Biological Psychiatry, Vol. 13, No. 6, 1978

Altered Metabolism of the Methionine Methyl Group in the Leukocytes of Patients with Schizophrenia

Latife Ismail,[1,2] Thornton Sargent III,[1,5]
Ernest L. Dobson,[1,3] and Myron Pollycove[4]

Received October 29, 1977; revised March 22, 1978

Previous work has indicated that abnormal methylation processes may be associated with schizophrenia. In this study, leukocytes from patients with schizophrenia were incubated with methyl-^{14}C-L-methionine and the evolved $^{14}CO_2$ measured. With increasing concentration of methionine, the evolved $^{14}CO_2$ was lower in the patients than in normal control subjects. The incorporation of ^{14}C into protein was the same in both groups, and when carboxyl-^{14}C-L-methionine was used the evolved $^{14}CO_2$ was the same in both groups, thus excluding the possibility that altered incorporation into protein or oxidation of the methionine molecule as a whole were responsible. The observed differences in methionine-methyl metabolism suggest that an abnormality in transmethylation processes or in oxidation of the methyl group to CO_2 is associated with schizophrenia. That this occurs in a peripheral tissue indicates that the abnormality is not restricted to the central nervous system.

INTRODUCTION

The transmethylation hypothesis of the etiology of schizophrenia, first stated by Osmond and Smythies (1952), proposed that a metabolic error results

This work was supported by the Biology and Medicine Division of the Department of Energy.
[1]Donner Laboratory and Lawrence Berkeley Laboratory, University of California, Berkeley, California.
[2]Present address: The Office of the President of the Turkish Federated State of Cyprus, Lefkosa, Mersin 10, Turkey.
[3]Deceased.
[4]Clinical Laboratories, San Francisco General Hospital, Department of Clinical Pathology and Laboratory Medicine, University of California School of Medicine, San Francisco, California.
[5]Correspondence should be directed to Dr. Thornton Sargent III, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.

0006-3223/78/1200-0649$05.00/1

in production of methoxylated psychotoxins from adrenalin (epinephrine). Many studies since that time have been designed to test the hypothesis. Pollin *et al.* (1961) first reported that feeding of methionine with an MAO inhibitor exacerbated the symptoms of some schizophrenics. Altogether, ten such studies have been reviewed by Cohen *et al.* (1974), who concluded that there were sufficient cases in all of the studies "to lend weight to the possibility that an aberrant transmethylation of biogenic amine precursors plays some role in the pathogenesis of schizophrenia." In one study of methionine loading, concommitant MAO-inhibitor treatment was not required to exacerbate the symptoms (Antun *et al.*, 1971), and in another, not included in the summary of Cohen *et al.*, schizophrenic symptoms were induced by methionine in folate-deficient patients, also without MAO inhibitors (Stahelin and Winchell, 1969). The methyl carbon of methionine is the major methyl donor in metabolic reactions (Meister, 1965). It appears in methylated metabolites; it is converted to methanol and thence to formaldehyde, both of which may be oxidized to CO_2; formaldehyde may condense with indolethylamines to form β-carbolines (Meller *et al.*, 1974), some of which are known to be psychotomimetic; and it is incorporated into other amino acids which, together with the labeled methionine itself, are incorporated into protein.

Rosengarten and Friedhoff (1976) have recently reviewed the theories and experimental reports involving methylation of neurotransmitters and the biosynthesis of hallucinogens as part of the etiology of schizophrenia. They have reviewed and discussed in considerable detail enzymes that have been described in mammalian systems which are capable of forming psychotomimetic substances *in vitro*. These include enzymes which *N*-dimethylate indolethylamine to form dimethyltryptamine; di-*O*-methylation of catecholamines by COMT and guaiacol-*O*-methyltransferase; mescaline-forming enzymes in brain and liver; and enzymes which form tetrahydrobetacarbolines. Demethylation reactions have been much less studied, but a recent report by Tyce (1977) has shown that *O*-methyl metabolites of catecholamines are demethylated *in vivo* by erythrocyte lysates. Many methoxylated biogenic amines have been considered as possible endogenously produced psychotoxins, including indoles (Mandell *et al.*, 1971) and catecholamine analogs (Shulgin *et al.*, 1969). Friedhoff and van Winkle (1962) first reported the presence of 3,4-dimethoxyphenethylamine (DMPEA) in the urine of schizophrenic patients. The presence of this methylated amine, presumably a product of dopamine, has been controversial ever since. A recent report by Braun *et al.* (1974), using highly sensitive methods in a longitudinal study, found that DMPEA occurred at some time in every patient with schizophrenia studied, and in none of the controls. Their finding of periodic rather than regular production of DMPEA would explain the contradictory reports of others, and keeps open the question of DMPEA as an abnormal metabolite in schizophrenia. The dynamics of certain metabolic processes may be determined by administering compounds with a specific site labeled with ^{14}C and

