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Serum Dopamine β-Hydroxylase*

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I. Introduction

Dopamine β-hydroxylase (dopamine β-monooxygenase, E.C. 1.14.17.1, DBH) catalyzes the conversion of 3, 4-dihydroxyphenylethylamine (dopamine) to norepinephrine (93). The release of DBH with catecholamines from sympathetic nerves

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(29, 61, 177, 203) and the adrenal medulla (190) and the observation that DBH is present in blood (66, 194, 196) raised the possibility that determinations of serum DBH activity might provide a measure of the release of catecholamines and of the function of the adrenal medulla and sympathetic nerves. This possibility resulted in a wave of enthusiasm for the measurement of DBH in the blood of experimental animals and of patients with a variety of diseases. The initial enthusiasm was followed by disappointment when measurements of serum DBH failed to yield dramatic new insights into adrenergic function and when the results of many studies proved to be contradictory or confusing. However, at the time that most of these experiments were performed little was known of the regulation of DBH activity in blood. Recent kinetic, pharmacological, and biochemical genetic studies in experimental animals and in man have begun to clarify the regulation of serum DBH and to result in a reevaluation of many of the assumptions made by earlier investigators. Even the basic assumption that serum DBH directly reflects exocytotic release of catecholamines has been called into question. New information may now make it possible to begin to evaluate serum DBH as an indicator of adrenergic status and function in a more critical fashion. The purpose of this review is to summarize present understanding of the biochemistry, the regulation, and the possible significance of DBH in the blood of man and of experimental animals and to point out areas in which information is lacking. The topics of biochemistry, assay methods, source, fate, and regulation of serum DBH as well as reported levels of serum DBH in human disease will each be discussed in turn. Although much is known about DBH in neural tissue and in the adrenal medulla, this information will be described only when relevant to an understanding of serum DBH. Brief reviews of various aspects of the topic of serum DBH have appeared previously (59, 97, 100, 133, 172, 200).

II. Biochemical Characteristics of DBH

A. Introduction

The biochemical properties of DBH have been determined in enzyme isolated from the adrenal medulla and pheochromocytomas and in homogenates of neuronal and adrenal tissue from a variety of species. A brief review of the biochemical properties of DBH in solid tissues is necessary as an introduction to a discussion of the biochemistry of serum DBH.

B. Biochemical Characteristics of Adrenal DBH

Bovine adrenal DBH is a tetrameric glycoprotein with a molecular weight of approximately 290,000 daltons (23, 93, 191). Each of the four subunits has a molecular weight of about 75,000, and it has been suggested that pairs of the subunits are linked by disulfide bridges to form dimers, two of which are joined by noncovalent bonds (23, 117, 191). There are from 3 to 7 mol of copper per mol of DBH, and the copper is essential for enzyme activity (93, 191). Purified bovine adrenal DBH contains approximately 4% carbohydrate by weight (191). This carbohydrate is composed of residues of mannose, glucosamine, galactose, glucose, fucose, and sialic acid. The observation that DBH is a glycoprotein has facilitated the purification of the enzyme since DBH binds to the lectin concanavalin A, and affinity chromatography with concanavalin A has proven useful in the purification of the enzyme (9, 168). The properties of DBH purified from human pheochromocytoma are similar to those of the bovine adrenal enzyme except for a reported tendency of the human pheochromocytoma enzyme to aggregate (186) and an increase in the quantity of the human tumor DBH that is precipitated by the lectin ricin (125) as compared with the bovine enzyme.

Bovine adrenal DBH is a mixed function oxidase that requires molecular oxygen and

ascorbic acid or some other electron source to catalyze the *beta*-hydroxylation of a variety of phenylethylamine substrates (93). The substrate specificity of the enzyme is relatively broad, and many aromatic alkyl amines may serve as substrates (93). Early studies demonstrated that the addition of dicarboxylic acids such as fumaric acid to the reaction mixture resulted in an increase in reaction velocity (93). Dicarboxylic acids appear to function as activators of the enzyme. Finally, DBH activity is enhanced in the presence of catalase. It is thought that catalase prevents the inactivation of the enzyme by peroxides (93).

DBH is associated with catecholamine-containing vesicles in the adrenal medulla and adrenergic nerves (82, 148, 182). Adrenergic nerves contain both small and large dense core vesicles (48, 60). Although DBH is associated with large dense core vesicles, it is not universally agreed that the enzyme is also associated with the smaller vesicles (15, 28). A variable proportion of the DBH may be released from isolated adrenal chromaffin granules and from adrenergic vesicles by lysis (13, 82). In most tissues, the majority of the enzyme is "membrane-bound." Some investigators have suggested that this observation is an artifact of the lysis procedure for large dense core adrenergic vesicles (94). Although it has been speculated that the "soluble" enzyme released during vesicle lysis might represent that released with catecholamines in vivo, the relationship of the enzyme activity released from vesicles in vitro during lysis to that released from an organ in response to nerve stimulation is not clear.

Tissue homogenates contain potent DBH inhibitors (31, 136). There are probably a variety of inhibitors with different characteristics in different tissues (152). Several approaches have been used to inactivate DBH inhibitors so that the enzyme activity can be measured accurately. These include the dilution of tissue homogenates and/or the addition to homogenates of reagents such as copper sulfate or N-ethylmaleimide (31, 136).

C. Biochemical Characteristics of Serum DBH

The biochemical properties of serum DBH are very similar to those of DBH isolated from the adrenal medulla. Serum enzyme activity is increased in the presence of catalase and of fumaric acid (196). Molecular oxygen and an electron donor such as ascorbic acid are required by the enzyme (196). Apparent K_m values for the amine substrate of the serum enzyme are similar to those for the enzyme in homogenates of solid tissue from the same species (196). The electrophoretic mobility of serum DBH, although different in different species, is similar to that of the enzyme in solid tissues from the same species (165). Finally, there are serum DBH inhibitors that can be inactivated by copper sulfate or N-ethylmaleimide (66, 196).

When human plasma is subjected to gel filtration chromatography on Sephadex G-200, approximately 80% of the DBH activity is associated with a species with an apparent molecular weight of 560,000 and 20% of the enzyme activity is associated with a species with an apparent molecular weight of 189,000 (163). When these apparent molecular weights are corrected by the use of ultracentrifugation sedimentation, the molecular weights of the two forms of serum DBH have been calculated to be approximately 289,000 and 147,000, respectively. The high molecular weight species may be a tetrameric form of the enzyme while the lower molecular weight species may represent a dimer. No interconversion of the two species is found on repeat gel filtration chromatography. Purified human serum DBH consists of four subunits with molecular weights of about 65,000 to 75,000 (56, 69, 126), and one report raises the possibility that these subunits may in turn consist of two 32,000 dalton subunits (126). In addition to affinity chromatography with concanavalin A, chromatography with octyl-Sepharose has been reported to be useful for the purification of the human serum enzyme (56). Approximately 13% by weight

of purified human serum DBH is carbohydrate (127), and 95% of the enzyme activity can be precipitated with concanavalin A (125). Incubation of purified human serum DBH with neuraminidase has been reported to release 1.9 sialic acid residues per subunit (125). This observation may have relevance to the metabolic fate of the circulating enzyme (130, 189). The characteristics of plasma DBH inhibitors have also been studied. Human plasma contains both a dialyzable, copper-sensitive inhibitor and a nondialyzable inhibitor that is not copper-sensitive (78). From a practical clinical point of view, human serum DBH activity is very stable. For example, one group has reported no change in the DBH activity of plasma samples incubated at 37°C for up to 7 days (45).

In summary, most of the biochemical properties of serum DBH are similar to those of the enzyme purified from the adrenal medulla. Both are tetrameric glycoproteins. The molecular weights of both are approximately 300,000 and the mechanism of the enzymatic reaction is similar with a requirement for molecular oxygen and for an electron donor such as ascorbic acid. Enzyme activity is increased in the presence of catalase and of a dicarboxylic acid such as fumaric acid. Finally, enzyme inhibitors that can be inactivated with N-ethylmaleimide or by copper sulfate are present in both solid tissues and in plasma. Knowledge of these biochemical characteristics is essential for an understanding of the assay procedures used to measure serum DBH activity.

III. Assay Procedures for Serum DBH

A. Introduction

The development of sensitive and specific assays of DBH activity led to the observation that the enzyme is present in serum. Subsequently many modifications of those original procedures as well as entirely different assay methods have been described. There is controversy as to whether the same relative DBH activities are measured with different procedures. Immunoassays for the measurement of serum DBH protein have also been developed, and the possibility has been raised that the measurement of immunoreactive DBH (IDBH) might yield more information about adrenergic function than the measurement of DBH enzymatic activity. There has also been disagreement whether there is a significant positive correlation between serum IDBH and DBH enzymatic activity.

B. Assay of DBH Enzymatic Activity

The assays for DBH that were available in the late 1960's were useful primarily for measurement of the activity of partially purified enzyme or the activity in adrenal medullary homogenates. The development of a very sensitive coupled radiochemical assay (66, 129) made it possible to measure accurately DBH activity in sympathetically innervated tissue and led to the observation that the enzyme is present in blood (66, 194, 196). This assay was based on the conversion of substrate to β-hydroxylated product by DBH, followed by the enzymatic methylation of the product of the DBH reaction with a radioactively labeled methyl group by the enzyme phenylethanolamine-N-methyltransferase (noradrenalin N-methyltransferase, E.C. 2.1.1.28, PNMT). Both tyramine and β-phenylethylamine have been used as substrates for the assay. Although tyramine is a better substrate for DBH than is phenylethylamine, in practice phenylethylamine is often used because it is possible to measure the broad range of activities in human serum samples at a single dilution and because it is not necessary to dry the samples overnight to remove a volatile radioactive contaminant that is present in the final organic solvent extract when tyramine is the substrate (129). In the course of the reaction catalyzed by DBH, tyramine is converted to octopamine and phenylethylamine is converted to β-hydroxy-β-phenylethylamine (phenylethanolamine) (Fig. 1). The reaction is performed

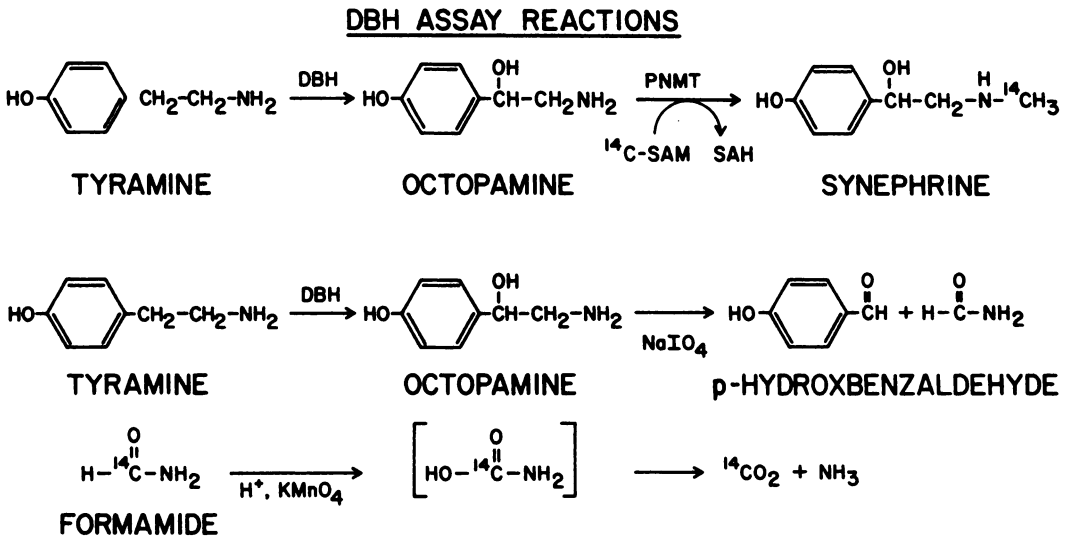

FIG. 1. Assay procedures for dopamine β-hydroxylase (DBH) enzymatic activity. The reaction sequences of several of the commonly used assay methods for the measurement of serum DBH enzymatic activity are shown.

