heat capacity of water. The capacity of such a cloud to extract heat from an object passing through it must, at the same time, be rather greater than would previously have been estimated. The frequency of occurrence of cloud temperatures low enough to furnish heat capacities significantly larger than normal values, lower, that is, than −25°C, is, however, not known to us.

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9 March 1973

Dopamine-β-Hydroxylase Deficits in the Brains of Schizophrenic Patients

Abstract. *Postmortem brain specimens from 18 schizophrenic patients and 12 normal controls were assayed for dopamine-β-hydroxylase (DBH), the enzyme responsible for the final step in norepinephrine biosynthesis. There was a significant reduction in the DBH activity of the schizophrenic group in all brain regions examined. Enzyme deficits in hippocampus and diencephalon were somewhat larger than that in pons-medulla. Since various extraneous factors, such as nonspecific deterioration, drug treatment, duration of hospitalization, cause of death, sex, and age could be ruled out, the deficits in DBH may be associated with the schizophrenic disease process. These findings are consistent with the hypothesis that noradrenergic "reward" pathways are damaged in schizophrenia.*

Recently, we proposed a neurochemical model of schizophrenia in which the fundamental symptoms and chronic course of the disease are assumed to result from a progressive deterioration of central noradrenergic pathways (*1*). Our work is based on Bleuler's (*2*) concept of the disease. To emphasize the inadequacy of central integrative processes, Bleuler coined the term "schizophrenia": "In every case we are confronted with a more or less clear-cut splitting of the psychic functions. If the disease is marked, the personality loses it unity . . . ." According to Bleuler, fragmentation of schizophrenic behavior and personality arises mainly from the fact that the thoughts, feelings, and actions "are not related and directed by any unifying concept of purpose or goal." In the related view of Rado (*3*), the crucial defect is in the organizing action of pleasure or reward; thus, schizophrenic "disorders may be viewed as so many experiments of Nature, showing what happens to central integration in the person whose pleasure resources are inherently deficient."

Research findings in many different fields (*4*) have resulted in the identification of central noradrenergic pathways tentatively associated with pleasure or reward and, hence, with the organization and control of goal-directed thinking and behavior (*5*). Thus, if the analyses of Bleuler and Rado are correct, it would be necessary only to posit the deterioration of this noradrenergic system to explain the chronic disturbance of goal-directed thinking and behavior in schizophrenia. In our model, it accordingly has been assumed that noradrenergic reward pathways and their rich terminal systems in diencephalon and limbic forebrain are the sites of critical damage. We report here postmortem studies of enzymes in human brain that were designed to evaluate the validity of this assumption. The finding that dopamine-β-hydroxylase (DBH) activity is significantly reduced in the brains of schizophrenic subjects is consistent with our proposal that the noradrenergic reward system is damaged in schizophrenia.

Damage to central noradrenergic neurons can be estimated by measuring the DBH in various regions of the brain. The DBH catalyzes the β-hydroxylation of dopamine (*6*), the last step in the biosynthesis of norepinephrine, and is "distributed in brain with a regional pattern of activity that parallels levels of norepinephrine" (*7*). Electrolytic or chemical damage to central noradrenergic tracts causes similar reductions in brain norepinephrine and DBH (*8*). Indeed, the enzyme is used as a marker in immunofluorescence studies to visualize the noradrenergic pathways (*9*). These and other results lead to the conclusion that "in brain, as in the periphery, DBH is intraneuronal and primarily restricted to those nerve cells synthesizing norepinephrine" (*8*). As a first approach, we therefore assumed that measurements of DBH in postmortem samples of human brain could serve as an indicator of the integrity of noradrenergic systems.

Brain specimens were obtained from eight male and ten female patients with a diagnosis of chronic schizophrenia who died at Norristown State Hospital, Norristown, Pennsylvania, at a mean age of 71.2 years (range, 45 to 97). The average duration of hospitalization of these patients was 34.4 years (range, 5 to 60). Control material was obtained through the Medical Examiner's Office, New York City, from six male and six female subjects with no known psychiatric history, who died suddenly in accidents or from heart attacks at an average age of 57.2 years (range, 26 to 80). Care was taken to exclude drug addicts, alcoholics, and suicides from the control group. Also excluded from statistical analysis was one control case with head injury who died after 12 days in coma; at autopsy, anoxic necrosis of the brain was found.