measuring the expired $^{14}CO_2$ resulting from metabolism at the labeled site. This technique is particularly applicable to reactions involving a single methyl group. Previous work with this method included separately labeling the methoxyl groups of DMPEA and of the psychotomimetic agent 2,4,5-trimethoxyphenylisopropylamine (TMA-2) (Sargent *et al.*, 1976). The rates and total fraction of demethylation at each labeled site provided suggestions as to the mechanism of demethylation and its possible involvement in psychotomimetic action. In another study, intravenous administration of methyl-^{14}C-L-methionine to schizophrenic patients showed significant differences, compared to normal subjects, in the dynamics of expired $^{14}CO_2$, which were interpreted as resulting from altered metabolic methylation processes (Israelstam *et al.*, 1970).

The methylation and demethylation of catecholamine and indolic neurotransmitters thus comprise a recirculating pool of methyl groups. Evidence is lacking, however, to show whether a defect in these processes is causally related to schizophrenia. The purpose of the work reported here was to determine whether differences in the metabolism of methyl groups could be detected by measuring the production of $^{14}CO_2$ from methyl-^{14}C-L-methionine, using leukocytes from patients with schizophrenia. Leukocytes were chosen for this study because they possess the major cell organelles and enzymatic systems, they are readily obtainable without risk from patients, and they can be utilized readily in a variety of *in vitro* studies.

MATERIALS AND METHODS

Clinical Material

The study included 11 patients with schizophrenia and 7 normal control subjects. All of the patients had been examined and diagnosed in accordance with the *Diagnostic and Statistical Manual of Mental Disorders* (APA, 1968). The seven control subjects were healthy adult volunteers who were not taking any drugs, and who were without history of psychiatric disturbances.

Reagents

1. Heparin: 1000 units/ml, with 0.9% benzyl alcohol as preservative (Lipohepin) for anticoagulation of blood samples (Riker Laboratories, Northridge, Calif.).

2. 3% Dextran (MW 100,000–200,000 clinical grade) in normal saline.

3. Hanks-fetal calf serum (FCS) solution: 10 ml Hanks, balanced salt solution (Grand Island Biological Co. [GIBCO], Grand Island, N.Y.), 20 ml

fetal calf serum (GIBCO), 1 ml sodium bicarbonate solution, 7.5% (GIBCO), q.s. to 100 ml with distilled water.

4. 0.9% saline.

5. 3.5% saline.

6. Special minimum essential medium (MEM): To 100 ml of methionine-free MEM (GIBCO) add 1 ml L-glutamine, 200 mM (GIBCO) and 1 ml penicillin, 5000 units/ml, and streptomycin, 5000 μg/ml (GIBCO).

7. L-methionine-methyl-^{14}C (specific activity 11 mc/mM) (New England Nuclear Corporation, Boston, Mass.).

8. L-methionine-carboxyl-^{14}C (specific activity 58 mc/mM) (Amersham/Searle, Arlington Heights, Ill.). Specific activity adjusted to 11 mc/mM by the addition of nonradioactive L-methionine.

9. 2 M acetate buffer, *p*H 3.8.

10. CO_2-trapping solution: One volume 2-aminoethanol to 2 volumes 2-methoxyethanol (Mallinckrodt/Nuclear, St. Louis, Mo.).

11. Scintillation fluid: 2 liter toluene, 1 liter 2-methoxyethanol and 16.5 g PPO (2,5-diphenyloxazole) scintillation grade (J. T. Baker Chemical Co., Phillipsburg, N.J.).

12. 15% trichloroacetic acid (TCA) solution.

13. Absolute ethanol.

14. Nuclear/Chicago Solubilizer (NCS) solution (Amersham/Searle).

All glassware was siliconized and sterilized by autoclaving.

Isolation of Leukocytes from Whole Blood

Blood from fasting patients and controls was drawn into heparinized plastic syringes, and was mixed with the 3% dextran solution in 40-ml centrifuge tubes in the ratio of 2:1 blood to dextran solution. After gently inverting the tubes seven times, the mixture was allowed to stand for 30–40 min at room temperature to permit adequate sedimentation of the red blood cells. The supernates were collected and centrifuged at 100 *g* at room temperature for 5–7 min. All subsequent centrifugations for this phase of the study were performed in this manner.