in the presence of catalase, fumarate, and ascorbic acid, and is terminated by the addition of a solution buffered to pH 8.6, far above the 5.0 to 5.5 pH optimum of DBH. At the same time that the DBH reaction is stopped, ^{14}C-S-adenosyl-*l*-methionine and partially purified bovine adrenal PNMT are added to the reaction mixture. PNMT catalyzes the N-methylation of the β-hydroxylated product with S-adenosyl-*l*-methionine as the methyl donor to form synephrine or N-methylphenylethanolamine from octopamine or phenylethanolamine, respectively (Fig. 1) (10). After the PNMT reaction is terminated by the addition of a borate buffer, pH 10, the radioactively labeled product is separated by organic solvent extraction, and radioactivity is measured in a liquid scintillation counter. Samples that contain known quantities of octopamine or phenylethanolamine are assayed in parallel to make it possible to express the results of the assay in terms of nanomoles of product formed. One advantage of the coupled radiochemical assay over the procedures used prior to its introduction is that "blank" values are significantly lower and the sensitivity of the assay is thus greater because the amine substrate of the DBH reaction is not itself radioactive.

Although the coupled radiochemical assay is very sensitive, it does have drawbacks. Increasing concentrations of the amine substrate for DBH (e.g., tyramine) inhibit the PNMT catalyzed step (129, 196). Therefore, the greatest sensitivity is obtained with nonsaturating levels of substrate, and the substrate concentrations most often used in practice have been very close to the K_m values. It is primarily for this reason that controversy has developed as to whether the relative DBH activity values obtained with this assay are comparable to those obtained with assay methods that use saturating concentrations of substrate (see below). Controversy has also developed as to whether copper sulfate or N-ethylmaleimide should be used to counteract the effects of serum DBH inhibitors. Although similar activities are obtained in the presence of both at optimal concentrations (138), copper sulfate inhibits DBH as its concentration is increased above these values (129). Therefore, a titration must be performed in each tissue studied to determine optimal concentrations of $CuSO_4$. In addition, $CuSO_4$ inhibits the PNMT step of

the reaction. Therefore, one modification of the assay involves the addition of the chelating agent disodium ethylenediamine tetraacetate (EDTA) to the mixture of ^{14}C-S-adenosyl-*l*-methionine, PNMT, and buffer used to terminate the DBH reaction (21). EDTA prevents the inhibition of PNMT by $CuSO_4$. EDTA also inhibits DBH, so the addition of this reagent helps to insure that the first stage of the reaction is terminated. Other modifications of the original procedure have been described (89), but most of them have not been widely adopted.

Even though the coupled radiochemical assay has been used extensively by investigators who measure DBH activity in tissue homogenates and in the serum of experimental animals, other assay procedures have been described and have found wide acceptance, particularly for the measurement of human serum DBH. The most popular of these and probably the most commonly used procedure for the determination of DBH activity in human blood is a spectrophotometric assay (138). In this assay tyramine is converted to octopamine by DBH, and the octopamine is converted to *p*-hydroxybenzaldehyde by sodium periodate treatment after isolation of octopamine by ion exchange chromatography (Fig. 1). The *p*-hydroxybenzaldehyde is measured spectrophotometrically. The advantages of this procedure include the lack of requirement for expensive radioactive isotopes or the necessity to purify PNMT. In addition, DBH activity can be measured in the presence of saturating concentrations of substrate since there is not a second enzymatic step that is inhibited by the amine substrate. The major disadvantage of the procedure is its relative lack of sensitivity. It was pointed out in the initial description of the assay that it could not be used for the measurement of DBH in the serum of experimental animals (138). In addition, the assay is not sufficiently sensitive to measure the enzyme activity in many adrenergically innervated tissues. Several modifications of this procedure have been described which attempt to increase its sensitivity. In one of these 2-^{14}C-tyramine is used as substrate. The tyramine is converted to radioactively labeled octopamine, which is then converted to ^{14}C-*p*-hydroxybenzaldehyde. The final radioactive product is separated by organic solvent extraction (137, 209). This method is reported to be sensitive enough to measure DBH activity in rat serum. However, one of the difficulties with this approach, a difficulty that plagued the DBH assays of the late 1960s, is the problem of high "blanks." To obtain acceptable blank values the radioactively labeled tyramine substrate must be purified by paper chromatography. Another modification of the periodate cleavage assay is based on the use of dual-wavelength spectrophotometry. The spectrophotometric assay is performed as originally described except for the addition of a step in which the *p*-hydroxybenzaldehyde is separated by organic solvent extraction and its absorbance is then measured by dual-wavelength spectrophotometry (91, 92). This procedure is reported to be sensitive enough to measure DBH activity in the serum of experimental animals and in homogenates of many sympathetically innervated tissues. Yet another modification of the periodate cleavage assay involves the use of 1-^{14}C-tyramine as substrate. This compound is converted to 1-^{14}C-octopamine by DBH, and radioactively labeled formamide is produced by periodate cleavage (Fig. 1). The ^{14}C-formamide is oxidized to $^{14}CO_2$ by $KMnO_4$, and the $^{14}CO_2$ is trapped prior to the measurement of its radioactivity (85). This procedure has also been reported to be sensitive enough to measure DBH activity in rat serum. Recently, high performance liquid chromatography has been used to separate products of the DBH reaction which are then detected fluorometrically (49, 58). Undoubtedly, additional assay procedures will appear in the future. An attempt has been made here to summarize the principles of only those procedures that are most widely used at the present.

The question of whether similar relative enzyme activities are measured with the two most commonly used assays, the coupled radiochemical procedure and the spectrophotometric assay, is an important one. A very high correlation of the relative enzyme activities measured with both procedures in 15 randomly selected blood samples was reported in one early experiment (116). In a larger study of 93 blood samples from consecutive randomly selected blood donors at the Mayo Clinic, DBH activity was measured with both methods (Fig. 2). The spectrophotometric method was performed exactly as described by Nagatsu and Udenfriend (138) and the coupled radiochemical assay was performed with phenylethylamine as substrate (196, 197, 201). The average activities of these samples were 47.4 ± 2.5 I.U. (mean ± S.E.M.) by the spectrophotometric method and 796 ± 45 nmol/hr/ml by the coupled radiochemical method (Fig. 2). The correlation coefficient for DBH activities measured with the two methods was 0.94 ($P < .001$) (Weinshilboum, unpublished observation). Based on an analysis of the data shown in Figure 2, the results of the two methods could be interconverted best by multiplying the results of the spectrophotometric method by 18.9 or by dividing the results of the coupled radiochemical assay by 18.9 (42). These data make it much less likely that differences in the results of experiments performed with the two assays can be attributed to systematic differences in relative enzyme activities due to the method of measurement.

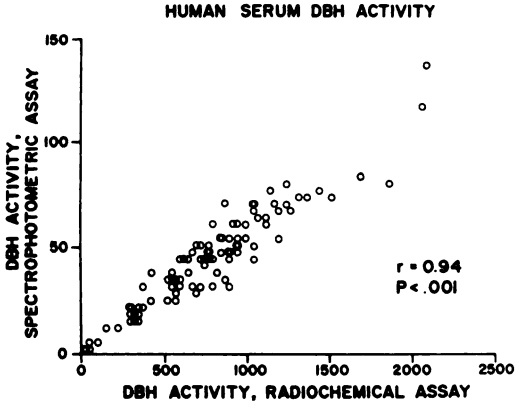

FIG. 2. Correlation of serum dopamine β-hydroxylase (DBH) activity measured with two different assays. Serum DBH activity was measured in 93 randomly selected subjects with the spectrophotometric and the coupled radiochemical assay. Each point represents the mean of duplicate determinations.

The term "serum" DBH has been used consistently to this point. DBH activity is also easily measured in plasma from blood anticoagulated with heparin. There is no difference between the enzyme activity measured in serum or in heparinized plasma (196), but, since DBH is a copper-containing enzyme that is inhibited by chelating agents (72), plasma from blood anticoagulated with chelating agents should not be used to assay enzyme activity. In the subsequent discussion "plasma" DBH will always refer to samples from blood anticoagulated with heparin.

In summary, there are several methods for the measurement of serum DBH enzymatic activity, but only two, the coupled radiochemical assay and the spectrophotometric method have been widely used. Each procedure has advantages and disadvantages. The spectrophotometric method has gained the widest use clinically for the measurement of enzyme activity in human serum samples. The relative lack of sensitivity of this method and the fact that it cannot be used for the determination of DBH activity in serum samples or tissue homogenates from experimental animals is a serious limitation. However, the spectrophotometric method is probably the method of choice for a laboratory that is engaged only in the routine measurement of human serum DBH activity. In a laboratory in which DBH activity will be measured in samples of serum or tissue homogenates from experimental animals, the coupled enzyme radiochemical assay offers sensitivity adequate for both animal experiments and for certain biochemical and genetic studies of the very low levels of enzyme activity in some human serum samples. Relative values obtained with these two different methods of assay are highly

correlated, at least in samples obtained under "basal" conditions.

