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MONOGENIC INHERITANCE OF CATECHOL-*O*-METHYLTRANSFERASE ACTIVITY IN THE RAT—BIOCHEMICAL AND GENETIC STUDIES*

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Abstract—Catechol-*O*-methyltransferase (COMT) activities in the livers and kidneys of Fischer-344 (F-344) inbred rats were only 58 and 59 per cent, respectively, of those in the same organs of inbred Wistar–Furth (W–F) rats. Breeding experiments were performed to study possible mechanisms of inheritance of COMT activity in these animals. COMT activities in livers and kidneys of F1 (hybrid) animals (N = 36) were intermediate between those of the parental strains, but were closer to W–F (N = 22) than to F-344 (N = 22) values. The results of studies of F2 generation (N = 147) and backcross (N = 171) animals were compatible with the autosomal recessive inheritance of low COMT activity. 25.8 per cent of the F2 animals fell into a "low" COMT group when enzyme activities in both liver and kidney were used for phenotypic classification. This result is compatible with autosomal recessive inheritance of the trait "low" COMT. Statistical estimates of the number of genes involved in the control of COMT activity in these rats were also compatible with monogenic inheritance. Because of the possibility that there might be genetically determined differences in the biochemical properties of COMT in F-344 and W–F rats, COMT activities in tissue homogenates of liver from the two strains were compared with regard to thermal stability, electrophoretic behaviour, apparent Michaelis–Menton (K_m) constant for substrates, and responses to three COMT inhibitors (tropolone, *S*-adenosyl-l-homocysteine, and calcium). No differences in thermal stability, R_f values after electrophoresis, apparent K_m values for substrates or 50 per cent inhibitory concentrations of the enzyme inhibitors for hepatic COMT were detected between the two strains of rats.

Catechol-*O*-methyltransferase (EC 2.1.1.6, COMT) plays an important role in the metabolism of both endogenous catecholamines and catechol drugs [1, 2]. The level of activity of COMT in the human erythrocyte (RBC) is under genetic control [3–5], and there is a direct correlation in man between RBC COMT activity and the activity of this enzyme in other organs such as the kidney and the lung [6]. These observations raise the possibility that individual genetically mediated variations in human COMT activity may result in individual variations in the metabolism of endogenous catecholamines and catechol drugs.

It would be helpful if an animal model of genetic differences in COMT activity were available for studies of the biochemical basis of the genetic regulation of COMT activity and for use in pharmacogenetic experiments. The COMT activities in the liver and kidneys of Wistar–Furth (W–F) rats are approximately twice the enzyme activities in the same organs of Fischer-344 (F-344) rats [7]. If the COMT activities in F-344 and W–F rats are genetically controlled, these animals might serve as one model for use in biochemical genetic and pharmacogenetic studies of COMT. The experiments described below were performed to study both the possible role of inheritance in the regulation of COMT activity in these two strains of rats and the biochemical basis of the differences in COMT activity in their livers and kidneys.

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MATERIALS AND METHODS

Animals

Fischer-344 and Wistar–Furth rats were obtained from Microbiological Associates, Inc., Rockville, MD. Unless stated otherwise, all animals were 10–11 weeks of age.

Breeding procedures

Standard matings were performed to obtain an F1 generation (W–F × F-344), an F2 generation (F1 × F1) and backcross animals. BF will be used to refer to the backcross of an F1 animal to an F-344 rat (F1 × F-344), and BW will be used to refer to the backcross of an F1 animal to a W–F rat (F1 × W–F). In all the experiments reciprocal crosses with respect to sex were performed. A total of 398 rats (44 P1, 36 F1, 147 F2, 83 BF and 88 BW) were studied in the course of the breeding experiments.

Tissue preparation

Breeding experiments. The animals were decapitated and exsanguinated; the livers and kidneys were removed and placed on aluminum foil on ice. The organs were homogenized in 4 vol. of 5 mM Tris–HCl buffer, pH 7.8, in a Polytron tissue homogenizer. The homogenates were centrifuged for 10 min at 10,000 *g* in a refrigerated centrifuge, and the supernatant from the initial centrifugation was centrifuged at 100,000 *g* for 60 min in a refrigerated ultracentrifuge. The supernatant obtained from the 100,000 *g* centrifugation was diluted in 5 mM Tris–HCl buffer, pH 7.8, which contained 0.25% bovine serum albumin (BSA). Ten μl of

the supernatant from the liver homogenates was added to 10 ml of this buffer, and 10 μl of the supernatant from the kidney homogenates was added to 7.5 ml of the buffer. Nine vol. of the diluted high-speed supernatant was added to 1 vol. of a suspension of Chelex-100 chelating resin, treated as described previously [8], and the slurry was mixed by gentle rotation for 1 hr to remove calcium, an inhibitor of COMT [9, 10]. The samples were then centrifuged at 5000 *g* for 10 min, and the supernatant was separated from the chelating resin. Two hundred μl samples of the supernatant were placed in 15 ml stoppered conical glass reaction tubes for measurement of COMT activity.

Biochemical experiments. Six male F-344 and six male W–F rats 9 weeks of age were decapitated, exsanguinated, and had their livers removed. Homogenates were prepared and were subjected to centrifugation through the 100,000 *g* step. Pooled samples of the 100,000 *g* supernatant (1 ml from each rat) were placed in plastic bottles and were frozen at −85°. These pooled samples were used for all subsequent biochemical studies. COMT activities in samples from each individual rat were also determined. Before each experiment the pooled supernatant samples were thawed and an aliquot was diluted with 5 mM Tris–HCl buffer, pH 7.8, which contained 0.25% BSA. Either diluted samples or pooled supernatant samples were used for the biochemical studies described below.

