PHARMACOLOGICAL REVIEWS
Vol. 24, No. 2


# Multiple Forms and Localization of Enzymes

CHAIRMAN: MERTON SANDLER, M.D.

## Characterization and Tissue Localization of Catecholamine Synthesizing Enzymes

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It was through the purification and study of enzymes that the biosynthesis and metabolism of catecholamines was elucidated. According to the biosynthetic scheme proposed by Blashko (3) four enzymes are involved in the biosynthesis of norepinephrine and epinephrine and all four enzymes were isolated and characterized in the last decade. In recent years it was shown that multiple forms of isozymes are involved in the regulation of physiologically important reactions. The multiple forms of enzymes involved in the synthesis and degradation of biogenic amines have not yet been extensively studied. In this presentation we will describe some procedures used for isolation and characterization of multiple forms of catecholamine synthesizing enzymes. In addition we will present studies on serum dopamine-β-hydroxylase (DβH) activity as well as on the cellular localization of aromatic L-amino acid decarboxylase (AADC), DβH and phenylethanolamine-N-methyl transferase (PNMT) in various tissues of mammals.

### Aromatic L-Amino Acid Decarboxylase (AADC)

*Purification and properties of the enzyme*

The enzyme which catalyzes the decarboxylation of dopa to dopamine also catalyzes the decarboxylation of all naturally occurring amino acids and therefore according to the suggestion of Lovenberg *et al.* (37) the enzyme was referred to as AADC. AADC was purified from bovine adrenal glands (22) and from hog kidneys (5). The final preparation of the adrenal enzyme yielded one broad band on polyacrylamide disc gel electrophoresis (fig. 1) which contained AADC activity. The distribution of AADC activity among the gel sections after disc gel electrophoresis is shown in figure 2. It can be seen that the pat-

This work was supported by U. S. Public Health Service grant MH-02717 and NSF grant GB 27603.

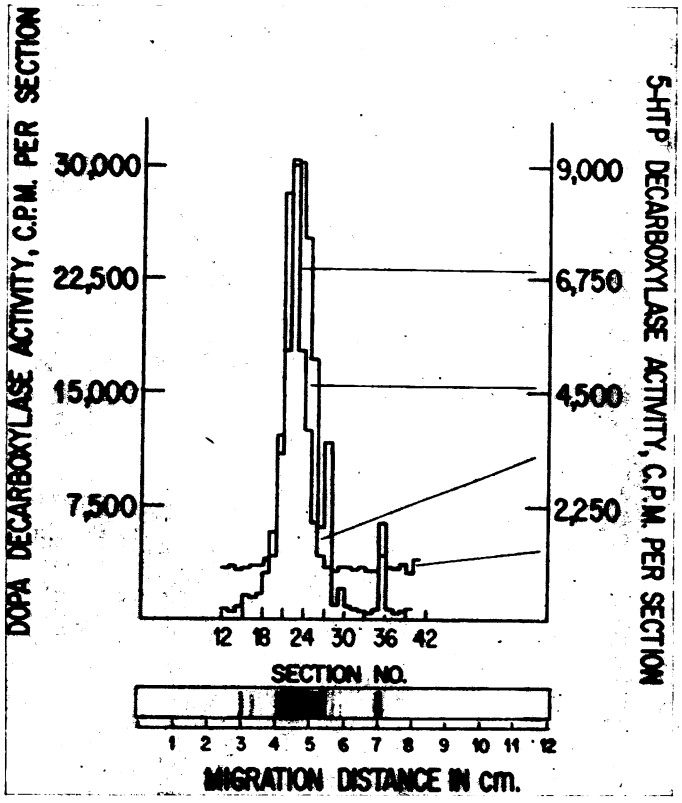


FIG. 1 (*left*). Photograph of polyacrylamide gel electrophoresis of purified AADC preparation. The sample (100 μg of protein) was run for 90 min, and amido black was used to stain the protein on the gel. Anodic end is shown down.

FIG. 2 (*right*). The distribution of dopa and 5-HTP activity after polyacrylamide gel electrophoresis.

tern of enzyme activity was similar whether dopa or 5-hydroxytryptophan (5-HTP) was used as a substrate. These results indicate that the same adrenal enzyme catalyzes the decarboxylation of both aromatic L-amino acids dopa and 5-HTP.

*Immunohistochemical studies*

After immunization of rabbits with purified AADC (enzyme preparation obtained after polyacrylamide gel electrophoresis) an antiserum to AADC was obtained (fig. 3). Bovine AADC antiserum inhibits AADC activity from different tissues of various species. The AADC activity from rats striatum and from rats brain stem is inhibited to the same extent by the bovine AADC antiserum (Ceasar, Anagnoste, Barone and Goldstein, unpublished data). It seems therefore that AADC is immunologically indistinguishable in these two regions.