C. Normal Values of Serum DBH Activity

It is important to have available "normal" values for serum DBH activity in man and experimental animals. Unfortunately, direct comparisons of the results of independent studies are often difficult because a variety of assay methods, substrates, and methods for the expression of enzyme activity have been used. Whenever an attempt is made to compare results it must be remembered that two different substrates, tyramine and phenylethylamine, have been widely used, that subsaturating levels of substrate are used in the coupled radiochemical assay, and that the results of a particular set of experiments may be expressed as μmol/min/L (I.U.), nmol/hr/ml, nmol/20 min/ml, etc. In addition, in some early reports the reaction was performed at a pH, 6.0, that resulted in lower activity than that found in subsequent studies performed at the pH optimum for the enzyme. An attempt has been made in Table 1 to summarize representative values of serum DBH activity that have been reported with different assay techniques and substrates. At least two observations may be made on the basis of the data shown in the table. First, the average serum enzyme activities in adult experimental animals is about 10 to 100 times less than that in adult human blood. The earliest studies of serum DBH showed that the rat serum enzyme activity is much less than that in adult human blood, and all experimental animals studied thus far have much lower serum DBH activity than does man (66, 196). Second, the values for large human population studies

TABLE 1

*Serum dopamine β-hydroxylase (DBH) activity in man and experimental animals**

Species	N	Activity (mean ± SEM)	Range	Units	Substrate	Method	Ref.
Man	54	42.6 ± 3.7	3–100	μmol/min/L	Tyramine	Spectrophotometric	138
Man	100	31 ± 3	2–100	μmol/min/L	Tyramine	Spectrophotometric	184
Man	114	35	0–105	μmol/min/L	Tyramine	Spectrophotometric	77
Man	90	561	17–1743	nmol/hr/ml	Tyramine	Coupled radiochemical	84
Man	93	675 ± 48	0–2145	nmol/hr/ml	Phenylethylamine	Coupled radiochemical	4
Man (age 6–12)	841	686 ± 15	0–2228	nmol/hr/ml	Phenylethylamine	Coupled radiochemical	202
Man	227	682 ± 28	0–2450	nmol/hr/ml	Phenylethylamine	Coupled radiochemical	202
Rat	5	0.37 ± .01		μmol/min/L	Tyramine	Spectrophotometric (dual wavelength)	92
Guinea pig	5	0.37 ± .08		μmol/min/L	Tyramine	Spectrophotometric (dual wavelength)	92
Japanese monkey	4	0.59 ± .01		μmol/min/L	Tyramine	Spectrophotometric (dual wavelength)	90
Rat (Sprague-Dawley)	6	20 ± 2.2		nmol/hr/ml	Tyramine	Coupled radiochemical	151
Dog	8	2.7 ± 0.4		nmol/hr/ml	Tyramine	Coupled radiochemical	154

* Serum DBH activities from studies of large numbers of human subjects and studies of several species of experimental animals are shown. The table includes data with regard to the assay method, the method of expression of enzyme activity, and the substrate used. Values expressed per 20 or 30 min of incubation have been corrected to equivalent values for a 1-hr incubation.

performed with the same assay methods and substrates are fairly similar although in occasional studies values quite different from those usually found have been reported (71). A third observation that cannot be made on the basis of the data shown in the table is that the distribution of human serum DBH values is not "normal" in shape but is skewed (116, 200, 201) (see Fig. 4, *middle panel*). This skewness can best be "corrected" by the use of the square root of DBH rather than the enzyme activity itself (200, 201). Such a transformation is useful for the statistical treatment of data from large population studies. The shape of the frequency distribution will prove to be important in the subsequent discussion of the role of inheritance in the regulation of the enzyme activity in man. It is also important to determine whether there are significant differences in human serum DBH activity among subjects of different ages, between men and women, or among different racial groups. There are very striking changes in serum DBH in the course of growth and development, and these changes are discussed in detail below. No significant male-female differences have been reported in any of the large population surveys that have been performed (84, 123, 201). Although several studies have shown that DBH activity is slightly lower in the blood of black than in that of white subjects (84, 123), one population survey of a large number of black and white subjects in the United States failed to demonstrate a significant racial difference in serum enzyme values (123). Finally, serum DBH values in normal subjects are quite stable over long periods of time. This observation was made in the earliest reports of serum DBH activity in man (196), and has been confirmed repeatedly in subsequent studies (71, 84, 146).

D. Immunoassay of Serum DBH

1. Introduction. Soon after DBH activity was observed in blood, the question of whether the quantity of circulating DBH protein is correlated with the serum enzymatic activity was raised. It is possible that the measurement of DBH protein might yield more information about the exocytotic release of catecholamines than the measurement of enzyme activity if a large percentage of the enzyme is inactivated during release from nerves or the adrenal medulla. The answer to this question, a question of whether there is a direct positive correlation of serum DBH enzymatic activity and serum DBH protein content, has been the subject of controversy. In retrospect one of the reasons that different laboratories obtained different results in their measurements of IDBH was that the relative lack of species crossreactivity of anti-DBH antibodies was not always appreciated. The two techniques that have been used for the measurement of serum DBH protein levels are immunotitration and radioimmunoassay.

2. Immunotitration of serum DBH. Immunotitration and immunoprecipitation use antibodies against DBH to titrate or to precipitate the enzyme activity. Increasing amounts of the antibody are added to serum samples, and the quantity needed to remove or inactivate a specific proportion of the enzyme activity is determined. The underlying assumption is that the quantity of antibody needed to titrate or to precipitate the enzyme is directly related to the quantity of enzyme protein present in the sample. Unfortunately, absolute quantitation of DBH is not possible with this technique, and the sensitivity of the method decreases with decreasing basal levels of enzyme activity since the procedure is based on the measurement of the enzyme activity that remains after titration. However, immunotitration does allow a relative quantitation of DBH protein and thus a determination of whether immunoreactive DBH (IDBH) and the enzymatic activity are correlated. In one early study antibodies against purified bovine adrenal DBH were used in an immunoprecipitation procedure. There was a direct positive correlation be-

tween the quantity of antibody needed to titrate DBH and the enzyme activity in blood samples from 50 randomly selected blood donors ($r = 0.9$, $P < .001$) (18). In a subsequent study antibodies against DBH purified from a human pheochromocytoma were used, and, once again, a direct positive correlation of IDBH with enzymatic activity was found ($r = 0.96$, $n = 61$, $P < .001$) (95). Finally, immunoprecipitation with an antibody against DBH purified from human adrenal glands also showed a significant positive correlation of serum enzymatic activity with IDBH values ($r = 0.94$, $n = 38$, $P < .001$) (33). All of these data, including those obtained with antibody to heterologous DBH, were compatible with the conclusion that there is a direct positive correlation between DBH enzymatic activity and the quantity of IDBH in human blood. Unfortunately, antibody to heterologous DBH did not give the same results as antibody to homologous DBH in radioimmunoassay studies (34, 40, 169, 170).

3. Radioimmunoassay of serum DBH. The first radioimmunoassay for serum DBH used antibody against purified sheep adrenal enzyme. When sheep adrenal DBH was used as antigen, average IDBH values of 0.59 ± 0.02 μg/ml were found in blood from five sheep (167). When this assay was used to measure human plasma IDBH, apparent values that ranged from 80 to 160 ng/ml were found (167). However, the authors of this study pointed out that it was not possible to quantitate human IDBH accurately because of the lack of a homologous antigen. In a later study DBH was purified from human pheochromocytoma, antibodies were prepared against this antigen, and a radioimmunoassay was developed. Human plasma IDBH levels with this radioimmunoassay ranged from approximately 5 to 40 μg/ml, and there was a direct positive correlation of IDBH with enzymatic activity measured with the coupled radiochemical assay ($r = 0.8$, $n = 60$, $P < .01$) (40). It was reported in 1974 that radioimmunoassay with antibody against bovine adrenal DBH and an antigen purified from human adrenal glands showed human serum IDBH values of 11.7 ± 34.4 μg/ml with "a complete lack of correlation" of IDBH with enzymatic activity (169). However, a subsequent study by the same authors found an "excellent" correlation between human serum IDBH and DBH enzymatic activity with a radioimmunoassay that used antibody against homologous enzyme ($r = 0.98$, $n = 52$) (170). The values for IDBH in this series ranged from 0.5 to 7.0 μg/ml. The authors speculated that the difference in results obtained with antibodies against homologous and heterologous protein were due to lack of crossreactivity with the human enzyme of antibody against bovine adrenal DBH. Finally, a radioimmunoassay study that used three separate anti-human DBH antibodies (one anti-adrenal and two anti-pheochromocytoma) showed a direct positive correlation of IDBH levels and enzyme activity with all three antibodies (34). The correlation coefficient for samples from 134 randomly selected subjects aged 16 to 18 years was 0.84 ($P < .001$). The average IDBH levels in these samples was 824 ± 38 ng/ml (mean ± S.E.M.). It now seems to be established that measurement of IDBH in man yields essentially the same information as the measurement of DBH enzyme activity under most conditions. Of course, the possibility still exists that in selected pathological states or under certain physiological conditions the correlation of serum IDBH with DBH activity will not hold.

E. Conclusion

Although much remains to be learned about the measurement of DBH activity and IDBH levels in blood, in man the results of the two most commonly used assay procedures, the coupled radiochemical assay and the spectrophotometric method, correlate well. The choice of an assay procedure depends on the specific needs of a given laboratory and the purpose of the experiments. When antibodies to homologous protein are used, there is an excellent correlation of IDBH levels with DBH en-

zymatic activity measured by either of the commonly used enzyme assay procedures.

IV. Source and Fate of Serum DBH

A. Introduction

The suggestion that serum DBH might serve as a measure of sympathoadrenal function was based on the assumption that the enzyme in blood originates from sympathetic nerves and/or the adrenal medulla. Sympathoadrenal "function" has been assumed to mean the exocytotic release of catecholamines. The assumption that serum DBH might reflect exocytosis resulted from the demonstration of the coupled proportional release of DBH and catecholamines during in vitro stimulation of the adrenal medulla and sympathetic nerves (29, 61, 177, 190, 203). Furthermore, it has often been assumed that only the "soluble" and not the "membrane bound" DBH is released and eventually finds its way into the circulation. A schematic representation of this hypothetical series of events is shown in Figure 3. It is important that the assumptions that underlie this scheme be tested experimentally. In addition, it is essential that the biochemical mechanism by which DBH is removed from the circulation be understood if serum levels of the enzyme activity are to be interpreted correctly. Technical and ethical considerations have limited studies of the source and fate of human serum DBH; so the majority of data have come from experiments with laboratory animals. Caution must be exercised in the extrapolation of these results to man because of differences between man and experimental animals in basal serum enzyme activities, in patterns of change in the enzyme activity during development, and in the genetic regulation of serum DBH.

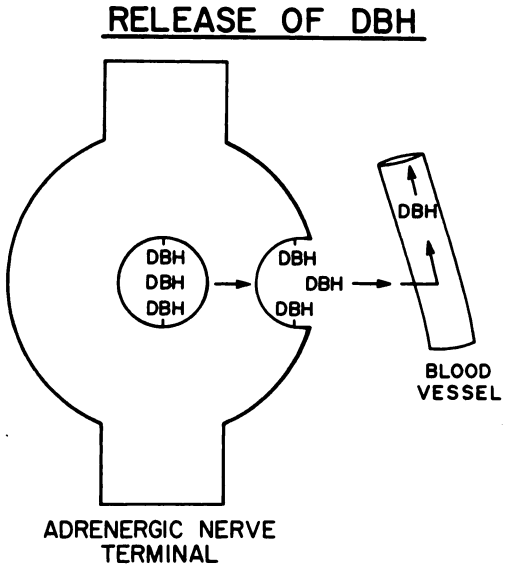

FIG. 3. The release of dopamine β-hydroxylase (DBH) from an adrenergic varicosity. A schematic representation of the release of DBH from an adrenergic varicosity is shown. Some of the assumptions that underlie this scheme have been challenged (see text for details).

