Identification of two molecular-mass forms of phenylalanine hydroxylase that segregate independently in rats

Specific association of each form with certain rat strains

Julian F. B. MERCER,* Andrew GRIMES, Ian JENNINGS and Richard G. H. COTTON
Birth Defects Research Institute, Royal Children's Hospital, Parkville, Vic. 3052, Australia

(Received 14 November 1983/Accepted 13 January 1984)

The nature of the different molecular-mass forms of phenylalanine hydroxylase in rat livers was examined by immunoprecipitation of the enzyme from crude liver extracts that had been radiolabelled by reductive methylation. The two forms of the enzyme were resolved by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and detected by fluorography. Segregation of the two forms of the enzyme was demonstrated in Sprague–Dawley rats, as would be expected if the two forms were products of allelic genes. In addition, hooded and albino Wistar rat livers contained only the slower-migrating form and Lewis rat livers contained only the faster-migrating form, and hence we suggest that the forms be referred to as W (for Wistar) and L (for Lewis). Peptide mapping showed that the W and L forms are closely related, and the difference between them appears to reside at one or other end of the polypeptide chain. The kidney contained the same forms as the liver in one-tenth the quantity, providing further evidence that the same phenylalanine hydroxylase gene is expressed in liver and kidney.

The enzyme phenylalanine hydroxylase (phenylalanine 4-mono-oxygenase, EC 1.14.16.1) catalyses the conversion of phenylalanine into tyrosine and is found in the liver of mammals and to a much lower extent in the kidneys (Kaufman, 1977; Goodwin, 1979; Hsieh & Berry, 1979). Analysis of purified phenylalanine hydroxylase by two-dimensional gel electrophoresis has revealed rather complex patterns of polypeptides (Choo *et al.*, 1979; Cotton *et al.*, 1980), with two molecular-mass forms (H and L) and several differently charged species. We are interested in determining the origin of this heterogeneity and in particular whether any of the phenylalanine hydroxylase polypeptides derive from different genes.

Phenylalanine hydroxylase isolated from rat liver originally was reported to consist of two molecular-mass subunits (M_r 49000 and 50000) (Fisher & Kaufman, 1973). Other workers, however, have failed to detect the two molecular mass forms (Nakata & Fujisawa, 1980; Webber *et al.*,

1980). This discrepancy, and previous work from this laboratory with human phenylalanine hydroxylase and rat hepatoma phenylalanine hydroxylase, suggested that the smaller- M_r form represented a proteolysis product (Choo *et al.*, 1979). Recently, however, we demonstrated that the two forms are produced by cell-free translation of rat liver mRNA, and hence they are encoded by different mRNA species (Mercer *et al.*, 1983a). In the present paper we report experiments that demonstrate that the two molecular-mass forms of the enzyme in the rat segregate independently and most probably are products of two allelic genes. Various strains of laboratory rat have one or the other or both forms of the enzyme in the liver and kidney, thus explaining the discrepant results of the past.

Experimental

Materials

KB^3H_4 ($>3\text{Ci/mmol}$) and $[^{14}\text{C}]$ phenylalanine were supplied by Amersham International, Amersham, Bucks., U.K. *Staphylococcus aureus* suspension (Pansorbin) was purchased from Calbiochem–Behring, San Diego, CA, U.S.A. Phenyl-

Abbreviations used: H form and L form, enzyme forms with larger M_r and smaller M_r ('higher molecular weight' and 'lower molecular weight') respectively; SDS, sodium dodecyl sulphate.

* To whom all correspondence should be addressed.

Sephacrose was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. *S. aureus* V8 proteinase was purchased from Miles Laboratories, Elkhart, IN, U.S.A., and papain was obtained from Boehringer Mannheim, Mannheim, West Germany. Sources of other reagents have been listed elsewhere (Choo *et al.*, 1979).