The pons-medulla, diencephalon, and

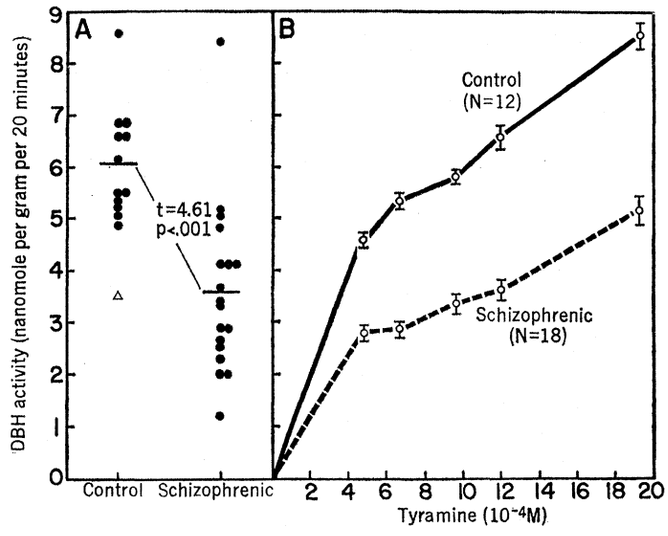

Fig. 1. The DBH activity in postmortem specimens of diencephalon from 18 schizophrenic patients and 12 normal controls. (A) Distributions of individual values of diencephalic DBH. Each point is the average of 30 assays (six determinations at each of five different tyramine concentrations); these values are used for all analyses of diencephalic DBH reported in the text. Horizontal lines indicate means of distributions. Triangle shows DBH activity from a control case which was excluded from statistical analysis because of anoxic necrosis of the brain. The highest value in the schizophrenic group represents the case that had been autopsied 8 days after death. (B) The DBH activity as a function of substrate concentration. Activity in schizophrenic group is significantly reduced ($P < .001$) as compared to control group at every concentration of tyramine. Bars indicate standard errors.

hippocampus were dissected out at autopsy and frozen on Dry Ice. The brain parts were stored at − 15°C in plastic bags for varying periods prior to enzymatic analysis; mean storage time ± standard deviation was 3.3 ± 3.1 months for control specimens and 2.7 ± 1.0 month for schizophrenic specimens. Periodic redeterminations of DBH in several cases revealed that the enzyme was stable under these storage conditions, although reduced activity was observed after storage for more than 1 year. Autopsies generally were performed after postmortem intervals of several hours at room temperature and 1 to 3 days at 4°C. Intervals between autopsy and death were as follows: Four controls and four schizophrenics were autopsied on day of death; four controls and six schizophrenics, 1 day after death; two controls and one schizophrenic, 2 days after death; one control and one schizophrenic, 3 days after death; three schizophrenics, 4 days after death; two schizophrenics, 6 days after death; one schizophrenic, 8 days after death; and for one autopsied control, the time of death was unknown.

To assess the effects of postmortem decomposition on brain DBH, we killed rats with ether and then exposed different groups prior to autopsy to roughly the same conditions of time and temperature as those encountered in our human studies. After 3 hours at room temperature, DBH was reduced by 27 percent. However, storage at 4°C for an additional 1 to 3 days led only to small further decreases in enzyme activity. [Mean DBH activity (nanomole of product per gram per 20 minutes) ± standard error of the mean in whole brain minus neocortex and cerebellum after various storage times was as follows: autopsied immediately, 27.6 ± 2.0; 3 hours at 22°C, 20.1 ± 1.3; 3 hours at 22°C and 1 day at 4°C, 17.3 ± 1.1; 3 hours at 22°C and 2 days at 4°C, 16.9 ± 0.7; 3 hours at 22°C and 3 days at 4°C, 15.0 ± 0.7.]

For human assays, 400-mg samples of pons-medulla and diencephalon, and 150-mg samples of hippocampus, were used. To ensure random sampling, a whole brain region was immersed in its plastic bag in liquid nitrogen and then fragmented by hammering. The fragments were sifted and particles 2 to 3.4 mm in diameter were retained. After weighing, the samples were homogenized and assayed for DBH according to the procedure of Molinoff *et al.* (*10*). Three samples of particles were analyzed in duplicate on different days for an individual brain region (six determinations). Diencephalic samples were assayed at five different tyramine concentrations. Each diencephalic data point used for statistical analysis thus represents the average of 30 determinations.

