain the experimental results. *a:* Indicates distribution of radioactivity significantly different from that for administration of tyrosine alone ($P < 0.025$); *b:* Same comparison, ($P < 0.001$).

fraction was concentrated under nitrogen to a final volume of 1 ml.

In order to determine the efficiency of the separation procedures, radioactive *p*-hydroxyphenylpyruvic acid, tyrosine, and tyramine were chromatographed on IRC 50 columns as described above. In each case a recovery greater than 90% was obtained with *p*-hydroxyphenylpyruvic acid being present in the *acidic fraction*, tyrosine in the *neutral fraction*, and tyramine in the *basic fraction*.

The *acidic*, *neutral*, and *basic fractions* were subjected to paper chromatography (Whatman 3 MM) using butanol, acetic acid, water (6:1.5:2.5 v/v) as solvent. The R_F values of tyrosine, tyramine, and *p*-hydroxyphenylpyruvic acid were determined in this system by using the reference ^{14}C compounds.

Incorporation of [^{14}C]tyrosine into protein was determined in the following manner. Ten milliliters of each carcass homogenate was brought to a final concentration of 5% with respect to trichloroacetic acid and centrifuged at 30,000 × *g* for 15 min. The precipitate was washed twice with 4 ml of 5% trichloroacetic acid and the pellet resuspended in 4 ml of 1 M NaOH. After stirring at room temperature for 15 min, 8 ml of 30% trichloroacetic acid was added and the suspension was centrifuged at 30,000 × *g* for 15 min and washed twice again with 4 ml of 5% trichloroacetic acid. The pellet was resuspended in 3 ml of 3:1 ethanol–ether, heated at 50° for 10 min, cooled, centrifuged, and washed with two 5-ml portions of ethanol–ether. The weight of protein present was obtained after 1-hr evaporation of the solvent at room temperature. The protein was dissolved by shaking at 55° in 2 ml of NCS (Amersham-Searle) solvent and the radioactivity determined.

p-Hydroxyphenylpyruvic acid was determined by the method of Diamondstone (9) and tyrosine by the method of Waalkes and Udenfriend (10). Radioactivity, except when otherwise noted, was determined by liquid scintillation counting in Instabray (Yorktown). Counting efficiency was determined by means of an internal standard and the results are expressed in dpm. The paper chromatograms were scanned for radioactivity with a Packard (model 7201) strip scanner.

Since uniformly labeled tyrosine was used in these experiments, the formation of tyramine by decarboxylation will result in a 11.1% loss of radioactivity, which represents the carboxyl group of tyrosine. Therefore, the counts measured in the *basic fraction* representing tyramine were corrected for the loss. With respect to the *neutral fraction* no such adjustment was made, since the radioactivity of this fraction presumably was tyrosine. The *acidic fraction* can contain a number of different compounds of the transaminase pathway. Thus, *p*-hydroxyphenylpyruvic acid would retain all of the radioactivity of the original tyrosine. However, the formation of homogentisic acid involves a decarboxylation and the product and its metabolites would contain 11.1% less radioactivity. We did not correct for this potential loss, since paper chromatography of *acidic fractions* showed that they contained mainly *p*-hydroxyphenylpyruvic acid. The *acidic fraction* was not further fractionated for these studies.

The total recovery of administered radioactivity following the described fractionation and separation procedures was 85% or greater. Statistical analysis was performed using Student's *t* test.

RESULTS

The metabolic fate of an orally administered tracer dose of uniformly labeled [^{14}C]tyrosine was studied in the mouse at times ranging from 2 to 8 hr following its administration. These studies were performed in the presence or absence of a decarboxylase inhibitor, Ro 4-4602. All animals had been treated with the monoamine oxidase inhibitor, pargyline, to prevent oxidative deamination of tyramine.

All the radioactivity contained in the *neutral fraction* cochromatographed with tyrosine on paper chromatography and the decarboxylase inhibitor did not significantly alter the amount of radioactivity present in this fraction (Fig. 1). Since the products of the transaminase pathway are all acids, these compounds should be present in the *acidic fraction* from the IRC 50 column and the amount of radioactivity present in this fraction should be a measure of the functional operation of the transamination pathway. The amount of radioactivity present in the *acidic fraction* increases as a function of time. At its maximum it accounted for 45% of the administered radioactivity. The decarboxylase inhibitor (Ro

4-4602) did not alter the amount of radioactivity associated with this fraction (Fig. 1). Thus it seems fair to conclude that Ro 4-4602 did not inhibit tyrosine transamination under these conditions. Since these animals were pretreated with the monoamine oxidase inhibitor pargyline, any tyramine that was formed by the decarboxylation of tyrosine would be expected to accumulate in the *basic fraction*. The amount of radioactivity increased in this fraction during the course of the experiment so that by 8 hr it contained almost 15.4% of the administered radioactivity (Fig. 1). Furthermore, over 95% of the total radioactivity contained in this fraction cochromatographed with authentic tyramine. As would be expected, following decarboxylase inhibition the amount of radioactivity in the *basic fraction* was drastically reduced.