Reductive methylation of tissue homogenates

The method is a modification of the procedure of Finger & Choo (1981). Fresh or frozen rat livers or kidney were homogenized, in 1.5 ml of saturated sodium borate, pH 9, per g wet wt. of tissue, in a Kontes ground-glass homogenizer on ice. The homogenate was centrifuged at 25 000 g for 45 min, and the protein concentration in the supernatant was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Tissue extract equivalent to 150 µg of protein in 6 µl of saturated sodium borate, pH 9, was treated with 2 µl of 0.1 M-formaldehyde, and then, after 30 s, 400 µCi of KB^3H_4 was added. After 30 min on ice, the reaction was stopped by addition of 10 µl of a saturated aqueous solution of glycine and 128 µl of 0.01 M-Tris/HCl buffer, pH 7.4, containing 20% (v/v) glycerol, 0.01 M-phenylalanine and 0.1 mM-EDTA. The solution was stored at -20°C until use. Typical incorporation of radioactivity was 2×10^5 – 4×10^5 c.p.m./µg of protein.

Immunoprecipitation and gel electrophoresis

Immunoprecipitation of labelled tissue extract equivalent to 50 µg of protein was carried out in 75 µl of medium containing 1.8% (v/v) Triton X-100, 1.8% (w/v) sodium deoxycholate and 0.4 M-NaCl. Rabbit antiserum (5 µl) that was specific for rat phenylalanine hydroxylase (Mercer *et al.*, 1983a) was added and the mixture incubated for 1 h at 4°C . In some cases pre-immune serum was used as a control, as well as competition with an excess (7 µg) of unlabelled phenylalanine hydroxylase being added with the antibody. A 10% (w/v) suspension of *S. aureus* was added, and the sample was shaken gently for 30 min at room temperature. The *S. aureus* was sedimented by centrifugation and washed four times by suspension in 1.0 ml of medium containing 0.5% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate and 0.5 M-NaCl. For analysis, the *S. aureus* pellet was heated for 2 min at 90°C in 50 µl of 62.5 mM-Tris/HCl buffer, pH 6.8, containing 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol and 0.5% (w/v) Bromophenol Blue. After the *S. aureus* had been sedimented by centrifugation the supernatant was applied to an SDS/12.5%-(w/v)-polyacrylamide-gel system as described by Laemmli (1970) and electrophoresed at a constant voltage of 160 V for

5–6 h. After electrophoresis, gels were fixed and stained overnight with 0.05% (w/v) Coomassie Brilliant Blue in methanol/water/acetic acid (5:5:1, by vol.). Gels were then destained for 2 h in ethanol/water/acetic acid (1:8:1, by vol.) and prepared for fluorography (Bonner & Laskey, 1974).

Rat breeding and liver biopsy procedure

The Royal Children's Hospital (R.C.H.) colony of Sprague-Dawley rats has been maintained by random breeding for at least 10 years, but their origin is not known. Rats were anaesthetized with fluorothane, and in each a 1 cm incision was made, through which a lobe of the liver could be exposed. Approx. 200 mg of liver tissue was removed, and after bleeding had stopped the lobe was replaced and the incision closed.

Purification of phenylalanine hydroxylase

Phenylalanine hydroxylase from Sprague-Dawley rat livers (of known phenylalanine hydroxylase type) was isolated by using the two-step procedure of Shiman *et al.* (1979) without the use of DEAE-cellulose. Further purification was achieved by the phenyl-Sepharose binding and elution procedure described by Shiman (1980).

Peptide mapping

The procedure of Cleveland *et al.* (1977) was used. Purified phenylalanine hydroxylase (40 µg) in 75 µl of 0.03 M-Tris/HCl buffer, pH 7.2, containing 15% (v/v) glycerol, 50 µM-EDTA, 10 mM-phenylalanine and 0.3 M-KCl was mixed with 25 µl of 0.5 M-Tris/HCl buffer, pH 6.8, containing 2.0% (w/v) SDS, 40% (v/v) glycerol and 0.004% Bromophenol Blue. The samples were then heated for 2 min at 100°C . Proteinase digestions were initiated by addition of 4 µg of *S. aureus* V8 proteinase or 0.1 µg of papain in 28 µl of 0.125 M-Tris/HCl buffer, pH 6.8, containing 0.5% (w/v) SDS, 10% (v/v) glycerol and 0.001% Bromophenol Blue. Samples (32 µl) were removed at the times indicated in the Figure legends, 2-mercaptoethanol and SDS were added to final concentrations of 10% (v/v) and 2% (w/v) respectively, and the mixtures were heated for 2 min at 100°C followed by analysis on SDS/15%-(w/v)-polyacrylamide gels. Peptide bands were detected by staining with 0.25% Coomassie Brilliant Blue.

