cessive water loss. However, at air temperatures below 35°C no change in EWL was elicited by heating the brain alone. Thus, the magnitude of the EWL response is dependent on a combination of brain temperature and other central and peripheral body temperatures. This is similar to findings in other reptiles (*1, 4*) as well as in mammals (*10*).

Although reptiles do not have the metabolic or evaporative capacity to continually maintain large temperature gradients between themselves and their environment, it is becoming increasingly evident that they do have much if not all the sensory and integrative capacity for temperature control possessed by the so-called higher vertebrates.

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Dopamine β-Hydroxylase Activity in Brains of Chronic Schizophrenic Patients

Abstract. *Postmortem brain specimens from nine chronic schizophrenic patients and nine controls were assayed for activity of dopamine β-hydroxylase, the enzyme responsible for the conversion of dopamine to norepinephrine. Unlike the results of previous reports, there was no statistically significant difference in enzyme activity between the patient and control groups. There were, however, significant negative correlations between dopamine β-hydroxylase activity and the time spent in the morgue before autopsy, and between enzyme activity of schizophrenics and dosage of chlorpromazine or its equivalent.*

Wise and Stein (*1*) reported that dopamine β-hydroxylase (DBH) activity is low in the autopsied brains of schizophrenic patients. This result is exciting because it is consistent with their hypothesis that brain norepinephrine concentrations are low in schizophrenia, as well as with the popular view that schizophrenia is associated with a hyperactive dopaminergic system. Wise and Stein attempted to control the variables that might have produced this abnormality. However, we report data that are in discrepancy with theirs.

Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at St. Elizabeths Hospital, Washington, D.C. Brain specimens from four chronic schizophrenics and nine controls were obtained by a similar arrangement with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members and from hospital and police records. The control brains were obtained from persons without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The mean age of the schizophrenics (seven males and two females) was 49.2 ± 6.3 [standard error of mean (S.E.M.)]. Four died suddenly after traumatic suicides; three, from cardiac arrest; one, from pulmonary aspiration; and one, suddenly from pulmonary edema. The mean age of controls (all male) was 42.3 ± 4.5. Six died suddenly of trauma; and three, from cardiac arrest.

The periaqueductal pons-mesencephalon, hypothalamus, and hippocampus were dissected out at autopsy and immediately placed on Dry Ice and subsequently stored at − 80°C for up to 1 year [Wise and Stein (*1*) found that DBH activity was stable for 1 year at − 15°C]. There was no difference in mean storage time for the schizophrenics and controls.

Samples (100 to 600 mg) from the dissected brain parts were homogenized in 40 volumes of 0.005*M* tris(hydroxymethyl)aminomethane acetate buffer (*p*H 7.0) containing 0.1 percent Triton X-100 and assayed by the method of Molinoff *et al.* (*2*). For optimal enzyme activity, the final copper sulfate concentrations for both normal and schizophrenic brain parts were as follows: hippocampus, $1.3 \times 10^{-5}M$; hypothalamus, $2.2 \times 10^{-5}M$; and pons, $1.6 \times 10^{-5}M$. All assays were performed by a person unaware of whether the samples were from schizophrenics or controls.

No significant differences between the two groups were found for DBH activity in any of the brain regions (Student's two-tailed *t*-test) (Table 1), nor was there a significant difference when all three regions were taken into account ($P > .50$, multivariate *t*-test) (*3*). Although the differences were not statistically significant, the regional means for the schizophrenics ranged between 77 and 89 percent of control values. The possible reasons for this were explored.

Wise and Stein indicated that the presence of phenothiazines was probably not a cause of the DBH differences between controls and schizophrenics. They gave rats chlorpromazine (20 mg per kilogram of body weight) daily for 12 weeks and found a small increase in DBH activity. In our study, seven of nine patients were taking phenothiazines at the time of death. Although the patients were also taking a number of nonneuroleptic drugs, there were significant ($P < .05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus ($r = -.60$) and pons ($r = -.65$) (*4*). This could mean that the neuroleptics tend to decrease brain DBH activity in schizophrenics, or that there is a negative correlation between severity of clinical disease (as determined by the need for higher drug dosages) and DBH activity.

Wise and Stein attempted to determine the effects of death-to-morgue

intervals on DBH activity (several hours may elapse between death and arrival in the morgue). To simulate these intervals they killed rats and allowed them to remain 3 hours at room temperature before assay. In these rats, DBH activity was reduced 27 percent. In a similar experiment performed in our laboratory, enzyme activity decreased 15 percent in 6 hours at room temperature (*5*). Since Wise and Stein's death-to-morgue intervals for human brains were "several hours" and ours were a mean of 4.2 hours for the controls and 3.0 hours for the schizophrenics, this would not seem to be an important factor, except for one control who did not reach the morgue until 27 hours after death. Since he had the lowest DBH activity of any of the subjects, the DBH activities for controls are presented with and without this subject's values (Table 1). This does not affect the failure to reach statistical significance. Two subjects with death-to-morgue times of 7 and 11 hours had DBH activities higher than the group means. One subject with a 3.5-hour death-to-morgue time had low activity. All other subjects had death-to-morgue times of about 1 hour.