The cell button was then washed twice with the Hanks-FCS solution in order to obtain partially purified leukocytes. The cells were resuspended in 4 ml of 0.9% saline. Lysis of the residual RBC was accomplished by the addition of 12 ml of sterile H_2O, and mechanical agitation was achieved by aspiration and expulsion through a serologic pipette. Hypotonicity and mechanical agitation were limited to 30 sec to minimize leukocyte damage. Isotonicity was restored by the addition of 4 ml 3.5% saline. Following the addition of 5 ml special MEM, the cell suspension was centrifuged. The red cell ghosts were

removed by aspiration of the cell button surface. The separated leukocytes then were resuspended in the special MEM, and were diluted to a concentration of $2.0 \pm 0.5 \times 10^7$ cells/ml. Counts of the separated cells were done using a hemocytometer. Differential white cell counts were carried out on both the whole blood and the isolated leukocytes. Granulocytes comprised 80–90% of the separated cells. All of the isolated leukocytes were viable, as demonstrated by the trypan blue dye exclusion test.

Incubation of the Cells

One-milliliter aliquots of the cell suspension in special MEM were transferred into 25-ml Erlenmeyer flasks which contained 2 ml special MEM, and the flasks were closed with an airtight rubber cap. After preincubation for 30 min at 37 C in a shaking water bath (50 cycle/min), methyl-^{14}C-L-methionine or carboxyl-^{14}C-L-methionine, 0.5 to 20 μc, which contained from 0.54 to 0.938 μmole/methionine, was injected into each flask. Cell suspensions from each subject were incubated at one to four different concentrations of methionine. In order to determine the amount of radiochemical contaminants in, or spontaneous decomposition of, the ^{14}C-methionine preparations, the labeled substrates were also incubated with special MEM without leukocytes. At the end of the 2-hr incubation period, metabolic activity of the cells was terminated by the addition of 2 ml of the acetate buffer to each flask. The CO_2 evolved was collected in the system described below.

$^{14}CO_2$ Collection

Approximately 8.5 ml of the CO_2 trapping solution was dispensed into preweighed 40-ml test tubes fitted with rubber stoppers through which two tubes passed: a dispersion tube extended to a level well below that of the CO_2 trapping solution, and a short tube extended to the air space above the trapping fluid. After the dispersion tube was connected to the incubation flask by plastic tubing, vacuum was applied to the space above the CO_2 trapping solution in order to maintain a continuous gentle air flow through the incubation flask and trapping solution. In order to monitor the trapping efficiency of this system for CO_2 a second tube containing trapping solution was connected in series with the first. Counts in this second tube were never significantly above background. At the end of the collection period, the tubes were reweighed, and 4-ml aliquots of the trapping solution were weighed into counting vials. Fifteen milliliters of scintillation fluid were added to each vial, and ^{14}C activity was quantitated in a liquid scintillation counter with sufficient counts to obtain a standard error of 2.5%.

Table I. Clinical and Therapeutic Data

Diagnosis	Age	Sex	Duration of illness (years)	Clinical manifestations[a]				Drug therapy	
				Auditory hallucination	Disturbance of affect	Thought disorder	Depression	Drug	Dose/day
1. Mental retardation & chronic undifferentiated	25	F	4	+	0	+	0	Haloperidol	2 mg
2. Schizo-affective	38	F	16	?	+	+	+	Fluphenazine Methyprylon	7.5 mg 300 mg
3. Chronic undifferentiated	40	M	7	?	+	+	+	Mesoridazine	100 mg
4. Childhood schizophrenia, mental retardation	19	M	10	+	+	+	+	Fluphenazine	5 mg
5. Schizo-affective, depressed	51	F	> 3	+	+	+	+	Amitriptyline Perphenazine Pentobarbital Trihexyphenidyl hydrochloride Diphenhydramine	75 mg 6 mg 100 mg 6 mg 150 mg
6. Residual schizophrenia	36	M	>14	+	+	+	+	Niacinamide	1 mg
7. Chronic undifferentiated	26	F	5	+	+	+	+	None	
8. Residual schizophrenia	33	M	15	0	0	+	+	None	
9. Schizo-affective circular	26	M	20	0	+	+	+	Trifluoperazine hydrochloride Diazepam	4 mg 15 mg
10. Residual schizophrenia	47	F	>10	0	0	0	0	None	
11. Chronic undifferentiated	22	F	3	+	+	+	+	Haloperidol Diazepam	4 mg 15 mg

[a] +, Present; 0, none; ?, unknown.