B. Source of Serum DBH

The biochemical and immunological characteristics of serum DBH are similar to those of the enzyme in the adrenal medulla. However, the biochemical similarity of serum DBH to the enzyme in solid tissues does not prove that the source of the circulating enzyme is the adrenergic neuron or the adrenal medulla. Removal of the adrenal gland or the adrenal medulla does not result in a significant decrease in serum DBH activity in the rat (195, 199). Treatment of rats with intravenous 6-hydroxydopamine in doses that destroy the sympathetic nerves in the heart and spleen results in about a 25% reduction in the serum enzyme activity, whether or not adrenal medullary tissue is present (195). Decreases of rat serum DBH activity of more than 40% occur when animals are treated with very high doses of 6-hydroxydopamine (22). Prolonged treatment of rats with guanethidine in doses that destroy sympathetic nerve terminals results in a 95% reduction in cardiac DBH activity and a 50% reduction in serum DBH activity (73). A factor common to these pharmacological experiments is the destruction of sympathetic nerve terminals. The results of experiments with both 6-hydroxydopamine and guane-

thidine are compatible with the conclusion that a large part of the serum DBH activity originates from sympathetic nerve terminals. However, these results do not entirely rule out an extraneuronal contribution to the circulating activity, nor do they prove that coupled release with catecholamines during exocytosis is the process by which all of the enzyme escapes from nerves into the blood.

Several investigators have studied the mechanism of DBH release into blood by measuring both plasma catecholamines and DBH in rats treated with drugs that alter adrenergic activity. For example, treatment of rats with bretylium for 4 hours results in a greater than 50% decrease of plasma norepinephrine in control and adrenalectomized animals, but no change in serum DBH activity (159). Treatment of rats for 48 or 72 hours with the ganglionic blocking drug chlorisondamine reduces plasma norepinephrine to virtually undetectable levels, but there is no change in plasma DBH activity (159). Exposure of rats to phenoxybenzamine, a drug that increases release of both norepinephrine and DBH from nerve stimulated preparations in vitro (87), presumably by blockade of presynaptic *alpha*-adrenoceptors (108), results in a 3-fold increase in plasma norepinephrine levels after 24 and 48 hours that is associated with a slight decrease in serum DBH activity (159). Although such data have raised doubts about exocytotic release with catecholamines as the source of basal serum DBH activity, they cannot be interpreted without knowledge of the half-life of the circulating enzyme. However, these results have recently been confirmed and extended with an experimental approach that allows estimates of the half-life of serum DBH to be made. The technique involves injection into rats of antibodies against rat DBH. A profound reduction in serum DBH activity occurs after the injection of an appropriate quantity of antibody, a reduction thought to result from the rapid clearance from the circulation of immune complexes between antibody and endogenous enzyme. The kinetics of DBH entry and exit from the circulation may then be estimated on the basis of the rate of return of serum DBH activity to normal levels (74). After treatment with chlorisondamine for 11 days at doses that result in a 3-fold reduction in plasma catecholamine values, there is no difference in the turnover of DBH determined with this technique, i.e., its rate of entry into the circulation is unchanged (75). Likewise, after 11 days of treatment with phenoxybenzamine, treatment that results in a doubling in plasma catecholamine levels, there is no change in serum DBH turnover (75). These results have led to the suggestion that although the source of most of the rat serum DBH is the adrenergic neuron, much of the enzyme may enter the blood by a process that does not involve the coupled exocytotic release of enzyme with catecholamines (75, 159). The interpretation of these data depends on the half-life of rat serum DBH—and thus on the rate and mechanism of its removal from the circulation as well as on the rate of entry. These subjects will be discussed next.

C. Fate of Serum DBH

An early attempt to study the fate of serum DBH involved the intravenous injection of ^{125}I-labeled purified ovine adrenal DBH into sheep (167). Half-life values of about 3 hours were calculated from the rate of disappearance of labeled enzyme after a 2-hour infusion. The highest levels of radioactivity after infusions were found in the lungs, kidneys, and liver (167). Two different techniques have been used to estimate the half-life of rat serum DBH. One approach has involved the intravenous injection of purified bovine adrenal DBH into rats followed by the repetitive removal of blood samples and the measurement of enzyme activity. The results of these experiments revealed a biphasic decline in DBH activity. There was an initial rapid phase with a half-life of 2.2 to 3.0 hours followed by a second phase with a half-life of 4.5 to

5.5 days (64). Exogenous DBH activity appeared in thoracic duct lymph very quickly in these experiments, and the DBH activities of lymph and plasma were similar within 2 to 3 hours (64). The results of studies performed with both cats and dogs are compatible with the conclusion that lymph may be one avenue for the entry of this large molecular weight protein into the blood (81, 164). The results of similar studies in man are difficult to interpret (1). Furthermore, no evidence of pulmonary inactivation of human serum DBH was found in a study of pulmonary artery and left ventricular enzyme levels in 14 subjects (181). A different approach to the determination of the half-life of rat serum DBH involves the procedure described above in which anti-rat DBH antibody is injected into animals. The estimated half-life of serum DBH in adult Sprague-Dawley rats measured with this technique is approximately 4.2 days (74). It has been suggested that the relatively short 3-hour half-life estimated in sheep might have represented the initial "mixing" or redistribution phase of the injected enzyme (64).

Very little is known of the biochemical events associated with the removal of DBH from the circulation. The metabolic fate of many other plasma glycoproteins is related to the structure of the carbohydrate portion of the molecule. For example, if a terminal sialic acid residue is removed from many of these proteins, their half-life in blood is shortened (130). Galactose is often exposed by the removal of terminal sialic acid, and a hepatocyte membrane binding site for galactose is thought to play an important role in the removal from the circulation of desialylated plasma glycoproteins (189). Whether similar biochemical factors affect the half-life of serum DBH is not known.

D. Conclusion

Sympathetic nerve terminals in the rat represent the source of much if not most of the circulating DBH. The half-life of the rat serum enzyme is approximately 4 days. Most experiments have shown significant decreases in serum DBH activity after treatment with drugs that destroy sympathetic nerve terminals, but very little change in the serum enzyme activity has been found after pharmacological treatment that alters the release of norepinephrine in response to nerve stimulation. These results have raised serious questions with regard to the assumption that basal serum DBH activity in the rat results entirely from the coupled exocytotic release of enzyme with catecholamines. However, the results of many experiments indicate that quantitatively small but significant increases in rat serum DBH activity occur during high levels of sympathetic nervous system activity (see below). Finally, there is very little information available with regard to the source, half-life, and fate of human serum DBH, and the relationship of the observations made in the rat to the situation in man is unclear.

V. Regulation of Serum DBH

A. Introduction

Information with regard to the source and fate of serum DBH does not give a complete picture of the regulation of the circulating enzyme activity. When serum DBH was first suggested as a measure of adrenergic function, it was assumed that the major source of variation in the enzyme activity would prove to be the rate of nerve stimulation induced release of DBH and catecholamines, presumably via exocytosis. It has subsequently become clear that a variety of factors are involved in the regulation of serum DBH and that the release of enzyme with catecholamines is probably only one factor among many that may contribute to variation in the enzyme activity in a given subject or to individual variations among a group of subjects. For example, there are dramatic changes in serum DBH activity in experimental animals and man during growth and development; genetic factors play an extremely important role in individual variations in man; and drugs, stress, and hormones can all alter the en-

zyme activity. In the following discussion the regulation of serum DBH by each of these factors will be reviewed.

B. Effects of Growth and Development on Serum DBH

1. Developmental changes in experimental animals. In 1974 it was reported that serum DBH activity is much higher in 3-week old than in 6- or 14-week old rats (135). Several subsequent studies demonstrated that the enzyme levels are 2 to 5 times as high in newborn as in adult Sprague-Dawley rats; that serum DBH values rise to a peak approximately 3 to 7 times adult levels at 14 to 18 days of age; and that the enzyme activity then declines rapidly to adult values by about 50 to 60 days of age (12, 98, 107, 151). This pattern contrasts with the changes in DBH activity in sympathetically innervated organs of the rat such as the heart or salivary glands in which activity increases from birth to about 2 weeks of age and then remains constant (107, 151). The same pattern of change in serum DBH activity between 14 and 60 days of age has been found in a variety of rat strains (151). No significant decrease in total plasma catecholamine levels occurs during this time interval. However, the blood for these studies was obtained by decapitation (151), a procedure now known to result in the acute elevation of plasma catecholamine levels (158).

Changes in rat serum DBH activity during growth and development might result from changes in the functional activity of the sympathetic nervous system, from changes in the biochemical properties of DBH (i.e., "fetal" and "adult" forms of DBH), from changes in the access of DBH to the blood, or from changes in its rate of removal from the circulation. No differences in the biochemical properties of DBH were detected in the blood of 2-week-old and 8-week-old rats (139), and, in one series of experiments, there was very little change in the apparent quantity of rat serum IDBH between 2 and 8 weeks of age, a time interval during which enzymatic activity decreases 5-fold. However, IDBH was measured in these experiments with an immunoprecipitation procedure that used heterologous (anti-bovine adrenal) antibody (151). Subsequent experiments with homologous (anti-rat adrenal) antibody have shown the expected proportionate and parallel decreases in IDBH and DBH enzymatic activity between 2 and 8 weeks of age (Grzanna and Coyle, unpublished observation). These results emphasize once again the dangers involved in the use of antibodies to heterologous DBH. Experiments in which young and older rats have been injected with antibody to homologous DBH have shown that the rates of degradation and the half-lives of serum DBH are similar in young and adult animals, but that the rate of entry of DBH into the circulation is four times faster in young than in older Sprague-Dawley rats (Grzanna and Coyle, unpublished observation). The biochemical or physiological mechanism responsible for the increased rate of entry of DBH into the circulation of young rats is not clear. It is clear that the pattern of change in serum enzymatic activity with growth and development in the rat is very different from that in man or in the only nonhuman primate that has been studied, the Japanese monkey (*Maracci fuscata fuscata*). Serum DBH activity in these monkeys increases approximately 10-fold between 3 months and 10 years of age (90). There is no dramatic decline in activity with maturation. The pattern of change in the Japanese monkey during growth and development is similar to that which takes place in man, but the final adult enzyme levels in these and other nonhuman primates are only about $^1/_{100}$ those in human blood (90).