It is also apparent from the data in Fig. 1 that the administration of Ro 4-4602 did not affect the absorption of radioactive tyrosine. The incorporation of tyrosine into proteins appeared to be increased 4 and 6 hr after administration of the decarboxylase inhibitor.

Experiments were then carried out in which increasing amounts of nonradioactive tyrosine were given along with the tracer dose of radioactive tyrosine. Pargyline-pretreated mice were given a constant amount of radioactive tyrosine as before along with 0.5, 1 and 1.5 g/kg of nonradioactive tyrosine. In addition, some mice at each dose level received the decarboxylase inhibitor. In each case the experiment was terminated 6 hr after the administration of tyrosine and the distribution of radioactivity in the various fractions was determined. Again the decarboxylase inhibitor did not reduce the amount of radioactivity contained in the *acidic fraction* (Fig. 2). In fact, a slight, possibly significant, increase was noted. The amount of radioactivity found in the *neutral fraction* was markedly elevated by the decarboxylase inhibition. The magnitude of this elevation increased as the dose of tyrosine was raised from 0.5 to 1.5 g. In the absence of the decarboxylase inhibitor, the amount of radioactivity in the *basic fraction* was quite large, representing 25% of the administered dose at the 0.5 g/kg level, 32% at the 1 g/kg and 42% at the 1.5 g/kg level. Paper chromatography of these fractions revealed only a single peak with an R_F identical to that of authentic tyramine and containing about 95% of the applied radioactivity. In animals which had been given the decarboxylase inhibitor, radioactivity was almost completely absent from the *basic fraction*. This would be expected if the tyramine was formed via decarboxylation of tyrosine.

The *neutral fractions* shown in Fig. 2 were assayed for tyrosine as well as for radioactivity. Although at each dose level of tyrosine the decarboxylase inhibitor elevated tyrosine, as determined chemically (10), the increase in radioactivity associated with the *neutral fraction* (presumably tyrosine) was even greater. This disparity increased as the oral dose of tyrosine was raised so that at the 1.5 g/kg level, treatment with the decarboxylase inhibitor resulted in a 2.9-fold increase in the radioactive content of the *neutral fraction* compared to only a 1.8-fold increase in chemically assayed tyrosine. These results indicated that, following decarboxylase inhibition, the *neutral fractions* contained a metabolite(s) of tyrosine. The *neutral fractions* were, therefore, subjected to paper chromatography. As shown in Fig. 3, the *neutral fraction* from animals which had received the decarboxylase inhibitor contained, in addition to tyrosine, a second radioactive spot, apparently a neutral metabolite. This material

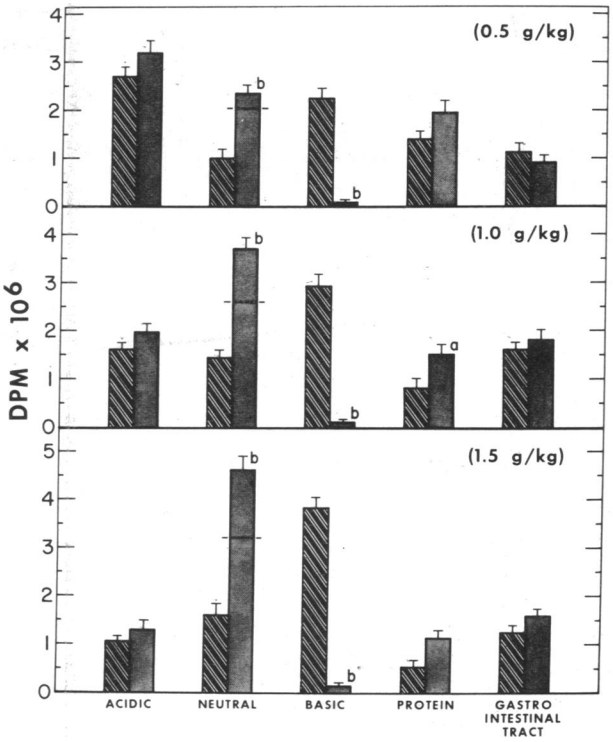

FIG. 2. Metabolic disposition of large amounts of orally administered tyrosine. The protocol is essentially the same as described under Fig. 1 with the exception that the radioactive tyrosine was given together with large amounts of nonradioactive tyrosine (0.5, 1, and 1.5 g/kg) and the animals were killed 6 hr after the initiation of the experiment. Four animals were used for each dose and the results are expressed as the mean dpm ± SEM. The amount of neutral material below the *dashed line* represents tyrosine, that above the *line* an unidentified metabolite(s) of tyrosine. *a:* Significantly different from tyrosine alone ($P < 0.01$); *b:* Significantly different from tyrosine alone ($P < 0.001$).