Wise and Stein also simulated the effects on DBH activity of the time between arrival in the morgue and autopsy. They stored rat brains at 4°C for up to 3 days and found a further 18 percent decrease in DBH activity. However, the human brains in their study were kept at 4°C for 1 to 8 days. Because of this, they matched morgue-to-autopsy times for controls and schizophrenics and still found the DBH activity of the schizophrenics to be lower. Our controls were in the morgue for 9.7 ± 1.9 hours, while the patients were there for 33.8 ± 8.2 hours, a statistically significant difference ($t = 2.73$, $P < .02$). Furthermore, for all brains there were significant negative correlations between morgue-to-autopsy time and DBH activity in the hippocampus ($r = -.4$, $P < .06$) and hypothalamus ($r = -.47$, $P < .06$), while that for the pons ($r = -.33$) was nonsignificant. The highest correlation ($r = -.70$, $P < .05$) was between DBH activity in the hypothalamus of schizophrenics and morgue-to-autopsy time (*4*). This suggests that the uneven distribution of morgue-to-autopsy times between the two groups may be responsible for the small nonsignificant differences in DBH activity in our samples. The brains studied by Wise and Stein came from two sources. Although there was no statistically significant difference in our study between the DBH activity of brains of schizophrenics from two different sources, it is possible some of the differences between their groups may be due to different storage techniques.

Table 1. The DBH activity in postmortem specimens from nine schizophrenic patients and nine controls. Enzyme activity is expressed as nanomoles of octopamine formed per gram of tissue per hour. Because the control who had the lowest DBH activity also had the longest death-to-morgue (D-M) time (27 hours), control means are reported with and without values for this subject. All *t*-tests were nonsignificant. The DBH values reported here for humans are three to eight times higher than those reported by Wise and Stein (*1*), although the values for whole rat brain are similar. Our values for rat brain are closer to those for humans than theirs are. The reasons for this discrepancy are not clear.

Region	DBH activity		
	All controls (mean ± S.E.M.)	Controls except one with long D-M time (mean ± S.E.M.)	Schizophrenic (mean ± S.E.M.)
Pons-mesencephalon	77.3 ± 19.5	84.8 ± 20.4	65.0 ± 14.3
Hypothalamus	140.8 ± 26.8	152.4 ± 27.4	118.3 ± 25.6
Hippocampus	39.8 ± 2.72	41.2 ± 2.66	35.5 ± 2.31
Whole rat brain	69.8 ± 2.75		

Studies from our laboratory (*6*) as well as from others (*7*) indicate that there is no difference in DBH activity in the plasma of schizophrenics and controls. In normal monozygotic twins, there is a higher correlation for plasma DBH activity than there is in dizygotic twins (*8*), which indicates that activity of this enzyme is under genetic control. Furthermore, even in monozygotic twins who are discordant for schizophrenia, plasma DBH activity is in the normal range and is highly correlated between twins (*9*).

This is in striking contrast to other diseases in which plasma DBH activity appears to be abnormal (*10*). Mice strains also differ in brain DBH activity (*11*).

Our data suggest that low DBH activity is not a generalized phenomenon in schizophrenia, and our brain data indicate that DBH activity is not very low in the central nervous system of chronic schizophrenics. Measuring postmortem brain enzyme activities is complicated, particularly when comparing normals to schizophrenics. There is no universal agreement about the diagnosis of schizophrenia. The environment of a schizophrenic is certainly different from that of other diagnostic groups, and, except for those who commit suicide or get into serious accidents, schizophrenics normally live to become elderly and have the same problems associated with age that other groups do.

Perhaps the negative correlations between enzyme activity and both morgue-to-autopsy time and neuroleptic dose will explain at least some of the differences between schizophrenics and controls reported by Wise and Stein, as they appear to do in our data. The Wise and Stein hypothesis, however, rests on the existence of a deficit in brain norepinephrine in schizophrenia. Methodology for testing this hypothesis more directly may soon be available (*12*).