Assay procedures

COMT Assay. COMT activity was measured by the method of Raymond and Weinshilboum [8] as modified to determine enzyme activity in rat tissue [11]. 3,4-Dihydroxybenzoic acid was used as a substrate and [^{14}C]methyl-*S*-adenosyl-l-methionine (SAM) was used as the methyl donor for this reaction. The 3,4-dihydroxybenzoic acid was converted to radioactively labeled 4-hydroxy-3-methoxybenzoic acid (vanillic acid) by COMT, and the reaction product was removed by organic solvent extraction prior to the determination of its radioactivity in a liquid scintillation counter. No substrate was added to "blank samples". A heated sample is an inappropriate blank for measurement of COMT activity with this assay because of the existence of an enzyme activity that results in the formation of radioactivity labeled methanol in the presence of [^{14}C]SAM [12]. One unit of enzyme activity represented the formation of 1 nmole of 4-hydroxy-3-methoxybenzoic acid/hr of incubation at 37°, either per g of tissue or per mg of protein.

Protein assay. Protein concentrations were measured by the method of Lowry *et al.* [13] with bovine serum albumin as the standard.

Disc gel electrophoresis

Polyacrylamide disc gel electrophoresis was performed as described by Davis [14]. Twenty μl of pooled high-speed supernatant fluid was added to 70 μl of a 32% sucrose solution containing bromphenol blue. The mixture was placed onto a 7% acrylamide gel, pH 8.9, and electrophoresis was carried out at 0.2 mA per tube for 1.5 hr at 4°. Gels were sliced into 1.6 mm sections, and each section was placed in 200 μl of 0.25% BSA in 5 mM Tris–HCl buffer, pH 7.8. Gel sections were stored in buffer at 4° overnight before measurement of COMT activity.

Kinetic analysis

Michaelis–Menten (K_m) values were determined by the method of Wilkinson [15] with a Fortran program written by Cleland [16]. A Control Data Corporation 3500 computer was used for these calculations.

Materials

Tris(hydroxymethyl)aminomethane base, 3,4-dihydroxybenzoic acid, *S*-adenosyl-l-methionine hydrochloride, and *S*-adenosyl-l-homocysteine were purchased from the Sigma Chemical Co., St. Louis, MO. Chelex-100 chelating resin, 50–100 mesh, was obtained from BioRad Inc., Richmond, CA. [^{14}C]methyl-*S*-adenosyl-l-methionine (sp. act. 58 mCi/m-mole) was purchased from the New England Nuclear Corp., Boston, MA. Dithiothreitol (Cleland's reagent) was obtained from CalBiochem, San Diego, CA. Tropolone was purchased from the Regis Chemical Corp., Morton Grove, IL.

RESULTS

Breeding experiments

Parental and F1 generations. COMT activity was measured in the livers and kidneys of 10- to 11-week F-344 and W–F rats of both sexes. The COMT activities are shown in Table 1. The results are expressed both as units per g of tissue and as units per mg of protein. The values listed in the table are very similar to those reported previously [7]. Liver and kidney COMT activities in both male and female F-344 rats are 55–65 per cent of the values in the same organs of W–F animals. The magnitudes of the differences in activity between strains were very similar when expressed either in terms of tissue weight or mg of protein. The similarity of results expressed in these two fashions was true of all the animals studied in the course of the breeding experiments, so all subsequent data will be expressed as activity per g tissue wet weight.

When COMT activity was measured in tissue from F1 hybrid animals, the enzyme activity was intermediate between that found in the two parental strains (Figs. 1 and 2). However, F1 values were always closer to those in tissue from W–F animals than to values in tissue from F-344 rats. This finding suggests partial or incomplete dominance of the W–F phenotype. The data for male and female animals have been displayed separately in the table and the figures because of the observation that hepatic COMT activity is slightly higher in females than in males and that renal COMT activity is slightly higher in male than in female animals. There were no differences in the COMT activities of F1 animals derived from reciprocal matings (i.e. female F-344 × male W–F and female W–F × male F-344). This observation makes sex-linked transmission of differences in enzyme activity extremely unlikely. Therefore, data from reciprocal matings were pooled in the figures and in the discussion. The results of matings of parental strains to yield an F1 generation give little information with regard to whether inheritance of a trait might be monogenic or polygenic. Matings of F1 animals with each other to yield an F2 generation and

Table 1. COMT activity in parental strains*

Strain	Age	Sex	N	Kidney ū/g × 10^{-3}	Kidney % W–F	Kidney ū/mg protein	Kidney % W–F	Liver ū/g × 10^{-3}	Liver % W–F	Liver ū/mg protein	Liver % W–F
F-344	11 weeks	F	11	7.52 ± 0.21	62	95 ± 3.3	66	30.7 ± 1.3	58	223 ± 9.6	59
		M	11	7.80 ± 0.15	55	93 ± 1.6	56	28.3 ± 0.54	60	219 ± 5.1	65
W–F	11 weeks	F	11	12.2 ± 0.35	100	145 ± 4.2	100	52.9 ± 1.6	100	377 ± 18	100
		M	11	14.2 ± 0.32	100	166 ± 4.9	100	47.2 ± 1.2	100	339 ± 12	100

* Liver and kidney COMT activities in Fischer-344 (F-344) and Wistar–Furth (W–F) rats expressed both as units per g tissue weight and units per mg of protein are shown. Values are mean ± S.E.M. The values in F-344 animals have also been expressed as a percentage of the values in the same organs of like-sexed W–F rats (% W–F).

backcrosses of F1 animals to the parental strains are the best methods to determine whether a trait behaves as if its inheritance were monogenic. The expected mendelian segregation ratios for backcrosses of F1 animals to parental strains are 1:1 for an autosomal monogenic trait. Expected ratios for F2 animals are 1:2:1 for an autosomal codominant trait, or 1:3 if a trait is inherited in an autosomal recessive fashion [17].