Assessment of Incorporation of ^{14}C into Cellular Protein

After the $^{14}CO_2$ collection period, the cells were centrifuged at 0 C and washed twice with ice-cold saline; 1 ml of chilled TCA solution was added and the reaction mixture allowed to stand for 30 minutes at 0 C. The resulting precipitate was centrifuged and washed once with the cold TCA solution, and once with 1 ml absolute alcohol. To the washed precipitate was added 1 ml NCS solution to digest the protein. After addition of 15 ml scintillation fluid, the ^{14}C content of the digest was quantitated in the manner described above.

RESULTS

Data describing the 11 schizophrenic patients as to their major diagnostic category, duration of disease, major clinical manifestations, and drug therapy are shown in Table I. Three of the patients were not on drug therapy at the time of the study.

The rates of oxidation of methionine-methyl carbon to CO_2 by leukocytes from all of the control subjects and schizophrenic patients are shown in Fig. 1. These rates of oxidation are shown for different methionine concentrations in the incubation medium, which range from 17.8 to 312 μM/liter. Included in Fig. 1 is a plot of the least-squares best-fit of each group, fitted to the function $ax/(b + x)$.

The rate of methionine-methyl oxidation showed a direct correlation to the methionine concentration in the incubation medium. However, the rate of oxidation by the leukocytes of the patients with schizophrenia was less than that of the normal group for each concentration of methionine. The difference was small at low concentrations, but progressively increased with increasing concentrations of methionine.

The rates of oxidation of the carboxyl carbon of methionine to CO_2 by leukocytes from two normal subjects and two schizophrenic patients are shown in Table II. These preliminary studies were performed with a concentration of carboxyl-^{14}C-L-methionine of 93.7 μmole/liter in the incubation fluid. A relatively wide difference in oxidation of the methyl carbon by the two groups (normals and patients) was found at this concentration (Fig. 1). However, unlike the difference between the patients and normals in oxidation of the methyl carbon by the leukocytes, no significant difference in oxidation of the carboxyl carbon of methionine was noted.

The incorporation of ^{14}C-methyl carbon into protein (and other TCA precipitable material) by leukocytes as a function of methionine concentration in incubating media is shown in Fig. 2 for both control subjects and schizophrenic patients. While a progressive increase in incorporation is noted with an

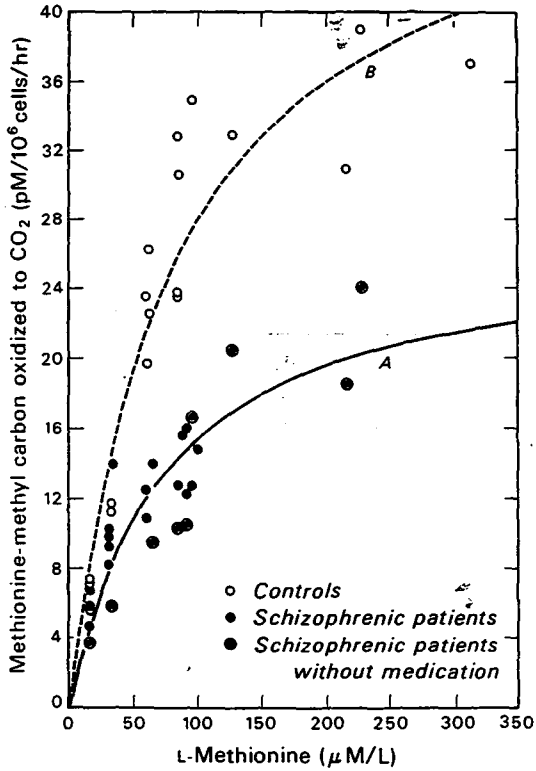

Fig. 1. The rates of oxidation of methionine-methyl carbon to CO_2 (pM/10^6 cells/hr) by leukocytes from control subjects, schizophrenic patients, and schizophrenic patients without medication, as a function of the concentration of methionine (μmole/liter) in the incubation medium. Curve A is the best fit for the schizophrenic patients, B for the control subjects.