2. Developmental changes in man. Human serum DBH activity increases sharply during the first years of life. In a group of 146 randomly selected subjects aged 1 day to 39 years, there was a striking increase in enzyme activity during the first 4 to 5 years of life with no subsequent change up to age 39 (197). The average serum DBH activity in 10 children under 6 months of age was only about one-fifth to one-sixth of that in adults. A study of 84 subjects from 1 day to

60 years of age showed that the activity increased an average of 12-fold from the first year of life to the age range 20 to 40 (53). DBH enzymatic activity in the umbilical cord blood of newborn infants has been measured with several assay techniques (58, 198, 204) and IDBH levels have been determined by radioimmunoassay with homologous antibody (198). The values for newborn infants have all been very low when compared with values in adult subjects. For example, in 32 consecutive, randomly selected cord blood samples obtained after normal full-term deliveries, the average enzyme activity measured by coupled radiochemical assay with phenylethylamine as substrate was 8.3 ± 1.8 nmol/hr/ml (mean ± S.E.M.) (198), a value that may be compared with an average of 686 ± 14.6 in 841 randomly selected children aged 6 to 12. IDBH measured by radioimmunoassay in eight randomly selected samples averaged 17.7 ± 4.2 ng/ml compared with an average level of 824 ± 38 ng/ml in 134 randomly selected adolescents (34). Population surveys of large numbers of school children (841 subjects aged 6 to 12 and 630 subjects aged 13 to 18) have shown that enzyme values by age 6 are comparable to those in adult subjects with the possibility of a small peak in early adolescence (192, 201, 202). Other population surveys have indicated that a slight decrease in activity might occur after age 50, although the number of older subjects studied is probably not adequate to reach a firm conclusion (147). In summary, human serum DBH activity and IDBH levels are very low at birth but increase in most subjects by several orders of magnitude during growth and development. The biochemical basis of this increase is unknown. The relationship of the increase in serum DBH to the genetic regulation of the human serum enzyme is discussed below.

C. *Effects of Inheritance on Serum DBH*

1. Introduction. Measurement of serum DBH activity in subjects with genetic diseases such as familial dysautonomia (see below) raised the possibility that inheritance might play a role in the regulation of the circulating enzyme activity. These observations stimulated biochemical genetic studies of serum DBH, studies that have shown that most of the variation of DBH activity in a randomly selected human population is due to the effects of inheritance. Much more is known about the genetic regulation of serum DBH in man than is known about the effect of inheritance on the enzyme in experimental animals.

2. Biochemical genetics of serum DBH in the rat. There are significant differences in serum DBH activity among inbred strains of rats, but the largest of these is less than 2-fold. The mechanism of inheritance of rat serum DBH activity appears to be complex (183 and Stolk, Hurst, Van Rippen, and Harris, unpublished observation). Although the results of genetic studies in rodents are only preliminary in nature, the differences between the situation in man and the rat strains studied thus far are striking. In man there are orders of magnitude differences in the levels of enzyme activity among individuals, and the trait of low enzyme activity is inherited in a simple monogenic (mendelian) fashion (202).

3. Biochemical genetics of serum DBH in man. Investigation of the biochemical genetics of human serum DBH has proceeded in a stepwise fashion. The possibility that inheritance might play a role in the determination of the enzyme activity was raised when significant correlations of serum DBH activities among relatives were reported. This observation led to the examination of distributions of enzyme activities in large populations. These distributions included a subgroup, approximately 3 to 4% of the population, with very low DBH enzymatic activity. The results of family studies were compatible with the monogenic (mendelian) inheritance of the trait of low serum DBH activity. Radioimmunoassay studies demonstrated that low enzymatic activity and low IDBH segregate together in families. Finally, other variant forms of the enzyme were described that did not segregate with the trait of low en-

zymatic activity but which also had a significant familial aggregation.

An early study of serum DBH activity in 317 randomly selected children aged 6 to 12 years demonstrated a highly significant sibling-sibling correlation of enzyme activity in 94 sibling pairs ($r = 0.57$, $P < .001$) (201). If a trait is determined entirely by inheritance, i.e., if its heritability is 1.0, the correlation coefficient for the trait in monozygotic twins would be expected to be 1.0 while that in siblings, dizygotic twins, and parent-child pairs would be expected to be 0.5 (17). A later study of DBH activity in randomly selected families reported correlation coefficients of 0.50 for siblings, 0.51 for father-child pairs, and 0.48 for mother-child pairs (147). It is difficult to separate the effects of inheritance from those of shared environment when only family data such as these are available. Therefore, it was significant that a separate group of investigators reported a correlation coefficient for plasma DBH activity in monozygotic twins of 0.96 and in dizygotic twins of 0.75 (166). The heritability of serum DBH activity has been estimated to be 0.90 on the basis of data from a separate twin study (112).

Although family and twin studies indicate that the heritability of plasma DBH activity is high, these data give no information about the mechanism of inheritance, i.e., whether inheritance is monogenic or polygenic. This question is of practical importance because of the possibility that the biochemical basis of a monogenically inherited trait might be elucidated much more easily than that of a trait determined by a large number of genetic loci. It was noted during a survey of DBH activity in 841 randomly selected children that while the average value for the entire population was 683 nmol/hr/ml, a subgroup of approximately 3 to 4% of the population had very low levels of activity (< 50 nmol/hr/ml, Fig. 4, *middle panel*) (201, 202). DBH activity was measured in blood from first degree relatives of individuals with very low enzyme activity in 22 families, and the results of pedigree and segregation analyses were compatible with the autosomal recessive inheritance of an allele for very low serum DBH activity (202). This is a com-

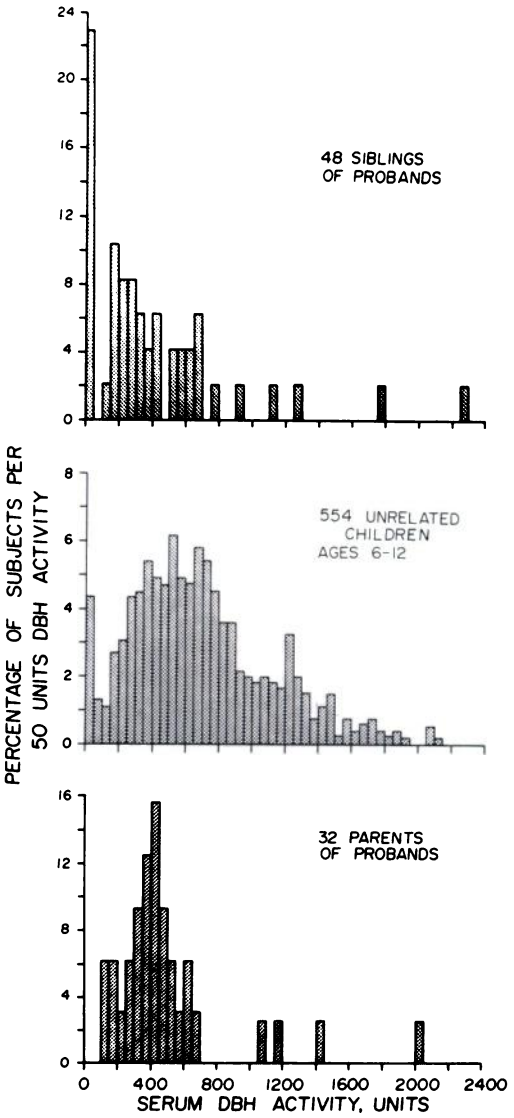

FIG. 4. Distributions of serum dopamine β-hydroxylase (DBH) activities. *Top panel*: Serum DBH values in siblings of probands with very low activity (< 50 nmol/hr/ml) from matings of two heterozygotes for the trait of low enzyme activity, DBH^{l}. Approximately one-fourth of these children have very low enzyme activity. *Middle panel*: Serum DBH activities in 554 unrelated, randomly selected children aged 6 to 12 are shown. This frequency distribution does not differ significantly from that for 227 unrelated adults. *Bottom panel*: Serum DBH activities in blood samples from parents of children with very low enzyme activity (< 50 nmol/hr/ml) who themselves had activity greater than 50 nmol/hr/ml are shown. [By permission of the Mayo Clinic Proceedings (183).]

mon variant with an estimated gene frequency of approximately 20%. About one-third of a randomly selected population is heterozygous for the trait. The enzyme activity in the blood of obligate heterozygotes for the allele for very low enzyme activity is intermediate between that in homozygous subjects and that in a randomly selected population (Fig. 4, *bottom panel*). In addition, in matings of two heterozygotes, 22% of the siblings of probands with low activity had very low activity, a figure much higher than the 3 to 4% found in a randomly selected population and very close to the 25% figure predicted for mendelian inheritance (Fig. 4, *top panel*) (202). Measurement of serum DBH activity in a large kindred have confirmed the autosomal recessive inheritance of the trait of low serum DBH (42), and this polymorphism has been estimated to be responsible for between 50 and 75% of the population variance of serum DBH activity in man (42). The allele for very low serum DBH activity has been referred to as "d" with the alternative allele designated "D" (202), but it has now been proposed that the alleles for low and higher enzymatic activity be designated DBH^L and DBH^H, respectively (36, 193), to conform to the recommendations of the Committee on Nomenclature of the Third International Workshop on Human Gene Mapping (20).

There are many biochemical mechanisms that might explain the inherited differences in serum DBH activity due to the allele pair DBH^L and DBH^H. Several of these mechanisms would result in a decrease in the quantity of DBH protein in the blood of subjects homozygous for the trait. This possibility has been tested experimentally by the measurement of IDBH by both immunoprecipitation and radioimmunoassay. There is a direct positive correlation between enzymatic activity and IDBH measured by both immunoprecipitation and radioimmunoassay in the blood of subjects whose genotype for the allele pair DBH^H and DBH^L has been determined by family studies (33, 34). Approximately 3 to 4% of a randomly selected population has very low levels of IDBH (< 100 ng/ml), and the traits of low IDBH (< 100 ng/ml) and of low DBH enzymatic activity (< 50 nmol/hr/ml) segregate together in family studies (34). These results are compatible with the conclusion that the allele for very low enzymatic activity, DBH^L, results in a decrease in the total quantity of DBH protein in blood.

Other variant forms of human serum DBH have been described that have not been as well characterized as the common polymorphism for very low enzymatic activity. For example, in the course of the radioimmunoassay experiments described above, one family was discovered in which the ratios of IDBH to enzymatic DBH of the mother and three of five children were much higher than those in any of the other 200 subjects tested (34). This "dissociation" of IDBH and enzymatic DBH values suggests the existence of a rare variant of DBH that is enzymatically less active than the usual form of the enzyme. Finally, there is a thermolabile variant of DBH that is found in approximately 10% of a randomly selected population. This variant also has a significant familial aggregation, but does not segregate with the trait of very low enzymatic activity (35, 37).

Genetic effects on the "realization" of an enzyme activity have been classified by Paigen (153) as those due to structural gene, regulatory gene, processing gene, or temporal gene effects. At birth all children have very low levels of both serum DBH enzymatic activity (< 50 nmol/hr/ml) and of IDBH (< 100 ng/ml), levels similar to those in adults homozygous for the allele DBH^L. There is a rapid increase in the mean enzymatic activity during the first years of life. Therefore, the effect of DBH^H is apparently a "temporal" gene effect as defined by Paigen, but the biochemical mechanism that underlies this effect is not known.

4. Conclusion. The results of biochemical genetic studies of human serum DBH activity require alteration in some of the early assumptions with regard to this enzyme activity in man. Included among these was the assumption that individual variations in DBH activity represent individual

variations in the exocytotic release of catecholamines. It is now clear that most of the variation in serum DBH activity in a human population is determined by the allele pair DBH^L and DBH^H (42). The relationship, if any, of this genetic variation to adrenergic function is unknown.

