the other the NH_2-terminal seven residues. An altered light chain electrophoretically identical to this adventitious one could be produced at will by incubating the whole myeloma protein at *p*H 4.8 (0.15*M* NaCl, 0.05*M* acetic acid, *p*H adjusted with NaOH) for 1 to 2 hours at 37°C.

One other instance of an association between facile hydrolysis and unusual chain length has been reported for the human myeloma protein Sac (*4, 14, 17*). The light chain of this protein has lost 65 internal amino acid residues within its variable region (*14*). Because the missing residues are internal, this loss is undoubtedly due to a deletion at the DNA level. This shortened light chain undergoes hydrolysis at the first peptide bond with loss of some of its NH_2-terminal residue (*4*). Moreover, the heavy chain in the same protein lacks almost its entire variable region, including the amino terminus (*17*). While it is possible that the loss in the heavy chain is due to deletion at the DNA level, such an explanation requires the coincidence of two rare deletions in the same cell line. It is simpler to attribute the "deletion" in the Sac heavy chain to peptide-bond hydrolysis facilitated in some way by the unusual length of the Sac light chain, in a manner analogous to the loss of one amino acid residue from the Sac light chain itself, or of four or seven residues from the unusually long MPC 11 light chain, as discussed in this report.

Laskov and Scharff (*1*) found a small difference between tryptic peptide chromatograms of MPC 11 light chains produced by a cloned cell line and by the original tumor line. Different degrees of conversion of the two preparations of light chain to the altered form described here would provide a simple explanation of this difference.

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16. The samples were fully reduced with 10 m*M* dithiothreitol in 8*M* urea before electrophoresis. Starch-gel electrophoresis was performed at *p*H 3.9 in 8*M* urea, 40 m*M* formic acid, 8 m*M* ethylenediaminetetraacetic acid, 8 m*M* NaOH, 2 m*M* dithiothreitol, at 4.2 volt/cm for 35.5 hours at 4°C. All samples showed a heavy chain band about 6 cm toward the cathode. The native light chain migrated 7.9 cm toward the cathode, while the altered light chain migrated 8.4 cm toward the cathode. If the amino acid at position 1 of MPC 11 is assumed to be Asp (aspartic acid) rather than Asn (asparagine), this electrophoretic behavior is consistent with the evidence cited in the text that the altered form is missing residues 1 to 4 or 1 to 7; for in that case the altered form would have about one less negative charge than the native light chain.
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Serum Dopamine-β-Hydroxylase Activity: Sibling-Sibling Correlation

Abstract. *Dopamine-β-hydroxylase activity is released into the blood with catecholamines from the adrenal medulla and sympathetic nerves. This enzyme activity has been measured in the blood of 317 normal children and 227 normal adults. A significant sibling-sibling correlation of serum dopamine-β-hydroxylase values was found in the 94 sibling pairs tested. Frequency distributions of serum enzyme values in both children and adults suggest the existence of two populations with regard to serum activity of this enzyme.*

Dopamine-β-hydroxylase (DBH) is the enzyme that catalyzes the conversion of 3,4-dihydroxyphenylethylamine (dopamine) to the neurotransmitter norepinephrine (*1*). The enzyme is localized to the catecholamine-containing vesicular structures in sympathetic nerve terminals and in the adrenal medulla (*2*). In response to stimulation of sympathetic nerves and the adrenal gland, DBH is released with catecholamines (*3*). A serum DBH activity described in both man and experimental animals is biochemically and immunochemically identical to the activity found in sympathetic nerves and in the adrenal medulla (*4*). This enzyme activity is increased in the blood of experimental animals and man under stress (*5*), and is decreased in the rat after partial chemical sympathectomy with the drug 6-hydroxydopamine (*6*). It has been suggested that serum DBH activity might be a useful measure of the level of function of the sympathetic nervous system (*4*). In many clinical studies, abnormal levels of circulating DBH activity have been described in patients with various diseases, including familial dysautonomia, neuroblastoma, torsion dystonia, and Down's syndrome (*7*). A wide range of values in control human subjects has been reported in all studies performed. Little is known of the factors that regulate serum or tissue DBH activity in man. We wish to describe the results of a study of serum DBH activity in large populations of normal children and adults, the results of which demonstrate a significant correlation of DBH activity between siblings and suggest that there may be at least two populations in humans with regard to serum levels of this catecholamine biosynthetic enzyme.

Blood samples from 317 children age 6 through 12 years were obtained as part of a survey of lipoprotein values in children attending the Rochester, Minnesota, schools. Blood was obtained at school in the early morning after an overnight fast. Blood samples from 227 adults were obtained from normal blood donors receiving no medications.

