A Test of the Transmethylation Hypothesis in Acute Schizophrenic Patients

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Keeping biochemical determinations and clinical judgments independent, the authors investigated three aspects of the transmethylation hypothesis. They found that 26 acutely schizophrenic patients were no more likely to have bufotenine or N,N-*dimethyltryptamine present in urine or elevated serum indolethylamine* N-*methyltransferase activity than 10 normal control subjects. The authors conclude that these are naturally occurring substances which are equally likely to be present in normal and schizophrenic subjects.*

THE TRANSMETHYLATION HYPOTHESIS of schizophrenia has received extensive attention in psychiatric investigations during the past two decades. In 1952 Osmond and Smythies (1) proposed that altered transmethylation of catecholamines could produce methylated amines with hallucinogenic properties, which might account for some forms of schizophrenia. The subsequent isolation of a methylated amine, 3,4-dimethoxyphenylethylamine, from the urine of schizophrenic patients by Freidhoff and Van Winkle (2) was therefore met with enthusiasm, although the status of the pink spot is now controversial and its etiological significance in schizophrenia is doubtful (3).

Several reviews have summarized the rationale and evidence for a relationship between schizophrenia and abnormal methylation of catecholamines or indoleamines (3–7). The reported findings include the following: 1) demonstration of indolethylamine *N*-methyltransferase (INMT) in brain (8–10), lung (11), and platelets (12); 2) findings of psychotomimetic amines, e.g., *N,N*-dimethylserotonin (bufotenine) and *N,N*-dimethyltryptamine (DMT), in the urine of schizophrenic patients but less or none of these substances in control subjects (3, 13–16); 3) reports of psychotic symptoms being produced when these psychotomimetic amines are administered to humans (17, 18); and 4) exacerbation of psychotic symptoms when methyl donors are administered to schizophrenic patients, especially when they are given with a monoamine oxidase inhibitor (3, 19, 20).

Despite the suggestive evidence of these investigations, the validity of the transmethylation hypothesis of the pathogenesis of schizophrenia remains unproven. Most studies have suffered from methodologic shortcomings, including poorly controlled and inadequately standardized biochemical assay techniques, failure to observe double-blind conditions, and absence of controls for factors of dietary intake, drug treatment, chronicity, and institutionalization. Attempts at replication of the reported findings are made more difficult by the fact that criteria for patient selection are often insufficiently described and comparability of subjects thus cannot be assured. These methodological problems were critically reviewed in 1971 by Wyatt and associates (3).

The study of hallucinogen-induced psychosis as a model for schizophrenia is also severely limited (21–23). Although hallucinogens can produce unusual perceptual experiences, these are recognized by clinicians as being different from the mental state in schizophrenia. Furthermore, subjects who have experienced both schizophrenia and drug-induced hallucinosis regard them as distinct experiences.

This report is based on two subject populations: drug-free acute schizophrenic patients and normal control subjects. Three aspects of the transmethylation hypothesis were tested: 1) urinary DMT would be present in schizophrenic patients but not in normal controls, 2) bufotenine would be detected in the urine of schizophrenic patients but not in the urine of normal control subjects, and 3) the activity of an *N*-methylating enzyme would be greater in serum from the schizophrenic patients.

GENERAL METHOD

The schizophrenic subjects in this investigation were hospitalized at the Clinical Center of the National Insti-

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tutes of Health on an inpatient unit designed for the treatment and investigation of acute psychoses. Patients were admitted to the center early in a psychotic episode if they met the following criteria: 1) the presumptive diagnosis was schizophrenia; 2) there was no evidence of organic disease; 3) they were between the ages of 18 and 60, and 4) they manifested delusions, hallucinations, thought disorders, or bizarre behavior. Subjects generally had adequate work and social function prior to the illness episode. Most patients had received brief courses of medication prior to admission.