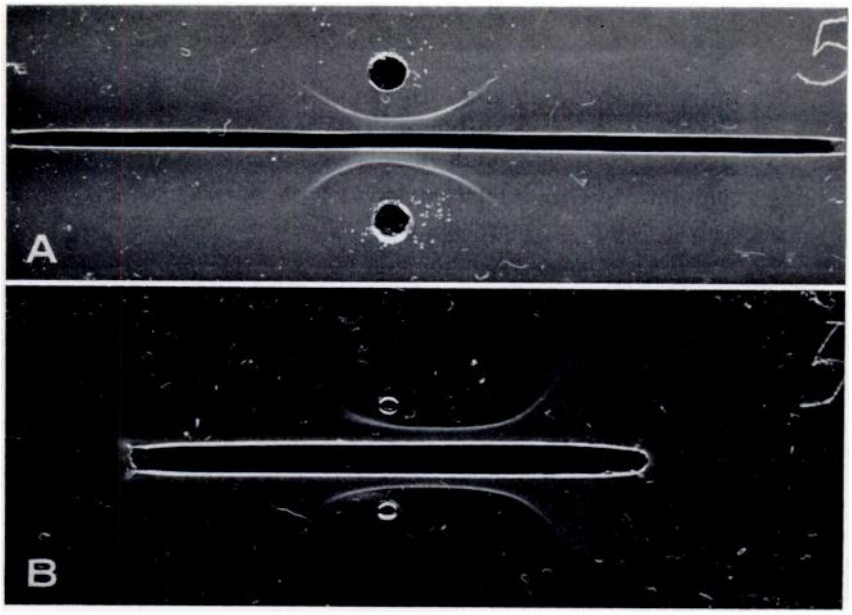


FIG. 3. Immunoelectrophoretic analyses. A, purified bovine AADC against rabbit anti-AADC; B, purified bovine DβH against rabbit anti-DβH.

The AADC antiserum was used in the immunohistofluorescent procedure for localization of the enzyme in various tissues (19, 27).

The following tissues were analyzed.

*Adrenal medulla* (hamster, mouse and guinea pig). A specific immunofluorescence of weak intensity was observed in the cytoplasm of practically all gland cells. No specific immunofluorescence was observed in the adrenal cortex (fig. 4).

*Kidney* (rat and guinea pig). A strong immunofluorescence was observed in the distal and proximal tubuli.

*Peripheral and central nervous system* (rat). A specific immunofluorescence of weak moderate intensity was observed in the norepinephrine cell bodies of the sympathetic ganglia and the dopamine cell bodies of substantia nigra (fig. 5) and the norepinephrine cell bodies of the locus ceruleus. Also the serotonin cell bodies exhibited a weak specific immunofluorescence. Unspecific fluorescence appeared in the ependyma and the glial cells.

## Dopamine-β-hydroxylase (DβH)

### *Purification and properties*

The enzyme DβH is a mono-oxygenase which catalyzes the terminal step in the biosynthesis of norepinephrine. The enzymatic dopamine-β-hydroxylation is coupled to a stoichiometrically equivalent oxidation of ascorbic acid as shown in the following equation (35).

$$\text{Dopamine} + \text{Ascorbate} + O_2 \rightarrow l\text{-Norepinephrine} + \text{Dehydroascorbate} + H_2O$$

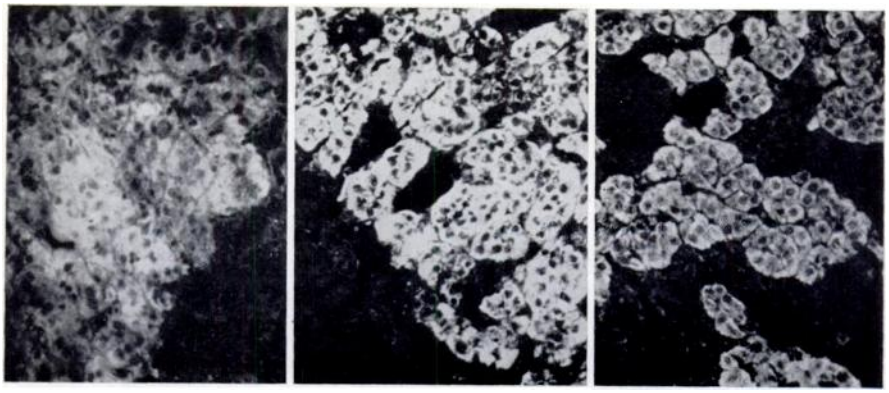

FIG. 4. Immunohistochemical localization of enzymes in adrenal glands. Indirect fluorescent antibody technique. *Left*, AADC in adrenal medulla from hamster. A specific immunofluorescence is observed in all adrenal medullary cells. In some gland cells a higher fluorescence intensity is observed than in other cells. × 120. *Middle*, DβH in adrenal medulla from rats. A specific immunofluorescence is observed in almost all medullary cells. × 150. *Right*, PNMT in adrenal medulla from rats. Only a certain population of cells contain PNMT. × 150. *Note:* The adrenal cortex lacks specific fluorescence.

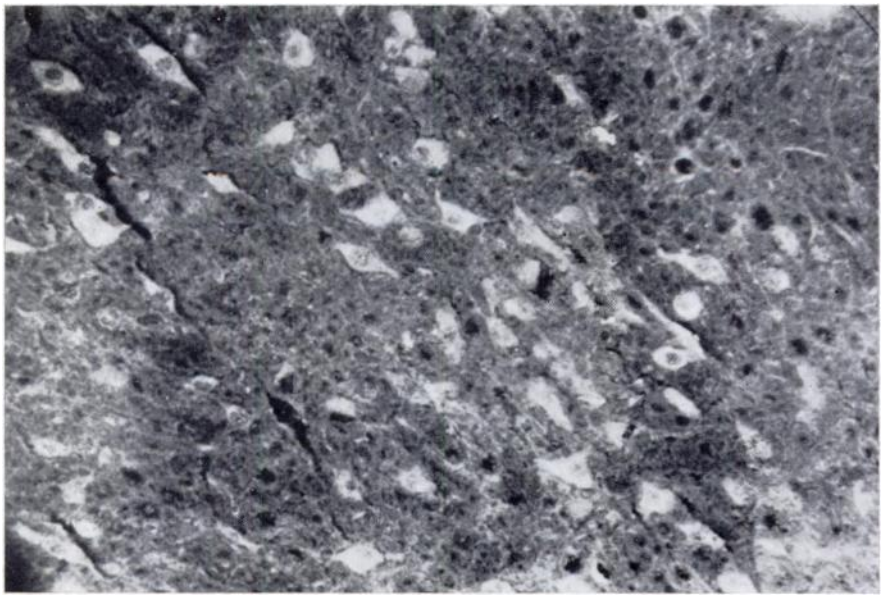

FIG. 5. Immunohistochemical localization of AADC in substantia nigra of rats. A specific immunofluorescence is observed in the cell bodies of substantia nigra. × 400.