Enzyme activity assay

The assay procedure was essentially as described by Choo *et al.* (1979). Before the assay the purified enzyme and crude extract were activated by incubation for 5 min at 25°C in the presence of 0.1 mM-phenylalanine (Shiman, 1980). The reaction was carried out in a total volume of 200 µl of 10 mM-potassium phosphate buffer, pH 6.8, containing

1 mM-phenylalanine, [^{14}C]phenylalanine (0.2 μCi), 0.2–0.5 unit of phenylalanine hydroxylase (1 unit converts 1 nmol of phenylalanine into tyrosine/min at 25°C), 2 mM-dithiothreitol, 1 mM-lysophosphatidylcholine and 0.25 mM-tetrahydrobiopterin. The reaction was carried out for 5 min at 25°C and terminated with 50 μl of 30% (w/v) trichloroacetic acid. The extent of conversion of [^{14}C]phenylalanine into [^{14}C]tyrosine was determined by t.l.c.

Results

Analysis of the M_r of phenylalanine hydroxylase in Sprague–Dawley rats

Our previous results with cell-free translation of rat liver mRNA from Sprague–Dawley rats indicated that each molecular-mass form was coded by a separate mRNA, and the L-form protein was isolated from two litters of neonatal rats (Mercer *et al.*, 1983a). The forms of phenylalanine hydroxylase present were studied further by reductive methylation of crude liver homogenates and immunoprecipitation of the phenylalanine hydroxylase by using a specific antiserum (Mercer *et al.*, 1983a). The immunoprecipitates were analysed by SDS/polyacrylamide-gel electrophoresis and fluorography, and typical results are shown in Fig. 1. The two forms (H and L) of phenylalanine hydroxylase were clearly resolved, and the identity of the immunoprecipitated bands was confirmed by using control serum and competition with pure phenylalanine hydroxylase (results not shown). Previously we had considered that the L form was more common in neonatal rats (Mercer *et al.*, 1983b), but the results in Fig. 1 show that there is no apparent correlation of the form of phenylalanine hydroxylase with the age of the rat, since the L form is found in a 100-day-old rat (lane 9) and the H form in a 3-day-old rat (lane 1). Note that the quantity of phenylalanine hydroxylase present in the liver of 3-day-old rats is much lower than in adults, and we have found that the phenylalanine hydroxylase activity increases rapidly in the first postnatal week (J. F. B. Mercer & A. Grimes, unpublished work). The rat livers contained either the H form (lanes 1, 3, 5 and 7), or the L form (lane 9), or equal quantities (confirmed by densitometry) of the H and L forms (lanes 2, 4, 6 and 8).

Molecular-mass forms of phenylalanine hydroxylase in livers and kidneys of rats from breeding experiments

To investigate the inheritance pattern of the two forms, breeding experiments were set up with the Sprague–Dawley rats in which the form of the enzyme had been identified by liver biopsies followed by reductive methylation, immunopre-

Fig. 1. SDS/polyacrylamide-gel-electrophoretic analysis of phenylalanine hydroxylase from Sprague–Dawley rat livers

Livers were excised from the rats and homogenized individually, and each extract was reductively methylated as described in the Experimental section. A portion (50 μg of protein) of the reductively methylated extract was immunoprecipitated with a phenylalanine hydroxylase-specific antiserum, the immunoprecipitated material was analysed by electrophoresis on an SDS/12.5%-polyacrylamide-gel and the labelled proteins were detected by fluorography. Rats of various ages were used: lane 1, 3-day-old; lane 2, 3-day-old; lane 3, 17-day-old; lane 4, 17-day-old; lane 5, 17-day-old; lane 6, 41-day-old; lane 7, 41-day-old; lane 8, 41-day-old; lane 9, 100-day-old.