Activity of DBH was determined by a sensitive radiochemical enzymatic assay (*4, 7, 8*). The only alteration in the assay procedure as previously described was that 1*M* acetate buffer, *p*H 4.9, was substituted for 1*M* tris(hydroxymethyl)-aminomethane hydrochloride buffer, *p*H 6. This change resulted in a final incubation *p*H of 5.2, the optimal *p*H for the determination of DBH activity in human serum in this assay system. One unit of enzyme activity represents the produc-

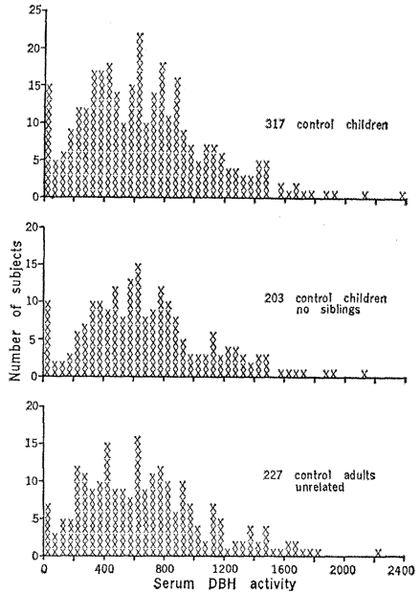

Fig. 1. Frequency distributions of serum DBH activity. The number of subjects with serum DBH activity in successive 50-unit increments is shown for three different populations: 317 normal control children, 203 of the same children with only one child from each family represented, and 227 normal adult control subjects.

tion of 1 nmole of β-phenyl-β-ethanolamine from β-phenylethylamine per milliliter of serum per hour (*9*). Bovine adrenal DBH was purified by the method of Geffen *et al.* (*10*). All results were evaluated by standard statistical methods.

Values of DBH were determined in the serum of 317 normal boys and girls age 6 through 12 years. It has been reported that circulating DBH activity increases in children with age. The greatest increase occurs during the first 2 to 3 years of life (*7, 11*). In this group of children, enzyme activity in girls did not change from age 6 to 12 years and in boys increased only approximately 50 units. Figure 1 shows the frequency distribution of serum DBH values. Of these 317 children, 4.7 percent had very low serum DBH activity (less than 50 units). This group of 15 children includes three out of the four children tested in one family and two other pairs of siblings. Serum DBH values in the remainder of the population are not distributed normally, but are skewed to the right. The skewness in the distribution is corrected by expressing the values as the square root of DBH activity. This group of 317 children includes many siblings. To eliminate the possibility that the distribution is biased by the inclusion of sets of siblings, Fig. 1 also includes a frequency distribution of the serum enzyme activity of 203 children in which only one child from each family was chosen randomly for inclusion.

Serum DBH activity was determined in blood obtained from 227 normal adult blood donors. These subjects ranged in age from 19 to 64 years, with a median age of 32.1 years. In this group there was no change in circulating DBH activity with age in either women or men, and there was no difference between values determined in women and men. Of this population, 3.1 percent had very low enzyme activity (less than 50 units). This frequency distribution is also skewed to the right.

A highly significant correlation ($r = .57$) was found between serum DBH activities in 94 sibling-sibling pairs represented in the population of normal children ($P < .001$). The correlation was established in terms of age- and sex-specific relative deviates about the sex-specific regressions of the square root of serum DBH activity with respect to age. This was done to correct for the small increase in enzyme activity with age in boys and for the lack of a normal distribution. Since the corrections were slight and the correlation was unchanged, for simplicity we have presented graphically the sibling-sibling scatter diagram in Fig. 2 in terms of uncorrected values of the square root of serum DBH activity. There was no difference in the degree of correlation between brother-brother, brother-sister, or sister-sister pairings. When random pairs of single children with no siblings were generated by using tables of random numbers, no correlation of serum DBH activity was found between members of nonsibling pairs.

Serum contains an inhibitor (or inhibitors) of DBH activity (*4*). Different levels of circulating inhibitor of DBH might explain the wide variation in DBH activity found in control subjects. To test this possibility, multiple random samples of serum with DBH activity that was (i) very low (less than 50 units), (ii) low in the normal range (200 to 350 units), and (iii) high in the normal range (850 to 1000 units) were assayed in the presence and absence of purified bovine adrenal DBH of activity comparable to that found in normal serum (450 units). These assays were done under the conditions routinely used to assay serum DBH in man (*9*). No differences were found in the degree of

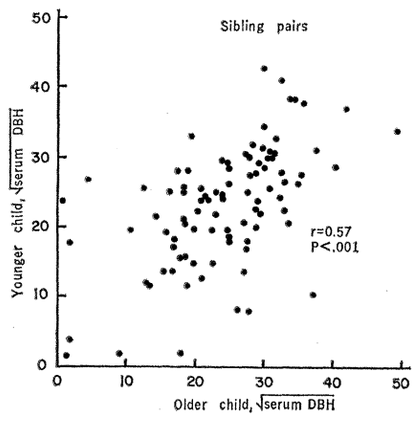

Fig. 2. Sibling-sibling correlation of serum DBH activity. The serum DBH activity in sibling pairs is plotted. The square root of the serum enzyme activity has been used to correct for the lack of a normal distribution.

inhibition of exogenously added bovine adrenal DBH in the presence of human serum samples containing very low, low normal, or high normal serum DBH activity (*12*). Furthermore, there was no inhibition of any of these samples under the conditions of assay. The wide range in serum DBH activity in normal controls cannot be accounted for on the basis of different levels of circulating inhibitor of the enzyme.