Table II. Oxidation of the Carboxyl Carbon of Methionine[a] by Leukocytes from Control and Schizophrenic Patients

Subject	Oxidation of carboxyl-carbon of methionine pM/10^6 cells/hr
Control	12.3
Control	12.1
Schizophrenic	12.0
Schizophrenic	12.1

[a] Methionine concentration in incubation fluid of 93.7 μmole/liter.

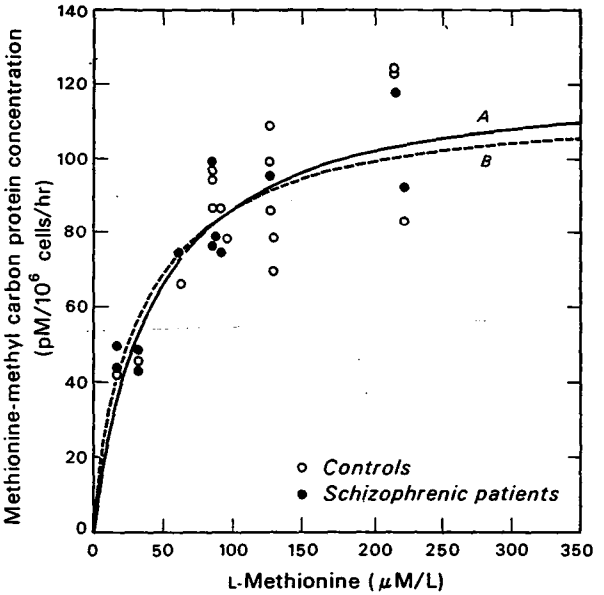

Fig. 2. The rate of incorporation into protein of methionine-methyl carbon (pM/10^6 cells/hr) by leukocytes from control subjects and schizophrenic patients as a function of the concentration of methionine (μmole/liter) in the incubation medium. The best fit curve for the schizophrenic patients is A; for the control subjects B.

increasing concentration of methionine, no significant difference is noted between normal and patient groups.

DISCUSSION

The decreased oxidation of the methyl-carbon of methionine which we have observed in the leukocytes of schizophrenic patients could be explained by any of the following: (i) decreased transmembrane passage of methionine into cells or intracellular organelles; (ii) a failure of one or more enzymes involved in methyl transfer reactions; (iii) a change in size of one or more of the metabolic pools involved with the oxidation of methionine; (iv) an increase in the pool size of methylated metabolites; (v) increased incorporation into protein, and (vi) an increased methionine pool size.

The fact that the oxidation of the carboxyl-labeled methionine was the same in the leukocytes of patients and normals speaks against (i) and (vi) as explanations, since each of these processes would, if altered, also change the

oxidation of the carboxyl carbon. An enlarged pool of methionine, as in (vi) or its metabolites as in (iii), is also unlikely because experimentally increasing the concentration of methionine in the medium resulted in an increasing difference between patients and normals in the rate of oxidation. If a pool size difference were responsible for the divergence of the curves in Fig. 1, such an artificial enlargement of the methionine pool would be expected to decrease rather than increase the divergence. An increased incorporation into protein (v) is eliminated by our findings that incorporation of methyl^{14}C-L-methionine into protein of leukocytes is the same in the patients with schizophrenia as in normals.

We are left with two possible explanations, (ii) or (iv), out of the six postulated. A failure of an enzyme, as in (ii), is what one would anticipate if schizophrenia is a genetically determined metabolic disorder. The enzyme systems involved in the methyl-transfer cycles have been reviewed by Mudd (1975); of particular interest is the methylation of acceptor molecules, as discussed earlier. The other half of this cycle, demethylation of such molecules, is less well understood, but has been observed in several *in vivo* systems. Demethylation of catecholamines or close analogs has been reported by Sargent *et al.* (1967, 1976), Frère and Verly (1971), Tyce (1977), and Zweig and Castagnoli (1977), although it has not been shown that this occurs by the action of an enzyme. Frère and Verly (1971) noted "As the *p*-*O*-methylamine compounds seem to be endowed with some toxic action for the central nervous system, their preferential destruction could be physiologically important to correct some noxious result of the catechol-*O*-methyltransferase inactivation of catecholamine." Failure of such a demethylating enzyme was proposed as a possible basis of schizophrenia by Sargent *et al.* (1967). This mechanism would satisfy both postulates (ii) and (iv), in the latter case by producing an enlarged pool of methylated metabolites, in which the ^{14}C-methyl group would not reenter the one-carbon pool and the oxidation to $^{14}CO_2$ would be correspondingly reduced, in agreement with the results reported here. Alternatively, failure of an inhibitory mechanism for a methylation process could also lead to an enlarged pool of methylated metabolites. The demethylation process and the presumed demethylating enzyme deserve further study.