The enzyme is non-specific and accepts a variety of sympathomimetic amines structurally related to dopamine as substrates (7, 25, 34).

DβH is a copper enzyme (4, 16, 30) and various chelating agents effectively inhibit the enzymatic activity *in vivo* and *in vitro* (24). Electron paramagnetic

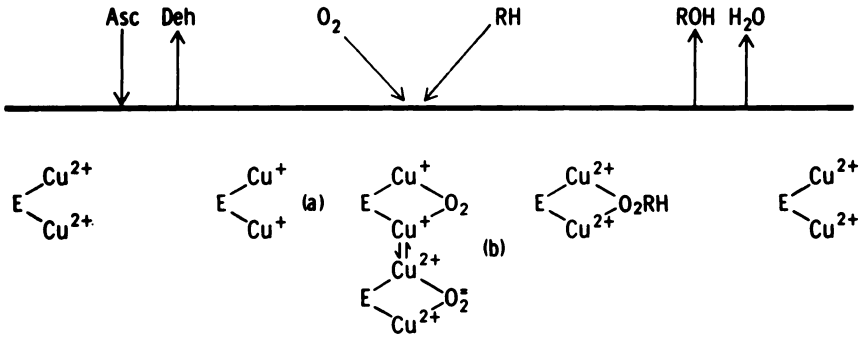


FIG. 6. Schematic presentation of enzymatic DβH. RH, tyramine or dopamine; ROH, product (octopamine or norepinephrine); Asc, ascorbate; Deh, dehydroascorbic.

resonance (EPR) and chemical studies have shown that the copper of the enzyme undergoes cyclic changes during the enzymatic hydroxylation reaction (4, 16, 21). The mechanism of the enzymatic dopamine-β-hydroxylation was further investigated with kinetic studies (29). The kinetic data as well as the EPR studies support the mechanism which is outlined in the scheme presented in figure 6. In this scheme the first product dehydroascorbate leaves the enzyme before the addition of the subsequent substrates (Ping-Pong). The subsequent substrates (dopamine or tyramine and oxygen) add to the reduced enzyme intermediate before either product is released. The kinetic pattern also indicates the obligatory addition of oxygen prior to the addition of dopamine. The interconversion of the central ternary complexes seems to be the rate-limiting step in the overall β-hydroxylation reaction. Fumarate and oxygen stimulate the enzymatic activity at low substrate (RH) concentrations and both change the $K_m$ of the substrate but not the $V_{max}$. Fumarate facilitates the interaction of the reduced enzyme intermediate with oxygen and most likely induces a conformational change of the enzyme (29).

The purification of DβH from bovine adrenals was described (4, 16, 35). More recently a shorter procedure was used for purification of DβH from bovine adrenal glands and human serum (23, 39).

### *Immunochemical studies*

After immunization of rabbits with purified bovine DβH or with human DβH (enzyme preparations purified on polyacrylamide disc gel electrophoresis) the corresponding antisera to DβH were obtained. Bovine DβH antiserum gives a single precipitin line with bovine adrenal enzyme on immunoelectrophoresis (fig. 3) but does not give a precipitin line with human serum DβH. Human DβH antiserum gives a precipitin line with human DβH serum but does not give a precipitin line with bovine adrenal DβH. However, bovine DβH antiserum inhibits human DβH activity and human DβH antiserum inhibits the activity of bovine DβH. These results indicate that DβH is heterogeneous among different species.

*Immunohistochemical studies* (18, 19, 27)

Bovine DβH antiserum was used in the immunohistofluorescent procedure for localization of the enzyme in various tissues of rats. The following tissues were analyzed.

*Adrenal medulla.* In rat the gland cells exhibited a green specific immunofluorescence which in most cells was of weak to moderate intensity. Several islands of cells, however, contained a strong specific immunofluorescence (fig. 4).

*Peripheral and central nervous system* (18). In contrast to AADC, DβH was only localized in the norepinephrine neurons but not in the dopamine neurons. Thus, with the immunofluorescence procedure it was possible to separate directly the norepinephrine bundles and cell bodies from the dopamine bundles and cell bodies.

The norepinephrine cell bodies of the sympathetic ganglia and of the pons and medulla oblongata, *e.g.*, those in the locus ceruleus exhibited a specific immunofluorescence of moderate intensity (fig. 7). The dopamine cell bodies showed no specific immunofluorescence. Furthermore, after transection of the central ascending norepinephrine axons, a strong specific immunofluorescence appeared in the norepinephrine axons of the cell body side but not in the dopamine axons. The primitive catecholamine cell system in the ganglia lacks specific DβH immunofluorescence and seems therefore to contain dopamine and not norepinephrine. Unspecific fluorescence appeared in the ependyma and the glial cells.

**Serum DβH Activity under Various Physiological and Pathological States**

A sensitive and specific procedure was developed for assay of DβH activity in tissues and serum (26, 38).

The principle of the assay is outlined in the following reaction scheme:

$$RCH_2CH_2NH_2 + O_2 + XH_2 \xrightarrow{D\beta H} RCHOHCH_2NH_2 + H_2O + X$$

$$RCHOHCH_2NH_2 + C^{14}H_3\text{-SAM} \xrightarrow{PNMT} RCHOHCH_2NHC^{14}H_3 + SAHCys$$

$XH_2$ = ascorbate; X = dehydroascorbate; $C^{14}H_3$-SAM = S-adenosylmethionine; S-AHCys = S-adenosylhomocysteine; R = phenyl.