The diagnoses in this study were made by the project's senior psychiatrist (W.T.C.), using criteria from *DSM-II* (24) and based on all relevant data obtained during a detailed three-week clinical assessment that included a semi-structured mental status examination, the Psychiatric Assessment Interview. This is a slightly abridged version of the Present State Exam, the reliability and applicability of which have been described elsewhere (25). The diagnosis of schizophrenia was further buttressed by the finding of an average of 6.2 points on a 12-point system with increasingly stringent criteria (26) for identifying schizophrenic patients: the approximate probabilities of 6 and 7 points being associated with a schizophrenic diagnosis are 11:1 and 40:1, respectively. Furthermore, 23 of these 26 patients were assigned to either a schizophrenic or paranoid psychosis category by CATEGO, a computerized classification based on a European diagnostic model (25, 27). All diagnosed schizophrenic patients admitted between August 1972 and August 1973 (N = 26, 15 women and 11 men) were included in the sample. Their mean age was 24.2 years (SE = 1.9). Nine of the 26 had a family history of mental illness.

The control group consisted of 7 college students (2 men and 5 women) and 3 male staff members. The students, normal volunteer subjects living on an inpatient unit at NIH, had no evidence of physical or mental disorder and no family history of mental illness. They ate the same low-monoamine diet that the patients received. The 3 staff members had no family or personal history of mental disorder and no evidence of organic disease. The control subjects had a mean age of 23.5 years (SE = 2.1).

Patients were off all medication between admission and the collection of urine and blood samples during their fourth week of hospitalization. Four complete 24-hour urine collections and 30 ml of serum were obtained. Another 30-ml serum sample was collected just before discharge, which again followed a drug holiday of at least 3 weeks. Nursing and medical staffs emphasized psychosocial treatment and management techniques, thus permitting the use of this drug-free research methodology.

Subjects in the normal control groups were generally free of medication, and a minimum 3-week drug-free period was assured in all cases. Urine and serum collections for control subjects were similar to those for schizophrenic patients, with the single exception that the interval between the two serum collections was 3 days rather than the several month interval in the illness and recovery phase patient studies.

All specimens were immediately coded and frozen, with patient and control samples intermixed. Batches of 20 to 25 specimens, still frozen, were flown from Bethesda, Md., to Galesburg, Ill., where biochemical analyses were carried out without information regarding the sample source (patient or control), the time of collection (admission or discharge), and whether samples were from the same or different subjects. Clinical ratings, diagnoses, and assessment of patients and controls were recorded without knowledge of biochemical results.

BIOCHEMICAL METHOD

The details of biochemical methods for the qualitative and quantitative assessments of the dimethylated tryptamines have been described in earlier publications (15, 28). The tertiary amine fraction was extracted from 90 percent of a concentrated 24-hour urine collection. Two 10-μl aliquots out of the 100 μl containing the tertiary amine fraction were used for two-dimensional thin-layer chromatography on silica gel G and cellulose with *o*-phthalaldehyde (29) and *P*-dimethylaminocinnamaldehyde (30), respectively, as spray reagents. The remainder of the fraction was converted to the trimethylsilyl derivative (31) and assayed for DMT and bufotenine by monitoring the selected ion at mass/electron charge 58 on a gas chromagraphic-mass spectrometric system (GC-MS) (15).

To determine INMT activity in serum samples, a mixture containing 50 mg of lyophilized serum, 1 mM *N*-methyltryptamine, and 25 μg of *S*-adenosyl[methyl-^{14}C]methionine (cpm 80,000) in a total volume of 0.25 ml of 0.5 M phosphate buffer, pH 7.9, was incubated at 37° C for 60 minutes. Appropriate serum blanks without *N*-methyltryptamine were incubated simultaneously. At the end of incubation, the pH of the mixture was adjusted to 11 with 4 drops of aqueous 2 N sodium hydroxide and extracted with 10 ml of ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate, evaporated to dryness, and redissolved in 100 μl of ethyl acetate. A 50-μl aliquot and an internal standard 1 μl (0.5 μg) of cold DMT were spotted on a silica gel G plate and the plate was developed in solvent system chloroform-methanol-ammonium hydroxide (12:7:1). The DMT spot was scraped and radioactivity was measured in a scintillation spectrophotometer (32). The second 50-μl aliquot was used for the identification of DMT by monitoring the selected ion during GC-MS (15).