F2 and backcross generations. (1) *Kidney data.* COMT activity was measured in the kidneys of 147 F2 and 171 backcross rats (Fig. 3). These assays were performed 4 months after the determinations of enzyme activity in tissues of parental strains and F1 animals. Therefore, the shapes of the frequency distribution histograms are probably as important as the average values in the interpretation of the results because of slight "drift" in the assay procedure during the months needed to breed the rats and to allow them to grow to 10–11 weeks of age. In both male and female animals the offspring of backcross matings of F1 rats to F-344 animals (BF) show two clearly separate groups. Fifty per cent of the female offspring and 58.5 per cent of the male offspring fall into a "low" COMT subgroup. The total of male plus female animals in the low enzyme activity subgroup for the backcross to the Fischers is 54 per cent (45/83). All of these values approximate closely the expected 1:1 ratio for a trait (low COMT) that is inherited in a monogenic fashion. In addition, in both male and female F2 animals, the frequency distribution histogram includes groups with low COMT

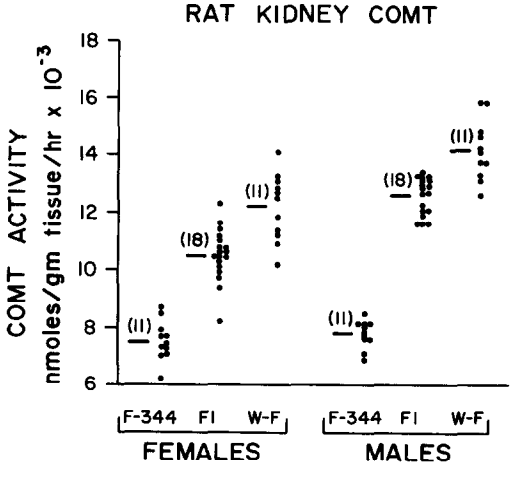

Fig. 1. Rat kidney COMT in F1 animals. The COMT activities in kidneys from female and male F1 generation rats are shown. Values for Fischer-344 (F-344) and Wistar–Furth (W–F) animals are shown for comparison. Bars represent mean values, and the numbers in parentheses represent the number of animals studied.

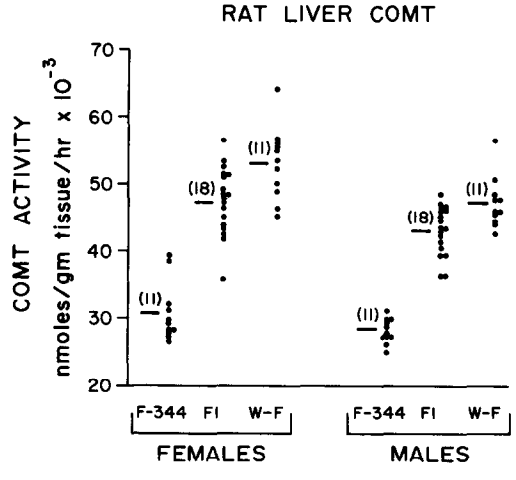

Fig. 2. Rat liver COMT in F1 animals. The COMT activities in livers from female and male F1 generation rats are shown. Abbreviations are the same as those used in Fig. 1. Values for F-344 and W–F animals are shown for comparison. Bars represent mean values, and the numbers in parentheses represent the number of animals studied.

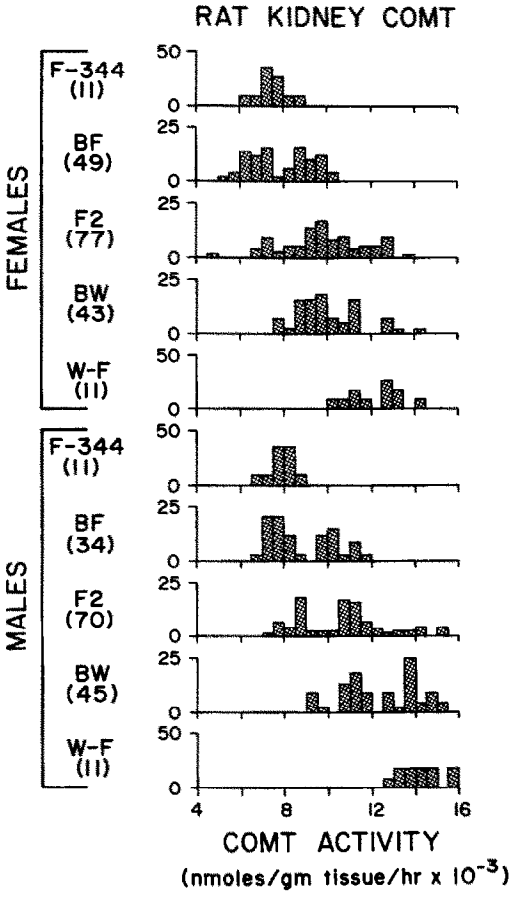

Fig. 3. Rat kidney COMT in F2 generation and backcross animals. COMT activities were measured in kidneys of F2 generation and backcross male and female rats. Values for F-344 and W–F animals are shown for comparison. BF refers to a backcross to F-344 animals and BW refers to a backcross to W–F animals. Other abbreviations are the same as those used in Fig. 1. Values on the ordinate represent the percentage of animals with COMT values by 0.5 ü increments. The numbers in parentheses represent the total number of rats of each type studied.