cipitation and analysis by SDS/polyacrylamide-gel electrophoresis. The livers and kidneys of the offspring resulting from each mating were removed at between days 20 and 30 after birth. The tissue homogenates were reductively methylated, and the phenylalanine hydroxylase type was determined. All the progeny (five) of a mating of an H-form male and an L-form female showed both H and L forms. Only the L form was found in the livers of six progeny of crosses between L-form parents, and in the kidneys of the three of these progeny that were examined. Two matings of two H+L-form parents yielded five progeny with only the H-form enzyme, four with both H and L forms and one with the L form only. In each experiment the identity of phenylalanine hydroxylase was confirmed by control precipitations with pre-immune

serum and by competition with excess unlabelled phenylalanine hydroxylase.

In all animals tested the form of enzyme present in the kidney was the same as in the liver and the quantity of enzyme estimated by densitometry was approximately one-tenth, matching well with results published for enzyme activity (Hsieh & Berry, 1979). These results suggest that the same phenylalanine hydroxylase gene is expressed in liver and kidney, but that the degree of expression differs in the two tissues.

Phenylalanine hydroxylase forms in various strains of rat

We analysed the forms of phenylalanine hydroxylase in a number of rat colonies, including various rat strains (Table 1). Among 32 randomly chosen individuals from our colony of Sprague-Dawley rats (R.C.H. strain), six were H form, ten were L form and 16 were H+L form. These frequencies fit a Hardy-Weinberg equilibrium for two allelic genes with frequencies of 0.44 (H) and 0.56 (L) in the population. These values seem close enough to 0.5 to fit with the possibility that our colony may have been derived by random breeding from two founders, one H form and one L form. In one other outbred colony of Sprague-Dawley rats we found five H form and one H+L form. All rats tested from a strictly inbred Sprague-Dawley line were H form. Thus the two forms are not restricted to the R.C.H. colony of Sprague-Dawley rats, but the frequency of each form in various colonies will depend on the genotypes of the founder rats and the breeding practices used.

The H₄-11-E-C₃ rat hepatoma contained only the H form (Choo *et al.*, 1979). This hepatoma was produced in an A.C.I. rat (Reuber, 1961), and so other A.C.I. rats (an inbred line) probably have the H form, although we have not established this. All

of the Wistar rats tested, both hooded and albino, contained the H form. Significantly, all the Lewis rats so far examined (only five individuals) contained the L form of phenylalanine hydroxylase. In view of the association of the H form with Wistar rats and the L form with Lewis rats, we suggest these forms be referred to as phenylalanine hydroxylase W (or W form) and phenylalanine hydroxylase L (or L form) respectively. This terminology is adopted for the remainder of this paper.

Purification and peptide mapping of the W and L forms of phenylalanine hydroxylase

To examine further the nature of the difference between the W and L forms of phenylalanine hydroxylase, we purified the enzymes from rats of known phenylalanine hydroxylase type (W, W+L or L). All forms were satisfactorily purified by phenyl-Sepharose chromatography (Shiman *et al.*, 1979; Shiman, 1980), and the SDS/polyacrylamide-gel-electrophoretic analysis of the purified enzymes is shown in Fig. 2. The proteins are quite pure (>98% by densitometry), and the mobility difference between the W (Fig. 2, lane 1) and L (Fig. 2, lane 3) forms is clearly apparent. Comparison with the standard markers gave apparent *M_r* values of 50 000 (W form) and 48 500 (L form), the same as previously reported (Mercer *et al.*, 1983a). The protein prepared from livers of heterozygote animals consisted of an equal quantity of W and L forms (Fig. 2, lane 2). A trace amount of protein with a mobility in between those of the W and L forms was detected in the W-form preparation (Fig. 2, lane 1); it is possible that this protein could be a proteolysis product of the W form.

To study the structural similarity of the W and L forms of phenylalanine hydroxylase, the purified enzymes were digested for various times with proteinases and the partial proteolysis products were

Table 1. *Molecular-mass forms of rat liver phenylalanine hydroxylase in various strains of rats*
The phenylalanine hydroxylase type was determined as described in the legend to Fig. 1. Sprague-Dawley, R.C.H., refers to the animals from our laboratory. The Sprague-Dawley, outbred and inbred, the Wistar and the Lewis rats were from various other laboratories. The cultured hepatoma cells (H₄-11-E-C₃) were derived from an A.C.I. rat.