In the first of the coupled reactions tyramine is converted by the enzyme to octopamine; in the second reaction the formed octopamine is further converted by added PNMT to N-methyl octopamine. S-adenosyl-1-methionine-methyl-$C^{14}$ serves as methyl donor and the radioactively labeled $C^{14}$-N-methyl octopamine is separated by solvent extraction and its radioactivity determined in a liquid scintillation counter.

The source of circulatory DβH is not clearly defined. The enzyme is localized in the norepinephrine containing granules of adrenal medulla and in the storage vesicles of the sympathetic innervated tissues (42). It is also present in blood vessel walls and accumulates adjacent to the walls (31). Evidence has been presented that DβH is released from adrenal medulla (45) and from sympathetic

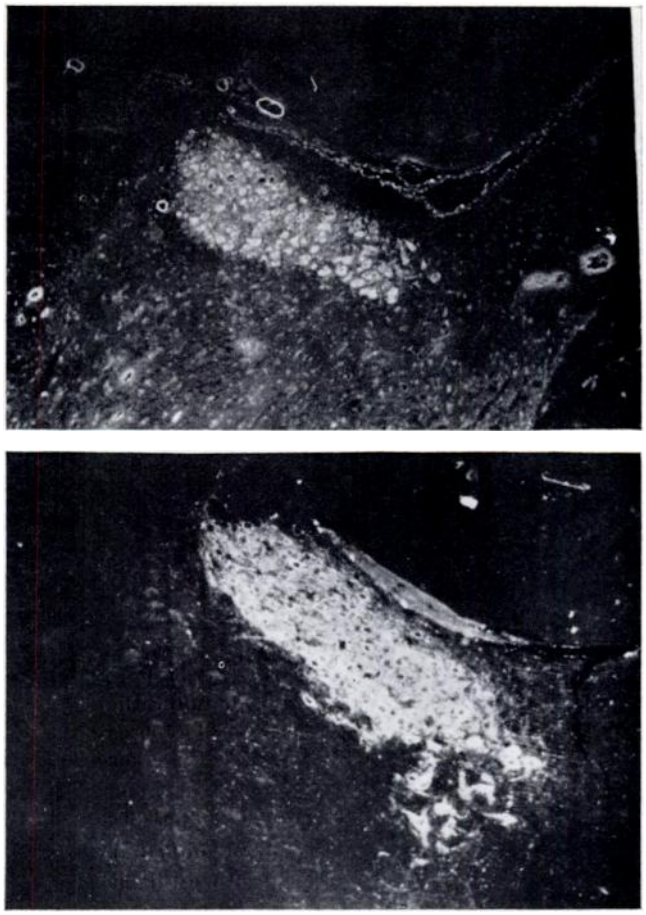

FIG. 7. *Top*, rat locus ceruleus. DβH immunofluorescence. Moderately to strongly green-fluorescent cell bodies are observed. The immunofluorescence is found diffusely all over the cytoplasm. × 160. *Bottom*, same area as in the upper picture. The histochemical fluorescence method for demonstration of catecholamines has been used. A strong specific catecholamine fluorescence is observed in the nerve cells of locus ceruleus. × 160.

nerves (48) by a process of exocytosis. Adrenalectomy in animals does not lower serum DβH levels (10) and it is also noteworthy that bilateral adrenalectomy decreases the urinary levels of epinephrine but not of norepinephrine (29). Therefore it appears that the circulating DβH derives from extramedullary tissues.

The steady state level of circulatory DβH may be pictured as follows:

$$\xrightarrow{\text{Release}} \text{Circulatory D}\beta\text{H} \xrightarrow{\text{Degradation}}$$

The level of circulatory DβH is a balance between rates of release from the sympathetic innervated tissues and degradation in some sites not yet known.

TABLE 1

*The Effect of experimentally induced changes in the sympathetic nervous system function on serum DβH activity in rats*

| Group | Treatment | Effects | Serum DβH |
|---|---|---|---|
| | | | *mμmol/ml* |
| Control | None | | 0.87 ± 0.03 |
| Control | Guanethidine* | Adrenergic blockade | 0.60 ± 0.29 |
| Hypophysectomy (7th day) | | Increased turnover of cardiac NE, decreased blood pressure. (33) | 1.49 ± 0.14 |
| Hypophysectomy (21st day) | | Increased turnover of cardiac NE, decreased blood pressure. (33) | 1.73 ± 0.15 |
| Hypophysectomy (21st day) | ACTH | Turnover of NE and blood pressure restored to normal. (33) | 1.12 ± 0.08 |

* Chronic treatment with guanethidine also induced decreases in serum DβH in man (15).

The levels of the serum enzyme are increased under increased sympathetic nervous system activity and are decreased by destruction of sympathetic nerve terminals (47). The results summarized in table 1 show the effects of experimentally induced changes in adrenergic activity on serum DβH levels. Guanethidine, a drug known to block adrenergic function causes a decrease in serum DβH activity in man and in rat (15). Hypophysectomy in rats causes an increase in the turnover rate of cardiac norepinephrine and a decrease in blood pressure (33) as well as a significant increase in circulatory DβH (17, 20). Chronic treatment of hypophysectomized rats with ACTH restored the turnover of cardiac norepinephrine (33) and the serum DβH levels to normal (17, 20).

At the present the mechanisms and sites involved in the degradation of serum DβH are unknown. It is conceivable that under various experimental and pathological conditions serum DβH levels are altered due to changes in the degradation rates of the enzyme. We have recently found that some children with leukemia have high serum DβH levels. At the present time we are investigating whether the high DβH levels in these patients are related to the pathology of this disease or to their treatment. Possibly the administered alkylating agents might inhibit the degradation processes of DβH.