activity, 22 per cent (17/77) of the females and 30 per cent (21/70) of the males. These groups contain 25.8 per cent (38/147) of the total F2 population when the separation is made at the midpoint of the nadirs on the histograms. The frequency distribution of enzyme activity for male offspring of the backcross of F1 rats to the W–F strain appears to show a bimodal distribution with 47 per cent of the animals in an apparent "high" COMT subgroup. In all other cases, however, it is not possible to distinguish a high enzyme activity subgroup. If inheritance of COMT in these animals is monogenic, this observation might result from the partial dominance of the W–F phenotype. Overall, these results are compatible with the autosomal recessive inheritance of low renal COMT in these strains of rats. The term "recessive" is used because the low activity subgroup can be clearly differentiated from the remainder of the animals studied. It should be understood that the meaning of the terms recessive and dominant is relative, and that they are used as a convenient convention. The importance from the point of view of studies directed toward evaluation of the biochemical mechanism of the differences in enzyme activity is that inheritance appears to be monogenic. This conclusion is also supported by estimates of gene number based on analysis of the variance of COMT activities in F1 and F2 animals (see below).

(2) *Liver data.* COMT activity was also measured in livers of F2 and backcross animals (Fig. 4). These data were much less easily interpreted than were the results obtained from kidney homogenates. The backcrosses of F1 animals to the F-344 parental strain resulted in an apparent low COMT subgroup that included 51.8 per cent (43/83) of the animals. Thirty-one per cent of the male F2 animals appear to be included in a low COMT subgroup, but it is not possible to detect a clear subgroup of low COMT activity F2 female animals. The fact that the liver is very vascular and might be variably contaminated with blood may be one possible explanation for the fact that the hepatic data, both when expressed per g of tissue or per mg of protein, are much less clear than the renal data. However, these data are

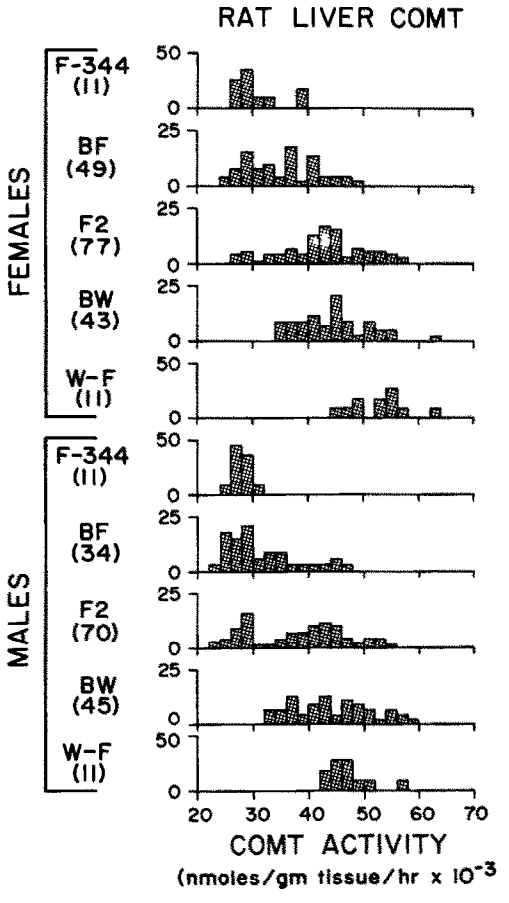

Fig. 4. Rat liver COMT in F2 generation and backcross animals. COMT activities were measured in livers of F2 generation and backcross male and female rats. Values for F-344 and W–F parental strain animals are shown for comparison. Abbreviations are the same as those used in Fig. 3. Values on the ordinate represent the percentage of animals with COMT values by 2 ü increments. The numbers in parentheses represent the total number of rats of each type studied.

compatible with monogenic inheritance when statistical estimates of gene number are made (see below).

(3) *Combined kidney and liver data.* Since this study includes data with regard to COMT activity in both liver and kidney, the possibility exists that the assignment of animals to phenotypic subgroups might be enhanced if the COMT activity in both organs were used to classify each individual rat. However, there is no reason to assume that the regulation of COMT activity in the two organs would be similar. Apparent differences in the regulation of COMT activity among organs have been described in inbred strains of mice [18, 19]. Therefore, it was determined whether there is a correlation of the relative level of COMT activity in the livers and kidneys of F2 animals. There is a significant positive correlation of liver with kidney COMT activity in these animals ($r = 0.704$, N = 147, $P < 0.001$, Fig. 5). In addition, 41/147 (28 per cent) of the rats fall into a "low" COMT group that includes animals with both kidney and liver COMT values less than the highest values found in any of the F-344 parental animals (9 ũ/g for kidney, 40 ũ/g for liver).