D. Other Sources of Regulation

1. Introduction. While the results of biochemical genetic studies have required that inheritance be assigned a much larger role in the regulation of serum DBH activity, the results of studies of the effects of pharmacological, humoral, and physiological manipulation have led to the conclusion that the circulating enzyme activity generally shows only quantitatively small changes in response to drugs, hormones, or short-term stress. The following discussion will examine the effects of these factors on serum DBH.

2. Circadian rhythm. A. EXPERIMENTAL ANIMAL STUDIES. There is a 24-hour rhythm of serum DBH in some strains of rat. The rhythm varies from strain to strain. In 50-day-old male Holtzmann rats, serum DBH activity is almost twice as great at 4 A.M. as at any other time in the day (11). However, a "dark phase" peak is not found in the blood of either 50- or 112-day-old Berkeley S1 rats (11). If 2-fold changes in rat serum DBH activity do occur during a 24-hour period in some strains, this fact will have to be taken into account in the design of experiments that use those strains.

B. HUMAN STUDIES. There have been several reports of a 24-hour rhythm of human serum DBH (46, 118, 149). A trough in enzyme activity occurs between midnight and 4 A.M. The magnitude of the change is small, only about 10% during a 24-hour period. The rhythm is present in blind subjects, and can be obliterated by either sleep deprivation or by having the subjects remain supine for 24 hours (46, 149). These latter observations have led to the suggestion that the 24-hour rhythm in man is related to activity and is not an intrinsic rhythm.

3. Drug and hormone effects. A. EXPERIMENTAL ANIMAL STUDIES. Rat serum DBH activity increases 100 to 150% 5 weeks after hypophysectomy (54, 106). One group of investigators has reported that there is no change in these elevated serum DBH levels after 3 days of treatment with ACTH (106), but another group found a significant decrease after 7 days of ACTH but not after dexamethasone treatment (54). Subcutaneous Pitressin for 4 days also resulted in a striking decrease in posthypophysectomy serum DBH activity (106), and Pitressin treatment reduced serum DBH activity in rats with inherited diabetes insipidus (212). These findings seem to indicate that both the adrenals and the posterior pituitary may play a role in the increase in serum DBH activity after removal of the pituitary. Even larger increases of rat serum DBH activity, 5- to 10-fold, occur in animals made diabetic by treatment with streptozotocin or alloxan (80). Although these observations have opened avenues for future investigation of the role of hormones in the regulation of serum DBH, it is not clear whether alterations in the rate of DBH entry into the blood or in the clearance of DBH are responsible for the changes in activity that occur with various types of endocrine manipulation. A variety of experiments have been performed in which drugs have been used to study the source and fate of DBH in laboratory animals, and the results of many of these experiments have already been described.

B. HUMAN STUDIES. Striking changes in human serum DBH activity occur in patients with thyroid disease or after treatment of patients with thyroid hormone or drugs that alter thyroid function. The average enzyme activity in 21 hyperthyroid patients was 19 I.U. compared with an average of 40 I.U. in 41 normal subjects. DBH activity increased from an average 22.3 I.U. to an average of 40.5 I.U. in six hyperthyroid patients during therapy that resulted in a decrease in serum thyroxin levels to normal (144). A separate study showed that the average DBH activity in 10 hypothy-

roid patients was almost twice that in 23 control subjects, and was significantly less in hyperthyroid patients than in control subjects (141). DBH values also rose as thyroxin levels fell during the treatment of hyperthyroidism in these patients.

Contradictory results have been reported with regard to changes in human serum DBH activity during the menstrual cycle. One group of investigators studied 12 women and reported that the enzyme activity peaks at values approximately 10% above average monthly levels soon after ovulation and decreases to values approximately 10% below the monthly average during the premenstrual period (105). However, no consistent relationship of either plasma norepinephrine or DBH to the menstrual cycle was found in a separate study of six subjects (213). The response of human serum DBH to hypoglycemia induced by insulin is also controversial. DBH activity has been reported to increase to 50% above control levels 1 hour after the intravenous injection of 0.15 I.U. of insulin per kg of body weight, but to be unchanged after the injection of 0.1 I.U. per kg (150). In the same study urinary epinephrine values rose to 5.6 and 4.7 times control levels, respectively, after the injection of the 0.15 I.U. and 0.10 I.U. per kg doses of insulin. However, another series of experiments failed to demonstrate a change in serum DBH activity after the injection of either 0.10 or 0.15 I.U. per kg (142). Finally, no differences were found between the average DBH activities of eight adrenalectomized patients and 41 control subjects (143).

Many experiments have been performed in which human serum DBH activity has been measured after treatment with drugs, usually drugs that alter adrenergic function. As might be expected, treatment of patients with known DBH inhibitors such as fusaric acid results in a striking decrease in the serum enzyme activity (121, 134). Of perhaps more interest are experiments in which drugs have been used as pharmacological probes to study either the source of human serum DBH or the effects of the drug on adrenergic activity. Contradictory results have been reported after treatment of patients with *beta*-adrenoceptor blocking agents such as propranolol. In one study it was reported that there was no change in the serum DBH activity of 15 hypertensive patients after 2 to 3 weeks of treatment with doses of propranolol that ranged from 80 to 750 mg per day even though blood pressure values decreased significantly (2). However, a small, approximately 10 to 20%, increase in DBH activity within 5 minutes after an oral dose of 120 to 240 mg of propranolol was reported by a separate group of investigators (38). Serum DBH activity in 10 subjects increased 23 to 36% 1 hour after the intravenous injection of 200 mg of theophylline (7), increased an average of about 10% 2 hours after treatment of eight subjects with 20 mg of oral *d*-amphetamine sulfate (119), and decreased an average of 20% in eight subjects after 2 days of treatment with fenfluramine, 120 mg per day (27).

In summary, with the exception of alterations in thyroid status or treatment with specific DBH inhibitors, most changes in human serum DBH activity in response to drugs and hormones have been quantitatively small and have often proved difficult to reproduce. The clear-cut changes that do occur, such as those in thyroid disease, have generally been interpreted as a reflection of an alteration in adrenergic function. However, little is known of the effects of these various treatments or diseases on removal of DBH from the circulation.

4. Effects of stress. A. INTRODUCTION. Initially it was anticipated that prompt changes in serum DBH activity of large magnitude would occur in response to acute stress. However, no changes in serum DBH have been found in many experiments designed to test this hypothesis under conditions that result in large increases in plasma or urinary catecholamines. In those experiments in which changes in the serum enzyme levels have been observed, the elevations have often been small in magnitude. As it has become clear that the half-life of

serum DBH in the rat is measured in days, the suggestion has been made that serum DBH might be a better measure of the chronic level of adrenergic activity rather than of the acute release of catecholamines by sympathetic nerves and the adrenal medulla (172).

B. EXPERIMENTAL ANIMAL STUDIES. Among the earliest experiments designed to test the effects of stress on serum DBH were studies with rats subjected to forced immobilization (199), a procedure that results in a prompt elevation in urinary catecholamine excretion (99). Quantitatively small (approximately 20%) but significant increases in serum DBH activity occur after a single 30-minute period of forced immobilization. Repeated daily 2½-hour periods of immobilization for 7 days result in an additional small increase in the enzyme activity. Adrenalectomy does not alter the basal levels of rat serum DBH activity nor does it change the elevation that occurs with forced immobilization (199). Rat serum DBH activity is increased 42% after 2 hours of swim stress and remains elevated for up to 24 hours (162). The return of DBH activity to basal levels after swim stress is biphasic, and the apparent half-life of the enzyme during the first phase is approximately 3 hours, a value similar to that reported for the "mixing" phase in the studies of the half-life of exogenous DBH in the rat. There have been at least two studies of the effect of acute hemorrhage on serum DBH in the dog. In one series of experiments between 40 to 60% of the blood volume was removed from each dog and plasma DBH and catecholamines were measured serially for up to 5 hours (154). Although large increases in plasma epinephrine and norepinephrine occurred within 30 minutes after hemorrhage, plasma DBH increased, but not significantly, during the entire 5 hours. In a separate series of experiments blood was removed until the blood pressure was reduced to 35 mm Hg; blood pressure was maintained at that level for 1 hour, and the blood was then reinfused (25). Plasma catecholamine levels increased up to 10-fold after hemorrhage while plasma DBH concentrations and total plasma DBH "content" doubled. The peak of DBH activity occurred later than that of catecholamines, and the increases in both catecholamines and DBH could be abolished by bilateral adrenalectomy. Although different forms of stress were used in experiments in the rat and the dog, the striking differences in the effect of adrenalectomy highlight our present lack of information with regard to species differences in serum DBH response to stress. Reports of differences in the response of rat, dog, and guinea pig serum DBH to the same stress (24) emphasize the need for experiments in several species and the danger of attempts to extrapolate results from one species to another.

C. HUMAN STUDIES. There have been many studies of the effect of acute stress on human serum DBH activity. These experiments have often used very similar stressful stimuli including the cold-pressor test, exercise, and acute change in posture. The cold-pressor test involves the immersion of one hand, both hands, or even both hands and both feet in ice water for 3 to 5 minutes, a maneuver that results in a striking rise in blood pressure. One early report described an average increase in serum DBH activity of about 15% in six subjects during the cold-pressor test (210). A separate group of investigators measured both enzyme activity and IDBH in six subjects during the cold-pressor test (51). An average increase in enzymatic activity of 18% and of 42% in IDBH was found. Another study showed that when the cold-pressor test was performed on 10 subjects, DBH activity rose 10 to 18% in six of the 10, did not change in two, and decreased in two subjects (−14% and −25%)(185). The latter investigators also reported that other serum constituents such as total protein and lactic dehydrogenase activity changed in parallel with DBH. These results were interpreted to indicate that short-term alterations in plasma volume during stress might result in quantitatively small changes in DBH activity, an

observation that has led to the suggestion that the results of such experiments be corrected for changes in total plasma protein. Some studies of human serum DBH during the cold-pressor test have shown little or no change in the enzyme activity (146, 175, 207) even though blood pressure increased and plasma norepinephrine rose to as much as twice control levels (207). A 20% increase in serum DBH was found in subjects at rest in a 10°C cold room for 30 minutes (55), but no change in serum DBH activity was found after the rather extreme stress of immersion to the neck in 10°C water for 1 hour—a maneuver that resulted in a decrease in core body temperature to 34.5°C (86).

Bicycle ergometry has been used frequently to investigate the effects of exercise on human serum DBH activity. One early study reported that serum DBH activity increased 15% in response to graded exercise (210). In a later experiment in which the work was gradually increased from 50 to 500 watts, an average 50% increase in the circulating enzyme activity was found in four subjects after 12 minutes of exercise (14). A separate group of investigators reported an average increase in serum DBH of 25 ± 3% (mean ± S.E.M.) among 34 volunteers subjected to work loads of 100 to 250 watts for 6 to 10 minutes (156). Subjects with relatively low basal enzyme activity had the same proportionate increase in serum DBH activity as did subjects with higher basal enzyme activity. When both DBH and plasma catecholamines were measured in 11 healthy volunteers during exercise ranging from 12.5 to 200 watts over a 12-minute period, DBH activity increased an average of 30% while plasma catecholamines increased 120 ± 71% (157). There was a significant correlation between the percentage increase in plasma DBH activity and the logarithm of the plasma catecholamine concentration ($r = 0.84$, $P < .001$).