There was a significant reduction in DBH activity in the schizophrenic group in all brain regions examined (Table 1). The deficits were somewhat larger in rostral regions (diencephalon and hippocampus) where the nerve terminals are localized than in caudal regions (pons-medulla) where the cell bodies are localized. Individual values of diencephalic DBH in the two groups were distributed with little overlap (Fig. 1A); only three schizophrenic values fell in the normal range. Significant deficits in diencephalic DBH were found at all tyramine concentrations tested (Fig. 1B).

To determine whether the reduction of DBH activity in schizophrenic brain was due to nonspecific deterioration, we measured the activity of lactate dehydrogenase, a key enzyme in carbohydrate metabolism with a general distribution in the body. The activity of this enzyme was similar in control and schizophrenic specimens [lactate dehydrogenase activity, assayed by a modification (*11*) of the method of Kornberg (*12*), and expressed as extinction units per 2 minutes per gram of diencephalon: control, 48.3 ± 1.0, $N = 12$; and schizophrenic, 47.0 ± 1.9, $N = 18$]. To control for differences in postmortem storage time prior to autopsy, we again computed the mean DBH of the two groups, after excluding the six schizophrenic cases that had been autopsied 4 to 8 days after death and the control case for which the storage time was not known. Although storage times were now well matched (control, 1.11 ± 0.25 day; and schizophrenic, 1.02 ± 0.23 day), diencephalic DBH was still significantly reduced in the schizophrenic subgroup [control, $N =$ 11, 6.15 ± 0.33 nmole g^{-1} (20 min)$^{-1}$ and schizophrenic, $N = 12$, 3.61 ± 0.31 nmole g^{-1} (20 min)$^{-1}$, $P < .001$]. Indeed, a significant deficit ($P < .02$) was obtained in a test involving only the eight cases that had received autopsies on the day of death.

We also attempted to rule out the possibility that the DBH deficit in the schizophrenic group was an artifact of treatment with antipsychotic drugs. Since various phenothiazine drugs had

Table 1. The DBH activity in postmortem specimens of brain from 18 schizophrenic patients and 12 controls. Enzyme activity is expressed as nanomoles per gram per 20 minutes. The concentration of tyramine used in all assays was $9.6 \times 10^{-4}M$; S.E.M., standard error of the mean.

Region	Control	Schizophrenic	
	Mean activity ± S.E.M.	Mean activity ± S.E.M.	Percentage of control
Pons-medulla	8.86 ± 0.86	6.09 ± 0.61*	70.1
Diencephalon	5.83 ± 0.30	3.38 ± 0.39†	57.9
Hippocampus	2.87 ± 0.52	1.42 ± 0.38‡	49.4

* Differs from control, $P < .02$. † $P < .001$. ‡ $P < .05$.

been administered at one time or another to nearly all of the schizophrenic patients, it was not possible to use our human material to determine whether or not these agents inhibit DBH in the brain. We therefore compared the activity of the enzyme in rats that had been treated with large doses of chlorpromazine with that in untreated controls. Thirteen rats received chlorpromazine hydrochloride (20 mg/kg) each day in their diet for 5 or 12 weeks; ten control rats received only the powdered diet. Chlorpromazine did not inhibit DBH even at these high doses; in fact, after 12 weeks of drug treatment, enzyme activity in the brain was elevated by 16.3 percent ($P < .02$) (*13*).

We next examined the possibility that the greater mean age of the schizophrenic patients contributed to the reduction in DBH. To compare individuals of equivalent age, we again analyzed the data of all normal ($N = 9$) and schizophrenic ($N = 14$) subjects who died between the ages of 45 and 80. Although the mean age at death of these matched subjects was nearly identical (control, 64.1 ± 3.9 years and schizophrenic, 66.8 ± 2.8 years), diencephalic DBH activity was still significantly reduced in the schizophrenic subgroup. The magnitude of the deficit after matching for age was nearly the same as that found originally [age-matched control, 5.89 ± 0.25 nmole g^{-1} (20 min)$^{-1}$ and age-matched schizophrenic, 3.96 ± 0.42 nmole g^{-1} (20 min)$^{-1}$, $P < .005$].