### Serum DβH Activity in Normal Population

Serum DβH activity varies widely among normal individuals, but is maintained at a relatively constant level by each individual. Mean values in the normal population rise progressively with each successive age group (fig. 8). There is no statistically significant increase after the 16 to 20 year age group (12, 13). A striking feature is the extremely low level characteristic of the first year of life, clustering at the lower limits of sensitivity of the assay method. Screening of a series of laboratory animals of different species has also revealed a low circulating level of DβH. A common factor in all these subjects is the

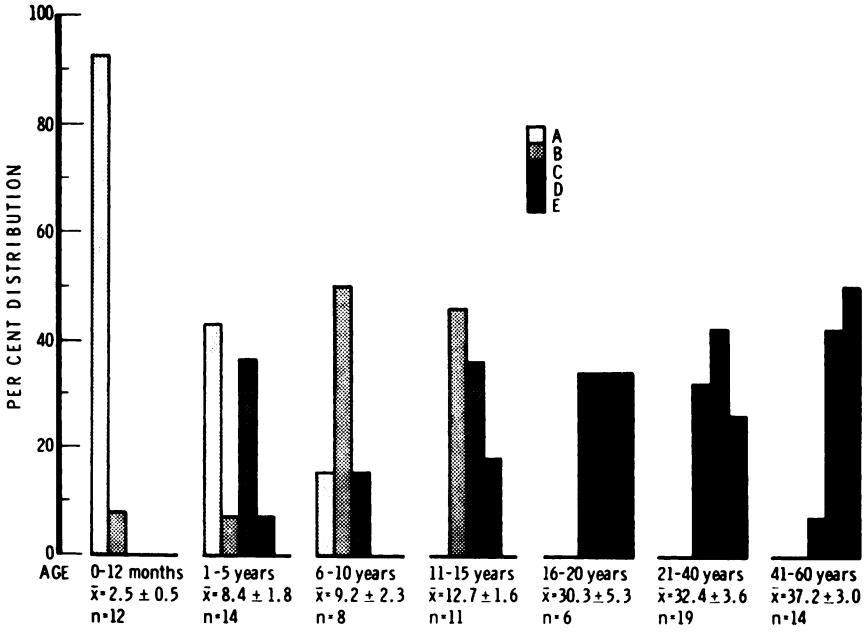


FIG. 8. DβH activity in normal subjects of various age groups. Subjects were grouped according to enzyme activity expressed in mμmol/ml serum. A, 0–5; B, 6–10; C, 11–20; D, 21–40; E, >41; x̄ mean ± SEM; n = number of subjects.

absence of erect posture. Prevention of postural hypotension is a function of the sympathetic nervous system (40). It is possible that the circulating level of DβH remains low until an erect posture is assumed. Another explanation might be that the vesicular system which stores norepinephrine and DβH might not be fully developed in infants and therefore serum DβH activities are low in this age group.

### Serum DβH Activity in Patients with Various Diseases

#### *Neuroblastoma*

DβH activity is present in mouse neuroblastoma C-1300 tumors. The activity is proportional to the weight of the tumor. Serum DβH activity is markedly increased in mice that bear the tumors (1, 11) (fig. 9). The catecholamine-DβH containing processes of the tumor cells terminate around very fine blood vessels (fig. 10). These findings indicate that the enzyme is released from the tumor into the blood vessels at the same time as catecholamines are released.

Serum DβH activity was also measured in neuroblastoma patients. Some neuroblastoma patients have serum DβH values in the range of the control subjects, while others have extremely high values (11). Patients with high serum DβH activity also excreted high amounts of the norepinephrine metabolite vanillmandelic acid, while patients with non-elevated serum DβH activity excrete high amounts of dopamine and its metabolite homovanillic acid. These

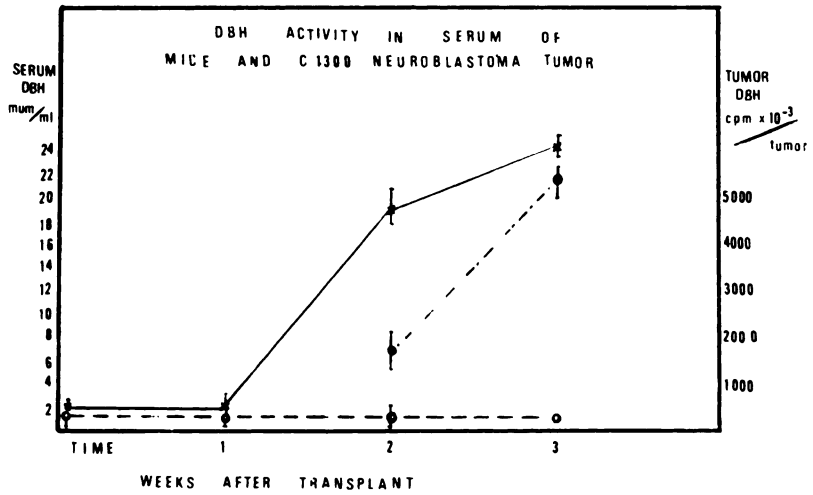


FIG. 9. DβH activity in mouse neuroblastoma C-1300 tumors and in serum at different times after implantation. ×——×, activity of enzyme in serum of A/J mice bearing C-1300 tumors; O····O, activity of enzyme in serum of A/J mice; °——°, activity in C-1300 neuroblastoma tumors.