Changes in posture or in volume status have also been used to study possible changes in human serum DBH activity in response to stress. In one early experiment no significant change in serum DBH activity was found after passive tilting (210). These results were later confirmed in eight subjects, and, although an average increase of about 10% in DBH activity occurred after these same eight subjects were fed a 10 mEq sodium diet for 4 days, this change was not significant (131). In a series of experiments that included acute changes in posture, sodium loading, and sodium depletion plus treatment with the diuretic furosemide, serum DBH in nine subjects increased an average of 22% in response to assumption of the upright posture and increased an average of 30% over baseline after sodium depletion plus treatment with furosemide (188). Conversely, serum DBH activity in seven subjects decreased an average of 20% during volume expansion by the intravenous infusion of 2400 ml of normal saline over a 2½-hour period (5). The hematocrits of these subjects decreased an average of 8% during the saline infusion. Many other stimuli have been used to study the effects of stress on human serum DBH, e.g., electroconvulsive therapy (43, 104), but, in general, the results have all been similar—either no changes or quantitatively small changes in the enzyme activity have been reported.

E. Conclusion

Many of the early assumptions about the regulation of serum DBH activity and the relationship of absolute serum DBH values to adrenergic function must be reevaluated. Initially it was assumed that the major factor that determines serum DBH values would be the coupled release of the enzyme with catecholamines, i.e., adrenergic function. It is now clear that in man most of the wide variation in enzyme activity among individual subjects is due to the effect of inheritance and that a single locus is responsible for at least half of the total population variation in enzyme activity. The biochemical basis of the genetic effects, and their relationship, if any, to adrenergic sta-

tus and function are unknown. On the other hand, acute stress or treatment with drugs that alter sympathetic nervous system function result in acute changes in serum DBH activity that are small in magnitude, at least as compared with the basal enzyme levels and as compared with changes in plasma catecholamine values. These facts have dampened enthusiasm for the use of serum DBH as a measure of acute alterations in adrenergic function in man, although the possibility that serum DBH might be a measure of chronic alterations in sympathetic nervous system function remains open and must be tested experimentally. The eventual interpretation of such chronic experiments might be complicated by the transsynaptic induction of DBH that occurs with prolonged stress (128). The situation in the experimental animals that have been studied is not much different from that in man, although genetic factors have not been shown to be as important in these species as they are in man and little is known about the variations among different species. Finally, the biochemical basis for the dramatic changes in serum DBH activity that occur in both man and the rat during growth and development is not understood.

VI. Serum DBH in Human Disease

A. Introduction

There has been great interest in the measurement of serum DBH in the blood of patients with a variety of diseases. It has usually been assumed in these studies that serum DBH is a direct measure of the function of the adrenergic nervous system, and that variations among subjects in enzyme activity reflect variations in the exocytotic release of catecholamines. Often little consideration has been given to the effects of inheritance or growth and development on variations in enzyme activity nor has the possibility been considered that disease might alter the metabolic clearance of the enzyme. Since the primary purpose here is to focus on the meaning of serum DBH activity and not to review the pathophysiology of a variety of diseases, the following discussion will not attempt to describe in detail all the diseases mentioned. For similar reasons, the many important experiments in which serum DBH has been used to evaluate adrenergic function in animal models of human disease, e.g., the spontaneously hypertensive rat, will not be described here.

B. Hypertension

There has probably been more interest in the measurement of DBH activity in patients with hypertension than in patients with any other disease. This situation is the result of both the importance of hypertension as a public health problem and of continued speculation that the sympathetic nervous system may play an important role in the pathogenesis of this disorder (26). It is not the purpose of this discussion to review the pathophysiology of hypertension. However, it must be pointed out that patients with high blood pressure are divided into those with secondary hypertension, patients in whom the etiology of the hypertension is understood, and those with "essential" hypertension, patients in whom the etiology is not understood (171). Since it is suspected that a variety of pathological processes may cause essential hypertension, there has been an intensive effort to subclassify patients with essential hypertension on either clinical or biochemical grounds. For example, patients may be classified clinically into those with "labile" or "borderline" hypertension as opposed to patients with fixed hypertension (88). Another approach involves the classification of patients into those with "low", "normal", and "high" renin hypertension according to the status of the renin-angiotensin-aldosterone system (109). Studies of serum DBH in hypertension must be viewed against this background. Initially these studies involved only comparisons of DBH activity in blood from patients with essential hypertension

and blood from randomly selected control subjects. Later experiments have included the subclassification of patients into those with labile hypertension or low renin hypertension and have also added dynamic tests of the status of the renin-angiotensin-aldosterone system and the sympathetic nervous system. In some recent studies simultaneous measurements of plasma catecholamines and serum DBH have been performed.

Large population surveys of randomly selected subjects have not shown a significant correlation of blood pressure values with DBH activity. Similar results have been reported in separate studies of 90 adults (84), of 841 children aged 6 to 12 (192, 202), of 68 normotensive adults (102), and of 253 subjects selected after stratification for race, sex, and age (123). Of perhaps more immediate relevance to the question of the pathophysiology of hypertension, no significant differences were found between the serum DBH activities of normotensive control subjects and of hypertensive patients in several large studies including the following: 90 normotensive and 70 hypertensive subjects (84); 93 normotensive and 53 hypertensive subjects (4); 68 normotensive and 106 hypertensive subjects (102); and 127 normotensive and 76 hypertensive subjects (71). There have been occasional reports of results that differ from these. For example, in one survey of 66 consecutive blood donors, DBH activity was significantly lower in subjects with high systolic blood pressure (greater than 130 mm Hg, 21 subjects) than in subjects with low systolic blood pressure (less than 110 mm Hg, 19 subjects) (103). There has been a report of a significant positive correlation of serum DBH with diastolic blood pressure and plasma catecholamine values among 28 hypertensive subjects (62). Unfortunately, these results were obtained with a radioimmunoassay performed with heterologous antibody, a procedure about which serious doubts have been raised (see discussion of assay techniques above). Overall the weight of the evidence based on the use of both the spectrophotometric assay and the coupled radiochemical assay indicates that basal DBH values are no different in normotensive subjects and patients with essential hypertension, patients in whom secondary causes of hypertension have been excluded. However, these data do not eliminate the possibility that a subgroup of patients with essential hypertension might exist in whom DBH values are abnormal, nor do they rule out the possibility that the "response" of serum DBH activity to stress might be altered in some hypertensive patients.

Several attempts have been made to measure serum DBH in "subgroups" of patients with essential hypertension. No significant correlation of serum DBH activity with plasma renin activity was found in one study of 20 hypertensive patients (3). However, in two separate studies serum DBH values were lower in patients with low renin hypertension than in patients with normal renin values. In one of these reports seven low renin patients had an average activity of 53.7 ± 14.9 I.U. (mean ± S.E.M.) compared with 70.8 ± 6.2 I.U. in 42 patients with normal renin values (110). However, these differences were not statistically significant. Another group of investigators reported that serum DBH values in seven patients with low renin hypertension were significantly lower than those in 17 patients with normal renin levels, 19.9 ± 4.3 I.U. versus 43.9 ± 5.0 I.U., respectively ($P < .05$) (143).

Serum DBH values have been reported to be elevated in patients with "labile" hypertension, a clinically defined subgroup, as compared with a randomly selected population (173, 184). Differences between normotensive subjects and hypertensive patients in the response of their serum DBH to stress have also been reported. Patients with essential hypertension have been reported to have "hyporesponsive" changes in DBH values during standing after treatment with the diuretic furosemide (14 ± 4% increase in 39 patients compared with 32 ± 2% increase in 20 control subjects) (30),

and to have "hyper-responsive" changes in DBH values during exercise on a bicycle ergometer (21 ± 2.4% increase in eight hypertensive patients and 11 ± 1.1% increase in eight control subjects) (155). Finally, no significant correlation of serum DBH values with either urinary catecholamine excretion nor plasma catecholamine levels has been found in several large studies of hypertensive patients (71, 102), although such a correlation has been reported to occur in patients with labile hypertension (173) and, as noted above, in a study that measured DBH by radioimmunoassay with a heterologous antibody (62). In summary, no correlation of DBH with blood pressure or basal catecholamine values and no significant differences in values between patients with essential hypertension and normotensive subjects have been found. The results of various "stress" tests are confusing. The possibility that serum DBH may be elevated in patients with "labile" hypertension or decreased in patients with low renin hypertension remain open questions. Serum DBH activity has also been measured in patients with secondary forms of hypertension.

DBH activity has been reported to be significantly decreased in patients with renovascular hypertension and hypertension associated with adrenocortical pathology and renal parenchymal disease (184). The relationship between renal disease and serum DBH is discussed below. DBH activity has been measured frequently in patients with pheochromocytoma. Part of the interest in serum DBH levels in pheochromocytoma results from controversy over whether catecholamine release from these tumors occurs by exocytosis (208). If release occurs by exocytosis, it has been assumed that a coupled proportional release of DBH and catecholamines will occur and that circulating levels of DBH will be elevated in patients with these tumors. Perhaps because many reports describe only one or a few cases, the results of these studies have been contradictory. There is general agreement that many patients have serum enzyme levels within the range found in normal subjects, but this is hardly surprising in view of the breadth of the normal range. There are some reports of no change in serum DBH levels after removal of a pheochromocytoma and some reports of dramatic, but gradual, declines in serum enzyme activity (6, 8, 63, 79). Larger series have generally demonstrated that there is a decrease in serum DBH activity in some patients after surgery to levels as low as 10 to 30% of preoperative values (8, 47, 96, 187). The half-life of the circulating enzyme in man has been estimated as between 8 and 12 hours from such data by one group of investigators (117). Since a postoperative decrease in serum DBH has not been observed in all patients, it has been suggested that the mechanism of catecholamine release may vary from tumor to tumor (8, 96, 187).

Serum DBH activity has also been measured in at least one important type of hypertension related to drug therapy, that which occurs during treatment with oral contraceptives. Parallel increases in DBH activity and blood pressure occurred in the subgroup of patients who experienced elevation in blood pressure when treated with daily estrogen-progesterone contraceptives (160). These results were thought to suggest a possible role for the sympathetic nervous system in oral contraceptive-related hypertension. Finally, several studies have shown that serum DBH values increase up to 50 to 60% during spontaneous or induced hypertensive episodes in quadriplegic patients (120, 132).

C. Renal Disease

Patients with hypertension associated with renal parenchymal disease have decreased serum DBH activity (184). It has also been reported that uremic patients in general have decreased serum DBH activity. The average enzyme activity in a group of 37 patients on chronic hemodialysis was 32 ± 17 I.U. (mean ± S.D.) compared with an average value of 50 ± 29 in 70 control subjects (179). An important problem in

the management of uremic patients is the occurrence of hypotension during hemodialysis in some of these patients. In one study of 10 patients who suffered from dialysis hypotension and 10 patients who did not, it was found that the serum DBH activity was higher, 42.8 ± 5.1 I.U. (mean ± S.E.M.), in patients who had difficulty with hypotension than in those who did not, 10.5 ± 2.6 I.U. (115). However, a separate study showed that DBH values were lower in anephric patients with hypotension than in anephric patients without hypotension (206).