Differences in the cause of death also fail to explain the enzyme deficit in the schizophrenic group. When this factor was controlled by considering only victims of heart attack (a total of five control and nine schizophrenic cases), there was still a significant reduction in the diencephalic DBH activity of the patients as compared to the controls [control, 5.59 ± 0.30 nmole g^{-1} (20 min)$^{-1}$ and schizophrenic, 3.48 ± 0.42 nmole g^{-1} (20 min)$^{-1}$, $P < .01$]. Furthermore, within the control group, the DBH activity in the diencephalon of the five heart attack cases did not differ substantially from that [6.45 ± 0.50 nmole g^{-1} (20 min)$^{-1}$] of the seven accident victims.

Sex was not a significant factor, although females had marginally greater enzyme activity than males in both groups [female control, 6.38 ± 0.58 nmole g^{-1} (20 min)$^{-1}$ and male control, 5.80 ± 0.33 nmole g^{-1} (20 min)$^{-1}$; female schizophrenic, 3.90 ± 0.62 nmole g^{-1} (20 min)$^{-1}$ and male schizophrenic, 3.23 ± 0.48 nmole g^{-1} (20 min)$^{-1}$]. No relation was found between DBH activity in the diencephalon and duration of hospitalization (correlation coefficient = .02), although periods of institutionalization in our sample of chronic schizophrenics ranged from 5 to 60 years. Furthermore, the mean DBH of 12 patients hospitalized for more than 30 years [mean hospitalization, 42.9 years; DBH, 3.53 ± 0.56 nmole g^{-1} (20 min)$^{-1}$] did not differ significantly from that of six patients hospitalized for less than 30 years [mean hospitalization, 17.3 years; DBH, 3.74 ± 0.52 nmole g^{-1} (20 min)$^{-1}$]. These results do not support the idea that the schizophrenic deficit in DBH is due to hospitalization, but final evaluation of this factor must await further study of nonhospitalized schizophrenics and hospitalized controls.

Some physiochemical properties of diencephalic DBH were examined in normal and schizophrenic subjects. The Michaelis constant (K_m) for tyramine was $6.7 \times 10^{-4}M \pm 1.0$ for the 12 control subjects and $7.3 \times 10^{-4}M \pm 1.4$ for the 18 schizophrenics. These values are closely similar and both are almost identical to that previously obtained from normal human blood serum (*14*). The K_m values for the ascorbic acid cofactor also were similar in the two groups (control, $1.4 \times 10^{-4}M \pm 0.1$, $N = 2$ and schizophrenic, $1.1 \times 10^{-4}M \pm .03$, $N = 4$). Diencephalic DBH from both groups showed linearity of reaction with respect to dilution and time up to 30 minutes of incubation. The initial velocity was proportionately lower in the schizophrenic group. Dialysis of control and schizophrenic homogenates for 2 hours in a $0.005M$ tris buffer, *p*H 6, containing 0.1 percent Triton X-100, did not change DBH activity.

The foregoing results suggest that the DBH deficit in schizophrenic patients cannot be attributed to extraneous factors such as nonspecific deterioration, drugs, age differences, cause of death, sex, or duration of hospitalization. The deficit in DBH might therefore be associated with the disease. Whether or not this deficit is specific in schizophrenia remains to be demonstrated. However, in preliminary tests at a single concentration ($19.2 \times 10^{-4}M$) of tyramine, the mean diencephalic DBH activity of seven heroin addicts did not differ significantly from that of the controls (*15*).

The DBH deficits in the schizophrenic group may be explained in several ways. For example, it is possible that schizophrenic DBH is different from and, hence, intrinsically less active than normal enzyme; however, similarities in the kinetic properties and assay parameters of the two enzymes do not support this idea. It is also unlikely that the deficit in the schizophrenic group is due to an excess of endogenous inhibitors. These would have been inactivated in the assay, and dialysis of homogenates did not affect the activity of the enzyme. The deficits could be the result of a defect in DBH synthesis, or they could reflect a more general pathological condition of noradrenergic neurons. The former possibility is not supported by the observation that the deficit varied in magnitude in different regions of the brain (Table 1), since a defect in synthesis probably would have led to a more uniform decrease in regional enzyme activity. Hence, we now favor the idea that noradrenergic neurons are disturbed, or damaged, or fail to develop normally in schizophrenia. Furthermore, because regions rich in nerve terminals exhibited larger reductions in DBH than regions rich in cell bodies, it seems possible that the noradrenergic terminal may be particularly susceptible to the schizophrenic disease process. Such vulnerability of terminals would be consistent with a suggestion that the schizophrenic process involves the formation of 6-hydroxydopamine or a closely related substance (*1*). 6-Hydroxydopamine has been shown in studies on animals to cause selective damage to cen-

tral noradrenergic nerve endings (*16*).

