and have positively identified 13 of the 14 oligonucleotides in the sequence presented in Fig. 2. The legend to Fig. 2 lists the T1 oligonucleotides identified by Duesberg *et al.* (*5*) and their position within our sequence. All but one of the AMV transformation-specific oligonucleotides fall within the cellular sequence inserted in AMV. Also, all but one of the helper-related oligonucleotides are found beyond the terminator signal TAG of the large open reading frame in the region shared with its helper virus. T1 oligonucleotide D-51, as in (*5*), located between positions 1316 and 1334 is present in both AMV and its helper virus. Comparison of our sequence with that of the RSV envelope region (*6*) reveals that the last 11 amino acids at the carboxyl terminus are shared by the two proteins, suggesting that the AMV gene is incomplete and utilizes the envelope terminator codon. This positions the 3′ terminus of the recombination event at position 1277.

A message generated from the AMV transforming region should direct the synthesis of the transforming protein with the predicted amino acid sequence (Fig. 2). This messenger RNA (mRNA) could be generated either by splicing with the leader sequence derived from the 5′ terminus of genomic RNA or by independent promotion. Splicing is generally used in the synthesis of viral subgenomic messages. Leader sequences identified in MC29 (*9*) and RSV (*6*) cloned proviruses contain the 5′ LTR, a noncoding region, and 18 nucleotides coding for six amino acids of the amino-terminal portion of the viral protein p19 (*6*, *9*). The splice donor portion of these sequences agrees with the consensus splice sequence of eukaryotic genes (*7*).

The alternative model for controlling the expression of the transforming gene would use the transcriptional signals found within the cellular insertion sequences in the region which lies between the polymerase gene and the open reading frame. This type of independent promotion would not utilize the transcription controls of the viral 5′ LTR. Within the regions containing 350 base pairs (bp) in front of the putative leukemogenic sequence we have identified transcriptional signals similar to those present in other eukaryotic genes (*10*, *11*). A six-base AT-rich sequence characteristic of eukaryotic promoters was identified at position 413 to 417, −56 bp from the capping site. Similarly, signals such as the −80 bp region and ribosomal binding sites have also been identified within this region (Fig. 2). The presence of these regulatory signals for transcription implies that the *amv* insert was generated by recombination directly with host DNA and not with a reverse transcript (complementary DNA) of the mRNA coded by chicken sequences homologous to *amv*.

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Schizophrenia: Dopamine β-Hydroxylase Activity and Treatment Response

Abstract. *Cerebrospinal fluid levels of dopamine β-hydroxylase, found to be relatively constant over time in individual patients, were significantly lower in schizophrenic patients who became nonpsychotic during neuroleptic treatment than in those who remained psychotic. Dopamine β-hydroxylase activity may delineate a subgroup of patients who have a dopamine-sensitive brain disorder.*

Many hypotheses about the etiology of schizophrenia have focused on changes in central catecholamine and indoleamine metabolism, and most evidence has indicated a role for dopamine (*1*). Evidence supporting the dopamine hypothesis of schizophrenia, which suggests that there is an increase in dopamine activity in specific central nervous system sites in schizophrenic patients, includes the pharmacological observations that the ability of neuroleptic drugs to block dopamine receptors correlates significantly with their antipsychotic potency (*2*) and that drugs that increase central dopamine function exacerbate schizophrenia (*3*). Many investigators have concluded from such data that the neuroleptic medications act by decreasing the central transmission of dopamine.

Alterations in brain dopamine in schizophrenia could derive from variations in its enzymes of synthesis or degradation. Dopamine β-hydroxylase (DBH), the enzyme that catalyzes the synthesis of norepinephrine from dopamine, has been localized in brain noradrenergic neurons, peripheral sympathetic nerves, and chromaffin granules of the adrenal medulla (*4*). Studies of the adrenal medulla and sympathetic nerve endings show that the enzyme is bound to storage vesicles and is released along with catecholamines by exocytosis (*5*). The serum of humans and other mammals contains DBH activity that arises from the DBH of sympathetic nerve endings (*6*). The DBH activity in cerebrospinal fluid (CSF) may originate from DBH released from brain noradrenergic neurons (*7*). A significant correlation between levels of DBH activity in the CSF and brain has been found in animals (*8*). In any individual, serum DBH activity is extremely constant over time (*9*), and it is likely that DBH activity is under significant genetic control (*10*).

Dopamine β-hydroxylase activity has been studied in serum (*11*), CSF (*12*, *13*), and postmortem brain tissue (*14*) of schizophrenic patients and controls, but differences between these two groups have not been consistently found. The heterogeneity of the clinical syndrome of schizophrenia itself may be the source of such discrepant results, since schizophrenia probably represents a variety of

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disorders, each having a different biological expression. One method of decreasing this heterogeneity is to separate schizophrenic patients by their clinical response to treatment with antipsychotic medication.