RESULTS

Subjects were scored as bufotenine- or DMT-positive if at least one of the four 24-hour urine collections contained the respective compound (see table 1). Bufotenine was identified significantly more often in the urine of the control group ($p<.05$, Fisher exact probability test). When detected in urine, bufotenine was quantitatively

similar in patients and controls. Mean levels of bufotenine in patients and controls were 1.67 and 1.73 μg/24 hours, respectively (silica gel method), or 1.14 and 1.71 μg/24 hours (GC-MS method). Unpaired t tests yielded no significant differences in either case ($p<.25$). There was also little difference in urinary DMT scores between patients and control subjects ($p<.6$, Fisher exact probability test). We compared the mean and standard deviations for the transmethylating enzyme INMT in the patient group (admission and discharge) and the control group (two specimens). One-way analysis of variance revealed no difference in INMT activity between the patients at admission (1,117±145 cpm), patients at discharge (1,219±192 cpm), and controls at first sampling (1,092±185 cpm) ($p<.25$). The second control sample was not significantly different from either patient sample or from the first control sample. Furthermore, a paired t test comparing the 13 patients who showed good clinical improvement between admission and discharge testing revealed no difference in enzyme activity ($p<.5$).

The relationship between the presence of urinary hallucinogenic compounds and the level of serum enzyme is summarized in table 2. All subjects were rank-ordered by level of transmethylating enzyme and divided into two groups, i.e., above and below the median. There was no significant difference in the number of bufotenine- or DMT-positive subjects in the two groups.

Data from the patient group were subjected to further analysis. The 26 patients were grouped according to whether they had been scored as 1) bufotenine positive or negative, 2) DMT positive or negative, and 3) transmethylating enzyme (INMT) activity above or below the median. An analysis of variance was performed using the 73 clinical variables in appendix 1. Few significant differences were found. Severity of psychopathology was greater in the bufotenine-negative group ($F=11.09$, $df=1/15$, $p<.005$). There was greater impairment of interpersonal relationships in the DMT-negative group ($F=12.59$, $df=1/9$, $p<.01$). The higher enzyme activity group had more nonsocial speech ($F=4.75$, $df=1/19$, $p<.05$), while the lower enzyme activity was associated with worse personal relationships ($F=4.70$, $df=1/19$, $p<.05$), more somatic concerns ($F=13.49$, $df=1/19$, $p<.005$), and greater impairment of insight ($F=5.17$, $df=1/19$, $p<.05$). Thus a wide range of clinical, premorbid, and sociodemographic variables were tested for a relationship to the presence of bufotenine or DMT in the urine or the level of INMT activity in the serum. Notable findings include the following: 1) a small, perhaps chance, number of significant associations between the biochemical and clinical variables, and 2) where significant differences were found, the patient group *without* the suspected biochemical abnormality generally had the higher psychopathology score.

TABLE 1
Comparison of Schizophrenic and Normal Subjects, by Presence of Bufotenine and DMT in Urine

Urinary Substance	Schizophrenic Patients	Normal Controls
Bufotenine		
Positive	6	8*
Negative	12	2
DMT		
Positive	4	4
Negative	8	5

*Bufotenine was identified significantly more often in the urine of the control group ($p<.05$, Fisher exact probability test).

TABLE 2
Relationship Between Serum Enzyme Activity and Urinary Bufotenine and DMT

Assay Assignment Grouping*	Enzyme Activity Grouping**	
	Above Median	Below or Equal to Median
Bufotenine		
Positive	6	7
Negative	7	6
DMT		
Positive	4	4
Negative	3	8

*No significant differences were found between the enzyme-activity-based groupings for presence or absence of either bufotenine or DMT.
**The median was derived from INMT activity on admission for all schizophrenia patients with either bufotenine or DMT assays and from first-sample INMT activity in all controls.

DISCUSSION

In this carefully controlled investigation, 26 drug-free acute schizophrenic subjects did not have bufotenine or DMT present in their urine more often than 10 control subjects. Serum INMT activity was similar in the two groups. These findings do not support the hypothesized abnormal transmethylation in the pathogenesis of schizophrenia.