Rat strain	No. of rats			Total
	H form only	H and L forms	L form only	
Sprague-Dawley, R.C.H.	6	16	10	32
Sprague-Dawley, outbred	5	1	0	6
Sprague-Dawley, inbred	6	0	0	6
Albino Wistar	6	0	0	6
Hooded Wistar	10	0	0	10
Lewis	0	0	5	5
H ₄ hepatoma (A.C.I.)	1	0	0	1

Fig. 2. SDS/polyacrylamide-gel-electrophoretic analysis of purified phenylalanine hydroxylase

Phenylalanine hydroxylase was purified from livers of rats of known phenylalanine hydroxylase type (W, W + L or L) by using the method of Shiman *et al.* (1979) as modified by Shiman (1980). Samples (10 µg) of each preparation were electrophoresed on SDS/12.5%-polyacrylamide gels, and the proteins were detected by Coomassie Brilliant Blue staining. Lane 1, protein isolated from W-form rats; lane 2, protein isolated from W + L-form rats; lane 3, protein isolated from L-form rats. At the side are indicated the M_r values for the protein standards (Pharmacia) used as markers: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soya-bean trypsin inhibitor, 20100; α -lactalbumin, 14400.

did not show any peptides that differed in M_r by 1000–1500, most being identical; however, a number of differences in the digestion patterns were noted. A peptide of M_r 37000 was present in the L form which did not appear on a digestion of the W form (compare the 10 min-digestion lanes in Fig. 3b). In this experiment the rates of digestion of the W and L forms by the V8 proteinase differed. The initial cleavage of intact protein to the first common cleavage product (M_r 43000) occurred very rapidly with the W form, but quite slowly with the L form, undigested protein still being detectable at 40 min (Fig. 3b). In other experiments, however, both forms were digested at the same rate.

Enzyme activity and thermal stability of the two forms of phenylalanine hydroxylase

To assess whether the two forms of phenylalanine hydroxylase differed in any functional sense, we determined the specific activity of the purified enzymes from W-, W + L- and L-form animals and also in crude liver extracts from different animals. The specific activity of the purified enzyme from W-form animals was found to be significantly higher than that of the enzyme from W + L-form and L-form animals (1.9 versus 1.3 units). However, the activities found in crude homogenates were somewhat variable and did not correlate with the phenylalanine hydroxylase type in the liver. This suggests that the activity difference detected in the purified preparations may have resulted from partial inactivation of the enzyme preparations during purification from the W + L- and L-form animals; however, since the assay results of liver extracts were variable, a small but real difference in activity would not have been detected. The thermal stability of the different forms was assessed by incubating the enzymes for various times at 50°C in the absence of phenylalanine. The activity of both W and L forms decreased at the same rate, the time for loss of 50% activity being approx. 18 min (results not shown).

Discussion

Our results clearly demonstrate that the two forms of rat phenylalanine hydroxylase segregate independently. The simplest interpretation of this behaviour is that they are coded for by allelic genes. The evidence for this conclusion is as follows: (1) individual animals contained either the W form, or the L form or both forms of the enzyme; (2) the W + L-form animals have an equal quantity of W and L forms, suggesting they are heterozygotes; (3) crossing W-form and L-form animals yielded all W + L-form progeny, and mating W + L-form animals produced pups that had a mixture of all possible phenylalanine

analysed by SDS/polyacrylamide-gel electrophoresis (Cleveland *et al.*, 1977). Fig. 3(a) shows that a 10 min digestion with 0.1 µg of papain produced a major peptide of M_r 31500 that was common to both W and L forms, and two smaller peptides of M_r 22500 (W form) and 21000 (L form), reflecting the apparent M_r difference in the intact molecules. Longer digestion produced an array of smaller peptides that appeared to be identical in both W and L forms. Digestion of W and L forms with *S. aureus* V8 proteinase (Fig. 3b)