Dopamine β-hydroxylase activity was measured in the CSF of 25 drug-free schizophrenic patients during hospitalization at the Clinical Center of the National Institutes of Health. Subsequently, these patients received neuroleptic medication in preparation for discharge. All gave written informed consent for their participation in the research procedures. Two psychiatrists, using Spitzer's research diagnostic criteria (*15*), evaluated the schizophrenic patients before admission and during hospitalization to confirm the diagnosis. A trained nursing staff completed the 15-point Bunney-Hamburg rating scale for psychosis twice daily for each patient (*16*). All patients were placed on the same diet, which was low in monoamines, alcohol-free, and caffeine-restricted, and all patients were drug-free for at least 2 weeks before each lumbar puncture (*17*). Cerebrospinal fluid DBH activity, which was evaluated three times over a 4-month period in each patient, remained markedly stable over time, regardless of the patient's clinical state. The coefficient of variation of CSF DBH activity for individual patients ranged between 3 and 18 percent with a mean ± standard deviation (S.D.) of 9 ± 6 percent. Therefore, the average value from all of the CSF samples for each patient was used as the indicator of that patient's CSF DBH activity. Because a significant positive relation was found between CSF protein and CSF DBH activity in both patients and normal controls, CSF DBH activity is expressed per milligram of CSF protein. The investigators who assayed the samples did not know the patient's treatment state.

The relation between CSF DBH activity and responsiveness to neuroleptic treatment was examined by separating the patients into two groups: one group consisted of 15 patients who became nonpsychotic during treatment (Bunney-Hamburg rating < 4); the other group (ten patients) remained psychotic (Bunney-Hamburg rating > 4), although they may have shown some improvement during treatment. The mean ± S.D. for the CSF DBH activity of the group that became nonpsychotic (0.016 ± 0.005 nmole/ml-hour per milligram of protein) was significantly lower than that of the group that remained psychotic (0.024 ± 0.005 nmole/ml-hour per milligram of protein) (two-tailed *t*-test of independent means: $t(23) = 3.79$; $P < .001$) (Fig. 1). The difference remained significant when not expressed per milligram of CSF protein [0.59 ± 0.37 compared to 1.09 ± 0.70 nmole/ml-hour; $t(23) = 2.28$; $P < .05$]. The mean duration of neuroleptic treatment did not differ significantly between the groups (50 ± 27 days compared to 46 ± 21 days). Patients who remained psychotic received higher doses of neuroleptic drug, as expressed in chlorpromazine equivalents (600 ± 273 compared to 885 ± 532 mg), but the difference was not significant.

We had reported earlier that CSF DBH activity in schizophrenic patients was not significantly different from that in normal controls; however, low levels of DBH in the CSF were significantly related to better social adjustment before the onset of illness, less symptomatology between hospitalizations, and better prognosis (*18*). Crow (*19*) attempted to draw a clear neurobiologic distinction between schizophrenic patients who have good clinical responses to neuroleptic treatment and patients who remain psychotic during such treatment. He proposed that there are two syndromes with distinct disease processes: (i) an acute schizophrenic syndrome with symptoms reversed by neuroleptic treatment, the illness thus being associated with increased dopaminergic neurotransmission, and (ii) a chronic deteriorating syndrome with symptoms not reversed by neuroleptic treatment, the illness being unrelated to dopamine transmission, but possibly related to structural brain changes.

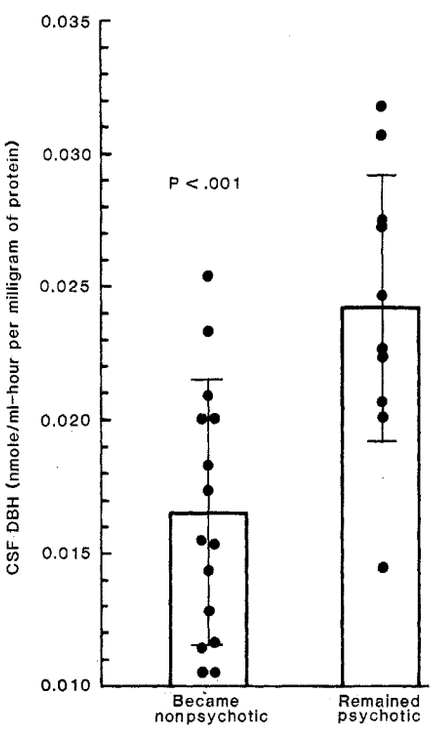

Fig. 1. Dopamine β-hydroxylase activity in the cerebrospinal fluid of schizophrenic patients who became nonpsychotic after chronic treatment with antipsychotic medication compared with that in patients who remained psychotic after treatment.