An attempt was made to remove the effect of sex and to place the relative enzyme activity of the two organs on the same basis by expressing the results as organ and sex specific relative deviates (Z values). When the correlation of Z values for kidney and liver COMT in the 147 F2 animals was determined in a fashion similar to that shown in Fig. 5, the correlation coefficient was increased to 0.817 ($P < 0.001$); 24.5 per cent (36/147) of the F2 animals had Z values less than the highest values for any F-344 rat, i.e. approximately the expected 25 per cent of the F2 rats had low COMT. Since the correlation data indicate that liver and kidney enzyme activities are regulated in a common fashion in these rats, the relative deviates for kidney and liver COMT for each F2 animal were summed to make possible phenotypic classification on the basis of data from both organs. The frequency distribution histograms of the summed values is shown in Fig. 6. Summed liver and kidney relative deviates for parental F-344 and W–F rats and for the backcross animals, calculated on the basis of the mean and S.D. for F2 rats, are shown in the figure for the sake of comparison only. The F2 frequency distribution histogram is clearly nonunimodal with 25.8 per cent (38/147) of the values in a low COMT subgroup made up of values less than those found in any F-344 rat (Z value −1.5). Once again a bimodal distribution is present for the backcross data for the low COMT activity F-344 animals, but not for the backcross to the high activity W–F rats.

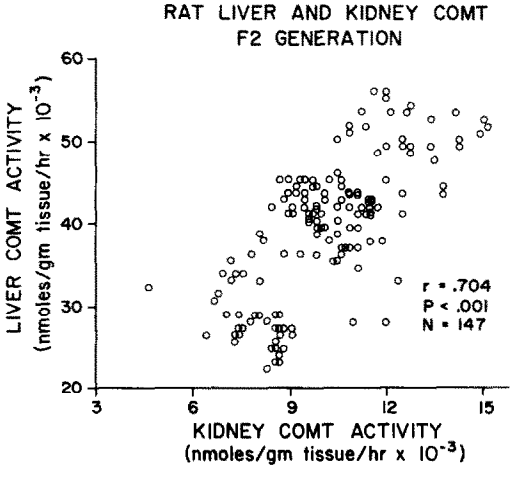

Fig. 5. Correlation of liver with kidney COMT activity in F2 animals. The correlation of liver with kidney COMT activity for F2 generation rats is shown. Data from both male and female animals are included.

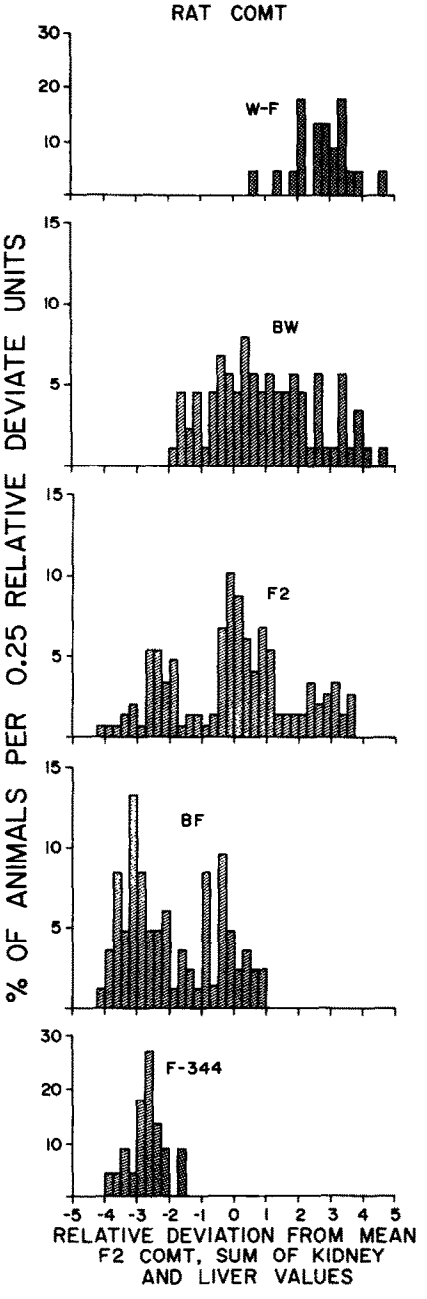

Fig. 6. Summed relative deviates for liver and kidney COMT. A frequency distribution histogram is shown of the summation of relative deviates of COMT activity in F2, backcross and parental strain animals to eliminate the effects of sex and differences in enzyme activities in the two organs (see text for full explanation).

Table 2. Estimates of number of genes responsible for inheritance of COMT in kidneys and livers of F-344 and W–F rats *

Sex	Organ	Estimated number of genes (N)
Male	Kidney	1.387
	Liver	0.737
Female	Kidney	1.096
	Liver	1.248

* Gene number was estimated by the method of Falconer [20].

Forty per cent of the BF rats have values greater than those found in any F-344 animals. All of these results are compatible with the autosomal recessive inheritance of an allele for low COMT activity, Co^l. Perhaps because of partial dominance of the high enzyme activity phenotype, heterozygotes for the two alleles at the *Co* locus cannot be clearly separated from animals homozygous for high COMT, Co^h Co^h.

Estimates of gene number. It is possible to make direct estimates of the number of genes involved in the determination of an inherited trait by an analysis of the variance of the trait in F1 and F2 generation animals [20]. When COMT activity levels in male and female animals for both liver and kidney were used for such analyses, in all cases the results were compatible with the hypothesis that the enzyme is controlled by a single locus, i.e. that only one gene is involved (Table 2). When similar calculations were performed using summed Z values for both liver and kidney to make it possible to pool data from both sexes and both organs, the estimate of the number of genes was 1.086. The assumptions that underlie these calculations, and the limitations of this approach have been discussed in detail elsewhere [20]. These results, like the segregation patterns, are compatible with monogenic inheritance of COMT activity in the livers and kidneys of these rat strains.