D. Cardiovascular Disease

Several groups have reported that on the first and second day after myocardial infarction serum DBH is elevated to 150% of enzyme levels on the 10th postinfarction day (44, 77, 140, 145). The significance of these observations is related to the possible role of the sympathetic nervous system in the etiology of cardiac arrhythmias, especially those that occur soon after myocardial infarction. Unfortunately, because of the wide individual variations in DBH activity, measurement of the enzyme level in a specific patient is not as useful as might be hoped since the baseline levels for that patient will be unknown when the information would be most helpful, on the first or second day after the myocardial infarction.

Serum DBH activity has been reported to be decreased in blood from patients with congestive heart failure of diverse etiologies. The average activity in the blood of 30 patients was 14.4 ± 2.7 I.U. (mean ± S.E.M.) while that in blood from 29 control subjects was 47.1 ± 4.7 (83).

E. Neurological Disease

Familial dysautonomia was one of the first diseases in which a significant alteration in serum DBH activity was reported. This disease is characterized by altered autonomic nervous system function and sensory disturbances, occurs primarily in Ashkenazic Jewish children and is inherited in an autosomal recessive fashion (16). Measurements of urinary catecholamine metabolites have raised the possibility of an impairment in the ability of children with this disease to convert dopamine to norepinephrine (178), a reaction catalyzed by DBH. Therefore, it was of interest when it was found that the average serum DBH activity in 19 patients over age 10 with familial dysautonomia was only about 60% of that in age-matched control subjects (197). In a subsequent study young patients with familial dysautonomia had slightly lower DBH activity than control subjects, and eight dysautonomic patients over age 16 had serum DBH activity that averaged 57% of that in the blood of control subjects (50). The observation of a significant decrease of serum DBH in the blood of subjects with familial dysautonomia was also confirmed in a later study in which both plasma catecholamine levels and DBH activity was measured (214). This latter study also showed that plasma DBH in dysautonomic patients does not increase after exercise or after standing as much as it does in control subjects.

Torsion dystonia is another inherited neurological disorder in which an abnormality in serum DBH activity has been reported. The torsion dystonias comprise a group of movement disorders that are classified on clinical and genetic grounds into autosomal recessive, autosomal dominant, and acquired forms (41). Average serum DBH activity has been reported to be almost twice as high in blood from patients with the autosomal dominant form of torsion dystonia as in control subjects or in patients with the autosomal recessive form of the disease (211). This observation has been challenged (39), and it remains uncertain whether serum DBH values are elevated in patients with this movement disorder. Other inherited diseases with neurological manifestations in which alterations in serum DBH have been reported include Down's syndrome, in which a significant decrease in serum DBH activity has been reported by several laboratories

(19, 52, 205); the dysequilibrium syndrome, an inherited disorder characterized by mental retardation and other neurological manifestations, in which low serum DBH has been reported (76); and Huntington's chorea, in which there have been reports of both elevated and normal levels of serum DBH activity (113, 122, 176). A very confusing situation exists with respect to the Lesch-Nyhan syndrome. There have been reports of both abnormally high (161) and abnormally low levels (101) of serum DBH activity in patients with this X-linked inherited disease characterized by neurological dysfunction, self-mutilation, and deficiency of the enzyme hypoxanthine guanine phosphoribosyltransferase (174). Serum DBH activity was 86% higher in a group of 17 subjects with migraine vascular headache than in a group of 40 control subjects (70), and 27 deeply comatose patients had enzyme activities that were decreased to approximately 60% of those in 51 control subjects (114).

F. Psychiatric Disease

Partly because of speculation that a decrease in DBH activity in the brain might be involved in the pathogenesis of schizophrenia (180), there has been interest in serum DBH activity in schizophrenic patients. Two early studies of relatively small numbers of schizophrenic patients, 22 and 12 respectively, failed to demonstrate differences in the enzyme activity in the blood of these patients as compared with control subjects (32, 67). Similar results were obtained in a larger group of 52 schizophrenic patients and 106 controls (204). All three of these studies used the coupled radiochemical enzyme assay, but the results were confirmed by a later study of 35 schizophrenic patients that used the spectrophotometric assay (124). It was surprising, therefore, when a study of 149 schizophrenic patients and 153 control subjects in which DBH activity was measured with a high-performance liquid chromatography method showed a significant decrease of serum DBH activity in schizophrenic patients, 16.2 ± 12.6 I.U. (mean ± S.D.) compared with control subjects, 42.5 ± 30.9 I.U. (57). The reason for this striking difference in results is not clear.

The question of the level of serum DBH activity in patients with affective disorders is also unsettled. One study of eight manic patients and 20 patients with unipolar primary affective disorder failed to demonstrate a difference between their average serum DBH activities and that in control subjects (67). Similar results were found in a study of 54 unipolar and 30 bipolar patients (204). There were no differences in the DBH activities of 28 patients with unipolar and 86 with bipolar affective disorder in another series and no relationship of serum DBH activity to mood was found in these patients during a 10-month prospective study (111). A separate study of 33 unipolar depressed, 19 bipolar depressed, and 24 bipolar manic patients reported normal DBH values in these groups, but when the depressed patients in the study were separated into psychotically and nonpsychotically depressed groups, the values in 22 unipolar psychotically depressed patients averaged 15.1 ± 17.4 I.U. (mean ± S.D.) compared with 26.4 ± 15.2 I.U. in 73 control subjects (124). The question of whether serum DBH values are normal in schizophrenic patients and in patients with primary affective disorders remains to be resolved.

G. Neoplastic Disease

Neuroblastoma, a common tumor of childhood, originates from neural crest cells. These tumors frequently have the ability to synthesize catecholamines, and serum DBH activities are elevated to 3 to 10 times the mean values in age-matched control subjects in up to half of these patients (65). Elevated DBH levels are found primarily in patients with increased urinary excretion of vanillylmandelic acid, a metabolite of norepinephrine. These two observations are consistent since DBH is required for the formation of norepinephrine. Elevated serum DBH activity has also been

reported in some patients with leukemia and hepatoma (68). Since most of these patients are treated with potent antineoplastic agents, the possibility exists that the removal of DBH from blood has been impaired either by the primary disease process or by the treatment.

H. Endocrine Disease

Thyroid disease, discussed above, is one example of endocrine pathology in which there is a marked alteration in serum DBH. There is also a striking decrease in the serum DBH of diabetic patients with severe diabetic neuropathy and idiopathic orthostatic hypotension. The average enzyme activity in four such patients was 6.7 ± 4.2 I.U. (mean ± S.E.M.) compared with an average 40.0 ± 4.0 I.U. in 40 control subjects (143). This situation contrasts sharply with that in the rat in which 5- to 10-fold elevations of serum DBH occur with streptozotocin induced diabetes (80), but may be analogous to the sympathetic neuropathy caused by the treatment of experimental animals with 6-hydroxydopamine or guanethidine (73,195).

I. Conclusion

A biochemical test may be used in a variety of ways in a clinical setting. It may be used to arrive at a diagnosis in an individual patient, to follow disease progress in an individual patient, or to increase understanding of the pathophysiology of a disease by using the test as an investigative tool in a large group of patients. Serum DBH activity has been measured in patients with the anticipation that it might prove useful in each of these ways. Although much data is now available with regard to serum DBH and human disease, measurements of this circulating enzyme activity have not fulfilled all expectations. One reason that has often been cited for disappointment in the measurement of serum DBH in human disease is the relatively small magnitude of the changes that are found. However, physicians are satisfied with the clinical significance of much smaller percentage changes in many tests, e.g., serum sodium concentration. The difference here is the wide individual variation in serum DBH values in healthy subjects, variation that is primarily controlled by inheritance. Changes in the DBH values of diseased patients usually occur within this normal range, a fact that makes it almost impossible to use single DBH measurements in an individual patient for purposes of diagnosis. This does not mean that serial determinations of serum DBH might not prove useful to follow the course of a disease or a treatment in an individual patient, especially when combined with other sources of information. The most common use of DBH in clinical investigation will probably continue to involve the comparison of values in large groups of patients with those in control groups, control groups that should be large and genetically appropriate. Finally, although it has occasionally been suggested that relative serum DBH values may reflect the relative enzyme activity in nervous tissue, there are no data presently available that support this conclusion.

VII. Overall Conclusions and Future Research

Much has been learned about serum DBH since this catecholamine biosynthetic enzyme was first found in blood. It is known that the biochemical and immunological properties of the circulating enzyme are similar to those of DBH in the nervous system and the adrenal medulla, that adrenergic nerves are the source of much of the enzyme in experimental animals, and that the half-life of the enzyme in the rat is long. Human serum DBH activity is much greater than that in any other species studied thus far, and the wide individual variations in human serum activity are determined primarily by inheritance. There are dramatic changes in serum DBH activity during growth and development. Increases in circulating DBH occur in man and experimental animals in response to short-term stress, but the magnitude of change is small when compared with baseline levels

of enzyme activity, a fact which might result from a long half-life of the serum enzyme. Finally serum DBH activity has been measured in a variety of human diseases, and several significant differences between patients and normal subjects have been reported.

However, much still remains to be learned about serum DBH. The assumption that the circulating enzyme originates entirely from coupled proportional release with catecholamines, presumably by exocytosis, has been questioned. It must be determined whether a process exists by which DBH may be released from sympathetic nerves independent of the release of catecholamines. The biochemical basis of genetic variations in human enzyme activity remains to be explored as does the biochemical basis of the changes in serum activity that occur with growth and development. The metabolic fate of the circulating enzyme is not known and the half-life of serum DBH in man is not known. Imaginative experiments are needed to study the disposition and half-life of the human enzyme, since this information is directly related to the possibility that serum DBH might be a better measure of long-term trends in adrenergic function than of acute responses of the sympathetic nervous system.

The initial wave of enthusiasm for the assay of serum DBH as an easy and precise measure of sympathoadrenal function has passed. This enthusiasm was based on the naive expectation that it would be possible to assess sympathetic nervous system function by the measurement of serum DBH with little understanding of the regulation of the enzyme itself. It has become obvious that a thorough understanding of the mechanism of release, the metabolism, the biochemistry, and the regulation of the circulating enzyme activity is essential for the interpretation of the results of experimental studies in animals and of clinical studies in man. Even though substantial advances have been made, important questions about serum DBH remain to be answered. When these questions have been answered the clinical data already available may be much easier to interpret and may offer clues for future experiments, the results of which will be less confusing than many of those that have appeared during the last decade. Under the best circumstances, serum DBH activity will almost certainly be only one piece of information among many that will be used to assess sympathoadrenal status and function. However, the time may yet come when the measurement of serum DBH activity may prove useful both in the research laboratory and in the clinic.

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