The lack of any consistent difference between those three patients who did not receive drugs and the eight patients who received a variety of drugs suggests that medication did not significantly affect the results. If transmethylation does play a role in schizophrenia, it also suggests that drug therapy does not alter transmethylation processes in the direction of normal.

These initial findings of altered methionine-methyl metabolism in the leukocytes of schizophrenic patients suggest an abnormality in transmethylation processes and/or an alteration in the metabolic pathways for the oxidation of this methyl group to CO_2. The fact that this abnormality was demonstrated in leukocytes suggests that the metabolic disorder associated with schizophrenia

may be manifested in the general metabolism of the body. If these preliminary findings are confirmed by further studies, they not only will support the hypothesis of a transmethylation defect as a biochemical basis for schizophrenia, but also suggest a possible new approach to the study of this disease.

ACKNOWLEDGMENT

We would like to thank Dr. Tod H. Mikuriya for diagnosis and referral of the patients. Thanks are also due to Patricia Garbutt, Catherine Allan, and Carol Bohlen for the blood withdrawals and cell counts.

REFERENCES

American Psychiatric Association (1968). *Diagnostic and Statistical Manual of Mental Disorders,* 2nd ed., APA, Washington, D.C.

Antun, F. T., Burnett, G. B., Cooper, A. J., Daly, R. J., Smythies, J. R., and Zealley, A. K. (1971). The effects of L-methionine (without MAOI) in schizophrenia. *J. Psychiat. Res.* 8: 63.

Braun, G., Kalbhen, D. A., Muller, J., and Vahar-Matiar, H. (1974). Nachweis und Bedeutung der intermittierenden Ausscheidung von 3,4-Dimethoxyphenylathylamin (DMPEA) im Harn von patienten mit akuter schizophrenie. *Arch. Psychiat. Nervenkr.* 218: 195.

Cohen, S. M., Nichols, A., Wyatt, R., and Pollin, W. (1974). The administration of methionine to chronic schizophrenic patients: A review of ten studies. *Biol. Psychiat.* 8: 209.

Frère, J. M., and Verly, W. G. (1971). *O*-Demethylation of noradrenaline and demethylation in rat blood. *Biochim. Biophys. Acta* 235: 85.

Friedhoff, A. J., and Winkle, E. V. (1962). Isolation and characterization of a compound from the urine of schizophrenics. *Nature* 194: 897.

Israelstam, D. M., Sargent, T., Finley, N. N., Winchell, H. S., Fish, M. B., Motto, J., Pollycove, M., and Johnson, A. (1970). Abnormal methionine metabolism in schizophrenic and depressive states: A preliminary report. *J. Psychiat. Res.* 7: 185.

Mandell, A. J., Buckingham, B., and Segal, D. (1971). Behavioral, metabolic and enzymatic studies of a brain indolethylamine *N*-methylating system, in *Brain Chemistry and Mental Disease,* Ho, B., and McIsaac, W. (eds.), Plenum Publishing Corp. New York.

Meister, A. (ed.) (1965). *Biochemistry of the Amino Acids,* Vol. II, Academic Press, New York, pp. 757-818.

Meller, E., Rosengarten, H., and Friedhof, A. J. (1974). Conversion of C^{14} S-adenosylmethionine to C^{14} formaldehyde and condensation with indoleamines: a side reaction in *N*-methyltransferase assay in blood. *Life Sci.* 14: 2167.

Mudd, S. H., and Poole, J. R. (1975). Labile methyl balances for normal humans on various dietary regimens. *Metabolism* 24: 721.

Osmond, H., and Smythies, J. (1952). Schizophrenia: A new approach. *J. Ment. Sci.* 98: 309.

Pollin, W., Cardon, P. V., Jr., and Kety, S. S. (1961). Effects of amino acid feedings in schizophrenic patients treated with iproniazid. *Science* 133: 104.

Rosengarten, H., and Friedhoff, A. J. (1976). A review of recent studies of the biosynthesis and excretion of hallucinogens formed by methylation of neurotransmitters or related substances. *Schizophrenia Bull.* 2: 90.

Sargent, T. W., Israelstam, D. M., Shulgin, A. T., Landaw, S. A., and Finley, N. N. (1967). A note concerning the fate of the 4-methoxyl group in 3,4-dimethoxyphenethylamine (DMPEA). *Biochem. Biophys. Res. Commun.* 29: 126.