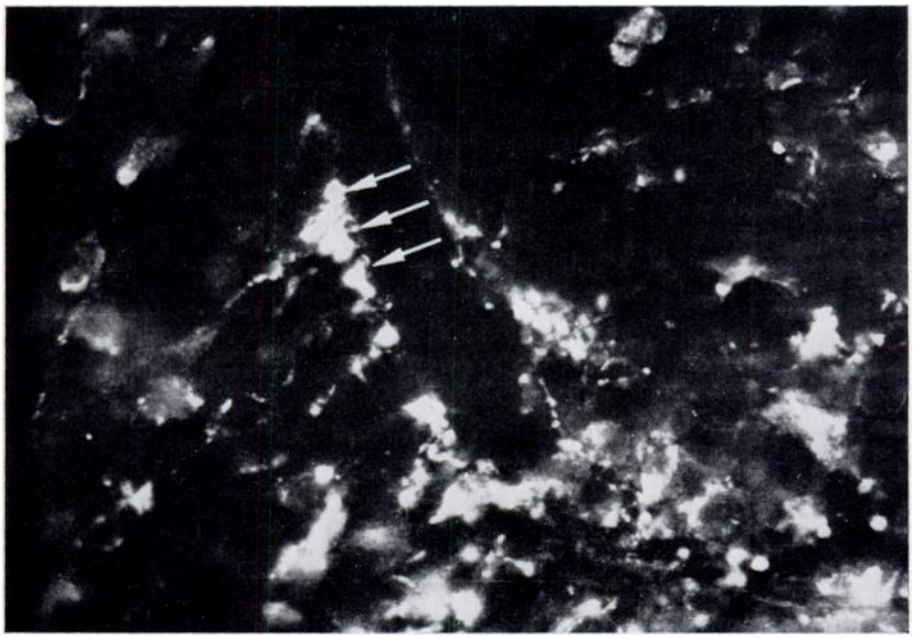

FIG. 10. Localization of catecholamine containing cells in mouse C-1300 neuroblastoma tumors by histochemical method of Falck and Hillarp. × 160. *Note:* 1–5% of the tumor cells exhibit specific catecholamine fluorescence. The cells have strong fluorescent cell processes which are relatively thick (2–3 μ) and seem mainly to terminate around the fine blood vessels of the tumor.

findings suggest that not all neuroblastoma tumors have the enzymatic capacity for norepinephrine production and that the catecholamine synthesis ceases in some tumors at the dopamine stage.

*Neurological and mental diseases*

There is a preponderance of high activities among patients with Huntington's chorea and low activities among patients with Parkinson's disease. The mean DβH activity in parkinsonian patients treated with L-dopa was higher than in untreated patients (36). It is conceivable that other factors not directly related to the etiology of these disorders may influence serum DβH levels. Elevated serum DβH levels in patients with Huntington's chorea might be due to stress of constant uncontrollable involuntary movements and the reduced serum DβH activity in untreated parkinsonian patients might be due to physical inactivity. Serum DβH activity was also measured in psychiatric patients which include bipolar manic subjects, neurotic depressive, endogenous depressives, schizophrenics and character disorders. The psychiatric patients did not show blood enzyme levels varying from normal controls within the same age group (43).

*Familial dysautonomia*

Familial dysautonomia occurs primarily in Ashkenazi Jewish children and appears to be inherited in an autosomal recessive manner. The disease is characterized by symptoms of altered autonomic nervous system function and sensory disturbances (8). About 25% of patients with dysautonomia have low serum DβH activity (12, 13, 46). The children with low DβH activity had at least one parent with the serum DβH activity lower than the mean value of the appropriate control subject (fig. 11). Although there is considerable evidence

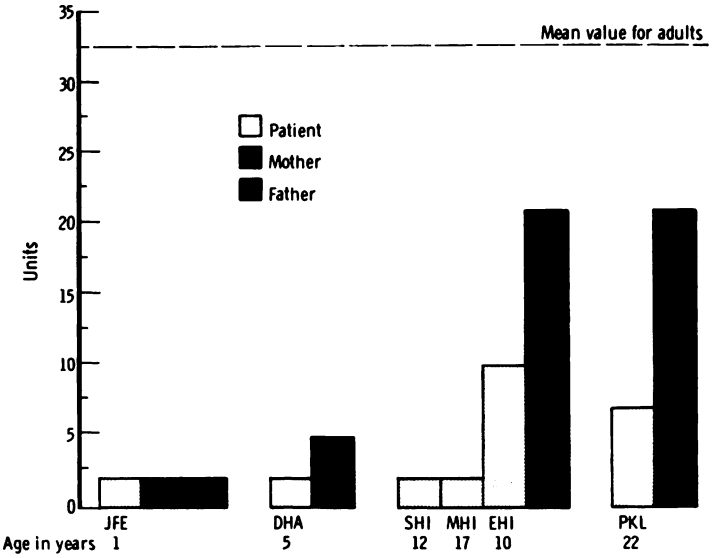


FIG. 11. Serum DβH activity in some dysautonomic children and in their parents.

for derangement of function of the sympathetic nervous system in familial dysautonomia, it is probably not a primary feature of the disease (44). In order to alleviate symptoms resulting from parasympathetic system inadequacy several dysautonomic patients were treated with urecholine. In five of the six patients treated with urecholine the serum DβH activity increased several-fold as compared with their levels prior to treatment (14). These results suggest that the reduced serum DβH activity in dysautonomia might be a reflection of reduced parasympathetic nervous system activity. Dysautonomic patients exhibit a supersensitive reaction to cholinergic agents and it is therefore possible that this effect is specific for these patients. The finding that urecholine increases serum DβH levels in dysautonomic children and that two of three siblings have low enzyme levels while the third has normal serum DβH levels (fig. 11) does not support the idea that a subgroup of "DβH negative" patients have a genetic defect involving DβH as the cause of the pathological process (46).