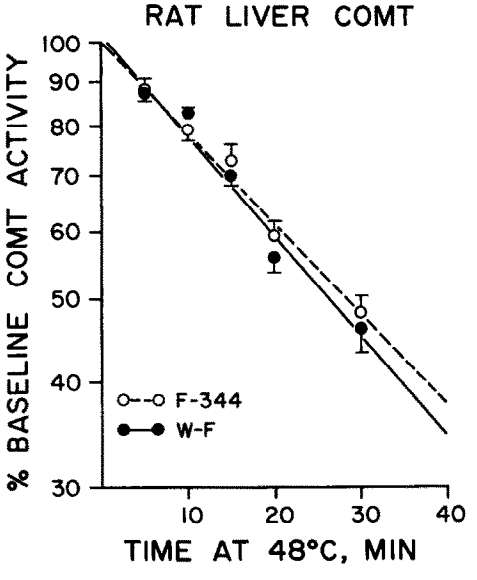

Fig. 7. Thermal stability of COMT. COMT activity was measured in pooled liver homogenates from F-344 (O– – – O) and W–F (●——●) rats after heating at 48° for variable time periods. Linear least square lines are shown. Each point represents the mean ± S.E.M. of three determinations.

Biochemical studies

Introduction. Included among the possible biochemical mechanisms which might explain the genetic observations made during the breeding experiments are (a) the existence of a difference in the quantity of COMT protein in the livers and kidneys of these two strains of rats, and (b) the existence of biochemically different forms of COMT in the two strains. These possibilities are not mutually exclusive. To begin to test the hypothesis that different biochemical forms of COMT might exist in F-344 and W–F rats, several biochemical procedures were used to compare the properties of COMT in the parental strains.

COMT in pooled tissue homogenates. The COMT activities in separate liver homogenates from six male F-344 and six male W–F rats were $26.9 \pm 1.4 \times 10^3$ nmoles/hr/g and $44.4 \pm 1.1 \times 10^3$ nmoles/hr/g respectively. Thus, the activity in the livers of F-344 animals was 61 per cent of the activity in W–F liver homogenates. These values are very similar to those of the male F-344 and W–F animals shown in Table 1. Pooled samples of hepatic homogenate (1 ml homogenate/rat, see Materials and Methods above) were used for all subsequent biochemical experiments. Although slight differences in activity were found from experiment to experiment, the relative activities of the pooled samples remained constant throughout the entire series of biochemical experiments.

Thermal stability studies. Relative thermal stability is a very sensitive means of detecting differences in enzyme structure [21]. Therefore, the thermal stabilities of COMT in pooled rat liver homogenates from F-344 and W–F animals were compared. In preliminary experiments, the homogenates were heated for 15 min at various temperatures between 45 and 52.5°. For both pooled homogenates more than 85 per cent of the basal activity remained after 15 min at 45° and less than 5 per cent remained after 15 min at 52.5°. Two separate experiments were then performed in which the homogenates were heated at 48 and 49° for varying times. The results of the 48° incubation, plotted in a semilogarithmic fashion, are shown in Fig. 7. Half-life ($T_{\frac{1}{2}}$) values for COMT activity were estimated by drawing linear least square lines. The estimated $T_{\frac{1}{2}}$ values for F-344 and W–F rats were 28.7 ± 1.5 min and 26.7 ± 1.5 min (mean ± S.E.M.) at 48°, and 20.9 ± 0.4 min and 21.4 ± 0.5 min at 49°. In neither case did the $T_{\frac{1}{2}}$ values for the two strains differ significantly.

Kinetic studies. COMT activities in pooled homogenates of liver from the two strains of rats were measured in the presence of varying concentrations of the two co-substrates of the reaction, *S*-adenosyl-l-methionine and 3,4-dihydroxybenzoic acid, and of magnesium, an activator of COMT [1]. Lineweaver–Burk plots of these data were made (Fig. 8) and apparent Michaelis–Menten (K_m) values were calculated from the plots (Table 3). There were no significant differences in the apparent K_m values for the co-substrates or for magne-