Low CSF DBH activity appears to delineate a subgroup of schizophrenic patients with the acute "reactive" syndrome (characterized by episodic psychotic behavior in response to stress) (*19*), which is very responsive to antipsychotic medication. Furthermore, although low CSF DBH activity is not specific for schizophrenia—equally low levels were found in some normal controls—such low levels may predict a vulnerability to psychotic decompensation (*20*); that is, low CSF DBH activity alone is insufficient to produce the illness, but it may predispose to a psychotic state.

A relation between drug-induced inhibition of DBH activity and the production of psychotic symptoms has been found in a number of studies [for example, (*21, 22*)]. Disulfiram exacerbates psychotic symptoms in some schizophrenic patients, and previously nonpsychotic alcoholic patients who developed schizophrenic-like psychotic reactions during disulfiram treatment for alcoholism had lower CSF DBH activity levels than those who did not have psychotic reactions (*21*). When fusaric acid, a specific DBH inhibitor, was given to manic patients, there was an increase in psychosis (*22*). Normal human subjects treated with L-dopa to stimulate catecholamine turnover, together with fusaric acid to block the metabolism of dopamine to norepinephrine, developed psychotic symptoms similar to those observed in early acute schizophrenia (*23*). Such reactions to DBH inhibition could be secondary to centrally increased dopamine levels in noradrenergic neurons acting as a "false" neurotransmitter directly on adrenergic systems (*24*) or acting on dopamine systems anatomically nearby.

Different physiological factors, such as protein synthesis, axonal flow, rate of release by exocytosis, neuronal reuptake, entry into and clearance from the CSF, and degradation, all could affect CSF DBH activity (*25*). The stability of DBH activity from one time to another in the same patient is therefore remarkable. Low DBH activity may be characteristic of a subgroup of schizophrenic patients, who—as evidenced by their responsiveness to neuroleptic treatment—may have a hyperdopaminergic disorder (*18*), as

distinguished from patients who are not responsive to neuroleptic treatment and who may have different or additional pathophysiology (*26*). Because DBH is found only in noradrenergic neurons and not in dopaminergic neurons, these findings emphasize the need to include central noradrenergic systems in the study of schizophrenia (*27*).

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Reduced Leucine-Enkephalin–Like Immunoreactive Substance in Hamster Basal Ganglia After Long-Term Ethanol Exposure

Abstract. *Golden Syrian hamsters were placed individually in cages with three drinking bottles—one empty, one containing water, and the third containing water and ethanol. Control hamsters received water only. After 1 year the experimental hamsters showed a significantly lower concentration of leucine-enkephalin–like immunoreactive substance in the basal ganglia than the control hamsters. This finding indicates that the action of ethanol involves endogenous peptidyl opiates.*

The effect of ethanol has been linked with opioids (*1*), and ethanol has been shown to alter neuronal functions, such as the synthesis, release, and degradation of certain neurotransmitters (*2*). Furthermore, ethanol has been described as effectively interfering with the synthesis of brain peptides (*3*). Concentrations of the peptidyl opiates β-endorphin and enkephalins, which are putative neurotransmitters, neuromodulators, or hormones (*4*), have been altered by short- and long-term treatments with various drugs (*5*). Evidence from animal and human studies indicates that ethanol and narcotic drugs interact at similar endogenous peptidyl opiate sites (*6*) and that these drugs could induce euphoria via the endorphinergic system (*7*). We now report that long-term ethanol consumption in hamsters significantly reduces the concentration of an enkephalin-like immunoreactive substance in the basal ganglia. This finding further supports the involvement of peptidyl opiates in the action of ethanol.

In our experiment we used the alcohol-preferring Syrian hamster (*Mesocricetus auratus*) (*8*). Experimental animals ($N = 10$) were placed individually in cages with three drinking bottles. One bottle was empty, the second contained water, and the third contained a solution of water and 5 percent ethanol (*9*). Control animals ($N = 10$) were placed individually in cages in which they had access to water only. After 1 month the concentration of ethanol was increased to 10 percent and kept there for the remaining 11 months of the experiment. The animals were maintained on a photoperiod with 12 hours of light and room temperature (23° to 25°C) throughout the experiment. Consumption of water and ethanol was measured daily.

At the end of the 12-month period, eight experimental and five control hamsters (*10*) were anesthetized with ether and killed by perfusing the brain through the left cardiac ventricle with a 2.5 percent paraformaldehyde Sorensen-buffer (*p*H 7.4) for approximately 10 minutes or until the liver color turned a grayish white.

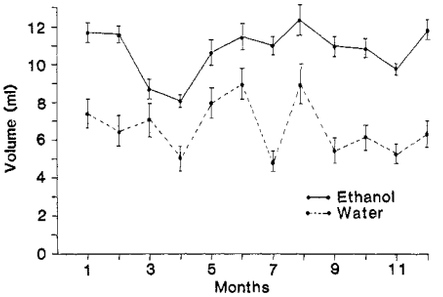

Fig. 1. Consumption of ethanol and water by the eight surviving experimental hamsters over the 1-year period. Values are means ± standard errors.

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