Although serum DBH activity has been measured in many clinical studies, the factors that influence the activity of this circulating catecholamine biosynthetic enzyme in normal subjects are not well understood. In man, as in experimental animals, stressful situations that result in increased urinary catecholamine excretion are associated with transient increases in serum DBH activity (*5*). The magnitude of the changes in serum DBH activity under these circumstances is not as great as is the range of normal values in a control population. Human serum DBH activity in children increases with age, particularly in the first 2 to 3 years of life (*7, 11*). It has been suggested that the rapid increase in serum DBH activity in the first years of life may be related to the functional or anatomical development of the sympathetic nervous system (*7, 11*). The results of the present study show that very little increase occurs after 6 years of age. The significant sibling-sibling correlations in serum DBH activity found in this study demonstrate that familial factors are also important in the regulation of the circulating levels of this enzyme. The relative contributions of heredity and

shared environment to these familial factors and the possible mechanisms of inheritance (single gene or polygenic) will have to be determined by family and twin studies. Familial factors might alter serum DBH activity in many ways, including changes in the quantity of releasable DBH, the rate of release, the access of DBH to the circulation, or the clearance of the enzyme from the blood. The data also suggest the possible existence of a separate subgroup of normal subjects with very low serum activity. The fact that this group includes approximately the same percentage of the adult population and of a population of children older than 6 years of age makes it less likely that the existence of such a subgroup among children represents only delayed maturation of the sympathetic nervous system. All of these data raise the possibility of significant familial differences in catecholamine biosynthesis in man.

The results of this study are also important in the interpretation of both previous and future clinical studies of serum DBH activity. The wide range of normal human serum DBH values, the previous demonstration that changes in these values in response to stress are relatively small (*5*), and the finding that familial factors significantly influence baseline levels of circulating enzyme activity all make it less likely that an isolated determination of serum DBH activity will be useful in establishing the level of sympathetic nervous system function in a particular individual. However, serial determinations of serum DBH activity in an individual or the comparison of single determinations in members of one population with determinations performed on blood from an appropriate control population might be useful clinically. The finding of very low serum DBH activity in blood from normal adults and children suggests that previous reports of very low enzyme activity in patients with diseases such as familial dysautonomia and Down's syndrome (*7*) must be interpreted with caution.

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13. Supported in part by a faculty development award in clinical pharmacology sponsored by the Pharmaceutical Manufacturers Association Foundation, Inc. (R.M.W), by PHS grant 5 S01 RR 05530-10 (R.M.W.), and by NIH grant HE 14196E (W.H.W.). We thank P. Hodgson for her assistance.

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Isolation of a Neurotropic Type C Virus

Abstract. *A neurogenic paralysis of the lower limb can be induced and serially transmitted in mice by a nontransforming type C virus strain that originated in an embryo of a wild mouse. The virus exerted a neurotropic effect on the anterior horn neurons.*

Stansly (*1*) encountered in BALB/c mice a lower limb paralytic disease associated with virus-induced reticular neoplasms and suggested that the paralytic agent and neoplastic virus could be the same. Apart from this report (*1*), pathogenic effects in vivo, other than cancer and possibly an autoimmune type kidney disease (*2*), have not been causatively associated with type C virus activity in mice or any other vertebrate species.

We isolated a type C virus (1504E) from the embryo culture of a wild mouse (*Mus musculus*) (*3*). Inoculation in vivo of purified concentrated 1504E virus from cells passaged in tissue culture into newborn wild mice and NIH Swiss laboratory mice led, after many months, to several instances of lymphoma, as might be expected. More surprising was the apparent induction of a neurogenic type of lower limb paralysis in many of the recipient wild and Swiss mice. We have described (*4*) a natural population of lymphoma-prone wild mice in which an identical lower limb paralysis, also experimentally transmissible, occurs spontaneously in association with elevated activity of indigenous type C virus (*5*).

The 1504E embryo culture was established 30 months ago from a single, whole, wild mouse embryo of about 18 to 19 days' gestation. The mother mouse was extensively infected with type C virus, as was evidenced by the recovery in vitro of infectious virus from many extracts of various organs, including uterus (*3*). Type C virus, which appeared spontaneously in two separate laboratories at the second subpassage of the 1504E embryo cell cultures, fulfilled necessary morphologic, immunologic, and biologic criteria for characterization as a nontransforming murine type C virus (*3*). It was infectious for NIH Swiss embryo cells in vitro but differed from the established strains of murine type C virus in that it produced unusually small syncytia in the XC plaque test (*6*) and was not neutralized by antiserums to Gross passage A, AKR, or FMR viruses. The 1504E culture, now in its 57th passage, is free of pleuropneumonia-like organisms and grows well with a doubling time of 3 to 4 days. The growth reaches saturation at a density of 7.5×10^5 cell/cm^2, and the cells continue to produce a type C virus without any diminution in titer. The cells were diploid in early culture but in later passage became heteroploid and showed the telo-