Fig. 3. *Peptide mapping of phenylalanine hydroxylase*

The W and the L forms of phenylalanine hydroxylase ($40\ \mu\text{g}$) were digested with either $0.1\ \mu\text{g}$ of papain (a) or $4\ \mu\text{g}$ of *S. aureus* V8 proteinase (b), and samples ($10\ \mu\text{g}$ of protein) were removed at the times indicated under the Figure (10, 20, 40 or 60 min) and the peptide fragments analysed by electrophoresis on an SDS/15%-polyacrylamide gel as described by Cleveland *et al.* (1977). Peptides were detected by using Coomassie Brilliant Blue staining. At the side are the M_r values for the same protein standards as used in Fig. 2.

hydroxylase types, but the numbers were too small to obtain the expected 1:2:1 proportions of W:W+L:L; (4) the two forms of phenylalanine hydroxylase are encoded by separate mRNA

species (Mercer *et al.*, 1983a). A less likely explanation, which cannot be excluded by our data, is that we are observing the segregation of two other co-dominant genes for proteins that direct transcrip-

tion to different promoters of the one phenylalanine hydroxylase gene. As far as we are aware, there are no precedents for this in the same tissue of different strains of an animal, but tissue-specific amylase-gene expression (Young *et al.*, 1981) and intracellular versus secreted forms of the same invertase gene in yeast (Carlson & Botstein, 1982) result from use of different transcription-initiation sites of the same gene. The two tissues of the rat that express phenylalanine hydroxylase (liver and kidney) show exactly the same forms, so no tissue-specific expression of the two forms is evident in this case.

The peptide-mapping evidence suggests that the two forms are closely related, since most peptides are identical. The first cleavage event with *S. aureus* V8 proteinase generates identical major fragments, presumably resulting from the loss of a 7000-dalton (W form) or 5500-dalton (L form) peptide that was not detected in the gel. This suggests that the amino acid residue(s) responsible for the mobility difference must reside within about 70 amino acids from the *N*- or *C*-terminus of the protein. Although the two forms of phenylalanine hydroxylase differ in apparent M_r on SDS/polyacrylamide gels, it is possible that they differ by only one amino acid, leading to an apparent molecular-weight change of 3% owing to an alteration in SDS binding. For example, a single neutral amino acid substitution of leucine by glutamine in α -crystallin chains results in a 3% mobility change on SDS/polyacrylamide-gel electrophoresis (De Jong *et al.*, 1978). Alternatively, the molecular-mass difference may be real and could be produced by a number of types of mutation, or different transcription sites of a single gene as discussed above. Complete analysis of the relationship between the W and L forms will require sequencing of peptides or complementary-DNA clones specific for each form.

The fact that different rat strains may have the W form (Wistar), the L form (Lewis) or both (Sprague-Dawley) explains why some workers have purified only one form whereas other workers have found both. Thus Nakata & Fujisawa (1980), using Wistar rats, found only one form in the liver, as expected from our results, whereas Fisher & Kaufman (1973) reported observing the two forms but did not state the strain of rat used. Reported purifications of phenylalanine hydroxylase from Sprague-Dawley rats have yielded only one form (Shiman *et al.*, 1979) or two (Robson *et al.*, 1982), as we have also reported (Mercer *et al.*, 1983a). The varied results with Sprague-Dawley rats are expected, since the strain carries both forms, but the frequency of each in various colonies will differ depending on the forms present in the founder rats and the breeding practices used. We found sig-

nificant differences in the frequency of the two forms in three different colonies of Sprague-Dawley rats. Sprague-Dawley rats have been found to be polymorphic at other loci, e.g. around the albumin locus (Esumi *et al.*, 1982) and in the α -amylase gene family (Crerar *et al.*, 1983). All of these findings are consistent with the fact that Sprague-Dawley rats are generally randomly bred and are presumably heterozygous at many loci (Russel-Lindsey, 1979). The Wistar rat is inbred (Russel-Lindsey, 1979), and this accords with our finding of only the W form in both albino and hooded Wistars. Of the five Lewis rats (also inbred; Russel-Lindsey, 1979) examined, all had the L form, which indicates that this should be present in other colonies of Lewis rats. The observation of the W form in the H₄ hepatoma was originally used to support the proteolytic origin of the L form (Choo *et al.*, 1979), but this observation can now be re-interpreted as indicating that the host A.C.I. rat livers contain only the W form. We did not observe any reproducible differences in activity or thermal stability of the two different proteins; however, more detailed kinetic work may reveal differences, and, if so, future investigation should always be carried out on rat strains of known phenylalanine hydroxylase type.