## Phenylethanolamine-N-methyl Transferase (PNMT)

### *Purification and properties*

The enzyme PNMT catalyzes the conversion of norepinephrine to epinephrine and is mainly localized in the adrenal glands. PNMT accepts as substrate not only primary phenylethanolamines (2) but also secondary phenylethanolamines and primary phenylethylamines (23, 32). The enzyme was purified from the bovine adrenal medulla (23, 28), and approximately 15 to 20% of the enzymatic activity is associated with the particulate fraction. The enzyme from the supernatant fraction was isolated in pure form and has a molecular weight of approximately 40,000 (6, 32). The enzyme occurs also in two higher molecular forms with molecular weights of approximately 80,000 and 160,000 (32). The low molecular form of the enzyme exists as several differently charged molecular species which can be separated on DEAE-Sephadex and on polyacrylamide disc gel electrophoresis (fig. 12) (32). Amino acid analysis reveal a relatively high content of dicarboxylic acid or their amides and the presence of hexosamine (32). It is possible that the charge isozymes may arise from each other by deamidation.

The binding of the two substrates, norepinephrine and S-adenosyl-L-methionine (SAM) to PNMT was investigated (32, 41). The results presented in figure 13 show that at 0°C both substrates bind to the bovine adrenal enzyme. Complex formation depends on the concentration of enzyme and on the concentration of each of the substrates. S-Adenosyl-L-homocysteine inhibits the binding of SAM while epinephrine inhibits the binding of norepinephrine more effectively than the binding of SAM. These results indicate that SAM and norepinephrine bind with PNMT at separate binding sites.

### *Immunochemical studies* (32)

Three samples of PNMT antisera were obtained. One antiserum sample was prepared with the purified enzyme preparation (PNMT antiserum), the other two samples were prepared with enzyme preparations after separate elution of

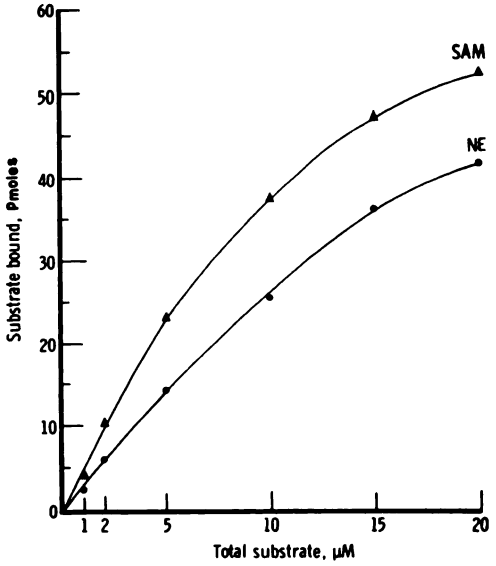


FIG. 12 (*left*). Photograph of polyacrylamide gel electrophoresis of purified PNMT. Experimental conditions the same as described in figure 1.

FIG. 13 (*right*). Complex formation between PNMT and its two substrates; S-adenosyl-L-methionine (SAM), norepinephrine (NE).

the two major isozymes (designated here as $B_1$ and $B_2$) after polyacrylamide disc gel electrophoresis (PNMT antiserum $B_1$, PNMT antiserum $B_2$). Figure 14 shows that PNMT antiserum gives a single precipitin arc with purified bovine PNMT on immunoelectrophoresis (IE). Higher quantities of antigen produce two precipitin arcs with the same antigenic mobility but with different diffusion rate. These findings suggest that the enzyme aggregates to higher molecular weight species. The two major charge isozymes ($B_1$ and $B_2$) are indistinguishable from each other on IE. The immunochemical analyses reveal heterogeneity of adrenal PNMT among different species. After IE the precipitin arc obtained with rats adrenal PNMT had a different antigenic mobility than the arc obtained with bovine adrenal PNMT.

The results in table 2 show that the amount of antiserum which inhibited homologous antigen 95 to 100 % inhibited the enzyme from human pheochromo-

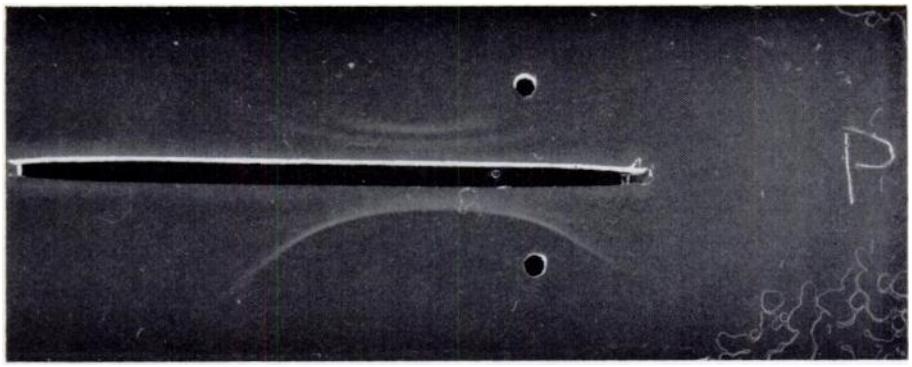

FIG. 14. Immunoelectrophoresis analyses of purified PNMT against rabbit anti-PNMT. Upper well 10 μg of PNMT. Lower well 7.5 μg of PNMT.