was not observed in animals which had not been given the decarboxylase inhibitor. This spot and the tyrosine spot were quantitatively eluted from the paper and their radioactivity measured; the sum of the two accounted for more than 95% of that applied to the paper chromatogram. When corrected for this metabolite the increase in radioactive tyrosine following decarboxylase inhibition was in good agreement with the increase in chemically assayable tyrosine. The contributions of tyrosine and this unknown metabolite(s) are depicted graphically in Fig. 2. It can be seen that with the decarboxylase inhibitor, as the dose of tyrosine was increased, the amount of both tyrosine and this unknown metabolite of tyrosine increased.

As also shown in Fig. 2, decarboxylase inhibition in animals given large amounts of tyrosine appears to increase the amount of radioactivity incorporated into protein. It does not affect the amount of tyrosine absorbed from the gastrointestinal tract.

DISCUSSION

In a previous report (8), we noted that although aromatic-L-amino-acid decarboxylase is important in the formation of neuronal catecholamines and serotonin, most of the enzyme is present in liver and kidney, not associated with nerves (11),

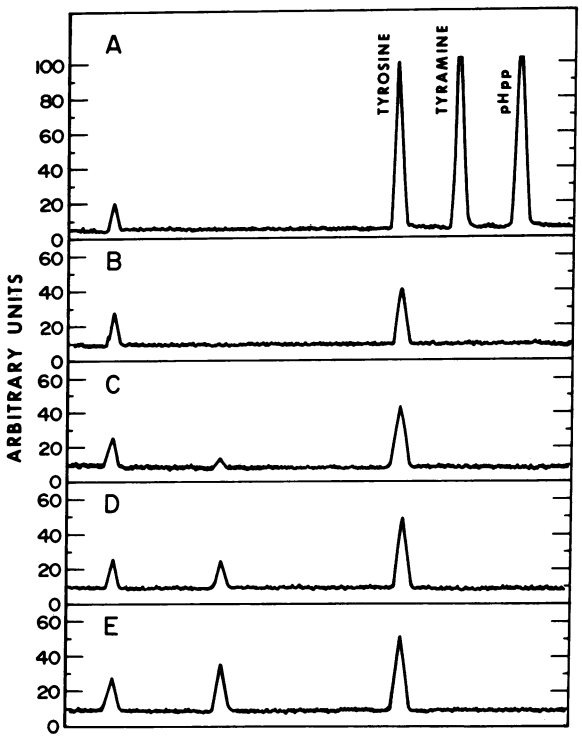

FIG. 3. Paper chromatograms of the *neutral fractions* obtained from IRC 50 columns. An aliquot of each *neutral fraction* shown in Fig. 2 was subjected to paper chromatography and scanned as described under *Methods*. The first peak in each chromatogram is a marker used for the calculation of R_F. The amount of radioactivity taken in each case was equivalent to approximately 1 ml of the original homogenate. Frame *A* shows the mobility of authentic tyrosine, tyramine, and *p*-hydroxyphenylpyruvic (pHpp) acid in this system. Frame *B* represents the *neutral fraction* from animals given 1.5 g/kg of tyrosine alone. Frames *C*, *D*, and *E* represent the fractions from animals given 0.5 g/kg, 1.0 g/kg, and 1.5 g/kg of tyrosine plus the decarboxylase inhibitor.

and plays an important role in the metabolism of the three dietary aromatic amino acids.

The present studies show that, as tissue concentrations of tyrosine are increased, decarboxylation becomes increasingly important and, at very high tissue levels, is the predominant route of metabolism (Table 1). Even under fasting conditions, using a tracer dose of tyrosine, large amounts of tyramine are formed. It should be noted that tyramine was made to accumulate in these experiments by administering a monoamine oxidase inhibitor. Without such an inhibitor, most of the tyramine would have been further metabolized. As the dose of tyrosine was increased from 0.5 to 1.5 g/kg, the amount of tyrosine metabolized via decarboxylation to tyramine rose from 3.5 to 18.9 mg. By contrast, the products of the transamination pathway remained fairly constant, increasing from 4.5 to only 5.2 mg.