Bufotenine has been reported in the urine of clinically normal subjects (3). In a recent study (34), however, the subjects were the parents and grandparents of previously studied autistic children (35). Our finding of urinary bufotenine and DMT in the control groups is noteworthy since our control subjects were carefully screened to assure the absence of both psychopathology and a family history of psychiatric disorders. The enzyme findings are not surprising in view of the previously documented presence of INMT in many human tissues studied in both normal and schizophrenic subjects (8–12, 36, 37).

CONCLUSIONS

The hallucinogenic substances DMT and bufotenine are present in the urine of some drug-free acutely psychotic schizophrenic patients. Comparison with normal

control subjects, however, reveals that these compounds are at least as prevalent in subjects who are drug free and without illness and have no family history of mental illness. An *N*-transmethylating enzyme measured in serum had similar activity in acute schizophrenics and normal controls. Multiple clinical variables related to diagnosis, mental status, and prognostic and sociodemographic features generally were not found to be significantly associated with the presence or absence of hallucinogenic amines in urine or transmethylating enzyme activity in serum. When associations were found, they were generally in a direction opposite to that which would be expected on the basis of the transmethylation hypothesis. Finding these substances in the normal control group as well as the schizophrenic patients clearly fails to support the hypothesis that DMT and bufotenine are abnormally produced in the schizophrenic population. Based on these data, one would assume that these are normally occurring substances which are neither qualitatively nor quantitatively more likely to be found in an acute schizophrenic population than in normal individuals. This study therefore lends no support to the hypothesized abnormal transmethylation in the pathogenesis of acute schizophrenia. Since this conclusion rests primarily on finding DMT and bufotenine in the urine of normal control subjects, one would expect that chronic schizophrenic patients would not be qualitatively different from normal subjects, although further controlled studies are needed to ascertain whether a quantitative difference exists.

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APPENDIX 1

Sociodemographic Variables Used in Analysis of Variance

VARIABLES

1. Age at admission
2. Hollingshead and Redlich education code (33)
3. Hollingshead and Redlich occupation code (33)
4. Social class
5. Family history of mental illness
6. Patient history of alcohol abuse
7. Patient history of drug abuse
8. Patient history of childbirth or abortion within six months prior to hospitalization
9. First-rank symptoms
10. 12 differential symptom systems (26)
11. Age at first psychotic symptom
12. Age at first hospitalization
13. Total duration of hospitalizations prior to NIH (months)
14. Number of prior hospitalizations

PROGNOSTIC SCALE

15. Quantity of useful work
16. Personal social relations
17. Treatment facilities
18. Action problems
19. Flat expression or emotion
20. Severity of subjective distress
21. Time since onset of symptom
22. Presence of thought disorder, hallucinations, delusions
23. Presence of depression, hypomania, or mania
24. Precipitating events
25. Total

ADMISSION SPECIAL FEATURES

26. Anhedonia
27. Excessive dependency
28. Absence of insight
29. Impairment of interpersonal relations
30. Impaired reality testing
31. Lack of impulse control
32. Impairment of stimulus barrier

ADMISSION PSYCHOPATHOLOGY SCALE

33. Cognitive disorganization
34. Severity of psychopathology
35. Type of psychopathology

PHILLIPS SCALE

36. Recent sexual adjustment
37. Social aspects of sexual adjustment
38. Social aspects of recent sexual life
39. History of personal relations
40. Recent adjustment in personal relations

DIMENSIONS

41. Depression
42. Anxiety
43. Reported restlessness
44. Observed restlessness
45. Retarded speech
46. Retarded movement
47. Hypomania
48. Somatic concerns
49. General suspiciousness
50. Observed belligerence
51. Reported belligerence
52. Obsessions
53. Unkempt appearance
54. Disorientation
55. Lack of insight
56. Depersonalization—derealization
57. Delusions of reference and persecution
58. Grandiose delusions
59. Delusions of passivity
60. Depressive and nihilistic delusions
61. Other delusions
62. Visual hallucinations
63. Auditory hallucinations
64. Other hallucinations
65. Bizarre behavior
66. Withdrawal
67. Incomprehensibility
68. Nonsocial speech
69. Flat affect
70. Labile affect
71. Incongruous affect
72. Delusions about hallucinations
73. Rapport