Finally, some therapeutic implications of our findings may be noted. The general aim would be to correct or compensate for the presumed pathology of noradrenergic systems. One could try to increase the formation of DBH, or to promote supersensitivity of noradrenergic receptors (*5*), or even, ideally, to stimulate the regeneration of damaged noradrenergic terminals as, for example, by suitable central administration of nerve growth factor (*17*). Alternatively, in analogy to the use of L-dopa to elevate the central levels of dopamine in Parkinson's disease (*18*), one could try to increase the concentration of norepinephrine in the brains of schizophrenic patients by administration of a precursor (*19*).

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20. We thank J. Axelrod and R. Weinshilboum for guidance in setting up the DBH assay and M. M. Baden and M. H. Book for advice in selecting and obtaining human brain specimens. We also thank D. J. Reis for providing samples of phenylethanolamine-*N*-methyltransferase and A. T. Shropshire, W. J. Carmint, N. S. Buonato, and B. A. Brehmeyer for technical assistance.

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Vasopressin: Possible Role of Microtubules and Microfilaments in Its Action

Abstract. *Colchicine, vinblastine, podophyllotoxin, and cytochalasin B inhibit the action of vasopressin and cyclic adenosine monophosphate on osmotic water movement across the toad bladder. The findings suggest that microtubules, and possibly microfilaments, play a role in the action of vasopressin, perhaps through involvement in the mechanism of release of secretory material from the bladder epithelial cells.*

Vasopressin (antidiuretic hormone) stimulates contraction of certain types of smooth muscle and promotes transcellular water movement—and in some instances sodium transport—across the epithelium of the distal portion of the mammalian renal tubule, and amphibian skin and bladder (*1*). Vasopressin appears to promote water movement across responsive epithelial cells by inducing an increase in the permeability of the membrane at the apical surface of these cells (*2*); this action of the hormone is evidently mediated by cyclic adenosine monophosphate (AMP) (*3*). However, the molecular mechanisms involved in the permeability change induced by vasopressin and cyclic AMP are unknown.

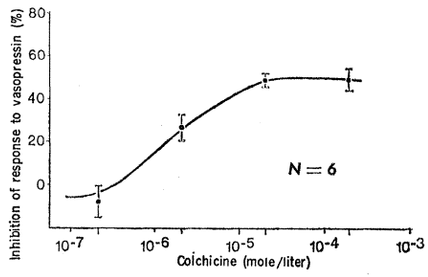

Fig. 1. The inhibitory effect of colchicine on osmotic water movement in response to vasopressin : dose-response curve. Colchicine was added 4 hours before the addition of vasopressin (20 munit/ml). Results are expressed as the percentage of reduction in the response to vasopressin in the colchicine-treated hemibladders relative to their paired controls, measured over a 2-hour period; each point represents the mean ± standard error of six to seven paired experiments.

The idea that an effect on common or analogous mechanisms may underlie the actions of vasopressin on smooth muscle and on epithelial tissues is appealing. Recent studies have suggested that microtubules and microfilaments are associated with several types of intracellular movement (*4, 5*), including chromosome movement (*6, 7*), the transport and release of secretory products (*8–10*), and cytoplasmic streaming (*4, 11, 12*); it has been postulated that these organelles participate in contractile mechanisms analogous to those occurring in muscle (*5, 7, 8, 11, 12*). We have therefore investigated the possibility that the action of vasopressin on the transcellular movement of water and sodium in epithelial tissues is mediated by a mechanism involving microtubules or microfilaments (or both).

The antimitotic agents colchicine, vinblastine, and podophyllotoxin, which exert disruptive effects on microtubules in vivo (*6, 13*) and interact with microtubule subunit protein in vitro (*14, 15*), have been used extensively as tools in the investigation of the role