The finding that, as the concentration of tyrosine in an animal is raised, the extent of decarboxylation increases, can be explained in terms of the K_m value of tyrosine for aromatic-L-amino-acid decarboxylase, which is of the order of 8 mM (6). Normally, the concentrations of tyrosine in plasma and tissue are about two orders of magnitude below this value. We have been able to demonstrate that whole

TABLE 1. *Relative proportions of transamination and decarboxylation with increasing tyrosine intake*

Dose of tyrosine (g/kg)	Transamination		Decarboxylation	
	% of tyrosine dose	mg	% of tyrosine dose	mg
Tracer*	45	—	14	—
0.5	30	4.5	25	3.5
1.0	17.5	5.3	32	9.6
1.5	11.5	5.2	42	18.9

The extent of transamination and decarboxylation was calculated from the radioactivity found in the *acidic* and *basic fractions*, respectively, 6 hr following the administration of the indicated amounts of tyrosine. The results are expressed as the percentage of the tyrosine dose administered and the absolute amount of tyrosine either transaminated or decarboxylated per mouse per 6 hr.

* The amount of radioactive tyrosine administered (9.1×10^6 dpm) was 67 μg/kg.

mouse extracts do indeed possess sufficient tyrosine decarboxylase activity to account for the amount of tyramine formed at the concentrations of tyrosine obtained *in vivo*.

Tyrosine aminotransferase has a reported K_m of about 1.5 mM (12). Therefore, one would have expected to see a similar increase in the products of transamination with increasing tyrosine intake and following elevation of body tyrosine levels by the decarboxylase inhibitor. This did not occur, although the tyrosine aminotransferase in a single mouse liver is capable of transaminating 114 mg of tyrosine within the 6-hr period of the experiment. It would appear that all of the potential tyrosine aminotransferase activity found *in vitro* is not functional *in vivo*. One possible explanation for this could be substrate inhibition of *p*-hydroxyphenylpyruvic oxidase. This enzyme is known to be subject to inhibition by its substrate both *in vivo* (13) and *in vitro* (14). With increasing doses of tyrosine, *p*-hydroxyphenylpyruvate is formed in increased amounts. This would inhibit *p*-hydroxyphenylpyruvic oxidase and block further tyrosine metabolism at this point. Since the transamination of tyrosine to *p*-hydroxyphenylpyruvic acid is reversible, a blockade of *p*-hydroxyphenylpyruvic oxidase would shift the equilibrium of the transamination toward the formation of tyrosine from *p*-hydroxyphenylpyruvic acid. Such an effect could explain why the metabolism of tyrosine through the transamination pathway was not increased when tyrosine levels were increased.

The limitation of the transaminase pathway at high tyrosine levels became further apparent when decarboxylase inhibitors were used. With decarboxylation almost totally inhibited there was little additional transamination. Instead, tyrosine accumulated as well as a neutral tyrosine metabolite. The latter has not yet been identified, but may represent the product of an enzyme which has a very low affinity for tyrosine.

The significance of the large turnover of tyramine and probably also of phenethylamine and tryptamine is not apparent. However, it does explain the presence of the large amounts of monoamine oxidase in liver and kidney which are required to metabolize these pharmacologically active amines.

1. Meyer, E. (1901) *Deut. Arch. Klin. Med.* **70**, 443–466.
2. Friedmann, E. (1908) *Beitr. Chem. Physiol. Pathol.* **11**, 304–305.
3. La Du, B. N. & Greenberg, P. (1951) *J. Biol. Chem.* **190**, 245–255.
4. Knox, W. E. & Lemay-Knox, M. (1951) *Biochem. J.* **49**, 686–693.
5. Schepartz, B. (1951) *J. Biol. Chem.* **193**, 293–295.
6. Christenson, J. G., Dairman, W. & Udenfriend, S. (1970) *Arch. Biochem. Biophys.* **141**, 356–367.
7. Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962) *J. Biol. Chem.* **237**, 89–93.
8. David, J. C., Dairman, W. & Udenfriend, S. (1974) *Arch. Biochem. Biophys.* **160**, 561–568.
9. Diamondstone, T. L. (1966) *Anal. Biochem.* **16**, 395–401.
10. Waalkes, P. G. & Udenfriend, S. (1957) *J. Lab. Clin. Med.* **50**, 733–736.
11. Klingman, G. I. (1965) *J. Pharm. Exp. Ther.* **148**, 14–21.
12. Jacoby, L. A. & La Du, B. N. (1964) *J. Biol. Chem.* **239**, 419–424.
13. La Du, B. N. & Zannoni, V. G. (1961) *Ann. N.Y. Acad. Sci.* **92**, 175–194.
14. La Du, B. N. & Zannoni, V. G. (1955) *J. Biol. Chem.* **217**, 777–787.