Several possible origins of the two forms can be considered: (1) they are only found in laboratory rat strains, and one derives from a mutational event during selective breeding or because one wild rat used to start the colonies carried a rare mutation; (2) the two forms are common in wild rats but are not present in other species; (3) the two forms are found in other species and hence have an earlier evolutionary origin. It will be necessary to examine the phenylalanine hydroxylase from wild rats and other species to try to resolve these possibilities.

We are grateful to Professor David Danks and Dr. Stuart Smith for helpful discussions and for criticism of this manuscript. This work was supported in part by grants from The National Health and Medical Research Council of Australia and the Royal Children's Hospital Research Foundation.

References

- Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83-88
- Carlson, M. & Botstein, D. (1982) *Cell (Cambridge, Mass.)* **28**, 145-154
- Choo, K. H., Cotton, R. G. H., Danks, D. M. & Jennings, I. G. (1979) *Biochem. J.* **181**, 285-294
- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102-1106
- Cotton, R. G. H., Jennings, I. G., Choo, K. H. & Fowler, K. (1980) *Biochem. J.* **191**, 777-783

- Crerar, M. M., Swain, W. F., Pictet, R. L., Nikovits, W. & Rutter, W. J. (1983) *J. Biol. Chem.* **258**, 1311–1317
- De Jong, W. W., Zweers, A. & Cohen, L. H. (1978) *Biochem. Biophys. Res. Commun.* **82**, 532–539
- Esumi, H., Takahashi, Y., Sato, S. & Sugimura, T. (1982) *Nucleic Acids Res.* **10**, 4247–4257
- Finger, J. M. & Choo, K. H. (1981) *Biochem. J.* **193**, 371–374
- Fisher, D. B. & Kaufman, S. (1973) *J. Biol. Chem.* **248**, 4345–4353
- Goodwin, B. L. (1979) in *Aromatic Amino Acid Hydroxylase and Mental Disease* (Youdim, M. B. H., ed.), pp. 5–76, John Wiley and Sons, New York
- Hsieh, M. C. & Berry, H. K. (1979) *J. Exp. Zool.* **208**, 161–167
- Kaufman, S. (1977) *Adv. Neurochem.* **2**, 1–132
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mercer, J. F. B., Hunt, S. M. & Cotton, R. G. H. (1983a) *J. Biol. Chem.* **258**, 5854–5857
- Mercer, J. F. B., Hunt, S. M., Grimes, A. & Cotton, R. G. H. (1983b) in *Manipulation and Expression of Genes in Eukaryotes* (Nagley, P., Linnane, A. W., Peacock, W. J. & Pateman, J. A., eds.), pp. 27–28, Academic Press, Sydney
- Nakata, H. & Fujisawa, H. (1980) *Biochim. Biophys. Acta* **614**, 313–327
- Reuber, M. D. (1961) *J. Natl. Cancer Inst.* **26**, 891–899
- Robson, K. J. H., Chandra, T., MacGillivray, R. T. A. & Woo, S. L. C. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4701–4705
- Russel-Lindsey, J. (1979) in *The Laboratory Rat* (Baker, H. J., Russel-Lindsey, J. & Weisbroth, S. H., eds.), vol. 1, pp. 2–30, Academic Press, New York
- Shiman, R. (1980) *J. Biol. Chem.* **255**, 10029–10032
- Shiman, R., Gray, D. W. & Pater, A. (1979) *J. Biol. Chem.* **254**, 11300–11306
- Webber, S., Harzer, G. & Whitely, J. M. (1980) *Anal. Biochem.* **106**, 63–72
- Young, R. A., Hagenbüchle, U. & Schibler, U. (1981) *Cell (Cambridge, Mass.)* **23**, 451–458