TABLE 2

*Specificity of PNMT inhibition by antibody*

| Enzyme Source* | Control Activity |
|---|---|
| | % |
| Bovine adrenals | 0–5 |
| Rats adrenals | 40–50 |
| Frogs adrenals | 120–130 |
| Human pheochromocytoma tumors | 45–50 |
| Mouse neuroblastoma tumors | 50–55 |
| Rats olfactory bulb area | 115–125 |

* Tissues were homogenized in 0.005 M potassium buffer pH 7.3. The homogenates were centrifuged at 40,000 × *g* for 20 min. The volumes in the supernatant were adjusted to contain approximately 0.1 × $10^{-3}$ units of enzyme activity. Aliquots of the supernatant were incubated with 25 μl of PNMT antiserum I for 1 hr at room temperature and bovine-serum albumin was added to maintain a roughly constant protein concentration. After the incubation period the mixtures were centrifuged at 40,000 × *g* for 30 min and aliquots of the supernatant were assayed for enzymatic activity.

cytoma, mouse neuroblastoma and rat adrenals 45 to 60%. No inhibition but rather a slight enhancement of activity was observed upon the addition of the antiserum to PNMT preparation from adrenals of frogs and from olfactory bulbs of rats. It appears therefore that the bovine adrenal antibody does not cross-react with the corticoid uninducible form of the enzyme and that the two forms are immunologically distinguishable.

*Immunohistochemical studies* (18, 19, 27)

Bovine PNMT antiserum was used in the immunohistofluorescent procedure for localization of the enzyme in various tissues. The following tissues were analyzed.

*Adrenal medulla.* In rat and mouse most of the gland cells showed a specific greenish immunofluorescence of moderate intensity which was localized to the

cytoplasm of the cells (fig. 4). In guinea pigs practically all of the gland cells showed a specific immunofluorescence. The cells of the adrenal cortex did not show any specific fluorescence.

By combining the immunofluorescence technique with the histofluorescence technique of Falck and Hillarp it was possible to localize the norepinephrine and PNMT-epinephrine cells on the same section in the adrenals (Hökfelt, Fuxe and Goldstein, unpublished data). The immunofluorescence in the PNMT-epinephrine containing cells is green while the norepinephrine containing cells show an intense yellow fluorescence upon condensation with formaldehyde.

Changes in adrenal PNMT levels after hypophysectomy were analyzed with three different methods; PNMT activity was assayed with the known radioassay procedure (2), the enzyme protein content was assayed with a recently developed immunoassay (M. Goldstein and T. H. Joh, unpublished data), and the enzyme levels in the glands were studied with the use of the immunohistochemical procedure. The PNMT activity, as well as the enzyme protein content, is markedly reduced in the adrenals of hypophysectomized animals. However, the immunohistochemical studies reveal only a slight reduction of the enzyme levels in the gland cells (17, 20) while the number and/or size of gland cells not only in the adrenal cortex but also in the medulla were reduced by 50 % 1 week after hypophysectomy. Thus, atrophic changes in adrenal medulla may contribute to the decrease in the levels of catecholamine synthesizing enzymes after hypophysectomy. These results demonstrate the importance of correlative biochemical and histochemical studies in investigations of the function of the adrenergic system.

## Summary

1. Three enzymes involved in catecholamine biosynthesis, namely, aromatic-L-amino acid decarboxylase (AADC), dopamine-β-hydroxylase (DβH) and phenylethanolamine-N-methyl transferase (PNMT) were purified from bovine adrenal glands. The purified enzymes were used to induce the production of the corresponding immunologically pure antienzymes in rabbits. The latter were utilized for immunochemical studies as well as for localization of catecholamine synthesizing enzymes in peripheral tissue and brain by the indirect immunofluorescent method.

2. Immunochemical studies reveal heterogeneity of DβH and PNMT among different species. Bovine PNMT occurs in different molecular forms and differently charged isozymes were separated from the low molecular form of the enzyme. The PNMT charged isozymes are indistinguishable from each other on immunoelectrophoresis. The corticoid inducible PNMT is immunologically distinguishable from the uninducible form.

3. AADC was localized in all medullary cells and in all catecholamine and serotonin containing cell bodies of the peripheral and central nervous system. DβH was localized in adrenal medullary cells, peripheral and central norepinephrine cell bodies and transected peripheral and central norepinephrine nerve fibers. The primitive catecholamine cell system in the ganglia lacks specific DβH immunofluorescence and seems therefore to contain dopamine and not norepi-

nephrine. PNMT was localized in the cytoplasm of most of the adrenal medulla glands in rats and mice and in all medullary cells in the guinea pig. By combining the immunofluorescence technique with the formaldehyde condensation technique it was possible to separate the norepinephrine containing cells from the epinephrine containing cells in the adrenal medulla of rats.

4. Serum DβH activity was assayed by a sensitive enzymatic procedure. The activity of this enzyme in serum depends on the rate of release of the enzyme from the sympathetic innervated tissues and on the rate of degradation in some sites not yet known. Evidence was presented that serum DβH levels are altered by changes in the sympathetic nervous system functions.

Human serum DβH activity increases with age; infants under 1 year of age have extremely low enzyme activity levels. The enzyme activity was investigated in patients with various disorders. Extremely high values were found in some neuroblastoma patients and low values in some patients with familial dysautonomia. The latter finding implies the involvement of the parasympathetic system in the regulation of serum DβH levels.

5. The effects of hypophysectomy on catecholamine synthesizing enzymes was investigated. The increase in the turnover rate of cardiac norepinephrine after hypophysectomy is associated with an increase in circulatory DβH. Chronic treatment with ACTH restores the turnover rate of cardiac norepinephrine and the serum DβH levels to almost normal levels. PNMT activity as well as the PNMT-protein content is markedly reduced in the adrenals of hypophysectomized animals and histochemical studies reveal a marked reduction in number and/or size of medullary cells. Thus, atrophic changes in adrenal medulla may contribute to the decrease in the levels of catecholamine synthesizing enzymes after hypophysectomy.

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