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4. Values for *t* and *P* were calculated again with drug dosage and morgue-to-autopsy times used as covariates. Whereas the *t* and *P* values for the pons, hypothalamus, and hippocampus, respectively, were initially 0.79 and $< .50$, 0.81 and $< .50$, and 1.1 and $< .30$, after the covariance they became 0.47 and $< .70$, 0.64 and $< .60$, and 0.4 and $< .70$.
5. Rats were killed by cervical dislocation and whole brains were assayed. DBH activities (mean ± S.E.M., expressed as nanomoles

of octopamine formed per gram per hour) were as follows: rats autopsied immediately, 69.8 ± 2.75; rats autopsied after 6 hours at 22°C, 59.5 ± 4.57.
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In biological research on schizophrenia, it is not unusual for workers with different perspectives to place different interpretations on the same set of facts. Wyatt *et al.* (*1*) find reduced activity of dopamine β-hydroxylase (DBH) in the brains of schizophrenics with deficits that range between 11 to 23 percent, depending on the region examined. Sampling from wider areas of brain, we (*2*) had found larger deficits of between 30 to 51 percent. Because their differences failed to reach statistical significance, Wyatt and his colleagues are inclined to attribute their smaller deficits to postmortem factors and to conclude that their data are "in discrepancy" with ours. For the reasons given below, we believe that the two reports are not necessarily in conflict, and that the results of both studies may be consistent with the hypothesis that schizophrenia is associated with a disturbance of DBH-containing (noradrenergic) neurons in the brain (*2*, *3*).

First, the small sample size and the use of an unnecessarily stringent two-tailed *t*-test reduced the probability of a statistically significant result in the Wyatt replication. Since a one-tailed significance test is appropriate when the direction of the expected difference is known, we recalculated the significance of the group differences in the three regions studied, using data presented in columns 2 and 3 of table 1 in (*1*). Our calculations indicated that the difference in the hippocampus (the region that yielded the largest difference in our study) would occur by chance only 6 times in 100. Wyatt and co-workers also find a significant negative correlation between dose of medication and the schizophrenics' DBH activity in the hypothalamus and pons. Since recommended drug dosage depends in part on the severity of illness (*4*), this finding suggests, as Wyatt *et al.* also recognize, that a positive relationship may exist between the severity of the illness and the deficit in DBH.

These facts, in themselves, are hardly persuasive, but they do open the possibility that a true deficiency in schizophrenics' DBH may have been obtained in the Wyatt study. Analysis of data from individual patients, generously provided to us by R. J. Wyatt, supports this idea and even suggests that the DBH deficiency may be related to diagnosis. Three patients with a diagnosis of "paranoid schizophrenia," all of whom died suddenly after traumatic suicides, were included among the nine schizophrenic cases. These paranoid subjects all had normal or higher than normal DBH scores. The six remaining patients had a diagnosis of "chronic, undifferentiated schizophrenia," and thus resembled the patients we studied. Severe DBH deficiencies in both hypothalamus and pons were found in five of these six "chronic" cases; mean values for the six patients were only 63.6 and 58.8 percent, respectively, of the control values reported in column 2 of table 1 in (*1*). These DBH deficits closely approximate those we obtained. And if Wyatt's sample size had been as large as ours, these group differences would have been statistically significant.

Our analyses convince us that postmortem factors cannot explain the DBH deficiency we observed. As already noted (*2*), we matched control and schizophrenic cases for morgue-to-autopsy time and still found a significant ($P < .001$) DBH deficiency (*5*). In recent animal studies, we simulated the effects on DBH activity of death-to-morgue intervals of up to 24 hours at room temperature. The DBH activity was reduced by only 23 percent after 24 hours, a result that confirms the relatively slow decline reported by Wyatt (*6*). In any event, death-to-morgue intervals were roughly equivalent for all of our schizophrenic patients and most of our controls (approximately 2 to 5 hours). In three control cases, however, this interval exceeded 24 hours. Although the DBH of these "delayed" cases was somewhat lower than that of the remaining controls (5.28 ± 0.13 versus 6.37 ± 0.37 nmole per gram per 20 minutes; difference not significant), it still was significantly higher than that of the 18 schizophrenics (3.61 ± 0.40 nmole per gram per 20 minutes; $P = .05$, single-tailed test).

Finally, Wyatt and his colleagues report absolute values of DBH that are three to eight times higher than those we reported. One reason for the apparent discrepancy may be that Wyatt sampled from relatively localized brain regions (such as hypothalamus) that contain higher densities of noradrenergic innervation than the larger, less densely innervated areas which we examined (such as diencephalon). To test this idea, we measured the hypothalamic DBH of four additional schizophrenic patients. Using Wyatt's assay we obtained a value of 93.5 ± 1.45 nmole per gram per hour for the four subjects. This hypothalamic value is nine times higher than the diencephalic value we had previously reported, and it corresponds precisely to the chronic schizophrenics' hypothalamic value (96.9 ± 34.5) reported by Wyatt (*7*).

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6. Our earlier finding (*2*) of a more rapid decline in postmortem DBH activity was obtained in rats killed by ether, which induces vigorous escape reactions. Rats were killed nontraumatically by CO_2 in the present study, and they were killed instantaneously by cervical dislocation in (*1*).
7. Disparate absolute values for "pons-mesencephalon" in (*1*) and "pons-medulla" in (*2*) may be similarly explained. The discrepancy in hippocampal values does not yield easily to this explanation, unless the dissections were different.
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