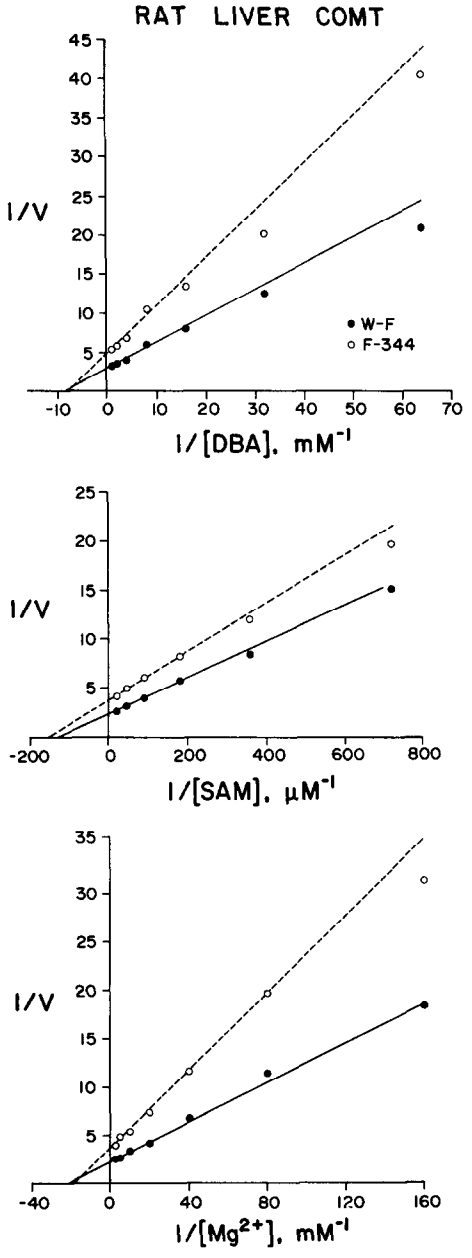

Fig. 8. Kinetic studies. COMT activity was determined in pooled liver homogenates from F-344 (○– – –○) and W–F (●——●) rats with variable concentrations of 3,4-dihydroxybenzoic acid (DBA), *S*-adenosyl-1-methionine (SAM) or magnesium. Lineweaver–Burk plots based on these data are shown. Each point represents the mean of three determinations.

sium in liver homogenates from the two inbred strains of rats.

COMT inhibitor studies. The effects of three different COMT inhibitors on the enzyme activities in liver homogenates of W–F and F-344 animals were compared. The three inhibitors used were tropolone, *S*-adenosyl-1-homocysteine and calcium. Each of these inhibitors is though to have a different mechanism of action [9, 22, 23]. The concentrations of each inhibitor necessary to inhibit the enzyme 50 per cent (IC_{50}

Table 3. Michaelis–Menten (K_m) constants for COMT from rat liver homogenates *

Substrate or Activator	Strain W–F K_m (μM)	F-344 K_m (μM)
DBA	100 ± 15	124 ± 13
SAM	8.02 ± 0.39	6.43 ± 0.50
Mg^{2+}	46.2 ± 4.4	53.1 ± 5.8

* DBA represents 3,4-dihydroxybenzoic acid and SAM represents *S*-adenosyl-1-methionine. All values are the mean ± S.E.M. of three separate determinations.

values) were identical for all three inhibitors for the two strains of rats (Fig. 9). Once again, these experiments failed to demonstrate a biochemical difference in the COMT activities in livers of F-344 and W–F animals.

Disc gel electrophoresis. The electrophoretic mobility of COMT activities in homogenates of W–F and F-344 rat liver were compared by disc gel electrophoresis. Tissue samples were subjected to electrophoresis in duplicate, and the R_f values for two samples from F-344 animals were 0.70 and 0.71. The R_f values for

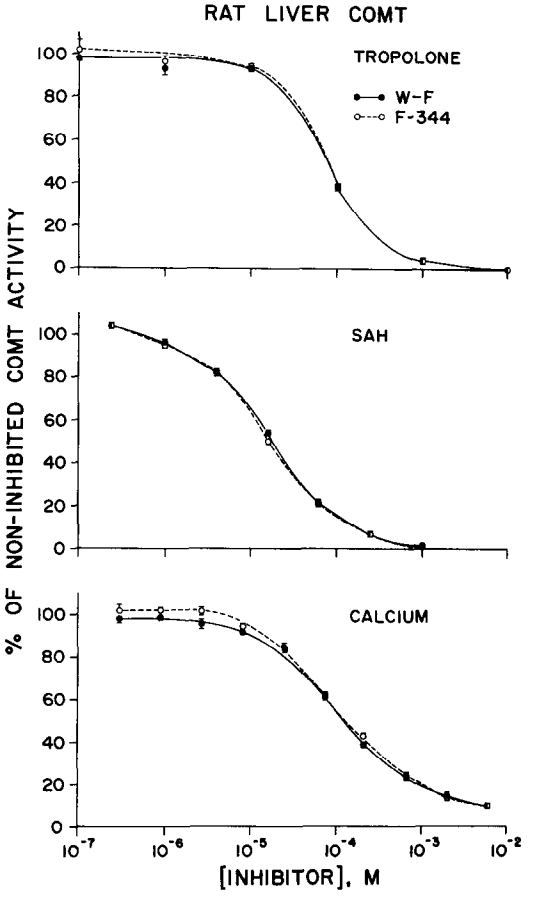

Fig. 9. COMT inhibitors. The effects of different concentrations of tropolone, *S*-adenosyl-1-homocysteine (SAH) and calcium on COMT activity in pooled liver homogenates from F-344 (○– – –○) and W–F (●——●) rats are shown. Each point represents the mean ± S.E.M. of three determinations.

COMT activity in W–F liver homogenates were 0.72 and 0.71. A serious problem encountered in electrophoretic studies of COMT activity is the instability of the enzyme. In this study only approximately 10 per cent of the enzyme activity applied to the gel was recovered after electrophoresis. Therefore, these results should not be regarded as definitive. However, the disc gel electrophoresis experiments, like the studies of thermal stability, K_m values and effects of inhibitors, failed to demonstrate differences in the biochemical properties of COMT in the livers of F-344 and W–F rats.

DISCUSSION

COMT activity in human erythrocytes is under genetic control [3–5]. There is a common allele for low erythrocyte COMT activity that is inherited in man in an autosomal recessive fashion [5], and the relative COMT activity in the human erythrocyte is directly correlated with relative COMT activity in other tissues such as the kidney and lung [6]. It would be useful if animal models were available for studies of the biochemical basis of genetic effects on COMT activity and for pharmacogenetic studies. Fischer-344 rats have about half as much COMT activity in their livers and kidneys as do Wistar–Furth rats, and these differences in activity are not due to differences in levels of endogenous enzyme activators or inhibitors [7]. Breeding experiments were performed to determine whether these differences in enzyme activity are under genetic control, and what the mechanisms of inheritance might be. The results were compatible with the autosomal recessive inheritance of low COMT activity in the liver and kidney of these two strains of rats. The possibility of autosomal codominant inheritance cannot be excluded on the basis of these studies. The term "recessive" is used to describe the inheritance of the trait of low COMT only because the subgroup of animals with low COMT could be clearly separated from the remainder of the population. We propose that the locus studied here be referred to as *Co* and that the alternative alleles for low and high activity be designated Co^l and Co^h respectively. If future experiments clarify the biochemical basis of the action of the locus and make it possible to classify it as a structural, temporal, processing or regulatory gene as defined by Paigen *et al.* [24], then the locus may be designated *Cos, Cot, Cop* or *Cor* respectively. This nomenclature is analogous to that suggested for genetically determined biochemical variants in the mouse by the Committee on Standardized Genetic Nomenclature for Mice [25].

Previous studies of COMT activity in inbred strains of mice with different levels of brain COMT have been carried only through the F1 generation [18, 19]. In both of the reported studies with mice, the enzyme activities in the brains of F1 animals were closer to those in the high COMT parental strain than to those in the low COMT parental strain, an indication of dominance of the high enzyme phenotype. Since the studies in mice were not carried through to F2 and backcross generations, it was not possible to determine whether inheritance was monogenic or polygenic. If inheritance is monogenic, the biochemical mechanism that underlies the genetic regulation is potentially more easily elucidated than if inheritance is polygenic.

Preliminary biochemical studies of COMT in the livers of W–F and F-344 rats demonstrated no detectable biochemical differences in the COMT activity in these strains. In a previous study of inbred mice with different levels of brain COMT, no differences in the K_m for substrate or in the optimal magnesium concentration between the two strains were found [18]. Failure to detect biochemical differences, of course, does not mean that such differences will not be demonstrated in the future. However, these results raise the possibility that differences in the quantity of COMT protein might account for differences in enzyme activity. This hypothesis can be tested by the development of antibodies to rat COMT which can be used for immunoprecipitation and radioimmunoassay studies. In addition, it might be possible to use these two strains of rats in pharmacogenetic studies of the effects of differences in the COMT activity on drug metabolism and the metabolism of endogenous catecholamines. Finally, it must be understood that data obtained from animal models cannot be extrapolated directly to the situation in man, and that biochemical and immunochemical studies of the molecular basis of the genetically mediated differences in human COMT activity must ultimately be performed with human tissue.

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REFERENCES

1. J. Axelrod and R. Tomchick, *J. biol. Chem.* **233**, 702 (1958).
2. M. E. Conolly, D. S. Davies, C. T. Dollery, C. D. Morgan, J. Paterson and M. Sandler, *Br. J. Pharmac.* **46**, 458 (1972).
3. R. M. Weinshilboum, F. A. Raymond, L. R. Elveback and W. H. Weidman, *Nature, Lond.* **252**, 490 (1974).
4. E. S. Gershon and W. Z. Jonas, *Archs gen. Psychiat.* **32**, 1351 (1975).
5. R. M. Weinshilboum and F. A. Raymond, *Am. J. hum. Genet.* **29**, 125 (1977).
6. R. M. Weinshilboum, *Life Sci.* **22**, 625 (1978).
7. R. M. Weinshilboum and F. A. Raymond, *Neuropharmacology* **16**, 703 (1977).
8. F. A. Raymond and R. M. Weinshilboum, *Clinica chim. Acta* **58**, 185 (1975).
9. R. M. Weinshilboum and F. A. Raymond, *Biochem. Pharmac.* **25**, 573 (1976).
10. D. R. Quiram and R. M. Weinshilboum, *Biochem. Pharmac.* **25**, 1727 (1976).
11. D. R. Quiram and R. M. Weinshilboum, *J. Neurochem.* **27**, 1197 (1976).
12. J. Axelrod and C. K. Cohn, *J. Pharmac. exp. Ther.* **176**, 650 (1971).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. B. J. Davies, *Ann. N.Y. Acad. Sci.* **121**, 402 (1964).
15. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
16. W. W. Cleland, *Nature, Lond.* **198**, 463 (963).
17. G. Mendel, *Verh. naturf. Ver. Brünn.* **4**, 3 (1865).
18. K. Schlesinger, J. Harkins, B. S. Deckard and C. Paden, *J. Neurobiol.* **6**, 587 (1975).
19. E. S. Gershon and W. Z. Jonas, *Biol. Psychiat.* **11**, 641 (1976).
20. D. S. Falconer, in *Methodology in Mammalian Genetics*

(Ed. A. Burdette), p. 193. Holden-Day, San Francisco (1963).
21. K. Paigen, in *Enzyme Synthesis and Degradation in Mammalian Systems* (Ed. A. Recheigl), p. 1. University Park Press, Baltimore (1971).
22. R. T. Borchardt, *J. med. Chem.* **16**, 377 (1973).
23. J. K. Coward, M. D'Urso-Scott and W. D. Sweet, *Biochem. Pharmac.* **21**, 1200 (1972).
24. K. Paigen, R. T. Swank, S. Tomino and R. E. Ganschow, *J. cell. Physiol.* **85**, 379 (1975).
25. Committee on Standardized Genetic Nomenclature for Mice, *Biochem. Genet.* **9**, 369 (1973).